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Exploring the role of glucagon in glucose homeostasis

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CHAPTER 1

General introduction and outline of the thesis

GLUCOSE HOMEOSTASIS

The pancreatic islets of Langerhans secrete the reciprocal hormones insulin and glucagon which are responsible for controlling of the glucoregulatory feedback-loop and tightly control blood glucose levels (Figure 1). Insulin is produced by the ß-cells of the pancreas and was first discovered in 1921 by Dr. Frederick Banting and Charles Best. Shortly after the discovery of insulin, glucagon hormone, which is secreted from pancreatic α-cells, was discovered by Kimball and Murlin [1].

INSULIN REGULATES CARBOHYDRATE, PROTEIN AND FAT METABOLISM IN THE BODY

Insulin binds to tyrosine kinase receptors at the cell surface. Insulin inhibits hepatic glucose production and stimulates glycogenesis, the process of glycogen synthesis for storage in liver and muscles. Glucose uptake is increased by insulin, which stimulates the transport of vesicles containing glucose transporters towards the cell membrane. Furthermore, insulin inhibits glucagon secretion from pancreatic α-cells, thereby decreasing hepatic glucose production (gluconeogenesis and glycogenolysis). Adipose tissue is exquisitely sensitive to the inhibitory effect of insulin on lipolysis. Insulin decreases the release of nonesterified fatty acids and glycerol from adipose tissue and gluconeogenic precursors from skeletal muscles, thus causing a decrease in precursor supply for hepatic gluconeogenesis.

GLUCAGON PROTECTS AGAINST HYPOGLYCEMIA

The main role of glucagon is to protect the body and in particular the brain from low glucose levels during periods of fasting. In healthy subjects, glucagon levels are only elevated in the fasting state, because low glucose levels are the most important physiologic stimulator of glucagon secretion. Glucagon mediates its effects by binding to and activating the glucagon receptor (GCGR), a member of the class B family of heptahelical GTP-binding protein coupled receptors [2]. Stimulation of these receptors results in activation of adenylate cylase and increased levels of intracellular cyclic adenosine monophosphate (cAMP). Glucagon receptors are mainly expressed in the liver and kidney with lesser amounts in heart, adipose tissue, adrenal glands, pancreas, cerebral cortex and gastrointestinal tract [2;3]. The role of GCGR in glucose homeostasis has been studied in mice lacking the receptor, which show slightly reduced plasma levels of glucose and insulin. Agonism at the glucagon receptor results in glycogenolysis, gluconeogenesis, proteolysis and lipolysis.

INCRETINS

There are two main incretin hormones in humans, GLP-1 (glucagon-like peptide-1) and GIP (glucose-dependent insulinotropic peptide, also known as gastric inhibitory peptide). Both hormones are secreted by endocrine cells that are located in the epithelium of the small intestine. Food intake, and also stimulation of the sympathetic nervous system (for example physical exercise), stimulate the secretion of GLP-1 and is rapidly inactivated by enzyme DPP-IV (Dipeptidyl peptidase-IV). The mechanism of incretin action is outlined in Figure 2. GLP-1 stimulates the production and secretion of insulin, the release of somatostatin, and glucose utilization by increasing insulin sensitivity. It inhibits glucagon release, gastric emptying, appetite, and food intake via the central nervous system [4].

BALANCE BETWEEN GLUCOSE PRODUCTION AND UTILIZATION

In the postabsorptive state plasma glucose levels are the result the balance between the rates of glucose production and glucose utilization [5]. Each of these processes is tightly regulated by the levels of hormones and substrates in blood. Glucose is produced by both the liver (90%) and the kidneys (10%). The kidneys take up ~10% of the glucose produced, so that in a net sense they do not supply glucose to the other tissues of the body. Therefore, the liver is responsible for providing glucose to both insulin-insensitive (neural tissues, formed elements of the blood, skin, smooth muscle, etc.) and insulin-sensitive (skeletal muscle and fat) tissues. The control of hepatic glucose production (HGP) by the liver serves as a primary regulatory event of glucose homeostasis. In normal physiology the liver maintains blood glucose homeostasis by rapid clearance of glucose from the portal vein in the absorptive state after a meal (glycogenesis), and by controlled production of glucose (gluconeogenesis & glycogenolysis) in the fasted state at a sufficient rate to maintain euglycemia (blood glucose level 4.5-6.5 mmol/L) [6].

DIABETES MELLITUS TYPE 2 (T2DM)

Type 2 diabetes mellitus (T2DM) is a characterized by a combination of resistance to insulin action in target tissues and inadequate compensatory insulin secretion. In the absence of a defect in ß-cell function, individuals can compensate for insulin resistance with appropriate hyperinsulinemia. However, in a later stage of the disease, a decline in pancreatic ß-cell function (relative insulin deficiency) ultimately leads to postprandial and fasting hyperglycemia

that characterizes T2DM. Insulin resistance prolongs the duration of postprandial hyperglycemia, which can be marked when both hepatic and extra-hepatic insulin resistance are present [7]. Studies using hyperinsulinemic euglycemic clamps show impaired suppression of hepatic glucose production by hyperglycemia [8] and by elevated insulin levels [9], indicating that intrahepatic changes in glucose metabolism and in responsiveness to hyperglycemia and insulin contribute to the increase in the hepatic glucose threshold and insulin resistance.

In addition, subjects with T2DM have elevated fasting glucagon concentrations that do not decrease appropriately, and can even paradoxically increase, after food ingestion [10-13]. During fasting conditions in T2DM patients, hyperglucagonemia sustains gluconeogenesis and glycogenolysis in the liver, contributing to increased fasting blood glucose levels [14]. Similarly, increased glucagon responses after food ingestion, result in inadequate suppression of hepatic glucose production, contributing to increased postprandial glucose levels [11]. This paradoxical glucagon response may be explained by an impaired suppressive effect of glucose on the α-cell in T2DM and by gastrointestinal factors as reduced incretin effect observed in patients with T2DM [15]. Knop *et al.* showed attenuated and delayed glucagon suppression in T2DM after oral ingestion of glucose, where intravenous administration of the same amount of glucose results in normal suppression of glucagon, supporting this hypothesis [15].

GLYCAEMIC CONTROL / TREATMENT OF T2DM

The Diabetes Control and Complications Trial [16] and the U.K. Prospective Diabetes Study [17;18] have documented that strict glycemic control effectively reduces the risk of developing microvascular (diabetic nephropathy, neuropathy, and retinopathy) and, to a lesser extent, macrovascular (coronary artery disease, peripheral arterial disease, and stroke) complications of diabetes. Current treatments for T2DM are focused on increasing insulin secretion or improving insulin sensitivity. Lifestyle modifications such as diet, are considered the first line of treatment to halt or delay further progression of the disease [19]. In addition to energy intake restriction, specific food components like amino acids and proteins can be applied to more directly modulate glycemic control. The possibility that substances other than glucose could stimulate insulin secretion was first reported by Cochrane *et al*. in 1956 [20]. Subsequently, many studies have demonstrated that the combined intake of carbohydrate and protein induced a higher insulin response than the intake of carbohydrate alone [21;22], both in healthy subjects [23-25] and in T2DM patients [26-31].

CURRENT INSULIN-BASED THERAPIES

Standard pharmacological, insulin-based, treatment regimens for T2DM are oral blood glucose lowering medication as biguanides, sulfonylureas, and/or thiazolidinediones. In the 1950's, the biguanide drug metformin was introduced for the treatment of diabetes as insulin sensitizer by increasing the efficiency of glucose transporters and lowering glycated hemoglobin (HbA1c) by 1–2% [32]. Gastrointestinal side-effects occur to varying degrees in up to 30% of patients. Sulphonylureas act mainly by stimulating insulin release from the ß-cells of the pancreas and may also improve insulin resistance in peripheral target tissues. These drugs reduce concentrations of HbA1c by 1–2% and fasting plasma glucose (FPG) concentrations by 3.3–3.9 mmol/L [33]. Hypoglycemia is the most worrisome side effect of the sulfonylureas. Thiazolidinediones (TZD), such as pioglitazone, have been associated with a 0.5 to 1.5 % reduction in HbA1c levels and 1.4 to 2.8 mmol/L reductions in FPG levels. TZD's are contraindicated in patients with (a history of) heart failure and should be used with caution in women at high risk of fractures [34]. Currently, TZDs are hardly prescribed any more in the Netherlands. In a more advanced stage of the disease, exogenous subcutaneous insulin therapy or a combination of subcutaneous insulin with oral drugs will be prescribed. Although insulin therapy results in a 1-2% reduction of HbA1c, it is accompanied by weight gain, a significant risk of hypoglycemia and an increased risk of cancer in patients taking long-acting insulin [32].

CURRENT INCRETIN-BASED THERAPIES

Almost 10 years ago, incretin-based therapies (GLP-1 analogs or DPP-IV inhibitors) were introduced on the market for overweight T2DM patients [35]. T2DM patients display an impaired incretin effect and higher GLP-1 levels can be achieved using GLP-1 analogs, GLP-1 receptor agonists produced by recombinant DNA technology or DDP-IV inhibitors which prolong the half-life of endogenous GLP-1 by preventing its enzymatic degradation. In 2005 exenatide was the first FDA (Food and Drug Administration, USA) approved drug that uses the 'incretin effect'. Currently available GLP-1 analogs exenatide and liraglutide have to be administered subcutaneously; exenatide twice daily and liraglutide once a day. Recently, exenatide has been developed in an extended-release formulation which can be used once weekly. Clinical trials with exenatide [36-39] showed significant reductions in HbA1c of approximately 1.0–1.2% when compared to placebo, and a modest reduction in fasting plasma glucose (FPG) of approximately 1.0–1.4 mM. The average weight loss amounted to 1.6 kg in the exenatide-treated groups. Liraglutide significantly lowered HbA1c by 0.8–1.5%, FPG with up to 2.6 mM, and induced a weight loss in the range of 2 to 3 kg compared to the placebo-treated group [39-41]. In LEAD 6 study both available

GLP-analogs were compared [42]. The mean reduction in HbA1c levels was significantly higher with liraglutide 1.8 mg once daily than with exenatide 10 mg twice daily (−1.12% versus −0.79%; p<0.001). The most common adverse events associated with GLP-1 mimetics are gastrointestinal. Safety concerns have been raised during the development of liraglutide; a small number of cases of pancreatitis have been reported [43], while the initial concerns regarding thyroid C-cell tumors have not been confirmed in humans [44]. In contrast to GLP-1 receptor agonists, DPP-IV inhibitors are orally available and have a longer duration of action, requiring only once daily dosing. Sitagliptin, vildagliptin, saxagliptine, and linagliptine as monotreatment and also combined with metformin are currently available in the Netherlands. These drugs control hyperglycemia, reduce HbA1c concentrations by ~1%, and improve pancreatic ß-cell function. DPP-IV inhibitors are generally safe and well-tolerated with a low risk of hypoglycemia, but do not reduce appetite or cause weight loss such as GLP-1 agonists [45]. The long-term safety and effects of GLP-1 analogs and DPP-IV inhibitors have not been established yet.

SGLT2 INHIBITORS IN THE TREATMENT OF TYPE 2 DIABETES

Agents that inhibit sodium glucose co-transporter 2 (SGLT2) in the kidney represent a novel class of drugs, which has become available for treatment of T2DM since 2012. The SGLT2 transporter protein is found only in renal epithelium cells of the proximal tubule, and mediates the majority (~90%) of glucose reabsorption along the nephron. Pharmacological inhibition of SGLT2 increases urinary glucose excretion and decreases plasma glucose levels in an insulin-independent manner [46]. Hypoglycemic episodes are less likely, because of the insulin independence of their action plus the fact that these compounds only lower the glucose re-absorption threshold without completely blocking renal glucose reabsorption. In November 2012 the first SGLT2 inhibitor dapagliflozine was introduced on the market in Europe, followed by canagliflozine in September 2013. In the USA canagliflozin became the first SGLT2 inhibitor (approved March 2013), followed by dapagliflozine which was approved in January 2014 by the FDA. Clinical trials on these two agents have shown significant and sustained HbA1c reduction of 0.5-1% when used as monotherapy or in combination with other antidiabetic agents [47]. Comparing other antidiabetic drugs, the major disadvantage of SGLT2 inhibitors is the increased risk of genital mycotic infections and urinary tract infections, particularly in women. Additionally, since the efficacy of SGLT2 inhibitors requires adequate filtered load of glucose in the kidney, their efficacy diminishes in renal impairment. Multiple other SGLT2 inhibitors are currently in clinical development such as small molecules empagliflozin, ipragliflozin, LX4211 (a novel dual inhibitor of SGLT1 and SGLT2 glucose transporters), and antisense oligonucleotide targeting the human SGLT2 transporter [48].

NOVEL MECHANISM(S) OF ACTION FOR THE TREATMENT OF DIABETES TYPE 2

Despite all currently available pharmacological therapies, treatment of patients with diabetes is not completely successful in restoring control of glucose metabolism. Thus, there remains a need for agents with novel mechanism(s) of action. Nowadays, there is more attention to the role of liver in the pathogenesis of diabetes, reduction of hepatic glucose production has been targeted as a strategy for diabetes treatment and can be achieved through counteracting the action of glucagon. Attenuation of the action of glucagon could be a viable therapeutic strategy for T2DM in combination with insulin-based antidiabetic drugs that are currently on the market (combined targeting of two sites within the liver).

GLUCAGON RECEPTOR ANTAGONISTS

Pharmacological antagonism of glucagon action may be a potential therapeutic approach for T2DM. Different mechanisms to lower glucagon levels are: inhibition of glucagon secretion, GCGR receptor blockers and (antisense) inhibition of GCGR expression. Peptide antagonists and monoclonal antibodies against the GCGR attenuated hyperglycemia in animal models [49-51], suggesting a potential to treat hyperglycemia in T2DM through the inhibition of glucagon function. Peptides were the earliest GCGR antagonists that were designed by structural modification of the native hormone [52] . However, oral bioavailability and long half-life appeared to be hurdles that have not been overcome. The development of small molecules against the GCGR has not been very successful due to limited drug selectivity, cross-species differences and lack of sustained effects after non-competitive blockade [53]. Only one phase 1 study has been published describing the acute effects of Bay 27-9955, a small molecule glucagon receptor inhibitor [54] that blunted hyperglucagonemia-induced hyperglycemia. However, long-term antidiabetic benefits, as well as side effects, of this compound in patients with T2DM remain unknown.

ANTISENSE THERAPY

Antisense oligonucleotides (ASOs) as therapeutical agents are relatively new, and only one antisense compound has been approved by the FDA and European authorities until now [55]. Many antisense compounds are currently in preclinical or clinical development phases. ASOs are short, single-stranded molecules which are complementary to a target messenger ribonucleic acid (mRNA). Upon Watson-Crick hybridization with their target mRNA, ASOs inhibit

translation through the activation of endogenous RNase H enzymes and other mechanisms, for example via alternative splicing (Figure 3) [56].

CLINICAL USE OF ANTISENSE OLIGONUCLEOTIDES (ASOS)

For clinical use of ASOs, chemical modified ASOs were used. The first generation oligonucleotides contain a phosphorothioate modification to protect the molecule from rapid degradation by nucleases. Second-generation 2'-O-methyl (2'-OME) and 2'-methoxyethyl (2'-MOE) oligonucleotides were developed to further increase nuclease resistance, thereby improving pharmacokinetics and to increase target affinity. Third-generation oligonucleotides represent a heterogeneous group of ASOs that are most often DNA and RNA analogs with modified phosphate linkages or riboses. These modifications also lead to improved nuclease resistance, affinity and pharmacokinetics. The different classes of ASOs have different toxicological, pharmacokinetic and pharmacological properties [57;58]. Studies with species-specific GCGR antisense drugs in rodent models of T2DM have demonstrated selective inhibition of hepatic and adipose tissue glucagon receptor expression and normalization of blood glucose levels without development of hypoglycemia or weight gain [59;60]. In addition to hepatic effects, in preclinical studies glucagon receptor antisense therapy increased the levels of active GLP-1 levels and improved pancreatic beta cell function [60].

In conclusion, the pathophysiology of T2DM is characterized not only by insulin resistance and ß-cell dysfunction, but also with elevated fasted and postprandial plasma glucagon levels. It has been suggested that the diabetic α-cell exhibits a reduced glucose sensitivity and/or insulin resistance. The overall aim of this thesis was to gain more insight in the role of glucagon in glucose homeostasis in health and disease, and to explore glucagon antagonism as therapeutic potential for the treatment of T2DM.

OUTLINE OF THIS THESIS

This thesis is comprised of a variety of human studies designed to investigate the role of glucagon in glucose homeostasis in health and disease.

CHAPTER 2 studied the effects of a single protein hydrolysate meal replacement (insuVida™) on postprandial serum glucose, insulin and glucagon levels in patients with type 2 diabetes.

The aim of the study in CHAPTER 3 was to characterize the applicability of the glucagon challenge test as a tool in diabetes research, by assessing the inter- and intra-individual variabilities of the glucagon challenge test and investigating the activity of the autonomic nervous system (ANS) during the challenge, as this might have an indirect impact on glucose homeostasis.

Furthermore, we determined whether human adipose tissue expresses glucagon receptor MRNA.

In CHAPTER 4 the effects of a glucagon challenge in T2DM patients were explored. A stable isotope glucose tracer technique was applied to determine hepatic glucose production. The influence of oral antidiabetic drugs on the response to hyperglucagonemia was investigated by using a cross-over study design. We compared the glucagon challenge data of healthy volunteers with T2DM.

Increased fasting and post-meal glucagon concentrations cause excessive hepatic glucose production (HGP) in patients with type 2 diabetes, suggesting that attenuation of hepatic glucagon action could be a promising therapeutic strategy for T2DM. CHAPTER 5 shows the results of the phase I double-blind, placebo-controlled, dose-escalation study, which evaluated the safety, tolerability, PK and pharmacodynamics of single and multiple dose administrations of placebo or antisense glucagon receptor antagonist (ISIS 325568) at 4 dose levels in healthy subjects. In the multiple dose cohorts at each dose level, 8 subjects received 8 doses over 6-weeks (3 IV doses in week 1 followed by 5 weekly SC doses) and underwent a glucagon challenge procedure (glucagon infusion that doubled both plasma glucagon and glucose levels) at baseline and at the end of 6-week treatment.

CHAPTER 6 describes the development of a semi-mechanistic model simultaneously describing glucagon, plasma glucose, insulin and glucagon receptor internalization. This model was build using data from our glucagon challenge study in healthy volunteers (chapter 3).

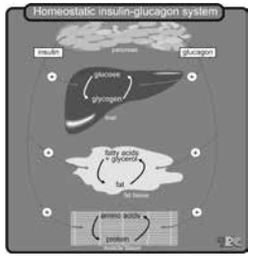
Finally, CHAPTER 7 combines the results and conclusions from the previous chapters and places these in a broader perspective. The role of glucagon in glucose homeostasis in health and disease is discussed and suggestions for future research are given.

FIGURE 1 Homeostatic insulin-glucagon system
Diagram of the homeostatic (insulin-glucagon) system. Insulin secretion is stimulated by high blood glucose levels and glucagon is stimulated by low blood glucose levels.

FIGURE 2 Enteroinsulinar axis
After food intake (1) the complex sugars are broken down in the small intestine into glucose molecules. L-cells in the distal intestinal wall secrete the incretin GLP-1 upon stimulation by food/glucose (2). This glucagon-like peptide-1 is released proportional to the amount of food post-prandial in the distal intestine. GLP-1 is key player in many processes after a meal. It stimulates the release of insulin by the ß-cells in the pancreas (3). Similarly, GLP-1 inhibits the release of glucagon by the α-cells (4). The rise in insulin will stimulate the glucose uptake from the blood into the cells (8). Further, GLP-1 inhibits gastric emptying (5) and induces the feeling of satiety (6) in order to reduce further carbohydrate intake. GLP-1 is metabolised by the enzyme dipeptidyl peptidase 4 (DDP-IV) which is present in the cell wall (7).

FIGURE 3 Antisense oligonucleotides
RNase H-dependent antisense mechanism. Single-stranded oligonucleotides are transported across the plasma membrane, by either poorly characterized natural processes or by the use of facilitators such as cationic lipids (step 1). Once in the cytoplasm, single-stranded oligonucleotides rapidly accumulate in the cell nucleus (steps 2 and 3), where they bind to their targeted RNA (step 4). Once bound to the RNA, RNase H recognizes the oligonucleotide/RNA duplex as a substrate, cleaving the RNA strand and releasing the antisense oligonucleotide (step 5). Although the cleavage of the RNA by RNase H is shown to occur in the nucleus, RNase H is also present in the cytosol, allowing for cleavage to occur in that cellular compartment as well.

See inside of the backcover for the figures in full colour.



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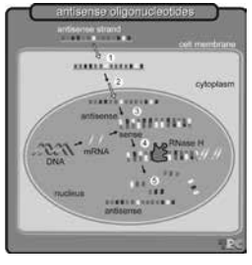

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REFERENCES

1 Dunning BE, Gerich JE: The role of alpha-cell dysregulation in fasting and postprandial hyperglycemia in type 2 diabetes and therapeutic implications. Endocr Rev 2007;28:253-283.
2 Gelling RW, Du XQ, Dichmann DS, Romer J, Huang H, Cui L, Obici S, Tang B, Holst JJ, Fledelius C, Johansen PB, Rossetti L, Jelicks LA, Serup P, Nishimura E, Charron MJ: Lower blood glucose, hyperglucagonemia, and pancreatic alpha cell hyperplasia in glucagon receptor knockout mice. Proc Natl Acad Sci U S A 2003;100:1438-1443.
3 Svoboda M, Tastenoy M, Vertongen P, Robberecht P: Relative quantitative analysis of glucagon receptor mRNA in rat tissues. Mol Cell Endocrinol 1994;105:131-137.
4 Drucker DJ, Nauck MA: The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. Lancet 2006;368:1696-1705.
5 Cherrington AD: Banting Lecture 1997. Control of glucose uptake and release by the liver in vivo. Diabetes 1999;48:1198-1214.
6 Agius L: New hepatic targets for glycaemic control in diabetes. Best Pract Res Clin Endocrinol Metab 2007;21:587-605.
7 Rizza RA: Pathogenesis of fasting and postprandial hyperglycemia in type 2 diabetes: implications for therapy. Diabetes 2010;59:2697-2707.
8 Mevorach M, Giacca A, Aharon Y, Hawkins M, Shamoon H, Rossetti L: Regulation of endogenous glucose production by glucose per se is impaired in type 2 diabetes mellitus. J Clin Invest 1998;102:744-753.
9 Basu R, Basu A, Johnson CM, Schwenk WF, Rizza RA: Insulin dose-response curves for stimulation of splanchnic glucose uptake and suppression of endogenous glucose production differ in nondiabetic humans and are abnormal in people with type 2 diabetes. Diabetes 2004;53:2042-2050.
10 Shah P, Basu A, Basu R, Rizza R: Impact of lack of suppression of glucagon on glucose tolerance in humans. Am J Physiol 1999;277:E283-E290.
11 Shah P, Vella A, Basu A, Basu R, Schwenk WF, Rizza RA: Lack of suppression of glucagon contributes to postprandial hyperglycemia in subjects with type 2 diabetes mellitus. J Clin Endocrinol Metab 2000;85:4053-4059.
12 Butler PC, Rizza RA: Contribution to postprandial hyperglycemia and effect on initial splanchnic glucose clearance of hepatic glucose cycling in glucose-intolerant or NIDDM patients. Diabetes 1991;40:73-81.
13 Mitrakou A, Kelley D, Veneman T, Jenssen T, Pangburn T, Reilly J, Gerich J: Contribution of abnormal muscle and liver glucose metabolism to postprandial hyperglycemia in NIDDM. Diabetes 1990;39:1381-1390.
14 Reaven GM, Chen YD, Golay A, Swislocki AL, Jaspan JB: Documentation of hyperglucagonemia throughout the day in nonobese and obese patients with noninsulin-dependent diabetes mellitus. J Clin Endocrinol Metab 1987;64:106-110.
15 Knop FK, Vilsboll T, Madsbad S, Holst JJ, Krarup T: Inappropriate suppression of glucagon during OGTT but not during isoglycaemic i.v. glucose infusion contributes to the reduced incretin effect in type 2 diabetes mellitus. Diabetologia 2007;50:797-805.
16 The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. The Diabetes Control and Complications Trial Research Group: N Engl J Med 1993;329:977-986.
17 Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). UK Prospective Diabetes Study (UKPDS) Group: Lancet 1998;352:854-865.
18 Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group: Lancet 1998;352:837-853.
19 Carroll MF, Gutierrez A, Castro M, Tsewang D, Schade DS: Targeting postprandial hyperglycemia: a comparative study of insulinotropic agents in type 2 diabetes. J Clin Endocrinol Metab 2003;88:5248-5254.
20 Cochrane WA, Payne WW, Simpkiss MJ, Woolf LI: Familial hypoglycemia precipitated by amino acids. J Clin Invest 1956;35:411-422.
21 Pallotta JA, Kennedy PJ: Response of plasma insulin and growth hormone to carbohydrate and protein feeding. Metabolism 1968;17:901-908.
22 Rabinowitz D, Merimee TJ, Maffezzoli R, Burgess JA: Patterns of hormonal release after glucose, protein, and glucose plus protein. Lancet 1966;2:454-456.
23 Calbet JA, MacLean DA: Plasma glucagon and insulin responses depend on the rate of appearance of amino acids after ingestion of different protein solutions in humans. J Nutr 2002;132:2174-2182.
24 Claessens M, Calame W, Siemensma AD, van Baak MA, Saris WH: The effect of different protein hydrolysate/carbohydrate mixtures on postprandial glucagon and insulin responses in healthy subjects. Eur J Clin Nutr 2009;63:48-56.
25 van Loon LJ, Saris WH, Verhagen H, Wagenmakers AJ: Plasma insulin responses after ingestion of different amino acid or protein mixtures with carbohydrate. Am J Clin Nutr 2000;72:96-105.
26 Frid AH, Nilsson M, Holst JJ, Bjorck IM: Effect of whey on blood glucose and insulin responses to composite breakfast and lunch meals in type 2 diabetic subjects. Am J Clin Nutr 2005;82:69-75.
27 Gannon MC, Nuttall FQ, Saeed A, Jordan K, Hoover H: An increase in dietary protein improves the blood glucose response in persons with type 2 diabetes. Am J Clin Nutr 2003;78:734-741.
28 Manders RJ, Wagenmakers AJ, Koopman R, Zorenc AH, Menheere PP, Schaper NC, Saris WH, van Loon LJ: Co-ingestion of a protein hydrolysate and amino acid mixture with carbohydrate improves plasma glucose disposal in patients with type 2 diabetes. Am J Clin Nutr 2005;82:76-83.
29 Manders RJ, Koopman R, Sluijsmans WE, van den Berg R, Verbeek K, Saris WH, Wagenmakers AJ, van Loon LJ: Co-ingestion of a protein hydrolysate with or without additional leucine effectively reduces postprandial blood glucose excursions in Type 2 diabetic men. J Nutr 2006;136:1294-1299.

30 Nuttall FQ, Mooradian AD, Gannon MC, BillinGTon C, Krezowski P: Effect of protein ingestion on the glucose and insulin response to a standardized oral glucose load. Diabetes Care 1984;7:465-470.
31 van Loon LJ, Kruijshoop M, Menheere PP, Wagenmakers AJ, Saris WH, Keizer HA: Amino acid ingestion strongly enhances insulin secretion in patients with long-term type 2 diabetes. Diabetes Care 2003;26:625-630.
32 Nicholson G, Hall GM: Diabetes mellitus: new drugs for a new epidemic. Br J Anaesth 2011;107:65-73.
33 Luna B, Feinglos MN: Oral agents in the management of type 2 diabetes mellitus. Am Fam Physician 2001;63:1747-1756.
34 Home PD, Pocock SJ, Beck-Nielsen H, Curtis PS, Gomis R, Hanefeld M, Jones NP, Komajda M, McMurray JJ: Rosiglitazone evaluated for cardiovascular outcomes in oral agent combination therapy for type 2 diabetes (RECORD): a multicentre, randomised, open-label trial. Lancet 2009;373:2125-2135.
35 Nauck MA, Vilsboll T, Gallwitz B, Garber A, Madsbad S: Incretin-based therapies: viewpoints on the way to consensus. Diabetes Care 2009;32 Suppl 2:S223-S231.
36 Buse JB, Henry RR, Han J, Kim DD, Fineman MS, Baron AD: Effects of exenatide (exendin-4) on glycemic control over 30 weeks in sulfonylurea-treated patients with type 2 diabetes. Diabetes Care 2004;27:2628-2635.
37 DeFronzo RA, Ratner RE, Han J, Kim DD, Fineman MS, Baron AD: Effects of exenatide (exendin-4) on glycemic control and weight over 30 weeks in metformin-treated patients with type 2 diabetes. Diabetes Care 2005;28:1092-1100.
38 Kendall DM, Riddle MC, Rosenstock J, Zhuang D, Kim DD, Fineman MS, Baron AD: Effects of exenatide (exendin-4) on glycemic control over 30 weeks in patients with type 2 diabetes treated with metformin and a sulfonylurea. Diabetes Care 2005;28:1083-1091.
39 Lund A, Knop FK, Vilsboll T: Glucagon-like peptide-1 receptor agonists for the treatment of type 2 diabetes: Differences and similarities. Eur J Intern Med 2014.
40 Peters KR: Liraglutide for the treatment of type 2 diabetes: a clinical update. Am J Ther 2013;20:178-188.
41 Rigato M, Fadini GP: Comparative effectiveness of liraglutide in the treatment of type 2 diabetes. Diabetes Metab Syndr Obes 2014;7:107-120.
42 Buse JB, Rosenstock J, Sesti G, Schmidt WE, Montanya E, Brett JH, Zychma M, Blonde L: Liraglutide once a day versus exenatide twice a day for type 2 diabetes: a 26-week randomised, parallel-group, multinational, open-label trial (LEAD-6). Lancet 2009;374:39-47.
43 Lee PH, Stockton MD, Franks AS: Acute pancreatitis associated with liraglutide. Ann Pharmacother 2011;45:e22.
44 Parks M, Rosebraugh C: Weighing risks and benefits of liraglutide --the FDA's review of a new antidiabetic therapy. N Engl J Med 2010;362:774-777.
45 Pratley RE, Salsali A: Inhibition of DPP-4: a new therapeutic approach for the treatment of type 2 diabetes. Curr Med Res Opin 2007;23:919-931.
46 Gerich JE: Role of the kidney in normal glucose homeostasis and in the hyperglycemia of diabetes mellitus: therapeutic implications. Diabet Med 2010;27:136-142.
47 Hasan FM, Alsahli M, Gerich JE: Invited review. SGLT2 inhibitors in the treatment of type 2 diabetes. Diabetes Research and Clinical Practice 2014;Accepted 19 February 2014. Epub.
48 Zanardi TA, Han SC, Jeong EJ, Rime S, Yu RZ, Chakravarty K, Henry SP: Pharmacodynamics and subchronic toxicity in mice and monkeys of ISIS 388626, a second-generation antisense oligonucleotide that targets human sodium glucose cotransporter 2. J Pharmacol Exp Ther 2012;343:489-496.
49 Brand CL, Rolin B, Jorgensen PN, Svendsen I, Kristensen JS, Holst JJ: Immunoneutralization of endogenous glucagon with monoclonal glucagon antibody normalizes hyperglycemia in moderately streptozotocin-diabetic rats. Diabetologia 1994;37:985-993.
50 Potterat O, Wagner K, Gemmecker G, Mack J, Puder C, Vettermann R, Streicher R: BI-32169, a bicyclic 19-peptide with strong glucagon receptor antagonist activity from Streptomyces sp. J Nat Prod 2004;67:1528-1531.
51 Yan H, Gu W, Yang J, Bi V, Shen Y, Lee E, Winters KA, Komorowski R, Zhang C, Patel JJ, Caughey D, Elliott GS, Lau YY, Wang J, Li YS, Boone T, Lindberg RA, Hu S, Veniant MM: Fully human monoclonal antibodies antagonizing the glucagon receptor improve glucose homeostasis in mice and monkeys. J Pharmacol Exp Ther 2009;329:102-111.
52 Hruby VJ: Designing peptide receptor agonists and antagonists. Nat Rev Drug Discov 2002;1:847-858.
53 McCormack JG, Westergaard N, Kristiansen M, Brand CL, Lau J: Pharmacological approaches to inhibit endogenous glucose production as a means of antidiabetic therapy. Curr Pharm Des 2001;7:1451-1474.
54 Petersen KF, Sullivan JT: Effects of a novel glucagon receptor antagonist (Bay 27-9955) on glucagon-stimulated glucose production in humans. Diabetologia 2001;44:2018-2024.
55 Roehr B: Fomivirsen approved for CMV retinitis. J Int Assoc Physicians AIDS Care 1998;4:14-16.
56 Crooke ST: Progress in antisense technology. Annu Rev Med 2004;55:61-95.
57 Chan JH, Lim S, Wong WS: Antisense oligonucleotides: from design to therapeutic application. Clin Exp Pharmacol Physiol 2006;33:533-540.
58 Kurreck J: Antisense technologies. Improvement through novel chemical modifications. Eur J Biochem 2003;270:1628-1644.
59 Liang Y, Osborne MC, Monia BP, Bhanot S, Gaarde WA, Reed C, She P, Jetton TL, Demarest KT: Reduction in glucagon receptor expression by an antisense oligonucleotide ameliorates diabetic syndrome in db/db mice. Diabetes 2004;53:410-417.
60 Sloop KW, Cao JX, Siesky AM, Zhang HY, Bodenmiller DM, Cox AL, Jacobs SJ, Moyers JS, Owens RA, Showalter AD, Brenner MB, Raap A, Gromada J, Berridge BR, Monteith DK, Porksen N, McKay RA, Monia BP, Bhanot S, Watts LM, Michael MD: Hepatic and glucagon-like peptide-1-mediated reversal of diabetes by glucagon receptor antisense oligonucleotide inhibitors. J Clin Invest 2004;113:1571-1581.