Charles University in Prague
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The latest advances in diabetes mellitus treatment

Diploma thesis

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Acknowledgments

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I, Katerina Hadjiyiangou, declare that this thesis is my original copyrighted work. All literature and other resources I used while processing are listed in the bibliography and properly cited.

Abbreviations:

BMI: Body mass index

CSII: Continuous subcutaneous insulin infusion

DM: Diabetes Mellitus

DPP-4: Dipeptidyl peptidase-4

FDA: Food and Drug Administration

GAD: Glutamic acid decarboxylase

GIP: Gastric inhibitory peptide

GLP-1: Glucagon like peptide-1

HbA1C: Glycated hemoglobin

MDI: Multiple daily injections

MET: Metformin

PP cells: Pancreatic polypeptide cells

SFUs: Sulfonylureas

T1DM: Type 1 Diabetes Mellitus

T2DM: Type 2 Diabetes Mellitus

TZDS: Thiazolidinediones

WHO: World Health Organisation

English abstract

Diabetes mellitus (DM) is the best known endocrine disease of the pancreas where the incidence of the affected individuals is increasing rapidly worldwide. It is caused by defects in both the pancreatic islets and in the pancreas to produce enough insulin resulting in impaired glucose homeostasis leading to higher than normal glucose levels in the blood (hyperglycaemia) which is considered to be the hallmark of diabetes. Consequently the main aim of diabetes management is to monitor the glycaemic status. Diabetes mellitus can be subdivided into two classes, type 1 diabetes mellitus (T1DM) which is also known as insulin-dependent diabetes mellitus and type 2 diabetes mellitus (T2DM) which is known as non-insulin-dependent diabetes mellitus. Management of either type of DM requires a number of lifestyle modifications such as increased exercise and decreased weight with the intention of improving metabolic control and enhancing the quality of life. Unfortunately, the majority of patients will eventually require administration of antidiabetic drugs.

The present study was performed with the intention of reviewing the currently available scientific literature about both types of DM and discuss the current and novel approaches of treatment, thus giving more emphasis on the novel advances. For the purposes of the study, we searched Medline, Pubmed, Google Scholar and the online library of Charles University to identify English language original and review articles about DM and the novel approaches of its treatment.

Well-established antidiabetic agents used for many years include sulphonylureas, thiazolidinediones, biguanides and alpha-glucosidase inhibitors which act mainly by stimulating and improving insulin secretion and associated action. Novel combinations and ways of insulin administration have been developed including oral and inhaled insulin in order to replace the need for frequent injections which complicate patients' life. Moreover much attention has been given to the incretin system as an alternative treatment option including both GLP-1 receptor agonists (exenatide, liraglutide) and DPP-4 inhibitors (saxagliptin, sidagliptin, vildagliptin).

Increased understanding of the pathophysiology of DM provided a narrow window of potential alternative therapies with the aim of eliminating the requirement of exogenous insulin administration and also reversing or preventing the development of immune-mediated

response that is observed in T1DM. These strategies include islet cell transplantation, pancreas transplantation, gene therapy, stem cells and also a number of different drugs as potential compounds for the prevention of diabetes such as oral insulin, abatacept, otelixizumab and glutamic acid decarboxylase which are currently in different phases of clinical trials.

Taking together all of our findings, it can be concluded that in order to have the best management for diabetes, there is a need for a close collaboration between scientists, clinicians and patients. Additionally, continued clinical trials need to be carried out with the intention of developing novel compounds which will be able to provide the best-long-term efficacy and safety profile, lowering at the same time the possible risk of hypoglycaemia and also to avoid the unwanted side effects observed with the currently existing antidiabetic drugs.

Český abstrakt

Diabetes mellitus (DM) je nejznámější endokrinní choroba pankreatu s celosvětově stoupající incidencí. Je způsobena poruchou pankreatických ostrůvků a pankreatu při tvorbě dostatečného množství inzulínu, což má za následek abnormální zvýšení hladiny glukózy v krvi (hyperglykémii), která je považována za známku diabetu. V důsledku toho je hlavním úkolem léčby diabetu sledování stavu glykémie. Onemocnění diabetes mellitus můžeme dělit na dvě skupiny, diabetes mellitus 1. typu (T1DM), který je rovněž znám jako diabetes mellitus závislý na inzulínu, a diabetes mellitus 2. typu (T2DM), který je znám jako diabetes mellitus nezávislý na inzulínu. Léčba obou typů DM vyžaduje mnoho změn v životním stylu jako je zvýšení tělesné aktivity a snížení váhy za účelem zlepšení metabolické kontroly a kvality života. Většina pacientů však nakonec vyžaduje terapii antidiabetickými léky.

Předložená studie byla vypracována se záměrem podat přehled současně dostupné vědecké literatury týkající se obou typů DM a prodiskutovat současné a nové přístupy k léčbě, přičemž se snaží o zdůraznění nových metod. Za účelem vypracování této studie jsme procházeli Medline, Pubmed, Google Scholar a elekronickou knihovnu Univerzity Karlovy, abychom sledovali anglicky psané původní studie a přehledné články o DM a nové přístupy k jeho léčbě.

Zavedené antidiabetické léky užívané po mnoho let zahrnují deriváty sulphonylurey, thiazolidindiony, biguanidy a inhibitory alfa-glukosidázy, které působí hlavně tím, že stimulují a zlepšují sekreci inzulínu a přidružené pochody. Byly vyvinuty nové kombinace a způsoby podávání inzulínu včetně aplikace perorální a inhalace inzulínu, aby byla nahrazena nutnost častých injekcí, které komplikují pacientův život. Nadto byla věnována pozornost inkretinovému systému jako alternativnímu způsobu léčby, která zahrnuje– jak agonisty receptorů GLP-1 (exenatid, liraglutid), tak inhibitory DPP-4 (saxagliptin, sidagliptin, vildagliptin).

Hlubší pochopení patofyziologie DM poskytlo přesnější pohled na potenciální způsoby alternativní terapie s cílem eliminovat nezbytnost exogenního podání inzulínu a rovněž pomoci zvrátit nebo znemožnit rozvoj imunitní odpovědi, která je pozorována u DM 1. typu. Tato strategie zahrnuje transplantaci buněk ostrůvků, transplantaci pankreatu, genovou terapii, kmenové buňky a také mnoho různých léků jako potenciální prostředků v prevenci diabetu,

jako jsou perorální inzulín, abatacept, otelixizumab a dekarboxyláza kyseliny glutamové, které jsou současně v různých fázích klinických zkoušek.

Po shrnutí všech výsledků našeho výzkumu je možné závěrem říci, že k dosažení optimální léčby diabetu je třeba úzké spolupráce mezi vědeckými pracovníky, klinickými lékaři a pacienty. Pokračující klinické zkoušky musí být navíc uskutečňovány s cílem vyvíjet nové látky, které by měly nejlepší dlouhodobou účinnost a bezpečnost tím, že by snižovaly případné riziko hypoglykémie a vyhnuly se zároveň nežádoucím vedlejším účinkům pozorovaným u antidiabetik užívaných v současné době.

1. Introduction

1.1. The pathophysiology of the endocrine pancreas

The pancreas is a complex digestive gland organ and as indicated on Figure 1 is located behind the stomach next to the small intestine.

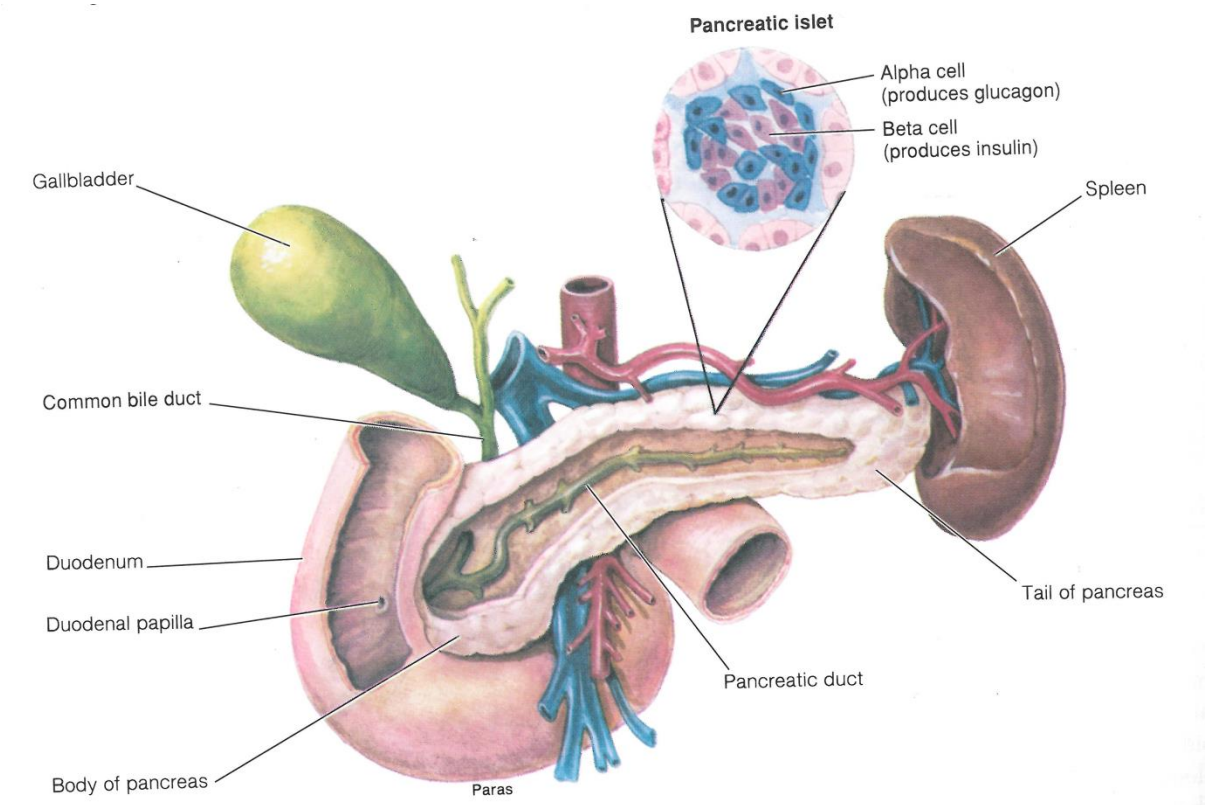


Figure 1: The pancreas

The pancreas is a complex digestive gland organ, located behind the stomach next to the small intestine (Figure adapted from: Graaf 1997)

This organ is made up from a combination of two glands: the exocrine and the endocrine pancreas (Figure 2) which secrete a number of different pancreatic cells both into the intestine and the bloodstream respectively (Lumelsky, Blondel et al. 2001, Zaret and Grompe 2008, Courtney, Pfeifer et al. 2011). These secretions are of major importance for the execution of different processes including control of body metabolism, digestion and glucose homeostasis (Zaret and Grompe 2008, Chandra and Liddle 2011).

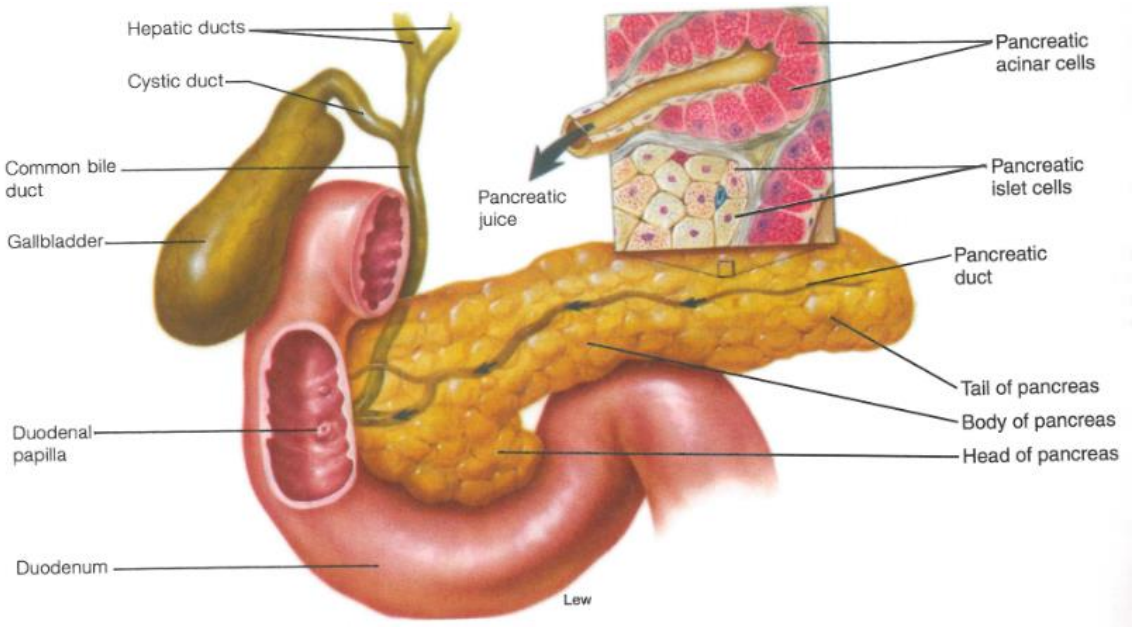


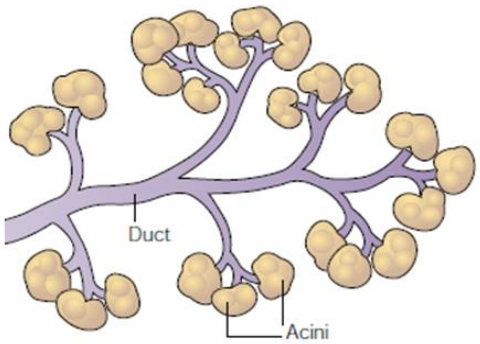
Figure 2: The pancreas

The pancreas is a combination of two glands: the exocrine and the endocrine pancreas whose main function is to secrete substances into the intestine and bloodstream, respectively (Graaf 1997, Weiss, Halangk et al. 2008).

(Figure adapted from: (Graaf 1997)).

During a meal, the exocrine pancreas is responsible for food digestion while at the same time the endocrine pancreas indicates to the body that the food is already digested and guided to other parts of the body (Bardeesy and DePinho 2002, Chanclon, Martinez-Fuentes et al. 2012).

Acinar cells and ductal cells (Figure 3) are the two major types of cells comprising the exocrine pancreas having as their main function the secretion and transportation of substances into the intestine and particularly into the duodenum (Bardeesy and DePinho 2002, Weiss, Halangk et al. 2008, Zaret and Grompe 2008).



(Adapted from: Bardeesy and DePihno, 2002)

Figure 3: The exocrine pancreas

The exocrine pancreas consists of 2 main cell types: the acinar cells and the ductul cells whose main function is to secrete and transport substances into the intestine

On the other hand, the endocrine pancreas is composed from 5 cell types (α, β, δ, ε and pancreatic polypeptide (PP) cells) which consist the islets of Langerhan and represent only approximately 1-2% of the whole pancreatic mass (Weiss, Halangk et al. 2008, Bramswig and Kaestner 2011). These cells synthesize a number of different hormones: α cells secrete glucagon and β cells secrete insulin, while α cells cooperate with β cells for regulating glucose (Bardeesy and DePinho 2002, Woods, Lutz et al. 2006). Aside from insulin, β cells co-secrete another hormone named amylin responsible for reducing food intake and gastric emptying while at the same time inhibits both pancreatic glucagon and enzyme secretion (Woods, Lutz et al. 2006). δ cells secrete the hormone somatostatin and PP cells secrete pancreatic polypeptide (Bardeesy and DePinho 2002, Woods, Lutz et al. 2006).

1.1.1. Insulin-regulation of secretion and mechanism of action

As aforementioned, the endocrine pancreas and particularly the β cells secrete the insulin hormone into the bloodstream in order to regulate glucose levels (Bardeesy and DePinho 2002, Woods, Lutz et al. 2006). Insulin is a principal hormone responsible for controlling metabolism in the liver, muscles and fatty tissue and under normal pancreatic function, circulates into the bloodstream where it stimulates glucose transport across the cell membrane (Oliveira, Lages et al. 2011) and glucose uptake by muscle cells in order to use it for energy (Goutham 2001, Vendelbo, Clasen et al. 2012). Additionally, some other major actions of this potent anabolic hormone include: the uptake of glucose, amino acids and fatty acids into the cells, inhibition of either the expression or activity of enzymes

that catalyze degradation, and also the increase in the expression or activity of enzymes which are responsible for catalyzing glycogen, lipid and protein synthesis. Another important action of insulin is the promotion of synthesis and storage of carbohydrates, lipids and proteins while at the same time it also inhibits both of their degradation and release into the circulation.

In many cases though, due to abnormal pancreatic function insulin fails to stimulate normal glucose uptake and the tissues fail to respond properly to the action of insulin (Goutham 2001, McClain, Lubas et al. 2002). Subsequently glucose levels into the bloodstream will significantly increase while at the same time in order to compensate for resistance the pancreas will keep producing more and more insulin (Goutham 2001). This phenomenon is known as insulin resistance and modern lifestyle is the commonest cause. Particularly, as indicated in Figure 4, increased nutrient supply resulting in overweight and obesity in combination with reduced physical activity causes increased production of hepatic glucose and reduced peripheral glucose uptake which are the basis of impaired insulin secretion, impaired glucose tolerance and subsequently insulin resistance (Reaven 1988, Stumvoll, Goldstein et al. 2005, Kahn, Hull et al. 2006, Prentki and Nolan 2006).

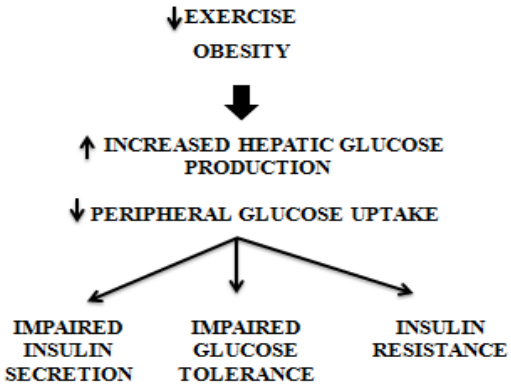


Figure 4: Insulin resistance

Reduced physical activity in combination to obesity cause increased hepatic glucose production and reduced peripheral glucose uptake. This results in impaired insulin secretion, impaired glucose tolerance and finally in insulin resistance.

1.2. Diabetes Mellitus

Diabetes represents the best known endocrine disease of the pancreas (Courtney, Pfeifer et al. 2011). In contrast to a healthy (Figure 5) organism where glucose with the help of insulin is absorbed from cells in the blood, in T2DM the pancreas however, produces

inadequate amounts of insulin and as a result glucose levels in the blood are increased and cells cannot utilise glucose for the production of energy (Figure 6). Defects in pancreatic islets and in the pancreas to produce sufficient insulin, causes impaired glucose homeostasis and principally higher than normal glucose levels in the blood (hyperglycemia) which is considered to be the hallmark of diabetes (Lumelsky, Blondel et al. 2001, Goldstein, Little et al. 2004, Caduff, Lutz et al. 2011). Subsequently, the corestone of diabetes care is to monitor the glycemic status and achieve the best possible blood glucose levels (Goldstein, Little et al. 2004).

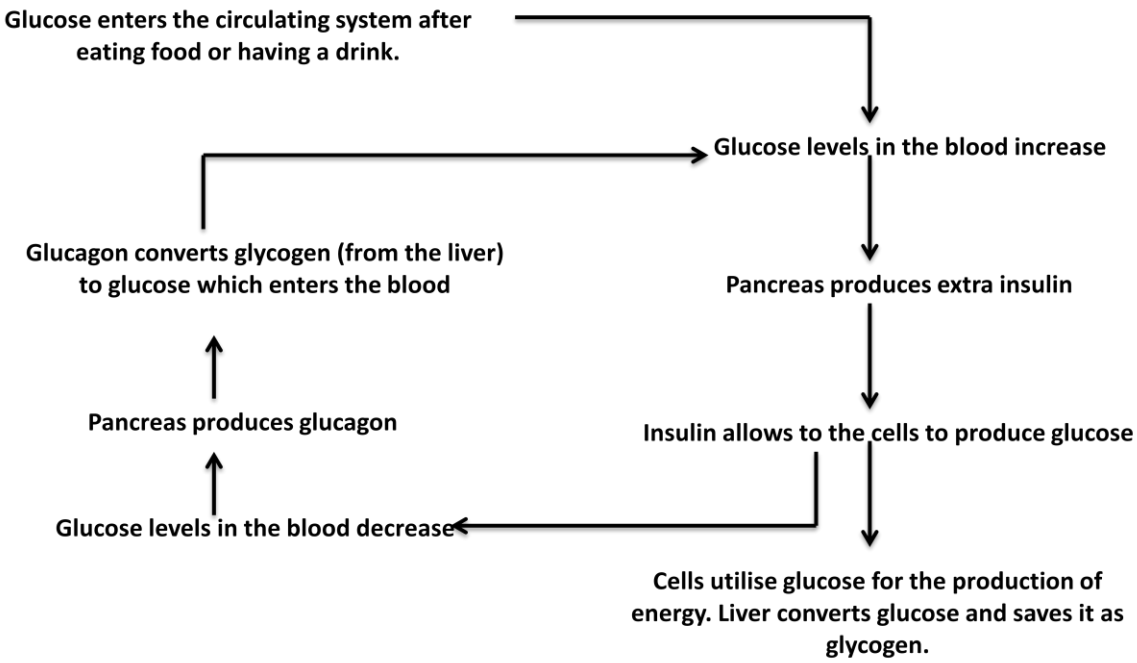


Figure 5: Healthy organism

After eating our meals or having a drink, glucose enters our circulating system and with the help of insulin it is absorbed from the cells in the blood. Part of glucose is saved in the liver as glycogen. When glucose levels in the blood decrease, glucagon converts glycogen to glucose and glucose levels in the bloodstream increase.

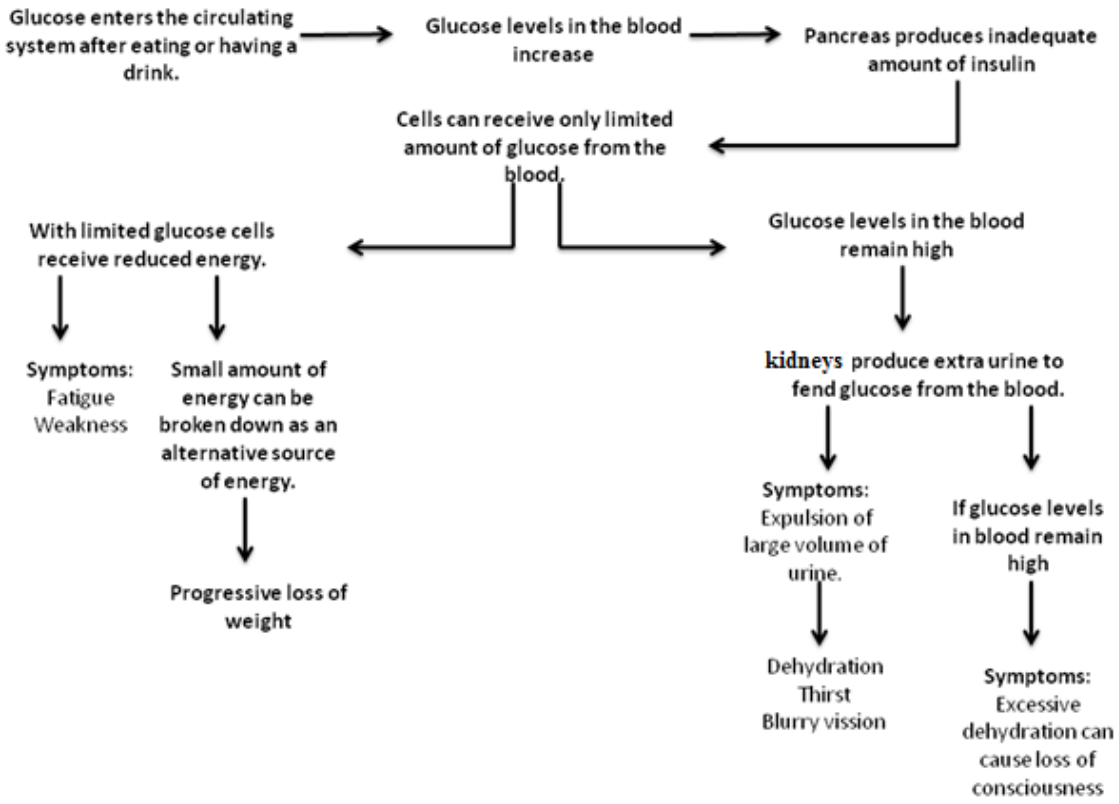


Figure 6: Pancreas produces inadequate amount of insulin

Glucose levels in the blood increase and cells cannot utilise glucose for the production of energy. This causes the symptoms of diabetes and if glucose levels remain high this can further affect patients´ eyes, kidneys or nerves.

The global number of affected individuals by DM is increasing rapidly in the 21st century reaching nearly the number of 300 million people worldwide (Shaw, Sicree et al. 2010, Zhang, Zhang et al. 2010, Tahergorabi and Khazaei 2012). According to the International Diabetes Federation, approximately 246 million individuals worldwide were affected by DM in 2008 while this number is projected to rise and reach over 380 million individuals by 2025 (Lancet 2008, Tahergorabi and Khazaei 2012). The World Health Organization (WHO) also provided some estimation about the prevalence of DM in USA expecting an increase in prevalence from 21.9 million to 30.3 million by 2025 (WHO 2002, Wild, Roglic et al. 2004). This increase is attributed mainly to obesity, an unhealthy diet, sedentary lifestyle, an ageing population and to an increased migration of susceptible patients (Wild, Roglic et al. 2004, Nicholson and Hall 2011). Main acute symptoms of DM include glycosuria, polyuria, polydipsia, acidosis and in more severe cases ketoacidotic coma.

There are a number of diabetes-associated complications which can develop in individuals suffering from DM including both microvascular and macrovascular complications affecting different parts of the body such as eyes, kidneys, peripheral nerves and large vessels (Chapman, Noble et al. 2002, Garnock-Jones and Plosker 2009, Gurzov and Eizirik 2011). These complications occur because of chronic hyperglycemia and their incidence can be reduced with glycemic control (Garnock-Jones and Plosker 2009, Gurzov and Eizirik 2011). In addition to the cardiovascular diseases, premature death, amputations, as well as blindness can occur as a result of diabetes (Zhang, Zhang et al. 2010).

As previously stated, there are two types of DM: T1DM and T2DM. Both types are associated with increased morbidity and mortality and are characterized by defects in pancreatic β-cell mass and by different degrees of insulin deficiency, either absolute or relative insulin deficiency (Chapman, Noble et al. 2002, Gurzov and Eizirik 2011).

1.2.1. Type 1 diabetes mellitus (T1DM)

T1DM is also known as insulin-dependent diabetes mellitus meaning that affected patients require a permanent insulin treatment (Migdalis 2011) and affects approximately 10-15% of patients (Gurzov and Eizirik 2011, Miller and St Onge 2011). This chronic disease may onset either in childhood or adolescence affecting approximately 1 in every 400 children or adolescents whereas on the other hand, the incidence of T1DM in children is expected to increase by 70% in Europe by 2020 (Atkinson and Eisenbarth 2001, Group 2008, Patterson, Dahlquist et al. 2009). Hyperglycemia, polydipsia, polyuria, weight loss, blurred vision and even coma comprise the main characteristics of T1DM (Miller and St Onge 2011).

Pathophysiology of T1DM

According to the (WHO) and the American Diabetes Association T1DM can be further subdivided into two categories. Type 1A diabetes where there is an autoimmune destruction of the pancreatic β cells and type 1B diabetes where there are not any detectable autoimmune antibodies (Alberti and Zimmet 1998, Imagawa, Hanafusa et al. 2000, Miller and St Onge 2011).

The commonest form is the immune mediated type (Type 1A), where both environmental and genetic factors cause an autoimmune attack (Figure 7), the immune system does not

recognise any longer proteins which are associated with the pancreatic islet β cells and subsequently are targeted for an immune response as they are considered as foreign (Wallensteen, Dahlquist et al. 1988, Scheen 2004, Courtney, Pfeifer et al. 2011). The contribution of environmental factors in the development of an autoimmune attack is supported by twin studies where not all of the monozygotic twins will develop T1DM (Barnett, Eff et al. 1981, Raskin and Mohan 2010, Yeung, Rawlinson et al. 2011). Autoimmune attack causes permanent destruction of the insulin producing pancreatic β cells, resulting in insulinopenia, hyperglycaemia and ketoacidosis (Gepts and Lecompte 1981, Eisenbarth 1986, Bach 1994, Ichii and Ricordi 2009).

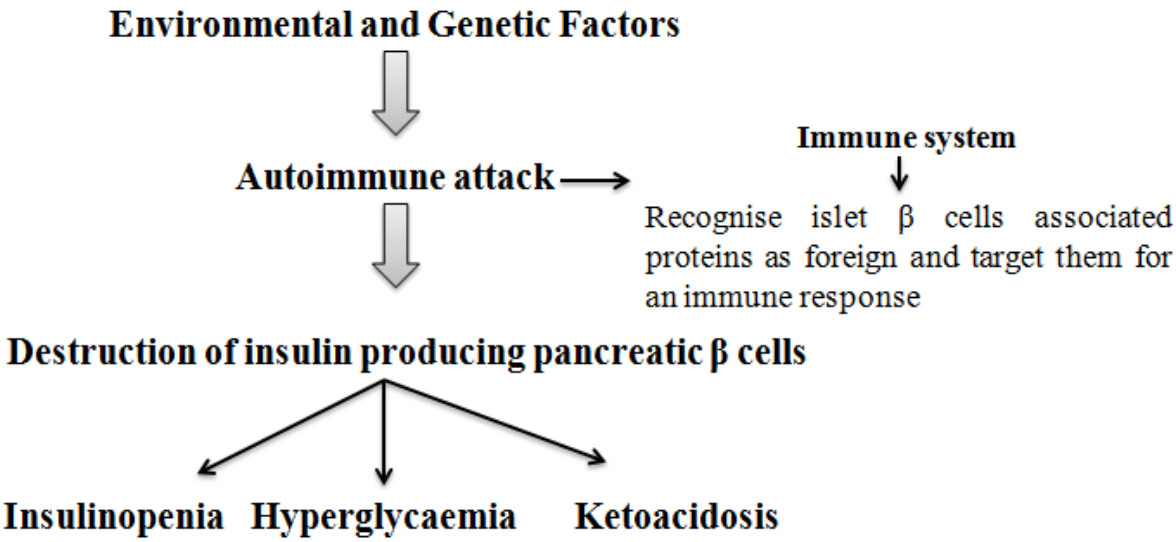


Figure 7: Immune mediated diabetes

Both environmental and genetic factors cause an autoimmune attack when the immune system recognise proteins associated with islet cells as foreign and target them for an immune response. This autoimmune attack causes destruction of the insulin producing pancreatic b cells leading to insulinopenia, hyperglycaemia and ketoacidosis.

By the time that a patient is diagnosed with T1DM, approximately 80% of the β cells have already been destructed and only some β cells remain (Gepts 1965, Wherrett, Bundy et al. 2011).

The main characteristic of T1DM is the absolute lack of endogenous insulin production resulting in a lifelong dependence on exogenous insulin administration which represents the first line treatment for T1DM aiming in monitoring blood glucose levels (Ichii and Ricordi 2009, Association 2011).

1.2.2. Type 2 Diabetes mellitus (T2DM)

T2DM is a complex, endocrine, metabolic disorder associated with high morbidity and mortality rates (Moutzouri, Tsimihodimos et al. 2011) and is also known as non-insulin dependent diabetes mellitus (Gonzalez, Johansson et al. 2009, Gurzov and Eizirik 2011). This metabolic disorder is basically caused by the body's ineffective use of insulin, affecting roughly 80-85% of patients (Gonzalez, Johansson et al. 2009, Gurzov and Eizirik 2011). The prevalence of T2DM is increasing dramatically worldwide, with global estimates predicting that the number of cases by the year of 2030 will reach 370 million (Zimmet, Alberti et al. 2001, Wild, Roglic et al. 2004). The increase in prevalence of T2DM seems to be much greater in developing countries than in developed countries with an incidence of 69% and 20% respectively (Shaw, Sicree et al. 2010). In developing countries, this increase is strongly associated to the modern lifestyle modifications including high energy diet and reduced physical activity leading to overweight and obesity (Zimmet, Alberti et al. 2001, Colagiuri 2010, Nolan, Damm et al. 2011).

Inactivity, overnutrition/obesity and hypertension are highly associated with T2DM where it is estimated that about 85% of T2DM patients are obese (Centers for Disease and Prevention 2004) and approximately 75% of them have hypertension (Sugerman, Wolfe et al. 2003). Obesity is the major cause of hyperglycaemia, impaired glucose tolerance, insulin resistance and altered lipid metabolism (Reaven 1988, Stumvoll, Goldstein et al. 2005, Kahn, Hull et al. 2006). In contrast to T1DM which is characterised by the rapid onset of the disease, in T2DM, there is a slow onset of the disease. In T2DM, under conditions of overnutrition, inactivity and insulin resistance, the function of islet β-cells declines and thus become unable to secrete enough insulin (Nolan, Damm et al. 2011). Subsequently, inadequate production of insulin from islet β-cells in response to overnutrition and insulin resistance together with impaired insulin action comprise the key defects and the cardinal metabolic features of T2DM (DeFronzo, Bonadonna et al. 1992, Kahn 2003, Stumvoll, Goldstein et al. 2005, Gurzov and Eizirik 2011).

The pathophysiology of T2DM is quite complex where multiple organ systems are affected, thus leading to a number of associated complications, including non-alcoholic fatty liver disease, vision disorders, heart disease, oral complications, possibly malignancies, kidney disease, infections and neuropathy (Larter, Chitturi et al. 2010, Renehan, Smith et al. 2010, Nolan, Damm et al. 2011).

A combination of environmental and genetic factors is involved in the pathogenesis of T2DM. A number of established risk factors include older people ageing 45 years or even greater, Western lifestyle characterized with overnutrition, obesity and reduced physical activity and high blood pressure (Bays, Frestedt et al. 2011). Moreover, another risk factor is to have family history of T2DM where there is an increased risk up to 3 fold to develop the disease in cases where there is a 1st degree relative affected (Pierce, Keen et al. 1995, Gloyn and McCarthy 2001) while increased risk for the development of the disease is also observed in the cases of monozygotic twins than in dizygotic twins (Wild, Roglic et al. 2004). Further evidence for the genetic implication in T2DM stems from the fact that the incidence of the disease is much higher in particular populations such as Nauruan Islanders, Pima Indians and Mexican Americans (Knowler, Bennett et al. 1978).

Management of T2DM

Therapy and management of T2DM usually starts by educating patients about the potential benefits of lifestyle modifications such as increased physical activity, weight reduction, and increased consumption of dietary fibers. Meanwhile, the simultaneous act of reducing the consumption of saturated fat also achieves the objectives of improved metabolic control, enhanced quality of life and therefore, consequent relief of the symptoms (Figure 8) (Carey, Walters et al. 1997, Hanson, Imperatore et al. 2002, Kosaka, Noda et al. 2005, Hamman, Wing et al. 2006). Furthermore, of major importance is to educate patients the significance of self-monitoring of blood glucose levels in order to be able to identify periods of hypoglycaemia (Wilson and Perry 2009, Noh, Graveling et al. 2011).

Despite the potent benefits of successful lifestyle modifications the majority of patients will require a combination of diet with pharmacological therapy including insulin and oral antidiabetics (Figure 12) (Krentz and Bailey 2005). Although lifestyle modifications and antidiabetic drugs can successfully provide adequate glycemic control during the early stages of the disease, due to the progressive nature of the disease where the ability of pancreatic β-cells to produce insulin declines progressively, some patients with advanced disease will require insulin administration as their main therapy similarly to T1DM patients (Hamaty 2011).

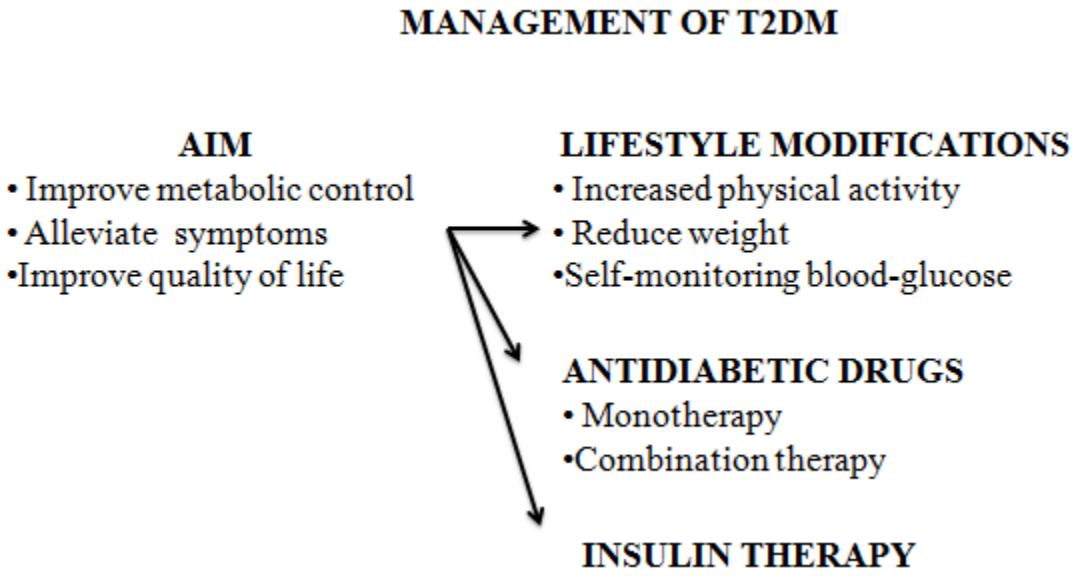


Figure 8: Management and treatment of T2DM

Due to the progressive nature of T2DM, there is a sequential approach followed for both of its treatment and management. All of them aiming towards improving metabolic control, alleviation of symptoms, and therefore improving in this way the underlying quality of life. The first step is to induce lifestyle modifications such as increased physical activity, reduced body weight and education of self-monitoring blood glucose levels. Subsequently, a number of antidiabetic drugs are used either as monotherapy or as combination therapy. In some patients, as the time progresses, the ability of β-cells to produce insulin declines and thus these patients require insulin therapy similar to T1DM patients.

2. Aims of the Thesis

The present study was performed with the intention to review the currently available scientific literature about both types of Diabetes mellitus, type 1 and type 2 and discuss the current and novel approaches for its treatment, giving more emphasis on the latest advances in Diabetes mellitus treatment.

3. Methodology

For the purpose of this study, we searched Medline, Pubmed, Google Scholar and the online library of Charles University with the intention of identifying English language original and review articles about Diabetes mellitus together with the associated novel approaches for its treatment.

The search terms we used, were “diabetes mellitus”, “type1 diabetes mellitus”, “type 2 diabetes mellitus”, “pancreas and diabetes mellitus”, “insulin resistance”, “insulin secretion”, “glycaemia”, “pancreatic islets”, “β-cell failure”, “insulin therapy”, “incretins”, “incretin therapy”, “islet cell transplantation”, “metabolic surgery”, “bariatric surgery”, “oral hypoglycaemic agents”, “stem cells for diabetes” and finally a combination of these terms.

Subsequently, we summarised and presented all of the novel findings on the area of Diabetes mellitus treatment.

4. T1DM Treatment

4.1 Insulin treatment for T1DM: current and latest advances

A brief history of insulin

The fact that the root cause of diabetes is the pancreas was reported for the first time in 1889 by Von Mehring and Minkowski (Owens, Zinman et al. 2001). This discovery formed the basis for the extraction and introduction of insulin a few years later, around 1892. Macleod J. was the first man who coined the term "insulin" and together with Frederick G. Banting and Charles Best tested this extract in dogs and subsequently 15mL into patient Leonard Thompson; the first man who received insulin treatment, rescuing him from death due to T1DM (Hirsch 2004). Insulin was rapidly adopted around the world revolutionizing the management of diabetes and saving simultaneously millions of lives (Owens, Zinman et al. 2001, Hirsch 2004) while this outstanding achievement of medicine earned the Nobel Prize in 1923 (Hirsch 2004).

Insulin administration in T1DM

The main characteristic of T1DM is the absolute deficiency of insulin (Association 2011) where endogenous insulin fails to meet the body's requirement (Miller and St Onge 2011). Consequently, T1DM patients are obligated to receive a permanent insulin treatment throughout their lives (Migdalis 2011).

Even though exogenous insulin administration represents the principal treatment of T1DM, in many cases initiation of insulin therapy is delayed mainly due to unwillingness, anxiety and fear of either the physician or the patient about difficulties considering the self-administration of injections as well as about the side effect and consequences of treatment including weight gain and hypoglycaemia (Korytkowski 2002, Meece 2006, Fonseca, Gill et al. 2011). Thus, is of major importance to consult DM patients about the significance of insulin therapy, ensuring comfortability about having diabetes and proper training of how to become fully capable of injecting insulin and self-monitoring blood

glucose independently (Silverstein, Klingensmith et al. 2005, Wennick and Hallstrom 2007, Olinder, Nyhlin et al. 2011).

The fundamental therapeutic goal of T1DM is to regulate properly blood glucose levels, achieving normoglycemia and also to delay, reduce or in many cases prevent the progression of diabetes and associated long-term complications such as nephropathy, neuropathy, retinopathy in addition to other microvascular and cardiovascular complications as it has been prooved that the more intense the treatment the greater the chances to delay or even prevent those complications (Atkinson and Maclaren 1994, Group 1998, Colagiuri, Cull et al. 2002, Hart, Redekop et al. 2005).

In order to achieve an effective T1DM treatment, insulin therapy needs to effectively be combined with pharmacotherapy, lifestyle changes, medical nutrition therapy and exercise (Association 2003, Hart, Redekop et al. 2005). Patients need to be encouraged to exercise on a regular daily basis including a daily walking in order to improve in this manner insulin sensitivity and reduce insulin resistance (Holloszy, Schultz et al. 1986). Moreover, patients should be advised to keep a healthy body weight and include foods high in fibers in their diet as this kind of nutrition is associated with reduced risk of hypertension, hyperlipidemia and cardiovascular complications (Reaven 1994, Goutham 2001). Prior to insulin therapy, either one or two antidiabetic drugs are used and if in the event these drugs fail to achieve the goal glycaemic control, then insulin therapy is initiated (Fonseca, Gill et al. 2011). A good early choice in terms of pharmacotherapy is metformin. This drug has beneficial effects about weight gain, lowers glycaemia and increases insulin sensitivity (Bailey and Turner 1996, Migdalis 2011).

4.1.1. Continuous subcutaneous insulin infusion or multiple daily injections?

Insulin analogies, purified and recombinant insulin

Both CSII and MDI as a therapeutic option aim to achieve two main goals: avoid hypoglycaemia weight gain and also to improve blood glucose control (Radenkovic 2011).

CSII is an insulin therapy introduced in 1976 by Pickup and Keen (Pickup and Keen 2002). This method of insulin therapy is the choice of treatment for T1DM and makes use of an insulin pump (Figure 9). The internal part of the pump consists of a disposable reservoir for insulin, an insulin promotion mechanism, a battery-powered pump and a

computer chip controller while on the external part there is the infusion set (under the skin) (Olohan and Zappitelli 2003, Didangelos and Iliadis 2011).

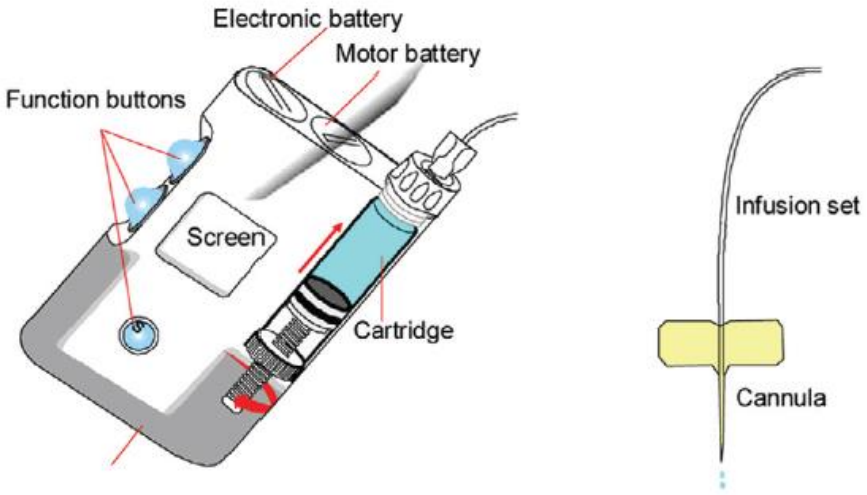


Figure 9: The insulin pump

It consists of an external and an internal part. The external part is made up of a disposable reservoir for insulin, an insulin promotion mechanism, a battery-powered pump and a computer chip controller while on the external part there is the infusion set (under the skin). (Figure adapted from (Olohan and Zappitelli 2003)).

The insulin pump can be arranged to infuse insulin in a subcutaneous mode throughout the day and night mimicking a healthy pancreas and particularly the natural secretion of insulin from the pancreatic β cells and consequently meet the insulin needs in people having insulin defficiency (Pickup and Keen 2002, Olohan and Zappitelli 2003, Didangelos and Iliadis 2011).

Using CSII as a therapeutic option has a number of marked advantages regarding efficacy and safety: 1) It significantly lowers the risk for hypoglycaemia and improves blood glucose control, 2) there is less weight gain caused by insulin therapy, and 3) it lowers the number of total insulin doses improving in this way the quality of life. Simultaneously, it delays and in many cases prevents some of the diabetes associated complications (Pickup, Mattock et al. 2002, Didangelos and Iliadis 2011, Radenkovic 2011).

On the other hand MDI is used as an alternative therapeutic option and makes use of both rapid-acting and long-acting insulins in order to achieve a good blood glucose control (Owens, Zinman et al. 2001). Rapid-acting insulin analogues include insulin lispro, insulin aspart and insulin glulisine while long-acting insulin analogues include insulin glargine and insulin detemir (Hirsch 2004, Werner and Chantelau 2011). Rapid-acting insulins are used in an attempt to provide a more natural insulin response and have the

advantage of maintaining the glucose rise early in the morning which is a common phenomenon in patients suffering with DM (Pickup and Keen 2002). The most important disadvantage of rapid acting insulin is their mode of absorption. They are absorbed relatively slow and late hypoglycaemia occurs as a result of the delayed absorption (Owens, Zinman et al. 2001, Zinman 2001, Chapman, Noble et al. 2002). In order to avoid this unwanted result, they are injected approximately 15-30 minutes prior meals (Owens, Zinman et al. 2001).

Insulin aspart

Insulin aspart is identical to the regular human insulin with the difference being the amino acid on the position 28 on the B chain of the regular human insulin which is proline, where insulin aspart is substituted with aspartic acid (Figure 10) (Chapman, Noble et al. 2002). This substitution makes insulin aspart to be absorbed more rapidly in comparison to regular human insulin after subcutaneous administration (Chapman, Noble et al. 2002). In contrast to regular human insulin which needs to be administrated usually 30 minutes before meal, this rapid acting human insulin analogue can be administrated immediately before meals, significantly improving postprandial glycaemic control (Chapman, Noble et al. 2002). Apart from postprandial glycaemic control which has improved, another benefit of this insulin analogue is that it has shown in many clinical trials to lower the incidence of hypoglycaemia in comparison to that of regular human insulin (Chapman, Noble et al. 2002). The commonest adverse event observed in a number of clinical trials in T1DM and T2DM patients treated with insulin aspart was hypoglycaemia while other commonly observed adverse events were similar to those caused with regular human insulin such as allergic reactions, headaches and upper respiratory tract symptoms (Home, Lindholm et al. 2000, Tamas, Marre et al. 2001).

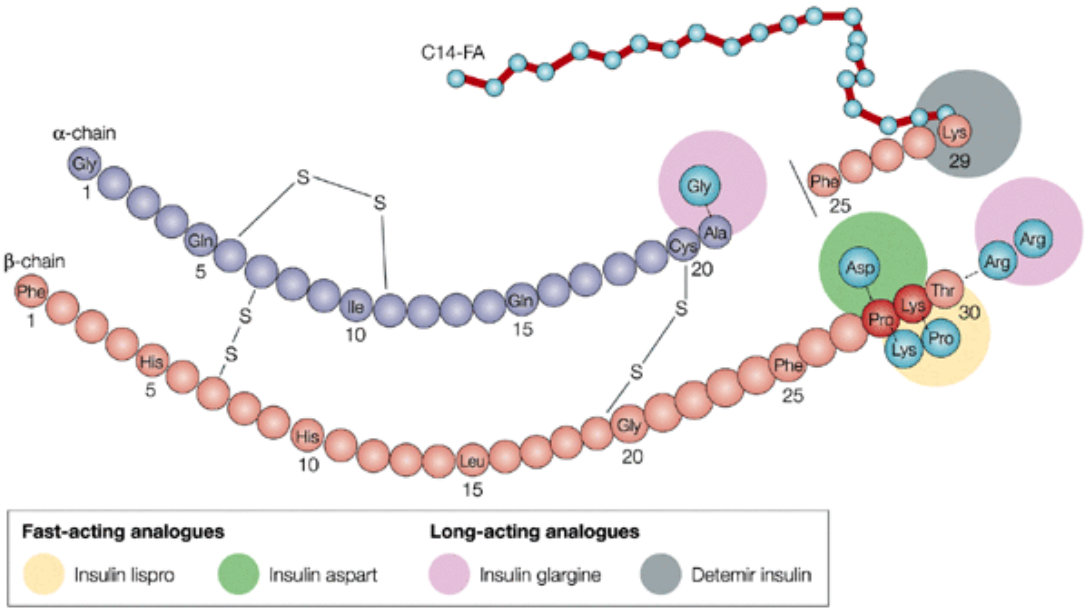


Figure 10: The structure of insulins aspart, lispro, glargine and detemir

Insulin aspart is identical to regular human insulin with the difference that the amino acid at the position 28 of the B chain of the regular human insulin, which is proline, is substituted with aspartic acid

Insulin Lispro is produced by DNA recombination technology where on positions 28 and 29 of the B chain there is lysine and proline respectively Insulin glargine is produced by DNA recombination technology. Three amino acids are modified for its production. Particularly, at position 21 of the A chain, asparagines is substituted with glycine while at the same time, 2 arginine residues are added on the B chain elongating it at the C terminus Insulin detemir structure is produced by the removal of an amino acid, threonine from position 30 on B chain and a substitution with a C14 fatty acid chain to lysine on B chain at position 29

(Figure adapted from (Owens 2002))

Insulin glulisine

Insulin glulisine is another rapid-acting human insulin analogue. It was formed with alterations in the human insulin analogue using the DNA recombinant technology where as indicated on figure 11, asparagine at position 3 of the B chain was replaced with lysine and lysine at position 29 of the B chain was replaced with glutamic acid (Hoogma and Schumicki 2006, Garnock-Jones and Plosker 2009, Sanofi-aventis 2009). When compared to regular human insulin, insulin glulisine shows faster glucose lowering effect while in terms of bodyweight there are no significant differences (Hoogma and Schumicki 2006, Garnock-Jones and Plosker 2009). In accordance to a number of different clinical studies, this insulin analogue shows good overall tolerability in both T1DM and T2DM patients (Hoogma and Schumicki 2006, Rayman, Profozic et al. 2007,

Kawamori, Kadowaki et al. 2009). As it happens with all of the insulins a common adverse event that is observed with insulin glulisine is hypoglycaemia (Rayman, Profozic et al. 2007). Finally, the dosage of Iinsulin Glulisine should be individualised for each patient according to both the blood glucose monitoring as well as to the previous insulin history of the patient (Sanofi-aventis 2009, Sanofi 2012).

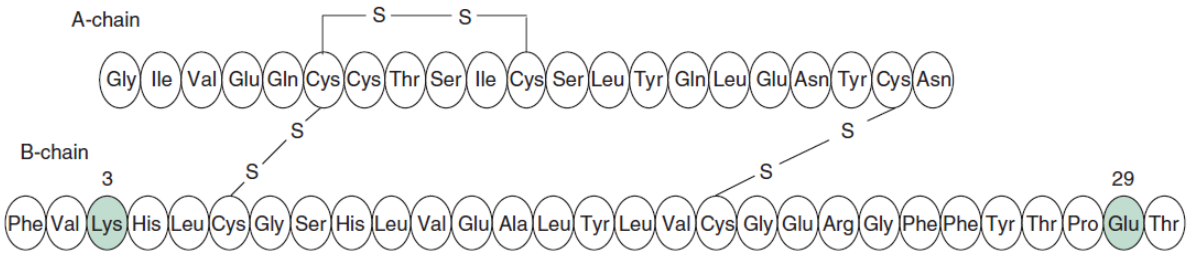


Figure 11: The structure of insulin glulisine

Insulin glulisine was formed with alterations on the human insulin using the DNA recombinant technology where asparagine on position 3 of the B chain was replaced with lysine and lysine on position 29 of the B chain was replaced with glutamic acid (Figure adapted from (Garnock-Jones and Plosker 2009)).

Insulin lispro

Insulin lispro is another human insulin analogue produced by DNA recombination technology. As indicated on Figure 10, there is a substitution on the B chain in positions 28 and 29 with lysine and proline respectively, where this exchange of the amino acids produces a rapid acting insulin analogue (http://www.drugbank.ca/drugs/DB00047 , Renner, Pfutzner et al. 1999, Simpson, McCormack et al. 2007).

Similarly to insulin aspart and insulin glulisine, insulin lispro needs to be administrated immediately before food and it is rapidly absorbed (http://www.drugbank.ca/drugs/DB00047). A number of different studies performed on both T1DM and T2DM patients provide evidence about its therapeutic efficacy and tolerability (Tanaka 2011). Furthermore, Renner et al., 1999, demonstrated that insulin lispro is quiet convenient, safe and efficient insulin to be used providing significant improvements in daily blood glucose levels as well as in postprandial glucose control with comparable hypoglycaemic episodes with other insulins. The only concern about it, is the fact that once its delivery accidentally is interrupted there is an increased risk of ketoacidosis (Renner, Pfutzner et al. 1999).

With the intention of overcoming the disadvantages of rapid-acting insulin, new long-acting insulins were added into the clinical practise with more predictable and rapid absorption achieving a significantly improved and constant control of blood levels(Owens, Zinman et al. 2001, Owens 2002). An additional advantage in comparison to rapid-acting insulin is that they can be administrated within a shorter time before a meal or even immediately before a meal, though not 15-30 minutes earlier. By this way it improves the quality of life providing increased flexibility of lifestyle whilst reducing the risk of hypoglycaemia (Owens, Zinman et al. 2001).

Insulin glargine

DNA recombination technology was also used for the production of another insulin analogue, insulin glargine (Owens 2002). As shown in figure 10, on A chain at position 21 there is a substitution of asparagine with glycine while 2 arginine residues are added on B chain, elongating it at the C terminus (Owens 2002). The modification of those 3 amino acids, results in insulin glargine to have a longer duration of action as well as a delayed and prolonged absorption once it is subcutaneously administrated on patients (Ratner, Hirsch et al. 2000, Kaur 2008). A number of different studies demonstrated that this insulin analogue is associated with decreased risk of hypoglycaemia and lower fasting blood glucose levels, and can be administrated once daily (Yki-Jarvinen, Dressler et al. 2000).

Insulin detemir

In order for insulin detemir to be formed, an amino acid, threonine is removed from position 30 on B chain. There is a substitution with a C14 fatty acid chain to lysine on B chain at position 29 (Markussen, Havelund et al. 1996, Owens 2002, Kaur 2008). This modification and substitution results in this insulin analogue to have a delayed absorption once it's subcutaneously administrated (Markussen, Havelund et al. 1996). It is administrated twice daily (Kaur 2008). Finally, according to a number of different studies, it has a number of well documented benefits including reduction in body weight, and good maintenance of glycaemic control (L. F. Meneghini, K. H. Rosenberg et al. 2007).

4.1.2. Inhaled insulin

Subcutaneous administration of insulin to diabetic patients has been one of the major problems, limiting patients' compliance as there is a need for frequent and multiple injections (Zarogoulidis, Papanas et al. 2011). In order to overcome this barrier, novel combinations and ways of insulin administration were developed, including inhaled insulin. Administration of drugs via the pulmonary route is advantageous over the subcutaneous administration because drugs administrated in this way have faster onset of action (Mandal 2005). The first inhaled drug which was approved was Exubera and was introduced in 2006 by Pfizer (Kaur 2008). Exubera is administrated via the pulmonary route in a powder form (Kaur 2008). Various inhaled insulin devices have been developed with many differences in terms of their size, mechanism and regulation of insulin administration where the ideal system should be small and portable in order to be convenient for the patients, friendly to be used and be able to closely mimic β-cell insulin secretion showing rapid onset of action (Kaur 2008, Rubin and Peyrot 2011).

There is a number of well-established factors that might affect the absorption of inhaled insulin. According to a few studies, smoking increases insulin bioavailability raising at the same time the risk of hypoglycaemia (Becker, Sha et al. 2006). BMI also interfere with the absorption of inhaled insulin as insulin doses need to be adjusted to the weight of the patient while there is evidence from some studies that in obese patients inhaled insulin was beneficial in comparison to subcutaneous insulin (Rave, Nosek et al. 2004, Heise, Nosek et al. 2007). Another important problem with this type of insulin is that it is possible small amounts of the drug to be lost via and between the inhaler and the mouth during inhalation (Kaur 2008) and finally the cost of inhaled insulin which is significantly higher than subcutaneous insulin (Kaur 2008). Common side effects include dry mouth, shortness of breath, sore throat with cough being the commonest and more usual (Siekmeier and Scheuch 2008).

4.1.3. Oral insulin

Another novel development for the treatment of T1DM, which has been approved by the FDA on 31st of December 2012 (Oramed 2012), is the oral insulin aiming to alleviate patients' lives that are dependent on daily insulin injections. Apart from the fact that having multiple daily injections is of low compliance, once insulin is provided with injection, it is unable to follow the normal physiological pathway and instead, it is

therefore delivered to systemic circulation (Rekha 2011). In contrast to insulin injections where only a small percentage of insulin reaches the liver which is the primary site of action, oral insulin follows the normal physiological pathway, by firstly traversing into the liver before crossing the bloodstream. (Gordon Still 2002, Oramed 2012).

4.2. Type 2 Diabetes mellitus (T2DM)

T2DM is a complex, endocrine, metabolic disorder associated with high morbidity and mortality rates (Association 2008, Moutzouri, Tsimihodimos et al. 2011) and is also known as non-insulin dependent diabetes mellitus (Gonzalez, Johansson et al. 2009, Gurzov and Eizirik 2011). It is basically caused by the body's ineffective use of insulin and affects roughly 80-85% of diabetic patients (Gonzalez, Johansson et al. 2009, Gurzov and Eizirik 2011). The prevalence of T2DM is increasing dramatically worldwide, with global estimates predicting that the number of cases by the year of 2030 will reach 370 million (Zimmet, Alberti et al. 2001, Wild, Roglic et al. 2004). The increase in prevalence of T2DM seems to be much greater in developing countries than in developed countries with an incidence of 69% and 20% respectively (Shaw, Sicree et al. 2010). In developing countries, this increase is strongly associated to the modern lifestyle modifications including high energy diet and reduced physical activity leading to overweight and obesity (Zimmet, Alberti et al. 2001, Colagiuri 2010, Nolan, Damm et al. 2011).

Inactivity, overnutrition/obesity and hypertension are highly associated with T2DM where it is estimated that about 85% of T2DM patients are obese (Centers for Disease and Prevention 2004) and approximately 75% of them have hypertension (Sugerman, Wolfe et al. 2003).Obesity is the major cause of hyperglycaemia, impaired glucose tolerance, insulin resistance and altered lipid metabolism (Reaven 1988, Stumvoll, Goldstein et al. 2005, Kahn, Hull et al. 2006). In contrast to T1DM which is characterised by the rapid onset of the disease, in T2DM there is a slow onset of the disease. In T2DM, under conditions of overnutrition, inactivity and insulin resistance, the function of islet β-cells declines and thus become unable to secrete enough insulin (Nolan, Damm et al. 2011). Subsequently, inadequate production of insulin from islet β-cells in response to overnutrition and insulin resistance together with impaired insulin action comprise the key defects and the cardinal metabolic features of T2DM (DeFronzo, Bonadonna et al. 1992, Kahn 2003, Stumvoll, Goldstein et al. 2005, Gurzov and Eizirik 2011).

The pathophysiology of T2DM is complex and multiple organ systems are affected leading to a number of associated complications, including non-alcoholic fatty liver disease, vision disorders, heart disease, oral complications, possibly malignancies, kidney disease, infections and neuropathy (Larter, Chitturi et al. 2010, Renehan, Smith et al. 2010, Nolan, Damm et al. 2011). A combination of environmental and genetic factors are involved in the pathogenesis of T2DM. A number of established risk factors include older people ageing 45 years old or even greater, Western lifestyle characterized with overnutrition, obesity and reduced physical activity and high blood pressure (Bays, Frestedt et al. 2011). Moreover, another risk factor is to have family history of T2DM where there is an increased risk up to 3 fold to develop the disease in cases where there is a 1st degree relative affected (Pierce, Keen et al. 1995, Gloyn and McCarthy 2001), while increased risk for the development of the disease is also observed in the cases of monozygotic twins than in dizygotic twins (Wild, Roglic et al. 2004). Further evidence for the genetic implication in T2DM stems from the fact that the incidence of the disease is much higher in particular populations such as Nauruan Islanders, Pima Indians and Mexican Americans (Knowler, Bennett et al. 1978).

4.2.1. Management of T2DM

Therapy and management of T2DM usually starts by educating patients about the potential benefits of lifestyle modifications such as increased physical activity, weight reduction and increased consumption of dietary fibres, while reducing simultaneously the consumption of saturated fat, achieving the objective of improved metabolic control and enhanced the quality of life and the ultimate relief the symptoms (Figure 8) (Carey, Walters et al. 1997, Hanson, Imperatore et al. 2002, Kosaka, Noda et al. 2005, Hamman, Wing et al. 2006). Also of major importance is to educate patients the significance of self-monitoring blood glucose levels in order to be able to identify periods of hypoglycaemia (Wilson and Perry 2009, Noh, Graveling et al. 2011).

Despite the potent benefits of successful lifestyle modifications, the majority of patients will require a combination of diet with pharmacological therapy including insulin and oral antidiabetics (Figure 12) (Krentz and Bailey 2005). Although lifestyle modifications and antidiabetic drugs can successfully provide adequate glycemic control during the early stages of the disease (due to the progressive nature of the disease where the ability of pancreatic β-cells to produce insulin declines progressively), some patients with advanced

disease will require insulin administration as their main therapy similarly to T1DM patients (Hamaty 2011).

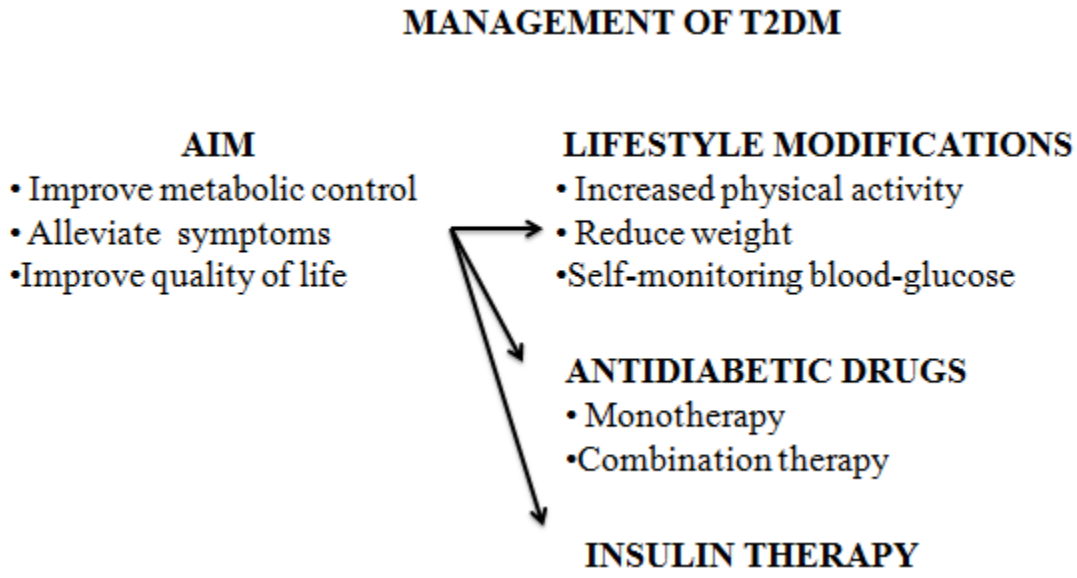


Figure 12: Management and treatment of T2DM

Due to the progressive nature of T2DM, there is a sequential approach followed for both of its treatment and management all of them aiming in improving metabolic control, alleviate symptoms improving in this way the quality of life. The first step is to induce lifestyle modifications such as increased physical activity, reduced body weight and education of self-monitoring blood glucose levels. Subsequently, a number of antidiabetic drugs are used either as monotherapy or as combination therapy. In some patients, as the time progresses, the ability of β-cells to produce insulin declines and thus these patients require insulin therapy similar to T1DM patients.

4.2.2. Insulin treatment

Insulin resistance together with hyperglycemia and insulin deficiency occur from the early stages of T2DM development (Reaven 1988, Wyne and Mora 2007). Subsequently, appropriate pharmacological management is required for dealing with these metabolic defects associated with T2DM aiming to decrease insulin resistance, reduce long-term complications of diabetes, improve glycemic control and stimulate endogenous insulin secretion (Caballero 2009).

Metformin is a good early choice and is quite often recommended as the first line pharmacotherapy for T2DM based on its beneficial and safety profile. Some of its beneficial effects include that weight neutral, which makes it a preferable first line agent in overweight patients, improves insulin sensitivity, decreases hepatic gluconeogenesis, reduces plasma triglycerides, inhibits glycogenolysis and also may improve a number of cardiovascular risk factors (Nicholson and Hall 2011). Additionally, it is inexpensive

(Holman 2007) and can be used either as monotherapy or in combination with other antidiabetic drugs or insulin (Ahluwalia and Vora 2011). In cases where metformin is used as a first-line agent, where glycaemic control is not achieved, then insulin may be added.

It has been established that the majority of T2DM patients will eventually require insulin treatment either alone or in combination with oral antidiabetic agents (Robert C. Turner 1999). Consequently, it would be beneficial for patients to start with insulin treatment with insulin as a therapeutic tool at the early stages of their disease rather than in later stages when antidiabetic agents fail to manage the metabolic effects of diabetes (Yki-Jarvinen 2001, Wyne and Mora 2007, Hamaty 2011).

The first insulin analogue that is successful in closely mimicking the physiological pancreatic β-cell insulin secretion observed in healthy individuals and that is recommended for insulin coverage for T2DM patients is insulin glargine (http://www.drugbank.ca/drugs/DB00047 , DeWitt and Hirsch 2003, Wang, Carabino et al. 2003).

4.2.3. Incretin effect

Over the last several years, increased understanding of the pathophysiology of diabetes has led to an expansion of treatment choices with the development of novel antidiabetic agents for the treatment of T2DM patients. These agents include GLP-1 receptor agonists, DPP-4 inhibitors, insulin analogues and other novel antidiabetic drugs (Ahluwalia and Vora 2011, Nicholson and Hall 2011).

Following food intake, a number of several different hormones are secreted by the gut. In humans among those hormones, the two most important ones are GLP-1 and GIP which are also termed as the incretin hormones (Robertson 2011). Both GIP and GLP-1 are presented in very low circulating levels during the fasting state while food intake causes their levels to significantly and rapidly increase (Gautier, Fetita et al. 2005). At the same time once these hormones are released, they stimulate pancreatic β-cells to release insulin after oral glucose ingestion (Robertson 2011). In normal physiological conditions both GLP-1 and GIP are responsible for limiting rises in postprandial blood glucose in contrast to pathological conditions where their absence causes pancreatic response to glucose to be diminished and this phenomenon is known as “the incretin effect” (Robertson 2011).

Later on, after several studies it was realized that this phenomenon, the incretin effect is compromised in T2DM (Nauck, Stockmann et al. 1986).

GLP-1 is a 30 amino acid peptide and is a product of the proglucagon gene (Holst 2007). It is produced as a main product in the enteroendocrine L-cells (Holst 2007). GLP-1 targets the key islet defects in T2DM and some of its main physiological actions (Figure 13) include: stimulation of glucose-induced insulin secretion in islets of Langerhans and in the perfused pancreas, stimulation of all steps of insulin biosynthesis providing in this way continual supplies of insulin for secretion, a potent stimulator of somatostatin secretion from isolated human islets while it is also able to suppress glucagon secretion in pancreatic islets maintaining in this way, elevated levels of blood glucose (Nauck, Wollschlager et al. 1996, Gautier, Fetita et al. 2005, Ahren 2011). Moreover, it is able to inhibit gastrointestinal secretion and motility particularly on gastric emptying (Holst 2002). Nauck et al., 1997, demonstrated that GLP-1 administration in healthy volunteers caused slowing of gastric emptying and glucose absorption leading to reduction of postprandial glucose concentration, indicating that probably the physiological role of GLP-1 is to slow gastrointestinal transit and decrease at the same time secretion of digestive enzymes (Nauck, Niedereichholz et al. 1997). Finally, it also exerts some effects on food intake by reducing caloric intake and enhancing satiety (Zander, Madsbad et al. 2002). In particular, it achieves to regulate food intake by increasing the feeling of fullness causing in this way termination of food ingestion (Drucker and Nauck 2006). Finally, it is also well documented that it has potential effects in both the cardiovascular and nervous systems (Drucker and Nauck 2006).

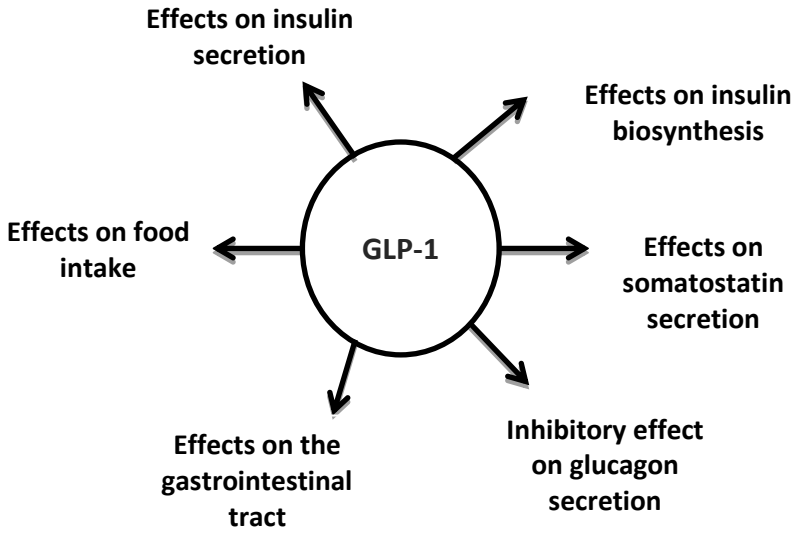


Figure 13: Physiological actions of GLP-1

GLP-1 exerts effect on insulin secretion and insulin biosynthesis, effect on food intake, on the gastrointestinal tract and somatostatin secretion while it also has an inhibitory effect on glucagon secretion.

Several studies have demonstrated the ability of GLP-1 to reduce hyperglycemia in T2DM patients and also to target both of the insulin and glucagon secretion when used as the first treatment (Ahren 2011).

GIP shares a close amino acid homology with GLP-1 and is basically a single 42 amino acid peptide, located on chromosome 17 in humans (Gautier, Fetita et al. 2005). It is secreted by K cells in a bioactive form and released from the upper small intestine followed ingestion of carbohydrates and lipids (Gautier, Fetita et al. 2005). Its receptors (GIP receptors) are expressed in pancreatic islets, heart, pituitary, gut and cortex and in several regions of the brain (Fehmann, Goke et al. 1995). GIP has been shown able to stimulate insulin secretion (Dupre, Ross et al. 1973). When smaller loads of nutrients are provided, the upper incretin hormone GIP is activated while when ingestion of larger meals would preferentially activate the distal incretin GLP-1 (Gautier, Fetita et al. 2005). Finally, in contrast to GLP-1, GIP does not affect either pancreatic alpha cell secretion of glucagon or gastric emptying (Trumper, Trumper et al. 2001).

DPP-4 is an aminopeptidase enzyme which is widely expressed in a number of several tissues and organs such as in the liver, endothelium, lungs and kidneys, while it also exists in a soluble circulating form (Drucker and Nauck 2006). The enzyme cleaves N-

terminal dipeptides from oligopeptides with a proline or alanine residues (Ahren, Schweizer et al. 2009).

4.2.4. Development of incretins as therapeutic agents

As aforementioned, the incretin effect is compromised in T2DM while according to a number of different studies, exogenous administration of GLP-1 could improve this disrupted incretin physiology (Campbell 2011). Despite its beneficial profile, GLP-1 has raised some clinical concerns regarding its short half-life (approximately 1-2 minutes) followed by its rapid enzymatic degradation by DPP-4 (Nicholson and Hall 2011). In an attempt to counteract this shortcoming and restore simultaneously the GLP-1 signal, GLP-1 receptor agonist and DPP-4 inhibitors have been developed and incorporated into clinical practice (Drucker and Nauck 2006, Nicholson and Hall 2011). GLP-1 receptor agonists are designed in a way in which they are able to resist to DPP-4 degradation while at the same time they produce similar effects to those produced by the native GLP-1 (Nauck 2009, White 2009, Campbell 2011). On the other hand, DPP-4 inhibitors prevent the degradation of GLP-1 by inactivating the enzyme responsible for its degradation, achieving in this way increase of insulin secretion and reduction of glucagon secretion (Drucker and Nauck 2006, Nauck 2009, Chaplin 2011).

4.2.4.1. GLP-1 receptor agonists

GLP-1 receptor agonists require subcutaneous administration and currently there are two commercially available and approved for use GLP-1 agonists: exenatide and liraglutide (Robertson 2011). Exenatide has been approved for use in the United States and Europe while liraglutide in the United States and Europe and Canada (Robertson 2011). A number of different studies and randomised clinical trials have been carried out in order to study the efficacy of both of the aforementioned GLP-1 receptor agonists for the treatment of diabetes either when used as monotherapy or as add-on therapy (Ross and Ekoe 2010).

Exenatide

Exenatide belongs to the injectable GLP-1 receptor agonists and is basically the synthetic form of exendin-4 which has been identified and isolated from the saliva of the lizard Heloderma suspectum (Gila monster) (Nicholson and Hall 2011). Exenatide has been available since 2005 (Chiu, Shih et al. 2012) and regardless of being a larger molecule

than human GLP-1, shows 53% homology with the human GLP-1 amino acid sequence (Chen and Drucker 1997, Kolterman, Kim et al. 2005, Drucker 2006). A benefit of exenatide in comparison to human GLP-1 is its increased resistance to degradation by DPP-4 (Drucker 2006). Comparable to human GLP-1, exenatide suppresses glucagon secretion in cases where it is inappropriately increased, slows gastric emptying, reduces food intake facilitating in this way weight loss and enhances glucose-dependent insulin secretion (Neumiller 2009).

Exenatide has been suggested as an adjunct to the ongoing therapy of patients with T2DM with the intention of improving glycemic control. It can be used either as monotherapy, even though this is not the most attractive option, or as combination therapy together with other oral antidiabetic agents (Verspohl, 2009). In terms of being used as combination therapy, exenatide has been approved as adjunctive therapy to metformin, sulfonylureas, thiazolidinediones or even with metformin and sulfonylureas together (DeFronzo 1999, Buse, Henry et al. 2004, Kendall, Riddle et al. 2005, Zinman, Hoogwerf et al. 2007). So far, the use of exenatide with insulin has not been studied and thus this combination cannot be recommended (Bond 2006).

It is administrated subcutaneously via a prefilled pen and is available in two doses: 5μg and 10 μg (Robertson 2011). In order to provide an adequate daily replacement, exenatide should be administrated twice a day usually within 60 minutes before both of the morning and evening meals (Robertson 2011).

A large number of clinical studies have demonstrated a number of different established beneficial effects caused after treating T2DM patients with exenatide. It is a well-tolerated and safe compound which is also resistant to degradation by DPP-4 (Drucker and Nauck 2006, Ahren 2011). Either administrated once daily or twice daily, exenatide exhibited glucoregulatory actions in a number of different studies and significantly marked reductions in HbA1c (Buse, Henry et al. 2004, Gautier, Fetita et al. 2005, Zinman, Hoogwerf et al. 2007). Other well recognised benefits of exenatide include slowing of gastric emptying; reduced food intake associated with weight loss and decreased fasting and postprandial plasma glucose levels (Neumiller 2009). Finally, the most commonly observed adverse events during the initial steps of therapy included nausea and mild to moderate hypoglycaemia (Ahren 2011).

Liraglutide

Liraglutide is a long-acting GLP-1 analogue with 97% homology to native GLP-1 (Verspohl 2009). It is not recommended as a first-line therapy for patients inadequately controlled with diet and exercise (Klonoff 2010, Campbell 2011) while it has been approved as an adjunct to diet and exercise with the intention of improving glycemic control in patients with T2DM (Klonoff 2010, Campbell 2011). Moreover it can be administered subcutaneously either as monotherapy or as combination therapy together with MET, SFU or TZDs. The absorption of liraglutide is accomplished within 9-12 hours after injection (Wajcberg and Amarah 2010).

Liraglutide, inhibits appetite (Nauck, Frid et al. 2009) and apoptosis of β-cells (Sturis, Gotfredsen et al. 2003) resulting in a number of different beneficial effects to patients receiving it as therapy. It produces weight reductions of approximately 3kg, improves b-cell function and increases b-cell mass, decreases systolic blood pressure and finally is associated with low hypoglycaemia incidence with no major episodes (Nauck, Frid et al. 2009). The most commonly observed adverse events associated with liraglutide therapy included nausea and vomiting which typically disappeared after several weeks of treatment (Nauck, Frid et al. 2009).

4.2.4.2. DPP-4 Inhibitors

In contrast to GLP-1 receptor agonists, DPP-4 inhibitors are all orally available with longer duration of action inhibiting the catalytic site of DPP-4 (Ahren 2011). Currently available DPP-4 inhibitors include sitagliptin, saxagliptin and vildagliptin which can be effectively be used either as monotherapy or as in combination therapy with other drugs.

Sitagliptin

Sitagliptin which is an orally active DPP-4 inhibitor is thought to inhibit approximately 80% of DPP-4 activity (Stonehouse, Okerson et al. 2008, Neumiller 2009) and was initially approved by the US FDA in 2006 (Gupta and Kalra 2011). A number of different clinical studies have demonstrated the half-life of sitagliptin varying from 8 to 14 hours (Herman, Stevens et al. 2005). It has been licensed to be used either as monotherapy in cases where metformin is inappropriate, in combination either with metformin or insulin and as triple therapy used with metformin plus a sulfonylurea or a glitazone (Amori, Lau et al. 2007, Chaplin 2011, Subbarayan and Kipnes 2011). It is administrated orally with

the recommended dosage being 100mg once daily as an adjunct to diet and exercise with the intention of improving glycemic control in patients with T2DM (Amori, Lau et al. 2007, Alexander, Sehgal et al. 2008, Ahren 2009, Chaplin 2011, Subbarayan and Kipnes 2011). In contrast to metformin, there are not long-term safety data for sitagliptin and therefore metformin is added as a second agent (Subbarayan and Kipnes 2011). Sitagliptin is well absorbed and tolerated demonstrating efficacy and safety either when used as monotherapy or as combination therapy.

According to different clinical studies, sitagliptin achieves significant reductions in fasting plasma glucose, improves b-cell function and lowers HbA1c when compared to placebo (Drucker and Nauck 2006, Goldstein, Feinglos et al. 2007). Moreover it is considered to be weight neutral as its weight effects were found to be minor increases or decreases (Raz, Hanefeld et al. 2006), and also has low risk for inducing hypoglycaemia (Goldstein, Feinglos et al. 2007).

Although sitagliptin is considered to be free from major drug interactions, a number of adverse events were noted in a small percentage of patients treated with sitagliptin including gastrointestinal events, nasopharyngitis, headache and respiratory infections (Raz, Hanefeld et al. 2006, Hermansen, Kipnes et al. 2007, Subbarayan and Kipnes 2011).

Saxagliptin

Saxagliptin is another potent selective DPP-4 inhibitor which was approved by both FDA and by the European medicines evaluation in 2009 for the treatment of patients with T2DM (Kania, Gonzalvo et al. 2011). Particularly this DPP-4 inhibitor has been designed for extended inhibition of the DPP-4 enzyme (Rosenstock, Sankoh et al. 2008) improving glycemic control in patients suffering with T2DM once it is added as an adjunct to both diet and exercise (Frederich, McNeill et al. 2012). Moreover it is taken once daily without major side effects (Chaplin 2011).

A number of different studies which were carried out to study the role of saxagliptin for the treatment of T2DM either as monotherapy or in combination-regimens, have demonstrated a favourable safety profile. The commonest established benefits of saxagliptin include significant reductions in both fasting plasma glucose and postprandial glucose levels, low risk of hypoglycaemia, good tolerability profile, improvement of b-

cell function, reductions in HbA1C and a neutral effect on body weight (Rosenstock, Sankoh et al. 2008, Chacra, Tan et al. 2009, Kania, Gonzalvo et al. 2011).

The most common adverse events reported in patients treated with saxagliptin were upper respiratory tract infection, urinary tract infection, headache, nausea, cough, nasopharyngitis and constipation (Rosenstock, Sankoh et al. 2008).

Vildagliptin

Vildagliptin is another novel, selective DPP-4 inhibitor designed to be orally administrated once daily with the intention of treating patients with T2DM (Neumiller 2009, White 2009). Similarly to the two aforementioned DPP-4 inhibitors sitagliptin and saxagliptin, vildagliptin has an effective and good tolerability profile (Bosi, Dotta et al. 2009) demonstrating benefits such as weight neutrality, significant reductions of blood glucose levels and Hba1C either used as monotherapy or in combination with other antidiabetic agents (Rosenstock, Sankoh et al. 2008) metformin (Bosi, Dotta et al. 2009) or insulin (Fonseca, Schweizer et al. 2007) and also shows low risk of hypoglycaemia (Ahren 2007). Despite its good and safe profile, some adverse events have been reported in a number of patients treated with vildagliptin including upper respiratory tract infections, headaches, nasopharyngitis, dizziness, and more rarely hepatic dysfunction (Neumiller 2009).

4.3. Current established agents for the treatment of T2DM

Although currently there is an extensive range of novel oral antidiabetic agents for the treatment of T2DM as aforementioned above, there are a number of well-established drugs used for many years (Figure 14). These antidiabetic agents can be subdivided into four main categories depending to their mode of action: Sulphonylureas which stimulate insulin secretion, thiazolidinediones which improve insulin action, biguanides which reduce hepatic glucose production and the alpha-glucosidase inhibitors which act by delaying the digestions and the absorption of intestinal carbohydrates (Nicholson and Hall 2011).

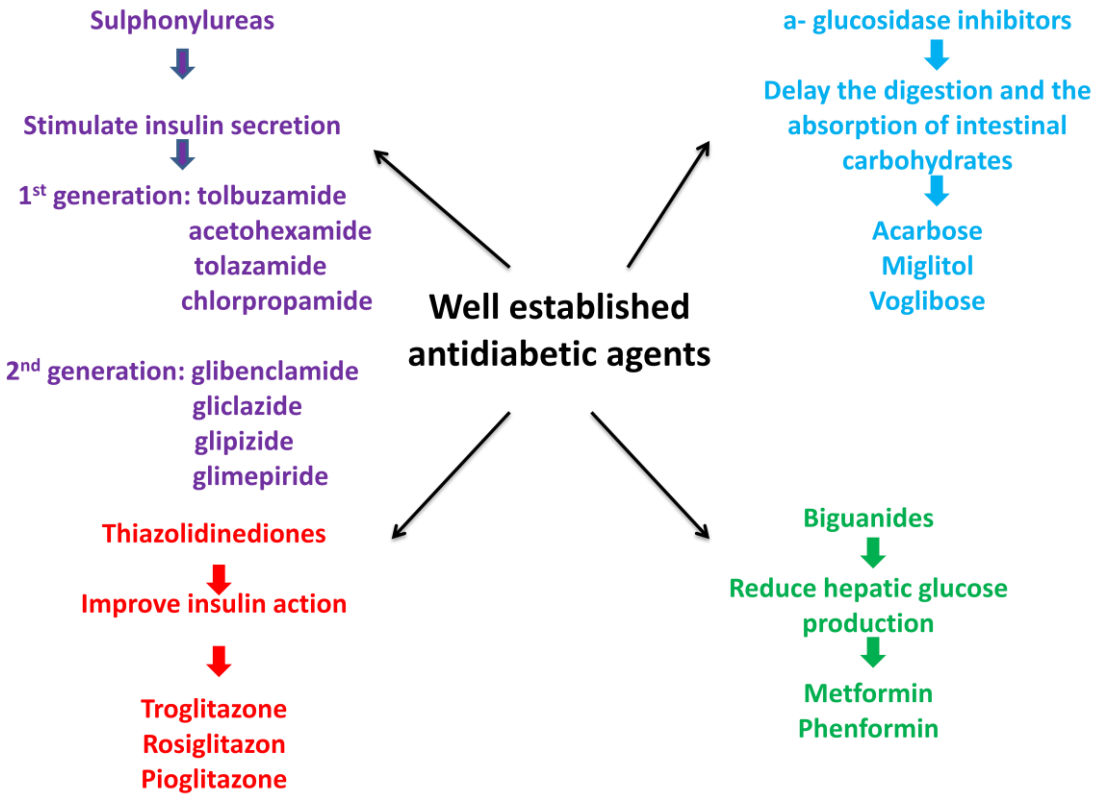


Figure 14: Well established antidiabetic agents

Well established antidiabetic agents include sulphonylureas which stimulate insulin secretion, biguanides which reduce hepatic glucose production, thiazolidinediones which improve insulin action and finally alpha-glucosidase inhibitors which act by delaying the digestions and the absorption of intestinal carbohydrates.

Sulphonylureas

Sulphonylureas are the first oral agents which have been used extensively for the treatment of T2DM for nearly half a century. (Nicholson and Hall 2011). These agents are divided into first and second generation sulphonylureas. Those belonging to the first generation were developed by the 1960s and include tolbutamide, acetohexamide, tolazamide and chlorpropamide (Krentz and Bailey 2005, Noh, Graveling et al. 2011). Due to the fact that according to a number of multicentre trials, these drugs were reported to cause some negative cardiovascular effects (Nicholson and Hall 2011), by 1970s were largely supplanted by the second generation sulfonylureas which are associated with a much lower incidence of adverse reactions and which include glibenclamide, gliclazide, glipizide and the latest one glimepiride

which was introduced by the late 1990s (Ballagi-Pordany, Koszeghy et al. 1990, Langtry and Balfour 1998).

Sulphonylureas mainly act by stimulating insulin secretion from the β-cells of the pancreatic islets (Nicholson and Hall 2011). Having duration of action between 12 to 24 hours and being well absorbed these agents remain until nowadays a popular choice for the treatment of T2DM and should be introduced at a low dose (Krentz and Bailey 2005).

Their efficacy has been evaluated in a number of retrospective and prospective studies where they showed blood-glucose lowering efficacy and ability to decrease the levels of HbA1C by 1-2% (Nicholson and Hall 2011). On the other hand, the commonest and most serious adverse effect of sulphonylureas is hypoglycaemia which can provoke cardiac ischaemia in patients suffering from coronary disease (Harrower 2000, Desouza, Salazar et al. 2003). Other well established adverse effects include increase in appetite and weight gain and subsequently are not the first choice for obese patients, gastrointestinal problems such as nausea, vomiting, diarrhea (Krentz, Ferner et al. 1994, Harrower 2000), while more rare and uncommon adverse events include fever, jaundice and blood dyscrasias (Krentz and Bailey 2005).

Biguanides

Biguanides are also referred as glucose-lowering agents as they manage to lower blood sugar. They include two drugs, metformin and phenformin which were introduced in 1950s but later on around 1970s phenformin was withdrawn from the market (Nicholson and Hall 2011). Metformin is considered to be an ideal first-line agent for the treatment of diabetes with a mostly favourable side-effect profile and a variety of metabolic effects (Nicholson and Hall 2011). It acts mainly by improving insulin sensitivity, enhancing peripheral glucose uptake, suppressing glucose production while it also decreases hepatic gluconeogenesis and achieves a reduction in plasma triglycerides (Nicholson and Hall 2011). Moreover, it is considered an ideal choice for obese patients as it does not cause weight gain but may cause even weight loss in some patients (Nicholson and Hall 2011) and most importantly it is not associated with hypoglycaemia when used as monotherapy but can do so when is used in combination with other antidiabetic agents such as insulin (Nicholson and Hall 2011). It is also one of the least expensive antidiabetic agents which should be administrated usually with or before meals in order to avoid any unwanted gastrointestinal events, starting with a dose of 500 or 850 mg once daily or 500mg twice daily with the dose being increased slowly (Nicholson and

Hall 2011). Metformin is contradicted in cases where the patient suffers from impaired renal function, cardiac or respiratory insufficiency, and liver disease or to those who are abusing alcohol (Nicholson and Hall 2011).

Alpha-glucosidase inhibitors

Alpha-glucosidase inhibitors block the activity of an enzyme of the small intestine called alpha-glucosidase, causing in this way glucose absorption to be delayed and thereby decreases meal-related blood glucose increases (Krentz and Bailey 2005, Nicholson and Hall 2011). The first alpha-glucosidase inhibitor was introduced in the market in the early 1990s and was acarbose followed by two other agents miglitol and voglibose (Krentz and Bailey 2005). They have a good safety record, without causing weight gain, reduce postprandial hyperinsulinaemia, lower plasma triglyceride concentration and decrease HbA1C usually by 0.5-1.0% (Lebovitz 1998, Holman, Cull et al. 1999). Moreover they do not cause hypoglycaemia when used as monotherapy but can do so when they are used in combination with other antidiabetic drugs such as insulin or sulphonylureas (Krentz, Ferner et al. 1994). On the other hand, limited gastrointestinal tolerability, relatively high cost, gastrointestinal effects like abdominal discomfort, flatulence and diarrhea comprise the commonest negatives of alpha-glucosidase inhibitors (Krentz and Bailey 2005). Finally, acarbose must be started in low doses and should be taken with meals however; it should not be taken when meals are missed (Nicholson and Hall 2011).

Thiazolidinediones

TZDs also known as insulin sensitizers, appear to improve insulin sensitivity of the whole-body by lowering HbA1C, reducing fasting and postprandial glucose concentrations and by increasing the efficiency of glucose transporters. Simultaneously, these insulin sensitizers also decrease hepatic glucose production and promote peripheral adipose tissue lipogenesis (Krentz and Bailey 2005, Natali and Ferrannini 2006, Fonseca and Kulkarni 2008, Noh, Graveling et al. 2011).

The first TZD approved for the treatment of T2DM was troglitazone and was introduced in 1997, but was withdrawn from the market only three years later in 2000 as it was found to cause hepatotoxicity resulting in fatalities (Nicholson and Hall 2011).

Two other TZDs were introduced, rosiglitazone and pioglitazone (Nicholson and Hall 2011). They have the ability to be rapidly and nearly completely absorbed, causing significant

reductions in HbA1C and are not associated with hypoglycaemia when used as monotherapy but may do so when used in combination with other antidiabetic agents (Krentz and Bailey 2005). Common associated adverse events include effects on the cardiovascular system, an incidence of myocardial infarction, newly arising or worsening oedema whose risk further increases with age and drug dose with the female gender being more predisposed to it (Nicholson and Hall 2011). Another adverse event is the low risk of hepatotoxicity (Scheen 2001). Due to these adverse events, and subsequently, due to increased risk of mortality, in 2012 Europe has withdrawn rosiglitazone and in the USA its licence was restricted (Colhoun, Livingstone et al. 2012). Pioglitazone was established as a valid second-line treatment for patients with T2DM, in situations where metformin was deemed unsuitable or ineffective (Nicholson and Hall 2011, Colhoun, Livingstone et al. 2012).

4.4. Other approaches of treatment

In contrast to T2DM which is mainly attributed to genetic and lifestyle factors resulting in high insulin resistance (Miller and St Onge 2011), T1DM is a chronic, progressive, autoimmune disease where the beta-cells of the pancreas are targeted for destruction resulting in a lifelong commitment to insulin replacement therapies as there is an inability to regulate blood glucose levels and other metabolites (Giannoukakis, Rudert et al. 1999, Wong 2010, Courtney, Pfeifer et al. 2011, Tooley, Waldron-Lynch et al. 2012).

Increased understanding about the pathophysiology of b-cell destruction provided a narrow window of potential therapeutic benefit by developing new therapeutic agents as well as establishing novel alternative therapies comprising the main aim to eliminate the requirement of exogenous insulin administration (Courtney, Pfeifer et al. 2011). A number of different strategies have been developed with the intention of either preventing or reversing the development of immune-mediated response observed in T1DM including b-cell regeneration, b-cell replacement and b-cell protection (Raskin and Mohan 2010). B-cell replacement refers to whole pancreas or islet transplantation, b-cell protection includes ways to prevent b-cell apoptosis or death and finally b-cell regeneration comprises islets from stem cells using stem cell technologies (Guz, Nasir et al. 2001, Russ, Sintov et al. 2011).

4.4.1. Islet cell transplantation

Islet cell transplantation is an extremely promising therapy for the treatment of patients with T1DM by re-establishing normoglycemia (Fiorina, Shapiro et al. 2008) as well as for

restoring endogenous insulin secretion in patients suffering with T2DM (Ricordi 2003, Fiorina, Shapiro et al. 2008). The first report of islet cell transplantation was that of Watson-Williams and Harshant, 1984 where they transplanted into a young patient suffering from diabetic ketoacidosis small fragments from a sheeps' pancreas, followed by significant progress and improvements in this field (Shapiro, Lakey et al. 2000, Ricordi and Strom 2004). It is a minimally invasive procedure (Shapiro, Lakey et al. 2000) including implantation of the islet preparations into the recipients liver, followed by their slow infusion with the usage of a closed bag and finally the entry tract is plugged with a haemostatic sealant (Baidal, Froud et al. 2003, Froud, Yrizarry et al. 2004, Fiorina and Secchi 2007). Another advantage of this method is that it does not require any significant surgery or general anaesthesia, showing approximately 70% insulin independence at the 1 year mark for recipients with islet transplants (Ichii and Ricordi 2009).

Possible complications after their infusion into the recipients liver may include bleeding, portal hypertension and portal vein thrombosis (Brennan, Shannon et al. 2004, Hafiz, Faradji et al. 2005) while the morbidity and mortality rates are reported to be very low (Villiger, Ryan et al. 2005). Frequent and severe hypoglycaemic events, history of severe hypoglycaemia awareness and clinical and emotional problems associated with the exogenous insulin therapy comprise the commonest indications making a patient an ideal candidate for this procedure (Fiorina, Shapiro et al. 2008).

4.4.2. Pancreas transplantation

Pancreas transplantation of either whole or half pancreas is considered to be a very effective procedure during which a healthy pancreas taken from a deceased donor is transplanted into a diabetic patient, whose pancreas no longer functions properly, giving excellent long-term results (Sutherland 1997). Moreover pancreas transplantation may achieve up to 80% of insulin independence after 1 year of transplantation, while in many cases, this procedure may also effectively result in cure of diabetes (Sutherland, Gruessner et al. 2001, Calne 2005).

According to the American Diabetes Association, 2003 this procedure is advised to be performed in patients approximately after 20 years of establishment of diabetes providing a number of beneficial effects such as eliminating the need for exogenous insulin and daily blood glucose measurements as well as the dietary restrictions, which significantly improve the life of diabetic patients. Furthermore, of major importance is the elimination of the most

commonly and serious observed complications such as hypoglycaemia and hyperglycemia (Association 2003). Despite its beneficial profile, pancreas transplantation in contrast to islet cell transplantation is a major surgical procedure encompassing the risks associated with any major surgical procedure which seems to improve steadily (Hampson, Freeman et al. 2010).

Pancreas transplantation is regarded as an acceptable therapeutic alternative to patients who meet the following criteria: 1. Have a history of frequent and severe metabolic complications such as hypoglycaemia and hyperglycaemia, 2. Failure to respond to insulin-based management, 3. Having either clinical or emotional problems with exogenous insulin therapy, 4. Those with established end-stage renal disease and finally 5. Those who have already had or are planned to have kidney transplantation (Association 2003).

4.4.3. Gene therapy

As discussed above, the main feature of T1DM is the absence of islets which subsequently leads to insulin deficiencies and hypeglycemia (Giannoukakis, Rudert et al. 1999, Wong, Hawthorne et al. 2010). A novel possible approach for replacing endogenous insulin production and thus achieve maintenance of euglycemia is the gene therapy. This is achieved by introducing a foreign gene into any cell type in the body with the intention of allowing to it to produce insulin (Halban, Kahn et al. 2001), and for this purpose, various gene transfer methods can be used, including both viral vectors and non-viral methods (Wong, Hawthorne et al. 2010).

Non-viral methods include: 1. calcium-phosphate co-precipitation, which has the advantage of being a simple and cheap method for genetically modifying pancreatic cells, 2. liposomes which are highly effective transfection agents of cells, having the advantage of being capable to be injected into the bloodstream which makes them a less invasive treatment (Torchilin 2006), 3. direct microinjection where DNA is directly injected into the cells. However this technique is labour intensive as each cell needs to be targeted individually and therefore, is unsuitable for targeting large number of cells and finally electroporation can also be used as a non-viral method for the creation of permeable membranes for gene transfer by high voltage applications to cells (Wong, Hawthorne et al. 2010).

On the other hand viral vectors can be used for gene therapy where in order to be effective a viral vector needs to be simple to produce in large numbers, be able to transducer both dividing and non-dividing cells and also allow for long-term expression of the transgene (Lu

2004). For this purpose, adenoviral vectors and lentiviral vectors are most commonly used (Robbins, Tahara et al. 1998). Adenoviruses appear to have a more advantageous profile in comparison to lentiviral vectors as they are capable of transuding both dividing and non-dividing cells (Yoon and Jun 2002) whereas using lentroviral transduction, cells which are currently dividing can only transduce (Yoon and Jun 2002). Moreover, according to the available literature adenoviruses have been shown to be able to infect insulin secreting cells (Leibowitz, Beattie et al. 1999) and also to transducer rodent islets (Sigalla, David et al. 1997).

Apart from viral and non-viral methods, hepactocytes and stem cells can also be used as genetic manipulations in T1DM for achieving euglycemia. In nature, hepatocytes are similar to β-cells and consequently are good candidates for genetic manipulations as they are already equipped with the machinery to respond to fluctuating glucose levels. Hepatocytes can facilitate the removal of glucose from the blood-stream and also can be modified with the purpose of producing insulin as glucose levels increase (Efrat 1998, Halban, Kahn et al. 2001).

Research on stem cells therapy is increasing exponentially worldwide, representing a promising tool for treating diabetes generating a huge amount of excitement, anticipation and hope to the patients suffering from this disease (Brignier and Gewirtz 2010, Bhandari, Seo et al. 2011). They have emerged as novel therapeutic tools in immunotherapy, gene therapy and regenerative medicine (Calne 2005) where both adult and embryonic stem cells can be used for this purposes. Moreover, there is a number of different tissue sources from which stem cells can be obtained such as blood, bone marrow, placenta, brain as well as from the umbilical cord and umbilical cord blood following the birth of a baby (Magatti, De Munari et al. 2008, Murphy, Rosli et al. 2010). Stem cells appear to be more advantageous in comparison to embryonic stem cells as they are easily available, are associated with a low risk of graft-versus host diseases and most importantly they are not associated with any ethical issues like embryonic stem cells whose use is considered somehow ethically unacceptable also more difficult to handle (Zhao and Mazzone 2010, Bhandari, Seo et al. 2011).

Stem cells have a number of different benefits and unique characteristics which make them an attractive option as therapeutic tools. They have the ability to differentiate into every cell type in the body forming a limitless number of cell types. There are limitless sources from

which they can be obtained and thus are abundantly available. Stem cells are able to self-renew and can always give rise to a differentiated progeny (Calne 2005, Brignier and Gewirtz 2010, Wong, Hawthorne et al. 2010, Bhandari, Seo et al. 2011). Moreover, there exists extensive evidence that insulin secreting cells can be obtained from embryonic stem cells (Soria, Roche et al. 2000, Lumelsky, Blondel et al. 2001). Taking together all of their advantages, it is of major importance to keep in mind that their use may achieve either to reduce or even eliminate the necessity for repeated administration of the therapeutic cells (Brignier and Gewirtz 2010).

According to the available literature, stem cell transplantation can improve the metabolic profiles of different diabetic animal models, even their treatment (Soria, Roche et al. 2000, Herzog, Chai et al. 2003, Tang, Cao et al. 2004). And their use can be used for the treatment of various acute and chronic diseases such as T1DM, Parkinson disease and heart failure (Brignier and Gewirtz 2010).

4.4.4. Drugs for the prevention of T1DM

There are a number of different drugs (Table 1) which are currently in different phases of clinical trials and which are considered as potential compounds for the prevention of T1DM.

Table 1: Drugs as potential compounds for the prevention of T1DM **Compound**	**Mode of action**
Oral insulin	Protective immunity
Abatacept	Prevents destruction of β-cells
Otelixizumab	Prevents destruction of β-cells
GAD	Prevents destruction of β-cells

Oral insulin

Due to the autoimmune nature of T1DM, the immune system attacks and destroys the insulin producing β-cells resulting in a decreased ability of the body to produce insulin (Raskin and Mohan 2010). In order to overcome this phenomenon, clinical trials tested the potential role of autoantigen administration with different ways including intranasal, oral and aerosol inhalation of insulin therefore suggesting a potential role for providing a protective immunity

by preventing the immune system from attacking and destroying the β-cells (Raskin and Mohan 2010).

Abatacept

Abatacept functions by inhibiting the activation of T-cells and more specifically decelerates the T-cell mediated autoimmune destruction of β-cells, preserving in this way their function (Raskin and Mohan 2010, Orban, Bundy et al. 2011). Taking together its action with its good safety and tolerability profile, this compound is considered to be an excellent candidate for assessing T1DM.

Otelixizumab

Monoclonal antibodies have also been tested as potential compounds for the management of T1DM. Otelixizumab is a specific monoclonal antibody which also down-regulates and blocks the function of the effector T-cells in order to prevent further autoimmune assault, preventing β-cell loss (Raskin and Mohan 2010, Miller and St Onge 2011). Results from different clinical studies indicate that otelixizumab administration for just a short period of time, results in a number of different metabolic benefits such as arrested deterioration of insulin production and prevention of exogenous insulin administration (Miller and St Onge 2011, Tooley, Waldron-Lynch et al. 2012), while it is also postulated that its combination with either insulin or other immune modulators may maximise its efficacy and provide further benefits on T1DM (Bresson, Togher et al. 2006).

GAD

Autoantigens are an alternative approach for T1DM with the intention of inducing immunologic tolerance in recent years (Ludvigsson 2009). One major autoantigen which has been recognised in T1DM is GAD- 65, while GAD-alum is a recombinant human which functions as an antigen-specific immune modulator (Raskin and Mohan 2010, Tooley, Waldron-Lynch et al. 2012). GAD stands for glutamic acid decarboxylase and is an enzyme presented in the pancreas.

Different studies were carried out in order to test the efficacy of those autoantigens and the results of those studies demonstrated a number of benefits which arise from their use including a slowing or prevention of the autoimmune destruction of the pancreatic islets, maintenance of residual insulin secretion, improvement of glucose control as well as probable

diminishment of both acute and long-term diabetic complications (Jun, Khil et al. 2002, Ludvigsson, Faresjo et al. 2008).

5. Conclusion and Future aspects

Diabetes is a complex endocrine and metabolic disorder leading to variable degrees of insulin resistance and pancreatic beta-cell function. T1DM is an autoimmune disease characterised by absolute insulin deficiency and destruction of the β-cells of the pancreas obligating patients to have a lifelong commitment toward permanent insulin treatment with the intention of regulating blood glucose levels and other metabolites. On the other hand, T2DM is the most common form and is also known as non-insulin dependent diabetes mellitus. Caused by the body's ineffective use of insulin, T2DM is associated with high morbidity and mortality rates.

The prevalence of this costly and chronic disease is increasing rapidly in the 21st century due to the modern lifestyle which is characterised mainly by obesity and lack of exercise. Taken together its incidence with its progressive natural history, variable pathogenesis and the resultant complications, there is undoubtedly an urgent need for the development of new treatment strategies which will make the life of patients much easier, alleviating at the same time their stress. Management of either T1DM or T2DM starts by globally educating patients the benefits of lifestyle modifications encouraging increased physical activity, increased consumption of dietary fibres, reduction of weight and also teaching the importance of self-monitoring blood glucose, all to achieve the intent of enhancing the quality of life, alleviation of stress and also relief of underlying symptoms.

Due to the variable pathogenesis of the disease, different acting pharmacological compounds need to be used at different stages of the disease. But unfortunately each of the antidiabetic drugs is partly associated with unwanted side effects such as weight gain, hypolgycemia, gastrointestinal events and heart failure and therefore novel compounds need to be developed in order to achieve and maintain glycaemic control, delay any microvascular or macrovascular complications and improve insulin action showing a more favourable profile.

Due to the fact that T2DM is non-insulin dependent, initial management focuses on lifestyle alterations such as increased exercise and dietary modifications. In cases where these modifications fail to sustain blood glucose levels, metformin or insulin are then used. Metformin has been established as a good first line pharmacotherapy for patients with T2DM due to its long-term safety and beneficial profile. Despite the different attempts to control and

manage blood glucose levels, the majority of patients will eventually require insulin administration either alone or in combination with other antidiabetic drugs. This can be either given as CSII or as MDI where both of them have the same aim, to improve blood glucose control and avoid hypoglycaemia. CSII has been shown to significantly improve blood glucose control, minimise the risk of hypoglycaemia and in many cases even to prevent some of the diabetes associated complications. MDI was developed as an alternative therapeutic option and comprises both rapid-acting (insulin lispro, insulin aspart, insulin glulisine) and long-acting (insuling glargine, insulin detemir) insulin analogues. However, subcutaneous insulin administration makes patients' lives more difficult as there is a need of continuous and multiple injections and therefore in order to overcome this problem, novel insulin combinations have been developed including inhaled and oral insulin.

The incretin system has been developed recently as an additional treatment option for glucose lowering purposes, holding a promise to overcome any barriers of the other antidiabetic drugs. This includes GLP-1 receptor agonists such as exentadide and liraglutide and DPP-4 inhibitors such as sitagliptin, saxagliptin and vildagliptin where both classes help patients to achieve their glycemic goals.

Despite the fact that today there is an extensive range of novel antidiabetic drugs for clinicians to prescribe to diabetic patients, there is a number of well-established compounds which have been used for many years and that will continue to be used into the future due to their important implication within the disease. There drugs are biguanides, SFUs, alpha-glucosidase inhibitors and TZDs.

Finally, alternative therapeutic options with the aim of either preventing or reversing the immune-mediated response observed in T1DM include islet-cell transplantation, pancreas transplantation, gene therapy and a few novel drugs currently under investigation. According to the available literature, islet-cell transplantation may facilitate to restore endogenous insulin secretion and establish normoglycemia. Pancreas transplantation has been shown to achieve up to 80% insulin independence approximately 1 year after its administration. Gene therapy which aims to help the body to produce insulin by introducing a foreign gene to the cells and finally oral insulin, abatacept, otelixizumab and GAD which are potential compounds for the prevention of T1DM, whose effectiveness is currently tested in a number of different clinical trials.

In conclusion, it can be said that in order to be able to provide the best management for diabetes, apart for educating patients for their condition and advising them to have lifestyle modifications and prescribing them different drugs, there is a need for continued collaboration between scientists, clinicians and patients. Additionally, there exists a requirement for continued clinical trials to consist of the intention with regard to developing novel compounds which will provide the best-long-term efficacy and safety profiles, lowering at the same time the possible risk of hypoglycaemia and avoiding unwanted side effects observed with the currently existing antidiabetic drugs.

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