

Type 1 Diabetes Mellitus: Redefining the Future of Cardiovascular Complications with Novel Treatments

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1. Introduction

Diabetes Mellitus (DM) is a disease that was identified centuries ago, in around 1500 BC (King and Rubin, 2003). The word 'Diabetes' means "running through" (Holt, 2004) which is used to describe the excessive output of urine in this disease. "Mellitus" meaning "sweet" identifies the nature of the urine in patients suffering from DM (Widmaier *et al.*, 2004). Thomas Willis was the first to differentiate DM from other polyurias in 1674 (Eknoyan and Nagy, 2005). In 1776, Matthew Dobson demonstrated that the sugar present in the urine was also present in the blood and was associated with its rise (Holt, 2004). In 1848, Claude Bernard's experiments on the liver showed that glycogen stored in the liver produced sugar and he hypothesized that glycogenolysis caused the disease. Although his discovery that "sugar production is a normal function of the animal" was revolutionary, it did not quite explain the etiology of the disease. In 1889, Oskar Minkowski confirmed that the ablation of the pancreas in dogs resulted in DM (Farmer, 1952). Frederick Banting and Charles Best were the medical scientists that discovered insulin, later in 1921, the lack of which, it was made clear, caused DM (Voet and Voet, 2004). They used the extract of a dog's fresh pancreas and demonstrated that upon administration of 10mL of extract to blood, blood glucose level is decreased from 0.3% to 0.17% (Rosenfeld, 2002). Some 30 years later, in 1953, Frederick Sanger was able to determine the complete amino acid sequence of the protein for the first time in history (Boron and Boulpaep, 2009).

Today, DM is defined as a carbohydrate disorder characterized by impaired insulin secretion and/or peripheral insulin resistance leading to hyperglycemia (Beers and Berkow, 1999). It is considered to be the third leading cause of death after heart disease and cancer in the United States (Voet and Voet, 2004) and its incidence is expected to rise to 366 million people by the year 2030 (Wild *et al.*, 2004).

Diabetic patients have symptoms such as, thirst, polyuria, blurring of vision and weight loss. In extreme cases, ketoacidosis may develop leading to coma and ultimately death (Alberti and Zimmet, 1998). Diabetes Mellitus is classified according to etiology to two major types:

1.1 Type 1 diabetes mellitus

Although both type 1 Diabetes Mellitus (type 1 DM) and type 2 Diabetes Mellitus (type 2 DM) are characterized clinically by hyperglycemia, they have their differences. Type 1 DM

occurs commonly during childhood or adolescence therefore also named juvenile onset DM and may develop diabetic ketoacidosis (Beers and Berkow, 1999). Of all diabetes cases, 10% are type 1 DM (Holt, 2004). It includes all autoimmune and idiopathic causes of insulin-secreting β cell destruction resulting in absolute insulin deficiency (Alberti and Zimmet, 1998). The patient is usually genetically predisposed to DM, however, an environmental insult, such as a virus is needed to trigger the pathological process of the disease (Wilson *et al.* 1991). It is evident that CD4+ and CD8+ T lymphocytes are activated in the pancreatic lymph node and later infiltrate the pancreas causing inflammation (insulinitis) (Yang and Santamaria, 2003). During this time, the body develops an immune response that sees pancreatic islet cells as 'nonself' and starts destroying its β cells (Wilson *et al.*, 1991). Specific causes of β cell destruction, such as cystic fibrosis or mitochondrial defects, are excluded from this classification (Alberti and Zimmet, 1998). Recently, type 1 DM was subdivided into type 1 A and type 1 B. Type 1A diabetes mellitus is described as a disease with immune mediated, selective destruction of insulin producing β cells, with the presence of anti-islet autoantibodies. Whereas type 1B diabetes mellitus, exhibits inflammation of the pancreas, but lack of anti-islet autoantibodies (Rhoades and Bell, 2009). Patients with type 1 DM require daily subcutaneous insulin administration as a treatment (Seifter *et al.*, 2005). Administration of exogenous insulin cannot be compared to the fine control of minute to minute insulin secretion that the pancreas provides (Hakim, 2002); for that reason, human islet cell transplantation has been accepted and applied as an alternative treatment for patients with type 1 DM (George, 2009).

1.2 Type 2 diabetes mellitus

Type 2 DM is also known "Adult-onset Diabetes" (Beers *et al.*, 2006), and represents 90-95% of diabetic patients (Creager *et al.*, 2003; Seifter *et al.*, 2005). This percentage is expected to increase in the year of 2025 to reach 300 million diabetic patients (Rhoades and Bell, 2009) due to sedentary lifestyle and increase in obesity in addition to age progression (Boron and Boulpaep, 2009; King *et al.*, 2005). As the intake of glucose increases insulin secretion is elevated. After a while, insulin secretion becomes inadequate due to peripheral resistance of insulin receptors (Beers *et al.*, 2006; King *et al.*, 2005). Insulin resistance decreases glucose-mediated insulin transport and metabolism (Rhoades and Bell, 2009), resulting in a defect of the compensatory insulin secretion (Mcphee *et al.*, 2008). It is worth noting that in type 2 DM reactive oxygen species are generated resulting in endothelial dysfunction, cardiovascular complications and renal disease (Hayashi *et al.*, 2010; Seifter *et al.*, 2005). Insulin resistance develops when insulin signaling pathway is interfered in adipose, skeletal and hepatic cells (Seifter *et al.*, 2005). As a result of insulin resistance, glucose transport inside adipocytes and skeletal muscle is reduced and the suppression of glucose output from the liver is impaired. (Rhoades and Bell, 2009)

2. Complications of diabetes mellitus

Chronic hyperglycemia causes blood vessels injury (Seifter *et al.*, 2005) which is divided into two types depending on the size of vessels injured. Small vessels injury in diabetes is a microvascular complication whereas injury of large blood vessels determines a macrovascular complication (Hayashi *et al.*, 2010, Kalani, 2008). Microvascular and macrovascular complications occur in DM both types 1 and 2 (Retenakaran and Zinman,

2008; Rhoades and Bell, 2009). These injuries cause on the long run acceleration in atherosclerotic formation (Seifter *et al.*, 2005). In type 1 diabetic patients, hyperglycemia has been correlated with a variety of events in cardiovascular pathology, initiated with endothelial dysfunction and progressed to develop arterial stiffness (Tabit *et al.*, 2010). Thus, a relation has been clearly established to link glycemia with cardiovascular events in type 1 DM, based on the fact that glycemia itself is the only factor mediating a risk factor for cardiovascular risk in the absence of other risk factors (Retenakaran and Zinman, 2008). One way of reducing cardiovascular risk and renal outcomes in type 1 DM is to initiate an early intensive therapy for its management.

2.1 Microvascular complications

Microvascular complications include nephropathy, retinopathy and neuropathy (Coccheri, 2007; Fowler, 2008; Seifter *et al.*, 2005). There is a direct clinical practice to link these complications with DM type 1 rather than type 2 (Retenakaran and Zinman, 2008). Of note, type 2 DM may manifest development of microvascular complication 7 years preceding diagnosis (King *et al.*, 2005).

2.2 Macrovascular complications

These define the pathophysiology of cardiac disease in type 1 DM which includes atherosclerosis, coronary artery diseases, stroke and peripheral arterial disease (Retenakaran and Zinman, 2008; Seifter *et al.*, 2005). These cardiovascular complications are mostly focused in clinical practice to type 2 DM (Fowler, 2008), and increased in rate as well in type 1 DM (Mcphee *et al.*, 2008); however, the mortality impact of cardiovascular diseases in both DM types 1 and 2 is similar (Juutilainen *et al.*, 2008) contributing to 80 % of the mortality and morbidity (Coccheri, 2007). More alarmingly, development of macrovascular complication might not manifest in type 1 DM until 10 years proceeding diagnosis (King *et al.*, 2005).

Endothelial and smooth muscle dysfunction has been associated with type 1 diabetic patients, which in turn might cause hypertension, a major risk factor for developing cardiovascular diseases (Retenakaran and Zinman, 2008). Also lipid abnormalities in type 1 DM, decrease in HDL and increase in LDL compositions, are being incidents of causing cardiovascular diseases (Retenakaran and Zinman, 2008). High levels of ketone bodies are produced from the body as an alternate fuel in the absence of glucose as a main source of energy in severe starving conditions, mainly hyperglycemia (Seifter *et al.*, 2005), or an acute multiplication of type 1 but not type 2 DM (Rhoades and Bell, 2009). Three types of ketones can be generated in the body these are acetone whose odor can be detected overtly *via* breath, acetacetic acid and β -hydroxybutyric acid whose levels may elevate in the blood and be excreted in urine, which is followed by cations and fluid loss ultimately leading to coma (Rhoades and Bell, 2009).

3. Renin angiotensin system

With the discovery of renin in 1898, Robert Tigerstedt and Per Bergman were able to fill a gap present in the understanding of fluid balance, hypertension and cardiovascular disease (Basso and Terragno, 2001; Phillips and Schmidt-Ott, 1999). The Renin Angiotensin System (RAS) plays an important role in maintaining normal blood volume and blood pressure. When salt and water intake is reduced, the role of RAS becomes critical (Rhoades and Bell, 2008). Upon low plasma volume, intrarenal baroreceptors found in the afferent arteriole

walls sense a decrease in stretch and neighboring granular cells increase renin synthesis and release (Boron and Boulpaep, 2009). The granular cells of the juxtaglomerular apparatus of the kidney produce renin (Widmaier *et al.*, 2004). At first, prorenin is synthesized in the granular cells; its 23 amino acid signal peptide is then cleaved to form prorenin (Sepehrdad *et al.*, 2007). This proenzyme undergoes further modification- clipping the 43 amino acid segment from the N-terminal - to produce renin (Pool, 2007). Despite the conversion of prorenin to renin, prorenin remains the predominant form in the systemic circulation and represents 90% of the total plasma renin in humans (Pimenta and Oparil, 2009). Renin belongs to the family of aspartyl proteases, including pepsin, cathepsin D and chymosin (Verdeccia *et al.*, 2008). Unlike other aspartyl proteases however, renin is able to work at neutral pH. Moreover, renin is specific for one substrate only- angiotensinogen (Sepehrdad *et al.*, 2007). Made up of two lobes with a cleft in between renin accommodates the liver-derived angiotensinogen in its active site, where a peptide bond of angiotensinogen is hydrolyzed and the decapeptide Angiotensin I (Ang I) is released (Verdeccia *et al.*, 2008). Furthermore, this conversion is catalyzed 4-fold upon the binding of renin to its receptor (Pool, 2007). Angiotensin converting enzyme (ACE) is the principal enzyme that converts Ang I to the octapeptide Ang II (60% conversion); some other enzymes such as chymases, cathepsin G and other serine proteases account for the rest of the conversion (Weir, 2007).

3.1 Cross talk between RAS and litus

Angiotensin II was always considered in close proximity with diseases and vascular complications. This is implied from data linking high levels of Ang II to DM and vasoconstriction (causing hypertension) (Karam *et al.*, 2005), in addition to glomerular damaging ending up with nephropathy (Coccheri, 2007). Other than its direct effect as a potent vasoconstrictor, it was denoted to alter Endothelin-1 (ET-1) production (Karam *et al.*, 2005), remodel cardiovascular structure (Parmar and Jadav, 2007), modulate heart and vessels (Nuwayri-salti *et al.*, 2007), augment transforming growth factor- β (TGF- β) and boost proliferative and inflammatory events (Wiggins and Kelly, 2009). In conjunction; modulation of insulin significance is obtained (Karam *et al.*, 2005), either by directly halting signaling mechanism of insulin per se or damaging structure and function of β -cells *via* local pancreatic RAS (Coccheri, 2007). Angiotensin II has 2 receptors: Ang II Type 1 receptor (AT1R) and Ang II Type 2 receptor (AT2R). But until now AT1R rather than AT2R is assessed in the pathophysiology (Karam *et al.*, 2005; Rao, 2010) directing therapeutic agents either to target Ang II formation or block its binding to AT1R *via* Angiotensin converting enzyme inhibitors (ACEIs) and Ang II Receptor Blocker (ARB) respectively (Karam *et al.*, 2005). AT1R blockers improved vascular smooth muscle cell vasoconstriction and declined the hypertrophy of cardiomyocytes (CM) counteracting Ang II deleterious effect (Al Jaroudi *et al.*, 2005) once bound to the G-coupled protein receptor (AT1 R) (Parmar and Jadav, 2007; Wiggins and Kelly, 2009). Quite interestingly, ACEIs ameliorated insulin sensitivity and mitigated new onset of DM type 2 (Hadi and Suwaidi, 2007; Scheen, 2004). Pooled together, these G-coupled transmembrane proteins mimic insulin receptor action (Nuwayri-Salti *et al.*, 2007). Among several evidences, AT2R is more related to apoptotic actions thereby antagonizing insulin's growth effect, resulting in one way or another to boost cardiac hypertrophy mainly in type 1 DM (Al Jaroudi *et al.*, 2005). To complete this picture, a balance should be assured between both Ang II receptors to modulate a normal cardiac status in type 1 DM along the endothelial cells and cardiomyocytes (Al Jaroudi *et al.*, 2005).

The idea of blocking RAS at its point of origin was initiated around 50 years ago (Pool, 2007). The origin of these direct renin inhibitors (DRIs) was either analogues of the prosegment peptide of renin or of the AGT's N-terminal amino acid sequence (Gradman and Kad, 2008). Many peptidomimetic synthetic drugs used for renin inhibition have been launched including pepsatitn (the first renin inhibitor) and other oral drugs, including remikiren, enalkiren and zankiren (Pimenta and Oparil, 2009), but their low efficiency related to large molecular size, first-pass metabolism, incomplete intestinal absorption, hydrophobicity and short half-lives attributed their poor oral activity and bioavailability, besides the high production cost (Pool, 2007; Waldmeier *et al.*, 2007). The drug Tekturna® (United States) or Rasilez® (Europe), known with the generic name of Aliskiren, ascribed to Dr. Alice Huxley (Gradman and Kad, 2008), manufactured by Novartis Pharmaceuticals, was the first of the nonpeptide DRIs to be approved by the Food and Drug Administration (FDA) (Azizi, 2008). Thereby becoming the commercially available renin blocker in markets, prescribed through United States and Canada for an effective essential hypertension treatment in March 2007 (Azizi, 2008), and approved by European Medicine Agency (EMA) in August 2007 (Pool, 2007; Azizi, 2008).

4. Endothelin system

In 1985, Hickey *et al.* discovered a potent vasoconstricting substance from cultured endothelial cells and named it endotensin or endothelial contracting factor. Later on, Yanagisawa *et al.* isolated the same substance from cultured porcine aortic and endothelial cells and renamed it endothelin. Endothelin (ET) is a naturally occurring polypeptide (Prasad *et al.*, 2009) produced by the endothelium (Shah *et al.*, 2009; Wikes *et al.*, 2003; Ying wu, 2003) and consists of 21 amino acids. It is present in three isoforms ET-1, ET-2 and ET-3 (Agapitov *et al.*, 2002; Prasad *et al.*, 2009). These three isoforms are encoded by different genes but have similar structure and function (Wilkes *et al.*, 2003). They have conserved sequences of amino acids mainly at the 6 C terminus and 4 cysteine residues that form 2 disulfide bridges between residues 1 to 15 and 3 to 11 and the main difference is at the N-terminus (Prasad *et al.*, 2009). They are vasoconstrictors synthesized by vascular, right atrial and left ventricular endothelial cells, vascular smooth muscle cells and fibromyocytes. In addition, they are synthesized in extra vascular tissues such as the lungs, spleen, pancreas and nervous system (Penna *et al.*, 2006). All studies done on endothelial systems focused on ET-1, because represents the majority of the circulating endothelins (Prasad *et al.*, 2009; Schneider *et al.*, 2002) and has important role in the regulation of the cardiovascular system (Penna *et al.*, 2006; Prasad *et al.*, 2009).

4.1 Endothelin-1

Endothelin-1 is the most common isoform correlated with the cardiovascular system (Kalani, 2008). It was identified in the late 1980s by Dr. Yanagisawa and his colleagues and found to be a very potent vasoconstrictor (Prasad *et al.*, 2009; Steiner and Preston, 2008; Thorin and Webb, 2009). It has ionotropic, chemotactic and mitogenic activities. It influences salt and water retention through its effect on RAS, vasopressin release and stimulation of sympathetic nervous system. So the ultimate role of ET-1 is to increase blood pressure (Agapitov *et al.*, 2002) and to maintain vascular tone (Thorin and Webb, 2009). It is produced mostly from endothelial cells in addition to fibroblasts, cardiacmyocytes (Agapitov *et al.*,

2002; Schorlemmer *et al.*, 2008), leukocytes, macrophages (Thorin and Webb, 2009), kidney, central nervous system and posterior pituitary (Agapitov *et al.*, 2002).

Endothelin-1 exerts a paracrine/autocrine effect. In the circulation, the levels of ET-1 are in picomolars lower than that needed to cause vasoconstriction (Prasad *et al.*, 2009). Also endothelial cells secrete more ET-1 toward the vicinity of vascular smooth muscle cells than into the lumen of the blood vessels (Agapitov *et al.*, 2002).

4.2 Endothelin-1 synthesis and clearance

Endothelin-1 synthesis begins with the cleavage of a 200 amino acid peptide called preproendothelin-1 (preproET-1) to form preendothelin-1 (preET-1) (Agapitov *et al.*, 2002; Penna *et al.*, 2006). PreET-1 is then cleaved by furin endopeptidase to form big endothelin-1 (big ET-1) of 38-39 amino acids that is further metabolized by Endothelin Converting Enzyme-1 (ECE-1) to generate ET-1 of 21 amino acids (Agapitov *et al.*, 2002; Penna *et al.*, 2006). Moreover, chymase enzyme was found to produce ET(1-31) by breaking down big ET-1 at Tyr31-Gly32 bond (Agapitov *et al.*, 2002).

Furthermore, the regulation of ET-1 synthesis is at the gene level which means at the level of ET-1 messenger RNA (mRNA). The ET-1 mRNA is up-regulated by several factors including: interleukins, insulin, Ang II, tumor necrosis factor alpha and growth factor while it is down regulated by hypoxia, shear stress and nitric oxide (Thorin and Webb, 2009). The clearance of ET-1 from the plasma involves different mechanisms including: (1) endocytosis in the lungs, (2) enzymatic degradation in the liver and the kidney and (3) ET-1 binding to ET_B receptor thus forming ET_B Receptor-Ligand complex that is broken down by endocytosis (Kalani, 2008).

4.3 Cross talk between endothelin system and renin angiotensin system

The Renin Angiotensin and the Endothelin Systems are the most potent identified vasoconstrictory systems and has been suggested to be correlated with each other (Rossi *et al.*, 1999; McEwan *et al.*, 2000). This was demonstrated by several cell culture studies in cardiac fibroblasts. These studies showed that AngII increases preproET-1 mRNA expression, ET-1 levels and thus causes cardiomyocyte hypertrophy through regulation of ET_AR. Also increased levels of ET_BR via Ang II have also been identified in cultured CM (McEwan *et al.*, 2000).

The potential sites at which RAS increases ET-1 levels may be at the preproET-1 gene that possesses a *jun* sequence which is defined as the regulatory region of preproET-1. It is the site at which transcriptional regulation takes place by factors acting through G-protein-phospholipase and C-protein kinase C pathway. Another site for interaction of RAS and ET system is at the chymase enzyme which is abundant in the myocardium where it was found to produce ET (1-31) from big ETs, bearing in mind that the main function of chymase enzyme in the heart is to produce Ang II from Ang I (Rossi *et al.*, 1999). Therefore, elevated levels of ET-1 can be reduced by blocking the RAS system. Studies on patients suffering from hypertension or diabetes showed decrease in the level of ET-1 when ACEI was administered (Schneider *et al.*, 2002).

Therapies targeting these complications include the use of ARBs which proved their value as antihypertensive drugs. Losartan, an ARB, was found to decrease the destructive changes caused by Ang II on cardiac muscle beside its (Berk, 1999; Fiordaliso *et al.*, 2000) capacity to reduce blood pressure. Recently, Al Jaroudi *et al.* proved that losartan in diabetic normotensive rats supplemented with insulin was able to prevent nearly totally myocardial degeneration caused by diabetes (Al Jaroudi *et al.*, 2005) (Fig. 1).

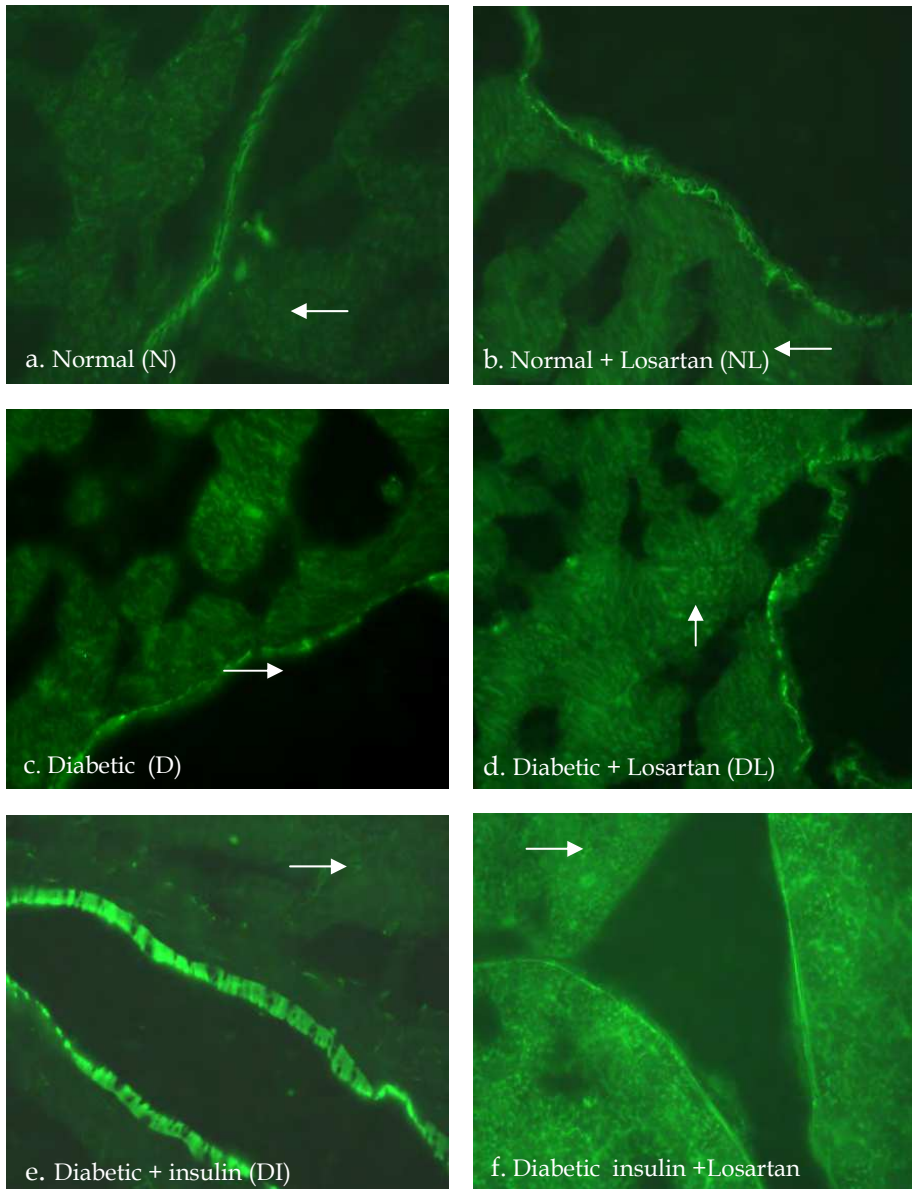


Fig. 1. Indirect immunofluorescence of CM and vascular endothelium from the different rat groups depicting insulin receptors. Noted is the increase of fluorescence in the NL group (Fig. b) when compared to the normal (N) (Fig. a) at the myocytes (arrow). There is also increase fluorescence of the myocytes of the (DL) group as compared to the (D) group, and of the (DIL) group as compared to the (DI) group (arrow).

Later, Karam *et al.* reported that the supplementation with insulin and administration of pharmacologic doses of losartan could improve cardiac contraction as well as coronary blood flow in the same normotensive rat model for type 1 DM as the one used by Jaroudi *et al.* through the modification of the affinity of ET type 1 receptor subtypes ET_AR and ET_BR to ET-1, a potent vasoconstrictor largely stimulated by Ang II (Karam *et al.*, 2005). In fact, Kakoki *et al.* have demonstrated that the ET_BRs are down-regulated by Ang II in the endothelial cells of the renal artery of diabetic rats through stimulation of increased ET-1 production (Kakoki *et al.*, 1999). On the other hand, these effects of Ang II are prevented by the ACE inhibitor Imidapril (Kakoki *et al.*, 1999). ACE inhibitors reduced also the plasma ET-1 levels in type 1 DM (Schneider *et al.*, 2002). Administration of Losartan to Ang II-treated rats restored the vasoconstrictive effect of ET-1 and decreased its tissue levels (D'Uscio *et al.*, 1997). Mc Ewan *et al.* showed that the blockade of the AT₁Rs by Losartan, in the presence of a high plasma Ang II levels, is accompanied by an increase in ET-1 production by the CM of Ang II-treated rats.

In addition to this, an increase in the ET_BRs mRNA but a decrease in the ET_ARs mRNA was also noted (Mc Ewan *et al.*, 2000). On the other hand, treatment with the selective ET_AR blocker LU135252 normalized the increased ET-1 level in the aorta, femoral artery and kidney, and the ECE activity in isolated aorta and femoral artery of rats treated with Ang II (Barton *et al.*, 1997). In cultured heart endothelial cells, Ang II stimulates ppET-1 mRNA and ET-1 production via Protein Kinase C (PKC) - dependent pathway. Calphostin, a PKC inhibitor, blocks Ang II effects (Chua *et al.*, 1993). Taken together, these results show that the ET and the RA systems are related to each other. They also demonstrate the beneficial role of ARBs and ACE inhibitors in the amelioration of the blood flow which can be altered in some disease states due to the ET-1 and Ang II constrictor effects.

5. Insulin

The discovery of insulin in 1921 was one of the most dramatic events in the history of diabetes therapy. Insulin is a small globular protein comprising of two polypeptide chains A (21 amino acid residues) and B (30 amino acid residues) held together by two disulfide bonds (Docherty and Steiner. 2003; Joshi *et al.* 2007). It is produced in the β -cells of the islets of Langerhans in the form of preproinsulin which is rapidly cleaved by the action of proteolytic enzymes into proinsulin. Further catalysis of proinsulin results in insulin and a 31 amino acid connecting peptide, C-peptide, both of which are stored for secretion in secretory vesicles in β -cells (Docherty and Steiner. 2003). The biosynthesis and secretion of insulin by β -cells primarily occurs in response to increased circulating glucose levels. During feeding, elevated glucose concentrations in the blood increase the plasma insulin level, which facilitates glucose uptake through GLUT-4 into muscle tissues for utilization as a source of energy and into adipose tissues for synthesis of glycerol. Insulin also exerts its action on liver cells as well thus promoting glycogen formation. Consequently, glucose utilization by these different tissues contribute to the decrease in the concentration of glucose in blood. On top of its profound effect in carbohydrate metabolism, insulin has a fat-sparing effect. Not only does it promote the synthesis of fatty acids in the liver, it also inhibits the breakdown of fats in adipose tissue thereby inducing fat accumulation. Hence, insulin is the major determinant of carbohydrate and lipid metabolism and has significant effects on protein metabolism. Insulin acts by binding to a receptor molecule embedded in the plasma membrane of its target tissues (Docherty and Steiner. 2003).

5.1 Insulin receptor

Insulin receptor is a member of the receptor tyrosine kinase family (De Meyts and Whittaker, 2002; Kanzaki, 2006; Klarlund *et al.*, 2003; Lawrence *et al.*, 2007; Stern, 1995; Ward *et al.*, 2008). It is a large cell-surface multi-domain glycoprotein that consists of two extracellular α -subunits (MW~125 kDa) and two transmembrane β -subunits (MW~95 kDa) (Kanzaki, 2006; Klarlund *et al.*, 2003; Lawrence *et al.*, 2007; Stern, 1995; Ward *et al.*, 2008) linked by disulfide bonds (Kanzaki, 2006; Lawrence *et al.*, 2007) into an $\alpha_2\beta_2$ heterotetrameric complex (Kanzaki, 2006). Extracellular α -subunits contain the insulin-binding site (Klarlund *et al.*, 2003) while intracellularly transmembrane β -subunits contain the insulin-regulated tyrosine kinase catalytic domain (De Meyts and Whittaker, 2002; Klarlund *et al.*, 2003; Ward *et al.*, 2008). Two nearly identical isoforms (A and B) of the insulin receptor exist due to tissue specific alternative splicing of the receptor m-RNA at exon 11. Isoforms A and B differ by the presence or absence of a 12 amino acid sequence at the carboxyl-terminus of the α -subunit (Anderson *et al.* 1993; De Meyts and Whittaker, 2002; Klarlund *et al.*, 2003; Lawrence *et al.*, 2007). Despite the known biochemical and physiologic differences of these isoforms, changes in their expression levels have not been consistently found in patients with DM (Klarlund *et al.*, 2003). Once insulin binds to the α -subunit of the insulin receptor, it induces a conformational change in the receptor that subsequently leads to the stimulation of the intrinsic tyrosine kinase activity in the β -subunits (Kanzaki, 2006; Klarlund *et al.*, 2003; Stern, 1995). This results in the transfer of phosphate groups from ATP to several tyrosine residues on the insulin receptor itself as well as phosphorylation of cellular proteins such as insulin receptor substrate-1 (IRS-1) and Shc (Klarlund *et al.*, 2003; Stern, 1995). The stimulation of the receptor tyrosine kinase allows transmission of the insulin signal to metabolic pathways such as glucose uptake upon translocation of GLUT-4 glucose transporters to the plasma membrane, glycogen synthesis, protein synthesis, and lipid metabolism within the cell. The various biological responses generated upon insulin receptor activation through insulin binding granted insulin a role in DM treatment due to its glucose lowering effects. Nevertheless, daily insulin injections continue to be a treatment for diabetic patients since 1922 despite the increasing worldwide prevalence of this disease (Sparre *et al.*, 2005). Furthermore, insulin treatment dramatically prolongs survival, but does not cure diabetes (Myers and Zonszein, 2002). Insulin treatment does not seem to be beneficial for many patients and is associated with weight gain, hypoglycemia, and failure of their glycemic control (Halimi, 2008). Therefore, new treatment modalities are intensively studied mainly the incretins, exemplified by GLP-1.

6. Definition of incretins

The intravenous infusion of glucose at a constant rate results in a biphasic insulin secretory response, in which the first peak rises rapidly followed by a slower second peak. In contrast, an oral administration of glucose followed by its gastrointestinal absorption, triggers a hormonal pathway that eventually leads to a far greater response of insulin secretion which can last as long as glucose is administered. This phenomenon is termed as the 'Incretin Effect' (Rhoades and Bell, 2008).

Incretins are hormones that are secreted by the gut upon feeding; their release alerts the pancreatic islets that nutrients will come through the gastrointestinal tract and islets start priming by vagal stimulation (Boron and Boulpaep, 2009). Glucagon-Like Peptide-1 (GLP-1) and Glucose Dependent Insulinotropic Peptide (GIP) are the major incretin hormones

produced by the L cells of the ileum and colon, and K cells of the duodenum and jejunum, respectively (Inzucchi and McGuire, 2008).

6.1 Synthesis of glucagon-like peptide-1

The proglucagon gene, located on chromosome 2 in humans, has the coding sequence of GLP-1. Pancreatic α -cells, intestinal L-cells and neural cells in the caudal brainstem and hypothalamus, express the proglucagon gene (Baggio and Drucker, 2007) and the mRNAs produced in the pancreas and intestine are identical. Its post-translational processing, however, differs in the two tissues. The post translational processing of proglucagon in pancreatic α - cells results in Glucagon, Glicentin-Related Pancreatic Polypeptide (GRPP), Intervening peptide-1 (IP-1) and the major proglucagon fragment (MPGF) (Holst, 2007). The latter codes for the production of GLP-1 (1-36 amide) and GLP-1 (1-37) and GLP-2 in the pancreas (Hui *et al.*, 2005). Whereas the intestinal L and brain cells produce GLP-1 (7-36 amide), GLP-1 (7-37), GLP-2, IP-2, and glicentin that if further cleaved produces GRPP and oxyntomodulin (Holst, 2007). The enzyme prohormone convertase (PC) 1/3 cleaves proglucagon to generate GLP-1 (Shin *et al.*, 2008). GLP-1 is an incretin hormone produced primarily by the L-cells in the mucosa of the ileum and the colon (Mannucci and Rotella, 2008). Small neurons found in the Nucleus Tractus Solitarius (NTS), caudal brainstem, also produce GLP-1 that plays the role of a neuromodulator (Berthoud, 2009). The central nucleus of the amygdala (CeA) and the paraventricular nucleus of the hypothalamus (PVN) are also sites of GLP-1 production (Kinzig *et al.*, 2002).

Recently, GLP-1 production was shown to exist in taste bud cells (Shin *et al.*, 2008; Berthoud, 2009) and in particular in α -gustducin and the sweet taste receptor subunit T1R3, that play an important role in mediating the glucose dependent secretion of GLP-1 (Shin *et al.*, 2008). GLP-1 is usually present in the circulation minutes after meal intake- far before food reaches the L-cells in the gut. Hence, its stimulation is believed to be controlled by both endocrine and neural signals (Drucker, 2007). The two active forms of GLP-1 are the 30 amino acid GLP-1 (7-36) amide and the 31 amino acid glycine extended GLP-1 (7-37) (Ban *et al.*, 2009; Mannucci and Rotella, 2008). GLP-1 (7-36) amide is far more abundant than GLP-1 (7-37) in the circulation (Mannucci and Rotella, 2008), but both have short half-lives (1.5-2 minutes) (Hui *et al.*, 2002). They are rapidly degraded into their inactive forms, GLP-1(9-36) amide and GLP-1 (9-37), respectively, by the enzyme Dipeptidyl-Peptidase-IV (DPP-IV) (Mannucci and Rotella, 2008) and eliminated through renal clearance (Hui *et al.*, 2002).

6.2 Effects of GLP-1

GLP-1 has numerous effects on glucose homeostasis. Upon interaction with its receptor on β -cells, GLP-1 increases the intracellular levels of cAMP and calcium, thereby releasing insulin (Drucker, 2007). Furthermore, sustained GLP-1 receptor (GLP-1R) signaling leads to enhanced gene transcription, insulin biosynthesis and β -cell proliferation (Elahi *et al.*, 2008; Drucker, 2007). GLP-1 was shown to increase the expression of glucose transporter 2 (GLUT2) (Elahi *et al.*, 2008), a hepatic glucose transporter that facilitates glucose transport in or out of the liver regulated by insulin (Eisenberg *et al.*, 2005) and glucokinase (Elahi *et al.*, 2008) the enzyme that phosphorylates glucose to glucose-6-phosphate (G6P) (Voet and Voet, 2004). GLP-1 has also inhibitory effects on Glucagon secretion from the α -cells, gastric emptying and food ingestion (Drucker, 2007). The role of GLP-1 in relaxing the proximal stomach and increasing gastric capacity has been demonstrated (D'Alessio and Vahl, 2004).

GLP-1 also exerts effects on the central nervous system: it promotes satiety and weight loss, inhibits food and water intake and improves memory and neuronal survival (Mannucci and Rotella, 2008; Drucker, 2007).

6.3 GLP-1 receptor

The GLP-1R belongs to the Guanine Nucleotide-Binding Protein (G-protein) coupled receptor family (GPCR) (Drucker, 2007). This seven transmembrane receptor protein is 90% identical to the rat GLP-1R and shows a 95% homology in amino acid. Its gene is found on the long arm of chromosome 6p21 in humans (Doyle and Egan, 2007). GLP-1 receptor is made up of 463 amino acid residues and has a molecular weight of approximately 65 kDa (Hui *et al.*, 2005). GLP-1 receptors are expressed in many tissues including, the central and peripheral nervous systems, heart, kidney, lungs and the gastrointestinal tract (Drucker and Nauk, 2006).

However, GLP-1 receptor expression in the pancreas is controversial. Studies have confirmed the detection of GLP-1 receptors in α , β and δ cells of the islets of Langerhans, whereas other studies indicate their expression exclusively in β cells (Doyle and Egan, 2007). In the heart, GLP-1 receptor expression was shown to exist in CM, microvascular endothelium, coronary smooth muscle cells and the highest in endocardium. In cardiac fibroblasts, however, there was no evidence in GLP-1R expression (Ban *et al.*, 2009). Low detection of GLP-1 receptor gene expression proves the existence of GLP-1 receptor in liver and muscles (MacDonald *et al.*, 2002). Abundant GLP-1R specific transcripts were found in lungs and detectable amounts in the heart (Ban *et al.*, 2009). When fragments of the N-terminus are denatured, GLP-1R loses its affinity for GLP-1. This asserts that GLP-1 receptor's N-terminus plays an important role in recognizing and binding of the GLP-1 (Doyle and Egan, 2007). Upon binding of the GLP-1 to its receptor numerous signaling messengers are activated; first, GLP-1 receptor can couple to G proteins, including G_{α_s} , G_{α_q} , G_{α_i} or G_{α_o} (Baggio and Drucker, 2007). Adenylate cyclase uses ATP to form cAMP via the stimulatory G protein. Following the increase of cAMP is a chain of events is triggered: ATP-sensitive K^+ channels shut and L-type voltage gated Ca^{2+} channels open; together with the efflux of Ca^{2+} molecules from intracellular Ca^{2+} stores, these ion channel alterations result in a subsequent intracellular rise in Ca^{2+} ions (Gomez *et al.*, 2002) and the ultimate exocytosis of granules that contain insulin. A sustained receptor signaling results in the activation of Protein Kinase A (PKA), gene transcription, insulin biosynthesis and β -cell proliferation (Drucker and Nauk, 2006). Additionally, the binding of GLP-1 to its receptor activates protein kinase B, which is linked to glucose transporting in muscles, glycogen synthesis and lipolysis in various tissues (Peyot *et al.*, 2009).

6.4 GLP-1 in diabetes mellitus and the heart

A recent study showed that GLP-1 receptor expression is downregulated in β -cells exposed to high glucose concentrations in vitro and hyperglycemia in vivo (Xu *et al.*, 2007). Diabetic individuals' β -cells exhibit attenuated sensitivity to GLP-1. In both type 1 and type 2 DM, there is a marked reduction in the incretin effect (Knop *et al.*, 2007). On the other hand, a study reported that GLP-1 levels are not decreased in type 2 diabetic patients (Lee *et al.*, 2010). The European GLP-1 Club held a meeting recently and debated this controversial issue and came to a conclusion that more data is required to determine the exact effect of DM on GLP-1 secretion from L-cells (Burcelin, 2008). GLP-1 has been suggested to

ameliorate left ventricular function, because of its antiapoptotic and insulin-like properties (Inzucchi and McGuire, 2008). In fact, one study confirmed that GLP-1 enhances the regulation of phosphatidylinositol 3 kinase (PI3K), that plays a key role in activating the antiapoptotic pathway, thus promoting cardioprotection in the ischemic rat hearts. Therefore, a direct effect of GLP-1 against apoptosis in cardiac cells is possible (Bose *et al.*, 2005).

6.5 Dipeptidyl-peptidase IV

DPP-IV grasped a great deal of the interest of the scientific, pharmaceutical, and medical community (Lambeir *et al.*, 2003). Every year, an increasing number of publications tend to elucidate the diverse compelling questions concerning the various properties of DPP-IV and its multiple functions in the different fields of Biology (Lambeir *et al.*, 2003). DPP-IV is a 766 amino acid serine protease that belongs to the prolyloligopeptidase family. It is a widely distributed cell surface peptidase expressed to different degrees in a variety of tissues such as the kidney, intestine, liver, placenta, uterus, prostate, skin, and capillary endothelium. Another soluble form of DPP-IV (sDPP-IV) also exists in the plasma and other body fluids (Drucker, 2007; Idris and Donnelly, 2007; Lambeir *et al.*, 2003). This proteolytic enzyme acts by specifically cleaving the N-terminal dipeptide of peptide hormones containing proline or alanine in the second position (Drucker, 2007; Idris and Donnelly, 2007; Lambeir *et al.*, 2003). Hence, DPP-IV truncates several biologically active peptides of medical importance. Furthermore, it has been implicated in glucose homeostasis through proteolytic degradation of the incretins (Drucker, 2007). In the case of GLP-1(7-36), proline and alanine are key determinants in incretin receptor activation therefore DPP-IV-mediated proteolysis results in the biologically inactive truncated molecules GLP-1(9-36) (Drucker, 2007; Idris and Donnelly, 2007). DPP-IV is a critical determinant of incretin inactivation (Drucker, 2007). Thus chemical inhibition of DPP-IV activity results in increased level of biologically active GLP-1 (Drucker, 2007). Therefore, extensive research studies were carried to create highly selective DPP-IV inhibitors.

6.6 DPP-IV inhibitors

DPP-IV inhibitors can be used as a potential treatment for diabetes due to their capability to potentiate the levels of active incretins (Lambeir *et al.*, 2003; Halimi, 2008; Inzucchi and McGuire, 2008) by reducing the enzymatic activity of DPP-IV by more than 80% for duration up to 24 hours (Inzucchi and McGuire, 2008). Oral inhibitors of DPP-IV reduce glycosylated hemoglobin (HbA_{1c}) (Drucker, 2007; Halimi, 2008; Moritoh *et al.*, 2008). Various studies reported the multiple metabolic effects of DPP-IV inhibitors including enhancement of glucose-dependent stimulation of pancreatic insulin release as well as attenuation of glucagon secretion (Drucker, 2007; Halimi, 2008; Lambeir *et al.*, 2003; Moritoh *et al.*, 2008). Furthermore, DPP-IV inhibitors demonstrated modestly effective glucose-lowering actions without being associated with hypoglycemia (Halimi, 2008; Lambeir *et al.*, 2003; Moritoh *et al.*, 2008) due to the fact that they are remarkably able to specifically end the insulin-secreting effect and glucagon inhibition once glycemic levels are normalized (Halimi, 2008). Moreover, loss of DPP-IV activity is associated with improved glucose tolerance, reduced glycemic excursion following oral glucose challenge, and increased pancreatic insulin content (Drucker, 2007). A cardinal role of DPP-IV inhibitors is their potential to significantly augment β -cell mass in streptozotocin (STZ)-injected diabetic rats (Moritoh *et*

et al., 2008) and enhance pancreatic islet cell function in animal models of type 2 diabetes and in diabetic patients (Lambeir *et al.*, 2003). In contrast to GLP-1 mimetics, there are no data indicating inhibition of gastric emptying or appetite or weight reduction due to a treatment with DPP-IV inhibitors (Inzucchi and McGuire, 2008). Thus, chronic treatment with orally administered DPP-IV inhibitors has neutral effects on body weight and food consumption (Halimi, 2008).

6.7 GLP-1 receptor agonists and GLP-1 analogues

In April 2005, the FDA approved the subcutaneous injectable exenatide with the brand name Byetta® to be launched as a GLP-1 analogue (Inzucchi and McGuire, 2008; Rhoades and Bell, 2009) with longer half life to maintain its glycemic control as diabetic medication (Behme *et al.*, 2003). Exenatide exhibits a half-life of 60-90 minutes and a single injection of exenatide makes its concentration in the blood last for 4-6 hours (Drucker and Nauk, 2006). Exenatide exhibits antidiabetic effects similar to those of GLP-1 and is shown to improve β cell functioning (Baggio and Drucker, 2007). It also reduces the glycosylated Hemoglobin-HbA1c- levels that reflect mean blood glucose levels of the last 6-8 weeks. In addition, exenatide affects gastric emptying, appetite and consequently causes weight loss (Inzucchi and McGuire, 2008). GLP-1 agonists have exhibited vasodilating and diuretic effects. Recently, Laugero *et al.* showed that Exenatide may display antihypertensive effects mediated by pathways independent from glucose control, but possibly by altering steroid hormones (Laugero *et al.*, 2009).

6.7.1 GLP-1 analogue, exendin-4

The short half-life of GLP-1 limits itself from performing as a good therapeutic agent, as it is rapidly degraded by the serine protease DPP-IV (Hirata *et al.*, 2009). The search for biologically active peptides in lizard venom, led to the discovery of Exendin-4, a naturally occurring GLP-1 analogue (Drucker and Nauk, 2006). Exendin-4 is a peptide naturally found in the saliva of heloderma suspectum - the Gila monster (Laugero *et al.*, 2009; Zhou *et al.*, 2008). Exendin-4 is a peptide made up of 39 amino acid residues that shares a 53% structural homology to GLP-1 (Zhou *et al.*, 2008). This long acting GLP-1 receptor agonist has an N-terminus almost identical to that of the GLP-1, except that the second amino acid residue is glycine in exendin-4 while alanine in GLP-1 (Hui *et al.*, 2005). This one amino acid difference makes it resistant to degradation by the enzyme DPP-IV, hence explaining its long half-life in vivo (Lovshin and Drucker, 2009). Studies have shown that GLP-1 receptor agonists like exendin-4, exhibit cardioprotective effects such as modifications in contractility, cardiac output and blood pressure (Ban *et al.*, 2009).

6.7.2 Mode of action of exendin-4

Endogenous GLP-1 action is inhibited upon degradation by DPP-IV (De Koning *et al.*, 2008; Mann *et al.*, 2007), an enzyme expressed in many organs mainly kidney, small intestine, liver, and lung (Inzucchi and McGuire, 2008). More apt, exendin-4 is synthetic, thus not recognized by DPP-IV enzyme, thereby not degraded (Rhoades and Bell, 2009). This resistance allows it to stay in the circulation longer mimicking GLP-1 role in bolstering insulin secretion but with 5 to 10 times more powerful insulinotropic effect (Hantouche *et al.*, 2010; Xu *et al.*, 1999). Moreover, it binds to GLP-1R with high affinity even after truncation of the first 8 amino acids at the N-terminal of the peptide unlike the endogenous GLP-1 (Mann

et al., 2007). GLP-1R is a G-coupled transmembrane receptor (GPCR) that is found in many organs, most importantly the pancreatic β -cells and ducts, heart, lung, kidneys (Ban *et al.*, 2008), brain and stomach (Hantouche *et al.*, 2010). Conceptual understanding of this receptor delineates its advantages in cardiac management (Ban *et al.*, 2008). On the initiation of treatment, slight adverse effects might arise as nausea and vomiting (Inzucchi and McGuire, 2008), which are dose-dependent and will vanish with time (Mcphee *et al.*, 2008). It can be used efficiently in type 1 DM (Behme *et al.*, 2003) in conjunction with insulin irrespective of hypoglycemic effects attributed to its glucose-dependent action; in spite of insulin low efficiency with type 2 DM (Inzucchi and McGuire, 2008).

6.8 Exendin-4 in the treatment of diabetes mellitus correlated with cardiovascular risk

In conjunction to insulinotropic action of GLP-1 role, exendin-4 also mimics the action of insulin itself, through reinforcing the heart to uptake glucose irrespective of insulin level in both diabetic and non-diabetic cases (Hantouche *et al.*, 2010) STZ-induced diabetic rats acquire cardiomyopathic damage on day 1 starting with apoptosis and ending with hypertrophied hearts (Al Jaroudi *et al.*, 2005), particularly myocardial atrophy (Poornima *et al.*, 2006). Thereby, associated with its glucose lowering effect, certain data emphasized a potential benefit of exendin-4 in recovery of heart failure and function of left ventricle (Inzucchi and McGuire, 2008). Just similar to that of GLP-1 in improving ventricular contractions (Hantouche *et al.*, 2010) upon activation of pancreatic GLP-1R to boost insulin synthesis and secretion (De Koning *et al.*, 2008). Treatment with exendin-4 was concomitant with no cardiovascular complications, inexorable renal progression, or escalating plasma lipid (De Fronzo *et al.*, 2005). In type 2 DM exendin-4 has completed a novel fruition in the arena of organ rejuvenation especially at the level of both α and β cells mass, implementing a new born anti-hyperglycemic drug for solving the diabetes conundrum (Xu *et al.*, 1999). This increase in pancreatic mass happens as a post hoc to β -cells neogenesis from either existing duct cells and/or replication of the already present β -cells (hyperplasia) and not from hypertrophy (escalating cell size) (De Koning *et al.*, 2008). Pooled together, this regimen was found to be concomitant with cardioprotection, judicious vascular and cardiac actions based on the fact that endogenous GLP-1 has the same sequence in humans, rats and mice (Ban *et al.*, 2008). Recently, it was found that exendin-4 ameliorates the sensitivity of insulin receptors towards insulin at both the CM and coronary endothelium (CE) level (Hantouche *et al.*, 2010). This effect is achieved by either enhancing the sensitivity of insulin towards insulin or augmenting β -cells to release more of the hypoglycemic hormone (De Koning *et al.*, 2008). Valuable efficacy of treatment with both exendin-4 and insulin alleviates cardiomyopathic regressions associated in type 1 DM at the level of receptor affinity enhancement and insulin secretion bolstering (Hantouche *et al.*, 2010) (Fig. 1 and 2).

Insulin affinity (τ) to its receptor was shown to be decreased in the diabetic state at the level of the CE as compared to normal. Exendin-4 treatment increases insulin receptor affinity at the CE in both diabetic and normal rat groups; whereas insulin tends to normalize the affinity irrespective of exendin-4 absence or presence (Table 1). Exendin-4 treatment probably augments insulin receptor affinity both in normal and diabetic state through exerting its insulinotropic actions (Nikolaïdis *et al.*, 2005) and/or by improving insulin sensitivity (Ebinger *et al.*, 2000). The dramatic increase in the affinity of insulin to its receptor in the normal group treated with exendin-4 might be attributed to both of the previously mentioned causes. Yet the rise in insulin receptor affinity in exendin-4-treated diabetic group is not as remarkable as in NE which may be due to the lack of β -cells in diabetic type 1 state thus abolishing the insulinotropic effect of exendin-4.

Rat Group	τ (min) \pm SEM
Normal (N)	0.279 \pm 0.004 ^{a'}
Normal + Exendin-4 (NE)	0.370 \pm 0.007 ^{b'}
Normal + DPP-IV inhibitor (N-Dp)	0.311 \pm 0.005 ^{c'}
Diabetic (D)	0.252 \pm 0.003 ^{d'}
Diabetic + Insulin (DI)	0.292 \pm 0.004 ^{e'}
Diabetic + Exendin-4 (DE)	0.327 \pm 0.005 ^{f'}
Diabetic + Insulin + Exendin-4 (DIE)	0.295 \pm 0.004 ^{g'}
Diabetic + DPP-IV inhibitor (D-Dp)	0.255 \pm 0.003 ^{h'}
Diabetic + Insulin + DPP-IV inhibitor (DI-Dp)	0.321 \pm 0.005 ^{i'}

Table 1. The calculated affinity constant (τ) of insulin to its receptor at CE (CHAPS-untreated) in normal and diabetic rats

Rat Group	τ (min) \pm SEM
Normal (N)	0.337 \pm 0.006 ^{a'}
Normal + Exendin-4 (NE)	0.424 \pm 0.009 ^{b'}
Normal + DPP-IV inhibitor (N-Dp)	0.234 \pm 0.003 ^{c'}
Diabetic (D)	0.368 \pm 0.007 ^{d'}
Diabetic + Insulin (DI)	0.331 \pm 0.006 ^{e'}
Diabetic + Exendin-4 (DE)	0.328 \pm 0.005 ^{f'}
Diabetic + Insulin + Exendin-4 (DIE)	0.331 \pm 0.006 ^{g'}
Diabetic + DPP-IV inhibitor (D-Dp)	0.376 \pm 0.007 ^{h'}
Diabetic + Insulin + DPP-IV inhibitor (DI-Dp)	0.382 \pm 0.007 ^{i'}

Table 2. The calculated affinity constant (τ) of insulin to its receptor at CM (CHAPS-treated) in normal and diabetic rats.

Therefore, in diabetes type 1, exendin-4 increases the insulin receptor affinity only by improving insulin sensitivity. As a conclusion, exendin-4 seems to have supplementary effects which might explain the increase in τ value at the level of the CE. First, it augments insulin release from pancreatic β -cells when present in the normal state. This is its insulinotropic effect. Second, it improves insulin sensitivity possibly by inducing a conformational change in the insulin receptor (Ebinger *et al.*, 2000). On the other hand, DPP-IV inhibitor (KR-62436, Sigma Chemical Company) treatment solely does not seem to be implicated in modulating insulin receptor affinity at the endothelial site in diabetic rats treated with DPP-IV inhibitor as compared to diabetics. Yet the effect of DPP-IV inhibitor at the CE becomes obvious in the presence of insulin, as seen in the diabetic group co-treated with insulin and DPP-IV inhibitor (DI-Dp) compared to diabetics (D) and in the normal group treated with DPP-IV inhibitor (N-Dp) with respect to normal. This could be due to some kind of a cross-talk between insulin and DPP-IV inhibitor which results in an increase of τ value in both N-Dp and DI-Dp. Moreover, insulin receptor affinity is not altered at the level of the CM in diabetics treated with DPP-IV inhibitor (D-Dp) and/or insulin (DI-Dp) when compared to diabetic rats (D) (Table 2). The major difference between the CE and CM

upon DPP-IV inhibitor treatment could be attributed to the fact that the already limited increase of GLP-1 by DPP-IV inhibitor is primarily imposed on endothelial cells which are the first site of encounter with systemic GLP-1. The difference in affinities observed between DPP-IV inhibitor and exendin-4 treatment might be due to the fact that DPP-IV inhibitor increases the systemically available GLP-1 levels; whereas exendin-4, besides its quantitative systemic effect, has a higher physiologic quality in term of being 5- to 10- fold more potent than GLP-1 (Saraceni and Broderick, 2007).

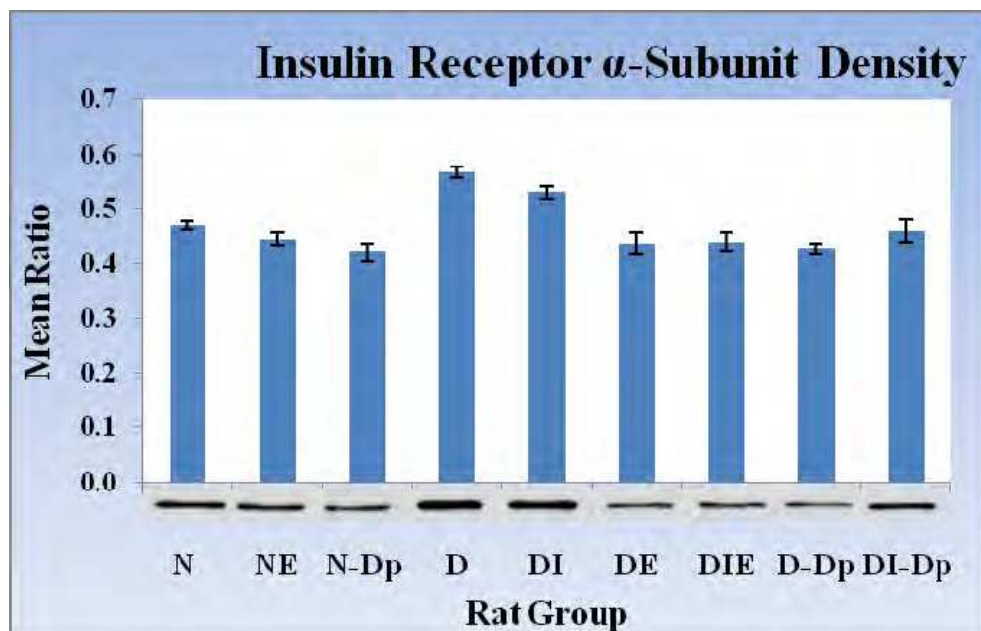


Fig. 2. Insulin receptor α -subunit (MW~125 kDa) density in the heart of the different rat groups.

Western blotting was performed on protein extracts from rat heart homogenates in order to assess the variation in the insulin receptor subunits density among the different treated and untreated normal and diabetic rat groups. Both insulin receptor α -subunit (IR- α) (Fig. 2) and insulin receptor β -subunit (IR- β) (Fig. 3) levels are augmented in diabetic state indicating that there is an increase in the level of cardiac insulin receptor protein in diabetics (D) compared with normal controls (N). These results indicate that cardiac insulin receptors are up-regulated in the heart of diabetic rats as a feedback mechanism probably due to the lack of insulin. This is consistent with the results demonstrated by Al Jaroudi *et al.* 2005 in the study done on insulin receptor regulation in the diabetic heart. Our results indicate that insulin administration to diabetic rats reduces the number of IR- α and IR- β to near normal values. Interestingly, exendin-4 treatment normalizes insulin receptor subunits density in diabetic rat hearts (Fig. 2 and 3). The regression in insulin receptor density with exendin-4 treatment is suggested to be attributable to the insulinomimetic effects of exendin-4 (Sokos *et al.*, 2006) which result in a cross-talk between GLP-1 and insulin signaling pathways (Ebinger *et al.*, 2000).

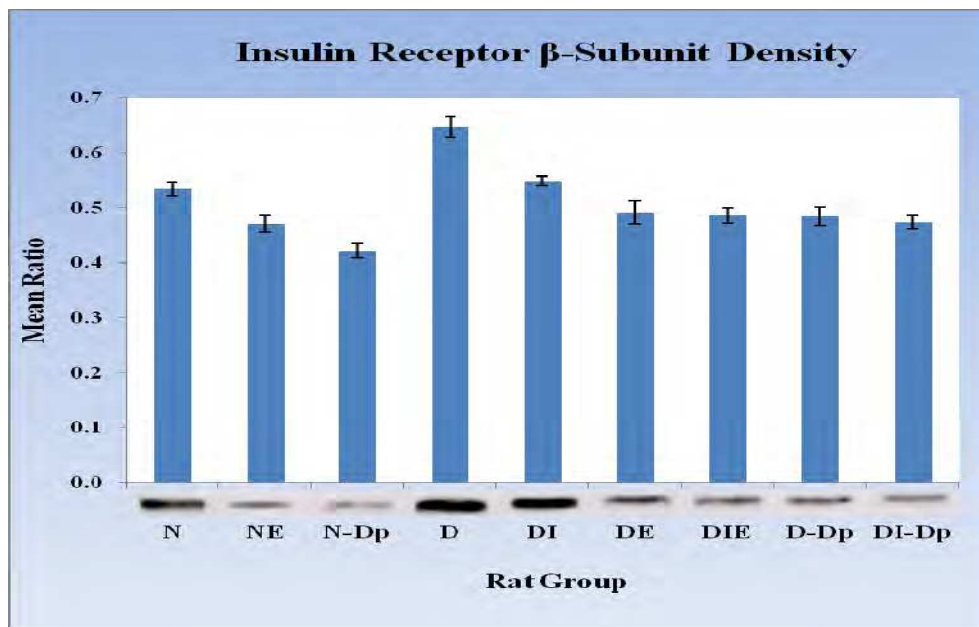


Fig. 3. Insulin receptor β -subunit (MW~95 kDa) density in the heart of the different rat groups.

There is no further attenuation in the level of insulin receptor upon insulin administration in combination with exendin-4 in diabetic rat groups. Thus, it is conceivable that GLP-1 is a key player in insulin receptor regulation in the diabetic state. GLP-1 also induces its insulinomimetic effects in the normal state thereby resulting in a slight decrease of insulin receptor density in NE. DPP-IV inhibitor induces similar effects as exendin-4 on IR- α and IR- β levels and this could be attributed as well to the insulinomimetic effects of GLP-1 (Fig. 2 and 3). There is a similar attenuation in receptor density in non-diabetic DPP-IV inhibitor treated rats (N-Dp). This reflects the actions of DPP-IV inhibitor treatment on the modulation of insulin receptor density not only in the diabetic but also in the normal state.

7. Conclusion

In conclusion, insulin, Losartan, exendin-4 and DPP-IV inhibitor have demonstrated their ability as promising candidates for treating type 1 diabetic patients. Moreover, exendin-4 and DPP-IV inhibitor appear to be promising in their efficacy and prolonged antidiabetic properties. Their actions on cardiovascular function, in both the preclinical and clinical realms, warrant future investigation.

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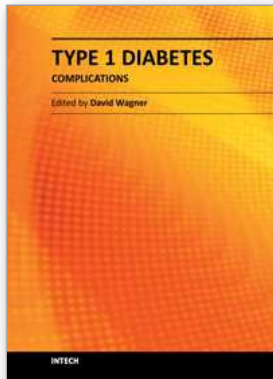
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This book is a compilation of reviews about the complication of Type 1 Diabetes. T1D is a classic autoimmune disease. Genetic factors are clearly determinant but cannot explain the rapid, even overwhelming expanse of this disease. Understanding etiology and pathogenesis of this disease is essential. The complications associated with T1D cover a range of clinical obstacles. A number of experts in the field have covered a range of topics for consideration that are applicable to researcher and clinician alike. This book provides apt descriptions of cutting edge technologies and applications in the ever going search for treatments and cure for diabetes.

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