Clinical effects of the glucagon-like peptide-1 receptor agonist exenatide in patients with type 2 diabetes

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"Curiosity is the essence of human existence and exploration has been part of humankind for a long time."

Eugene Andrew Cernan (born March 14, 1934)
Commander of Apollo 17

Aan mijn ouders…

Contents

Chapter 1

General introduction and rationale for this thesis

Only recently, our appreciation of type 2 diabetes mellitus has evolved from 'a relatively benign old people's condition' to a disease that globally threatens the life and well-being of a growing and ever younger proportion of the population (1, 2). Due to changing lifestyles, particularly in the developing world, obesity, in adults and children alike, is increasing at an alarming rate (3, 4). The World Health Organization has calculated that in the year 2030 over 350 million people will be diagnosed with type 2 diabetes world wide (3). In the Netherlands this number is expected to increase from 670.000 people in 2008, to approximately 1.3 million in 2025 (5).

Type 2 diabetes is characterized by progressive pancreatic beta-cell failure against a background of obesity-related insulin resistance (6, 7). Data from the United Kingdom Prospective Diabetes Study (UKPDS) showed that irrespective of the therapy used (8), glycemic control worsens over time as a result of progressive loss of pancreatic beta-cell function (9), whereas the severity of insulin resistance remains relatively stable (10, 11). The aforementioned progressive nature of type 2 diabetes makes the treatment of patients with type 2 diabetes challenging (12, 13). In addition to lifestyle interventions, that appear most effective but hard to implement and sustain (14), oral anti hyperglycemic agents are usually the first pharmacological step in the treatment of type 2 diabetes (15, 16). Unfortunately, as pancreatic beta-cell function deteriorates at an estimated rate of approximately 4% per year (11), a combination of multiple glucose lowering agents, and eventually insulin therapy, are often needed to achieve and maintain blood glucose at target values (13). Although, traditionally available therapeutic options reduce blood glucose concentrations, and subsequently improve pancreatic beta-cell function by (temporary) alleviating glucose toxicity (17), none of these available anti hyperglycemic agents changes the progressive nature of type 2 diabetes (**Figure 1.1**) (13).

Type 2 diabetes is associated with a high rate of microvascular (18, 19) and macrovascular (18, 20-22) morbidity and mortality, which imposes a tremendous burden on individuals and societies, with regard to quality of life, healthcare resources and socioeconomic aspects (4). Data from the UKPDS shows that the development of microvascular disease is reduced when glycemic control is improved and has helped establish treatment targets for glycemia in type 2 diabetes (23). Current therapeutic interventions aim to lower HbA_{1C} concentrations as close as possible to the normal, physiological range (approximately less than 6.5%) without imposing a high risk of severe hypoglycemia (13). Taken all together, the now widely used therapeutic options for patients with type 2 diabetes patients make up an unmet medical need.

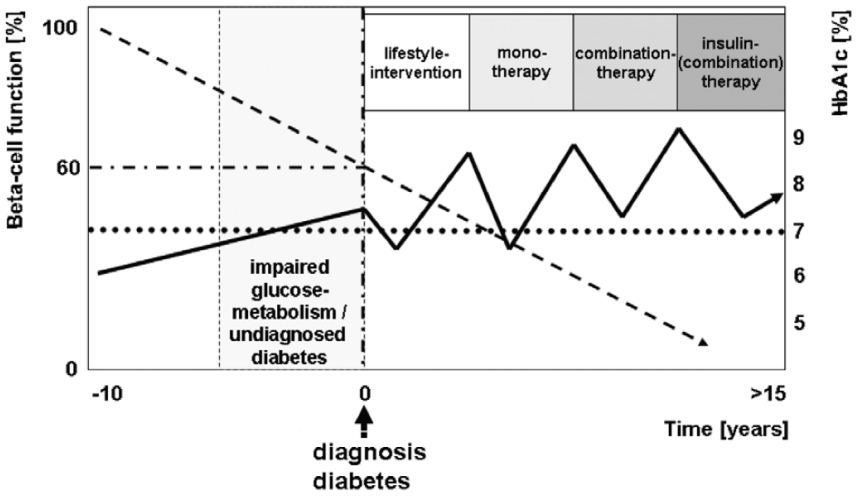

Figure 1.1 Current therapeutic implications of progressively declining beta-cell function in type 2 diabetes. At the time of diagnosis, beta-cell function is reduced by 40–50% in type 2 diabetes and continues to decline over the years (hatched line), irrespective of treatment modality. According to the present guidelines, HbA_{1c} above 7% (dotted line) should trigger therapeutic action, which may result in HbA_{1c}-lowering, however, this effect is only temporary and soon the next therapeutic step is needed (solid line). Adapted from Van Genugten (24).

Over the last decade scientific research focusing on the pathophysiology and the development of new pharmacotherapeutic options for type 2 diabetes, has greatly expanded (25). Traditionally, peripheral insulin resistance, increased hepatic gluconeogenesis, and pancreatic beta-cell failure were considered core elements in the evolution of type 2 diabetes (1). However, the pathophysiology of type 2 diabetes is complex, and disease progression is linked to factors independent of insulin deregulation and reduced first-phase insulin response (12). New discoveries in both the molecular background of type 2 diabetes, and pharmaceutical sciences have provided us with a colorful pallet of therapeutic options, in addition to traditionally used agents as metformin, sulfonylurea and insulin. These agents target all organs that contribute to the complex nature of type 2 diabetes (**Figure 1.2**) (1, 2, 26). In alignment with the changing view on pathophysiology, focus of interest has shifted from insulin resistance as a therapeutic target, to deranged islet cell homeostasis, in particular the preservation and improvement of pancreatic beta-cell mass and progressive loss of beta-cell function (26).

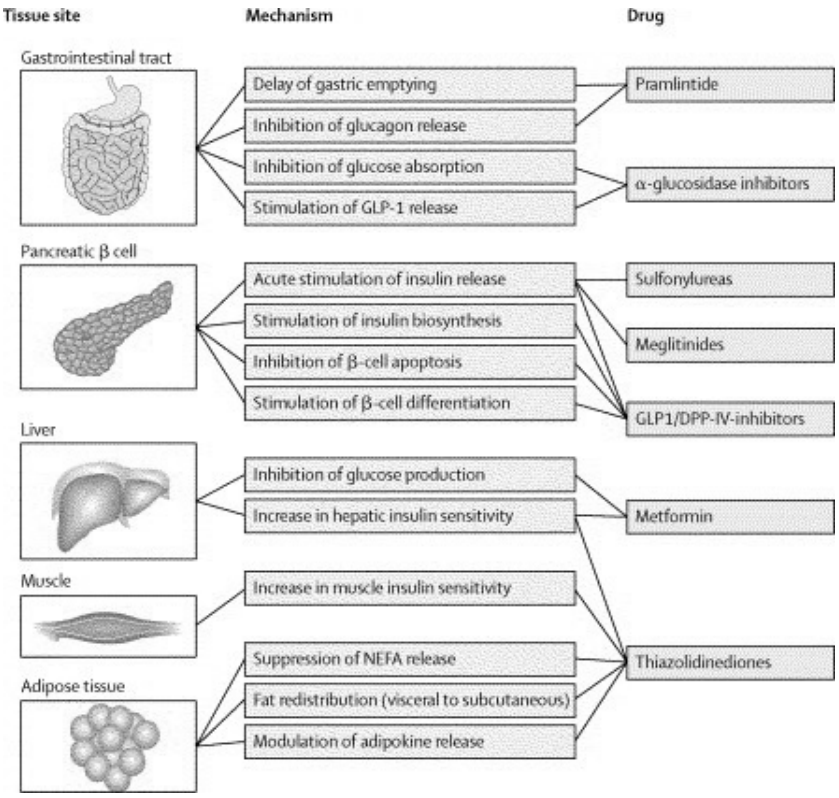

Figure 1.2 Pharmacological treatments of type 2 diabetes according to the site of action. Adapted from Stumvol (1).

It has been hypothesized that a treatment successfully addressing multiple elements of the underlying defects in type 2 diabetes would be an ideal intervention (12). To achieve this, a more holistic therapeutic approach may be needed enclosing treatment for both the beta-cell function and cardiovascular complications. This may involve the early initiation of >1 pharmacotherapeutic agent to target the underlying defects of the disease (13, 27). Additionally, future therapeutic interventions may include treatments addressing the decreased activity of incretins, as seen in patients with type 2 diabetes (28).

The incretin concept

During the early days of the 20th century the idea rose that after ingestion of a meal, a gastro-intestinal 'messenger' stimulated the enhancement of carbohydrate processing in the pancreas (29). To test this early hypothesis, already then, experiments were conducted studying gut extracts as a

possible therapy for diabetes. Moore et al. treated three patients with diabetes mellitus with an extract ('given by the mouth') derived from the mucous surface of the upper three or four feet of the small intestine of a pig (30). Shortly afterwards, this presumed endocrine pancreas stimulating, gastro-intestinal humoral activity was named 'incretin' by La Barre (31). Following the discovery of the radio-immunoassay of insulin in 1956 (32), the incretin concept (re)gained popularity again. Intravenous glucose infusions appeared to result in a smaller pancreatic insulin response compared to jejunal infusion of the same amount of glucose (33). The connection between the gut and the islets of Langerhans in the pancreas was called 'entero-insular axis' (34). Finally, Creutzfeldt defined an incretin as a substance released upon nutrient ingestion, which at physiological concentrations stimulates pancreatic insulin release in the presence of a raised blood glucose concentration (35).

The identity of factors responsible for the entero-insular axis remained unclear until the identification and characterization, glucose-dependent insulinotropic polypeptide (GIP) in the 1970s (36, 37). Initially, GIP appeared to be an inhibitor of gastric acid production; hence it's early name gastric inhibitory polypeptide (38). Due to the fact that the observed increase in insulin secretion was only seen in the presence of hyperglycemia, the proteins name was changed from gastric inhibitory polypeptide into glucose dependent insulinotropic polypeptide (39). Removal of GIP from gut extracts by immune-absorption method did not eliminate the incretin effect. This gave evidence for the existence of other factors with incretin-like activity (40). About a decade later, the second incretin hormone to be discovered was glucagon-like peptide-1 (GLP-1). GLP-1 is a product of the proglucagon gene and shares a 50% similarity with the amino acid sequence of glucagon (41). The proglucagon gene is expressed in the islet of Langerhans, the L-cells in the small intestine and specific areas of the central nervous system, predominantly the nucleus of the solitary tract (42, 43). L-cells are specialized entero-endocrine cells, located in ileum and colon, and somewhat less frequent in the distal jejunum and duodenum (44, 45). Proglucagon is processed in a tissue specific manner: in the pancreas predominantly into glucagon and into GLP-1 and GLP-2 in the L-cells, brainstem and hypothalamus (42). GLP-1 has 2 bioactive isoforms, GLP-$1_{(7-37)}$ and GLP-$1_{(7-36)}$-amide. Both GLP-1 and GIP are part of the glucagon-like superfamily and have both structural and functional similarities. GLP-1 release is regulated by endocrine, neuronal and nutrient related stimuli in a biphasic manner. Oral intake of a meal, containing carbohydrates or fat results in a rapid increase in the plasma GLP-1 concentration with a peak after approximately one hour and account for 50–70% of total meal-related

insulin secretion. It is thought that GLP-1 release is under the influence of indirect endocrine and nerve induced signals, as the GLP-1 producing L-cells are predominantly located in the more distal part of the gastrointestinal tract, and GLP-1 secretion occurs before nutrients have reached the majority of the L-cells in the ileum and colon (44, 46, 47). Taken together with the finding that the rapid phase of GLP-1 secretion is preserved in patients after ileal resections or proctocolectomy, it is hypothesized that the early release of GLP-1, which accounts for the majority of the incretin effect in normal individuals, is triggered by local luminal nutrient-sensing pathway in addition to neuronal stimuli (48). Biological characteristics of both GIP and GLP-1 are shown in **Table 1.1**.

Glucose-dependent insulinotropic polypeptide	**Glucagon-like peptide-1**
42-amino acid peptide released from the duodenum	30/31-amino acid peptide released from distal small bowel and colon
Stimulates insulin secretion	Stimulates insulin secretion
Minimal effect on gastric emptying	Inhibits gastric emptying
Stimulates glucagon secretion	Inhibits glucagon secretion
No regulation of satiety and body weight	Inhibits food intake and weight gain
Promotes expansion of beta-cell mass in preclinical models	Promotes expansion of beta-cell mass in preclinical models
Normal GIP secretion in type 2 diabetes	Reduced or normal secretion of GLP-1 in type 2 diabetes
Defective GIP response in type 2 diabetes	Preserved but impaired GLP-1 response in type 2 diabetes

Table 1.1 Comparison of the biological properties of glucose-dependent insulinotropic polypeptide (GIP) with glucagon-like peptide-1 (GLP-1). Adapted from Diamant (49).

The incretin concept, regained focus of interest as a therapeutic option for patients with type 2 diabetes over the last decade (44, 50). Nauck and coworkers showed, in their landmark study, that the insulin secretory response to an oral glucose load was more enhanced when compared to an isoglycemic (i.e. in the presence of similar circulating glucose concentrations) intravenous glucose infusion. This phenomenon is called the incretin effect (51). In patients with type 2 diabetes, the incretin effect is significantly impaired and it was shown that postprandial GLP-1, but not GIP, secretion is attenuated (51). GLP-1 administration stimulates insulin secretion in both healthy and type 2 diabetes individuals, in a glucose-dependent manner, whereas the insulinotropic effect of GIP infusion in patients with type 2 diabetes is lost (44). This observed loss of insulin potentiating effect of GIP played an important role in pursuing GLP-1, and not GIP, as an agent for the treatment of type 2 diabetes. In addition, in both healthy individuals and patients with type 2 diabetes, GLP-1 slows down gastric emptying (52, 53),

inhibits glucagon (54) and somatostatin (55) secretion, promotes satiety and reduces food intake (56), and reduces body weight (57, 58) (For review see: (24, 44, 45, 59)). Experimental data, shows that GLP-1 increases beta-cell mass (60-65) and clinical studies show that exogenous GLP-1stimulates glucose-dependent insulin secretion by pancreatic beta-cells (41, 66, 67). Collectively, these characteristics render GLP-1 as an extremely attractive option in the treatment of patients with type 2 diabetes (68-70).

Therapeutic potential of GLP-1 and GLP-1-based therapies

GLP-1 exerts its action through G-protein coupled receptors located in the islets of Langerhans, gastrointestinal tract, pituitary gland, hepatocytes, and heart (28). GLP-1 decreases the blood glucose concentration through various mechanisms, which act upon several organs (**Figure 1.3**).

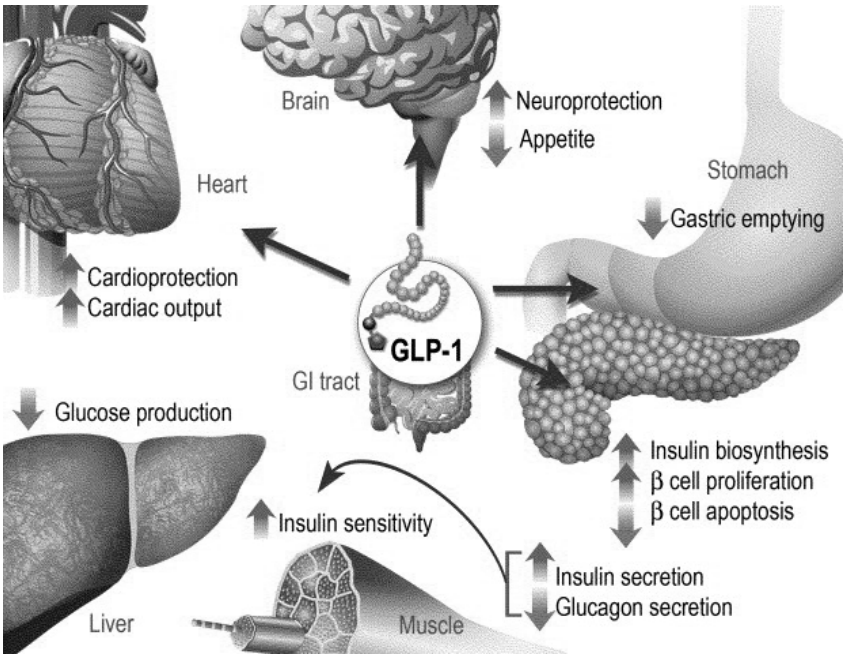

Figure 1.3 The actions of GlP-1 at different organs. Adapted from Drucker (45).

Primarily, GLP-1 potentiates insulin secretion and production by the pancreatic beta-cell, in a glucose-dependent manner (28). GLP-1 lowers glucagon secretion, slows down gastric emptying and induces satiety, resulting in less food intake and a reduction of body weight. Additionally, GLP-1 may be beneficial in attenuating the endothelial dysfunction, which

underlies many of the cardiovascular complications of diabetes (71). Taken together, the biological actions of GLP-1 seem to replenish the phenotype as seen in patients with type 2 diabetes (**Table 1.2**).

Type 2 diabetic phenotype	Actions of GLP-1
Impaired beta-cell function	↑ Insulin secretion and biosynthesis Improved beta-cell function Up regulated other genes essential for beta-cell function
Reduced beta-cell mass	↑Beta-cell proliferation/differentiation ↓Beta-cell apoptosis *(shown only in animal studies and in vitro)*
Glucagon hyper secretion	↓Glucagon secretion
Overeating, obesity	↓Gastric emptying, ↑satiety, ↓appetite →↓Food intake & ↓ body weight
Macrovascular complications	Beneficial cardiovascular effects (direct and indirect)
Insulin resistance	Improvements in insulin sensitivity

Table 1.2 The therapeutic potential of glucagon-like peptide (GLP)-1 in type 2 diabetes. The various actions of GLP-1 match the defects of the type 2 diabetes phenotype. Adapted from Van Genugten (24).

In both healthy subjects and patients with type 2 diabetes, continuous subcutaneous and intravenous GLP-1 administration lowers the plasma glucose concentration. However, this effect depends on the continuous administration of GLP-1 as it disappears 2 hours after cessation of GLP-1 infusion. In their landmark study, Zander et al compared a 6-week continuous subcutaneous GLP-1 (via an insulin-pump) to saline administration in a group (n=10) of type 2 diabetic patients. At 6 weeks, GLP-1 significantly reduced both fasting and postprandial plasma glucose concentrations, as well as the HbA_{1c} value (by 1.3%) from baseline (58). Furthermore, GLP-1 reduced appetite and food intake, resulting in 1.9 kg weight reduction as compared to placebo. Beta-cell function and insulin sensitivity improved during the 6-week treatment period.
However, circulating GLP-1 is quickly inactivated by the ubiquitous enzyme dipeptidyl peptidase (DPP)-4, a proteolytic enzyme that cleaves the two N-terminal amino acids of biologically active peptides (72-74). After cleaving GLP-1 is removed from the circulation by hepatic clearance and renal excretion (75, 76). Due to its degradation by DPP-4, GLP-1 only has a half-

life of several minutes (73). Because of its fast degradation by DPP-4 and its short half-life, necessitating continuous infusion, native GLP-1 is not a preferred therapeutic option. Therefore, two new pharmacotherapeutic approaches have been developed to harness the therapeutic potential of GLP-1 in clinical practice: 1) DPP-4 inhibitors which increase the circulating concentration of endogenous GLP-1 (77) and 2) DPP-4 resistant GLP-1 receptor agonists, which potently stimulate the GLP-1 receptor at pharmacological levels (**Table 1.3**) (78). By now, multiple compounds of both these two new classes have been approved by the FDA and the EMA and have become available for the treatment of patients with type 2 diabetes mellitus over the last years.

Dipeptidyl peptidase-4 inhibitors

Dipeptidyl peptidase-4 is an enzyme expressed on many cells that rapidly degrades circulating endogenous GLP-1 (73). Inhibition of this enzyme has the potential to address the abnormal pathophysiology of type 2 diabetes. DPP-4 inhibitors increase circulating endogenous GLP-1 concentrations by 2-fold, changes within the physiologic range. Over the last several years a number of oral DPP-4 inhibitors have been developed of which five are currently approved for the treatment of patients with type 2 diabetes: sitagliptin in 2006, vildagliptin in 2007, saxagliptin in 2009, alogliptin in 2010 (Japan only), linagliptin in 2011 (77). DPP-4 inhibitors improve glycemic control, reduce both fasting and postprandial glucose concentrations, and lower the HbA_{1c} concentration, without weight gain and with an apparently benign adverse event profile (**Table 1.3**) (77). As DPP-4 inhibitors are not part of this thesis, readers are referred to several recently published review papers on DPP-4 inhibitors (77, 80-84).

	GLP-1 receptor agonists	DPP-4 inhibitors
Mode of administration	Subcutaneous	Oral
Timing of administration	Once/twice daily, once weekly	Once or twice daily
GLP-1 receptor activation	Supra physiological Continuous	Close to physiological Retained diurnal pattern
Mediators of effect	GLP-1 receptor activation	GLP-1 receptor activation Other bioactive peptides?
Insulin secretion	Stimulation	Stimulation
Glucagon secretion	Inhibition	Inhibition
Gastric emptying	Inhibition	No effect
Appetite	Induction of satiety	No effect
Fasting glucose*	Reduction by ~ 1 mmol/l	Reduction by ~ 1 mmol/l
Prandial glucose	Marked reduction	Slight reduction
HbA_{1c}*	Reduction by ~ 1%	Reduction by ~ 0,8%
Body weight	Reduction	No change
Hypoglycemia	Infrequent	Comparable to placebo
Adverse events	Nausea, vomiting	No, suggestion of higher incidence of upper respiratory tract infections

Table 1.3 Characteristics and effects of GLP-1 receptor agonists and DPP-4 inhibitors. *Absolute numeric reduction dependent on baseline values. Adapted from Ahren (79).

Exenatide: a glucagon-like peptide-1 receptor agonist

Exendin-4 is a polypeptide derived from the salivary gland of the Gila monster lizard (heloderma suspectum) (85). During its feeding exendin-4 is released to the Gila monster's blood stream where it is thought to play a role in energy metabolism and inducing satiety (86). Exendin-4 has a bigger effective receptor affinity than GLP-1, and is therefore a potent GLP-1 receptor agonist (87, 88). Although exendin-4 has a 53% similarity in amino acid sequence, it is not strictly regarded a GLP-1 analogue as it is not a product of the proglucagon gene. Therefore, the more appropriate name GLP-1 receptor agonist (GLP-1RA) has been introduced. Animal and *in vitro* models of type 2 diabetes, exenatide has shown to share the glucose-regulating properties of GLP-1 (**Table 1.4**).

Exenatide's main clinical features are: 1) glucose-dependent increase in insulin secretion; 2) suppression of inappropriately high glucagon secretion; 3) reduction in gastric emptying rate; 4) centrally induced satiety associated with lower food intake, and 5) body weight loss. Additionally, animal and *in vitro* studies have shown an increase in pancreatic beta-cell number, as a

result of stimulation of cell formation from precursors and inhibition of their apoptosis (89). Due to the different amino acid sequence, as compared to endogenous GLP-1, exendin-4 is not degraded by the enzyme DPP-4. As a result exenatide has a longer half life (2.4 hours vs. a few minutes) which enables its therapeutic use (85).

	GLP-1	Exenatide
Insulin secretion	↑	↑
Insulin gene expression*	↑	↑
Glucagon secretion	↓	↓
Gastric emptying	↓	↓
Food intake	↓	↓
Body weight	↓	↓
Glucose utilization	↑	↑
Beta-cell neogenesis*	↑	↑
Beta-cell proliferation*	↑	↑
Beta-cell differentiation*	↑	↑
Neuro endocrine effects*	↑	↑

Table 1.4 Biological characteristics of glucagon-like peptide-1 (GLP-1) and the GLP-1RA exenatide. Adapted from Diamant (49). *Only in the preclinical models.

Synthetic exendin-4 is commercially available as exenatide (brand name: βyetta®). This compound requires to be injected subcutaneously using a pen device approximately one-hour prior to a major meal, twice daily. Exenatide was approved by the FDA in 2005, and the EMA in 2006 for use as an adjunctive therapy, exenatide is indicated to improve glycemic control in patients with type 2 diabetes not well-controlled with metformin, a sulfonylurea, thiazolidinediones, or a combination of metformin and sulfonylurea or thiazolidinediones, but who have not been able to achieve adequate control of blood glucose levels (**Table 1.5**).

Placebo and comparator-controlled clinical studies have demonstrated that 10 μg exenatide twice daily improves glycemic control and reduces body weight in patients with type 2 diabetes (**Table 1.6** and **Table 1.7**) (90-100). Some of these studies also showed amelioration of surrogate measures of beta-cell function such as fasting pro-insulin to insulin ratio in post-hoc analysis (92, 95, 96). In a subset of the studied population, additional beta-cell parameters were obtained from modeling analysis of glucose and C-peptide concentrations during standardized mixed-meal tests. Exenatide improved a number of beta-cell function parameters, including glucose sensitivity (101). In the open-label extension of the 3 pivotal trials, exenatide treatment was associated with an increase in HOMA-B from baseline by approximately 50% at 2 year and by approximately 70% at 3 years (102,

103). Accordingly, improvements have been demonstrated in first and second phase glucose-stimulated insulin secretion (104).

	Exenatide	Liraglutide	Sitagliptin	Vildagliptin	Saxagliptin
Monotherapy	No	No	Yes	No	No
Add-on to metformin	Yes	Yes	Yes	Yes	Yes
Add-on to sulfonylurea	Yes	Yes	Yes	Yes	Yes
Add-on to thiazolidinediones	Yes	No	Yes	Yes	Yes
Triple therapy with metformin and sulfonylurea	Yes	Yes	Yes	No	No
Triple therapy with metformin and thiazolidinediones	Yes	Yes	Yes	No	No
Add-on to insulin	No	No	Yes	No	No

Table 1.5 Indications of approved incretin-based therapies (EMA). Table with courtesy of prof. M. Diamant

Author Year (Ref.)	Treatment	n	Follow-up (week)	Age (y)	BW (Kg)	BMI (Kg/m2)	Diabetes duration (y)	HbA1c (%)	FPG (mmol/L)	Comments
Buse	Placebo BID	123	30	55±11	99±18	34±5	6±5	8.7±1.2	10.8±3.2	Added to SU alone
2004 (92)	Exenatide BID	129		56±11	95±18	33±6	7±7	8.6±1.2	9.9±2.8	
DeFronzo	Placebo BID	113	30	54±9	100±19	34±6	7±6	8.2±1.0	9.4±2.2	Added to MET alone
2005 (94)	Exenatide BID	113		52±11	101±20	34±6	5±5	8.2±1.0	9.3±2.6	
Kendall	Placebo BID	247	30	56±10	99±19	34±5	9±6	8.5±1.0	10.0±2.7	Added to MET and SU
2005 (96)	Exenatide BID	241		55±10	98±21	34±6	9±6	8.5±1.1	9.9±2.4	
Heine	Insulin Glargine QD	267	26	58±10	88±18	31±5	9±6	8.3±1.0	10.4±2.9	Added to MET and SU
2005 (95)	Exenatide BID	282		60±9	88±17	31±4	10±6	8.2±1.0	10.1±2.6	
Zinman	Placebo	112	16	57±10	97±19	34±5	8±6	7.9±0.8	8.8±1.9	Added to TZD/MET or MET
2007 (100)	Exenatide BID	121		56±11	98±19	34±5	7±5	7.9±0.9	9.1±2.6	
Barnett	Insulin glargine QD	68	16	55±8	86±16	31±4	7±5	8.9±1.0	11.8±3.2	Cross-over design
2007 (90)	Exenatide BID	70		55±8	84±16	31±4	8±5	9.0±1.0	12.2±3.2	Added to MET or SU
Nauck	Insulin aspart BID	248	52	58±9	83±16	30±4	10±6	8.6±1.1	11.3±2.8	Added to MET and SU
2007 (98)	Exenatide BID	253		59±9	86±16	30±4	10±6	8.6±1.0	11.0±2.7	
Bergenstal	Insulin aspart QD	124	24	52±11	97±25	34±7	8±6	10.1±1.8	10.9±3.7	Added to MET and SU
2009 (91)	Insulin aspart BID	124		53±10	94±24	34±7	10±6	10.3±1.9	11.2±4.2	
	Exenatide BID	124		53±1	97±24	34±7	9±6	10.2±1.5	11.7±3.7	
Liutkus	Placebo	54	26	54±9	93±18	33±5	6±5	8.3±0.9	9.0±2.0	Added to TZD/MET or MET
2010 (97)	Exenatide BID	111		55±8	95±18	34±6	6±4	8.2±0.9	9.2±2.8	
Buse	Placebo	122	30	59±10	93±21	33±6	12±7	8.5±1.0	8.3±2.3	Added to IG alone;
2011 (93)	Exenatide BID	137		59±9	95±20	34±6	12±7	8.3±0.9	7.9±2.1	IG/TZD/MET; IG/MET

BW = bodyweight; BID = twice daily; FPG = fasting plasma glucose; HbA_{1c} = glycated hemoglobin; n = number of patients; NR = not reported; QD = once daily; QW = once weekly; SU = sulphonylurea.; MET = metformin; TZD = thiazolidinedione; IG = insulin glargine

Table 1.6 Patient characteristics of controlled clinical trials with 10 µg exenatide BID in patients with type 2 diabetes mellitus.

Author Year (Ref.)	Treatment	n	Follow-up (week)	Mean ΔHba$_{1c}$ (%)		Hba$_{1c}$ < 7,0 (%)	Mean ΔFPG (mmol/L)		Mean ΔBW (Kg)	
				Vs control	Vs baseline		Vs controle	Vs baseline	Vs controle	Vs baseline
Buse	Placebo BID	123	30		+0.1±1.0	9		+0.4±3.3		-0.6±3.3
2004 (92)	Exenatide BID	129		NR	-0.9±1.2[a]	41[a]	NR	-0.6±3.4[a]	NR	-1.6±3.4[a]
DeFronzo	Placebo BID	113	30		+0.1±1.1	13		+0.8±2.1		-0.3±3.2
2005 (94)	Exenatide BID	113		NR	-0.8±1.1[a]	46[a]	-1.4 (NR)[a]	-0.6±2.1[a]	NR	-2.8±5.3[a]
Kendall	Placebo BID	247	30		+0.23±1.1	9		+0.8±3.1		-0.9±3.1
2005 (96)	Exenatide BID	241		NR	-0.77±1.2[a]	34[a]	NR	-0.6±3.1[a]	NR	-1.6±3.1[a]
Heine	Insulin Glargine QD	267	26		-1.11±NR[b]	48		-2.9±NR[b]		+1.8±NR
2005 (95)	Exenatide BID	282		+0.02 (-0.12 to 0.16)	-1.11±NR[b]	46	1.5 (1.1 to 1.5)[a]	-1.4±NR[b]	-4.1 (-4.6 to -3.5)[a]	-2.3±NR
Zinman	Placebo	112	16		+0.1±NR	16		+0.1±2.1		-0.2±NR
2007 (100)	Exenatide BID	121		-1.0 (-1.2 to -0.7)[a]	-0.9±NR	62[a]	-1.7 (-2.2 to 1.2)	-1.6±2.2	-1.5 (-2.2 to -0.9)[a]	-1.8±NR
Barnett	Insulin glargine QD	68	16		-1.36±0.84[b]	38		-4.1±1.7		+1.0±0.4
2007 (90)	Exenatide BID	70		-0.01 (-0.17 to 0.15)	-1.36±0.84[b]	40	+1.2 (+0.7 to +1.7)[a]	-2.9±1.7	-2.2 (-2.8 to -1.7)[a]	-2.0±0.4
Nauck	Insulin aspart BID	248	52		-0.89±0.9	24		-1.7±0.2		+2.9±3.1
2007 (98)	Exenatide BID	253		-0.2 (-0.3 to +0.0)	-1.04±1.1	32[a]	-0.1 (-0.6 to +0.4)	-1.8±0.2	-5.4 (-5.9 to -5.0)[a]	-2.5±3.2
Bergenstal	Insulin aspart QD	124	24	+0.7(-1.0 to -0.3)[a]	-2.3±1.5	26	NR	-2.9±0.3[a]	NR	+2.8±3.6
2009 (91)	Insulin aspart BID	124		+0.9(-1.2 to -0.6)[a]	-2.8±1.8	37[a]	NR	-3.5±0.3[a]	NR	+4.1±5.4
	Exenatide BID	124			-1.8±1.6	20		-1.2±0.3		-1.9±3.8
Liutkus	Placebo	54	26		-0.1±1.7	36		NR		-0.8±5.1
2010 (97)	ExenatideBID	111		-0.7 (-1.1 to -0.4)[a]	-0.8±2.1	49	-1.0 (-1.8 to -0.3)[a]	NR	-0.7 (-1.7 to 0.3)	-1.4±6.3
Buse	Placebo	122	30		-1.0 (-1.2 to -0.9)	35		-1.5 (-1.8 to -1.2)		+1.0 (+0.2 to +1.7)
2011 (93)	Exenatide BID	137		-0.7 (-0.9 to -0.5)[a]	-1.7 (-1.9 to -1.6)	60a	-0.1 (-0.5 to 0.3)[a]	-1.6 (-1.9 to -1.3)	-2.7 (-3.7 to -1.4)[a]	-1.8 (-2.5 to -1.1)

[a] Statistically significant different from control. BW = bodyweight; BID = twice daily; FPG = fasting plasma glucose; FSI = fasting serum insulin; FSP = fating serum proinsulin; HbA$_{1c}$ = glycated hemoglobin; n = number of patients; NA = not applicable; NR = not reported; QD = once daily; QW = once weekly; SU = sulphonylurea.; MET = metformin; TZD = thiazolidinedione; IG = insulin glargine

Table 1.7. Effects of 10 µg exenatide BID on glycemia and bodyweight in patients with type 2 diabetes mellitus. Data represent mean±SD or 95% confidence interval

Summary and rationale for conduct of the core studies and outline of this thesis

The core study presented in this thesis was collectively initiated and designed by the investigators from the three study sites: 1) Diabetes Center, Department of Internal Medicine, VU University Medical Center, Amsterdam, the Netherlands; 2) Lundberg Laboratory for Diabetes Research, Sahlgrenska University Hospital, Göteborg, Sweden; 3) Department of Medicine, Helsinki University Central Hospital, Helsinki, Finland. The study was performed between September 2004 and December 2009.

Type 2 diabetes mellitus is characterized by insulin resistance and by a progressive beta-cell defect. The latter abnormality is thought to be primarily responsible for the progressive nature of the disease. The decline in insulin secretory capacity requires periodic adjustment of blood glucose lowering therapy. The management of hyperglycemia in type 2 diabetes starts with education, whereby advice is provided to facilitate patients to change their dietary habits and increase their physical activity and reduce body weight. When these measures fail, and patients do not achieve adequate blood glucose control, oral blood-glucose lowering agent monotherapy with metformin is usually begun. It has become increasingly common to combine two or more oral blood-glucose lowering agents for patients who fail to achieve adequate glycemic control on monotherapy. At the point glycemic control cannot be achieved or maintained with one or more oral blood-glucose lowering agents, the addition of insulin therapy is generally accepted as the next step. Although the long duration of action of insulin glargine supports convenient, once-daily administration with improvement in overall glycemic control, insulin glargine use (as well as other forms of insulin therapy) still has negative aspects, such as the potential for overall increase in hypoglycemia over that observed with oral blood-glucose lowering agent therapy alone, and weight gain (105). In addition, insulin glargine does not target postprandial hyperglycemia and/or dyslipidemia (105) that may be associated with accelerated development of cardiovascular disease (CVD) (106). The potential beneficial effects of exenatide make it a promising candidate to improve glycemic control in patients with type 2 diabetes inadequately controlled on metformin with a reduction in the known side effects of weight gain and hypoglycemia associated with insulin therapy.

At present, there are no pharmacological therapies available that have been demonstrated to slow the decline of beta-cell function in patients with established type 2 diabetes. However, data exist from a number of preclinical studies supporting the premise that exenatide and/or GLP-1 have effects on beta-cells that go beyond the acute effects of enhancing glucose-dependent insulin secretion (79). The published scientific literature examining both in vitro and in vivo actions of exenatide and GLP-1-related peptides, now provides strong support that these peptides play an important role in the maintenance of beta-cell mass and function, through complementary actions to stimulate beta-cell proliferation and neogenesis, together with inhibition of beta-cell apoptosis (45). These findings are also consistent with work performed in a number of animal models, in which exenatide or GLP-1 have been shown to increase beta-cell mass and/or function (45). This leads to the important question of whether long-term exenatide usage in patients with type 2 diabetes may have similar effects, and if so, may restore some of the beta-cell functionality (or slow the progression of further loss) that is lost as part of the progression of the disease. The present study is designed to test this possibility; utilizing stepped euglycemic/hyperglycemic clamp experiments to assess beta- cell responses to glucose and arginine, a non-glucose insulin secretagogue.

The primary purpose of the study presented in this thesis is to assess the effects of long-term treatment period with exenatide or insulin glargine in modifying the parameters of beta-cell function in patients with type 2 diabetes who have not achieved a goal HbA_{1c} of ≤ 6.5% using metformin.

In addition to its effects on the beta-cell and enhanced glucose-dependent insulin secretion, exenatide also slows gastric emptying. This slowing may be beneficial in terms of postprandial glucose and lipid control, which may be important in decreasing the development of CVD in this population. Clinical, epidemiological and experimental evidence indicates that postprandial plasma glucose- and triglyceride responses, which are abnormally elevated and prolonged in patients with type 2 diabetes, are associated with increased CVD risk (107). In particular, postprandial triglyceride-rich lipoproteins seem to activate coagulation and inflammation, both of which have been implicated in the development of atherosclerosis (108). Therefore, the ability of exenatide to decrease the postprandial elevations in glucose and lipids may represent a key benefit differentiating exenatide and insulin glargine for patients who are not adequately controlled with oral blood-glucose lowering agents alone.

The general outline of this thesis is as follows: **chapter 2** describes the effects on hyperglycemic clamp derived measures of beta-cell function, glycemic efficacy, body weight following one-year of treatment, and during the course of a 12-week off-drug period. In **chapter 3** the effects of one-year treatment on postprandial glycemia, lipid profiles, and oxidative stress are shown. The effects on the postprandial beta-cell response, measured using mathematical modeling, are given in **chapter 4**. The effects of one-year treatment with either exenatide or insulin glargine on body composition, and circulating CVD risk markers are presented in **chapter 5. Chapter 6** gives the results on hyperglycemic clamp derived measures of beta-cell function, glycemic efficacy, body weight following the total 3.5-year duration of the study. Effects on bone mineral density and measures of calcium homeostasis are depicted in **chapter 7.** In **chapter 8** we demonstrate that exenatide treatment may play a beneficial role in the treatment of non-alcoholic liver steatosis.

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Chapter 1

Chapter 2

One-year treatment with exenatide improves beta-cell function, compared with insulin glargine, in metformin treated type 2 diabetes patients: a randomized, controlled trial

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Abstract

Background: Traditional blood glucose lowering agents do not sustain adequate glycemic control in most type 2 diabetic patients. Preclinical studies with exenatide have suggested sustained improvements in beta-cell function. We investigated the effects of 52-weeks treatment with exenatide or insulin glargine followed by an off-drug period on hyperglycemic clamp derived measures of beta-cell function, glycemic control and body weight.

Methods: Sixty-nine metformin-treated patients with type 2 diabetes were randomized to exenatide (n=36) or insulin glargine (n=33). Beta-cell function was measured during an arginine stimulated hyperglycemic clamp at week 0, week 52 and after a 4-week off-drug period. Additional endpoints included effects on glycemic control, body weight, and safety.

Results: Treatment induced change in combined glucose and arginine stimulated C-peptide secretion was 2.46 fold (95% CI 2.09 to 2.90, $p<0.0001$) greater following 52-week exenatide treatment as compared to insulin glargine. Both exenatide and insulin glargine reduced hemoglobin (Hb) A_{1C} similarly: -0.8±0.1% and -0.7±0.2% respectively (P=0.55). Exenatide reduced body weight compared to insulin glargine (difference -4.6 kg, $p<0.0001$). Beta-cell function measures returned to pre-treatment values in both groups after 4-week off-drug. HbA_{1c} and body weight rose to pre-treatment values 12-weeks after discontinuing either exenatide or insulin glargine therapy.

Conclusions: Exenatide significantly improves beta-cell function during 1-year of treatment as compared to titrated insulin glargine. Following cessation of both exenatide and insulin glargine therapy beta-cell function and glycemic control returned to pre-treatment values, suggesting that ongoing treatment is necessary to maintain the beneficial effects of either therapy.

Introduction

Type 2 diabetes mellitus is characterized by beta-cell dysfunction against a background of obesity-related insulin resistance (1). When lifestyle measures and oral blood glucose lowering medications fail to sustain glycemic control, current guidelines advise the use of basal insulin (2). Data from the United Kingdom Prospective Diabetes Study suggest that glycemic control progressively worsens over time and this deterioration has been attributed to a progressive loss of beta-cell function which occurs irrespective of whether metformin, sulfonylureas or insulin are used (3). Therefore, therapeutic approaches, which may prevent or delay the decline of beta-cell function in type 2 diabetes are eagerly awaited.

Exenatide is synthetic exendin-4, first identified and isolated from the salivary secretions of the Gila monster (*heloderma suspectum*). Exendin-4 shares 53% amino acid sequence identity with human glucagon-like peptide (GLP)-1 and binds directly to GLP-1 receptors. Placebo- (4-7) and comparator-controlled (8-10) clinical studies have demonstrated that exenatide improves glycemic control and reduces body weight in patients with type 2 diabetes. These studies also showed amelioration of surrogate measures of beta-cell function (4-6, 8). Accordingly, improvements have been demonstrated in first and second phase glucose-stimulated insulin secretion, and in meal-derived indices of beta-function compared to placebo (11, 12). In animals exenatide has shown to sustain improvements in beta-cell function or even increase beta-cell mass (13).

Previously, we showed that 26-week exenatide therapy lowered hemoglobin (Hb) A_{1c} similarly to insulin glargine in patients with type 2 diabetes who were treated with metformin and a sulfonylurea (8). However, at present, no data exist regarding the relative effects of these treatments on beta-cell function, nor is it known whether the effects of either therapy are sustained after discontinuation.

The aim of the current study, was to assess the effects of treatment with exenatide or insulin glargine on beta-cell function, glycemic control, body weight, and safety, after 52-weeks of treatment and during a 12-week off-drug period.

Research design and methods

The study was performed between September 27th 2004 and September 13th 2007 at three study sites, in Sweden, Finland and the Netherlands. In total, 69 patients were randomized using a permutated block randomization scheme stratified by site and screening HbA_{1c} to receive exenatide or insulin glargine, in addition to ongoing metformin treatment (**Figure 2.1**).

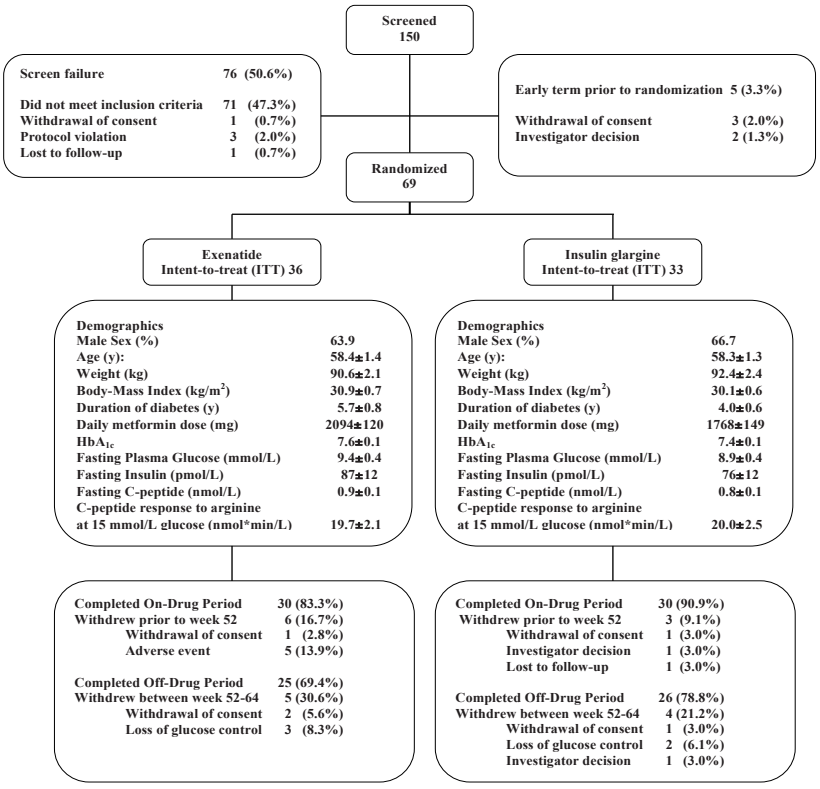

Figure 2.1 Protocol flow chart and baseline characteristics of the study population. Data represent mean±SEM.

Inclusion criteria were: age 30-75 years, HbA_{1c} 6.5-9.5%, body mass index 25-40 kg/m^2, and metformin treatment at a stable dose for at least 2 months. No other blood glucose lowering agents were allowed within 3 months prior to screening. No changes in other agents known to affect beta-cell function (such as ACE inhibitors and angiotensin receptor blockers) were allowed during the study. The study protocol was approved by each

site's ethics review committee and was in accordance to the principles described in the declaration of Helsinki. All participating patients gave their written informed consent prior to screening.

Patients randomized to exenatide (n=36) initiated treatment at a dose of 5 μg twice daily (BID), injected 15-min before breakfast and dinner, for a period of 4 weeks, followed by dose increase to 10 μg BID. Exenatide was titrated to a maximum dose of three times (TID) 20 μg, or the maximum tolerated dose, when HbA_{1c} ranged 7.1-7.5% at two consecutive visits, or when HbA_{1c} was ≥7.6% at any given visit. Patients randomized to insulin glargine (n=33) started at an initial dose of 10 IU once daily (QD), injected at bedtime. Patients were instructed to increase the daily dose based on their fasting self-monitored blood glucose (SMBG) levels, according to a pre-specified algorithm (14). When fasting SMBG was ≥5.6 mmol/L on three consecutive days the insulin dose was increased by 2 units until finally fasting SMBG would range between 4.5 and 5.5 mmol/L. If a hypoglycemic event (<3.3 mmol/L) occurred, patients were instructed to refrain from increasing insulin glargine dose for seven days and to contact the study physician. When necessary, the importance of proper titration of insulin was emphasized.

Study endpoints

Insulin secretion and sensitivity was measured during a combined euglycemic-hyperinsulinemic and hyperglycemic clamp procedure (**Figure 2.2**) (15, 16). First and second phase C-peptide secretion was calculated as $AUC_{180\text{-}190min}$ and $AUC_{190\text{-}260min}$. Arginine stimulated C-peptide secretion (AIR_{arg}) was calculated as the incremental $AUC_{260\text{-}270min}$ above the fasting C-peptide concentration. Arginine was administered during hyperglycemic clamp to measure maximum insulin secretory capacity at a steady-state glucose concentration of 15 mmol/L (17). Clamps were performed prior to randomization, following 52-weeks of treatment, and after a 4-week off-drug period. Following an overnight fast, an indwelling cannula was inserted into an antecubital vein for infusion of glucose and insulin. To obtain arterialized venous blood samples, a cannula was inserted in a retrograde fashion into a dorsal hand or wrist vein and maintained in a heated box at 50° Celsius. During the clamp at week 52, patients randomized to exenatide, were given the study drug 15 minutes prior to the onset of the hyperglycemic clamp and patients randomized to insulin glargine received their last insulin dose the night before at bed time.

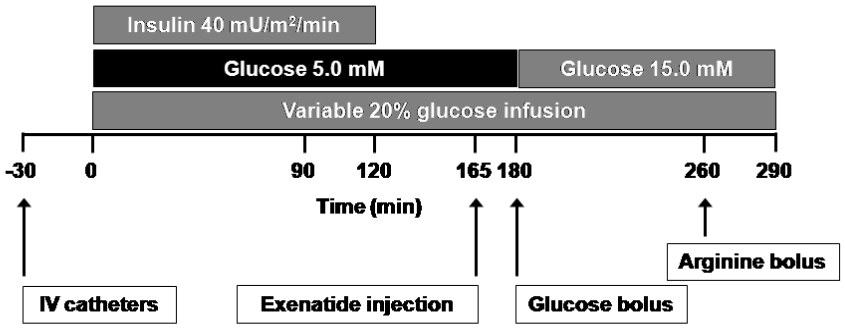

Figure 2.2 Clamp design.

HbA_{1c} (normal range: 4·3 to 6·1%, DCCT standardized Biorad assay) was measured using the, fasting plasma glucose, and safety parameters were measured prior to randomization and during each follow-up visit until the end of the 12-week off-drug period by a central laboratory (Quintiles, Livingston, UK). Patients were instructed to record seven-point (fasting, 2 hours after breakfast, before lunch, 2 hours after lunch, before dinner, 2 hours after dinner, and at bedtime) SMBG profiles using an OneTouch Ultra blood glucose meter (Lifescan, Milpitas, CA) prior to each visit. Plasma glucose concentrations during the clamp were measured using an YSI 2300 STAT Plus (YSI, Yellow Springs, OH) in Sweden and the Netherlands, and using a Beckman-Coulter Glucose Analyser 2 (Beckman-Coulter, Fullerton, CA) in Finland. C-peptide samples were analyzed at the VU University Medical Center using an immunoradiometric assay (Centaur; Bayer Diagnostics, Mijdrecht, the Netherlands).

Statistical analysis

The study's primary efficacy endpoint is the treatment effect on the beta-cell function as measured by the ratio of week 52 combined glucose and arginine-stimulated insulin secretion during a hyperglycemic clamp. A sample size of 26 patients per group was required to provide 90% power to detect a between group significant difference in arginine-stimulated insulin secretion between the two treatment groups, assuming the mean iAUC at baseline is 200 pmol*min/L for both groups and at week 52, 1100 pmol*min/L and 300 pmol*min/L for the exenatide and insulin glargine groups respectively (11, 18).

All outcome measures are compared between the two treatment groups using an analysis of covariance (ANCOVA) model. The dependent variable

used in the model is the $\log_e$ transformed ratio to pre-treatment for the beta-cell function parameters (AIR_{arg}, 1st phase, 2nd phase). For all other endpoints the dependent value used is the mean at the corresponding visit. The model includes factors for treatment group (exenatide/glargine), site (NL/SE/FIN), and baseline HbA_{1c} stratum (≤8.5%/>8.5%), and the pre-treatment variable of the corresponding dependent variable as a covariate
Statistical analysis was done using SAS software (SAS Institute, Inc., Cary, North Carolina). All inferential statistical tests were conducted at a significance level of 0.05 (two-sided). Unless otherwise stated, data are presented as mean (±SEM).

Results

Patient disposition and baseline clinical characteristics

Patient disposition and baseline clinical characteristics are shown in **Figure 2.1**. Sixty patients completed the 52-week treatment period. Of the patients randomized to exenatide 62.1% (n=18) were treated with exenatide 10 μg BID at 52-weeks of treatment. Five (17.2%) patients were using 20 μg TID, 2 (6.9%) 10 μg TID, 1 (3.4%) 15 μg BID, and 1 (3.4%) 15 μg TID. The daily exenatide dose was reduced to 5 μg BID in two patients (6.9%). Despite this increase in daily exenatide dose none of these patients reached the HbA_{1c} target of <7.1%. At 52-weeks the mean±SEM insulin glargine dose used was 33.6±3.5 units per day. The corresponding fasting SMBG in the insulin glargine treated group was 5.6±0.2 mmol/L.

Hemoglobin A_{1c} and fasting plasma glucose

Exenatide and insulin glargine treatment resulted in a similar reduction in HbA_{1c} (0.8±0.1% and 0.7±0.2%, respectively; p=0.55) with both groups achieving a mean HbA_{1c} of 6.8% at 52-weeks. The insulin glargine group showed a significantly greater reduction in FPG as compared to exenatide (-2.9±0.4 versus -1.6±0.3 mmol/L; p<0.0001; respectively), while SMBG profiles demonstrated significantly greater reductions in postprandial glucose excursions in the exenatide treated patients (**Figure 2.3C-D**). During the off-drug period, both HbA_{1c} and FPG increased in both groups and were not significantly different compared to pre-treatment values after 12 weeks off-drug (**Figure 2.3A-B**).

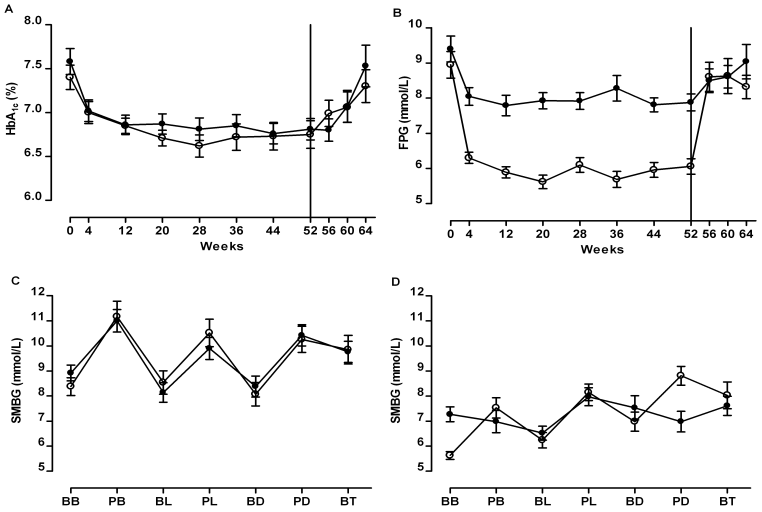

Figure 2.3A-D Time course for HbA_{1c} (A) and fasting plasma glucose (B). Data are mean (SEM). Black circles = exenatide; white circles = insulin glargine. Self-monitored blood glucose concentrations before (C) and after (D) 52-weeks treatment. Data are mean (SEM). Black circles = exenatide; white circles = insulin glargine.

Body weight and insulin sensitivity

Fifty-two weeks of exenatide treatment resulted in a lowering of body weight of -3.6±0.6 kg, while treatment with insulin glargine resulted in a body weight increase of +1.0±0.8 kg (between group difference, -4.6±1.1 kg; p<0.0001; **Figure 2.3E).** During the 12-week off-drug period body weight trended toward baseline values with both therapies (between group difference: -2.4±1.1 kg; p=0.03). At baseline, insulin-mediated glucose uptake did not differ between the two treatment groups (**Figure 2.3F**). Treatment with exenatide and insulin glargine improved insulin sensitivity to the same extent by 0.9±0.3 and 1.1±0.3 mg/min/kg, respectively (p=0.49). After 4-week discontinuation of study medication, the M-value was not significantly different from pre-treatment values in the insulin glargine treated group, while it remained significantly higher in the exenatide-treated group (between group difference 0.8±0.4 mg/min/kg, p=0.03).

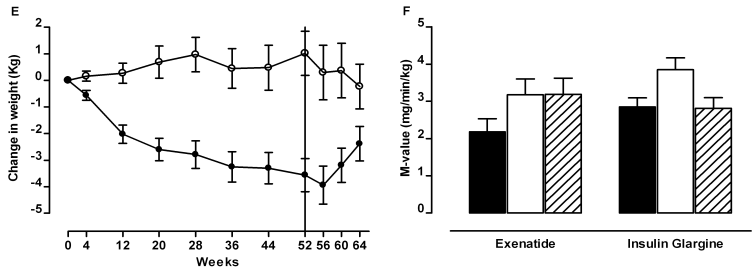

Figure 2.3E-F Change in body weight (E) and insulin sensitivity measured as M-value (F). Data are mean (SEM). Black circles = exenatide; white circles = insulin glargine; black bars = pre-treatment; white bars = 52-weeks on-drug; hatched bars = 4-weeks off-drug.

Hyperglycemic-clamp derived measures of beta-cell function

At baseline, both glucose and arginine stimulated C-peptide secretion did not differ between the two treatment groups (**Table 2.1; Figure 2.4A-D**). After 52-weeks of treatment, the exenatide group demonstrated a significant increase in all measures of beta-cell function. Accordingly, exenatide treatment significantly increased first and second phase glucose-stimulated C-peptide secretion by 1.53±0.11 and 2.85±0.22 fold, respectively ($p<0.0001$), compared to insulin glargine. The C-peptide response to arginine during hyperglycemia increased 3.19±0.24 fold from pre-treatment in the exenatide group compared to 1.31±0.07 fold increase in the insulin glargine group (between group difference 2.46±0.20 fold; $p<0.0001$). After 4 weeks discontinuation of the study medication, measures of beta-cell function returned to pre-treatment values in both groups.

Adverse effects and tolerability

The most frequently observed adverse event in exenatide-treated patients was mild-to-moderate nausea (50%). Other gastrointestinal adverse events were reported more commonly in exenatide treated patients including vomiting, diarrhea and abdominal distension. Biochemically confirmed hypoglycemia (<3.3 mmol/L) was observed more frequently in the insulin glargine group (24.2%), as compared to the exenatide treated patients (8.3%). There was no severe hypoglycemia with either treatment. Other adverse events observed more frequently in the insulin glargine group included influenza and gastroenteritis. One patient randomized to exenatide developed pancreatitis which resolved after withdrawal of study medication.

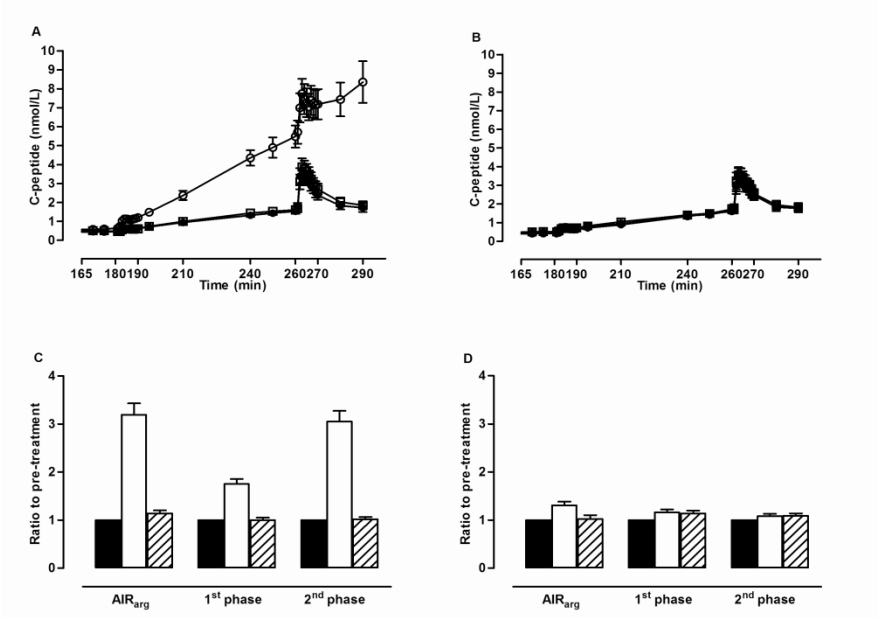

Figure 2.4A-D C-peptide concentrations during hyperglycemic clamp and ratio-to-pre-treatment in the exenatide (A and C) and insulin glargine (B and D) treated group. Data represent mean (SEM) in **Figure 2.3A-B** and geometric mean (SEM) in **Figure 2.3C-D**. AIR_{arg}, C-peptide response to arginine at 15 mmol/L glucose concentration; 1st phase, first-phase C-peptide response to glucose; 2nd phase, second-phase C-peptide response to glucose. See methods section for calculations of beta-cell function measures. Black circles and black bars = pre-treatment; white circles and white bars = 52-weeks on-drug; black squares and hatched bars = 4-weeks off-drug.

				On-drug ratio to pre-treatment (week 52)			Off-drug ratio to pre-treatment (week 56)		
	Baseline (week -2)	On-Drug (week 52)	Off-Drug (week 56)	Geometric mean	Between-group difference	P-value	Geometric mean	Between-group difference	P-value
1st phase									
Insulin glargine	5.4±0.6	6.1±0.5	6.1±0.6	1.17±0.06			1.13±0.05		
Exenatide	5.4±0.6	9.4±1.0	5.0±0.6	1.78±0.11	1.53±0.11	<0.0001	1.00±0.05	0.90±0.06	0.1188
2nd phase									
Insulin glargine	77.4±8.8	80.7±6.9	86.2±9.1	1.08±0.05			1.10±0.05		
Exenatide	78.5±8.3	235.6±23.0	79.5±9.1	3.05±0.22	2.85±0.22	<0.0001	1.01±0.04	0.92±0.06	0.1996
AIR_{arg}									
Insulin glargine	20.0±2.5	24.8±2.2	21.4±2.5	1.31±0.07			1.03±0.08		
Exenatide	19.7±2.1	62.2±7.0	22.0±2.6	3.19±0.24	2.46±0.20	<0.0001	1.12±0.06	1.08±0.10	0.4052

Table 2.1 Measures of beta-cell secretory function during hyperglycemic clamp and ratio to pre-treatment in the exenatide (n=30) and insulin glargine (n=30) treated group. Data represent mean (SEM). Ratio from pre-treatment are presented as geometric mean (SEM). AIR_{arg}, C-peptide response to arginine at 15 mmol/L glucose concentration (nmol*min/L); 1st phase, first-phase C-peptide response to glucose (nmol*min/L); 2nd phase, second-phase C-peptide response to glucose (nmol*min/L). See methods section for calculations of beta-cell function measures.

Discussion

This study demonstrates that 52-weeks of treatment with exenatide significantly improves beta-cell function as compared to insulin glargine in metformin-treated type 2 diabetic patients. Additionally, exenatide treatment achieved similar improvements in glycemic control, reduced body weight and resulted in fewer hypoglycemic events. Both exenatide and insulin glargine resulted in similar improvements in whole body insulin sensitivity. After cessation of both treatments, endpoint measures returned to pre-treatment values.

In type 2 diabetes defects in beta-cell function include an absent first phase insulin response and a gradually diminishing second phase response to glucose (1). This progressive loss of beta-cell function is regarded to be the main factor responsible for the gradual increase of glycemia over time, regardless of the therapy used (3). Acute and chronic exposure to exenatide has shown to improve beta-cell function (4-6, 8). However, there have been no studies comparing beta-cell function after long-term exposure to exenatide or other glucose lowering therapies. In the current study, one-year exenatide therapy significantly improved beta-cell function compared with titrated insulin glargine, in the presence of comparable improvements in glycemic control.

A number of prior studies have demonstrated that exenatide is non-inferior to insulin regimens as a glucose lowering treatment option in inadequately controlled patients with type 2 diabetes (8-10). These previous insulin comparator trials have been criticized for not achieving optimal insulin doses in the comparator arm of the study (19) despite mean reductions in HbA_{1c} levels that were within the range of reductions observed in comparable insulin trials. In the current study, insulin titration resulted in a mean daily insulin dose of 34±19 units. Although this insulin glargine dose is lower than that utilized in other studies of type 2 diabetes (20, 21) SMBG targets were achieved in the current study suggesting that insulin doses were appropriately titrated in the majority of patients. Additionally, the intensive treatment with both therapies reduced mean HbA_{1c} values after 52 weeks to 6.8%. In the LANMET study (22) where, comparable to our study, insulin glargine was added to metformin monotherapy in patients with type 2 diabetes, a higher dose of insulin glargine (68 U/day) was used. These apparent differences in insulin glargine dose may be attributed to the relatively good baseline HbA_{1c} in our population compared to the LANMET participants (HbA_{1c} 9.5%).

One limitation of the current study should be highlighted; exenatide was given 15-minutes prior to the start of the hyperglycemic clamp study. We therefore cannot discriminate between acute and long-term effects of exenatide on beta-cell function. The current study does, however, support the observation that longer-term treatment with exenatide does not attenuate the known acute effects of this therapy on beta-cell function while active insulin therapy did not improve beta-cell function to the same degree. As the primary study objective was to determine the effects of both active exenatide and insulin therapy on beta-cell function in type 2 diabetic patients, we decided to perform the tests on therapy, in accordance with previous studies (18, 23, 24). These findings support the idea that longer-term exenatide treatment could have an enduring effect on beta-cell function.

In the current study, treatment with both exenatide and insulin glargine resulted in a similar reduction in $HbA_{1c.}$ Of interest, the two therapies achieved this result through different ways: exenatide primarily affected postprandial glucose excursions, with a modest effect on fasting glucose, whereas insulin glargine predominantly reduced fasting plasma glucose, without influencing postprandial glucose elevations. The resultant average glucose concentrations are similar in both treatment groups, as reflected by the HbA_{1c} concentration. Albeit modest, the improved glycemic control in either group may have lowered the hyperglycemia associated oxidative stress burden, which in part may explain the modest improvement of beta-cell function in the insulin glargine group (25). Obviously, patients treated with exenatide showed a greater improvement in acute beta-cell function, which is considered as the result of binding to the beta-cell glucagon-like peptide-1 (GLP-1) receptor (13). These findings are consistent with work in a number of animal models, in which exenatide or GLP-1 have been shown to increase beta-cell mass and to reduce apoptosis (13). Collectively, these observations lead to the question whether long-term exenatide administration to patients with type 2 diabetes may restore some of the beta-cell functionality that is lost as part of the natural history of the disease.

In our study the improved beta-cell function at 52-weeks was lost 4-weeks after cessation of either study treatment, and this was accompanied by an increase in plasma glucose and HbA_{1c} to pre-treatment values in both study arms. Interestingly, insulin sensitivity remained significantly improved after 4-weeks cessation of treatment in the exenatide treated group only. This may suggest additional effects of exenatide, possibly mediated by weight loss, which may be of longer duration. Whether longer-term exposure to

exenatide can alter functional beta-cell mass in the absence of active exenatide treatment will require further study. These effects may be dependent on other factors including diabetes duration, the amount of functional beta-cells present at the initiation of therapy and overall achieved metabolic control. To study a possible preserving effect on beta-cell function, an additional two-year extension trial is currently ongoing.

Both titrated exenatide and insulin glargine were generally well tolerated with >80% of patients completing one-year of therapy in both groups. These therapeutic approaches differed in that the most common side effect with exenatide were of gastro-intestinal origin, occurring in a similar proportion of patients as reported in previous studies (4-10). Mild-to-moderate nausea (47%) was the most commonly reported adverse event with exenatide. In contrast, hypoglycemia was the most common adverse event reported in 25% of the insulin glargine treated patients.

In conclusion, this study uniquely demonstrates that one-year of treatment with exenatide significantly improved, beta-cell function and reduced body weight in the face of similar improvement in glycemic control compared to insulin glargine. Following cessation of therapy, the beneficial effects on beta-cell function, glycemic control and body weight were not sustained, suggesting that active treatment is necessary to maintain these beneficial effects of exenatide in patients failing oral blood glucose lowering therapy.

Acknowledgments

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improves 24-h glycemia and alpha- and beta-cell function and reduces endogenous glucose release in patients with type 2 diabetes. Diabetes 2004;53:1187-1194.
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Chapter 3

One-year treatment with exenatide vs. insulin glargine: effects on postprandial glycemia, lipid profiles, and oxidative stress

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Abstract

Background: The objective of the present study was to investigate the effects of one-year treatment with exenatide or insulin glargine, followed by a 5-week off-drug period, on postprandial lipidemia, glycemia and measures of oxidative stress.

Methods: Sixty-nine metformin-treated patients with type 2 diabetes were randomized (using a permuted block randomization scheme stratified by site and baseline HbA_{1c} stratum (≤ 8.5% or > 8.5%) of which 60 completed (exenatide n=30; insulin glargine n=30) the pre-treatment and on-drug meal test. Postprandial glucose, lipids and lipoproteins, and oxidative stress markers were studied at week -1, 51, and after a 5-week off-drug period following a breakfast and lunch mixed-meal containing 50g fat, 75g carbohydrates, and 35g protein.

Results: 51-week exenatide treatment resulted in a significant reduction of prandial glucose, triglycerides, apo-B48, calculated VLDL-C, FFA and MDA excursions whereas insulin glargine predominantly reduced fasting glucose, FFA and MDA. Changes in markers of oxidative stress were related to changes in postprandial glucose and triglyceride excursions, independent of treatment arm. All postprandial measures returned to pre-treatment values in both groups after 5-week cessation of study treatment.

Conclusions: Exenatide showed beneficial effects on postprandial glycemia and lipidemia, and these effects were related to changes in the oxidative stress markers MDA and oxLDL during 1-year of treatment as compared to insulin glargine. Following cessation of both exenatide and insulin glargine measures returned to pre-treatment values, suggesting that ongoing treatment is necessary to maintain the beneficial effects of either therapy.

Introduction

Patients with type 2 diabetes have a two to fourfold increased risk of cardiovascular disease (CVD) as compared to non-diabetic subjects (1). Dyslipidemia is an established risk factor for CVD in patients with type 2 diabetes, as well as in non-diabetic subjects (2). Diabetic dyslipidemia is a specific cluster of potentially atherogenic plasma lipid and lipoprotein abnormalities including increased plasma triglycerides, reduced HDL cholesterol concentrations, and small, dense LDL particles (2).

In addition to fasting dyslipidemia, exaggerated postprandial hypertriglyceridemia characterize patients with type 2 diabetes when compared to age- and weight- matched controls (3). After a meal there is an abundance of circulating Triglyceride Rich Lipoproteins (TRLs), which comprise both intestine-derived, apolipoprotein (apo)-B48-containing chylomicrons and liver-derived, apo-B100-containing very-low-density lipoprotein (VLDL) particles (4). Postprandial dyslipidemia is characterized by high concentrations of chylomicrons and VLDL remnants in plasma.

Both postprandial hyperglycemia and hypertriglyceridemia lead to mitochondrial free radical production and subsequent oxidative stress, which in turn may contribute to the development of microvascular and macrovascular complications (5).

Exogenous insulin therapy reduces fasting hypertriglyceridemia in patients with type 2 diabetes (6). The concentration of HDL cholesterol remains either unchanged or increases slightly during exogenous insulin therapy (7), but insulin therapy has been shown to change the composition of HDL particles in an anti-atherogenic direction (8).

Exenatide is a GLP-1 receptor agonist, which stimulates endogenous insulin secretion and suppresses glucagon secretion in a glucose-dependent manner, slows gastric emptying and reduces appetite (9). These actions contribute to a robust lowering of postprandial glucose concentrations (10). Short-term, placebo controlled, treatment of patients with type 2 diabetes with exenatide has been shown to reduce postprandial triglyceride concentrations (11) but there are, to our knowledge, no data on the effects of exenatide on postprandial lipoprotein metabolism and markers of oxidative stress.

The aim of the current study was to assess the effects of treatment with exenatide or insulin glargine on postprandial glycemia, lipid profiles and markers of oxidative stress after one year of treatment and during a 5-week off-drug period in metformin-treated patients with type 2 diabetes.

Research design and methods

Subjects

Details on study design were reported previously (12) (**Figure 3.1A**). Briefly, 69 patients were randomized (using a permuted block randomization scheme stratified by site and baseline HbA_{1c} stratum (≤ 8.5% or > 8.5%) at the three study sites, in Finland, Sweden and the Netherlands. Sixty patients (exenatide n=30; insulin glargine n=30) completed both the pre-treatment and on-drug meal test and are included in the current analysis (evaluable population); 47 patients participated in the off-drug meal test (exenatide n=25; insulin glargine n=22). HMG-CoA reductase inhibitors (statins) were allowed when used in a stable dose ≥2 months prior to screening; no other lipid-lowering agents or change in statin dosage were allowed.

Patients randomized to exenatide initiated treatment at a dose of 5 μg twice daily (BID) for a period of 4 weeks, followed by dose increase to 10 μg BID. If needed, exenatide was titrated to a maximum dose of three times (TID) 20 μg, or the maximum tolerated dose, when the HbA_{1c} value at two consecutive visits ranged between 7.1 and 7.5%, or when the HbA_{1c} was ≥7.6% at any given visit. Patients randomized to insulin glargine started with an initial dosage of 10 U once daily (QD) at bedtime, followed by self-adjustment of the daily dose according to a fixed-dose treat-to-target (FPG <5.6 mmol/L) algorithm, as previously described (12). At 52 weeks the mean±SEM insulin glargine dose used was 33.6±3.5 U per day. The corresponding fasting SMBG in the insulin glargine treated group was 5.6±0.2 mmol/L (12). The study protocol was approved by each site's ethical review committee and was in accordance to the principles described in the declaration of Helsinki. Patients were recruited at the local sites and through advertising. All participating patients gave their written informed consent prior to screening. This study is registered with ClinicalTrials.gov number NCT00097500.

Standardized mixed-meal test

The overall meal test design is shown in **Figure 3.1B**. Patients arrived in the study center after an overnight fast. A cannula was inserted into the non-dominant dorsal hand or wrist vein and maintained in a heated box at 50°C for blood collections. Two sequential mixed meal tests (breakfast and lunch, 4 hours apart; each test meal containing 50g of fat, 75g of carbohydrates, and 35g protein) were performed prior to randomization, after 51 weeks of treatment, and after a 5-week off-drug period. Subjects were requested to eat the test meal within 10 minutes. During the meal test at week 51,

patients received their study medication at the protocol-specified time, i.e. patients randomized to exenatide were given the study drug 15 minutes prior to breakfast and patients randomized to insulin glargine received their last insulin dose the night before at bedtime.

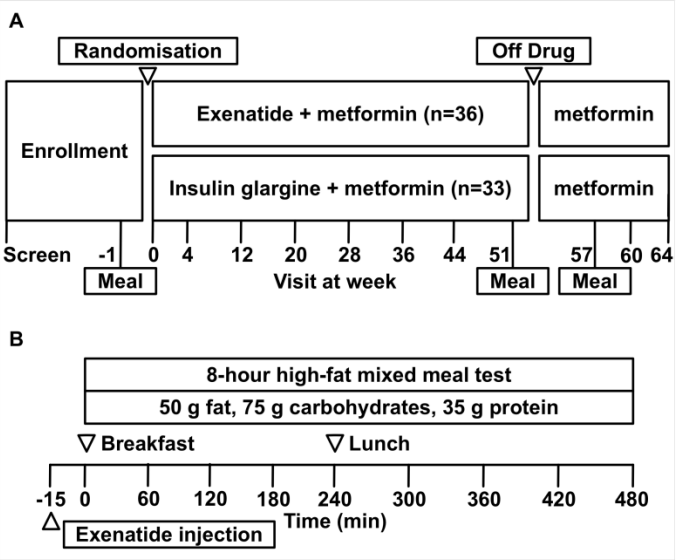

Figure 3.1A-B Study profile (A) and meal test design (B).

Blood sampling and analysis

Plasma glucose concentrations were measured at bedside using an YSI 2300 STAT Plus (Yellow Springs Instruments, Yellow Springs, OH) in Sweden and the Netherlands, and using a Beckman Coulter Glucose Analyzer 2 (Beckman Coulter, Fullerton, CA) in Finland. Serum ((s-) for analysis of lipids) and EDTA plasma ((p-) for analysis of oxidative stress markers) were separated by centrifugation (1300-1500 g) and stored at -80°C until analysis. Total cholesterol (s-TC) was measured using a cholesterol oxidase method (Roche Diagnostics, Penzberg, Germany). Enzymatic colorimetric assays were used to measure s-HDL-C, s-LDL-C, triglycerides (s-TG) (all Roche Diagnostics, Penzberg, Germany), and free fatty acids (s-FFA) (Wako Chemicals, Neuss, Germany). Immunoturbidimetric methods were used to measure s-apo-AI (Thermo Electron, Vantaa, Finland), s-apo-AII and s-apo-CIII (Wako Chemicals, Neuss, Germany), and s-apo-B (Thermo Electron, Vantaa, Finland). Malondialdehyde (HbA_{1c}(normal range: 4.3 to 6.1%, DCCT standardized Biorad assay), FPG, and serum safety parameters were preformed by a central laboratory (Quintiles International, Livingston, United Kingdom).

Statistics

All outcome measures, which include the change from pre-treatment (week -1) incremental AUC (iAUC) and ratio to pre-treatment (week -1) area under the curve (AUC) for postprandial concentrations are compared between the two treatment groups using an analysis of covariance (ANCOVA) model. The model includes factors for treatment group (exenatide/glargine), site (NL/SE/FIN), and baseline HbA_{1c} stratum (≤8.5%/>8.5%), and the pre-treatment variable of the corresponding dependent variable as a covariate. The AUC and iAUC were calculated using the trapezoidal rule. Log-transformation was applied to the AUCs before fitting the ANCOVA model. Within treatment group comparison was performed using the paired t-test. Partial correlations between markers of oxidative stress and glucose or triglyceride excursions adjusting for treatment groups were calculated. Statistical analysis was performed using SPSS 16.0 for Mac OS X (SPSS, Chicago, IL, USA). All inferential statistical tests were conducted at a significance level of 0.05 (two-sided).

Results

Prior to treatment no differences between the exenatide and insulin glargine-treated groups were observed (**Table 3.1**). Unless otherwise stated, data are presented as mean±SEM.

	Exenatide (n=36)	**Insulin Glargine (n=33)**
Age (years)	58.4±1.4	58.3±1.3
Male sex (n (%))	23 (63.9)	22 (66.7)
Caucasian race (n (%))	36 (100)	32 (97.0)
Duration of type 2 diabetes (years)	5.7±0.8	4.0±0.6
Daily metformin dose (mg)	2058±117	1798±147
Statin use (n (%))	16 (44.4)	20 (60.6)
Body weight (kg)	90.6±2.1	92.4±2.4
BMI (kg/m^2)	30.9±0.7	30.1±0.6
HbA_{1c} (%)	7.6±0.1	7.4±0.1
Fasting plasma glucose (mmol/L)	9.4±0.4	8.9±0.4
Completed study (n (%))	30 (83.3)	30 (90.9)
Discontinued study (n (%))	6 (16.7)	3 (9.1)
Discontinued study due to adverse events (n (%))	5 (13.9)	1 (3.0)

Table 3.1 Pre-treatment characteristics and patient disposition. Data represent mean±SEM or n (%).

Effects on postprandial plasma glucose concentrations

Postprandial plasma glucose and insulin profiles are shown in **Figure 3.2A-B**. One-year treatment with exenatide significantly reduced postprandial glucose excursions, whereas insulin glargine did not, which resulted in a significant between-group difference in least-squares (LS) mean $iAUC_{0\text{-}8h}$±SEM change from pre-treatment: -13.14±2.10 mmol*h/L; p<0.001. The observed effect was predominantly a result of the effect of exenatide on the breakfast meal (between-group difference LS mean $iAUC_{0\text{-}4h}$±SEM: -8.65±1.16 mmol*h/L; p<0.001). No between-group difference in glucose excursions following the lunch meal was observed (between-group difference LS mean $iAUC_{4\text{-}8h}$±SEM: 0.74±1.25 mmol*h/L, p=0.559). As exenatide predominantly lowers postprandial glucose excursions and insulin glargine

lowers fasting glucose concentrations (10) no between-group difference was observed in the total meal test glucose exposure (between-group ratio of geometric LS mean AUC±SEM ratio to pre-treatment: 0.96±0.04; p=0.310). No correlation was found between (changes in) body weight and postprandial glucose excursions.

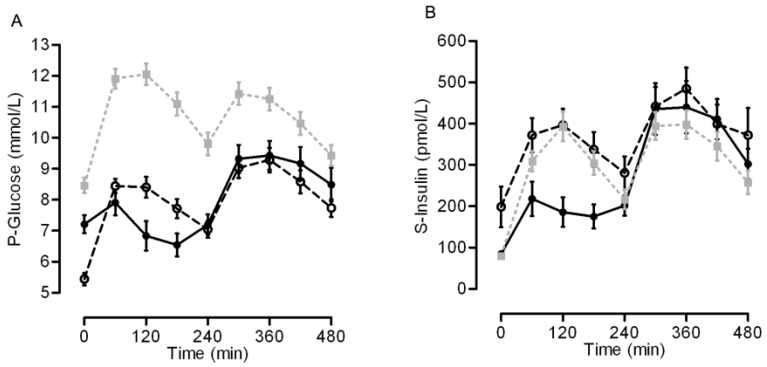

Figure 3.2A-B Postprandial concentration profiles of glucose (A), insulin (B) before and after 51 weeks of treatment. Grey squares/line = pre-treatment all subjects; black circles, solid line = exenatide; open circles, hatched line = insulin glargine. Data represent mean±SEM.

Effects on postprandial serum lipids and lipoprotein concentrations

No between-group differences in fasting lipid parameters were observed after 1 year intensified treatment (data not shown). Exenatide administration had clear effects on lipid profiles following the mixed meal test (**Figure 3.2C-F**), and had a predominant effect on s-TG, s-apo-B48 and s-FFA concentrations after the breakfast meal (between-group difference LS mean $iAUC_{0\text{-}4h}$±SEM change from pre-treatment: 2.3±0.4 mmol*h/L; p<0.001, -16.6±3.0 µg*h/mL; p<0.0001, and -897±294 µmol*h/L; p=0.004, respectively). These strong post-breakfast effects were sustained after exposure to the lunch meal (**Table 3.2**), although the curves did show a rise in these parameters towards the end of our 8-h postprandial follow-up period (**Figure 3.2C-F**).

Additionally, exenatide-treated patients showed a smaller postprandial decline in HDL-C, and a smaller increase in calculated VLDL-C (**Figure 3.2E**), compared to insulin glargine-treated patients (between-group difference $iAUC_{0\text{-}8h}$±SEM change from pre-treatment: +0.33±0.11 mmol*h/L; p=0.005, and -0.98±0.34 mmol*h/L; p=0.006, respectively).

No between-group differences on 8h postprandial s-TC, s-LDL-C, s-apo-A1, s-apo-A2, s-apo-B100, s-apo-C3 concentrations and LDL particle size were observed (**Table 3.2**). No correlations were found between (changes in) body weight and postprandial lipid and lipoprotein excursions.

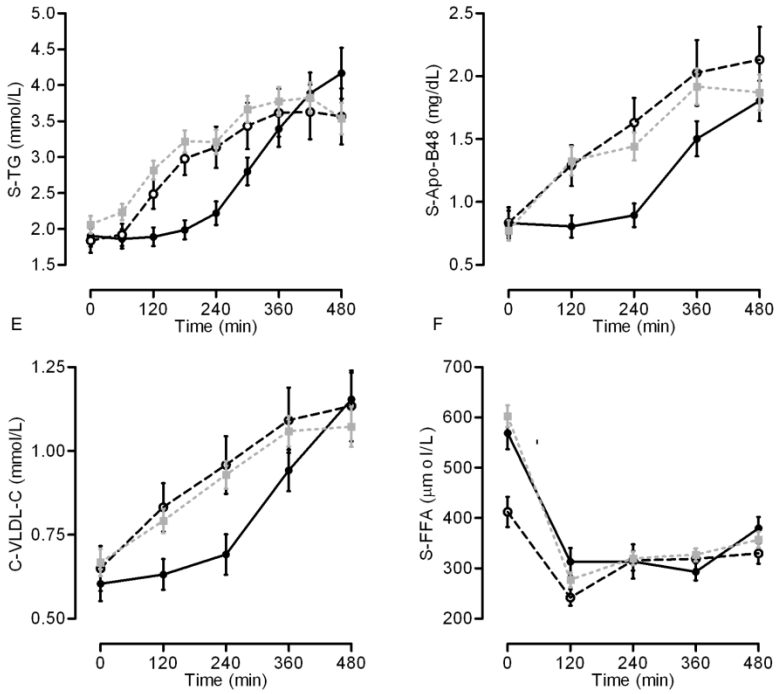

Figure 3.2 C-F Postprandial concentration profiles of triglycerides (TG) (C), apolipoprotein-B 48 (apo-B48) (D), very low-density cholesterol (VLDL-C) (E), and free fatty acids (FFA) (F) before and after 51 weeks of treatment.

	Mean $iAUC_{0-8h}$			On-drug change from pre-treatment (week 51)			Off-drug change from pre-treatment (week 57)		
	Pre-Treatment (week -1)	On-Drug (week 51)	Off-Drug (week 57)	LS mean change±SEM	Between-group difference	P value	LS mean change±SEM	Between-group difference	P value
P-Glucose (mmol*h/L)									
Insulin glargine	21.5±2.0	21.7±2.1	17.7±2.6	+2.2±1.8			-1.4±2.1		
Exenatide	17.5±2.0	6.7±1.3*	15.9±2.0	-10.9±1.7	-13.1±2.1	<0.001	-1.0±1.9	+0.4±2.4	0.856
S-Triglycerides (mmol*h/L)									
Insulin glargine	8.9±0.8	9.0±0.9	8.9±1.4	-1.0±1.0			-2.3±1.3		
Exenatide	8.3±1.2	5.4±1.0#	9.7±1.3	-4.1±1.0	-3.1±1.2	0.014	-0.3±1.1	+1.9±1.5	0.199
S-FFA (10^2µg*h/L)									
Insulin glargine	-18.8±1.9	-14.3±1.9#	-16.3±2.3	+4.5±2.5			-1.6±2.2		
Exenatide	-22.4±2.2	-24.7±2.3	-16.5±2.1	-4.5±2.4	-9.0±2.9	0.004	+0.8±2.0	+2.4±2.6	0.354
S-HDL-C (mmol*h/L)									
Insulin glargine	-0.6±0.1	-0.8±0.1	-0.6±0.1	-0.1±0.1			+0.2±0.1		
Exenatide	-0.7±0.1	-0.5±0.1	-0.7±0.1	+0.3±0.1	+0.3±0.1	0.005	+0.1±0.1	-0.1±0.2	0.154
Calculated VLDL-C (mmol*h/L)									
Insulin glargine	2.0±0.2	2.4±0.3	2.3±0.3	-0.1±0.3			-0.4±0.4		
Exenatide	2.2±0.3	1.4±0.2	2.6±0.4	-1.0±0.3	-1.0±0.3	0.006	+0.2±0.3	+0.6±0.4	0.161

Table 3.2 Between-group comparison of integrated postprandial glucose, lipid and oxidative stress marker concentrations (incremental AUC). Data represents least-squares (LS) mean change from pre-treatment±SEM. Within treatment group comparison: #p<0.05; *p<0.001.

	Mean iAUC$_{0\text{-}8h}$			On-drug change from pre-treatment (week 51)			Off-drug change from pre-treatment (week 57)		
	Pre-Treatment (week -1)	On-Drug (week 51)	Off-Drug (week 57)	LS mean change±SEM	Between-group difference	P-value	LS mean change±SEM	Between-group difference	P-value
S-Apo-B48 (µg*h/mL)									
Insulin glargine	60.2±7.0	61.8±7.4	52.2±10.2	-4.3±7.9			-8.8±8.3		
Exenatide	56.3±7.0	23.9±7.0*	64.0±6.6	-39.1±7.5	-34.8±9.2	<0.001	+3.1±7.4	+11.9±9.6	0.221
S-Apo-B100 (mg*h/dL)									
Insulin glargine	-2.2±6.8	-2.4±6.3	1.1±9.4	-9.6±7.8			8.5±9.8		
Exenatide	6.8±8.1	-1.4±6.6	16.1±6.6	-8.4±7.4	+1.2±9.1	0.893	17.5±8.8	+9.0±11.4	0.435
P-Malondialdehyde (µmol*h/L)									
Insulin glargine	14.5±2.2	16.0±2.3	13.6±3.2	5.2±2.3			+2.9±2.8		
Exenatide	8.8±2.3	-0.3±1.7*	8.7±2.3	-9.3±2.1	-14.5±2.6	<0.001	+0.8±2.5	-2.2±3.3	0.507
P-oxLDL:LDL-C (U/mmol)									
Insulin glargine	13.6±3.2	15.2±5.9	11.2±4.0	-3.0±6.7			-5.9±4.2		
Exenatide	19.0±3.2	2.5±4.6	11.6±2.7	-13.5±6.6	-10.5±7.8	0.184	-4.8±3.7	+1.0±4.7	0.825

Table 3.2 cont. Between-group comparison of integrated postprandial glucose, lipid and oxidative stress marker concentrations (incremental AUC). Data represents least-squares (LS) mean change from pre-treatment±SEM. Within treatment group comparison: #p<0.05; *p<0.001.

Effects on postprandial circulating markers of oxidative stress

Post-meal profiles of oxidative stress markers are shown in **Figure 3.3**. Exenatide treatment led to significantly lower p-MDA profiles, whereas insulin glargine did not have such an effect (mean $iAUC_{0\text{-}8h}$ change from pre-treatment: -9.1±2.1 µmol*h/L, p<0.001 and +1.5±2.4 µmol*h/L, respectively, p=0.527), resulting in a significant between-treatment group difference (**Table 3.2**). As shown with postprandial glucose this effect was mainly due to a strong effect following the breakfast meal (between-group difference LSmean $iAUC_{0\text{-}4h}$±SEM change from pre-treatment -8.8±1.4 µmol*h/L, p<0.001). The postprandial OxLDL-to-LDL-C ratio was significantly reduced following exenatide treatment whereas insulin glargine did not affect this oxidative stress marker (mean $iAUC_{0\text{-}8h}$ change from pre-treatment: -16.3±6.6 U*h/mmol, p=0.019 and +2.6±6.9 U*h/mmol, respectively, p=0.711). However, no significant between-group difference in postprandial OxLDL:LDL-C excursions was observed (between-group difference LS mean $iAUC_{0\text{-}8h}$ change from pre-treatment -10.5±7.8 U*h/mmol, p=0.184; **Table 3.2**).Partial correlation analysis, adjusted for treatment group, showed significant linear associations between the plasma glucose and p-MDA concentrations at the time of the peak glucose concentration (t=120 minutes) and between the triglyceride and OxLDL-to-LDL-C ratio at the lowest TG concentration (t=240 minutes). For both measures of oxidative stress (r=0.92; p<0.001 and r=0.44, p=0.001 respectively; **Figure 3.3**).

Effects on fasting plasma glucose and serum lipid concentrations

As shown previously (12), both exenatide and insulin glargine treatment reduced FPG concentrations. As a result of the treat to FPG <5.6 mmol/L the observed decrease was statistically significant greater in the glargine treated patients. In both treatment groups the decrease in FPG was accompanied by a decrease in plasma MDA concentrations. There was a strong correlation between FPG and p-MDA concentrations after 52-weeks: r= 0.82, p<0.001. No between-group differences in any of the fasting lipid parameters were observed after 1 year intensified treatment (**Table 3.3**).

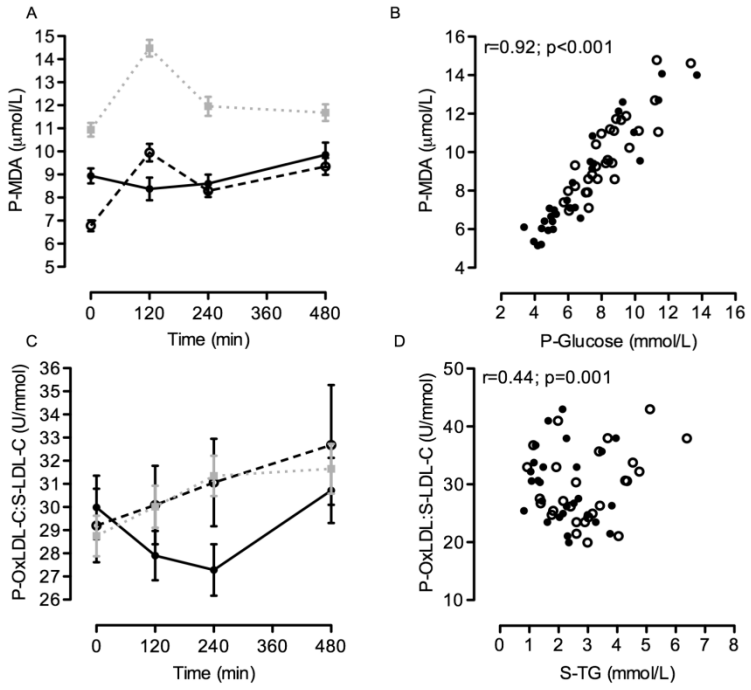

Figure 3.3 Postprandial concentration profiles for malondialdehyde (MDA) (A) and oxidized low-density lipoprotein (OxLDL) to low-density lipoprotein cholesterol (LDL-C) ratio (C) and relationships between peak postprandial glucose and MDA excursions at t=120 minutes (B) and relationships between nadir postprandial plasma triglycerides and oxidized low-density lipoprotein-to-low-density lipoprotein cholesterol ratio at t=240 minutes during the on-drug meal test (week 51). Grey squares/line = pre-treatment all subjects; black circles, solid line = exenatide; white circles, hatched line = insulin glargine. Data represent mean±SEM, partial correlation coefficient (r) and p-value.

Effects after 5-week cessation of study treatment

Following 5 weeks discontinuation of exenatide and insulin glargine treatment, all postprandial measures returned back to pre-treatment values, with no statistically significant between-group difference remaining.

	Geometric mean±SEM			Percentage change from pre-treatment (week 51)		Percentage change from pre-treatment (week 57)	
	Pre-Treatment (week -1)	On-Drug (week 51)	Off-Drug (week 57)	LS mean change±SEM	Between-group difference	LS mean change±SEM	Between-group difference
P-Glucose (mmol/L)							
Insulin Glargine	7.95±0.28	5.34±0.19	7.86±0.43	-34% (-38%;-29%)		-1% (-9%; +8%)	
Exenatide	8.59±0.33	7.06±0.26	7.83±0.36	-15% (-21%;-8%)	+29% (+18%;+40%)	-6% (-13%; +2%)	-5% (-14%; +5%)
S-Triglycerides (mmol/L)							
Insulin Glargine	2.04±0.16	1.70±0.15	1.74±0.15	-10% (-23%; +6%)		-11% (-23%; +4%)	
Exenatide	1.80±0.16	1.80±0.13	1.62±0.14	+1% (-13%; +17%)	+12% (-7%; +35%)	-6% (-17%; +7%)	+6% (-11%; +25%)
S-Insulin (pmol/L)							
Insulin Glargine	63.9±6.4	145.4±18.5	72.2±9.7	+124% (+84%; +172%)		-4% (-19%; +15%)	
Exenatide	69.3±9.1	67.5±7.6	57.3±7.1	-2% (-19%; +18%)	-56% (-65%; +82%)	-20% (-31%; -6%)	-17% (-32%; +2%)
S-Total Cholesterol (mmol/L)							
Insulin Glargine	4.51±0.51	4.34±0.15	4.52±0.18	-0% (-6%; +5%)		+0% (-5%; +7%)	
Exenatide	4.59±0.21	4.56±0.20	4.57±0.21	+1% (-4%; +6%)	+2% (-4%; +8%)	+3% (-2%; +9%)	+3% (-4%; +10%)
S-LDL-C (mmol/L)							
Insulin Glargine	2.62±0.13	2.51±0.15	2.71±0.16	-3% (-11%; +7%)		+2% (-7%; +13%)	
Exenatide	2.75±0.18	2.74±0.17	2.75±0.15	+2% (-7%; +11%)	+4% (-6%; +17%)	+5% (-3%; +14%)	+3% (-8%; +15%)
S-HDL-C (mmol/L)							
Insulin Glargine	1.13±0.05	1.16±0.05	1.15±0.06	+4% (-2%; +10%)		+3% (-4%; +11)	
Exenatide	1.15±0.06	1.15±0.05	1.21±0.06	+2% (-3%; +8%)	-2% (-8%; +5%)	+8% (+2%; +15%)	+3% (-8%; +15%)
Calculated VLDL-C (mmol/L)							
Insulin Glargine	0.62±0.07	0.55±0.06	0.53±0.07	-1% (-20%; +23%)		-8% (-29%; +20%)	
Exenatide	0.55±0.07	0.54±0.05	0.54±0.05	-1% (-19%; +21%)	-0% (-23%; +29%)	-6% (-24%; +17%)	+2% (-24%; +38%)
S-FFA (µg/L)							
Insulin Glargine	5.56±0.22	3.75±0.31	5.26±0.38	-28% (-39%; -15%)		+7% (-8%; +24%)	
Exenatide	6.19±0.38	5.57±0.35	4.89±0.33	-1% (-16%; +15%)	+37% (+13%; +67%)	-8% (-19%; +5%)	-14% (-27%; +3%)

Table 3.3 Between-group comparison of meal test fasting glucose, lipid and oxidative stress marker concentrations. Data represents geometric mean±SEM and adjusted least-squares (LS) mean percentage change (95% Confidence Interval (CI)) from pre-treatment. Bold confidence intervals represent statistical significant ($p<0.05$) changes from pre-treatment values.

	Geometric mean±SEM			Percentage change from pre-treatment (week 51)		Percentage change from pre-treatment (week 57)	
	Pre-Treatment (week -1)	On-Drug (week 51)	Off-Drug (week 57)	LS mean change±SEM	Between-group difference	LS mean change±SEM	Between-group difference
S-Apo-A1 (mg/dL)							
Insulin Glargine	127.5±4.1	126.7±3.6	125.7±4.0	1% (-3%; +5%)		-2% (-6%; +3%)	
Exenatide	127.0±4.9	127.9±3.8	130.4±4.6	+2% (-2%+6%)	+1% (-4%+6%)	+5% (+1%; +9%)	+7% (+2%; +12%)
S-Apo-A2 (mg/dL)							
Insulin Glargine	28.5±0.9	28.0±.08	27.8±0.8	+0% (-3%; +4%)		-2% (-6%; +3%)	
Exenatide	28.9±1.0	28.4±0.8	28.7±1.1	+0% (-3%; +3%)	-0% (-4%; +4%)	+2% (-2%; +6%)	+3% (-2%; +9%)
S-Apo-B48 (µg/mL)							
Insulin Glargine	7.29±0.89	6.76±0.79	6.25±0.85	+4% (-19%; +34%)		-8% (-30%; +20%)	
Exenatide	5.34±0.56	6.96±0.77	5.96±0.74	+25% (-2%; +59%)	+20% (-11%; +63%)	+12% (-11%; +42%)	+22% (-12%; +68%)
S-Apo-B100-(mg/dL)							
Insulin Glargine	104.2±4.3	96.7±4.9	100.2±5.0	-5% (-12%; +3%)		-4% (-11%; +4%)	
Exenatide	102.5±4.6	100.4±4.6	99.6±4.4	-1% (-7%; +7%)	+4% (-4%; +14%)	+0% (-6%; +7%)	+4% (-4%; +14%)
S-Apo-C3 (mg/dL)							
Insulin Glargine	14.1±1.0	12.5±0.8	13.1±1.0	-7% (-16%; +3%)		-1% (-13%; +11%)	
Exenatide	13.5±1.0	12.5±0.7	12.9±0.9	-6% (-15%; +4%)	+1% (-10%; +14%)	+2% (-9%; +14%)	+3% (10%; +19%)
P-Malondialdehyde (µmol/L)							
Insulin Glargine	10.09±0.36	6.67±0.23	9.30±0.43	-37% (-41%; -32%)		-12% (-20%; -3%)	
Exenatide	11.340.45	8.78±0.31	9.90±0.40	-19% (-25%; -12%)	+29% (+17%; +41%)	-7% (-15%; +1%)	+5% (-6%; +17%)
P-oxLDL:LDL-C (U/mmol)							
Insulin Glargine	28.1±1.2	28.1±1.4	27.1±1.5	+0% (-8%; +10%)		-7% (-14%; +4%)	
Exenatide	28.0±1.2	27.2±1.2	27.0±1.1	-3% (-11%; +6%)	-3% (-13%; +8%)	-9% (-14%; -1%)	-4% (-14%; +7%)
P-LDL size (nm)							
Insulin Glargine	20.8±0.1	20.7±0.1	20.7±0.1	-0% (-1%; +0%)		-0% (-1%; +0%)	
Exenatide	20.8±0.1	20.7±0.1	20.7±0.1	-0% (-1%; +0%)	+0% (-1%; +1%)	-0% (-1%; +0%)	+0% (-1%; +1%)

Table 3.3 cont. Between-group comparison of meal test fasting glucose, lipid and oxidative stress marker concentrations. Data represents geometric mean±SEM and adjusted least-squares (LS) mean percentage change (95% Confidence Interval (CI)) from pre-treatment. Bold confidence intervals represent statistical significant ($p<0.05$) changes from pre-treatment values.

Adverse effects and tolerability

The most frequently observed adverse event in exenatide-treated patients was mild-to-moderate nausea, observed in 50% of the patients treated with exenatide. Biochemically confirmed hypoglycemia (<3.3 mmol/L) was observed more frequently in the insulin glargine group (24.2%), as compared to the exenatide treated patients (8.3%). There was no severe hypoglycemia with either treatment (12).

Discussion

Our study is the first to show that one-year exenatide treatment reduces postprandial serum triglycerides, apolipoprotein-B48, calculated VLDL-C and FFA excursions to a greater extent than with insulin glargine, against a background of similar glycemic control. Exenatide also reduced the postprandial oxidative stress markers p-MDA and p-oxLDL. These data confirm our earlier finding that exenatide primarily reduces postprandial glucose excursions, whereas insulin glargine lowers fasting glucose concentrations (10), and extend these with the strong observed correlation between plasma glucose and MDA concentrations, predominantly following the first meal after the exenatide injection.

Postprandial hyperglycemia, dyslipidemia and oxidative stress have all been associated with risk of cardiovascular disease, and are known to be present in patients with type 2 diabetes (1). Previous studies have shown postprandial hypertriglyceridemia to be associated with endothelial dysfunction and elevated oxidative stress parameters in patients with type 2 diabetes and recent data suggest that apo-B48 and apo-B100-containing lipoproteins are equally atherogenic (2).

GLP-1 receptor agonists influence postprandial glucose and lipid metabolism through several mechanisms (15). In the current study, exenatide significantly decreased postprandial glucose, triglycerides and apo-B48 (the structural lipoprotein in chylomicrons) excursions compared to insulin glargine. In the postprandial state, intestinal lipids are absorbed from the small intestinal lumen as fatty acids and cholesterol, esterified to triglycerides and cholesterol esters, and secreted as chylomicrons to the surrounding lymph ducts. Animal studies have demonstrated that GLP-1 slows intestinal uptake of triolein and decreases intestinal lymph flow (16). Additionally, a recent study showed that the GLP-1 receptor signaling is essential for postprandial lipoprotein synthesis and secretion, and reduces intestinal secretion of triacylglycerol, cholesterol and apo-B48 (17). Also, fatty acid binding protein 2 (FABP2) is required for the formation of apo-B48-containing chylomicrons and is influenced by various intestinal hormones, such as GLP-1 (18). Combined, these data suggest that,

additional to the effect on gastric emptying, GLP-1 reduces intestinal lipid production and absorption, preventing the postprandial increase in triglyceride levels (16,17). Unfortunately, as a result of blood volume restrictions by our ethical review board we did not measure glucagon concentrations in the current study. The glucagon-suppressing effects of GLP-1RA such as exenatide are well-known and have been reported from many different studies (9).

We found postprandial calculated VLDL-C concentrations to be lower in exenatide-treated patients without a between-group difference in postprandial apo-B100 concentrations. Hepatic overproduction of apo-B100-containing VLDL1 particles is regarded as the dominant feature of diabetic dyslipidemia driven primarily by liver fat and hyperglycemia (19). Surprisingly, in both treatment arms, no improvement in postprandial apo-B100 levels was observed (**Table 3.2**). Calculated VLDL-C should be interpreted as non-HDL/LDL particle cholesterol, which includes chylomicron cholesterol. As we showed a vast reduction in the apo-B48 concentration, the reduction in calculated VLDL-C may merely be a result of this observed decrease in apo-B48, an observation that is strengthened by the unchanged apo-B100 concentrations in both treatment groups.

Low fasting HDL-C is an important feature of diabetic dyslipidemia and cardiovascular risk factor, which is reduced further during the postprandial period (1). This study shows that exenatide diminishes the postprandial reduction in HDL-C without having an effect on apo-A1, the predominant lipoprotein in HDL particles. The between-group difference in postprandial triglycerides may contribute to this difference in HDL-C. Plasma triglycerides drive the exchange of lipids between triglyceride-rich lipoproteins (such as VLDL) and HDL particles. Also, triglycerides in HDL particles are a good substrate for hepatic lipase and the hydrolysis produces smaller particles and free HDL apo-A1 that is subsequently cleared by the kidneys (2).

This study confirms our earlier findings that exenatide lowered postprandial glucose excursions compared to insulin glargine, using a 4-hour mixed-meal test (10). We extended this finding by showing that the total glucose excursion remained significantly lower with exenatide compared to insulin glargine during an 8-hour mixed-meal test, even after ingestion of an additional lunch meal, 4 hours after the (pre-breakfast) exenatide dose. Even though glucose excursions following the lunch meal were equal in both the exenatide and insulin glargine group, the total glucose exposure during the whole 8-hour meal test was significantly lower in the exenatide treated patients. Decreased gastric emptying, a reduction in endogenous (hepatic) glucose production and potentiation of beta-cell function have been

attributed to reduce postprandial glucose excursions (9, 20). Although, the total 8-h p-glucose, s-TG, and s-apo-B48 excursions (measured as iAUC) remain lower in the exenatide treated patients, a rise following the second, lunch meal appeared to be present, resulting in higher concentrations in the exenatide group after 8-h of follow-up. An explanation for this phenomenon may be that the lipid particles are sequestrated in the gut during the first meal and gradually become available during the second meal (20). Whether, and for how long, this increase continues after the second meal need further study using 24-h meal tests.
In addition, recent findings attribute a role to portal vein GLP-1 receptors in increasing postprandial glucose clearance independent of islet hormones (21). Postprandial improvement in several model-derived measures of beta-cell function has been described for exenatide (22) as well as for other incretin-based therapies (23,24). Whether these improvements in postprandial beta-cell function extend to after a second lunch meal, 4-h after exenatide administration, and possible explaining mechanisms as the Staub-Traugott effect (25) need further investigation.

Both postprandial hyperglycemia and hypertriglyceridemia lead to mitochondrial free radical production and subsequent oxidative stress, which in turn may contribute to the development of microvascular and macrovascular complications (5). Reducing postprandial glucose and lipid excursions has been suggested to be included in the treatment guidelines for type 2 diabetes (26). Our data show that attenuating postprandial with exenatide and fasting glucose excursions with insulin glargine are related to beneficial effects on malondialdehyde, as is the case for triglyceride and oxidized LDL concentrations. Also, this observed correlations suggest that lowering of glucose and triglyceride excursions may positively affect malondialdehyde and oxidized LDL concentrations. The observed findings may result in favorable effects on individual cardiovascular risk, which is suggested by a recent meta-analysis of exenatide clinical trial data (27).

In summary, the current study showed that one-year treatment with exenatide, as compared to insulin glargine, had beneficial effects on postprandial glycemia, dyslipidemia and measures of oxidative stress in patients with type 2 diabetes. Whether long-term treatment with GLP-1 receptor agonists, such as exenatide, leads to a decrease in diabetes-related cardiovascular complications can only be answered by a prospective long-term outcomes study.

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Chapter 5

Exenatide affects circulating cardiovascular risk biomarkers independently of changes in body composition

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Abstract

Background: To study the effect of exenatide on body composition and circulating cardiovascular risk biomarkers.

Methods: Metformin-treated patients with type 2 diabetes (N=69) were randomized to exenatide or insulin glargine and treated for one-year. Body composition was evaluated by Dual-Energy X-ray Absorptiometry. Additionally, body weight, waist circumference and cardiovascular biomarkers were measured.

Results: One-year treatment with exenatide significantly reduced body weight, waist circumference, total body and truncal fat mass by 6%, 5%, 11% and 13%, respectively. In addition, exenatide increased total adiponectin by 12%, and reduced hsCRP by 61%. Insulin glargine significantly reduced endothelin-1 by 7%. These changes were statistically independent of the change in total body fat mass and body weight.

Conclusions: Exenatide treatment for 1 year reduced body fat mass and improved the profile of circulating biomarkers of cardiovascular risk. No significant changes were seen with insulin glargine except a trend for reduced endothelin-1 levels.

Introduction

Abdominal obesity is associated with both type 2 diabetes and metabolic complications (1), including elevations in several circulating biomarkers of cardiovascular risk (2). Most pharmacologic glucose-lowering treatments increase body weight (3). Therefore, treatments that not only reduce glycated hemoglobin A_{1c}, but also improve other associated changes such as abdominal obesity are urgently needed (4).

We previously reported in that exenatide improves glycemic control to the same extent as insulin glargine, while exenatide decreased and insulin glargine raised body weight (5). Herein we present additional data on associated changes in body composition and circulating levels of biomarkers of cardiovascular risk following one-year's treatment.

Research design and methods

Details on study design were reported previously (5). Patients were randomized to exenatide (n=36) or insulin glargine (n=33) added to their ongoing metformin therapy (**Figure 5.1**). The study protocol was approved by each site's ethical review committee and was in accordance with the principles described in the declaration of Helsinki and all participating patients gave their written informed consent prior to screening. This study is registered with ClinicalTrials.gov number NCT00097500.

Dual-Energy X-ray Absorptiometry (DEXA) scan

Lean body and fat mass was assessed using Dual Energy X-ray Absorptiometry (DEXA) scans (Delphi A, Hologic, Waltham, MA) at baseline, and after treatment. Trunk (abdominal) and limb (hip/leg) regions of interest were determined from a total body scan. Waist circumference was measured at the midline of the interval between iliac crest and lowest rib using the mean of two measurements prior to the DEXA scan.

Biochemical analyses

Cardiovascular risk biomarkers were collected at baseline and after one-year of treatment. Serum was separated by centrifugation and stored at -80°C until analysis. All serum samples were analyzed in the Lundberg Laboratory for Diabetes Research using a single batch. Total adiponectin, high molecular weight (HMW) adiponectin, resistin, leptin, high-sensitive C-reactive protein (hsCRP), interleukin (IL)-6, monocyte chemotactic protein (MCP)-1, endothelin-1 were determined by commercial ELISA's (R&D Systems, Abingdon,UK).

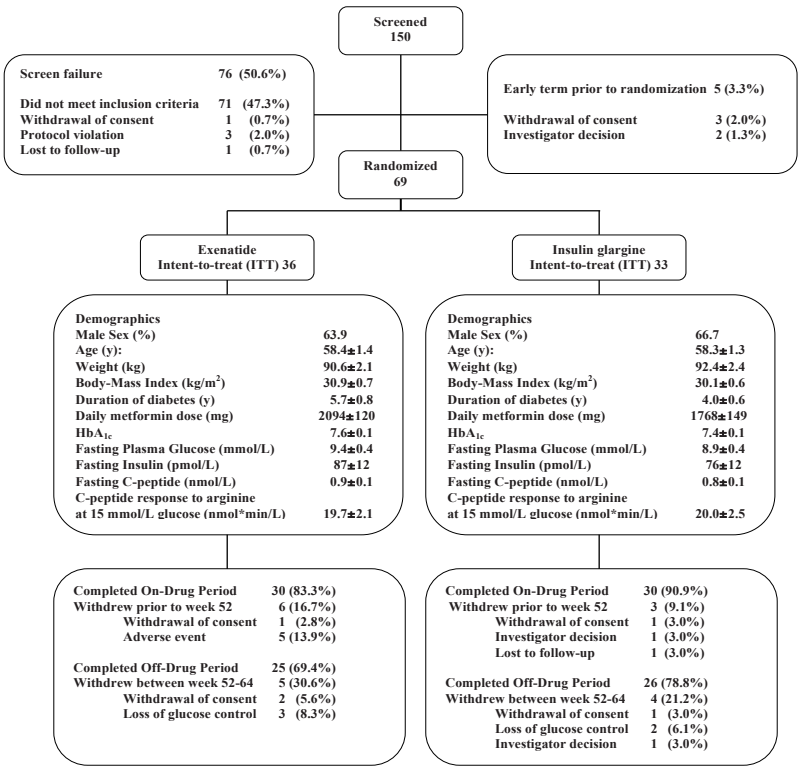

Figure 5.1 Protocol flow chart and baseline characteristics of the study population. Data represent mean±SEM.

Statistical analysis

Non-normally distributed data was log-transformed prior to statistical analysis, after which the approximated the normal distribution. All outcome measures are compared between the two treatment groups using an analysis of covariance (ANCOVA) model including factors for treatment, investigative site and baseline HbA_{1c} stratum (≤8.5% or >8.5%) and baseline values of corresponding outcome measure as a covariate (5). Statistical analysis was performed using SPSS 16.0 for Mac OS X (SPSS, Chicago, IL, USA). All inferential statistical tests were conducted at a significance level of 0.05 (two-sided).

Results

One-year treatment with exenatide resulted in a statistically significant reduction in total body fat mass (**Table 5.1**), mainly in the abdominal region, as illustrated by the decrease in trunk fat mass and waist circumference, in contrast to insulin glargine. Neither treatment significantly affected lean body mass.

In univariate analysis, the reduction in body weight in the exenatide arm was significantly correlated with the changes in leptin (r=0.580, p=0.001) and hsCRP (r=-0.590, p=0.001). No statistical significant univariate correlation was found between changes in body weight and other biomarkers. Interestingly, changes in all circulating biomarkers did not correlate with the changes in total body fat mass (total adiponectin: Pearson's r=-0.224, p=0.106; HMW adiponectin: r=0.057, p=0.694; leptin: r=0.229, p=0.106; hsCRP: r=-0.023, p=0.872).

Following multivariate analysis, statistical adjustment for body weight change, exenatide increased total adiponectin, and decreased hsCRP concentrations, whereas insulin glargine did not (**Table 5.1**). Insulin glargine reduced endothelin-1 concentrations, whereas exenatide did not have such an effect. No statistically significant effects of either treatment on HMW adiponectin, IL-6, MCP-1, and resistin was observed.

The crude between treatment group differences remained statistically significant after additional multivariate adjustment for total body fat mass change: total adiponectin +16% (95%CI:+5%; +28%), p=0.004; leptin -20% (-34%;-2%), p=0.028; hsCRP -48% (-69%;-13%), p=0.015; and body weight change (**Table 5.1**): total adiponectin +17% (95%CI:+6%;+30%), p=0.004; leptin -19% (-34%;-0%), p=0.045; hsCRP -52% (-71%;-19%), p=0.008.

			Percentage change from baseline		
	Baseline	Endpoint	LS mean (95% CI)	Between group difference	*p-value*
Total Fat Mass (kg)					
Insulin Glargine (n=28)	29.9±1.6	28.5±1.9	-1% (-7%; +5%)		
Exenatide (n=29)	27.8±1.4	25.4±1.6	-11% (-18%; -5%)	-10% (-16%; -4%)	0.003
Total Lean Mass (kg)					
Insulin Glargine (n=28)	60.1±1.7	60.6±1.8	0% (-1%; +2%)		
Exenatide (n=29)	57.8±2.1	58.1±2.4	0% (-2%; +1%)	-1% (-3%; +1%)	0.480
Trunk Fat Mass (kg)					
Insulin Glargine (n=28)	17.8±0.9	16.6±1.1	-1% (-8%; +5%)		
Exenatide (n=29)	16.3±0.8	14.8±1.0	-13% (-18; -7%)	-11% (-18%; -4%)	0.002
Body Weight (kg)					
Insulin Glargine (n=29)	94.1±2.5	93.8±2.7	-1% (-3%; +1%)		
Exenatide (n=30)	90.3±2.4	86.4±2.6	-6% (-8%; -3%)	-5% (-7%; -2%)	0.001
Waist Circumference (cm)					
Insulin Glargine (n=29)	106.9±1.9	107.4±2.0	+1% (-1%; +3%)		
Exenatide (n=30)	106.1±1.9	100.6±2.1	-5% (-7%; -3%)	-6% (-8%; -4%)	<0.001
Leptin (ug/L)					
Insulin Glargine (n=29)	7.79±1.29	8.41±1.53	+7% (-9%; +25%)		
Exenatide (n=30)	8.50±1.32	7.45±1.17	-15% (-26%; -1%)	-20% (-33%; -3%)	0.020

Table 5.1 Body composition, circulating cardiovascular risk biomarkers and percentage change from baseline. Data represent mean±SEM (body composition measures) or geometric mean±SEM (cardiovascular biomarkers) and body weight change adjusted least-squares (LS) mean percentage change (95% Confidence Interval (CI)) from baseline.

	Percentage change from baseline				
	Baseline	Endpoint	LS mean (95% CI)	Between group difference	*p-value*
Total Adiponectin (ng/mL)					
Insulin Glargine (n=29)	4648±461	4508±436	-2% (-10%; +8%)		
Exenatide (n=30)	4848±432	5314±466	+12% (+3%; +22%)	+14% (+3%;+26%)	0.015
HMW Adiponectin (ng/mL)					
Insulin Glargine (n=29)	1277±221	1321±236	-1% (-22%; +25%)		
Exenatide (n=30)	1571±255	1850±273	+16% (-8%; +45%)	+17% (-11%; +54%)	0.264
hsCRP (mg/L)					
Insulin Glargine (n=29)	1.42±0.27	1.38±0.35	-20% (-47%; +21%)		
Exenatide (n=30)	1.81±0.25	1.30±0.22	-53% (-31%; -31%)	-42% (-64%; -4%)	0.033
Il-6 (pg/mL)					
Insulin Glargine (n=29)	1.96±0.21	2.17±0.20	-1% (-22%; +26%)		
Exenatide (n=30)	2.11±0.22	2.10±0.25	-6% (-25%; +18%)	-5% (-28%; +26%)	0.721
MCP-1 (pg/mL)					
Insulin Glargine (n=29)	1.22±0.07	1.24±0.07	-1% (-10%; +9%)		
Exenatide (n=30)	1.18±0.09	1.21±0.11	-2% (-10%; +8%)	-1% (-11%; +11%)	0.894
Resistin (ng/mL)					
Insulin Glargine (n=29)	330±15	329±20	-2% (-10%; +7%)		
Exenatide (n=30)	316±14	311±16	+1% (-7%; +9%)	+3% (-7%; +13%)	0.611
Endothelin-1 (ng/mL)					
Insulin Glargine (n=29)	2.57±0.18	2.46±0.19	-5% (-9%; +0%)		
Exenatide (n=30)	2.53±0.19	2.53±0.19	-1% (-4%; +4%)	+4% (-1%; +10%)	0.103

Table 5.1 cont. Body composition, circulating cardiovascular risk biomarkers and percentage change from baseline. Data represent mean±SEM (body composition measures) or geometric mean±SEM (cardiovascular biomarkers) and body weight change adjusted least-squares (LS) mean percentage change (95% Confidence Interval (CI)) from baseline.

Discussion

This study showed that exenatide reduced body fat mass and improved the profile of circulating cardiovascular biomarkers. The changes in the different biomarkers could not be fully attributed to the observed changes in body fat mass and body weight. Direct effects of GLP-1 receptor agonists on adipocyte function have been described in both animal experimental studies as in *in-vitro* studies in normal human adipocytes (for review see reference 6), however as a significant univariate correlation between change in body weight (not with fat mass) change and cardiovascular biomarkers was present, our relatively small population may influence the statistical power of our study.

Animal studies have also demonstrated beneficial effects of exenatide on visceral fat mass (7) and circulating adiponectin (8), leptin (9), and CRP (10) concentrations. However, to the best of our knowledge, controlled clinical studies on the long-term effects of GLP-1 receptor agonists on body composition and biomarkers of cardiovascular risk have not previously been reported.
A recent 3-month study comparing exenatide to insulin glargine in 56 patients with type 2 diabetes has a design comparable to our one-year study. Similar to our findings, this study showed that exenatide treatment was associated with reduced hsCRP, without affecting the IL-6 levels (11).

Sub-analysis of the LEAD-3 data reported that liraglutide, treatment for 52 weeks compared to glimepiride reduced DEXA measured total fat tissue mass (12). Lean tissue mass was also reduced after one-year treatment, but as glimepiride also reduced lean tissue mass, this reduction was not statistically significantly different between the groups. 26-week data from the LEAD-2 study was used to show that the observed reduction in fat mass was mainly a result of a reduction in visceral fat (12). Unfortunately, this study did not report the effects of body composition on circulating biomarkers. Serum leptin, hsCRP, and IL-6 concentrations did not change in a 14-week placebo-controlled study with liraglutide 1.9 mg (13).

Of particular interest in our study was the finding that the changes in biomarkers of cardiovascular risk appeared to be independent of the changes in body fat mass. Recently, Chung and colleagues reported exendin-4 directly increased adiponectin mRNA levels and secretion in 3T3-L1 adipocytes (14). In that study, exendin-4 also decreased mRNA levels of IL-6 and MCP-1 (14). Additionally, we (15), and others (10), have previously reported beneficial effects of exenatide on hepatic steatosis, which also may contribute to a reduction in CRP.

In conclusion, we found that exenatide treatment for one-year led to a reduced total fat mass, including visceral fat, while lean body mass was not significantly altered. Additionally, the circulating levels of adiponectin, leptin, and hsCRP showed an improved profile, which appeared to be independent of the changes in fat mass. In contrast, no significant changes in body composition or circulating biomarkers were seen with insulin glargine.

Acknowledgments

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Chapter 6

Effects of exenatide on measures of beta-cell function after 3-years in metformin-treated patients with type 2 diabetes

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Abstract

Background: Previously we showed that exenatide enhanced insulin secretion after 1-year treatment, relative to insulin glargine, with a similar glucose-lowering action. These effects were not sustained after a 4-week off-drug period. Here we report the results after additional 2 years of exposure.

Methods: Sixty-nine metformin-treated patients with type 2 diabetes were randomized to exenatide or insulin glargine. Forty-six patients entered the 2-year extension study in which they continued their allocated therapy. Thirty-six completed (exenatide n=16; insulin glargine n=20) the 3-year exposure period. Insulin sensitivity (M-value) and beta-cell function were measured by euglycemic hyperinsulinemic clamp followed by hyperglycemic clamp with arginine stimulation at pre-treatment, week 52, and 4 weeks after discontinuation of study medication at week 56, and week 172. First-phase glucose stimulated C-peptide secretion was adjusted for M-value and calculated as the disposition index (DI).

Results: At 3-years, exenatide and insulin glargine resulted in similar levels of glycemic control: 6.6±0.2% and 6.9±0.2%, respectively (P=0.186). Exenatide compared to insulin glargine significantly reduced body weight (-7.9±1.8 kg; P<0.001). After the 4-week off-drug period, exenatide increased the M-value by 39% (p=0.006) while insulin glargine had no effect (p=0.647). Following the 4-week off-drug period, the DI, compared to pre-treatment, increased with exenatide, but decreased with insulin glargine (+1.43±0.78 and -0.99±0.65, respectively; P=0.028).

Conclusions: Exenatide and insulin glargine sustained HbA_{1c} over 3-year treatment, while exenatide reduced, and insulin glargine increased body weight. Following 3-year treatment with exenatide the DI was sustained after a 4-week off-drug period. These findings suggest a beneficial effect on beta-cell health.

Introduction

Type 2 diabetes is characterized by progressive beta-cell dysfunction against a background of obesity-related peripheral and hepatic insulin resistance (1). Current treatment guidelines promote a step-wise approach, starting with lifestyle and metformin, and adding a next agent, as soon as target HbA_{1c} values cannot be sustained below 7% (2). None of the presently advocated pharmacological interventions, most of which were already used in the UKPDS (3), address the underlying pathophysiological factors of type 2 diabetes, especially beta-cell function (4). Due to this progressive decline of beta-cell function, in the presence of additional glucose toxicity, the majority of patients will require polypharmacy and eventually insulin therapy to maintain acceptable glycemic control (4). Therefore, novel treatment options specifically addressing the beta-cell function defect are eagerly awaited.

Exenatide is the first-in-class glucagon-like peptide-1 receptor agonist (GLP-1RA), which improves blood glucose in patients with type 2 diabetes by many different mechanisms (5). Exenatide predominantly lowers postprandial glucose by a glucose-dependent stimulation of insulin-secretion, inhibition of an inappropriate glucagon secretion and by slowing down gastric emptying (6). Additionally, exenatide promotes satiety, decreases food intake and reduces body weight (6).

Previously, we showed exenatide, as compared to insulin glargine, improved pancreatic beta-cell secretory function against a background of similar glycemic control (7). However, these findings were not sustained after a 4-week off-drug period, thus it was not possible to demonstrate disease modification (7).

The aim of this extension study was to assess the long-term effects of exenatide and insulin glargine on glycemic control, body weight, and safety, after an additional 2-year treatment period and during a 12-week off-drug period. During the off-drug period, clamp derived measures of beta-cell function and insulin sensitivity were assessed after 4 weeks.

Research design and methods

The study was performed between September 2004 and December 2009 at three study sites, in Sweden, Finland and the Netherlands. The one-year data were reported previously (7). In total, 150 patients were screened of which 69 patients were randomized using a permutated block randomization scheme stratified by site and screenings HbA_{1c} to receive exenatide or insulin glargine, in addition to ongoing metformin treatment. Inclusion criteria were: age 30-75 years, HbA_{1c} 6.5-9.5%, body mass index 25-40 kg/m^2, and metformin treatment at a stable dose for at least 2

months. No other blood glucose lowering agents were allowed within 3 months prior to screening. The study protocol was approved by each site's ethics review committee and was in accordance with the principles described in the declaration of Helsinki. All participating patients gave their written informed consent prior to screening. This study is registered with ClinicalTrials.gov number NCT00097500.

Experimental design

Patients randomized to exenatide (n=36) initiated treatment at a dose of 5 μg twice daily (BID), injected 15-min before breakfast and dinner, for a period of 4 weeks, followed by dose increase to 10 μg BID. Exenatide was titrated to a maximum dose of three times (TID) 20 μg, or the maximum tolerated dose, when HbA_{1c} ranged 7.1-7.5% at two consecutive visits, or when HbA_{1c} was ≥7.6% at any given visit. Patients randomized to insulin glargine (n=33) started at an initial dose of 10 IU once daily (QD), injected at bedtime. Patients were instructed to increase the daily dose based on their fasting self-monitored blood glucose (SMBG) levels (<5.6 mmol/L), according to a pre-specified treat-to-target algorithm (8). When necessary, the importance of proper titration of insulin was emphasized.

After completing the one-year main treatment period of the study, patients were asked to return to their randomly assigned study medication for an additional 104-weeks of treatment, followed by a 12-week off-drug period (**Figure 6.1**). Forty-six patients gave their written consent to participate in the extension phase and continued their allocated treatment with exenatide (n=21) or insulin glargine (n=25). During the extension phase patients visited the study centers at 12-week intervals until the end of the treatment period (week 168). At this point they stopped the exenatide or insulin glargine treatment and continued their ongoing metformin treatment, which they had been using in an unchanged dose during the total 168-week treatment period. After a 4-week off-drug period, patients returned to the center for their final combined euglycemic hyperinsulinemic and hyperglycemic clamp with arginine stimulation. The final study visit was at week 180 (approximately 3.5 years after randomization).

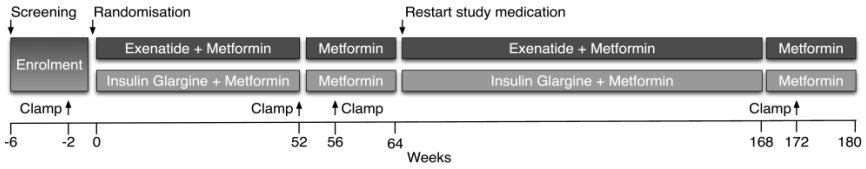

Figure 6.1 Study design.

Combined euglycemic hyperinsulinemic and hyperglycemic clamp with arginine stimulation

Insulin sensitivity and C-peptide secretion measures were measured during a combined euglycemic hyperinsulinemic and hyperglycemic clamp procedure, as described previously (**Figure 6.2**) (9, 10). Clamps were performed prior to randomization, following 52-weeks of treatment, after a 4-week off-drug period (week 56), and finally after a 4-week off-drug period following the total 3 years of treatment (week 172) as described previously (7). During the clamp at week 52, patients randomized to exenatide, were given the study drug 15 minutes prior to the onset of the hyperglycemic clamp and patients randomized to insulin glargine received their last insulin dose the night before at bed time. During the clamp at week 56 and week 172, patients did not receive either exenatide or insulin glargine. Arginine was administered at t=260 minutes, during the hyperglycemic clamp to estimate maximum insulin secretory capacity at a steady-state glucose concentration of 15 mmol/L (11). Whole body insulin-mediated glucose uptake (M-value) was calculated as the mean glucose infusion rate during the last 30 minutes of the euglycemic-hyperinsulinemic clamp between 90-120 minutes (9). First and second phase C-peptide secretion was calculated as area-under-the-curve (AUC) 180-190min and AUC190-260min, respectively. Arginine-stimulated C-peptide secretion (AIRarg) was calculated as the incremental AUC260-270min above the C-peptide concentration prior to the start of the hyperglycemic clamp (t=175 minutes). The disposition index (DI), a measure of beta-cell function, adjusted for insulin sensitivity, was calculated by multiplying the first-phase incremental C-peptide secretion with the M-value (AIRgluc*M) (12).

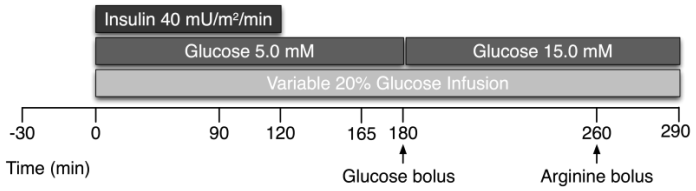

Figure 6.2 Clamp design. At week 52, exenatide injection at t=165 minutes, insulin glargine at bed time. At week 56 and week 172, no exenatide- or insulin glargine injection.

Biochemical analysis

HbA_{1c} (normal range: 4.3 to 6.1%, DCCT standardized Biorad assay), fasting plasma glucose (FPG), and safety parameters were measured prior to randomization and during each follow-up visit until the end of the 12-week off-drug period by a central laboratory (Quintiles, Livingston, UK). Plasma

glucose concentrations during the clamp were measured using an YSI 2300 STAT Plus (YSI, Yellow Springs, OH) in Sweden and the Netherlands, and using a Beckman-Coulter Glucose Analyzer 2 (Beckman-Coulter, Fullerton, CA) in Finland. C-peptide samples were analyzed at the VU University Medical Center using a single batch immunoradiometric assay (Centaur; Bayer Diagnostics, Mijdrecht, the Netherlands).

Statistical analysis

The extension study's primary efficacy endpoint was the treatment effect on the beta-cell function, measured as the 1st-phase glucose-stimulated C-peptide secretion adjusted for the M-value. Non-normally distributed data were base-e transformed prior to statistical analysis. Outcome measures were compared between the two treatment groups using an analysis of covariance (ANCOVA) model. The dependent variable used in the model was the change from pre-treatment for the beta-cell function parameters (AIRarg, 1st phase, 2nd phase). For all other endpoints the dependent value used was the mean at the corresponding visit. The model included factors for treatment group (exenatide/insulin glargine), site (NL/SE/FIN), and baseline HbA_{1c} stratum (≤8.5%/>8.5%), and the pre-treatment variable of the corresponding dependent variable as a covariate. If the parameter did not approximate the normal distribution after base-e transformation a non-parametric test was used (DI statistics using Mann-Whitney test). Statistical analysis was performed using SPSS 16.0 for Mac OS X (SPSS, Chicago, IL, USA). All inferential statistical tests were conducted at a significance level of 0.05 (two-sided). Unless otherwise stated, data are presented as mean (±SEM).

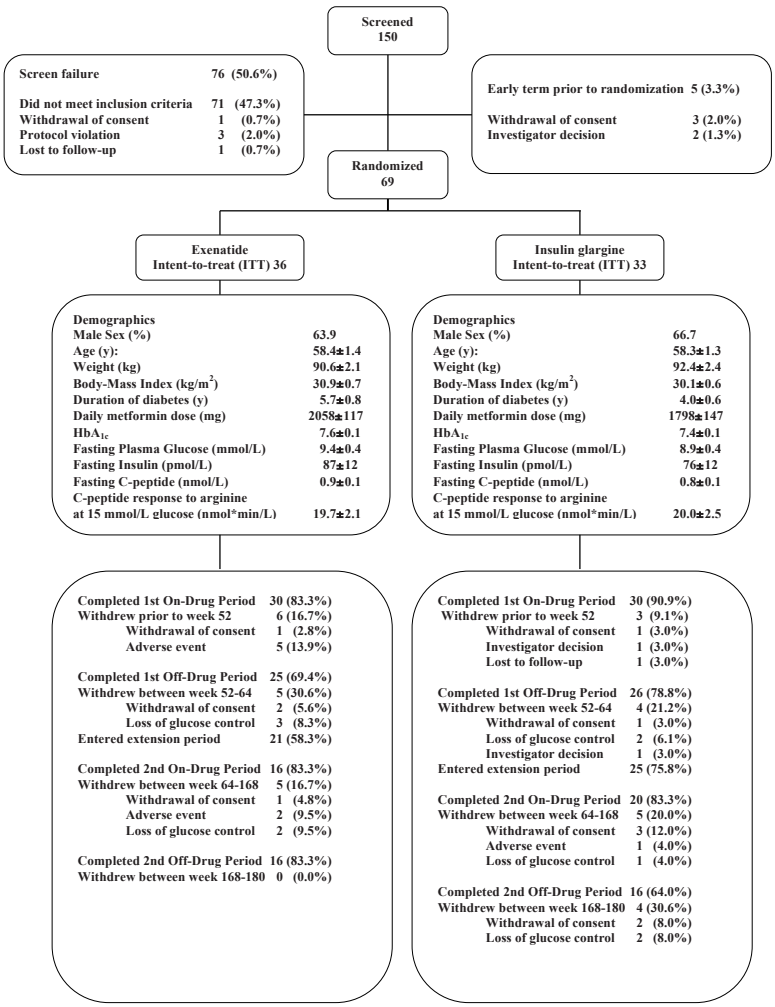

Figure 6.3 Protocol flow chart and baseline characteristics of the study population. Data represent mean ± SD.

Results

Patient disposition and baseline clinical characteristics

Extension phase patient disposition and baseline clinical characteristics are shown in **Figure 6.3**. No significant between treatment group differences were presents at baseline. At the end of the one-year main study phase five patients withdrew their consent and did not participate in the extension

phase (one in the insulin glargine group and four in the exenatide group), because of the demanding study procedures. Thirty-six patients completed the 168-week treatment period. Of the patients randomized to exenatide 69% (n=11) were treated with exenatide 10 μg BID at 168-weeks of treatment. 1 (6.25%) patient was using 20 μg BID, 1 15 μg TID, 1 a combination of 4QD/8BID, and 1 a combination of 6QD/8QD. The daily exenatide dose was reduced to 5 μg BID in 1 (6.25%) patient. At 168-weeks the mean±SEM daily insulin glargine dose used was 33.7±4.0 units.

Glycemic control

At three-years glycemic control was still comparable for exenatide and insulin glargine treatment: the HbA_{1c} values were 6.6±0.2%, and 6.9±0.2% at 168-weeks (between group difference: P=0.186, **Figure 6.4A**). Due to the treat-to-target titration, the insulin glargine group showed a significantly greater reduction in FPG as compared to exenatide (-2.0±0.4 versus -0.2±0.5 mmol/L; P<0.0001, respectively, **Figure 6.4B**). After 12 weeks off-drug both HbA_{1c} and FPG increased in both groups to pre-treatment values (**Figure 6.4A-B**).

Body weight and insulin sensitivity

After 168-weeks of treatment exenatide reduced body weight by -5.7±1.3 kg, while treatment with insulin glargine resulted in a body weight increase of 2.1±1.3kg (between group LSmean difference, -7.9±1.8 kg; P<0.001 **Figure 6.4C**). During the 12-week off-drug period body weight slightly trended back toward baseline values in both treatment groups, leaving a statistical significant difference at the end of the study in favor of exenatide (between group difference at week 180: -5.5±1.7 kg; P=0.004 **Figure 6.4C**).

Before randomization, whole-body insulin-mediated glucose uptake did not differ between the two treatment groups. Three-year treatment with exenatide improved the M-value by 39% (p=0.006) while insulin glargine had no effect (p=0.647).

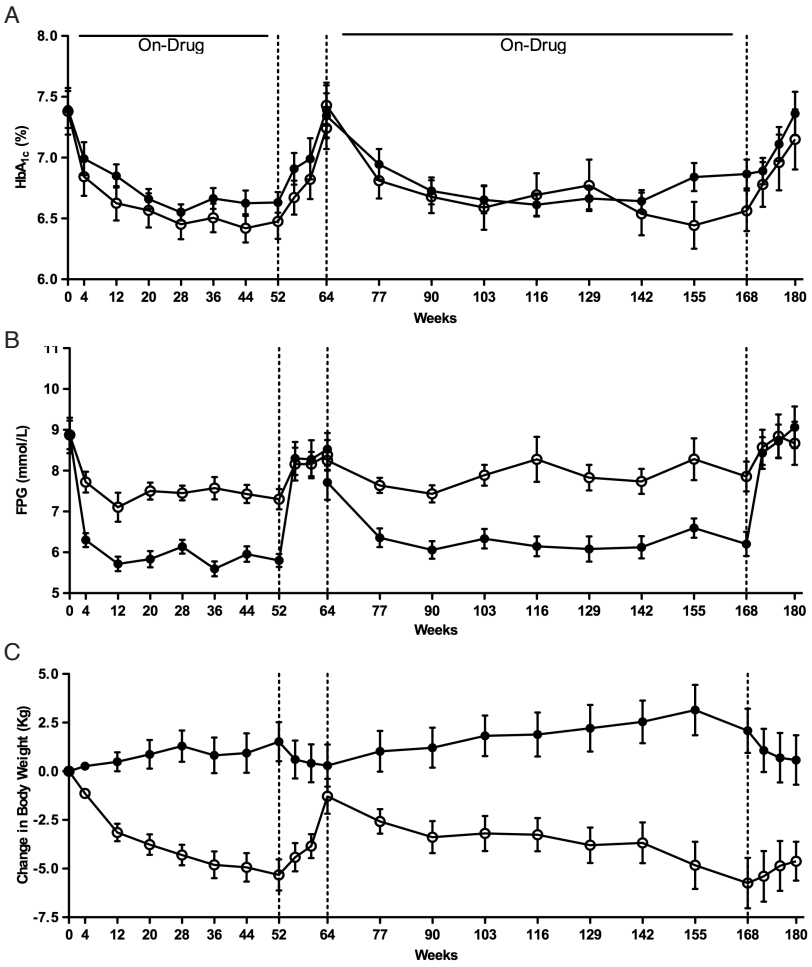

Figure 6.4A-C Time course for HbA_{1c} (A), fasting plasma glucose (B), and change in body weight (C). Data are mean (SEM). White circles = exenatide; black circles = insulin glargine. Vertical hatched line at week 52, 64 and 168 represents cessation and restart of study medication.

Measures of beta-cell function

At week 0, both glucose and arginine stimulated C-peptide secretion did not significantly differ between the two treatment groups (**Figure 6.5A**). Exenatide significantly improved beta-cell function during 52-week of active treatment compared with titrated insulin glargine (**Figure 6.5B, Figure 6.5E-F**). After 4-week cessation of both exenatide and insulin glargine therapy, beta-cell function returned to pretreatment values at week 56 (**Figure 6.5C, Figure 6.5E-F**), as reported previously (7).

Following three-year treatment with either exenatide or insulin glargine and a 4-weeks off-treatment period the glucose- and arginine stimulated C-peptide secretion, as compared to baseline remained similar: 1.02 ±0.11 and 1.06 ±0.10 for exenatide and insulin glargine, relative to baseline, respectively (**Figure 6.5E-F**) between treatment group comparison P=0.665). Interestingly, first and second phase glucose stimulated C-peptide responses were significantly lower in the exenatide treated patients after 3-years of treatment when compared to insulin glargine (**Figure 6.5D**): 1st phase relative to pre-treatment exenatide 0.88±0.09, insulin glargine 1.08 ±0.10, P=0.038, and 2nd phase relative to pre-treatment exenatide 0.97±0.08, insulin glargine 1.17 ±0.08, P=0.017. However, the DI change from pre-treatment showed a sustained effect on beta-cell function 4-weeks after cessation of treatment in the exenatide treated patients whereas a reduction was observed in the insulin glargine treated patients (**Figure 6.5G-H**: +1.43±0.78 and -0.99±0.65 respectively; between group difference P=0.028). This is in contrast to the one-year data, where no sustained effect on the DI was observed after cessation of treatment. No statistical significant between treatment group difference was observed in the DI calculated over the second phase C-peptide secretion (+36.89±17.51 and +25.02±14.24 for exenatide and insulin glargine respectively; between group difference P=0.763).

Adverse events and tolerability

During the extension phase, most common adverse events with exenatide treatment were gastrointestinal in nature (42.9%) and mild-to-moderate in intensity: nausea: 38.1%, vomiting 9.5%, abdominal distention 4.8%, and diarrhea 4.8%. In the exenatide treated group, 2 patients (9.5%) withdrew their consent as a result of nausea or vomiting. 19% of exenatide-treated subjects experienced treatment-emergent minor hypoglycemia, defined as a self-measured blood glucose concentration <3.0 mmol/L. At week 168, 31% of exenatide-treated subjects (5/16) had detectable anti-exenatide antibody titers, with the majority (4/5) of titers in the low range (<1/25 titer). The anti-exenatide antibodies had no predictive effect on the magnitude of an individual's glycemic response or the incidence of adverse events. Most reported adverse events with insulin glargine treatment were: treatment-related minor hypoglycemia 28%, gastrointestinal disorders 16%, vomiting 8.0%. No major hypoglycemia and no treatment-related withdrawal due to hypoglycemia were observed in both the exenatide and insulin glargine group. In the insulin glargine treated group, one patient (4.0%) withdrew his consent as a result of a cerebrovascular incident.

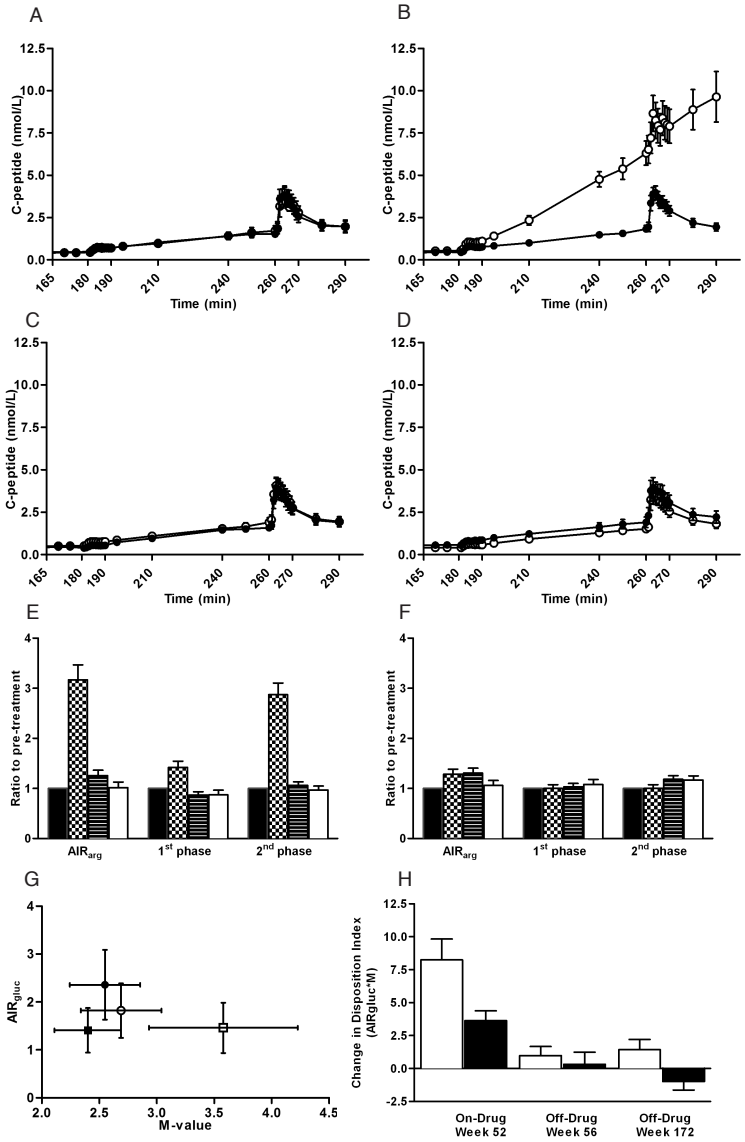

Figure 6.5A-H Beta-cell function parameters during 3-years of exenatide (n=16) and insulin glargine (n=20) treatment. Serum C-peptide concentrations during hyperglycemic clamp are shown at week 0 (**Figure 6.5A**), week 52 (**Figure 6.5B**), week 56 (**Figure 6.5C**), and week 172 (**Figure 6.5D**). White circles = exenatide; black circles = insulin glargine. Beta-cell secretory capacity ratio-to-pre-treatment in the exenatide (**Figure 6.5E**) and insulin glargine (**Figure 6.5F**) treated group. Black bars = week 0 (pre-treatment); blocked bars = week 52 (on-drug); horizontal lined bars = week 56 (off-drug); white bars = week 172 (off-drug). Mean Disposition Index the exenatide and insulin glargine treated group (Figure6.5G). Black square =

exenatide week 0 (pre-treatment); white square = exenatide week 172 (off-drug); black circle = insulin glargine week 0 (pre-treatment); white circle = insulin glargine week 172 (off-drug). Disposition Index change from pre-treatment (**Figure 6.5H**). White bars = exenatide, black bars = insulin glargine. Data represent mean (SEM) **Figure6.5A-D** & **Figure 6.5G-H**, and geometric mean (SEM) in **Figure 6.5E-F**. AIR_{arg}, C-peptide response to arginine at 15 mmol/L glucose concentration; 1st phase, first-phase C-peptide response to glucose; 2nd phase, second-phase C-peptide response to glucose. See methods section for calculations of beta-cell function measures.

Discussion

The main result of this 3-year follow-up study in patients treated with exenatide is the sustained improvement in first-phase glucose-stimulated C-peptide secretion, adjusted for prevailing insulin sensitivity (the DI), 4 weeks after discontinuation of treatment. No significant effect was seen in the insulin glargine treated patients, despite achievement of similar glycemic control. Additionally, exenatide treatment was associated with continued weight loss and improvement in whole-body insulin sensitivity. Both exenatide and insulin glargine were generally well tolerated with nausea and minor hypoglycemia being the most frequently reported adverse event in the exenatide and insulin glargine group, respectively.

Although human data is not available, exposure to GLP-1 and GLP-1RA in the pre-clinical setting results in beta-cell proliferation, islet neogenesis, and inhibition of beta-cell apoptosis in (human) cell lines, primary rodent islets and in vivo in different rodent species (13). It has therefore been hypothesized, although human islet-cell biology differs widely from that in rodents, that long-term GLP-1RA treatment may enhance beta-cell mass or health in humans, thereby potentially modifying the progressive course of type 2 diabetes (14). The current study, particularly the 3-year data presented herein, reports that exenatide treatment may lend some support to this idea, whereas following one-year exenatide exposure; the treatment-related improvement of beta-cell function was lost after 4-week drug cessation (7).

The current 3-year treatment data shows a small, but statistically significant, effect on the DI following a 4-week off-therapy period. Our results therefore suggest that a 3-year treatment with a GLP-1RA (such as exenatide) is necessary to delineate an effect on beta-cell function. This beneficial effect was not enough to sustain glycemic control. Additional factors, such as duration of type 2 diabetes, and achieved glycemic control and body weight reduction may play a role in the ultimate efficacy of the GLP-1RA. An even longer treatment or intervention at an earlier stage of the disease may

probably be necessary, given the chronic nature of the disease. Additionally, our results confirm findings in diabetic fatty Zucker rats, that prolonged exenatide treatment in humans does not result in tachyphylaxis (15).

Prolonged exposure to elevated glucose and lipid concentrations is detrimental to beta-cell function (16). These combined glucolipotoxic effects result in impaired insulin secretion and beta-cell apoptosis, and may contribute to the loss of beta-cell function in the pathogenesis of type 2 diabetes (17). Our results show a similar reduction in hyperglycemia, i.e. glucose toxicity, in the exenatide and insulin glargine group after 3-year treatment. Although we did find a 0.2% greater HbA_{1c} reduction in the exenatide treated group, this finding did not reach statistical significance, due to the decreased number of participants left in the study. Since glycemic control was similar for both treatments, this improvement cannot be solely attributed to an improvement of glycemic control, and therefore a GLP-1RA related factor should be considered.

In as much as beta-cell function integrity is determined by the combined effects of variables related to beta-cell stress versus beta-cell health, it is important to note that 3-year exenatide versus insulin glargine treatment resulted in body weight reduction of approximately 8 kg, with concomitant improvement of insulin sensitivity. Body weight reduction per se has been shown to improve beta-cell function in subjects with and without type 2 diabetes (18, 19). Interestingly, the improvement in beta-cell function reported in our manuscript cannot be explained fully by the reduction in body weight alone. Most patients treated with exenatide experienced a reduction in body weight. However, about half of the patients treated with exenatide a combined improvement in body weight and disposition index was observed. Additionally, there appeared to be no statistical correlation between the body weight reduction and the disposition index improvement in both exenatide and insulin glargine treated patients. Unfortunately, additional post-hoc analysis is not possible due to the small sample size.

We recently demonstrated that exenatide predominantly reduces truncal fat mass, whereas lean body mass was not affected (20). The observed 39% improvement in M-value may at least partly be due to a lowering of the (truncal) body fat mass. Obesity-related insulin resistance is a key feature of type 2 diabetes and is associated with metabolic and cardiovascular complications (16). The landmark study by Zander and colleagues was the first to report a beneficial effect of continuous GLP-1 infusion on clamp-measured beta-cell function as well as insulin sensitivity, in the presence of concomitant weight loss of 1.9 Kg, in spite of the mere 6-week duration (21). Subsequent clinical studies, using the euglycemic hyperinsulinemic, insulin-

modified frequently sampled intravenous glucose tolerance test (FSIGT), or HOMA-IR, do not provide a clear view of the effects GLP-1RA on insulin sensitivity, and suggest that such an effect may be secondary to the weight reduction (22, 23).

Since insulin sensitivity and beta-cell secretory function are closely interrelated it is essential to measure both when studying (long-term) therapeutic interventions that may affect insulin secretion and body weight (1). The DI adjusts for the interaction between changes in insulin sensitivity and insulin secretion, as differences in insulin sensitivity must be balanced by reciprocal changes in insulin release in order to maintain glucose tolerance (and prevent hypoglycemia). In the case of exenatide, given the observed weight-loss related improvement in insulin sensitivity (+39%), one may have expected that, if beta-cell secretory function had remained unaltered, the C-peptide response to rather decline. However, inasmuch as the C-peptide remained substantially unaltered (compared to pre-treatment), one may conclude that beta-cell function following 3-year of exenatide exposure was improved (1). Additionally, no correlation was observed between treatment-related changes in DI and body weight (**Figure 6.6**).

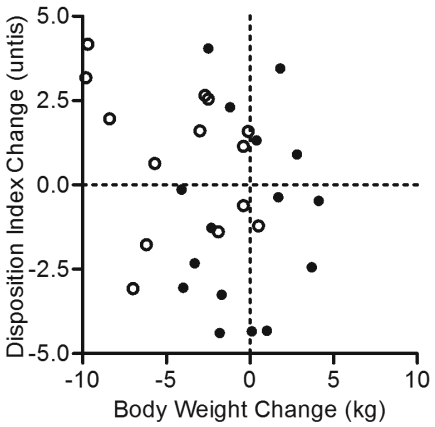

Figure 6.6 Scatter plot showing the relation between treatment-induced change in body weight and change in disposition index, calculated for the week 172 hyperglycemic clamp test. White circles = exenatide; black circles = insulin glargine.

Recently, DeFronzo and associates demonstrated a similar phenomenon following 20-week exenatide mono-therapy, as compared with the combination of exenatide and rosiglitazone (22). The DI was similar in the exenatide alone and exenatide/rosiglitazone groups, although the amount of insulin secreted in response to glucose alone or with arginine during the hyperglycemic clamp was markedly reduced in the group receiving exenatide/rosiglitazone combination therapy. These findings are in agreement with our results. Interestingly, DeFronzo and colleagues also

showed an improvement in the DI calculated from the second phase C-peptide secretion, a finding we did not observe in our study. In contrast to the former study, we did not administer exenatide prior to the hyperglycemic clamp. This differential study design may account for the observed difference. Additionally, a DI that is not derived from the rapid C-peptide response to intravenous glucose may not present correct physiology (1, 12).

The strength of our study is the long-term follow-up and the use of state-of-the-art gold-standard methodology to quantify of insulin sensitivity and beta-cell function in the study population. Additionally, as patients did not receive study medication prior to the hyperglycemic clamp at the end of the extension phase, the effects of a 3-year treatment period on beta-cell health were measured rather than the acute effects of exenatide administration. One limitation of our study is the relatively modest proportion of randomized patients who completed the entire study. Fifty-two percent (36/69) of all randomized patients completed the entire 3-year treatment period. Of the original 51 patients who completed the 64-week main study, 46 agreed to participate in the extension phase. From these, 78% completed the additional 2-year treatment period, with similar numbers remaining in each of both treatment arms. Most patients withdrew their consent and did not participate in the 2-year extension phase of the study because of the demanding nature of the study protocol, including long-term follow-up, which included a total of 30 visits (during 3.5 years) to the study center. The proportion of patients who did enter the additional 2-year treatment period, show characteristics comparable to those participating in large intervention studies in patients with type 2 diabetes (24, 25). Only 3 patients discontinued the 2-year extension phase because of loss of glycemic control (exenatide n=2, insulin glargine n=1). Finally, 3 patients dropped-out as a result of an adverse event: one patient randomized to insulin glargine experienced an ischemic stroke, and two patients randomized to exenatide dropped-out as a result of nausea following reinitiating of exenatide treatment during the first weeks of the extension period. Generally, both exenatide and insulin glargine were well tolerated during the 3-year study period, and reported adverse events were mainly mild-to-moderate in intensity, confirming previous long-term study tolerability results (6). No renal function deterioration and no pancreatitis were observed. Interestingly, no patients randomized to exenatide withdrew their consent during the 3-month off-drug period, whereas four patients in the insulin glargine group did. After cessation of insulin glargine, patients continued their SMBG measurements. 30.6% of patients randomized to insulin glargine did not want to continue with the off-drug period of the study as they observed an increase in SMBG,

confirming the important role self-monitoring of blood glucose plays in the treatment of patients with type 2 diabetes.

In conclusion, 3-year exenatide treatment in metformin-treated patients with type 2 diabetes resulted in sustained improvement in beta-cell function and progressive weight reduction. Long-term follow-up in a wide variety of patients at earlier stages of type 2 diabetes is needed to study possible disease modifying effects of GLP-1RA.

Acknowledgments

The authors thank the patients for participating in the study. This study was sponsored by Amylin Pharmaceuticals, Inc. and Eli Lilly and Company. The study was collectively initiated and designed by the investigators from the three study sites. The investigators had full access to the trial data and had control over the statistical analysis and interpretation of the study results. Parts of this study were presented in abstract form at the 70th Scientific Sessions of the American Diabetes Association, Orlando, Florida, 25-29 June 2010.

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Chapter 7

Exenatide treatment did not affect bone mineral density despite body weight reduction in patients with type 2 diabetes

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Poster presentation at the 70th Scientific Sessions of the American Diabetes Association, Orlando, Florida, 27-29 June 2010

Abstract

Background: preclinical studies suggest that incretin-based therapies may be beneficial for the bone, however, clinical data are largely lacking. We assessed whether the differential effects of these therapies on body weight differed with respect to their effect on bone mineral density (BMD) and markers of calcium homeostasis in patients with type 2 diabetes.

Methods: sixty-nine metformin-treated patients with type 2 diabetes were randomized to exenatide twice daily (n=36) or insulin glargine once daily (n=33). Total body BMD, measured by dual-energy X-ray absorptiometry and serum markers of calcium homeostasis were assessed before and after 44-weeks treatment.

Results: exenatide versus insulin glargine decreased body weight by 6%. Endpoint BMD was similar in both groups after 44-weeks therapy (LSmean±SEM between group difference -0.002±0.007 g/cm^2; P=0.782). Fasting serum alkaline phosphatase, calcium, and phosphate remained unaffected.

Conclusion: forty-four weeks exenatide or insulin glargine had no adverse effects on bone density in patients with type 2 diabetes, despite differential effects on body weight.

Introduction

Diabetes is associated with an increased risk of bone fractures (1). Although patients with type 2 diabetes have a higher bone mineral density compared to non-diabetic age-matched controls, this does not confer a lower fracture risk (1). Higher bone mineral density was protective in obese individuals with impaired glucose metabolism or those with recently diagnosed diet-treated patients with type 2 diabetes (1). However, in more advanced diabetes fracture risk was increased, due to factors including hyperglycemia and neuropathy, all of which may alter bone quality while leaving bone density unchanged (1). To date, the use of metformin, sulphonylurea and insulin does not seem to negatively affect bone health (1, 2).

Recently, the thiazolidinediones have been implicated in increased bone fracture risk in patients with type 2 diabetes (3). Body weight reduction is advocated in most type 2 diabetes patients to improve their cardiovascular risk profile, however, it may decrease bone density and increase bone turnover (4,5). Therefore, type 2 diabetes therapies should not only lower cardiovascular risk but also be safe for the bone, also given their chronic use.

Preclinical data suggest that gut hormones are associated with bone turnover, such that enhanced activity of these hormones has anabolic effects on the bone (6-8). We previously showed that 1-year treatment with the glucagon-like peptide (GLP)-1 receptor agonist exenatide reduced, while insulin glargine increased body weight in patients with type 2 diabetes (9).

Here, we assessed whether the differential effects of these therapies on body weight differed with respect to their effect on bone mineral density and markers of calcium homeostasis in these patients.

Research design and methods

Details on study design were reported previously (9). Sixty-nine metformin-treated patients with type 2 diabetes (45 males, 24 females; mean±SD: age 59±8y; HbA_{1c} 7.5±0.8%; weight 91.5±13.1kg; BMI 31±4kg/m2) were randomized to exenatide (n=36) or titrated insulin glargine (n=33) added to their ongoing metformin therapy. The study protocol was approved by each site's ethical review committee and was in accordance to the principles described in the declaration of Helsinki and all participating patients gave their written informed consent prior to screening. This study is registered with ClinicalTrials.gov number NCT00097500.

Bone mineral density was assessed using Dual Energy X-ray Absorptiometry scans (Delphi A, Hologic, Waltham, MA) at baseline, and after 44 weeks. Fasting serum alkaline phosphatase, calcium, and phosphate were

measured, as markers of bone metabolism and calcium homeostasis were determined using standard ELISA's on the same day, prior to scanning.
All outcome measures are compared between the two treatment groups using an analysis of covariance (ANCOVA) model as reported previously (9). The model includes factors for treatment group (exenatide/glargine), site (NL/SE/FIN), and baseline HbA_{1c} stratum (≤8.5%/>8.5%), and the pre-treatment variable of the corresponding dependent variable as a covariate. Statistical analysis was performed using SPSS 16.0 for Mac OS X (SPSS, Chicago, IL, USA). All inferential statistical tests were conducted at a significance level of 0.05 (two-sided).

Results

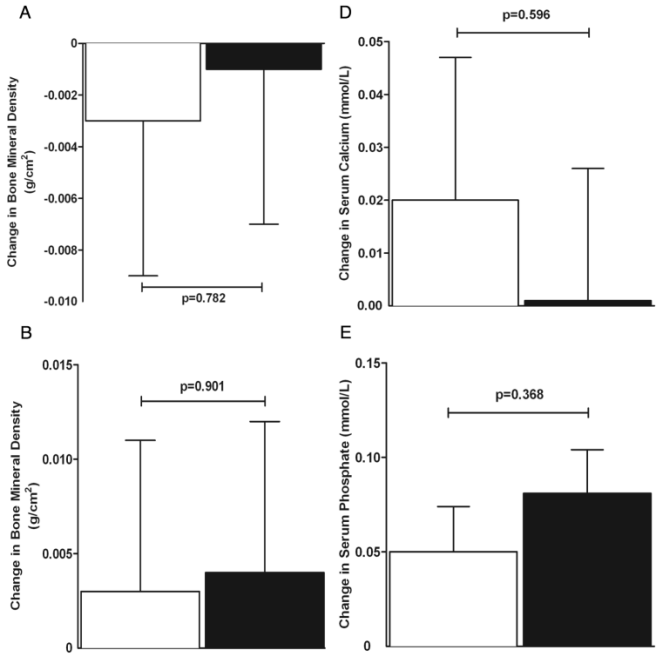
At baseline no between treatment group differences in outcome measures was present (**Table 7.1**).

	Exenatide (n=36)	**Insulin Glargine (n=33)**
Age (years)	58.4±1.4	58.3±1.3
Sex, M/F (n)	23/13	22/11
Caucasian race (n(%))	36 (100)	32 (97.0)
Duration of type 2 diabetes (years)	5.7±0.8	4.0±0.6
Daily metformin dose (mg)	2058±117	1798±147
Body weight (kg)	90.6±2.1	92.4±2.4
BMI (kg/m^2)	30.9±0.7	30.1±0.6
HbA_{1c} (%)	7.6±0.1	7.4±0.1
Fasting plasma glucose (mmol/L)	9.4±0.4	8.9±0.4
Bone mineral density (g/cm^2)	1.189±0.026	1.230±0.024
Calcium (mmol/L)	2.37±0.11	2.36±0.10
Phosphate (mmol/L)	1.14±0.16	1.10±0.19
Alkaline phosphatase (IU/L)	2.37±0.11	2.36±0.10
Completed study (n (%))	30 (83.3)	30 (90.9)
Discontinued study (n (%))	6 (16.7)	3 (9.1)
Discontinued study due to adverse events (n (%))	5 (13.9)	1 (3.0)

Table 7.1 Pre-treatment characteristics and patient disposition. Data represent mean±SEM or n (%).

At 44 weeks, exenatide versus insulin glargine, significantly decreased body weight (LSmean change from baseline±SEM: exenatide -3.5±0.9, insulin glargine +0.3±0.9 kg; difference -3.8 kg; P<0.001). No effects on total body bone mineral density was observed with either exenatide or insulin glargine. Endpoint bone mineral density was similar in both groups after 44-weeks therapy (adjusted LSmean±SEM: exenatide 1.213±0.006 g/cm2, insulin glargine 1.215±0.006 g/cm2; difference -0.002±0.007 g/cm2; P=0.782) (**Figure 7.1A**). Additionally, adjustment for gender did not alter the results (**Figure 7.1B-C**). Serum alkaline phosphatase, calcium, and phosphate also remained unaffected following 44-weeks treatment (adjusted between group LSmean±SEM difference: +0.26±2.87 IU; p=0.929, +0.02±0.04 mmol/L; p=0.596 and -0.03±0.03 mmol/L; p=0.368, respectively) (**Figure 7.1D-F**). No bone fractures were reported during the follow-up of our study.

As reported previously, exenatide and insulin glargine treatment was generally well tolerated (9). Mild-to-moderate hypoglycemia (exenatide 8.3%; insulin glargine 24.2%) and nausea (exenatide 50%; insulin glargine 0%) were the most frequently reported adverse events. There were no major hypoglycemic events observed during this study.



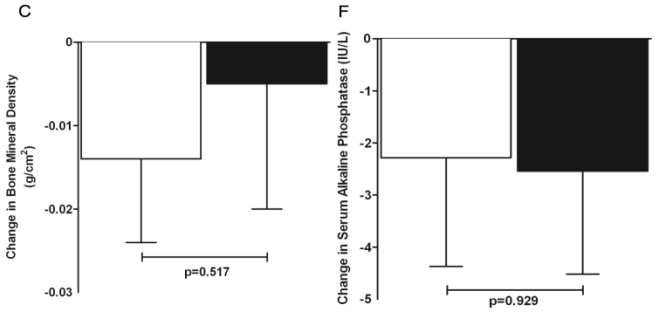


Figure 7.1A-F Bone mineral density in all patients (A), in male patients (B), in female patients (C), calcium (mmol/L) (D), phosphate (mmol/L) (E), and alkaline phosphatase (IU/L) (F) before and after 44 weeks of treatment. White box = exenatide; black box = insulin glargine. Data represent mean±SEM.

Discussion

Our study is the first to show that 44-week exenatide treatment does not affect bone mineral density and markers of bone metabolism and calcium homeostasis in patients with type 2 diabetes in spite of the body weight reducing effects of exenatide. Indeed, many studies have shown that 5-10% weight loss is associated with a decrease in bone mass and an increase in bone resorption, especially in obese postmenopausal women (4,5). Whereas insulin may reduce bone resorption and its weight-gain promoting effects may indirectly increase bone mineral density (6), the mechanisms underlying the bone mass-sparing effects of exenatide in the face of body weight reduction are unclear. Long-term exposure of type 2 diabetic patients to exenatide did not increase bone fracture risk, despite the progressive weight loss: at 82 weeks weight reduction was progressive and averaged 4.4 kg, with a mean of 11.9 kg in the highest weight-loss quartile (i.e. 11.4% of baseline body weight) (10).

Pre-clinical studies have shown gut hormones, in particular GLP-2 and glucose-dependent insulinotropic peptide (GIP), to play an important role in bone metabolism (9). Postprandial GIP and GLP-2 elevations suppress meal-related bone resorption (6). GIP also increases bone density in ovariectormized rats, a rodent model for postmenopausal osteoporosis, and functional GIP-receptors have been found on osteoblast-like cells (6). Unfortunately, the design of our study did not include measurement of GIP or GLP-2.

More recent data suggest GLP-1 to be involved in bone metabolism as well (7). GLP-1 receptor knock-out versus wild-type control mice showed increased bone resorption and had lower bone mineral density (6). Three-day exendin-4 treatment exerted osteogenic effects in rat models of insulin

resistance and type 2 diabetes, as quantified by markers of bone turnover and micro-CT assessment of bone structure (8). More recently, a GLP-1 receptor was found to be present in an osteoblastic cell line, that seemed independent of the cAMP-linked GLP-1 receptor (7). However, these findings need to be confirmed *in vivo*, particularly in humans.

In conclusion, 44-week exenatide treatment did not lead to significant changes in total body bone mineral density in men and women with type 2 diabetes, despite a marked reduction in body weight. Additionally, serum markers of bone metabolism and calcium homeostasis also remained unaffected. Long-term studies are needed to assess whether prolonged GLP-1 receptor agonist treatment can off-set the weight-loss related decline in bone mineral density, but more importantly, whether it may confer protection against bone fractures in type 2 diabetic patients.

Acknowledgments

We thank the subjects for participating in the study. This study was sponsored by Amylin Pharmaceuticals, Inc. and Eli Lilly and Company. The study was collectively initiated and designed by the investigators from the three study sites. The investigators had full access to the trial data and had control over the statistical analysis and interpretation of the study results.

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Chapter 8

Incretin mimetics as a novel therapeutic option for hepatic steatosis

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Liver Int 2006;26:1015-1017

Abstract

Background: Fat accumulation in the liver or non-alcoholic fatty liver disease (NAFLD) is regarded as a key pathogenic factor and component of the metabolic syndrome. It was reported that administration of the incretin mimetic exenatide reversed hepatic steatosis in an obese mouse model. We had the opportunity to study the effect of additional exenatide administration on liver fat content in a patient with type 2 diabetes.

Case report: A 59-year-old male with poorly controlled type 2 diabetes was treated with exenatide in addition to metformin monotherapy. Following 44 weeks of exenatide treatment mean the liver fat measured by liver spectroscopy declined from 15.8% to 4.3%. This dramatic decrease in liver fat was accompanied by significant beneficial changes in several cardiovascular disease risk factors and improvement of all liver enzymes, in particular alanine aminotransferase, the most important marker of liver steatosis.

Conclusion: This case report suggests that the incretin mimetic exenatide decreases hepatic fat accumulation and may play a role in the future treatment of NAFLD, and the associated insulin resistance and cardiovascular risk factors in an ever-growing high-risk population.

Introduction

The metabolic syndrome, a cluster of cardiovascular disease factors, is present in the majority of obese type 2 diabetic patients (1). Currently, fat accumulation in the liver or non-alcoholic fatty liver disease (NAFLD) is regarded as a key pathogenic factor and component of this syndrome (2). Therapies aimed at reducing liver fat have been shown to concomitantly improve the risk profile in this high-risk population. For example, both lifestyle interventions resulting in weight loss and administration of thiazolidinediones, which is associated with weight gain, have been associated with decreased liver fat and improvement of (hepatic) insulin sensitivity (3, 4). Furthermore, these studies suggested that the presence of risk factors for cardiovascular disease, in particular low HDL cholesterol and high triglycerides, is strongly related to the amount of liver fat.

Glucagon-like peptide 1 (GLP-1) is an incretin hormone that is secreted by the L cells of the intestine upon meal ingestion. In type 2 diabetic patients, GLP-1 lowers blood glucose levels in a glucose-dependent manner, by potentiating meal-induced insulin production and secretion by the pancreatic beta-cells, by slowing gastric emptying and by inhibiting glucagon secretion. Also, GLP-1 induces weight loss, possibly because of central inhibition of appetite, and may also improve insulin sensitivity (5). However, GLP-1 has a very short half-life as it is degraded by the ubiquitous enzyme dipeptidyl-peptidase-4 (DPP-4). The long-acting, DPP-4-resistant GLP-1 receptor agonist or incretin mimetic exendin-4 (exenatide, synthetic exendin-4) is a 39 amino-acid peptide that binds with high affinity to the GLP-1 receptor. Recently, exenatide was introduced, to the market in the United States as a blood-glucose-lowering-agent. The full scope of the effects of incretin mimetics still needs to be elucidated and the almost ubiquitous presence of the GLP-1 receptor in the human body implies many potential actions. Recently, it was reported that exenatide administration in an obese mouse model reversed hepatic steatosis. This effect was attributed to increased fatty acid oxidation and/or inhibition of de novo lipogenesis (6). At present, it is not clear whether incretin mimetics also affect liver fat content in obese type 2 diabetes patients.

Case report

A 59-year-old Caucasian male with type 2 diabetes, treated with metformin, was prescribed exenatide 20 ug twice daily, because of poor control (HbA_{1c}: 8.7%; reference values: 4–6%). He was a retired craftsman and had no history of alcohol abuse or viral hepatitis. At baseline, his body weight was 88.5 kg (BMI 28.7 kg/m2), his waist circumference was 98.5 cm and his

blood pressure was 157/87 mmHg. The fasting laboratory values were as follows: plasma glucose 14.5 mmol/l, total cholesterol 4.82 mmol/l, HDL cholesterol 1.04 mmol/l, LDL cholesterol 3.29 mmol/l, triglycerides 1.46 mmol/l, alanine aminotransferase (ALT) 46 IU/l, aspartate aminotransferase (AST) 18 IU/l, gamma-glutamyltranspeptidase (GGT) 28 IU/l (reference values: 6–48; 10–45; 7–51 IU/l, respectively) and insulin resistance estimated by homeostasis assessment model (HOMA-IR) of 6.36. Following 44 weeks of exenatide therapy, HbA_{1c} decreased to 8.4% and fasting plasma glucose to 9.9 mmol/l. His body weight fell by 4.7%, from 88.5 to 84.3 kg, and his waist circumference by 2.5 cm.

Liver fat accumulation was quantified in the fasting state using proton magnetic resonance spectroscopy (1H-MRS), a method that has been shown to correlate excellently with liver fat as measured in biopsy samples (7). At three positions (right anterior, right posterior and medial or left anterior), a 15 cm^3 spectroscopic volume of interest was positioned, avoiding major blood vessels, intra-hepatic bile ducts and the lateral margin of the liver. Areas of resonances from protons of water and methyl and methylene groups in fatty acid chains of the hepatic triglycerides were evaluated with LCModel (8). Surprisingly, the mean liver fat (average of the three volumes of interest) declined from 15.8% before to 4.3%, i.e. by 73%, after 44 weeks of exenatide therapy (**Figure 8.1**). This dramatic decrease in liver fat was accompanied by significant beneficial changes in several cardiovascular disease risk factors: blood pressure decreased to 140/85 mmHg, fasting LDL cholesterol to 2.67 mmol/l, triglycerides to 0.69 mmol/l, HOMA-IR to 2.51, while HDL cholesterol increased to 1.27 mmol/l. In accordance with the MRS findings, all liver enzymes, and in particular ALT, the most important marker of liver steatosis (9), improved. This value decreased from 46 to 20 IU/l, while AST decreased from 18 to 13 IU/l, and GGT from 28 to 23 IU/l.

Discussion

Experimental evidence indicates that exenatide, by activating the GLP-1 receptors, effectively reverses hepatic steatosis. This is the first report describing a marked reduction in liver fat accumulation following treatment with the incretin mimetic exenatide in humans. Although this effect may be indirect, because of concomitant systemic metabolic improvement, it is very likely that exenatide may also act directly on the liver, as a GLP-1 receptor is present on liver cells (6). A direct effect is even more likely in the present case, as additional exenatide treatment in our patient did not lead to significant improvement of glycemia, nor did it result in relevant weight loss,

both of which are treatment effects that are known to reduce liver fat (3, 4). In vitro studies in hepatocytes demonstrated that exposure to synthetic exendin-4 resulted in an up-regulation of PPARgamma mRNA, a key element in â-oxidation of free fatty acids, and in a down-regulation of sterol regulatory element binding protein (SREBP-1c) and stearoyl-CoA desaturase (SCD1), both critical regulators of de novo hepatic lipogenesis. As exenatide stimulates insulin secretion, and in turn inhibits lipolysis, the delivery of FFA to the liver will be diminished. This probably also contributes to the lowering of liver fat content.

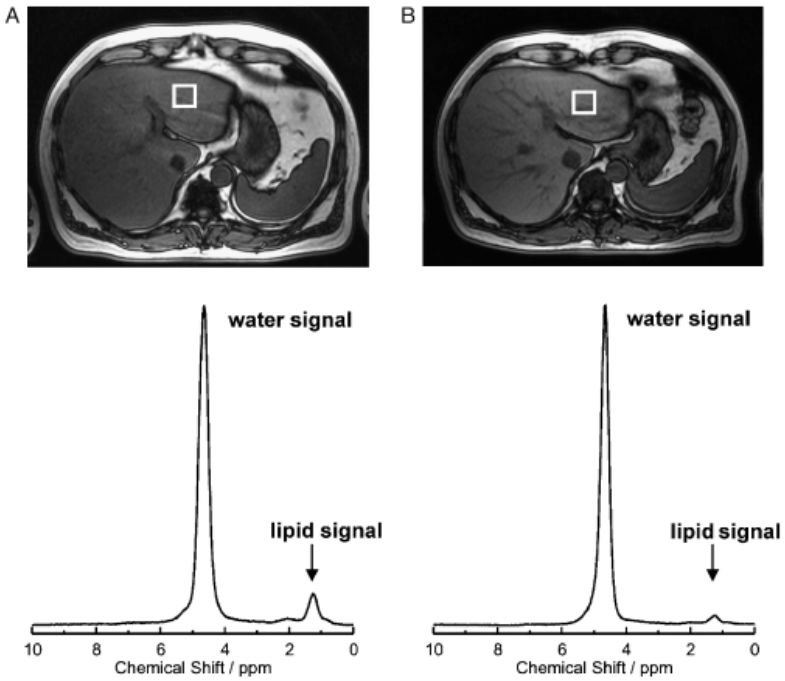

Figure 8.1 Abdomen magnetic resonance imaging scans indicating volume of interest in the anterior left hepatic lobe from which proton magnetic resonance spectra (CH_2 peak at 1.3 p.p.m. is the main signal of lipids) were obtained before (A) and after (B) exenatide treatment.

Incretin mimetics have already been demonstrated to influence beneficially a variety of abnormalities in type 2 diabetes, including defective insulin secretion, hyperglucagonemia as well as excessive body weight and appetite (5). Now it seems that incretin mimetics also have salient effects on NAFLD and the associated cardiovascular risk factors. Future long-term intervention studies are required to confirm these findings.

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Chapter 9

Summary and conclusion

This final chapter summarizes the main findings of the work presented in this thesis and also provides some methodological considerations and backgrounds. The primary objective of the studies presented in this thesis was to assess and compare the effects of long-term treatment with the GLP-1 receptor agonist exenatide versus insulin glargine on different parameters of pancreatic beta-cell function, in patients with type 2 diabetes who had not achieved an HbA_{1c} of ≤ 6.5% using metformin mono-therapy. In addition, treatment effects on postprandial lipid levels, body composition, circulating cardiovascular risk biomarkers, and safety were assessed.

Primary study endpoints: beta-cell function parameters

Type 2 diabetes is characterized by a progressive beta-cell defect in the presence of obesity-related insulin resistance (1, 2). The former is thought to be primarily responsible for the progressive nature of the disease (3, 4). The capacity to secrete insulin is thought to be related to functional beta-cell mass, which is determined by the balance of the rate of beta cell neogenesis and apoptosis (5), and is showed to be reduced in patients with type 2 diabetes (6). Until beta-cell volume can be quantified reliably and non-invasively, we will need to rely on the ability of glucose, with or without other secretagogues, to potentiate insulin release as the best surrogate estimate of the number of beta-cells (7). However, an attempt to estimate beta-cell mass based simply on the use of a functional test as a surrogate measure cannot, by nature, be complete and will therefore be imprecise (7).

A number of different functional indexes have been proposed to estimate beta-cell function in vivo (7-9). Hyperglycemic clamps and experiments in isolated pancreatic islets have demonstrated that glucose administration induces insulin secretion in a biphasic pattern: an initial component (first phase), which develops rapidly but lasts only a few minutes, followed by a sustained component (second phase) (8, 10). Loss of first phase glucose stimulated secretion and a reduction in second phase glucose stimulated, and combined glucose and arginine stimulated insulin secretion are characteristic features of type 2 diabetes mellitus (10, 11). It is known that a decrease in first phase glucose stimulated insulin secretion is found in the early stage of type 2 diabetes and also in patients with impaired glucose tolerance (12, 13). Detailed assessment of beta-cell function will therefore be useful to identify early stages of type 2 diabetes, but also to evaluate (the rate of) disease progression, and to monitor the effects of pharmacological interventions (14).

Evidence supporting the rationale and design of the current study included the demonstration in preclinical models of diabetes, and in *in vitro* assays, that GLP-1 and the GLP-1 receptor agonist exenatide improved beta-cell function, promoted beta-cell health and preserved or increased beta-cell mass (15-19). Initial, often short-term, studies in healthy humans and patients with type 2 diabetes, showed improvement of (surrogate) markers of beta-cell function following exenatide treatment (20-26).

Beta-cell function measures using the hyperglycemic clamp

In **chapter 2** we studied whether 52 weeks of treatment with exenatide or insulin glargine significantly improved beta-cell function, measured with the hyperglycemic clamp with additional arginine stimulation. Furthermore, durability of treatment effect on beta-cell function was assessed after a 4-week off-drug period. We showed that exenatide administration, relative to insulin glargine, significantly improved first and second phase glucose-stimulated insulin secretion by 53% and 185%, respectively. The combined glucose and arginine stimulated insulin secretion increased by 146%, when compared to insulin glargine, while on active treatment (**Table 2.1**). This beneficial effect on beta-cell function was accompanied by an improvement in glycemic efficacy (**Figure 2.3**).

Pancreatic beta-cells contain at least two pools of insulin secretory granules, that differ in release pattern, which account for biphasic insulin secretion. A small pool available for immediate exocytosis and a reserve pool which accounts for the vast majority of granules, and needs to be mobilized to being available for secretion (8, 27). The prevailing hypothesis is that granules from the readily releasable pool account for the first phase of glucose-stimulated insulin secretion, and that mobilization of a subsequent supply of new granules from the reserve pool accounts for the second phase (27-29). GLP-1 receptor agonists, such as exenatide, bind to a specific G-protein coupled receptor resulting in the activation of adenylate cyclase and an increase in cAMP generation (30, 31). In the beta-cell, cAMP binds and modulates activities of both protein kinase A (PKA) and cAMP-regulated guanine nucleotide exchange factor II (Epac2), thereby increasing intracellular Ca^{2+} concentrations and enhancing glucose-dependent insulin secretion (10, 30, 32). It is postulated that Epac2 signaling increases the size of the readily releasable pool and PKA signaling may increase the size of the reserve granule pool (10). Through these mechanisms exenatide treatment may have contributed to the increase in first and second phase insulin secretion as described in **chapter 2**.

Ward and Porte introduced the glucose-dependent arginine stimulation test (33). It is a method which gives thorough information on islet function as it measures both basal and "maximal" alpha-cell and beta-cell secretion (34). Unfortunately, we did not measure glucagon release as a measure of alpha-cell (dys)function in our study. It has been shown that combined glucose and arginine stimulated insulin release increases when arginine is administered at higher clamp glucose concentrations (33). During the design of our study we deliberately chose to test glucose-dependent arginine stimulation at moderately elevated glucose concentrations (15 mmol/L), as used by Ward (33) and Larsson (34). An intravenous glucose infusion rate to achieve higher glucose concentrations, for example 25 mmol/L, during the hyperglycemic clamp was expected not to be feasible while on active exenatide treatment. Therefore, the reported acute insulin responses to arginine (AIR_{arg}) in **chapters 2** and **6** cannot be interpreted as the real "maximum acute insulin responses" (AIR_{max}) in our population.

Following our initial one-year study the improved beta-cell function at 52 weeks was lost 4 weeks after cessation of exenatide and insulin glargine, suggestion an acute pharmacological effect of either treatment (**Figure 2.4**, **Table 2.1**). Interestingly, insulin sensitivity remained significantly improved after 4 weeks cessation of exenatide treatment. We did not observe this finding in the insulin glargine group (**Figure 2.3F**). This observation led to the idea that extra-pancreatic effects of exenatide, such as weight loss and the associated improvement in insulin sensitivity, might last longer. We concluded that the possible effects on preservation of beta-cell function might be dependent on other (additional) factors including: 1) diabetes duration, 2) the amount of functional beta-cells present at the initiation of therapy, 3) overall metabolic control achieved and 4) treatment duration. To study the possible preserving effect of exenatide on beta-cell function, patients were asked to participate in an additional 2-year extension trial, and restart their originally assigned treatment at the end of our original one-year study. The results of this extension study are presented in **chapter 6**.

At the end of the 3-year treatment period, exenatide and insulin glargine treatment was stopped and patients were only treated with their original metformin dose. As a result of strict regulations by the ethical review board we were not able to measure beta-cell function both on and off active exenatide or insulin glargine treatment. To answer the durability questions, which were raised following the one-year treatment period, we chose to only measure beta-cell function following a 4-week off-drug period. After 3 years of treatment, beta-cell secretory function measured as first and second phase glucose-stimulated insulin secretion showed a statistically significant

reduction in exenatide treated patients as compared to the insulin glargine treated patients (**Figure 6.5)**. Interestingly, insulin sensitivity remained significantly improved during the 4-week off-drug period in the exenatide treated patients. As insulin sensitivity and insulin secretion are closely interrelated this means that the proper assessment and interpretation of beta-cell function parameters requires the incorporation of both insulin sensitivity and the insulin response measures (35). The following paragraph will discuss this relationship in greater detail.

The relation between insulin sensitivity and insulin secretion

The relationship between insulin sensitivity and insulin secretion follows a hyperbolic function, such that the product of the two variables (called the disposition index) remains a constant (35, 36) (**Figure 9.1**). By acknowledging this hyperbolic relationship, beta-cell function measures should include measures of glucose-stimulated insulin first phase insulin release and insulin sensitivity (37). In response to the decrease in insulin sensitivity as observed in obesity (36), puberty (38) and pregnancy (39), human beta-cells increase insulin release to levels four to fivefold higher than in insulin sensitive individuals (2). As long as this compensatory insulin secretion is sufficient, resulting in a normal disposition index, glucose tolerance remains unaffected. Individuals predisposed to type 2 diabetes show a reduced beta-cell compensatory response to obesity-related insulin resistance and will subsequently develop impaired glucose tolerance, and type 2 diabetes (40).

In **chapter 6** we showed that 3-year long treatment with exenatide results in a 7.9 kg reduction in body weight as compared to insulin glargine (**Figure 6.4C**). Probably as a consequence of this, insulin sensitivity remained improved by 39% in the exenatide treated patients after 4-week cessation of treatment. Insulin secretion responses to glucose and arginine cannot be interpreted without taking the changes in insulin sensitivity into account. Following 4-week cessation of exenatide treatment the disposition index increased significantly. In the insulin glargine treated patients, the disposition index decreased (**Figure 6.5E-H**).

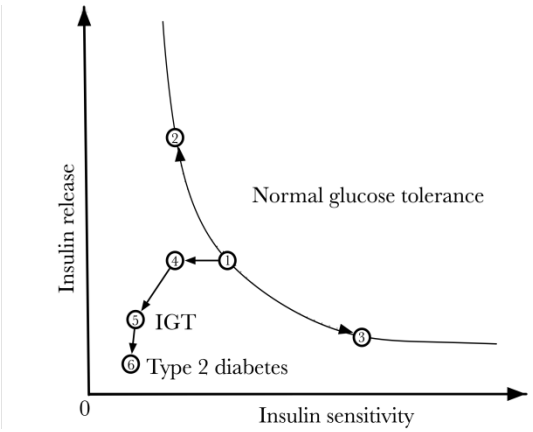

Figure 9.1 In people with normal glucose tolerance (1) a non-linear, hyperbolic relation between insulin sensitivity and beta-cell insulin release exists. The product of insulin release and insulin sensitivity is called disposition index (DI), and is assumed to be a constant. Healthy subjects react to a decrease in insulin sensitivity by a reciprocal increase in beta-cell insulin release (2), and vice versa (3). Patients with impaired tolerance (5) and type 2 diabetes (6) are not able to compensate for the decrease in insulin sensitivity. In situation 2, beta-cell insulin release is increased but the DI is normal. In 4, beta-cell insulin release appears normal but the DI is reduced. This hyperbolic relationship means that assessment of beta-cell function requires knowledge of both insulin sensitivity and the insulin response. Impaired glucose tolerance (IGT). Adapted from Kahn (2), Cobelli (8), and Stumvoll (1).

Body weight reduction per se has been shown to improve beta-cell function in subjects with and without type 2 diabetes (41, 42). It is possible that factors secreted from adipose tissue, such as free fatty acids and adipocytokines such as IL-6, TNF-α, or adiponectin, may directly or indirectly impact beta-cell function (43). In **chapter 5** we showed exenatide beneficially affected some of these adipocyte-derived proteins. In our 3-year extension study, the improvement in disposition index appears to be driven by the improvement in insulin sensitivity (**Figure 6.5G**). Interestingly, the improvement in disposition index reported in **chapter 6** cannot be fully explained by the reduction in body weight (and subsequent improvement in insulin sensitivity) alone. Most patients treated with exenatide experienced a reduction in body weight. However, about half of the patients treated with exenatide a combined improvement in body weight and disposition index was observed (**Figure 6.6**). Additionally, there appeared to be no statistical correlation between the body weight reduction and the disposition index improvement in both exenatide and insulin glargine treated patients. A more specific GLP-1 receptor agonist related factor could therefore not be

excluded. Unfortunately, more post-hoc analyses were not possible due to the small sample size.

As acknowledged in **chapter 2**, residual beta-cell function and mass may be an important determinant of the efficacy of GLP-1 receptor agonist therapy (44). Based on homeostasis model assessment (HOMA) data from the UK Prospective Diabetes Study (UKPDS), it has been suggested that in patients with type 2 diabetes, beta-cell function is already reduced by 50% at the time of diagnosis (45), and that loss of beta-cell function begins 10–12 years before diagnosis (14, 46). The data presented in **chapter 2** and **chapter 6** may suggest that a temporary improvement in pancreatic beta-cell function may be achieved following prolonged treatment with a GLP-1 receptor agonist. The observed improvement in disposition index was not accompanied by a lasting improvement in glycemic efficacy measures; the HbA_{1c} concentration returned to pre-randomization levels following the 12-week off-drug period (**Figure 2.3A** and **Figure 6.4A**). It has been shown that glucose control is closely related to pancreatic beta-cell function in humans (47). It may be that the improvement of the DI was not big enough in these patients with established type 2 diabetes to affect glycemic control. Pharmacological interventions aimed to preserve beta-cell function probably need to be initiated at an earlier stage. Additional studies are needed in patients at high-risk, for example patients with impaired glucose tolerance, to test this hypothesis (48).

Beta-cell function measured using the mixed-meal test

As mentioned in **chapters 2** and **chapter 6**, the euglycemic-hyperinsulinemic clamp and the hyperglycemic clamp are currently regarded gold-standard methods for measuring insulin sensitivity and beta-cell function respectively. However, these techniques also have disadvantages. The clamp technique is technically challenging, a burden to patients and time consuming. Additionally, the invasive nature of the clamp may not mirror 'real life' situations. Although fasting indices (such as the HOMA (49)) are frequently used as surrogate estimates of insulin sensitivity and insulin secretion, fasting concentrations only reflect a single point on the complex glucose-insulin dose-response curve, and thus cannot provide insight regarding the dynamics of the beta-cell in response to changing glucose concentrations such as typically occur in daily life (8). Therefore, for epidemiologic studies, and for the follow-up of individual changes in insulin sensitivity and insulin secretion, measurements derived from simpler methods such as the oral glucose tolerance test (OGTT) are often used (50, 51). The insulinogenic index, calculated as the ratio of the 30-minute

increment in insulin to glucose concentration (52), is widely used to estimate glucose responsiveness of the beta-cell following the OGTT. Despite its simplicity, this index is able to detect anomalies in beta-cell function in many circumstances (52). Nevertheless, it needs to be acknowledged that correlations between OGTT derived and intravenous derived measures of beta-cell function are not perfect (*r* values between 0.5-0.7) (53). Being a composite index, it does not reflect specific mechanisms of insulin secretion (for instance, the first phase insulin secretion as assessed with the hyperglycemic clamp) (9). In the interpretation of the OGTT beta-cell indices, it must be kept in mind that giving glucose through the oral route results in a potentiated secretory response due to the activation of the entero-insular axis, which does not occur with the hyperglycemic clamp (9). By design, the hyperglycemic clamp uses intravenous administration of glucose and other secretagogues (i.e. amino acids or GLP-1) to test beta-cell secretory function (33, 54, 55). Since the primary goal of most studies is to determine how alterations in beta-cell function and insulin action influence human metabolism, ideally these should be assessed under physiologic conditions by using simple tests, i.e. mixed-meals that provide carbohydrates, fats and amino acids (8).

Over recent years, several mathematical models have been developed to quantify the beta-cell response following an OGTT or standardized mixed-meal test (8, 56). A common aspect of these models is a dose-response function representing the relationship between the circulating glucose concentration and subsequent insulin secretion rate (ISR) (8, 53, 56). The simplest models are confined to just the dose response relationship between the glucose and insulin concentration (57), whereas others are more sophisticated (58-62). Insulin and C-peptide are equimolarly secreted from the beta-cell and pass through the liver, where insulin, but not C-peptide, undergoes hepatic extraction (63). Therefore more recently developed models rely on C-peptide deconvolution (64) rather than insulin concentrations per se. Although more models exist, two are now frequently used in the literature (58, 59, 61, 62). In these models insulin secretion is represented as the sum of two main compartments: 1) the glucose/insulin dose-response relations, and 2) one accounting for the observation that rapid changes in glucose concentration enhance insulin release (53) (for review see: (8, 56)). These models share common features, the models mainly differ by the way they interpret the potentiation of insulin secretion (8, 53). For the study presented in **chapter 4** we have chosen for the model by Mari et al. (61, 62) (**Figure 9.2**). Since this model has been used frequently in other studies using GLP-1 (65), exenatide (26), liraglutide (66), and the DPP-4

inhibitor vildagliptin (67, 68), it facilitates the comparison with other incretin-based therapies.

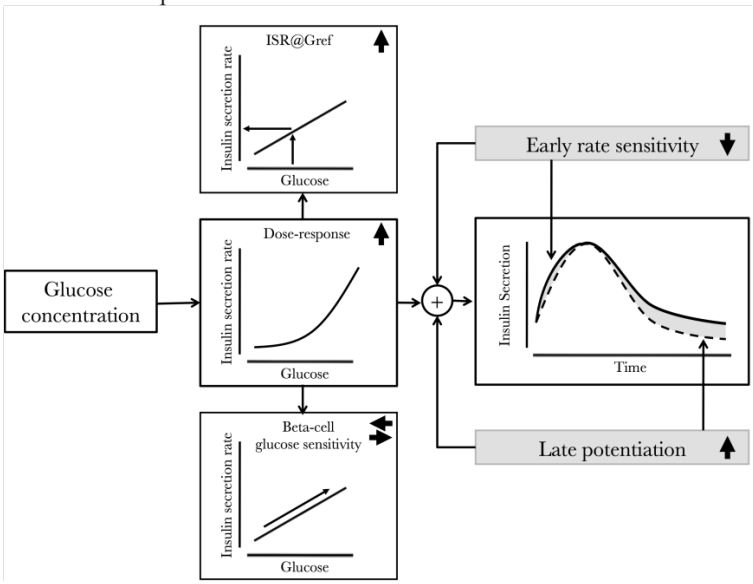

Figure 9.2 Block diagram of the beta-cell model and illustration of the role of the insulin secretion components during an oral glucose tolerance test. The central compartment of the model is the glucose/insulin dose-response relation, which describes the rise and fall of insulin secretion that parallels the rise and fall of the glucose concentration. From the glucose/insulin dose-response relation 2 parameters are calculated: 1) the insulin secretion rate at reference glucose concentration (ISR@Gref) and 2) beta-cell glucose sensitivity which is represented by the slope of the glucose/insulin dose-response relation. The dose-response relation is modulated by the potentiation factor, which is a positive function over time and has been constrained to have a time average of 1 during the experiment. The potentiation factor explains the sustained insulin secretion usually seen at the end of an OGTT or meal test when the circulating glucose concentration already has returned to baseline values. Finally, rate sensitivity is introduced into the model: an early insulin secretion component, which quantifies the sensitivity of the insulin response to the initial rapid glucose rise. The black arrows represent the effects of one-year treatment with exenatide on the specific model-derived measure as compared to insulin glargine. Adapted from Mari (56).

The main findings of the study assessing one-year treatment with exenatide, as compared to insulin glargine, on meal derived measures of beta-cell function are presented in **chapter 4**. One-year treatment with exenatide resulted in a significantly greater upward shift in the glucose/insulin dose-response relation, and increased potentiation after both breakfast and lunch. Notwithstanding the fact that the potentiation factor has been criticized for being a time-varying correction term that mathematically compensates for

the difference between the observed ISR and the ISR derived from the glucose/insulin dose response relation (8), Ferrannini postulates that potentiation should be considered as an independent parameter of beta-cell function (69). The strong initial increase in potentiation (**Figure 4.4C**) may represent the strong stimulating effect on insulin secretion following the exenatide injection prior to the breakfast meal. Additionally, both exenatide and insulin glargine treatment equally improved glucose sensitivity, representing the ability of the beta-cell to adjust the ISR to the change in prevailing glucose concentration. Finally, the beneficial effects on the glucose/insulin dose response relationship were sustained after a 5-week off-treatment period in both the exenatide and insulin glargine treated groups. As the improvement in beta-cell glucose sensitivity, and the lasting effect on glucose/insulin dose response relationship after cessation of treatment, was found in both treatment groups a more general mechanism, possibly reduction of glucose toxicity (70) may explain these findings.

Interestingly, the rate sensitivity significantly decreased in exenatide-treated patients when compared to insulin glargine. Rate sensitivity appreciates the relationship between the early, fast rise in plasma glucose concentrations and the subsequent change in insulin secretion. When the time derivative of the glucose concentration is negative, the derivative component equals 0 (61, 62). The observed delta in glucose concentration is negative in the exenatide group (**Figure 4.3A**). This reduction in postprandial glucose concentration may account for the between-group difference in rate sensitivity, as this parameter is automatically set to be 0 in many exenatide treated patients. Insulin glargine did not have such an effect on the postprandial glucose concentration, and hence on rate sensitivity. These findings are consistent with observations made in similar experiments with GLP-1 infusion (65) and the GLP-1 receptor agonist liraglutide (66).

Secondary study endpoints: cardiovascular disease risk markers

Today, it is well recognized that type 2 diabetic patients have an increased risk of developing micro and macrovascular disease. Mortality from cardiovascular disease (CVD) is 2-4 fold increased in persons with diabetes compared with the general population (71). Although it has been shown that an intensified multifactorial intervention reduces macrovascular and microvascular events by about half, it is still considerably higher than in the background population, leaving room for much improvement (72, 73). Hyperglycemia is associated with cardiovascular risk in patients with type 2 diabetes. However, there is less compelling evidence that glucose lowering

therapy reduces CVD risk. Data from the original United Kingdom Prospective Diabetes Study (UKPDS) cohort did not exhibit a reduction in CVD events, apart from a small subgroup of obese patients treated with metformin that experienced a CVD benefit. (74). However, the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications trial (DCCT/EDIC) showed that glucose lowering was associated with a long-term cardiovascular complications benefit in patients with type 1 diabetes (75, 76). Over the last few years data from ACCORD (77), ADVANCE (78), and the Veterans Affairs Diabetes Trial (79), did not demonstrate a significant reduction of cardiovascular events in the groups randomized to intensive glucose-lowering therapy as compared to the standard therapy group. More recently the United Kingdom Prospective Diabetes Study Post-Trial (UKPDS post-trial) showed a benefit of glycemic control after 10 years of post-trial follow-up (a so-called 'legacy effect') (80). These observations support the concept to treat patients to target as early as possible following diagnosis (81,82).

Effects on postprandial dysmetabolism

Both postprandial hyperglycemia and hypertriglyceridemia lead to mitochondrial free radical production and subsequent oxidative stress, which in turn may contribute to the development of both micro and macrovascular complications (81, 83-85) (**Figure 9.3**). It has been suggested to include the reduction of postprandial glucose and lipid excursions as treatment targets in the treatment guidelines for type 2 diabetes (86).

To our knowledge, studies on the effects of exenatide on postprandial cardio-metabolic parameters have used a single meal study design (23, 24, 26, 88-92). It is known that postprandial glucose and lipid responses differ following subsequent meals in patients with the metabolic syndrome and type 2 diabetes (82, 84, 93). To simulate the real-life daily food intake we used two consecutive meals in our study.

In **chapter 3** the effects of one-year exenatide treatment on postprandial glucose and lipids are presented. One-year exenatide resulted in a significant reduction of prandial glucose, triglycerides, apo-B48, VLDL-C, HDL-C, free fatty acid and MDA excursions (**Table 3.2**). Additionally, we found a statistically significant correlation between the reduction in postprandial glucose and lipid excursions, and different markers of oxidative stress (**Figure 3.3**).

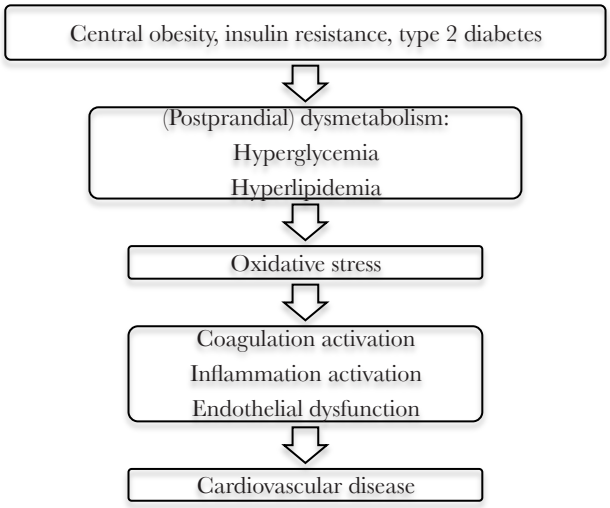

Figure 9.3 Type 2 diabetes is characterized by high circulating levels of atherogenic lipid particles due to an increased supply of fatty acids to the liver and defective hepatic clearance of lipoproteins. In the postprandial state, the lipid abnormalities are further exaggerated, with an additional adverse effect of meal-induced hyperglycemia. These postprandial metabolic derangements increase the production of reactive oxygen species causing oxidative stress. Collectively, postprandial dysmetabolism and the associated oxidative stress may link type 2 diabetes to cardiovascular disease. Adapted from Tushuizen (87).

Gastric emptying is an important determinant of postprandial glucose and lipid excursions in healthy subjects and type 2 diabetes (94, 95). Studies using the acetaminophen technique (96-99) and scintigraphy (100) have shown that exenatide slows gastric emptying in both healthy subjects and subjects with type 2 diabetes. Unfortunately, we did not measure gastric emptying rates in our study. However, is appears to be reasonable to assume that gastric emptying significantly contributed to the reduction in postprandial glucose and lipid excursions. The reduction in postprandial glucose excursions was previously shown to be related to the slowing of gastric emptying during exenatide treatment (100). However, other data suggest that, exenatide may lower postprandial glycemia via a novel mechanism independent of islet hormones and slowing of gastric emptying (101). Although the effects of exenatide on the second meal were attenuated as compared to the first meal, the total glucose and triglyceride excursion remained significantly lower with exenatide compared to insulin glargine during the entire 8-hour mixed-meal test, even after ingestion of an additional lunch meal, 4 hours after the (pre-breakfast) exenatide dose. Combined, our meal test findings may suggest a beneficial effect on the

cardiovascular risk profile of exenatide as compared to insulin glargine as epidemiological (102) and interventional studies (103) have suggested that postprandial glucose are stronger predictors of CVD than fasting plasma glucose in patients with diabetes.

Effects on body composition

Estimates indicate that approximately 60% of all cases of type 2 diabetes can be attributed to weight gain (104). Pharmacological interventions in patients with type 2 diabetes should therefore also target obesity (105). The clustering of cardiovascular risk factors with abdominal obesity is well established (106). The term metabolic syndrome captures a wide spectrum of factors thought to increase the risk of CVD and type 2 diabetes. This syndrome now includes amongst others: abdominal obesity, hypertension, abnormal plasma glucose, microalbuminuria, elevated levels of cytokines, and insulin resistance (107, 108).

Computed tomography (CT) and magnetic resonance imaging (MRI) technologies are regarded the gold standard for measuring body composition in vivo. However, as they are expensive and cumbersome, dual-energy X-ray absorptiometry (DEXA) provides a reliable alternative for regional body composition analysis in large-scale follow-up studies (109). Effects of exenatide and insulin glargine administrations on body composition assessed with DEXA are discussed in **chapter 5**. We showed that exenatide reduces body weight mainly as a result of a reduction of truncal fat mass and waist circumference. Additionally, circulating concentrations of leptin, adiponectin, and hsCRP were beneficially influenced. Adiponectin and CRP are thought to be independent key molecules in type 2 diabetes related cardiovascular disease (110, 111). Although the combination of a reduction in truncal fat mass and waist circumference may suggest a reduction in metabolically active visceral fat mass, a recent uncontrolled study did not show an effect of exenatide treatment on visceral fat measured with computed tomography (112). Therefore additional studies to assess the effects of exenatide on body composition and body fat distribution are needed.

Disproportionate accumulation of intra-abdominal and/or hepatic fat may explain variations in serum triglyceride, HDL-cholesterol, and hepatic insulin sensitivity, supporting the assumption that both fat deposits are important determinants of these components of the metabolic syndrome (113). According to the American Association for the Study of Liver Diseases, non-alcoholic fatty liver disease (NAFLD) is defined as fat accumulation in the liver exceeding 5% to 10% (114). In **Chapter 8** we

describe the possible effects of exenatide treatment on hepatic fat content in one patient participating in our study. Following approximately 10 months of exenatide treatment the liver fat content measured by liver MRI-spectroscopy declined from 15.8% to 4.3%.

Diabetic dyslipidemia is a cluster of potentially atherogenic lipid and lipoprotein abnormalities that are metabolically interrelated. Recent evidence suggests that a fundamental defect is an overproduction of large very low-density lipoprotein (VLDL) particles, which initiates a sequence of lipoprotein changes, resulting in higher levels of remnant particles, smaller and denser LDL particles, and lower levels of high-density lipoprotein (HDL) cholesterol (82, 115, 116). In addition, a fatty liver may further contribute to the CVD risk by a higher production of glucose, CRP, and coagulation factors (117). **Chapter 3** and **chapter 5** present data on the effects of exenatide on metabolic factors that are thought to be related to the amount of liver fat. Hepatic overproduction of apo-B-containing VLDL particles is regarded as the dominant feature of diabetic dyslipidemia driven primarily by liver fat and hyperglycemia (115). Although the reduction in truncal fat mass may play an important role in the in the observed reduction in atherogenic lipid particles and circulating CRP levels, we showed that the reduction in CRP was partially independent of changes in body weight and truncal fat mass changes. This may be attributed to a possible reduction in liver fat content following exenatide treatment.

The combined findings of **chapter 3**, **chapter 5** and **chapter 8** may suggest favorable effects on cardiovascular risk in type 2 diabetes treated with exenatide. A recent meta-analysis of exenatide clinical trial data supports this interpretation of our findings (118).

Secondary study endpoints: treatment safety

The exenatide therapy associated adverse event profile as reported in this thesis is similar to earlier published studies. The most common adverse events associated with exenatide treatment were mild-to-moderate gastrointestinal side effects (including nausea, diarrhea, vomiting), which are dose-dependent, more common during drug initiation and decrease over time (119). The risk of hypoglycemia is not increased when exenatide is combined with metformin (88). During our study no major hypoglycemic events occurred in either randomization arm.

In our study one patient randomized to exenatide developed pancreatitis while on active treatment, which resolved after cessation of the study medication. This adverse event is referred to in **chapter 2**. Cases of acute pancreatitis have been reported in patients treated with exenatide BID (120,

121), liraglutide (122), and vildagliptin (123). Recently published data showed that exenatide use was not associated with an increased risk of acute pancreatitis (124). Additionally, exenatide does not evoke pancreatitis and attenuates chemically induced pancreatitis in normal and diabetic rodents (125).
Type 2 diabetes is associated with an increased risk of bone fractures (126). Thiazolidinediones have been suggested in increased bone fracture risk in female patients with type 2 diabetes (127). Body weight reduction is advocated in most type 2 diabetes patients to improve their cardiovascular risk profile, however, it may decrease bone density and increase bone turnover (128, 129). Therefore, type 2 diabetes therapies should not only lower cardiovascular risk but also be safe in terms of bone health, also given their chronic use. In **chapter 7** we show that exenatide treatment does not affect bone mineral density despite the significant reduction in body weight.

Final conclusion

In addition to an acute pharmacological effect, the studies presented in this thesis showed that exenatide treatment, as compared to insulin glargine, beneficially affects metabolic parameters, which are thought to play an important role in the pathogenesis of type 2 diabetes (**Figure 9.4**).

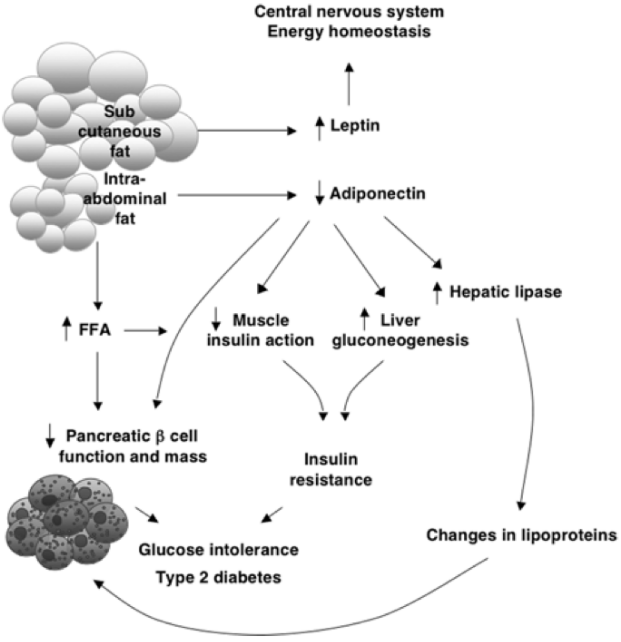

Figure 9.4 Model for the effects of body fat content on insulin sensitivity and beta-cell function in the pathogenesis of type 2 diabetes. Adapted from Cnop (130).

Taken together the following hypothesis could be suggested. Prolonged, multi-year, exenatide treatment leads to:

- a reduction in body weight by lowering truncal fat mass (**chapter 5**);
- subsequently, circulating adipocytokines (i.e. leptin and adiponectin) and inflammatory biomarkers, and insulin sensitivity will improve (**chapter 2, chapter 5** and **chapter 6**);
- exenatide treatment reduces liver fat content (**chapter 8**);
- together these metabolic effects lead to a more favorable (prandial) lipid and lipoprotein profile and a reduction in oxidative stress (**chapter 3**);
- which in turn lead to an amelioration of beta-cell function and glucose tolerance (**chapter 2, chapter 4,** and **chapter 6**).

However in our studies, the glucometabolic improvements were not persevered following a 12-week off-drug period suggesting multi-year treatment may be necessary for these effects to happen. Beta-cell preserving pharmacotherapeutic interventions should preferably start early to ensure that sufficient residual beta-cell function is still present. It has been proposed to start early in the disease pharmacological intervention to prevent the further deterioration of beta-cell function using a combination of metformin, thiazolidinediones, and exenatide, preferably in patients with impaired glucose tolerance (i.e. pre-diabetes) (48, 105). Against this background, future long-term pharmacotherapeutic interventions in patients with pre-diabetes are needed to study whether GLP-1 receptor agonists (such as exenatide) might favorably alter the progressive nature of type 2 diabetes.

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Chapter 10

Samenvatting voor de geïnteresseerde leek
List of abbreviations
Affiliation of co-authors
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Samenvatting voor de geïnteresseerde leek

Gedurende de laatste decades is diabetes mellitus type 2 (DM2) veranderd van een relatief onschuldige aandoening van de, met name de oudere, mens in een ziekte die een gevaar is voor de volksgezondheid. Door verandering in levensstijl neemt het aantal mensen met DM2 zeer snel toe, niet alleen in de westerse wereld, maar juist ook in opkomende economieën. De Wereldgezondheidsorganisatie van de Verenigde Naties (WHO) heeft berekend dat er in het jaar 2030 wereldwijd 350 miljoen mensen met DM2 zullen zijn. Voor Nederland is berekend dat het aantal mensen met DM2 zal toenemen van 670.000 in 2008 naar ongeveer 1.3 miljoen in 2025.

Wat is diabetes mellitus type 2?

DM2 is een progressieve ziekte van de koolhydraatstofwisseling. De koolhydraatstofwisseling is een belangrijk proces, waarmee het lichaam energie maakt. In dit proces spelen een aantal in het bloed circulerende voedingsstoffen en hormonen (signaalstoffen) een belangrijke rol. Glucose (suiker) is een voedingsstof die in de lichaamscellen wordt gebruikt om energie te verkrijgen. Om glucose vanuit de bloedbaan in de lichaamscel te krijgen is het hormoon insuline nodig. Insuline wordt aangemaakt door de zogenaamde beta-cellen in de alvleesklier. In de normale situatie zal na het eten van een maaltijd de hoeveelheid glucose in het bloed stijgen. De alvleesklier zal hierop een stijging van de hoeveelheid glucose in het bloed waarnemen. Deze stijging is voor de alvleesklier het signaal om insuline uit te scheiden. Door het loslaten van insuline in de bloedbaan zal glucose de cel in worden getransporteerd, waarna de glucose kan worden gebruikt voor de productie van energie. Bij mensen met DM2 is deze koolhydraatstofwisseling op twee manieren verstoord:

- zij zijn ongevoeliger voor de werking van het hormoon insuline
- zij kunnen onvoldoende insuline produceren om deze ongevoeligheid te compenseren.

Insulinegevoeligheid en insuline-afgifte

In de westerse consumptiemaatschappij eten we meer voedingstoffen dan nodig is voor normaal functioneren van het menselijk lichaam. Het overschot aan voedingsstoffen wordt door het lichaam opgeslagen in vetcellen en daar bewaard voor tijden waarin minder voedingsstoffen beschikbaar zijn (perioden van schaarste en honger). Het gevolg hiervan is dat het lichaamsgewicht, en met name het lichaamsvetgehalte, toeneemt. Naast deze opslagfunctie voor energie maken vetcellen allerlei signaalstoffen

aan. Deze signaalstoffen zijn belangrijk voor de regulering van o.a. de voedingstoestand van het lichaam. Een aantal van de, door vetcellen gemaakte, signaalstoffen hebben echter een schadelijk effect als zij in overvloed gemaakt worden. Een van deze (schadelijke) effecten is dat zij de werking van het hormoon insuline verminderen. Wij noemen dit insuline ongevoeligheid of insulineresistentie. Door deze ongevoeligheid is meer insuline nodig om dezelfde hoeveelheid glucose uit het bloed de cel in te transporteren en het bloedglucosegehalte binnen de normaalwaarden te houden. Onderzoek laat zien dat mensen met overgewicht en obesitas hogere insuline waarden in het bloed hebben in vergelijking met mensen zonder overgewicht. Op deze manier zijn zij in staat normale bloedglucose waarden in stand te houden. Dit geldt voor de meeste mensen met overgewicht: door meer insuline te produceren en af te geven zullen deze mensen normale bloedglucose waarden houden en geen DM2 ontwikkelen. Echter niet alle mensen zijn in staat om voldoende insuline vanuit de alvleesklier te produceren en op deze manier voldoende te compenseren voor de ontstane ongevoeligheid voor het hormoon insuline. Wat hiervan de precieze oorzaak is, is nog niet bekend, maar een erfelijke aanleg lijkt een belangrijke rol te spelen. Als gevolg van het onvermogen de insulineresistentie te compenseren met een verhoogde uitscheiding van insuline door de alvleesklier zal uiteindelijk het bloedglucosegehalte stijgen en DM2 ontstaan.

Traditionele behandeling

De hoeksteen van de behandeling van DM2 is verandering van leefgewoonte. Hiermee bedoelen we minder eten en meer bewegen. Hierdoor zal lichaamsgewicht afnemen en, als gevolg hiervan, zal de gevoeligheid van het lichaam voor het hormoon insuline weer toenemen. Gezamenlijk leidt dit tot een afname van het bloedglucosegehalte. Echter voor veel mensen met DM2 zal deze aanpak onvoldoende effect hebben en zal een behandeling met geneesmiddelen noodzakelijk zijn.

Traditioneel beschikbare geneesmiddelen voor de behandeling van DM2 proberen het bloedglucosegehalte binnen de daarvoor geldende normaalwaarden te houden. Dit doen zij op een aantal manieren. Sommige geneesmiddelen proberen de gevoeligheid van het lichaam voor het hormoon insuline te vergroten, andere geneesmiddelen proberen de hoeveelheid insuline die door de alvleesklier wordt afgegeven te vergroten. Het gevolg van beide manieren is dat het bloedglucosegehalte zal dalen. Studies in grote groepen mensen met DM2 laten echter zien dat deze traditionele geneesmiddelen het progressieve karakter van DM2 niet ongedaan kunnen maken. Gedurende het ziektebeloop zal op den duur de

dosering van de gebruikte geneesmiddelen moeten worden verhoogd. Vaak moeten meerdere geneesmiddelen worden gecombineerd om het bloedglucosegehalte binnen de daarvoor geldende normaalwaarden te houden. Deze aanpak, in combinatie met de eerder genoemde verandering van leefgewoonte, kan lang voldoende resultaat hebben. Helaas zal voor een groot deel van de mensen deze behandeling niet voldoende zijn, waardoor uiteindelijk insuline zal moeten worden geïnjecteerd. Daarnaast laten studies zien dat de huidig beschikbare traditionele geneesmiddelen de aan DM2 gerelateerde complicaties, zoals hart- en vaatziekten, onvoldoende voorkomen. Daarom zijn nieuwe geneesmiddelen noodzakelijk die het progressieve karakter en de complicaties van DM2 kunnen voorkomen.

Glucagonachtig peptide-1 (GLP-1)

Zoals eerder aangegeven zal na een maaltijd het glucosegehalte in het bloed stijgen, waarna de alvleesklier het hormoon insuline aan de bloedbaan zal afgeven. Dit is een gecompliceerd proces, waar meerdere signaalstoffen, maar ook b.v. het zenuwstelsel, een rol in spelen. Een van de signaalstoffen die een belangrijke rol speelt in de regulatie van de insulineproductie en -afgifte na een maaltijd is de signaalstof glucagonachtig peptide-1, ook wel GLP-1 genoemd. GLP-1 wordt na de maaltijd in delen van de darm aan de bloedbaan afgegeven. In de alvleesklier zal dit afgegeven GLP-1 de aanmaak en afgifte van insuline stimuleren. Wetenschappelijk onderzoek heeft laten zien dat de GLP-1 productie bij mensen met DM2 is verminderd vergeleken met gezonde mensen. Echter, de werking van GLP-1 in de alvleesklier van mensen met DM2 is ongestoord. Dit heeft er toe geleid dat er GLP-1 achtige stoffen zijn ontwikkeld die als geneesmiddel kunnen worden gebruikt voor de behandeling van mensen met DM2. Het in dit proefschrift gebruikte geneesmiddel exenatide is zo'n, van GLP-1 afgeleide, stof.

Exenatide

Exenatide lijkt erg op GLP-1 zoals dat door de mens wordt gemaakt. Het is echter op een aantal punten verschillend, waardoor het een langere werkingsduur heeft (i.v.m. menselijk GLP-1). Wetenschappelijk onderzoek bij knaagdieren met DM2 heeft laten zien dat exenatide mogelijk de insulineproductie door de beta-cellen in de alvleesklier zou kunnen herstellen. Of behandeling met exenatide het progressieve karakter van DM2 ook in mensen gunstig zou kunnen beïnvloedden was bij aanvang van het in dit proefschrift beschreven onderzoek nog niet geheel duidelijk. Echter, eerder verricht wetenschappelijk onderzoek heeft laten zien dat behandeling van mensen met DM2 met exenatide leidt tot een acute

toename van insulineproductie door de alvleesklier. Als gevolg hiervan zal exenatidebehandeling in mensen met DM2 het bloedglucosegehalte verlagen. Naast deze gunstige effecten op de insulineproductie en bloedglucosegehalte toonde onderzoek ook aan dat mensen die met exenatide behandeld werden, minder gingen eten en hierdoor lichaamsgewicht verloren. Vermindering van lichaamsgewicht is in mensen met DM2 een belangrijke stap voor het verminderen van de kans op hart- en vaatziekten. Zoals eerder gezegd, is het met traditionele geneesmiddelen niet mogelijk het progressieve karakter van DM2 een halt toe te roepen. Na de ontdekking van de nieuwe, op GLP-1 gebaseerde, geneesmiddelen rees de vraag of dit met deze nieuwe groep geneesmiddelen wel tot de mogelijkheden behoorde.

Bovenstaande leidde tot het in dit proefschrift beschreven onderzoek. De belangrijkste onderzoeksvragen van de studies beschreven in dit proefschrift waren:

1. Leidt behandeling met exenatide, gedurende 1, dan wel 3 jaar, in mensen met DM2 tot een verbetering in insuline-afgifte?
2. Zo ja, blijft deze verbetering in insuline-afgifte bestaan na het staken van de behandeling met exenatide?

Daarnaast hebben wij geprobeerd antwoord op vier additionele vragen te verkrijgen.

1. Leidt behandeling met exenatide tot een gunstige beïnvloeding van de koolhydraat- en vetstofwisseling na een maaltijd?
2. Leidt behandeling met exenatide tot een verandering in lichaamssamenstelling en -vetgehalte?
3. Leidt behandeling met exenatide tot een gunstige beïnvloeding van stoffen in het bloed die samenhangen met het risico op hart- en vaatziekten?
4. Is behandeling met exenatide veilig?

Om deze onderzoeksvragen te beantwoorden werden 69 mensen (45 mannen en 24 vrouwen) met DM2 door loting toegewezen aan een behandeling met exenatide (36 mensen) of insuline glargine (33 mensen). Alleen mensen die behandeld werden met het orale bloedglucose verlagende geneesmiddel metformine werden tot de studie toegelaten. Daarnaast moest het bloedglucosegehalte van de deelnemers, ondanks de behandeling met metformine, bij aanvang van de studie niet binnen internationaal gestelde behandeldoelen (HbA1c <6.6%) zijn.
Er werd gekozen om exenatide te vergelijken met het langwerkende insulinepreparaat glargine. Insuline glargine wordt in de regel

voorgeschreven als de behandeling met orale geneesmiddelen alleen onvoldoende behandeleffect laten zien.
Deelnemers werden gedurende 1 jaar behandeld met exenatide of insuline glargine. Aan het einde van de behandelperiode werd de studiemedicatie gestaakt en werden de deelnemers gedurende 12 weken alleen nog behandeld met metformine. Voor en na de behandelperiode, en gedurende de 12 weken met alleen metformine behandeling, werd het effect van de behandeling op de bovengenoemde onderzoeksvragen gemeten.

In **hoofdstuk 2** werd het effect van 1 jaar behandeling met exenatide op insuline-afgifte, bloedglucosegehalte, lichaamsgewicht en insulinegevoeligheid onderzocht. In vergelijking met insuline glargine zagen wij een toename van de insuline-afgifte en een afname van het lichaamsgewicht. Het bloedglucosegehalte verbeterde in gelijke mate in de exenatide en de insuline glargine behandelde mensen. Er werd geen verschil in bloedglucosegehalte waargenomen tussen de twee behandelgroepen. Na het staken van de behandeling met exenatide, of insuline glargine, was er geen effect op de insuline-afgifte meer waarneembaar, gemeten vier weken na het stoppen van de studiemedicatie. Interessant was de bevinding dat de insulinegevoeligheid in de mensen behandeld met exenatide verbetert in vergelijking met de mensen behandeld met insuline glargine. Dit effect was mogelijk een gevolg van de nog steeds waarneembare afname in lichaamsgewicht, vier weken na het staken van de studiemedicatie. Het bloedglucosegehalte verslechterde geleidelijk gedurende de periode waarin de studiemedicatie was gestopt. Twaalf weken na het stoppen van de studiemedicatie was het bloedglucosegehalte gelijk aan het gehalte voorafgaand aan de studie. De conclusie van hoofdstuk 2 is dat 1 jaar exenatidebehandeling in mensen met DM2 leidt tot een toename in de insuline-afgifte, een afname in lichaamsgewicht en een verbetering in bloedglucosegehalte, maar dat deze toename niet blijvend is na staken van de studiemedicatie. Als mogelijkheid hiervan worden o.a. DM2 ziekteduur of een te korte behandelperiode genoemd. Om deze laatste mogelijkheid te onderzoeken is een langere behandelduur van in totaal drie jaar op de insuline-afgifte onderzocht. Resultaten van deze drie jaar durende behandelperiode worden beschreven in hoofdstuk 6.

In **hoofdstuk 3** werd het effect van 1 jaar exenatide behandeling op de koolhydraat- en vetstofwisseling na een vetrijke maaltijd onderzocht. Deelnemers kregen een hamburgermaaltijd als ontbijt en als lunch. Na inname van de maaltijden onderzochten wij de verandering in het bloedglucose- en bloedvetgehalte, als mede de mate van zuurstofschade veroorzaakt door deze verandering. Zuurstofschade is een riscofactor voor

het ontstaan van hart- en vaatziekten. Exenatide behandeling verlaagde het bloedglucose- en bloedvetgehalte na de maaltijd in vergelijking met insuline glargine. Ook de hoeveelheid zuurstofschade nam af na behandeling met exenatide en deze afname was statistisch gekoppeld aan de effecten op het bloedglucose- en bloedvetgehalte.

De in hoofdstuk 2 beschreven effecten op de insuline-afgifte werden in een laboratoriumopstelling gemeten. De vraag was of de beschreven gunstige effecten van exenatide op de insuline-afgifte ook aanwezig zouden zijn wanneer gemeten na een maaltijd. De effecten van exenatide op de insuline-afgifte gemeten na een maaltijd werd onderzocht in **hoofdstuk 4**. In vergelijking met insuline glargine namen verschillende aspecten van maaltijd gerelateerde insuline-afgifte toe na 1 jaar behandeling met exenatide. Deze bevindingen bevestigde de in hoofdstuk 2 beschreven resultaten en breidde deze verder uit. Interessant was dat in zowel de exenatide als in de insuline glargine behandelde mensen met DM2 een blijvend effect waarneembaar was op de relatie tussen bloedglucose en insuline-afgifte, 5 weken na het stoppen van de studiemedicatie.

In **hoofdstuk 5** werd het effect van 1 jaar exenatide behandeling op de lichaamssamenstelling en bloedrisicofactoren op hart- en vaatziekten bestudeerd. In vergelijking met insuline glargine was een afname in lichaamsgewicht waarneembaar in exenatide behandelde deelnemers. Aanvullend onderzoek naar de veranderingen in lichaamssamenstelling liet zien dat de afname in lichaamsgewicht met name veroorzaakt werd door een afname van het vetgehalte in de romp. Doordat ook de buikomvang in deze deelnemers afnam, mogen wij concluderen dat deze afname waarschijnlijk wordt veroorzaakt door een afname van het buikvetgehalte. Buikvet is een risicofactor voor het ontstaan van hart- en vaatziekten. Naast de beschreven afname in lichaamsvetgehalte zagen wij ook een gunstige verandering van in het bloed circulerende stoffen die samenhangen met het risico op hart- en vaatziekten. Adiponectine, een eiwit dat een gunstige werking heeft op de insulinegevoeligheid, nam toe. CRP, een ander eiwit dat door de lever geproduceerd wordt en een maat is voor systemische ontsteking, nam af. Deze resultaten suggereren dat exenatide mogelijk een gunstige invloed heeft op het ontstaan van hart- en vaatziekten in mensen met DM2. Echter, een definitief antwoord op deze vraag kan alleen gegeven worden na een langdurige studie met deze specifieke onderzoeksvraag.

In **hoofdstuk 6** werden de effecten van exenatide en insuline glargine op insuline-afgifte, bloedglucosegehalte, lichaamsgewicht en insulinegevoeligheid, na een behandelperiode van 3 jaar onderzocht. In

tegenstelling tot de in hoofdstuk 2 beschreven effecten op insuline-afgifte na 1 jaar behandeling, zagen wij na 3 jaar behandeling een kleine afname in insuline-afgifte, 4 weken na het stoppen van de studiemedicatie. Echter, omdat er, als gevolg van de exenatidebehandeling, een aanzienlijke gewichtsafname had plaatsgevonden, was ook de insulinegevoeligheid toegenomen in de met exenatide behandelde deelnemers. Insuline-afgifte en insulinegevoeligheid zijn twee mechanismen die elkaar direct beïnvloeden. Als de insulinegevoeligheid afneemt zal de insuline-afgifte in gezonde mensen toenemen om te voorkomen dat het bloedglucosegehalte (te veel) stijgt. Tegenovergesteld, als er teveel insuline afgegeven wordt neemt de insuline gevoeligheid af om te voorkomen dat het bloedglucosegehalte te veel daalt. Deze relatie wordt beschreven in de dispositie index. In mensen met DM2 is deze relatie tussen insulinegevoeligheid en insuline-afgifte (en in de dispositie index) verstoord. In hoofdstuk 6 hebben wij laten zien dat als wij de afname in insuline-afgifte corrigeerden voor de toename in insulinegevoeligheid er een toename was van de dispositie index. Dit effect was niet waarneembaar na behandeling met insuline glargine. Omdat na 12 weken stoppen met de studiemedicatie in beide behandelgroepen het bloedglucosegehalte terug was op het niveau van voor de behandeling mag worden verondersteld dat deze verbetering in de dispositie index onvoldoende was om een blijvend gunstig effect op de koolhydraatstofwisseling te bewerkstelligen. Of dit op een andere manier wel mogelijk is, moet toekomstig aanvullend onderzoek aantonen.

In **hoofdstuk 7** werd aangetoond dat de behandeling met exenatide geen nadelige effecten heeft op de botmineraaldichtheid. Nadelige effecten op de gezondheid van de botten werd in eerder onderzoek met de bloedglucose verlagende geneesmiddelengroep thiazolidinedione aangetoond. Het is hierdoor belangrijk om bij nieuwe middelen voor de behandeling van DM2 aan te tonen dat zij geen negatieve effecten hebben op o.a. de botmineraaldichtheid.

Leververvetting wordt geassocieerd met een toegenomen risico op hart- en vaatziekten. In **hoofdstuk 8** werd het effect van exenatide op het levervetgehalte onderzocht in een van de deelnemers die in de studie exenatide kreeg toegewezen. Exenatidebehandeling verlaagde het levervetgehalte van 15,8% naar 4,3%, een waarde onder de grens waarboven gesproken wordt van leververvetting. Ondanks dat in dit hoofdstuk slechts een deelnemer beschreven wordt, laten de resultaten zien dat exenatide mogelijk een rol kan spelen in de behandeling van mensen met leververvetting.

Suggesties voor toekomstig onderzoek

De resultaten van het in dit proefschrift beschreven onderzoek laat zien dat behandeling van mensen met DM2 met exenatide leidt tot een:

- Toename van de insuline-afgifte;
- Verlaging van het bloedglucosegehalte;
- Verlaging van het lichaamsgewicht en lichaamsvetgehalte;
- Toename van de insulinegevoeligheid;
- Gunstige beïnvloeding van diverse risicofactoren voor hart- en vaatziekten.

Echter de hierboven beschreven (gunstige) effecten verdwenen enige tijd na het staken van de behandeling met exenatide. Toch heeft het onderzoek beschreven in dit proefschrift laten zien dat in theorie de insuline-afgifte (gemeten als dispositie index) mogelijk blijvend gunstig kan worden beïnvloed met behulp van GLP-1 gerelateerde stoffen zoals exenatide. De in dit proefschrift gebruikte studiepopulatie was echter mogelijk al te lang bekend met DM2. Hierdoor is mogelijk de potentie tot herstel van de insuline-afgifte onvoldoende. Toekomstig onderzoek bij mensen met een voorstadium van DM2 is nodig om te bestuderen of behandeling met exenatide de overgang naar DM2 kan uitstellen of eventueel kan voorkomen. Tevens is toekomstig onderzoek nodig om te bepalen of langdurig gebruik van exenatide aan DM2 gerelateerde complicaties kan voorkomen en veilig is.

List of abbreviations

ACE	Angiotensin converting enzyme
AIR	Acute insulin response
AIRarg	Acute insulin response to arginine
ALT	Alanine aminotransferase
ANCOVA	Analysis of covariance
AST	Aspartate aminotransferase
AUC	Area under the curve
bGS	Beta-cell glucose sensitivity
BID	Twice daily
BMD	Bone mineral density
BMI	Body mass index
BW	Body weight
CI	Confidence interval
CRP	C-reactive protein
CVD	Cardiovascular disease
DCCT	Diabetes control and complications trial
DEXA	Dual energy x-ray absorptiometry
DI	Disposition index
DPP-4	Dipeptidyl peptidase 4
ELISA	Enzyme-linked immunosorbent assay
EMA	European medicines agency
FABP2	Fatty acid binding protein 2
FDA	Food and drug administration
FFA	Free fatty acid
FPG	Fasting plasma glucose
FSIGT	Frequently sampled intravenous glucose tolerance test
GGT	Glutamyltranspeptidase
GI	Gastrointestinal
GIP	Glucose-dependent insulinotropic polypeptide
GLP-1	Glucagon-like peptide-1
GLP-1RA	Glucagon-like peptide-1 receptor agonist
Hb	Hemoglobin
HbA1c	Glycated hemoglobin
HDL	High-density lipoprotein
HMG-CoA	3-hydroxy-3-methylglutaryl-coenzyme A
HMW	High molecular weight
HOMA	Homeostasis Model Assessment
hsCRP	High-sensitive C-reactive protein
iAUC	Incremental area under the curve
IG	Insulin glargine

IL	Interleukin
IQR	Inter quartile range
ISR	Insulin secretion rate
LDL	Low-density lipoprotein
LEAD	Liraglutide effect and action in aiabetes
LS	Least squares
MCP	Monocyte chemotactic protein
MDA	Malondialdehyde
MET	Metformin
mRNA	Messenger ribonucleic acid
N	Number of patients
NA	Not applicable
NAFLD	Non-alcoholic fatty liver disease
NR	Not reported
oxLDL	Oxidized low-density lipoprotein
p-	Plasma
PFR	Potentiation factor ratio
PPAR	Peroxisome proliferator-activated receptors
QD	Once daily
QW	Once weekly
s-	Serum
SCD1	Stearoyl-CoA desaturase-1
SD	Standard deviation
SEM	Standard error of mean
SMBG	Self-monitored blood glucose
SREBP	Sterol regulatory element binding protein
SU	Sulphonylurea
TC	Total cholesterol
TID	Three times daily
TZD	Thiazolidinedione
UKPDS	United Kingdom Prospective Diabetes Study
VLDL	Very-low-density lipoprotein

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Curriculum Vitae

Mathijs Christiaan Michaël Bunck werd op 7 januari 1976 geboren te Amsterdam. Na het behalen van zijn van zijn atheneumdiploma aan het Hervormd Lyceum West te Amsterdam werd hij in september 1994 direct ingeloot voor de studie geneeskunde aan de faculteit der geneeskunde van de hoofdstedelijke Vrije Universiteit. In december 2001 rondde hij zijn co-schappen af en behaalde hij cum laude zijn artsendiploma. Van maart 2002 tot september 2004 werkte hij als arts-assistent en klinisch onderzoeker bij het, door prof. dr. Louis Gooren geleidde, Genderteam van het VU medisch centrum. In september 2004 startte hij met het in dit proefschrif beschreven promotieonderzoek in het Diabetescentrum, VU medisch centrum onder leiding van prof. dr. Michaela Diamant en prof. dr. Robert Heine. Per 1 januari 2010 is hij, onder supervisie van prof. dr. Hanne Meijers-Heijboer, begonnen met de opleiding tot klinisch geneticus in het VU medisch centrum in Amsterdam. Mathijs is sinds 2005 gehuwd met Marjolein Driehuis. Zij wonen, samen met hun Labrador Retreiver Guusje, in Zeist.