

**PHYSIOLOGICAL, MORPHOLOGICAL AND
MOLECULAR BIOLOGICAL STUDIES OF THE
EFFECT OF GLUCAGON-LIKE PEPTIDE-1 AND
EXENATIDE IN THE DIABETIC RAT PANCREAS**

by

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A thesis submitted in partial fulfilment for the requirements for the degree of
Doctor of Philosophy at the University of Central Lancashire, Preston, UK
(in collaboration with the United Arab Emirates University, Al-Ain, UAE)

June/2012

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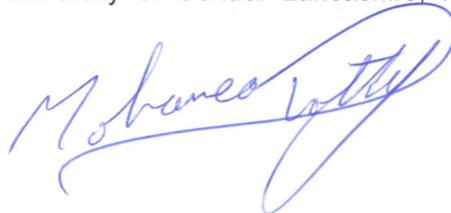
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Abstract

Diabetes mellitus (DM) is a major health problem currently affecting over 225 million people worldwide. It is often described as a major metabolic disorder, which can result in numerous long-term complications including retinopathy, nephropathy, neuropathy and cardiomyopathy. DM is due to a deficiency of insulin or insulin resistance. Of the 225 million diabetic patients, around 5-10% suffer from type 1 DM (T1DM) and the remaining 90-95% suffer from type 2 diabetes (T2DM). T1DM is due to insulin deficiency whereas T2DM is due to either a reduction in insulin secretion or insulin resistance. Patients with both T1DM and T2DM normally require insulin and hypoglycaemic drugs, respectively. Changes in life style habits, regular exercise and healthy diets can also help to control blood glucose in T2DM patients. Mediators that can help to increase the health of pancreatic islets to synthesize and secrete insulin will be of tremendous benefit to diabetic patients. This study investigated the beneficial effects of incretins, substances such as glucagon-like peptide-1 (GLP-1) and its synthetic agonist, exenatide on the diabetic rat pancreas compared to healthy, age-matched controls. These incretins exert their beneficial effects by repairing the pancreatic islets. Thus, increasing pancreatic beta (β) cell mass and in turn it will help to synthesize and secrete insulin into the circulation. The rationale of this study was to find out how these two incretins can improve insulin secretion both *in vivo* and *in vitro* employing the rat model of T1DM following injection with streptozotocin (STZ). The project employed six groups of rats, with three groups serving as age-matched, healthy controls and the other three groups rendered diabetic. One set of rats from each group was untreated while the rats from the other four groups were given either GLP-1(50 nmol/kg body weight) or exenatide (1 μ g/kg body weight) over 10 weeks. The project measured body weight, levels of blood glucose and insulin. The plasma levels of liver and kidney markers were also determined. The *in vitro* study measured insulin secretion from pancreatic fragments, the distribution of insulin- and glucagon-positive cells in pancreatic islets, granules, co-localization of different peptides in the islets, biochemical, and molecular biological changes, which may occur in the pancreas during the experimental period.

For the *in vivo* study, the results have shown mild gain in body weight and no change in blood glucose levels in both treated and untreated age-matched normal control rats. Furthermore, the results show a significant reduction in blood glucose levels in diabetic rats treated with either GLP-1 or exenatide, but the beneficial effect was more pronounced following GLP-1 treatment. The results also show no changes in glucose handling between normal treated or normal untreated rats following blood glucose tolerance test (GTT). However, in diabetic rats, the results show that the GTT reveals a better glucose tolerance in these diabetic animals treated with either GLP-1 or exenatide but the effect was more significant with GLP-1 compared with untreated diabetic rats. The present study shows that diabetic rats secreted significantly less insulin in the blood than normal healthy rats and a significant increase in serum insulin was detected in both normal and diabetic rats treated with either GLP-1 or exenatide compared to untreated controls. The results also show significant reductions in the liver enzymes, aspartate transferase and alanine transferase in the diabetic rats. A similar beneficial effect on kidney function was obtained owing to a small reduction in blood urea nitrogen, serum creatinine and serum uric acids in both normal and diabetic rats treated with either GLP-1 or exenatide. In the lipid profile study, the results show a mild reduction in serum cholesterol and a marked reduction in serum triglyceride in both normal and diabetic rats treated with either GLP-1 or exenatide.

The results from the *in vitro* study show that either GLP-1 or exenatide can evoke marked dose-dependent release (secretion) of insulin from pancreatic tissue fragments of normal and diabetic rats, indicating that there is a clear role for either GLP-1 or exenatide in inducing insulin secretion.

In this study, an attempt was also made to investigate both the number and distribution of endocrine cells in the control and diabetic rat pancreas using immunohistochemistry. The results show a significant increase in the number of cells containing either insulin or GLP-1 in both normal and diabetic treated rats. However, in the case of exenatide, catalase and glutathione reductase-positive cells were only significantly increased in diabetic rats, but the increase was not significant in normal rats treated with either GLP-1 or exenatide. These results show that the significant increase in number of catalase

and glutathione-positive cells in diabetic rats treated with either GLP-1 or exenatide reveal the beneficial antioxidant effect of both GLP-1 and exenatide in treatment of oxidative stress, which usually occurs in DM. On the other hand, there was a significant decrease in glucagon-positive cells in both normal and diabetic rats treated with either GLP-1 or exenatide.

The immunohistochemical and immunofluorescent studies also revealed that insulin-positive cells were distributed both in the central and peripheral portions of the islets of Langerhans in normal pancreas. In contrast, glucagon-positive cells were located in the peripheral part of the islets of Langerhans. After the onset of diabetes, the number of insulin-positive cells was reduced significantly. In contrast, the number of glucagon-positive cells increased significantly with abnormal pattern of distribution compared to normal pancreas. The pattern of distribution of both GLP-1 and exenatide has indicated co-localization not only with insulin, but also with glucagon. Furthermore, catalase and glutathione reductase-positive cells were distributed homogeneously all over the islet of Langerhans with no specific co-localization with specific type of endocrine cell.

In the gene expression study, the results show significant increases in the levels of mRNA of pancreatic duodenal homeobox-1, heat shock protein-70, glutathione peroxidase, insulin receptor and glucagon like peptide-1 receptor in both normal and diabetic rats treated with either GLP-1 or exenatide. However, the increase was not significant in mRNA gene expression of either insulin receptor or glucagon like peptide-1 receptor in normal rats treated with GLP-1. On the other hand, the gene expression results show that glucagon mRNA level was significantly decreased in both normal and diabetic rats treated with either GLP-1 or exenatide.

In conclusion, the results of this study have clearly demonstrated that both GLP-1 and exenatide have marked beneficial effects on pancreatic islet cells, especially β - and α -cells, which produce insulin and glucagon, respectively. The two incretins seem to repair the diabetic pancreas, which in turn secretes more insulin and less glucagon.

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List of Abbreviations

Abbreviations	Meanings
AC	Adenylate cyclase
ADA	American diabetes association
ADP	Adenosine diphosphate
ALT	Serum alanine transferase
AST	Serum aspartate transferase
ATP	Adenosine triphosphate
Bcl-2, IAP-2	Anti-apoptotic survival proteins
BMI	Body mass index
BSA	Bovine serum albumin
BUN	Blood urea nitrogen
Ca ²⁺	Calcium ion
cAMP	Cyclic adenosine monophosphate
CAT	Catalase
CCK	Cholecystokinin
cDNA	Complementary deoxyribonucleic acid
CNS	Central nervous system
CREB	cAMP response element binding protein
cSrc	Cytoplasmic tyrosine kinase
D	Diabetic
DAG	Diacylglycerol
DCs	Dendritic cells
DM	Diabetes mellitus
DPP-IV	Dipeptidyl peptidase-IV
EGF receptor	Epidermal growth factor receptor
ELISA	Enzyme-linked immunosorbent assay

Epac	Exchange protein activated by cAMP
ER	Endoplasmic reticulum
EXN	Exenatide
FBG	Fasting blood glucose
FMHS	Faculty of Medicine and Health Sciences
FoxO1	Factor forkhead box O1
G-6-P	Glucose-6-phosphate
GABA	γ -Aminobutyric acid
GDM	Gestational diabetes mellitus
GDP	Guanosine diphosphate
GIP	Glucose-dependent insulinotropic polypeptide
GLCG	Glucagon
GLP-1	Glucagon like peptide-1
GLP-1R	Glucagon like peptide-1 receptor
GLP-2	Glucagon like peptide-2
GLUT	Glucose transporter
GPCRs	G protein-coupled receptors
GPx	Glutathione peroxidase
GRP	Gastrin-releasing peptide
GSH	Glutathione reductase
GTP-binding	Guanosine triphosphate-binding
GTT	Glucose tolerance test
HbA1c	Glycosylated haemoglobin
HD	Homeodomain
HDL	High-density lipoprotein
HHEX	Homeobox hematopoietically
HI	Hyperinsulinism in infancy
HLA	Human leukocyte antigens
HMIT	H ⁺ -coupled myo-inositol transporter

HNF-1 α	Hepatocyte nuclear factor-1 α
HSP-70	Heat shock protein-70
IDDM	Insulin dependent diabetes mellitus
IDE	Insulin-degrading enzyme
IGF-IR	Insulin-like growth factor-I receptor
IGTT	Intraperitoneal glucose tolerance test
IL-2R α	Interleukin-2 receptor α
INS	Insulin
IP ₃	Inositol trisphosphate
IP ₃ R	Inositol trisphosphate receptor
IPGTT	Intraperitoneal glucose tolerance test
IR	Insulin receptor
IRSs	Insulin receptor substrates
K _{ATP} channel	Potassium adenosine triphosphate channel
KCNJ11	Potassium rectifying channel J 11
K _m	Michaelis constant of substrate kinetic
K _v channel	Potassium voltage channel
MafA	V-maf musculoaponeurotic fibrosarcoma oncogene
Merg	Merged cells
MODY	Maturity onset diabetes of the young
mRNA	Messenger ribonucleic acid
Mt	Mitochondria
mTOR	Mammalian target of rapamycin
N	Noramyl
NADPH	Reduced nicotinamide adenine dinucleotide phosphate
NeuroD1	Neurogenic differentiation-1
NFAT	Nuclear factor of activated T cells
NF-B	Transcription factor
NF-kB	Nuclear factor kappa-B

NGF/p75NTR	Nerve growth factor/p75 neurotrophin receptor
NGS	Normal goat serum
NIDDM	Non-insulin dependent diabetes
NO	Nitric oxide
PBS	Phosphate buffer saline
PC	Prohormone convertase
PCR	Polymerase chain reaction
PDX-1	Pancreatic duodenal homeobox-1
PI	Phosphatidylinositol
PI-3K	Phosphatidylinositol-3 kinase
PIP2	Phosphatidylinositol bisphosphate
PKA	Protein kinase A
PKB	Protein kinase B
PKC	Protein kinase C
PKC- δ	Protein kinase C- δ
PLC	Phospholipase C
PLC- ζ	Phospholipase C- ζ
PLs	Phospholipases
PP	Pancreatic polypeptide
PPAR- γ	Peroxisome proliferator-activated receptor- γ
rAd-GLP-1	Recombinant adenoviral vector expressing GLP-1
Rho	Monomeric GTP binding protein family
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RQ	Relative quantification
RyR	Ryanodine Receptor
SDS	Sequence detection systems
SEM	Standard error of the mean
SGLT	Sodium-dependent glucose transport

SNPs	Single nucleotide polymorphisms
SS	Somatostatin
STZ	Streptozotocin
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
T3DM	Type 3 diabetes mellitus
TCF7L2	Transcription factor 2
TM	Transmembrane domain
TMB	Tetramethylbenzidine
UAE	United Arab Emirates
UAEU	United Arab Emirates University
UCLAN	University of Central Lancashire
VDC channel	L-type voltage dependant calcium channel
α TC1-6	α -Cell line

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Dedicated to:

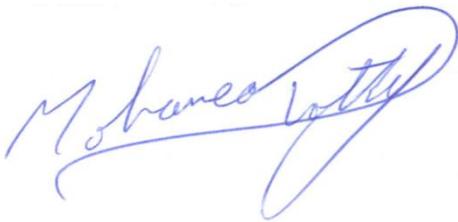
My Mother and Father

My Wife and Children

My Brother and Sister

Declaration

I declare that while registered as a candidate for the degree for which this submission is made that I have not been a registered candidate for another award by any other awarding body. No material contained in this thesis has been used in any other submission for academic award

A handwritten signature in blue ink, appearing to read 'Mohamed J. Alkhatib', is written in a cursive style.

CHAPTER ONE

GENERAL INTRODUCTION

1.1 The pancreas

The pancreas is located in the upper part of the abdominal cavity. It is attached to the duodenum, the first part of the small intestine via the common pancreatic bile duct. The gland is also extended towards the spleen (Figure 1.1-A). The pancreas is an elongated nodular organ of about 15-25 cm length and approximate 80 g in weight. The pancreas is formed by a broad head attached to the duodenum, a cylindrical body and a small tail near the spleen (Martini and Nath, 2009).

The pancreas is a mixed gland, composed of a major exocrine part and minor endocrine portion (Figure 1.1). The exocrine portion of the pancreas is composed of about 98% and this part is studied mainly by the Gastroenterologists while, the endocrine part is formed of approximately 2% of the pancreatic architecture and studied by the Endocrinologists. The exocrine pancreas is formed of a collection of acini that secrete digestive enzymes and alkaline buffer and open into the pancreatic duct which release its secretions into the duodenum. Pancreatic juice helps in the digestion of carbohydrates, fats and proteins and it also neutralize the acidic chyme from the stomach. Pancreatic juice secretion is regulated by cholinergic, adrenergic and peptidergic nerves as well as by the gut hormones, cholecystokinin (CCK) and secretin (Hug et al., 2003; Liddle, 2006; Chu et al., 2006; Amber and Bloom, 2007). In the endocrine pancreas a scattered groups of pancreatic islet cells, known as islets of Langerhans secrete pancreatic hormones. Normally, the pancreas contains approximately one million pancreatic islets, which are supplied with a capillary network that conveys its hormones directly into the blood circulation (Martini and Nath, 2009). The islets of Langerhans, include four distinct cell types that are innervated heavily with autonomic and peptidergic nerve endings (Tan, 2008).

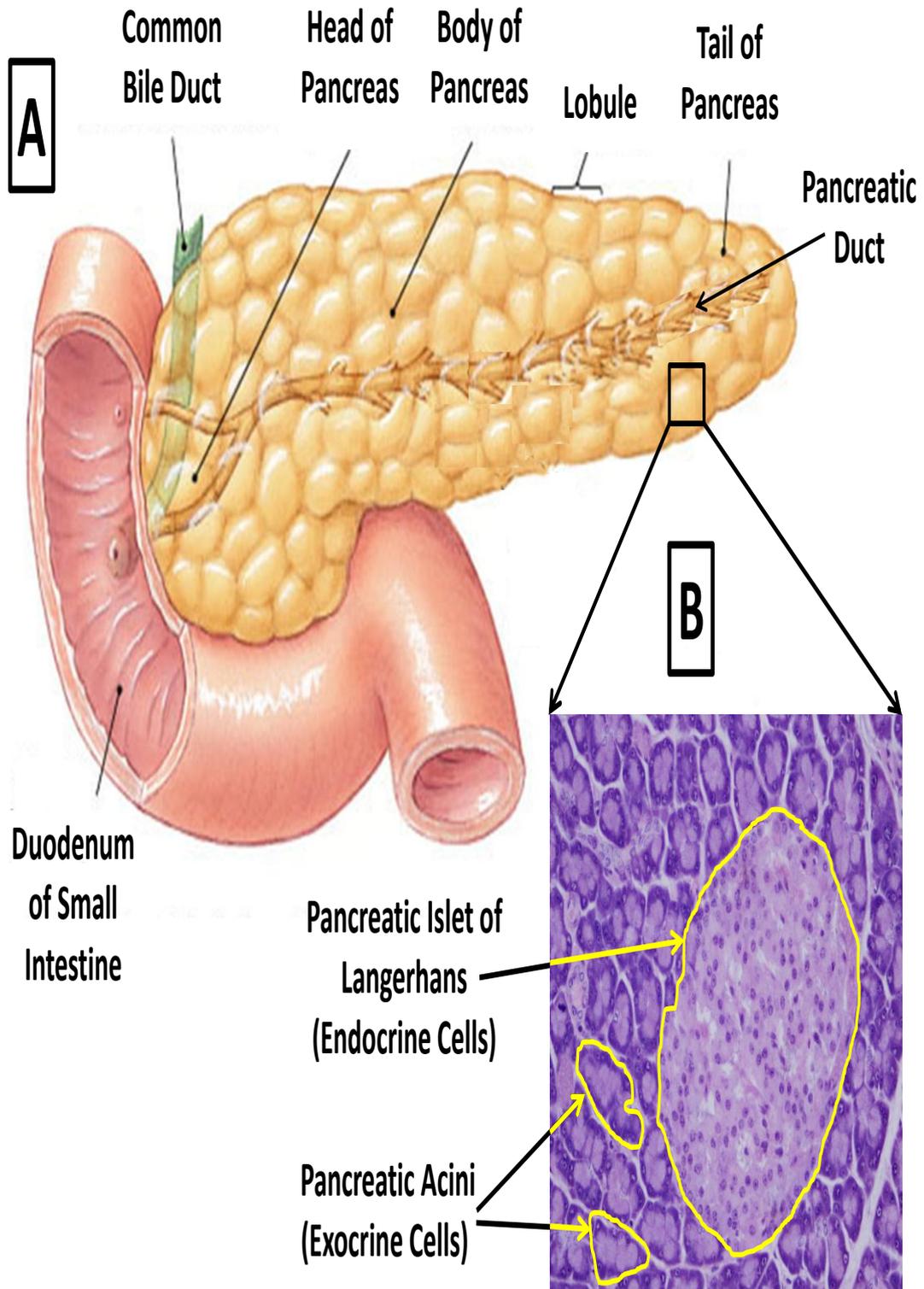


Figure 1.1: Diagrams showing (A) the position of the pancreas and its relationship with the duodenum and (B) a histological picture of the endocrine and exocrine components of the pancreas (Modified from Martini and Nath, 2009).

1.1.1 The endocrine pancreas

Since this thesis is related mainly to the endocrine pancreas, then much emphasis will be made on the endocrine pancreas. The endocrine part of the pancreas is composed of tiny units of islets with a rich supply of blood capillaries. The islets are scattered randomly within the exocrine pancreas. Paul Langerhans initially discovered pancreatic islet cells in 1869 and they carried his name (Bishop and Polack, 1997). The release of hormones from islets of Langerhans is controlled by rich innervations via cholinergic, adrenergic and non-cholinergic, non-adrenergic nerves (Toda and Herman, 2005). The medium-sized islets of Langerhans of human pancreas are formed from about 2500 cells. In most mammals, the islets contains 4 major types of cells (α , β , δ and PP) with a central core of insulin-producing β -cells and a peripherally located glucagon-producing α -cells, somatostatin-secreting δ -cells and pancreatic polypeptide-synthesizing PP-cells (Eberhard et al., 2008). The human pancreatic islet is a little bit different because β -cells mainly in the central area while α -, δ - and PP-cells can be either found within the central core or in the peripheral part of the islets (see Figure 1.2) (Brissova et al., 2005). The islets are not only composed of endocrine cells but also endothelial cells in contact with blood capillaries and dendritic cells. The dendritic cells reveal phagocytic actions. They may also be involved in the initiation of T1DM and play an important role in β -cells transplantation rejections (Bertelli and Bendayan, 2005). However, the islets of Langerhans comprise only about 2% of the total pancreatic mass; they are heavily supplied with blood vessels, which, account for 10% of the entire pancreatic blood circulation (Richards et al., 2010).

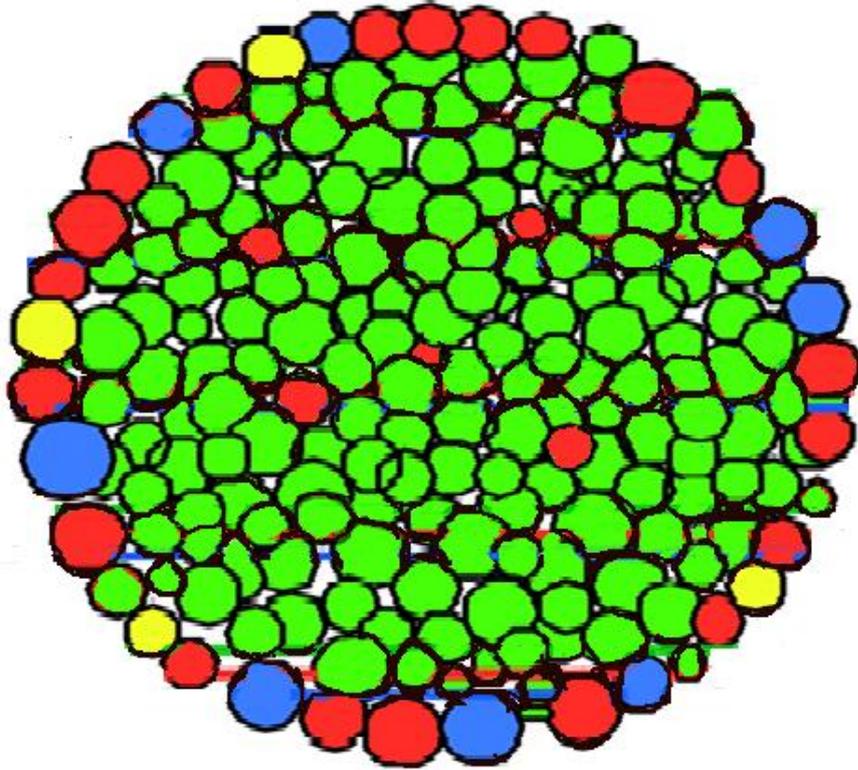


Figure 1.2: Schematic representation of optical sectioning of pancreatic islets. Islet cell types are illustrated in four different colours: β -cells, green; α -cells, red; δ -cells, blue; PP-cells, yellow (Modified from Brissova et al., 2005).

1.1.2 Cells types in pancreatic islets of Langerhans

Each islet contains basically four different cell types (Figure 1.2) and they include:-

Beta-cells: These cells produce insulin and they are the most common type of endocrine cells in the pancreas. The β -cells are situated mostly in the central area, also some are located in the peripheral regions of the islet. The percentage of β -cells is around 70% of the whole cell population in a definite islet of Langerhans (Adeghate and Ponery, 2003).

Alpha-cells: Glucagon-producing cells compose approximately 20% of all cells in the endocrine pancreas. They are detected in the outer part of the islets of Langerhans (Adeghate and Ponery, 2003).

Delta-cells: Somatostatin pancreatic producing δ -cells are positioned mostly in the marginal portion of the islets of Langerhans with about 8% of the islet cells (Adeghate and Ponery, 2003).

Pancreatic polypeptide-cells: Pancreatic polypeptide (PP) producing cells are found mainly in the outer area of the pancreatic islets of Langerhans comprising of about 2% of the total islet cells (Adeghate and Ponery, 2003).

1.1.3 Innervation of the pancreas

The pancreas is supplied by adrenergic, cholinergic and as well as peptidergic nerves. These nerves contain acetylcholine, noradrenaline, serotonin and a variety of peptides such as neuropeptide-Y, vasoactive intestinal polypeptide, galanin and leucine-enkephalin (Rossi et al., 2005). The nerve cells in the pancreas contain not only parasympathetic cholinergic and sympathetic adrenergic but also other neurotransmitters and neuropeptides. These categories of pancreatic innervations are involved in pancreatic functions in many ways and play a role in resolving pain in certain pancreatic illness like chronic pancreatitis (Rabinovich et al., 2006).

1.2 Pancreatic hormones

1.2.1 Insulin

Normal body growth, development and maintenance of nutrient balance are among the powerful anabolic actions of insulin. Insulin controls the metabolism of carbohydrates, lipids and amino acids within various body tissues. However, liver, muscle, and adipose tissue are the essential affected tissues. The traditional general function of insulin is to regulate carbohydrate metabolism and its specific action is to control plasma sugar level. One of the lethal causes of the diabetic patients with insulin deficiency is the disturbance of lipid metabolism, which leads to atherosclerosis and acidosis (Kumar

and Clark, 2007). In T1DM, there is a pronounced reduction of insulin concentration that causes a potent inhibition in the production and secretion of pancreatic enzymes with related reduction in food digestion. Inadequate insulin secretion or insulin resistance may ultimately cause the establishment of DM (Patel et al., 2006).

1.2.2 Insulin structure

Human insulin is a type of protein hormone of low molecular weight of about 5,800 KD as seen in figure 1.3. The structure of insulin molecule consists of 51 amino acids of two polypeptide chains (A and B). The A-chain is the smaller one and consists of 21 amino acids and the second larger B-chain is formed from 30 amino acids. Both A- and B-chains are attached together through two disulphide links found between cysteine amino acids. In the A-chain a similar internal disulphide link is found between two cysteine amino acids. The main function of these disulphide links is to give the three-dimensional form of insulin molecule and guarantee the precise physiological action of insulin hormone (Weiss, 2009).

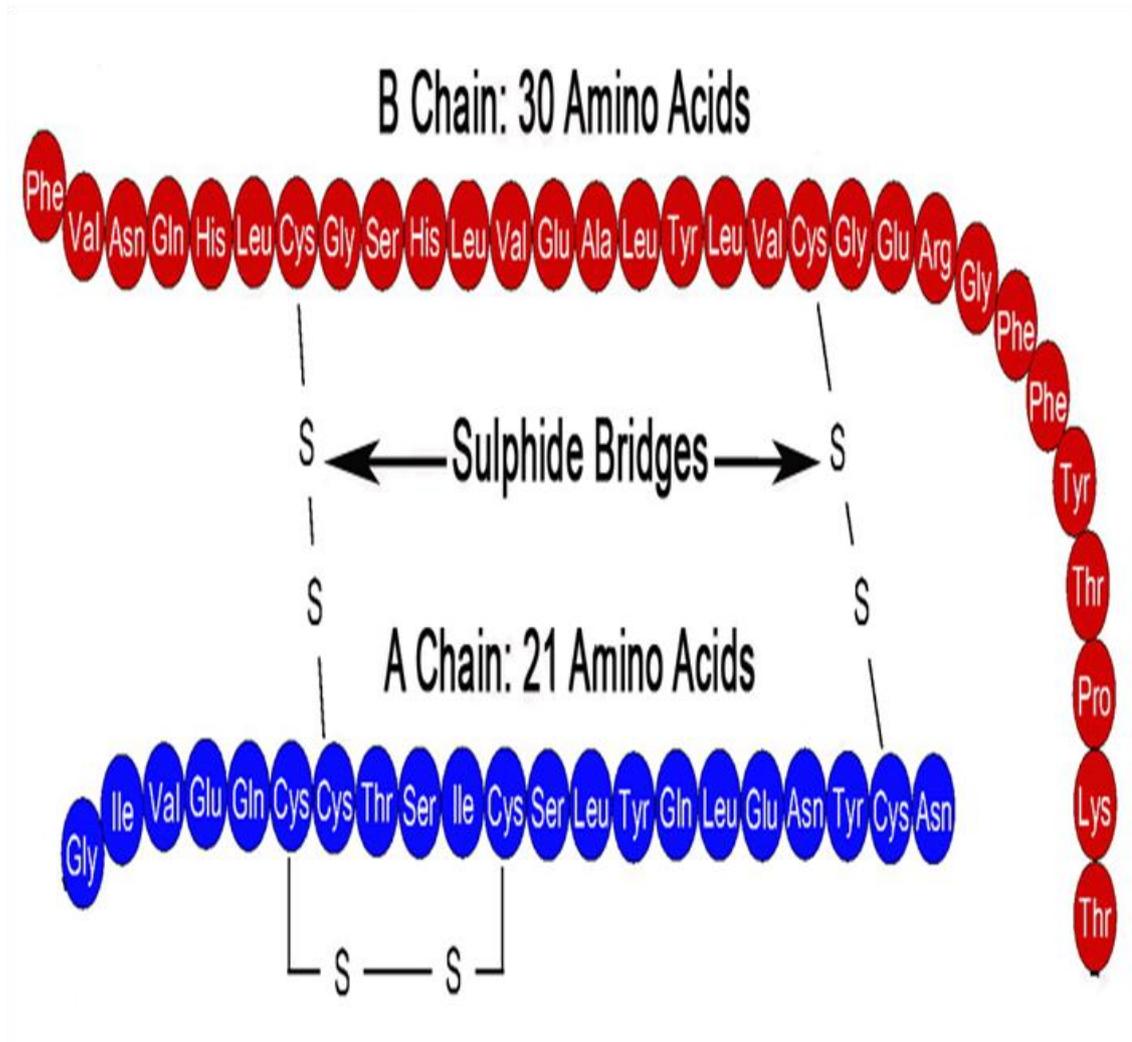


Figure 1.3: Diagram showing the structure of human insulin molecule (Modified from Guyton and Hall, 2005).

1.2.3 Insulin synthesis

There are several stages in the regulation of insulin synthesis in β -cell of the endocrine pancreas. This control system involves the transcriptional, translational and post-translational steps. The mechanism of insulin production is shown in figure 1.4. According to the human genome, insulin gene is found on the small arm of chromosome number 11 (Xu et al., 2009).

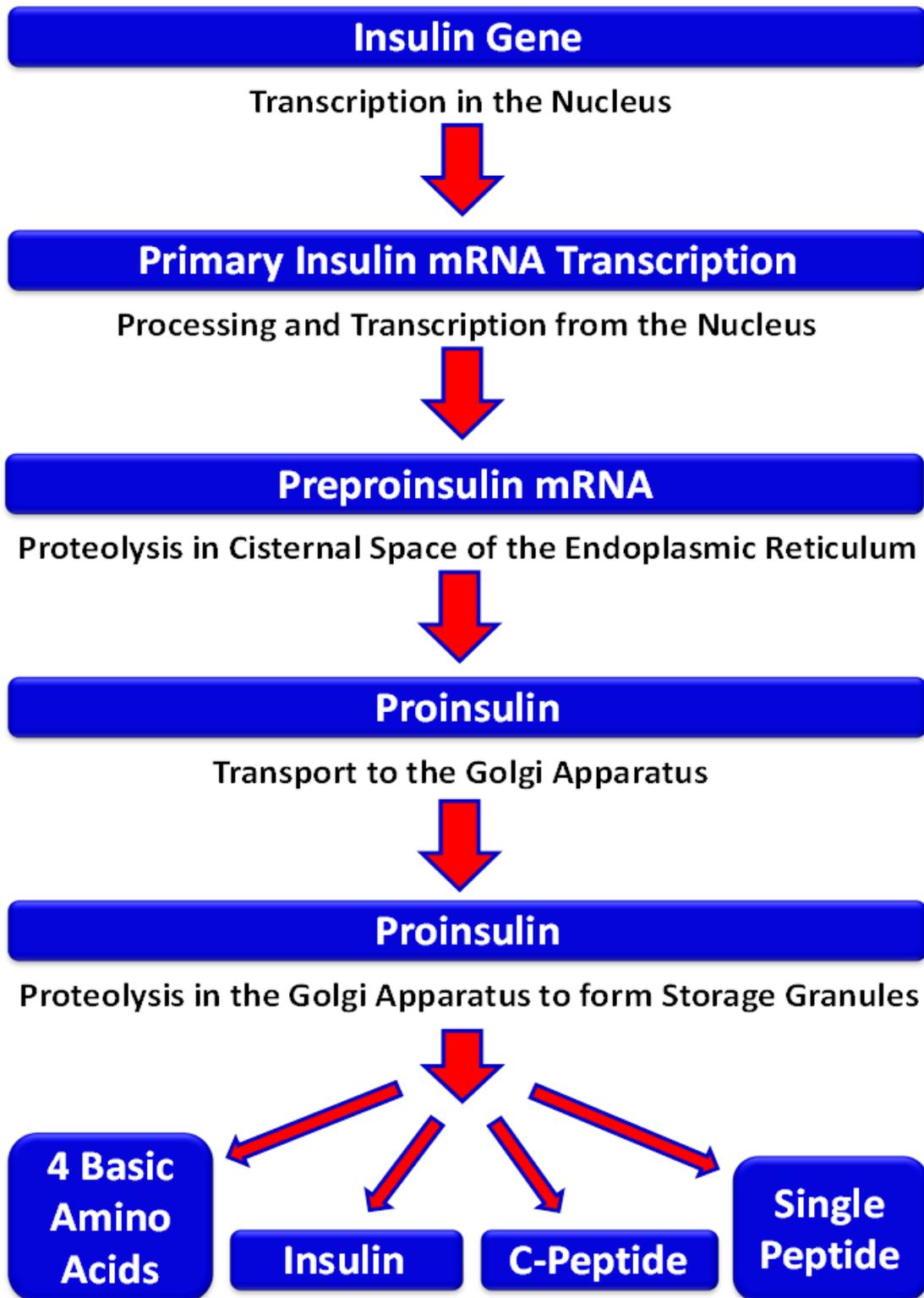


Figure 1.4: Schematic diagram showing multiple steps in the transcriptional and translational synthesis of insulin (Modified from Montague, 1993).

The essential function of the β -cell is to produce, store and secrete insulin. The fully formed insulin granule is made of two A and B polypeptide chains, which are not produced as single polypeptide chains but are formed by precise proteolytic processing of a bigger precursor, proinsulin (Steiner, 2004).

Insulin gene transcription via RNA polymerase forms a mRNA which is processed in the nucleus to generate a mature mRNA that is translated into pre-proinsulin in the cytoplasm. The translation of the mRNA into amino acid sequence of pre-proinsulin takes place in the ribosomes. Pre-proinsulin translation starts with the signal peptide, which is recognized via binding of signal recognition part to the ribosomes, and attaches it to the endoplasmic reticulum (ER) to initiate the synthesis of the molecule of pre-proinsulin. The pre-proinsulin is transported from ER membrane into its cisternal cavity to be changed to proinsulin, which then achieves chain folding and binding with disulphide bonds to develop an enclosed microvesicle, which is then conveyed to the Golgi apparatus. In the Golgi apparatus, proinsulin is packaged into storage granules (Wicksteed et al., 2001).

Through a proteolysis process, the recently synthesized proinsulin in Golgi apparatus is slowly changed to insulin granules, which finally, under the effect of certain proteases are converted to mature insulin granules with equivalent quantities of C-peptide, four basic amino acids and single peptide (see Figure 1.5). The maturely formed storage granules are transported from the Golgi apparatus to β -cell cytoplasm waiting for the secretory signals. These secretory granules contain mainly mature insulin with minor quantity of unconverted proinsulin (Steiner et al., 2005).

The most effective signal on insulin synthesis by β -cell is the blood glucose concentration. Low fasting blood glucose level (2-4 mM) leads to decreased synthesis level of proinsulin. On the other hand, increased level of blood glucose above the threshold concentration (4-6 mM) causes a remarkable enhancement of proinsulin production and under different body needs, a sufficient store of insulin is constantly accessible (Steiner et al., 2009).

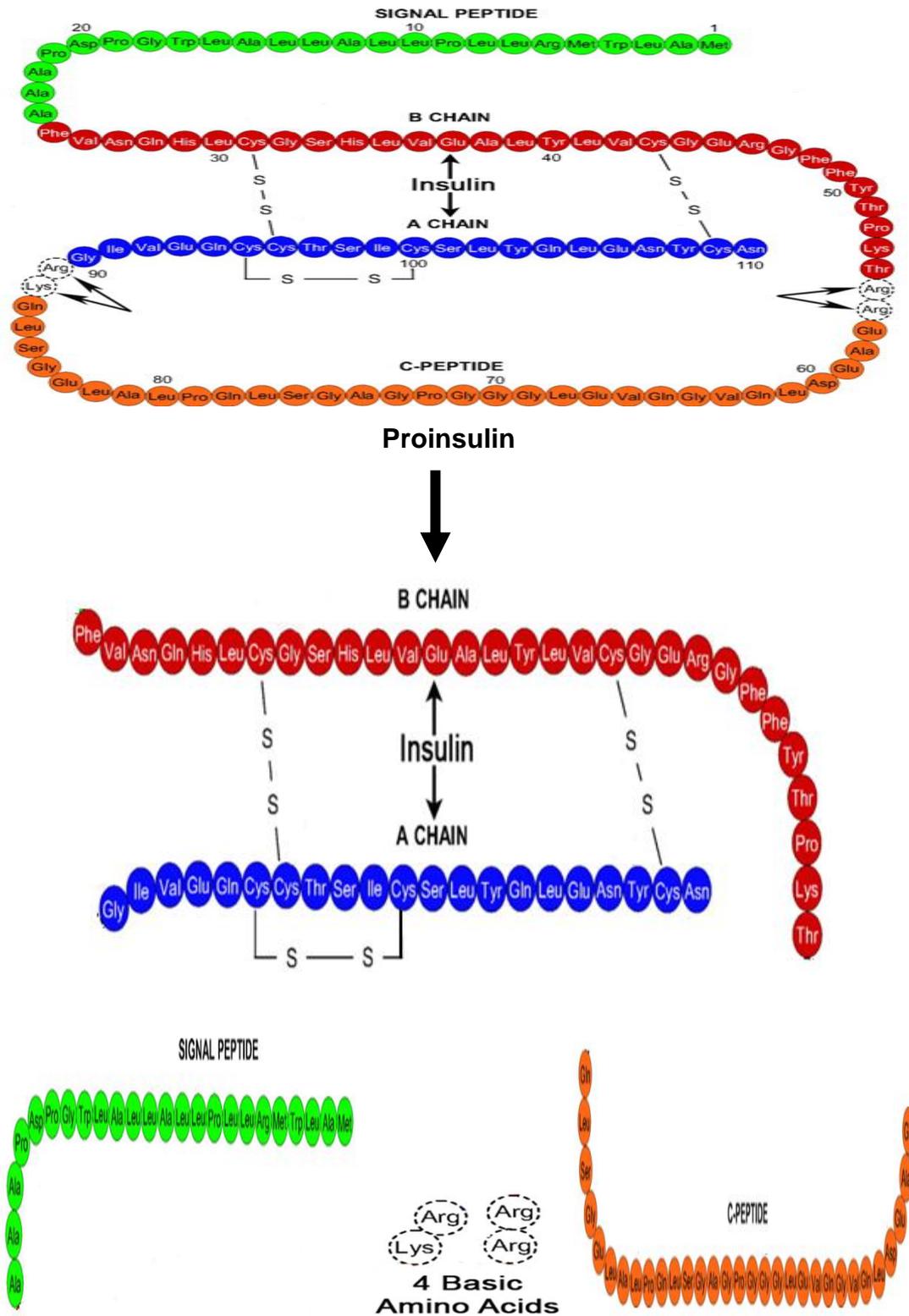


Figure 1.5: Diagram showing the conversion of proinsulin to signal peptide, insulin, C-peptide, and four basic amino acids (Modified from Montague, 1993).

Mammalian insulin gene expression is mostly associated with β -cell of the pancreas. The insulin promoter area is located 400 bp, upstream from the insulin gene, where the transcription initiation process starts. Insulin gene transcription may be controlled via tissue-specific expression and metabolic factors. Several transcription factors operate through this area, creating a transcriptional network, which ensures precise control of insulin gene transcription. The greatest critical acting DNA factors associated with transcriptional initiation are related to the A3, C1 and E1 sites (Poitout et al., 2006).

The production and release of insulin via the pancreatic β -cells is highly critical in preserving normoglycaemia. This is achieved by accurate regulation of insulin synthesis and secretion via β -cells under the influence of variations in blood glucose concentrations. The production of insulin is regulated by blood glucose concentration at the transcriptional and post-transcriptional stages. Although great number of transcription factors correlate with the role of insulin gene transcription, three β -cell-specific transcriptional factors, pancreatic duodenal homeobox-1 (PDX-1), neurogenic differentiation-1 (NeuroD1) and V-maf musculoaponeurotic fibrosarcoma oncogene homologue A (MafA), are reported to have an essential effect on glucose stimulation of insulin gene transcription and β -cell activities in the pancreas (Poitout et al., 2006). These three transcription regulators enhance insulin gene expression in accordance to elevated blood glucose levels. Fluctuations in blood glucose concentration change the activity of these transcription factors at different stages. Likewise, variations in calcium concentrations, food accessibility and hormone secretion also stimulate changes in the activity of these three transcription regulators (Chalmers and Cooper, 2008). These comprise modifications of the expression rates, DNA-binding action, transcription capacity, changing the location of some cellular organelles, and reactions between different proteins (Andrali et al., 2008). Glucose sensitivity to the insulin promoter is essentially controlled by A3, E1 and C1 boxes, which bind to the transcription factors PDX-1, NeuroD1 and MafA, respectively (Kataoka, 2007). These three transcription regulators enhance insulin gene expression under the effect of hyperglycaemia (Song et al., 2007).

Glucose modifies the activity of these three transcription regulators via different mechanisms. Alteration of glucose levels has a critical role in changing the localization of PDX-1 in β -cell either to decrease or increase the rate of insulin gene expression through their effect on DNA-binding and the interaction of PDX-1 with different cell proteins. At low glucose (1-3 mM), concentrations PDX-1 is mostly found in the nucleus periphery of β -cell and few unphosphorylated PDX-1 molecules bind to co-repressors protein at A box of insulin gene promoting area that leads to suppression of insulin gene transcription. However, high glucose concentrations (10-30 mM), activate the phosphorylation and translocation of PDX-1 to bind to co-activator protein at A box that enhances insulin gene transcription (Stanojevic et al., 2004).

In case of NeuroD1, it is suggested that NeuroD1 changed its position under the effect of various glucose levels. At low glucose levels (1-3 mM), NeuroD1 is mostly located in the cytoplasm of the β -cell. While under elevated glucose concentrations (10-30 mM), this can lead to an increase in the entrance of NeuroD1 into the nucleus. Inside the nucleus, NeuroD1 phosphorylation occurs and its binding to co-activator protein at E1 box within the insulin promoter causes enhancement of insulin gene transcription (Andrali et al., 2007).

The expression of MafA gene requires elevated glucose levels in β -cell. Therefore, MafA transcription is reduced at low glucose levels (1-3 mM). However, elevated glucose concentrations (10-30 mM), lead to an increase in MafA translocation to the nucleoplasm and its binding to C1 box at the promoter area of insulin gene that elevates insulin gene transcription (Vanderford et al., 2007).

1.3 Glucose transporters

There are thirteen members of the facilitative glucose transporter family (GLUT-1-12) and H⁺-coupled myo-inositol transporter (HMIT). In addition, the sodium-dependent glucose transport is mediated by Na⁺/glucose co-transporters (SGLT). There are about

six individuals of this family, however, only SGLT-1 and SGLT-2 have been extensively studied (Wright and Turk, 2004). The GLUTs basic structure is composed of twelve transmembrane-domains areas with intracellular situated carboxyl- and amino-termini (Figure 1.6) (Gorovits and Charron, 2003). GLUTs operate with facilitative diffusion gradient of sugar through the cell plasmalemma and reveal diverse substrate kinetic properties (K_m), specificities and tissue expression systems. The specific substrate kinetic (K_m) or Michaelis constant is an indicator of the affinity of the glucose transporter receptor for glucose molecules; a lower K_m value suggests a higher affinity. This affinity determines the degree of glucose and other sugars uptake into different cells. Each GLUT exerts variable degrees of insulin dependence and affinity (Joost et al. 2002). The expression of different sugar transporter isoforms in different body tissues, and cells are summarized in table 1.1 (Wood and Trayhurn, 2003; Zhao, and Keating, 2007).

All the GLUT family members have ‘sugar transporter signatures’. GLUT family members (Figure 1.6) may be classified into three subclasses (I-III) according to the sequence and motifs arrangement (Joost and Thorens, 2001).

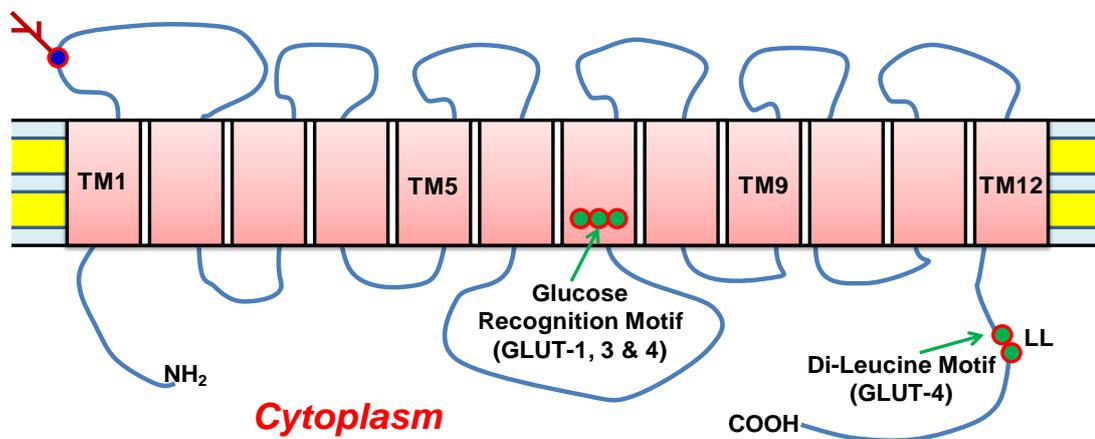


Figure 1.6: Diagram showing the glucose transporter of twelve hydrophobic transmembrane domains (TM rectangles) which are linked by hydrophilic loops. The COOH and NH₂ termini are directed intracellularly (Modified from Gorovits and Charron, 2003).

Table 1.1: Summary of the facilitative transporters family (GLUTs) (Modified from Wood and Trayhurn, 2003; Zhao, and Keating, 2007).

Facilitative Transporter	Class	Tissue Expression	Substrate & Affinity (Km)	Insulin Sensitive	Function (Transport)
GLUT-1	I	Erythrocytes, Brain, Liver, Skeletal muscle, Adipose tissue	Glucose 1-2 mM	No	Basal glucose uptake
GLUT-2	I	Liver, β -cells, Intestine, Kidney	Glucose 15-20 mM	No	Glucose & Fructose sensing & transport by liver & pancreas
GLUT-3	I	Brain (Neurons)	Glucose 1-2 mM	No	Basal glucose uptake
GLUT-4	I	Adipose tissue, Skeletal muscle, Heart, Brain	Glucose 5 mM	Yes	Insulin-stimulated glucose uptake
GLUT-5	II	Intestine, Kidney, Sperm, Skeletal muscle, Adipose tissue	Fructose 10-13 mM	No	Transport of fructose from intestinal lumen into the cells & into sperm
GLUT-7	II	Small intestine, Colon, Testis	(Glucose & Fructose) < 0.5 mM	Unknown	Glucose & Fructose
GLUT-9	II	Liver, Kidney	Urate \approx 0.6 mM	Unknown	Urate
GLUT-11	II	Skeletal muscle, Heart, Liver, Trachea, Lung, Brain	Glucose & Fructose	No	Glucose (Short isoform) - Fructose (Long isoform)
GLUT-6	III	Brain, Spleen, Leucocytes	Glucose 5 mM	No	Glucose
GLUT-8	III	Brain, Testis, Mammary Gland	Glucose 2 mM	No (Yes in Blastocytes)	Glucose
GLUT-10	III	Liver, Pancreas Skeletal muscle, Heart,	Glucose 0.3 mM	Yes	Glucose
GLUT-12	III	Heart, Prostate, Muscle, Small intestine, Adipose tissue	Glucose	Yes	Glucose
HMIT	III	Brain	H ⁺ -Myo-Inositol ~100 mM	Unknown	H ⁺ -Myo-Inositol

Na ⁺ /Glucose Cotransporters	Tissue Expression	Substrate & Affinity (Km)	Function (Transport)
SGLT-1	Intestine, Kidney	Glucose 0.2 mM	Glucose reabsorption in intestine and kidney
SGLT-2	Kidney	Glucose 2 mM	Glucose reabsorption in kidney

1.3.1 Class I facilitative transporters

The class I facilitative transporters have GLUT-1-4, and these have been widely categorized in relation to tissue distribution, structure and function. GLUT-1 is found mainly in the brain and erythrocytes and to a smaller extent in skeletal muscle, adipose tissue and liver. GLUT-1 is located in the plasmalemma of cells, as it is responsible for maintaining a basal rate of glucose uptake. The K_m of GLUT-1 is low (1-2 mM) with high glucose affinity (Zimmerman et al., 2001). In contrast, GLUT-2 has a high K_m value (15-20 mM) and therefore a low affinity for glucose. GLUT-2 is found in the plasmalemma of cells and it is also involved in preserving a basal glucose uptake rate. GLUT-2 is mostly expressed in β -cells, liver and kidneys. In the pancreatic β -cells, GLUT-2 is responsible for the glucose-sensing system. GLUT-2 is expressed in the liver under hormonal regulation and it permits for glucose transport in and out of hepatocytes. GLUT-2 is expressed on the proximal convoluted tubules and enterocytes in the kidney, where it plays a role in fructose and glucose secretion and reabsorption. GLUT-2 is also found in basolateral and brush border membranes of the small intestine (Stolarczyk et al., 2010). GLUT-3 exhibit a great affinity for glucose (K_m 1-2 mM) and it is located in the plasmalemma of cells. GLUT-3 is expressed in brain tissues where there is ultimate need for reserving a basal rate of glucose uptakes as an essential source of fuel for brain cells (Simpson et al., 2008). GLUT-4 is an insulin-responsive glucose transporter and it is expressed in skeletal muscle, adipose tissue, heart and brain. As muscle is an essential storage site for glucose and adipose tissue for triglyceride (into which glucose can be transformed for storage). The main function of GLUT-4 in these tissues is to lower the postprandial increase in blood glucose concentrations. After a meal, insulin stimulates the mobilization of GLUT-4-containing vesicles from cytoplasmic stores to the cell plasma membrane. Integration of GLUT-4 to the plasmalemma leads to fast elevation of glucose influx of about 10-20-fold (Bryant et al. 2002). A decreased expression of GLUT-4 in adipose tissue is detected in different diabetic animal models and human. GLUT-4 knockout mouse models show the involvement of GLUT-4 in the pathophysiology of T2DM (Abel et al. 2001).

1.3.2 Class II facilitative transporters

Class II GLUTs include GLUT-5, GLUT-7, GLUT-9 and GLUT-11. GLUT-5 is a fructose transporter of low affinity (K_m 10-13 mM). GLUT-5 is found mainly in the small intestine, kidneys and sperms. GLUT-5 mediates diffusion of fructose from the brushborder membrane of the intestinal lumen into the intestinal epithelial cells. GLUT-5 is also expressed in skeletal muscle and adipose tissue. The expression of GLUT-5 in human muscle is associated with the muscle ability to use fructose as a source of glycolysis and glycogenesis without involvement of GLUT-4 and GLUT-1 (Ferraris, 2001). GLUT-7 is expressed in the small intestine, colon and testis. GLUT-7 has a great affinity (< 0.5 mM) for glucose and fructose. The closest GLUT to GLUT-7 is GLUT-5. The accumulation of GLUT-7 in the small intestine matches the concentration of carbohydrate in the diet. However, the expression of GLUT-7 in the colon was not changed by the concentration of carbohydrate in the diet (Cheeseman, 2008). GLUT-9 is found in the liver and kidney. GLUT-9 regulates urate homeostasis by its double role in urate reabsorption in the kidney and urate degradation in the liver. Defect in GLUT-9 is one of the main causes of hyperuricaemia-induced gout (So and Thorens, 2010). GLUT-11 has two isoforms and each one is tissue-specific. The short isoform of GLUT-11 has reduced-affinity to glucose transport and is competed for by fructose. This type of GLUT-11 is found mainly in heart and skeletal muscle (Doege et al. 2001). The long isoform of GLUT-11 is found in liver, trachea, lung and brain. All of which help to elevate fructose transport (Wu et al. 2002). GLUT-5 is a fructose sensitive GLUTs and its structure is very close to GLUT-11. This explains the possible role of GLUT-11 as a compensatory glucose transport in GLUT-4 (-/-) muscle (Doege et al., 2001).

1.3.3 Class III facilitative transporters

Class III of facilitative transporters contain five members: GLUT-6, GLUT-8, GLUT-10, GLUT-12 and HMIT (Joost et al. 2002). GLUT-6 has a low affinity for glucose

(Km 5 mM). GLUT-6 is expressed in the brain, spleen and leucocytes (Doege et al. 2000). GLUT-6 is insensitive to insulin-stimulated glucose transport cytoplasmic translocation to the cell plasma membrane (Lisinski et al., 2001). GLUT-8 is present in the testis and brain (Ibberson et al. 2000). However, initiation of GLUT-8 translocation by insulin has been detected only in blastocytes. GLUT-8 may also play a main role in the delivery of glucose to mature sperm. GLUT-8 expression in mammary gland is intense throughout late pregnancy to early lactation, indicating its role in glucose uptake for milk production (Membrez et al., 2006). GLUT-10 is expressed in the liver and pancreas (McVie-Wylie et al. 2001). GLUT-10 is also found in the insulin-sensitive tissues such as skeletal and heart muscles. GLUT-10 is associated with the development of T2DM (Dawson et al. 2001). GLUT-10 deficiency may lead to arterial tortuosity syndrome and this relation may explain its mechanisms in causing microangiopathy normally seen in T2DM (Coucke et al., 2006). High affinity glucose transporters has been reported (Km 5, 2, and 0.3 mM) for GLUT-6 (Doege et al. 2000), GLUT-8 (Ibberson et al. 2000) and GLUT-10 (Dawson et al. 2001), respectively. GLUT-12 transporter is found in the heart, small intestine, prostate, adipose tissue and insulin-sensitive tissues. GLUT-12 transporter is a part of insulin-responsive glucose transport system, which influences its translocation to the cell membrane (Rogers et al. 2002). GLUT-12 is also expressed in the distal tubules and collecting ducts of the nephron and may play an important role in glucose reabsorption in the kidneys (Linden et al., 2006). On the other hand, HMIT is a H⁺-coupled myo-inositol transporter (Km ~100 mM) with no transport effect on glucose. HMIT is found mainly in the brain. HMIT exists in neuronal cytoplasm and may lead to the movement of myo-inositol the cell membrane resulting in cell depolarization, stimulation of protein kinase C or elevated intracellular calcium levels (Uldry et al., 2004). The intracellular actions for this transporter may be relevant to mood control in the brain (Di Daniel et al. 2009).

1.3.4 Sodium-dependent glucose transporters

At the small intestine epithelial cell brush border and the proximal convoluted tubules of the kidney, glucose is either absorbed or reabsorbed against its concentration gradient as a co-transporter with Na^+ down its concentration gradient (Figure 1.7). These transport systems are in continuous association with $\text{Na}^+/\text{K}^+/\text{ATP}$ pump machinery (Zhao and Keating, 2007).

SGLT-1 shows a strong glucose and galactose affinity ($K_m \sim 0.4$). SGLT-1 is largely expressed in the brush border membrane of the epithelial cells of the intestinal lumen and it is essentially involved in the absorption of the glucose and galactose in the meal (Suzuki et al., 2001). Expression of SGLT-1 has been detected in numerous body tissues such as the kidney, the liver and the mammary gland, which are associated with glucose uptake via different types of facilitative transports (Zhao et al., 2005). Not only sugar transporter but also, SGLT-1 plays an active role as a co-transporter for water and thus, it may be a multifunctional co-transporter (Wright et al., 2003).

SGLT-2 is expressed mainly in the kidney (K_m 2 mM) where it reabsorbs glucose from the luminal membrane of the proximal convoluted tubules of the nephron by Na^+ coupling via down inward gradient flow of Na^+ across the luminal epithelium. The process is continued by active transport of Na^+ through the basolateral membrane back to the blood circulation (Figure 1.7). Glucose is passively transported outside the cell down its concentration gradient via GLUT-2 (90%) and the remaining 10% by GLUT-1 (Wright, 2001). SGLT-2 inhibitors could be a novel therapeutic target for the treatment of T2DM (Chao and Henry, 2010).

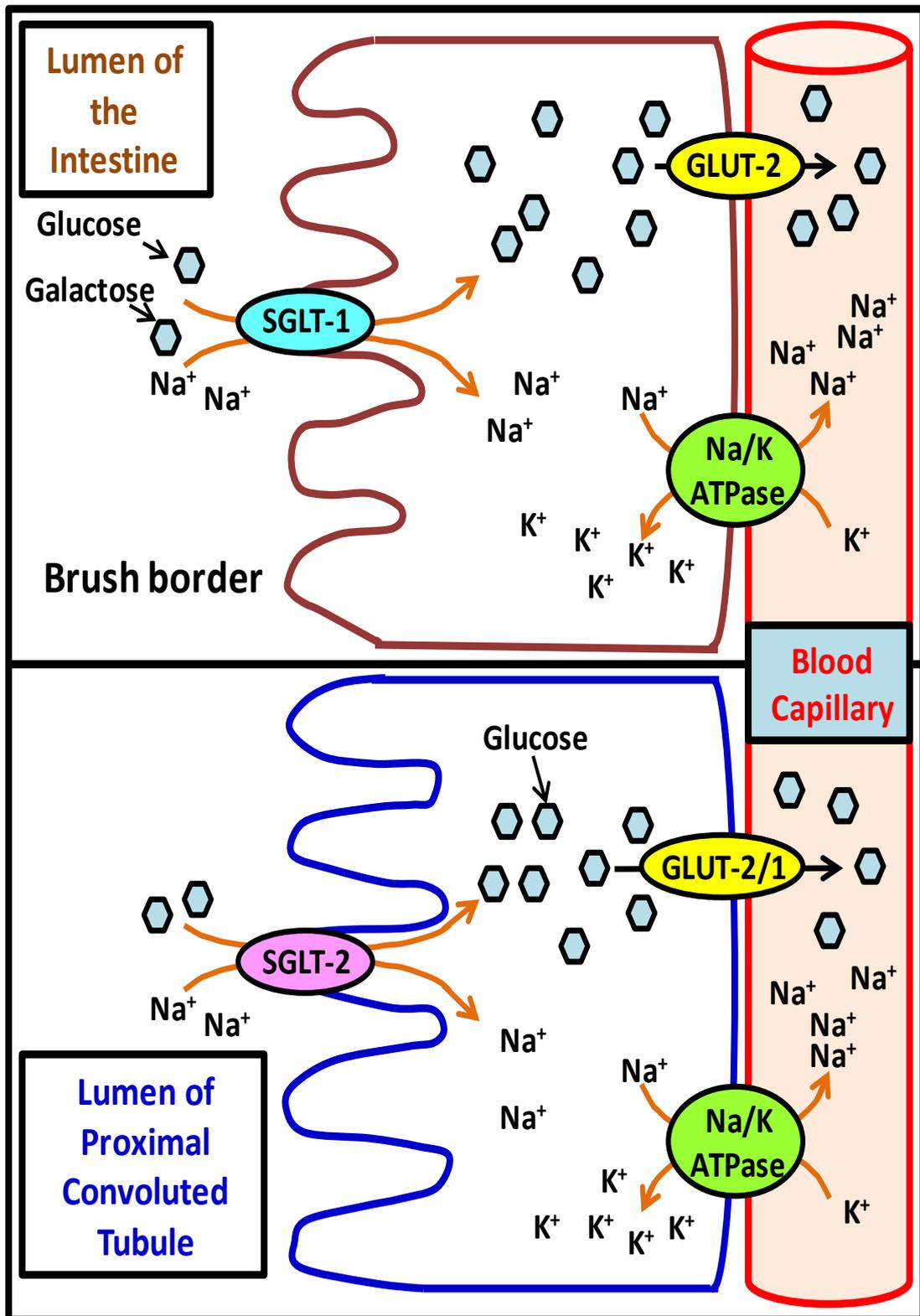


Figure 1.7: Schematic diagrams showing sodium-dependent glucose transporters (Modified from Caccia et al., 2007; Nair and Wilding, 2010).

1.4 Glucose metabolism

The concentration of plasma glucose is tightly controlled throughout different normal body activities. The normal range of glucose in the plasma is 3.5-8.0 mM/L (63-144 mg/dl) regardless of extreme conditions such as intake of large quantities of food, starvation and intense physical activities (Arciero et al., 1999). The process of glucose uptake by different body tissues is under the influence of two factors including the physiological requirements of the tissue and accessibility of glucose. The two systems in which glucose uptake occur are facilitated transport (a passive way) down the glucose concentration gradient and secondary active diffusion against glucose concentration gradient (an active way that indirectly needs adenosine triphosphate, ATP, as a source of energy). The liver controls glucose tolerance via glucose uptake and then converting it to glycogen. The liver later releases glucose into blood circulation between meals according to the need of peripheral tissues. The liver may also change glycerol and protein as non-carbohydrate molecules via gluconeogenesis into glucose (Beauvieux et al., 2008).

The brain is the main organ for glucose use. The rate of glucose uptake via the brain is about 1 mg/kg body weight/minute. This is a compulsory process and it is insulin independent (Mealey and Ocampo, 2007). Large quantity of glucose is also used up by muscle and adipose tissue. Muscle cells store glucose as glycogen and adipose tissue use glucose as a substrate for fuel or for the production of triglycerides (Gomes et al., 2009).

Although influenced by other hormones, insulin is a key determinant peptide in the regulation of glucose metabolism. At decreased plasma insulin concentration (e.g. 10 IU μl^{-1}), glucose release is at highest level and its use is at lowest. On the other hand, at high insulin level (e.g. 300-500 IU μl^{-1}), glucose use and uptake are at the highest level. While, at intermediate plasma insulin level (e.g. 40-50 IU μl^{-1}), hepatic and peripheral glucose release is reduced. Glucagon, adrenaline and growth hormones have an antagonistic effect on insulin action. They increase the production of glucose and reduce its utilization by the body (Kumar and Clark, 2007).

Insulin is not needed for the entrance of glucose into many body cells including the liver, kidney, retina, lens, Schwann cells of peripheral nerves, placenta, red blood cells, seminal vesicles and cells of the ovaries (Wiernsperger, 2005). As insulin is not needed for glucose uptake to the mentioned cells, high levels of glucose could be taken by these cells during hyperglycaemia especially during DM. The main type of facilitative glucose transporter (GLUT-1) in the plasma membrane of brain and liver cells is non-insulin dependent and it is responsible for maintaining a basal rate of glucose uptake for these tissues. The Km value of GLUT-1 is low (1-2 mM) with high glucose affinity (Zimmerman et al., 2001). Raised cytosolic glucose level and a sufficient amount of reduced nicotinamide adenine dinucleotide phosphate (NADPH) can lead to aldose reductase to form sorbitol, which unlike glucose, cannot diffuse through cell membranes and consequently it is kept stocked inside the cell (Kumar and Clark, 2007). This is worsened when sorbitol dehydrogenase is either reduced or absent in such tissues as the retina, lens, kidney and peripheral nerve cells. As a result, sorbitol accumulates in the mentioned cells, leading to elevated osmotic pressure and cell swelling due to holding of much water. Many of the pathological changes related to DM can be referred to this phenomenon, with cataract formation, peripheral neuropathy and vascular problems leading to nephropathy, retinopathy and cardiomyopathy (Maritim et al., 2003).

1.4.1 Stimulation of insulin secretion by β -cells

The pancreatic β -cell is a greatly specialized cell that synthesizes, stores and secretes insulin under the effect of increased blood glucose level. To achieve this function, β -cell has a sensory system for detecting blood glucose level in order to regulate the rate for either the synthesis or the release of insulin. β -cell glucose sensing system depends on glucose metabolic products that form a second messenger which in turn stimulates the exocytosis of insulin granules (Leroux, et al., 2001).

Glucose is the main secretagogue for insulin release. Glucose enters into the β -cell cytoplasm at high rate via GLUT-2 and it is quickly phosphorylated into glucose-6-

phosphate (G-6-P) by glucokinase enzyme. Glucokinase acts as a key factor for glucose sensation and it determines the speed of glycolysis. At the end of glycolysis, pyruvate is formed and it enters into the Krebs's cycle in mitochondria (Mt). This in turn leads to an elevation of the ATP/ADP ratio, which in turn activates the ATP-sensitive K^+ channels closure. Decrease of K^+ levels in the cytoplasm results in both the initiation and propagation of action potentials and membrane depolarization, thus enhancing the opening of voltage-dependent Ca^{2+} (VDC) channels and increases Ca^{2+} entry into the β -cell. The elevated cytosolic Ca^{2+} level enhances the binding of insulin-containing secretory vesicles to the cell plasma membrane (figure 1.8). Then, insulin granules are released by a process called exocytosis (Dunne, et al., 2004).

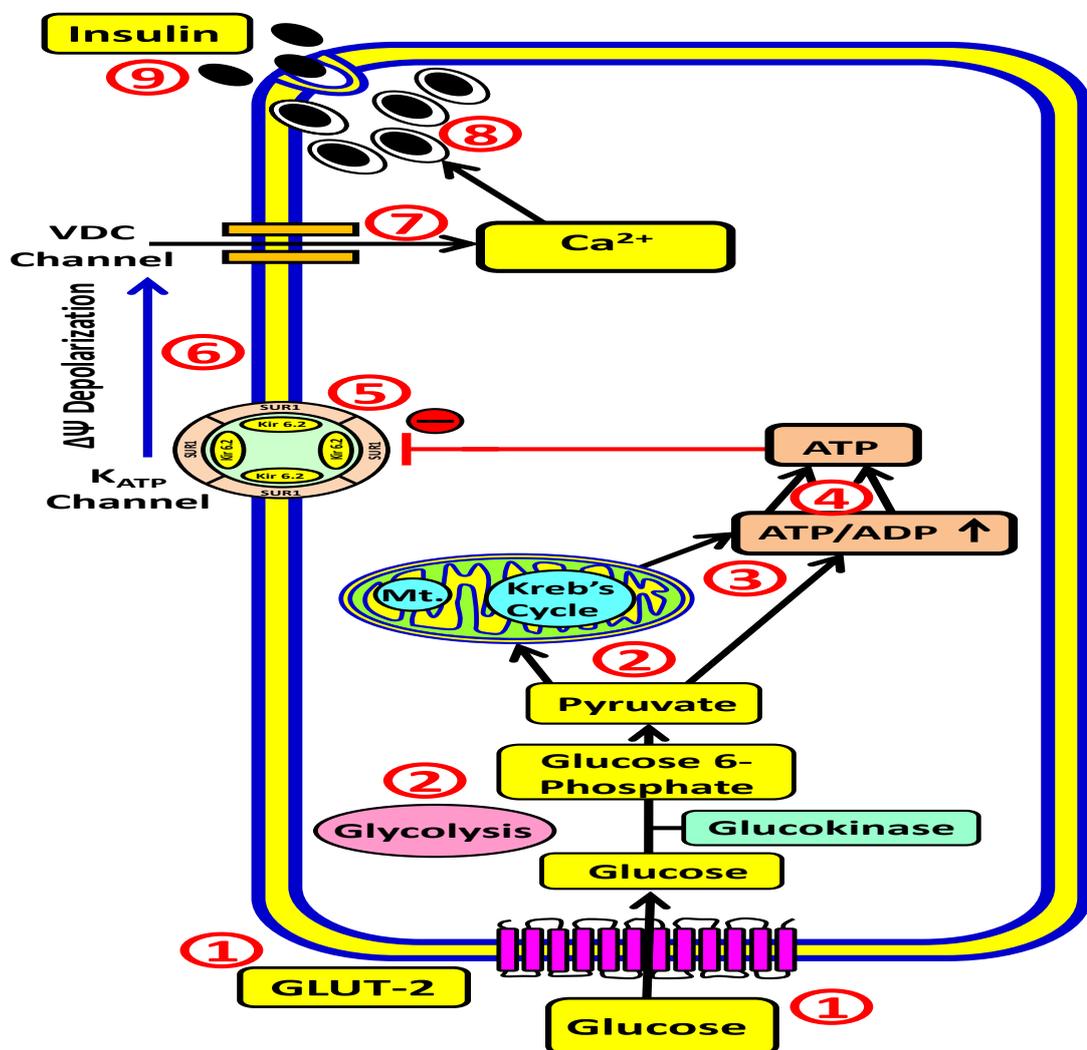


Figure 1.8: Schematic diagram showing the process of glucose-stimulated insulin secretion from an endocrine pancreatic β -cell (Modified from Jensen et al., 2008).

1.4.2 Insulin receptor (IR)

Insulin receptor (IR) is a heterotetrameric cell membrane binding protein receptor that consists of two extracellular α -subunits that bind insulin and two transmembrane β -subunits, with tyrosine kinase domain (Saltiel and Kahn, 2001). Figure 1.9 shows the relationship between insulin receptor and glucose transporter. IR is a member of tyrosine kinase receptor. IR is a glycoprotein and it is located on chromosome 19. The α -subunits of IR have three sub-domains ligand binding sites. Minimally, two epitopes overlap to create binding surfaces to insulin molecule, and in this way, insulin can bind asymmetrically with the two α -subunits receptors (Meyts et al., 2007). The β -subunit contains a part that is extracellular and binds to α -subunits by disulphide bonds, a part, which contains the trans-membrane portion of the receptor, and a region that is intracellular. The $\alpha\beta$ -dimeric receptor is capable of binding with insulin granules; however, the $\alpha\alpha\beta\beta$ -tetrameric receptor binds insulin with higher affinity. The β -subunits have the intrinsic tyrosine-protein-kinase enzymatic action (Amessou et al., 2010). IR gene is expressed all over the body tissues and cells, the concentrations of IR mRNA and its protein, differ between cells and tissues types and are controlled by variant elements and environmental factors (Amessou et al., 2010). The chief insulin target tissues are liver, adipose tissue, and skeletal muscle. IR is found in further tissues like brain, kidney, heart, pancreatic acini, pulmonary alveoli, placental vascular endothelium, erythrocytes, granulocytes, monocytes, and fibroblasts. The wide distribution of insulin receptor in numerous tissues of the body shows that IR has a distinctive effect in different organs (Belfiore et al., 2009). Insulin granule binds with the α -subunit to enhance tyrosine phosphorylation of the IR β -subunit (see Figure 1.9). The stimulated IR then phosphorylates its substrates (tyrosine phosphorylation). Insulin receptor substrates (IRSs) have four forms, IRS-1, IRS-2, IRS-3 and IRS-4 (White, 2002). The phosphorylated proteins activate downstream effectors cascades that induce various signalling reactions (Saltiel and Kahn, 2001). IRS-1 shows an essential effect in metabolic activities of insulin, while IRS-2 contributes mainly in controlling of glucose levels (Withers et al., 1999). After binding between insulin and its receptor, GLUT-4 glucose transporter moves towards the cell plasmalemma. Insulin enhances glucose uptake by muscle and adipose tissue via activating the translocation of GLUT-4. Insulin

binding to its IR initiates tyrosine phosphorylation of IRS-1/2 downstream signalling in the cytoplasm leading to stimulation of phosphatidylinositol 3-kinase (PI-3K), protein kinase B (PKB) and protein kinase C- ζ (PKC- ζ) downstream cascades (Figure 1.9). These protein kinases activate the translocation GLUT-4 into the cell membrane, which later contribute to glucose influxes into the cytoplasm (Huang and Czech, 2007). Finally, the IR complex is then moved into the cytosol where insulin is degraded and the IR move back to the cell membrane to be re-used (Kumar and Clark, 2007).

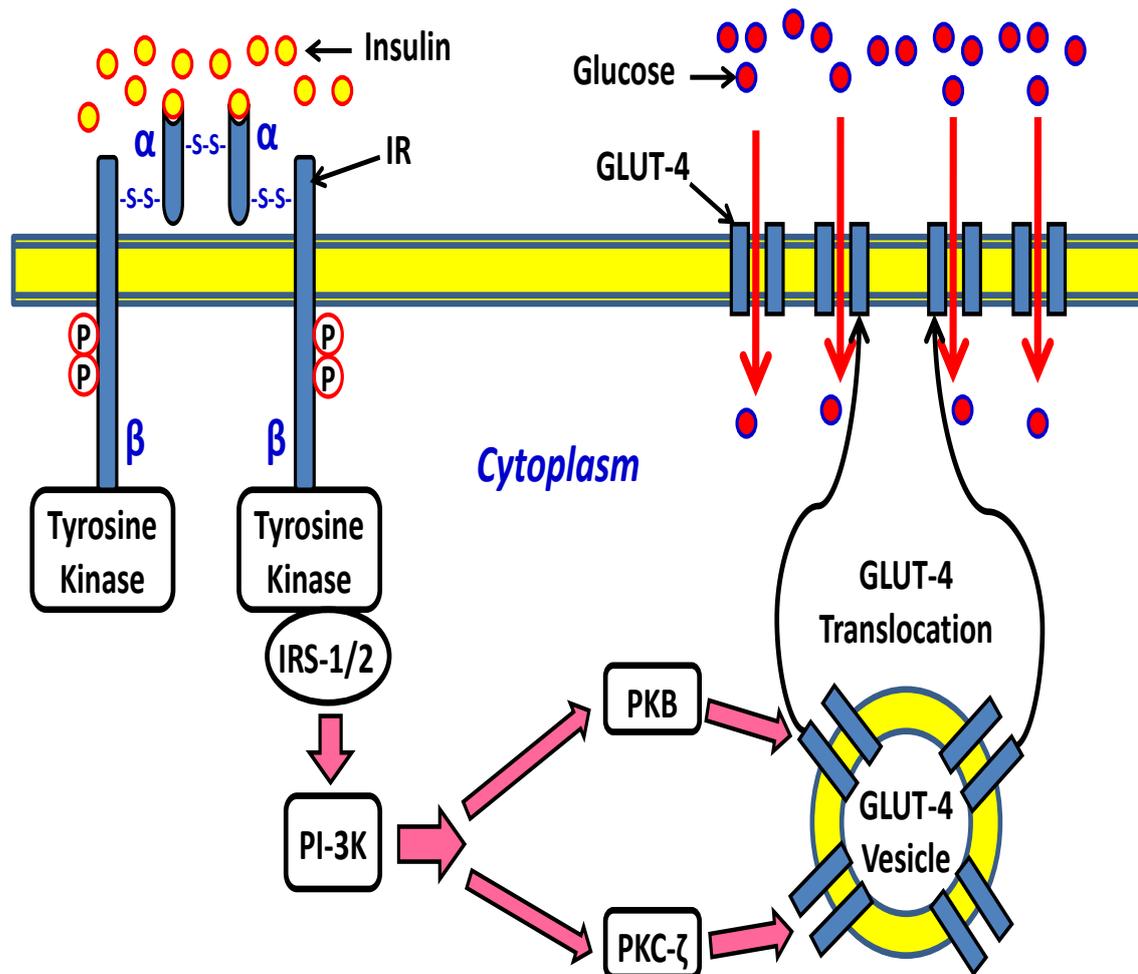


Figure 1.9: Diagram showing the relationship between the effect of insulin binding to its IR in the plasmalemma of adipocytes and muscle cells and cascade of events resulting in translocation of GLUT-4 to the cell membrane and increase in glucose uptake. IRS-1/2, insulin receptor substrate 1 or 2; PI-3K, phosphatidylinositol 3-kinase; PKB and PKC- ζ , protein kinase A and C- ζ (Modified from Schulingkamp et al., 2000).

1.5 Glucagon

Glucagon is produced in the endocrine pancreas from α -cells of islets of Langerhans. Glucagon is a polypeptide hormone formed of a single polypeptide chain of 29 amino acids (Figure 1.10) (Guyton and Hall, 2005). Proglucagon gene is expressed into glucagon in the pancreas while in the intestine it processed into GLP-1 and GLP-2. Glucagon has antagonistic effect to insulin action. At low glucose concentration, glucagon is released leading to a reduction in glucose oxidation and an increase in blood glucose concentration. Glucagon also increases blood glucose level mostly through enhancing the breakdown of glycogen in the liver via glycogenolysis. On the other hand, glucagon elevates blood glucose via enhancing lipolysis in both liver and adipose tissue. To a lesser extent, glucagon promotes proteolysis (Kumar and Clark, 2007). At regular situation, glucagon does not induce glucose uptake via either muscle or adipose tissue. Muscle glycogen acts as an energy store for muscular action and glycogen consumption is regulated by muscle contraction through catecholamines. Glucagon generally, has no effect on muscle glycogen (Carneiro and Amaral, 1983; Roach, 2002; Rivera et. al., 2007).

Glucagon enhances energy production more than storage especially from glucose, to maintain normal blood glucose level at different body needs. Glucagon exerts its effect on liver by binding to the hepatic cell membrane receptor and initiates a sequence of intracellular reactions through cAMP as a second intracellular messenger to induce or inhibit either kinase or phosphatase enzymes. These in turn inhibit glycogenesis, promote glycogenolysis and elevate glucose synthesis. At the same time, glucagon stimulates gluconeogenesis and reduces glycolysis. On the other hand, glucagon promotes β -oxidation of non-esterified fatty acids and reduces triglyceride production causing ketogenesis. Glucagon also inhibits coenzyme A reductase that leads to reduction of cholesterol production in liver cells. Glucagon has other effects on different organs of the body. Glucagon elevates cardiac output by stimulation of the adenylate cyclase enzyme of heart cells. Glucagon increases sodium levels in urine by reducing tubular reabsorption in the kidney and finally, it enhances lipase action in adipose tissue (Tan, 2008).

**H-His-Ser-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Asp-
Ser-Arg-Arg-Ala-Gln-Asp-Phe-Val-Gln-Trp-Leu-Met-Asn-Thr-OH**

Figure 1.10: A diagram showing the amino acid sequence of glucagon (Modified from Guyton and Hall, 2005).

1.5.1 Glucagon synthesis and secretion

Glucagon is produced mainly by pancreatic α -cells. It is also synthesized by different cells of gastrointestinal tract (stomach and intestine). Glucagon secretion is induced by various types of amino acids like alanine and arginine with different rate. Some other compounds, which can stimulate glucagon secretion, include catecholamines and gut hormones like gastrin, cholecystokinin and gastroinhibitory peptide. The autonomic nervous system (sympathetic and parasympathetic) also controls the secretion of glucagon by enhancement of its secretion at low blood glucose level and inhibits its release during hyperglycaemia (Kumar and Clark, 2007).

1.5.2 The interaction between insulin and glucagon

The pancreatic hormones control the metabolism of glucose, non-esterified fatty acids and amino acids. Both insulin and glucagon have antagonistic actions on substrate fluctuations, which are controlled by the insulin-glucagon ratio. The normal molar ratio is 2:1 however, it is converted to 1:2 at increased demand for mobilization of endogenous substrates, in case of fasting or extended exercise. The decrease in insulin release and the increase in glucagon release can lead to glycogenolysis and gluconeogenesis in the liver and lipolysis in adipose tissue in addition to increasing the liver metabolism of non-esterified fatty acids needed for β -oxidation (Tan, 2008).

After a meal, the insulin-glucagon ratio is elevated to about 10:1, mainly due to enhanced insulin release. This leads to activation of glucose uptake associated with oxidation and conversion of glucose to glycogen in either liver or muscle. Postprandially, there is no need for non-esterified fatty acids since in the adipose tissue lipolysis is suppressed (Cahova et al., 2007).

The pancreatic endocrine action is controlled through many factors including glucose. This controlling action involves the parasympathetic nervous system that enhances insulin and glucagon release, while the sympathetic pathway which enhances glucagon but reduces insulin release. The pancreas responds differently according to various body conditions (Quesada et al., 2008).

1.6 Somatostatin

Somatostatin is a pancreatic hormone synthesized in the form of prosomatostatin from δ -cells of islets of Langerhans. The 14-amino acid peptide of somatostatin (SS14) is a powerful suppressor of the release of insulin, glucagon and pancreatic polypeptide. The cells of the intestine also synthesise prosomastatin but process it in another way into 28 amino acid (SS28). SS28 suppresses the movements of gall bladder, stomach and intestine. Additionally, SS28 decreases gut secretions (Tostivint et al., 2008).

In the case of increased body physical activity with reduced blood glucose level, somatostatin decreases the secretion of growth hormones and thyroid stimulating hormone. Somatostatin reduces the volume of blood reaching to the stomach and spleen and inhibits the secretion of hydrochloric acid from the stomach and digestive enzymes from the pancreas (Bosch et al., 2009).

There are many stimulants for the release of somatostatin from either δ -cells of the pancreas or intestine. They include glucose, ketone bodies, amino acids, cholecystokinin, secretin and gastrin hormones. Somatostatin release is induced by insulin only at increased blood glucose levels. As somatostatin has inhibitory effect on insulin production and secretion, so insulin can inhibit its own release via inducing

somatostatin secretion (Brunicardi et al., 2001). On the other hand, decreased blood glucose level induces somatostatin release under the effect of glucagon-mediated secretion. Generally, it appears that there is a negative feedback relationship of somatostatin with either glucagon or insulin (Muroyama et al., 2004; Strowski and Blake, 2008). An elevated count of δ -cells has been detected in either T1DM or T2DM. This condition is associated with disturbed glucose-induced somatostatin release. In diabetic patients, both counts of δ - and α -cells are increased, but the elevation of α -cells is much more than that of δ -cells. In addition, the ratio of δ/α -cell is decreased due to increased α -cell hyperproliferation. This in turn can lead to disturbance in δ/α -cell ratio and causes impairment in glucose-stimulated somatostatin secretion. Somatostatin inhibitory effect on glucagon secretion is abolished and this condition worsens the hyperglycaemia in diabetes. The basal level of somatostatin secretion is normal in T2DM while it is elevated in T1DM. This difference may be due to the abolished secretion of insulin in T1DM as an essential controlling element for the normal secretion of somatostatin from pancreatic δ -cells (Strowski and Blake, 2008). Somatostatin may be an additional treatment of diabetes though its suppressing effect on glucagon releases (Madsen et al., 2002).

1.7 Pancreatic polypeptide

Pancreatic polypeptide (PP) is a peptide formed from 36 amino acids. PP is released from the pancreatic polypeptide cells in islets of Langerhans and is also secreted from the islet cells directly linked to acini cells. In contrast to glucagon, PP is mainly more in the head of the pancreas compared to the tail. PP is expressed chiefly in pancreatic endocrine cells and at low levels in gut cells (Ekblad and Sundler, 2002). The essential function of PP is to reduce the release of pancreatic bicarbonate and proteins. PP is released under the effect of the autonomic nervous system. In addition, PP leads to dilatation of the gallbladder and a reduction of hepatic bile release (Hennig et al., 2002). PP demonstrates a suppressory effect on pancreatic exocrine release. PP increases the secretion of glucocorticoid and changes gastric acid release and gastrointestinal movement. The quantity of PP secretion is altered with the condition of

digestion. The level of PP secretion is reduced during fasting and is elevated greatly after a meal. The secretion of PP is stimulated by the reduced concentration of blood glucose. PP can be stimulated by vagal cholinergic reflex because vagotomy and treatment with muscarinic receptor antagonists inhibit this action, thus supporting a role for the vagal-cholinergic system in PP release. Accordingly, PP may induce vagal stimulus to pancreas and many organs in the gut. PP is also involved in modifying ingestion mode to control energy equilibrium. PP secretion after a meal leads to reduction of food ingestion via decreasing the rate of stomach-emptying (Katsuura et al., 2002).

1.8 Diabetes mellitus (DM)

1.8.1 History of DM

DM is one of the oldest recognized diseases. The ancient Egyptians described the clinical symptoms similar to DM more than 3000 years ago. The word, diabetes was first used in 81-133AD. Then, the term mellitus (honey sweet) was firstly discovered by ancient Indians. Mellitus was established in 1675 following the detection of sweetness in the patient's urine and blood. The evidence of the existence of increased sugar level in patient's urine and blood as a source of sweetness was only established in 1776. The detection of the pancreas pathogenesis as a cause DM was firstly recognized in 1889 (Ahmed, 2002).

1.8.2 Epidemiology of DM

About 6% of the world's population are suffering from DM. There is a significant increase in the prevalence of DM (Townsend, 2000). The estimated number of diabetic people all over the world was about 225 million in the year 2010. This number is expected to rise to 350 million in 2030 (Zimmet, 2003; Benera et al., 2009). DM is classified mainly into T1DM and T2DM. Globally, 95% of all diabetic patients have T2DM while 55 suffers from T1DM (Adeghate, 2001). In the UK, around 3 million

people are diabetic and another 1 million are undiagnosed. It costs the UK National Health Service around £6 billion annually to diagnose, treat and care for diabetic patients. If this figure is extended worldwide then it will cost the world's economy around £560-£670 billion annually to fund diabetic cases. DM is a multifactorial disease, which is caused by several environmental and genetic factors leading to insulin dysfunction or decreased secretion. Physical inactivity, obesity, infection, drugs and toxic chemicals, are among the most common factors that lead to the development of the disease (Giovannucci et al., 2010).

T1DM is not mainly associated with hereditary. While, hereditary is the most important factor that leads to development of T2DM in association with environmental and life style factors. Modern inactive life style, environmental pollution and stress increase the susceptibility of the related genes and explain the high prevalence of diabetes now (Kumar and Clark 2007). There is a different prevalence of T1DM according to the age and it infrequently appears during in the first 6 months of birth. The frequency of T1DM increase greatly starting after 9 months of age with prolonged increase at age 12-14 years, and followed by a decrease in its prevalence. The occurrence of T1DM over the last 50 years in the United States has increased from about 5% in 1940 to 20% in 1980 (Ma and Chan, 2009). Several previous studies on the prevalence of T1DM according to the geographical distribution in the world showed that the occurrence rates of T1DM are smallest in Japan, the Caribbean, and Southern Europe. However, the largest occurrences are in the Scandinavian countries, especially Finland (Adeghate et al., 2006). The prevalence of T1DM in the white American population is greater than Northern Europe countries, but considerably less than in Sweden and Finland. The occurrence of T1DM in African Americans was less than in white Americans (Maahs et al., 2010). The prevalence of T1DM throughout the previous 20 years show that T1DM arises in the majority of races and ethnicity however, the largest incidence is detected among white population (Adeghate et al., 2006). The variations within races could be related to genes. The big differences between the white populations are similar to that detected between different races. All variations explain the effect of genetic and environmental factors in the aetiology of diabetes. Another study demonstrates the role of climate in the aetiology of T1DM (Liese et al., 2006).

The general incidence of T2DM in population aged 20-74 years in United States was 6.6% in 1980. Only one-half of these cases are diagnosed as T2DM. The incidence of T2DM was to a less extent more in women than in men, excluding the age group 65-74 years. The incidence of T2DM between black populations was more than between white population for both sexes and at all ages (Fagot-Campagna et al., 2005).

The predominance of T2DM in the European population is moderately less than that detected in the American populations. In European populations, the incidence of T2DM is slightly below the half of the incidences detected in American populations (Parra et al., 2004). The aetiology of T2DM in Saudi Arabia is about the same like that recorded in United States populations. However, in Central Asia the incidence of T2DM between men of 50 years old and over is about the same detected in European men (Wild et al., 2004).

The large variation in the prevalence of DM between white populations, mostly among those in Europe and North America, indicates the role of environment in the progress of DM. The role of environment is evidenced by the fact that the incidence of T2DM is higher in Chinese living out of Asia than the Chinese living inside China. As an example for this, there is 21% increased prevalence of T2DM between Chinese livings in the island of Mauritius than the recorded rate among native population (Adeghate et al., 2006).

1.8.3 Definition and description of diabetes

DM is a chronic metabolic illness associated with hyperglycaemia (Kumar and Clark 2007). DM influences the metabolism of carbohydrates, protein, fat, water, and electrolytes, causing structural alterations in tissues of numerous organs and systems particularly the vascular system (Adeghate et al., 2006). DM leads to disorder in lipid metabolism particularly, an elevated vulnerability to lipid peroxidation (Sedhrouchni et al., 2002). Symptoms of DM such as weight loss, thirst, polyuria and sweet-smelling urine were recorded from very long time starting from the ancient Greek, Roman, and Egyptian Physicians (Adeghate et al., 2006; Frank, 1957).

According to the American Diabetes Association (ADA, 2010), DM is a mixture of metabolic disorders associated with elevated blood glucose levels. This sustained hyperglycaemia, if not controlled for many years may lead to long-term complications such as nephropathy, neuropathy, retinopathy and cardiovascular disorders (Larsen, 2009).

The aetiopathogenesis of DM may include autoimmune damage of pancreatic β -cells or impairment in production and release of insulin by other factors, leading to abnormal carbohydrate, fat, and protein anabolism and catabolism (Cejkova et al., 2009).

Symptoms observed in diabetic patients include, polydipsia, polyuria, occasional weight loss, and polyphagia. Poorly controlled DM may lead to severe conditions such as ketoacidosis or non-ketotic hyperosmolar crisis (Kumar and Clark 2007).

1.8.4 Classification of DM

In 2010, the American Diabetes Association (ADA, 2010) described a recent diagnostic and classification principles concerning the diagnosis of disturbed fasting plasma glucose concentration. The new diagnostic and classification principles may be summarized as follows:

1. T1DM (pancreatic β -cell damage, usually resulting in total insulin deficiency).
2. T2DM (increased deficiency in insulin secretory capacity associated with insulin resistance).
3. Type 3 diabetes (the relationship between T2DM and Alzheimer's disease is lately cited as diabetes type 3, T3DM).
4. Gestational DM (GDM) (a diagnosis of diabetes is detected usually at pregnancy).
5. Other types of DM may be caused by other factors such as genetic abnormalities in pancreatic β -cell function, impaired genetic coding of insulin action, cystic fibrosis, and toxic drugs and chemicals.

1.8.4.1 Type 1 diabetes mellitus (T1DM)

1.8.4.1.1 Immune-mediated diabetes

T1DM was formerly referred to as insulin dependent diabetes mellitus (IDDM) or juvenile-onset diabetes. It recorded in about 5-10% of all patients suffering from diabetes. T1DM is caused mainly via cell-mediated autoimmune damage to pancreatic β -cells. This is detected by the presence of immune markers that points to β -cell destruction. These immune markers include among others; islet cell autoantibodies, insulin autoantibodies, and glutamic acid decarboxylase autoantibodies. More than one of these immune markers are found in about 85-90% of individuals with high fasting plasma glucose. In addition, T1DM has strong associations with human leukocyte antigens (HLA) (Raha et al., 2009).

Immune-mediated destruction of pancreatic β -cells usually occurs in juvenile and children, but it can affect any age group. The likelihood of immune destruction of pancreatic β -cells depends on a number of factors that include genetic predispositions and environmental factors (Ritchie et al., 2003).

1.8.4.1.2 Idiopathic diabetes

Some diabetic patients have regular insulin deficiency and are prone to develop ketoacidosis, they are classified as idiopathic T1DM because of lack of evidence of autoimmunity characterized by T1DM (Russell and Coustan, 2005).

1.8.4.2 Type 2 diabetes mellitus (T2DM)

Most diabetic patients have T2DM (90-95%), which is due to insulin resistance or reduced insulin release. T2DM was formerly called non-insulin dependent diabetes (NIDDM) or adult-onset diabetes. Since this group of patients have insulin, injection of insulin is not needed for survival. The precise origin of T2DM is not known but it is associated with obesity, which has been shown to be involved in insulin resistance. Slim patients with T2DM have also been shown to have large quantity of visceral fat

especially in the abdomen. Body weight reduction and physical activity is an adjunct therapy in the treatment of T2DM, in addition to pharmacological treatment. Unfortunately, T2DM usually goes undetected for many years until the hyperglycaemia becomes chronic to induce diabetes complications (Hossain et al., 2007; Rydén et al., 2007).

1.8.4.3 Type 3 diabetes mellitus (T3DM)

Glucose uptake because of brain metabolism demand is disturbed in Alzheimer's illness (Li and Holscher, 2007). Reduced glucose transporters are concurrent to disturbed hyperphosphorylation in Alzheimer's illness (Liu, et al., 2008). Consequently, destruction of insulin signalling leads to abnormal glucose homeostasis and several other deteriorating effects (Li and Holscher, 2007). Recently, a clear relationship linking T2DM and Alzheimer's illness has been established. Both Alzheimer's and T2DM have numerous similar molecular procedures, which share a related destructive process (Li and Holscher, 2007). Etiopathogenesis of both Alzheimer's and T2DM are multifactorial diseases and not fully identified. The aetiology of Alzheimer's and T2DM may be influenced by the co-reaction between the genetic and environmental elements (Wiwanitkit, 2008). The genetic role of Alzheimer's and T2DM illness is a polygenic source. Many epidemiological investigations correlate T2DM with an elevated hazard of Alzheimer's illness. Several roles may explore the relationship between Alzheimer's and T2DM such as decreased insulin secretion associated with insulin resistance, inflammatory factors, and oxidative toxicity (Haan, 2006). Due to the correlation between both of T2DM and Alzheimer's, a new categorization of diabetes is classified as T3DM (Wiwanitkit, 2008).

1.8.4.4 Gestational diabetes mellitus (GDM)

Gestational DM occurs when glucose metabolism is impaired at pregnancy. GDM causes complications in about 4% of examined pregnancies in the USA. However, this prevalence may vary from one region to the other. Impaired glucose homeostasis is

usually seen in pregnancy, but more common in the third trimester (Kucuka and Doymazb, 2009).

1.8.4.5 Other specific types of diabetes mellitus

1.8.4.5.1 Genetic defects of the β -cell

Although several genes cause DM, some forms of diabetes are induced by monogenetic impairments in pancreatic β -cell action. These types of DM are usually associated with hyperglycaemia at a young age before maturity. They are called maturity onset diabetes of the young (MODY). Patients with MODY have impaired insulin release but intact insulin function. MODY is inherited as a dominant autosomal disease. There are at least defects in six genetic loci on different chromosomes with mutations on chromosome 12 in a hepatic transcription factor called hepatocyte nuclear factor-1 α (HNF-1 α). Mutations have also been observed in the glucokinase gene on chromosome 7, leading to abnormal glucokinase enzyme. Glucokinase is a glucose sensor and responsible for the conversion of glucose to glucose-6-phosphate (G-6-P). G-6-P is important in the stimulation of insulin secretion from pancreatic β -cell (Manna, 2007).

1.8.4.5.2 Genetic defects in insulin action

Diabetes can also be caused by a genetically pre-determined abnormality of insulin action. This type of defect is associated with mutations of insulin receptor leading to conditions ranging from hyperinsulinemia, modest hyperglycaemia to complicated diabetes. Patients with insulin receptor mutations may develop *acanthosis nigricans*, virilization (in women) and polycystic ovaries (Mealey and Ocampo, 2007). Since it is difficult to characterize the defect in the structure and function of the insulin receptor in subjects with insulin resistant lipotrophic DM, it is presumed that the lesions occur at the post-receptor level (Rosen et al., 2000). Hyperinsulinism in infancy (HI) is an infrequent disease and it leads to death at newborn and primary childhood. There are

several reasons of HI and they are mainly associated with impairment of ATP-sensitive K^+ channel in β -cells. The disturbance may be due to gene mutation of ATP-sensitive K^+ channel structure or its enzymatic cascades, which cause a deficient insulin secretion and eventually diabetes (Dunne et al., 2004).

1.8.4.5.3 Drug- or chemical-induced diabetes

It has been shown that several drugs such as Vacor (rodent poison) and pentamidine can destroy the β -cells. Numerous drugs and hormones, including glucocorticoids and interferon- α can lead to diabetes establishment or even cause marked reduction in insulin secretion (Manna, 2007).

1.9 Effect of different factors on diabetes.

1.9.1 Gender differences in T1DM

A study on individuals between 15-29 years showed an increased rate of T1DM in males relative to females. This could be due to different levels of sex hormones or discrepancy of environmental factors (Virk et al., 2010).

1.9.2 Seasonal variation and viral infection in T1DM

Some investigations showed that T1DM reach the uppermost frequencies in the autumn and winter relative to other seasons. This could be related to specific viruses, which are predominant in winter and autumn compared to other seasons. It may also be due to availability of certain kind of foods predominant in winter and autumn or because of weather, sunshine period, vitamin D levels or temperature variations that all induce the immune-mediated responses which may cause β -cell disturbance and development of T1DM (Svensson et al., 2009).

1.9.3 Immunological aspects in T1DM

Many investigations showed that autoimmune progressions are associated with the aetiology of T1DM. Autoimmune illnesses, like Addison's disease, pernicious anaemia, and autoimmune thyroid disease all are implicated in T1DM. About 70% of T1DM have islet cell antibodies that are found in the patient's blood may be months or years prior to the real time of diabetes detection (Chatenoud and Bluestone, 2007). High titre of these antibodies reacts with pancreatic islet β -cells. Increased T-lymphocyte concentrations with elevation in insulin autoantibodies are also detected in patients of T1DM (Zvezdanovic et al., 2006).

1.9.4 Nutritional factors and toxic agents in T1DM

Food given throughout the neonatal and primary infancy stage, like the drinking of cow's milk containing certain protein with decreased breast-feeding in babyhood period, could elevate the vulnerability for T1DM. During childhood, an increased ingestion of nitrites and N-nitroso agents may lead to high risk of T1DM (Knip et al., 2005).

1.9.5 Gender differences in T2DM

Many studies have indicated that the incidence of T2DM is detected in females in higher levels than in males especially in China (Liu et al., 2010). On the contrary, men are more susceptible to T2DM compared to women in India (Kaur et al., 2010a).

1.9.6 Physical inactivity in T2DM

Steady physical activity enhances insulin sensitivity and glucose balance (Kriska et al., 2001). There are many studies, which showed that physical activities can help either to avoid or even to suppress the development of T2DM in subjects with impaired glucose tolerance. At the same time, lifestyle improvement, through diet and physical exercise, may lead to a reduction of about 50% in diabetes incidence. Furthermore, physical

training improves blood glucose tolerance in T2DM that is associated with a reduction of about 0.6% of glycosylated haemoglobin (HbA1c). Therefore, as a part of T2DM treatment, physical activity programme should be added to any therapeutic strategy to improve glucose tolerance and to reduce the development of diabetic complications (Sanz et al., 2010).

1.9.7 Body weight, nutrition and obesity in T2DM

Many recent studies have suggested a direct relationship between obesity and T2DM. At the same time, increased percentage of body fat showed an important association with T2DM. Body mass index is tightly concerned with elevated risk of T2DM. The intra-visceral fat mass is much more significant in predicting DM than subcutaneous fat (Huffman and Barzilai, 2009; Kaur et al., 2010). Ingestion of energy rich food, high monosaccharides and abundant with saturated fats and poor in compound carbohydrates and fibres could be associated with the progression of obesity and T2DM (Misra et al., 2010).

1.9.8 Severe and prolonged stress in T2DM

Modern life style is associated with severe and sustained stress. This is also accompanied with disturbance of blood glucose balance and may enhance the risk of T2DM (Hummasti and Hotamisligil, 2010). The progress of diabetes under continuous stress can lead to increased secretion of either adrenaline or glucocorticoid hormones causing glucose disturbance (Adeghate, et al., 2006).

1.9.9 Drugs and abnormal insulin secretion in T2DM

Drugs such as corticosteroids and some contraceptive steroid pills could lead to blood glucose imbalance and T2DM in vulnerable persons (Livingstone and Collison, 2002). Mainly, in T2DM there is a decreased insulin release comparative to the blood glucose

concentrations. This is associated with massive β -cells destruction under the effect of drugs abuse (Gavin et al, 2010).

1.10 Diabetes and molecular genetics

DM is a type of disease that may be developed due to various causes, genetic and environmental, including physical inactivities, obesity, infection, drugs and toxic chemicals. T1DM is not necessarily a genetic disease while, the chances of having it can be inherited. However, T2DM is mainly genetically induced and at the same time, it may be induced by different environmental factors. It is clear that interaction between both the susceptible genes and the environmental factors have a critical role for the aetiology of DM (Adeghate et al., 2006). The exact role of genetic factors in the aetiology of DM is not fully established and it is multifactorial. There is a tight role of environmental factors and the age of onset of diabetes for the expression of the diabetic genotype (Al-Mutairi et al., 2007).

Genes related to T1DM are strongly associated with disturbance in immune system. However, T2DM demonstrates powerful genetic effects with much contribution to heredity. T2DM patients demonstrate at least one-gene defect (Fajans, et al., 2001). These defects include two wide classes. The first class is the genes that control the pancreatic β -cells and their insulin secretion. These comprise glucokinase and hepatocyte nuclear factor-1 α (HNF-1 α) genes. The second class of genes, which manage the influence of insulin on its specific target cells, such as peroxisome proliferator-activated receptor- γ (PPAR- γ) and adiponectin genes (Romao and Roth, 2008).

1.10.1 Molecular genetics of T1DM

In T1DM, the genetic vulnerability may be inherited. For example, about 30-50% similar susceptibility for T1DM are detected in case of identical twins (Al-Mutairi et al., 2007). T1DM is a multi-factorial illness induced by autoimmune damage of the β -

cells of the pancreas, resulting in reduced synthesis of insulin (Devendra, et al., 2004). The damage to β -cells is triggered via infiltration of the islets of Langerhans with dendritic cells (DCs), macrophages and T lymphocytes (either $CD4^+$ or $CD8^+$). This damage affects only insulin-producing β -cells, but not glucagon (α) or somatostatin (δ) cells. T1DM is involved mainly young children in the first one and a half decades of their life. The detection of the disease is made mainly prior to 18 years old. The autoimmune disorder begins before the actual diagnosis of the disease, as indicated via the early discovery of autoantibodies against T1DM-specific antigens in patient serum (Miao et al., 2007). The three chief concerned autoantigens are insulin, glutamic acid decarboxylase-65 and insulin autoantigen-2. The detection of these autoantibodies is an indicator of β -cells damage that is mediated by T-lymphocyte. Each of $CD4^+$ (helper) and $CD8^+$ (cytotoxic) T-lymphocytes are vital in the progression of T1DM. The first step in the destruction of β -cell starts with the detection of extracellular antigens, which induce cytokine discharge and inflammation, while the next step is the reaction of inflammatory products with intracellular antigens resulting in β -cell death (Ounissi-Benkhalha and Polychronakos, 2008). At the clinical diagnosis time of T1DM, the majority of the β -cell insulin-release capability is completely abolished. More information of the genetic basis of T1DM, may significantly assist in the treatment of the disease (Ounissi-Benkhalha and Polychronakos, 2008).

T1DM is associated with many genes and it is linked to the human leukocyte antigens (HLA) gene on chromosome 6 (Devendra and Eisenbarth, 2003), which is responsible for around 50% of the familial clustering of T1DM via enormous changes affecting the normal haplotype function. Many other vital loci related to T1DM with less influence than HLA, include insulin gene on chromosome 11 (Undlien et al., 1995), PTPN22 on chromosome 1 (Ladner et al., 2005), CTLA4 on chromosome 2 (Ueda, 2003), the interleukin-2 receptor α (CD25, encoded by IL-2R α) locus on chromosome 10 (Lowe et al., 2007), IFIH1 (also known as MDA5) on chromosome 2, PTPN2 on chromosome 1, CYP27B1 on chromosome 12 (Todd et al., 2007), and CLEC16A (KIAA0350) on chromosome 16 (Hakonarson et al., 2007).

1.10.2 Molecular genetics of T2DM

T2DM has a significance environmental impact such as diet and lifestyles that can seriously be involved in the pathogenesis (or course) of the illness. Body mass index (BMI) of more than 30 kg/m² will be defined as obesity, which leads to amplifying the risk factors for T2DM. Besides, there is a strong genetic involvement, which raises the risk of disease progress to four folds in the affected individuals. Therefore, T2DM is considered as a classic, polygenic, multi-factorial illness (Frayling, 2007).

The discovery of genetic elements, which influence T2DM are important target goals to explore the exact relationship between T2DM and heredity (Neel, 1976). Lately, the investigations in several studies for genes susceptible for T2DM have established about 52 genes with promising proofs to cause T2DM (Kaput et al., 2007). These investigations have detected common variants in only three specific gene loci out of these promising genes with greater risk of T2DM. The three variants include the potassium rectifying channel J 11 (KCNJ11) of β -cells, peroxisome proliferator-activated receptor-gamma (PPAR- γ) and the transcription factor 2 (TCF7L2) of liver cells. Uncommon, but dangerous mutations in any one of these three genes may lead to monogenic types of T2DM (Owen and McCarthy, 2007). In addition, another investigation reported different variant in Zn transporter gene (SLC30A8) of β -cells. Furthermore, two other single nucleotide polymorphisms (SNPs) that could be of high risk for T2DM include insulin-degrading enzyme (IDE) and homeobox hematopoietically (HHEX) (Sladek et al., 2007).

Numerous additional genes revealed a strong relationship with T2DM. Some examples of these genes include insulin gene polymorphism on chromosome 11, glucose transporter genes on chromosomes 1 and 12, human leukocyte antigens (HLA) gene on chromosome 6, lipoprotein gene on chromosome 6, apo-lipoprotein genes on chromosomes 2 and 11, haptoglobin gene on chromosome 16, and insulin receptor gene on chromosome 19 (Barroso et al., 2003).

1.11 Diabetes and insulin resistance

The causes of insulin resistance in T2DM and the related metabolic disorder are not totally known. The stress that people have on a daily basis may lead to the accumulation of stress factors during the course of many years. These stress elements, can enforce the body of the affected person to compensate by activating different neuro-hormonal axes. The autonomic nerves, hypothalamic-pituitary-adrenal and renin-angiotensin pathways are collectively implicated in impaired nutrient metabolism and insulin function. Previous studies have revealed the possible recent mechanisms, pharmacological drugs and bioindicators, implicated in neurendocrine dysfunctions leading to insulin resistance. This has enabled scientists to understand the complicated and multi-factorial causes of insulin resistance (Sjostrand and Eriksson, 2009).

1.11.1 Factors which can induce insulin resistance

Insulin resistance correlates with obesity, T2DM, high blood pressure, inflammation, certain endocrine disturbance and polycystic ovarian disease (Reaven, 2004). In insulin resistance, there is a deficiency of target cell response to the regular hormonal level. Insulin resistance is mainly associated with glucose intolerance due to diminished action of insulin at glucose levels (Pessin and Saltiel, 2000). Pancreatic β -cells increase insulin secretion in case of decreased insulin sensitivity to overcome its reduced action. As a result of insulin resistance, abnormal elevation of insulin secretion will lead to hyper-stimulatory action of insulin on its various target cells and tissues in different degrees due to the distinctive action of insulin in each tissue. At the same time, the interaction between insulin and different hormones including epinephrine, leptin and growth hormone may change the ultimate effect of insulin (Muller, 2002). Elevated concentrations of non-esterified fatty acids are mainly associated with severe insulin resistance and disturbed glucose release in skeletal muscle. Saturated fatty acids (palmitate) and mono-unsaturated fatty acids (palmitoleate) play distinctive roles in insulin signalling and glucose entrance into skeletal muscle cells. High food content of mono-unsaturated fatty acids can enhance the influx and metabolism of glucose in

skeletal muscle cells under either normal or insulin resistant condition (Dimopoulos et al., 2006).

Glucotoxicity has prolonged influences on both insulin secretion and the initiation of insulin resistance within insulin-sensitive target tissues. Insulin resistance is essentially established before the detection of sustained elevated blood glucose levels (Kawahito et al., 2009). Moreover, prolonged blood glucose levels might contribute to the deterioration of the diabetic condition through intensifying insulin resistance (Zhao et al., 2004).

Liver: The liver is considered as one of the essential storage organs of glucose in the human body. Thus, the liver is an essential organ in controlling glucose balance, as liver uptakes glucose at hyperglycaemia and discharges glucose during hypoglycaemia (Brunner et al., 2009). It was reported that treatment of Zucker diabetic fatty rats with hypoglycaemic agent can markedly reduce insulin resistance in the rat liver (Fujimoto et al., 2006). In addition, other studies revealed that sustained elevated blood glucose levels can disturb the inhibition of glucose release from the liver during acute hyperglycaemia (Shao et al., 2005).

Skeletal muscle: Skeletal muscle is an additional tissue capable for the storage of glucose as a glycogen. One of the main features of T2DM is the insulin resistance of skeletal muscle (Pratipanawat et al., 2002). Long persistence increased blood glucose level in rodents can lead to insulin resistance in skeletal muscle (Kawanaka et al., 2001). These previous reports were verified via isolated muscles (Itani et al., 2003). Unexpectedly, drug treatment for restoring the normal blood glucose level in Zucker diabetic fatty rats did not achieve an improvement of insulin resistance in skeletal muscle. Some studies have shown that normoglycaemia does not improve insulin resistance in skeletal muscle. This indicates that elevated blood glucose level is not the single cause of insulin resistance in skeletal muscle (Nawano et al. 2000).

Adipose tissue: The adipose tissue is also implicated in blood glucose regulation but come after liver and skeletal muscle. Adipose tissue has the ability to store glucose in the form of triglyceride as an essential supply of energy for different body tissues (Rosen and Spiegelman, 2006). Severe hyperglycaemia stimulates adipose tissue to

release many substances like leptin and cytokines (Walker et al., 2007). Insulin resistance in adipose tissue may become worse under a prolonged hyperglycemia. Similar to skeletal muscle, insulin resistance may occur via the destruction of IRS-1 and GLUT-4 signalling systems (Renstrom et al., 2007).

1.12 Complications of DM

DM is a chronic endocrine illness coupled with series of micro-and macrovascular complications. It influences the metabolic activities of carbohydrates, fat, protein, minerals and electrolytes, that leads to alterations in the construction of the blood vessels (Kumar, and Clark, 2007; Adeghate, 2001). Increased blood glucose level is the corner stone in diabetic patients that deteriorates most of the body organs, tissues, and various cells, like retinal capillary endothelial cells, renal glomerular mesangial cells, neuronal cells and Schwann cells in peripheral nerves. These cells are the most susceptible cells and they reveal many pathological illnesses under the effects of increased blood glucose levels (D'Souza et al., 2009).

Hyperglycaemia provokes oxidative toxicity and reduces antioxidant protective system, causes elevated free radical synthesis (D'Souza et al., 2009). Deficient insulin sensitivity can lead to oxidative toxicity in diabetic patient and there is a marked confirmation in a reduction of antioxidant protective mechanism and in a deficiency of antioxidant action in diabetes. This condition has a considerable influence in the inhibition of insulin gene expression and insulin release (Kajimoto and Kaneto, 2004).

1.13 Management and treatment of DM

Recently, T2DM treatments begin with food control, adjustment of body weight, increase of physical activities, oral drugs supplementation and finally insulin injection (Bailes, 2002). Only food limitation and increased physical activity program could be

sufficient to avoid or to treat T2DM. Inductions of physical activities lead to enhancement of blood glucose uptake by specific tissues and reduction of blood glucose concentration. However, if physical exercise, as mono-therapy does not achieve the target of normoglycaemia, then it must be accompanied with food modification and drug treatments (Sato, 2000). Nevertheless, food control is considered as the essential primary treatment of T2DM. Diet treatment has a vital tool in avoiding DM and it also plays a significant effect in DM treatment. Food management must be consisting of an adequate mixture of carbohydrates, proteins, lipid, minerals and vegetables (Hagura, 2000).

Individuals suffering from T1DM are insulin dependent. Insulin are normally injected subcutaneously as essential therapy for T1DM (Griffen et al., 2006). Insulin is manufactured in different forms, like fast affecting insulin, which is vastly absorbed. The structural difference between normal human insulin and the synthetic form of insulin is the conversion or replacement of few amino acids to slow down its absorption or to extend its action. Rapid insulin has to be given at eating period. While, short effecting insulin is exerting its effect within one hour after subcutaneous injection. While long acting insulin may act for more than twelve hours (Dawn, et al., 2003).

T2DM treatment depends mostly on different hypoglycaemic drugs (Moller, 2001). There are about five categorizes of available drugs. Sulfonylureas show their hypoglycaemic effect through enhancing insulin release from pancreatic β -cell. The mechanism of action of sulfonylureas is via its binding with ATP-sensitive potassium channels and thus preventing the release of potassium from the cell leading to influx of calcium via the voltage-dependent calcium channels. The elevated cytoplasmic calcium levels lead to the secretion of insulin. Meglitinide is a non-sulfonylureas drug that is linked to different β -cell receptors promoting a similar action to sulfonylureas in enhancing insulin secretion (Ganesh et al., 2010).

Metformin is one of the biguanides anti-diabetic drug categories and it exerts its effect by inhibiting glucose release from the liver via suppressing gluconeogenesis and glycolysis. Metformin does not lead to a condition of hypoglycaemia since it has no effect on insulin release (Ganesh et al., 2010). Thiazolidine increases glucose entrance

into different tissues including skeletal muscle (Oiknine, and Mooradian, 2003). A last class of anti-diabetic drugs is the α -glycosidase inhibitors like miglitol and acarbose. Their effect is mainly through postponing carbohydrate absorption in the epithelial cells in the lining wall of the small intestine (Braunstein, 2003).

1.14 Incretins

The idea that secretions of the gastrointestinal tract contribute to the control of endocrine release was established in the 20th century (Moore, 1996). The definition “secretin” was initially employed to denote elements that take part in regulating pancreatic secretion. The functional relationship between the gut and the endocrine part of the pancreas was noted in the mid-1960s, when it was possible to measure insulin in plasma. Investigators noted that the response to insulin secretion was higher when glucose was supplemented orally (D’Alessio et al., 2005).

Incretins include gastrointestinal hormones known as glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP)-1 (Drucker, 2003). Incretins may elevate the plasma insulin after eating via different ways defined in general as the incretin action. This increased insulin release is detected following oral, as compared to intravenous, glucose supplementation and can induce about 80% of related insulin release according to the dose of glucose ingested (Vetter et al., 2009).

In healthy person, the incretin action retains the normal glucose concentrations after carbohydrate ingestion whatever the amount of glucose ingested, as incretins can increase insulin release from the β -cells of the pancreas in a dose dependent way. Patients with T2DM are suffering from blunting or even complete loss of the incretin action (Svec, 2010). Table 1.2 compares the differences between GIP and GLP-1 incretin hormones in relation to their properties and biological effects.

Table 1.2: Different properties and biological actions between GIP and GLP-1. DPP-IV, dipeptidyl peptidase-IV; GIP, glucose-dependent insulintropic polypeptide; GLP-1, glucagon-like peptide-1 (Modified from Holst, 2008).

GIP	GLP-1
42 amino acid peptide	30/31 amino acid peptide
Released from duodenum and jejunum by K-cells	Released from jejunum and ileum by L-cells
NH ₂ -terminal inactivation by DPP-IV	NH ₂ -terminal inactivation by DPP-IV
Potentiates glucose-induced insulin secretion	Potentiates glucose-induced insulin secretion
Minimal effect on gastric emptying	Inhibits gastric emptying
No effect on glucagon secretion	Inhibits glucagon secretion
No regulation of satiety and body weight	Inhibits food intake and weight gain
Promotes expansion of β -cell mass	Promotes expansion of β -cell mass
Normal GIP release in diabetic patients	Reduced GLP-1 release in diabetic patients
Defective insulintropic effect in T2DM	Preserved insulintropic effect in T2DM

1.14.1 Incretins synthesis and metabolism

GIP consists of 42 amino acids while GLP-1 consists of 30/31 amino acids. Both GLP-1 and GIP are parts of the glucagon peptide family. They are produced as a result of proglucagon gene expression, which produces GLP-1, GLP-2, glucagon and other proglucagon-derived peptides. According to the site of proglucagon gene, it is found in either pancreatic or intestinal cells, the differential posttranslational processing will produce various types of peptides according to the precursor cells as shown in figure 1.11 (Baggio and Drucker, 2007).

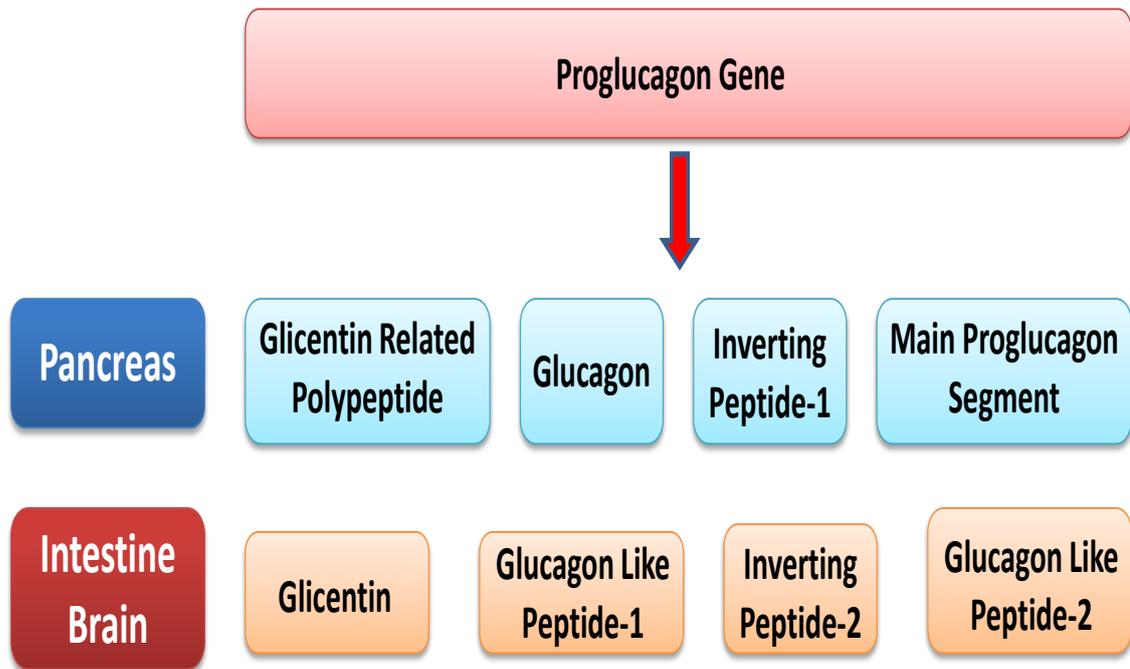


Figure 1.11: Differential posttranslational processing of proglucagon in the pancreas, intestine and brain (Modified from Holst, 2007).

The proglucagon gene is expressed in the pancreatic α -cells, the L-cell of the jejunum and ileal portions of small intestine and in some of the neurons of the brain stem (Holst, 2007). GLP-1 is synthesized as two peptides: GLP-1 (7-36) amide and GLP-1 (7-37) (see Figure 1.11) (Mortensen et al., 2003). GLP-1 and GIP may be inactivated via splitting at the second terminal amino acid, alanine, by their inhibitory enzyme DPP-IV (Deacon, et al., 2000). The plasma levels of GIP and GLP-1 are reduced rapidly by DPP-IV (Deacon, 2005), and by kidney elimination. The plasma half-lives of GIP and GLP-1 are 6 and 2 minutes, respectively (Girard, 2008).

1.14.2 Incretins release is affected by a number of factors

The secretion of both GLP-1 and GIP is stimulated by food ingestion, hormonal and neural factors which raise their levels quickly following food intake. Remarkable increases in their plasma levels are prominent after about 10-15 minutes. The secretion of either GLP-1 or GIP is stimulated after food intake by either carbohydrates, fats or

proteins (Shrayyef and Gerich, 2010). Direct contact between food and the intestinal mucosal cells can evoke the secretion of these incretins leading to fast increase in the plasma insulin levels (Deacon, 2005). Before the nutrients reach the L-cells to stimulate incretins release, the neural control leads to a more rapid stimulation of incretin release. Stimulation of cholinergic muscarinic, β -adrenergic and peptidergic receptors found on L-cells can enhance GLP-1 secretion (Anini and Brubaker, 2003). Many investigators have reported that gastrin-releasing peptide (GRP) and acetylcholine play an important physiological role in the initiation of GLP-1 secretion (Lim, and Brubaker, 2006). Laboratory studies have demonstrated that after food intake, GIP is released by K-cells and leads to stimulation of afferent nerves of the vagus, resulting in GLP-1 release through vagal efferent and gut neurones (Girard, 2008). An increased level of GIP has an activating influence on GLP-1 secretion from L-cells (Hansen, and Holst, 2002). Somatostatin, released from adjacent δ -cells in the intestine, has a reducing effect on incretin secretion (Deacon, 2005), and somatostatin antagonist leads to a great increase in GLP-1 release (Hansen, et al., 2000).

1.14.3 Physiological actions of incretins

Incretins reveal their physiological actions through the stimulation of their specific G-protein-coupled receptors (GPCRs). The G-protein receptors are expressed in pancreatic β - and α -cells beside numerous other tissues and organs such as intestine, adipose tissue, brain, nervous system, kidneys, heart and lungs. These wide distribution of incretins-receptors demonstrate that the physiological actions of incretins are much more than insulin release (Wideman and Kieffer, 2004; Girard, 2008).

GPCRs are seven-transmembrane domain receptors (TM1-TM7) with extracellular N-terminal domain and intracellular C-terminal domain (see Figure 1.12). "G-protein" represents a membrane-associated heterotrimeric G-proteins. These proteins are triggered via GPCRs and are formed from three subunits α , β and γ (Hurowitz et al., 2000). Several ligands can bind to GPCRs and activate membrane, cytoplasmic and nuclear targets. GPCRs cooperate with heterotrimeric G-proteins in conjunction with α , β and γ subunits that are guanosine diphosphate (GDP) coupled in the inactive form.

Ligand coupling initiates a conformational change in the receptor, which activates the separation of GDP from α subunit, then guanosine triphosphate-binding (GTP-binding) to G_α and the separation of $G_{\beta\gamma}$ from G_α subunits (Pierce et al., 2002). GPCR ligand may bind to one of four subfamilies of G-proteins α subunits; $G_{\alpha s}$, $G_{\alpha i}$, $G_{\alpha q}$ or $G_{\alpha 12}$ as seen in figure 1.12. Every G-protein binding may stimulate or inhibit numerous downstream cascades. $G_{\alpha s}$ activates protein kinase A (PKA) and stimulates adenylyl cyclase (AC) which in turn changes adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP) and increases the concentrations of cAMP (Hall, 2000). While, $G_{\alpha i}$ suppresses AC and depresses cAMP concentrations. $G_{\alpha i}$ also can activate phosphatases and phosphodiesterases (Billington and Penn, 2003). However, members of the $G_{\alpha q}$ family couple to and stimulate protein kinase A (PKA) and phospholipase C- ζ (PLC- ζ). In turn, PLC- ζ splits phosphatidylinositol bisphosphate (PIP₂) to diacylglycerol (DAG) and inositol triphosphate (IP₃) which elevate Ca²⁺ release (Rhee, 2001). The $G_{\alpha 12}$ subfamily can regulate the action of GTP-binding proteins to a monomeric GTP binding protein family (Rho), which is a subfamily of the Ras superfamily and Rho is involved in cell proliferation, gene expression and apoptosis (Kozasa et al., 2011). The G_β and G_γ subunits act as a dimer to stimulate several signalling molecules, such as, phosphoinositol-3 kinase (PI-3K), phospholipases (PLs), ion channels and AC (Mills and Moolenaar, 2003; Marinissen and Gutkind, 2001).

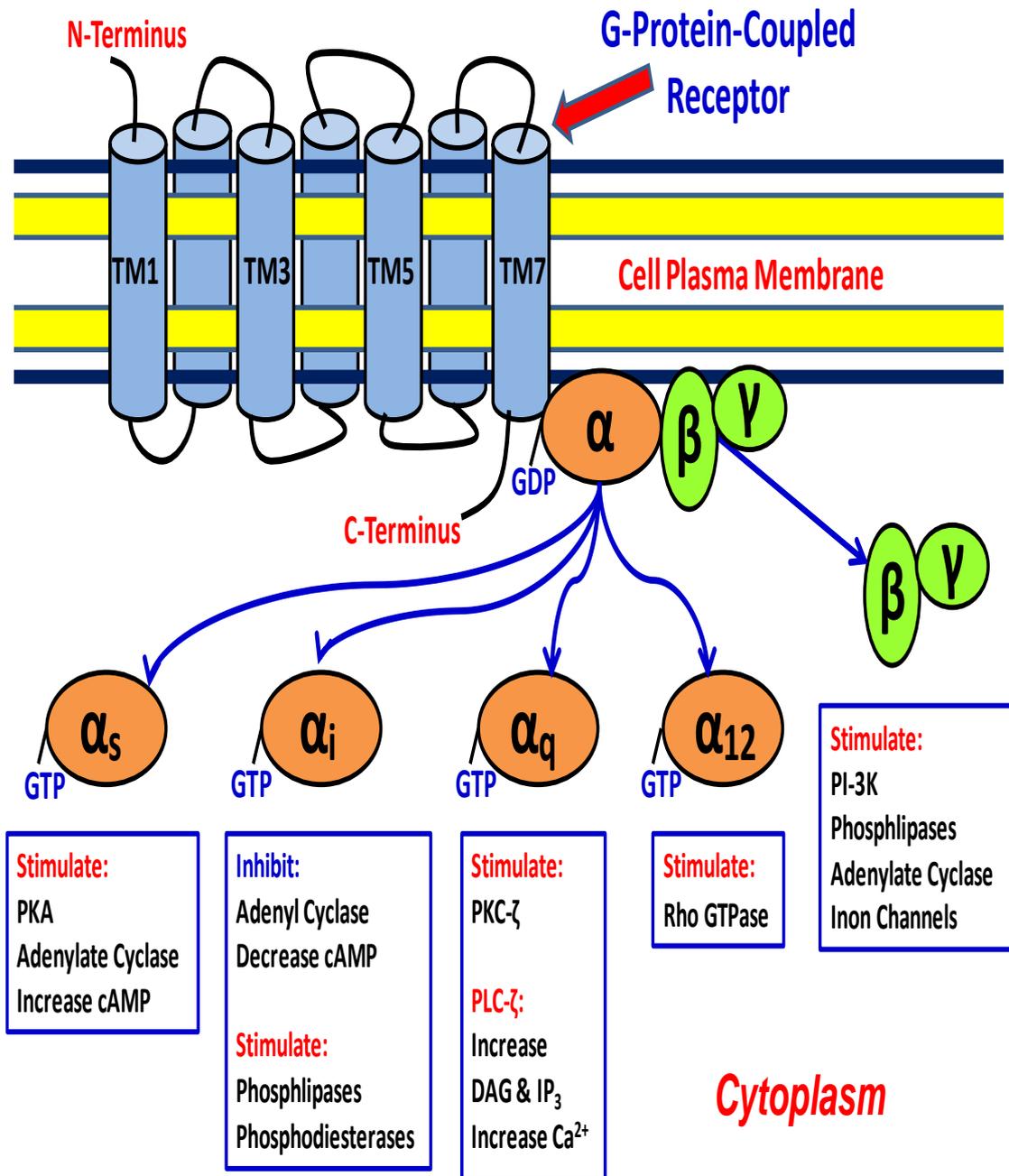


Figure 1.12: Schematic diagram of the G proteins couple to G-protein-coupled receptor showing different downstream cascades regulating many cellular processes. Abbreviations: guanosine diphosphate (GDP); guanosine triphosphate (GTP); protein kinase A (PKA); protein kinase C- ζ (PKC- ζ); a monomeric GTP binding protein (Rho GTPase); diacylglycerol (DAG); inositol triphosphate (IP₃); cyclic adenosine monophosphate (cAMP); phosphoinositol-3 Kinase (PI-3K) (Modified from Dorsam and Gutkind 2007).

1.14.4 Effects of GLP-1 on insulin biosynthesis

GLP-1 can activate insulin gene transcription and other steps in the translation of insulin and eventually insulin release. These actions are essential in the regulation of optimum level of insulin throughout production-secretion phases (Holst, 2006).

1.14.5 Inhibitory effects of GLP-1 on glucagon secretion

In addition to the ability of GLP-1 to stimulate insulin release, it is capable of suppressing glucagon release from the pancreatic α -cells. The exact way by which GLP-1 leads to suppression in glucagon release is not completely clear. However, there are the hypotheses, which suggest that this inhibition may be introduced indirectly through the secretion of insulin and somatostatin into the blood circulation, although a direct action of GLP-1 cannot be ruled out because of the presence of GLP-1 receptors on the glucagon-producing α -cells (Holst, 2006).

The ability of GLP-1 to inhibit glucagon release is a key component in the regulation of blood glucose. For example, in patients with T1DM, GLP-1 reduces the plasma glucose level with associated inhibition of the action of glucagon probably via the suppression of glucagon-induced hepatic glucose release via reduction of either glycogenolysis or gluconeogenesis (Nauck et al., 2002).

1.14.6 Effects of GLP-1 on the gastrointestinal tract

GLP-1 has been shown to inhibit gut motility and secretion (Gautier et al., 2008). GLP-1 is also capable of slowing the rate of gastric emptying and therefore reducing the degree at which glucose is absorbed. This in turn results in a reduction in postprandial blood glucose level. The ability of GLP-1 to inhibit gut motility and secretion may be mediated via autonomic nerves including the vago-vagal circuit (Bucinskaite et al., 2009). This effect of GLP-1 on gastrointestinal tract motility and secretion may be designed to modulate the absorptive capability of the gut though inhibition of enzymes

production and slowing down the movement of the chyme. All of these effects can help in the reduction of postprandial glucose levels (Meier and Nauck, 2005).

1.14.7 Effects of GLP-1 on food intake

GLP-1 is capable of decreasing caloric intake and stimulating satiety under the control of central nervous system. Laboratory investigations show that intracerebroventricular injection (administration) of GLP-1 inhibited ingestion. This effect was abolished with exenatide treatment (Stanley et al., 2005). Other studies in Rhesus monkeys, db/db mice and Zucker diabetic fatty rats showed a significant reduction in food ingestion following the supplementation of GLP-1 analogues (Meier et al., 2002).

The way by which GLP-1 suppresses food ingestion is still unclear. However, it is possible that GLP-1 stimulates vagal afferent fibres, which in turn regulate neuronal GLP-1 transmission in the central nervous system. This assumption is strengthened by the presence of GLP-1 in the neurones of hypothalamic region, which is involved in the regulation of food ingestion (Schick et al., 2003). Moreover, GLP-1 may also act via the area postrema and subfornical organ on the brain nuclei, which are implicated in food homeostasis (Baumgartner et al., 2010).

In addition, the ability of GLP-1 to reduce food intake may be attributed to the fact that GLP-1 can inhibit gastric emptying. Inhibition of gastric emptying may induce the feeling of fullness resulting in lowering the interest for food intake (Meier et al., 2002).

1.15 Therapeutic potential of incretins in the treatment of DM

In healthy individuals, there is a dynamic balance between β -cell mass and body needs for insulin release in different body activities and under normal or pathological conditions. However, disturbance of this equilibrium due to insulin deficiency and/or insulin resistance can lead to DM. In diabetic patient, β -cell dysfunction may cause a

decrease in insulin release, which correlated clearly with deficiency in glucose demand for insulin and this contributes to glucose intolerance (Zimmet and Alberti 2006). There is a vital need for a new treatment of DM. This treatment is not only for preserving the function of the remaining β -cell but also, to induce β -cell neogenesis and to decrease β -cell apoptosis. Using incretins could be an ideal approach to meet this target. Treatment of diabetic patients with incretins may improve β -cell proliferation and inhibit β -cell apoptosis, which may lead to enhancing insulin release and to increase the active β -cell mass. In this way, incretins can open a new era for advanced progress in establishing new drugs to meet this challenge in the next decades for treatment of T2DM. In addition, this can be a significant contribution to the traditional arsenal of anti-diabetic drugs (Karaca et al., 2009).

Both GIP and GLP-1 are quickly destroyed by the enzyme, dipeptidyl peptidase-IV (DPP-IV) (Baggio and Drucker, 2007). DPP-IV breaks down the functional incretin at the second amino acid alanine (N-terminal) causing inactivation of the incretin (see Figure 1.13), (Gautier et al., 2008). DPP-IV is extensively found in different body tissues such as the pancreas, gut, brain, spleen, kidneys, lungs, placenta, white blood cells (lymphocytes), endothelial cells and adrenal glands (Gautier et al., 2008). Most of GLP-1 is inhibited rapidly after its release. The half-life of GLP-1 is about 2 minutes (Gautier et al., 2008). This reduced half-life restricts the treatment effect of GLP-1. Changes of the amino acids at the N-terminus of GLP-1 will increase its functional half-life because the new structure will be resistant to DPP-IV. In this way, the newly modified GLP-1 structure will significantly enhance its receptor stimulation and prolong its biological efficacy (Green and Flatt, 2007).

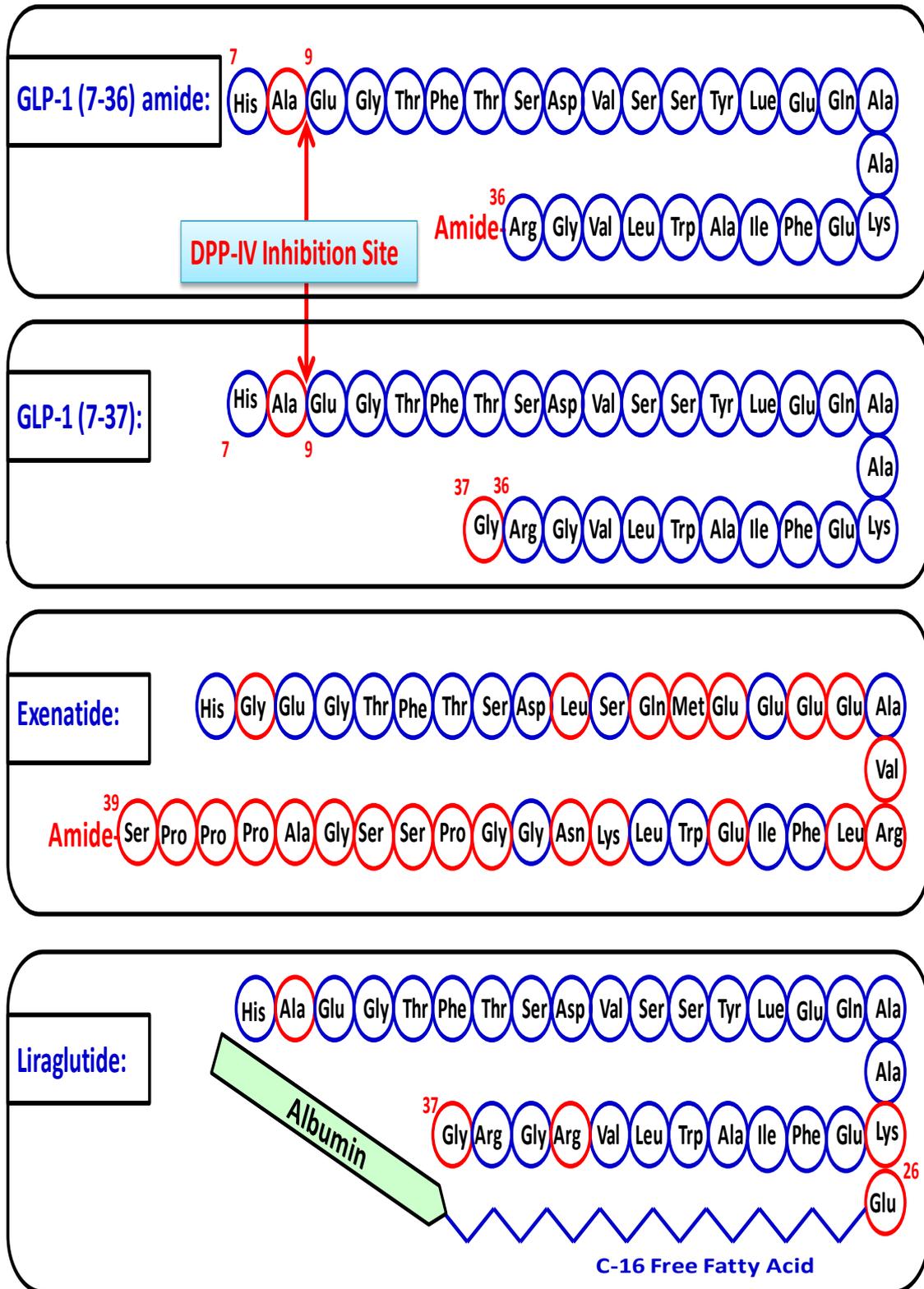


Figure 1.13: Structures of GLP-1 (7-36) amide, GLP-1 (7-37), exenatide, liraglutide, and DPP-IV inhibitor (Modified from Nauck, 2009).

1.15.1 Incretins-based therapy: potential use of incretin mimetics

1.15.1.1 Exenatide

Exenatide is an artificial form of the natural exendin-4 peptide (Figure 1.13). Exenatide was firstly extracted from the salivary juice of the lizard, gila monster (*Heloderma suspectum*) (Baggio and Drucker, 2007). The structural similarity between exenatide and GLP-1 is about 53% similarity of amino acid chain. Exenatide functions as an incretin mimetic compound via binding and stimulating the GLP-1 receptor. Exenatide structure has a glycine amino acid at the second location of its polypeptide chain instead of alanine in natural GLP-1. Therefore, it is resistant to DPP-IV inhibition and has a prolonged active half-life more than GLP-1 (Barnett, 2007).

Exenatide retains pancreatic β -cell mass and activities via enhancing the expression of the essential β -cell genes which help in increasing β -cells propagation and neogenesis. Exenatide also inhibits apoptosis of β -cells (Gedulin et al., 2005). Exenatide has strong effects on β -cell sensitivity to glucose, which enhances insulin secretion mostly under increased level of glucose. Insulin release is decreased in response to reduced plasma glucose concentrations (Barnett, 2007).

1.15.1.2 Liraglutide

Liraglutide is a fatty acid derived GLP-1 agonist. It is formed from 16-carbon free fatty acid molecule linked to the amino acid lysine at position 26 as shown in figure 1.13. This binding covers the splitting site of liraglutide and thereby protected from DPP-IV action (Green and Flatt, 2007). Liraglutide reduces fasting and after-meal blood glucose levels in T2DM patients (Degn et al., 2004). Liraglutide therapy leads to prolongation of stomach evacuation time (Juhl et al., 2002) and has a glucose-dependant enhancement effect on insulin secretion by β -cells. Liraglutide elevates β -cell count and reduces apoptosis (Wajchenberg, 2007). At postprandial, liraglutide decreases glucagon secretion in a glucose-dependent way (Juhl et al., 2002).

1.15.1.3 Other incretin mimetics

Prolonged acting exenatide LAR is supplemented as one dose weekly currently presenting hopeful trial results (Drucker et al., 2008). Exenatide LAR reduces glycosylated haemoglobin (HbA1c), fasting blood glucose and body weight (Kim et al., 2007).

Taspoglutide is a GLP-1 mimetic drug given as a single dose per week, which decreases the HbA1c and reduces body weight (Nauck et al., 2009).

Albiglutide is an albumin-linked peptide drug of GLP-1 similar action (Tomkin, 2009). It has a 7 days prolonged effective period (Bush et al., 2009). The action of albiglutide can lead to a decrease in the average fasting and postprandial blood glucose concentrations per day with minimal hazard of hypoglycaemia (Matthews et al., 2008). Albiglutide also enhances GLP-1 receptor-dependent, which is accompanied with blood glucose normalization and suppression of gut movement. In addition, it increases insulin release and decreases plasma glucose concentration (Baggio et al., 2004).

1.15.2 DPP-IV and DPP-IV inhibitors therapy

DPP-IV enzyme is formed from 766 amino acid. It is a serine-type protease enzyme, which inhibits GLP-1 action (Lambeir et al., 2003). DPP-IV antagonists exert their action in reduction of blood glucose levels in T2DM via prolongation of incretin activity (Flatt et al., 2008).

The effective oral DPP-IV antagonists inactivate more than 90% of blood DPP-IV action for more than a day *in vivo* study (Pratley and Gilbert, 2008). These drugs enhance incretin action through inhibiting its fast degradation. Therefore, DPP-IV antagonists can be used to treat T2DM patients (Pratley, 2008).

DPP-IV suppression leads to improving glucose tolerance by multiple effects. The main action is to enhance pancreatic islet-cells activities. DPP-IV suppression is conducted through the subsequent steps: (1) elevation of fasting and after meal GLP-1 concentrations, (2) reduction of fasting and after meal blood glucose levels, (3)

enhancement of β - and α -cells activities in T2DM, (4) elevation of β -cells occupation area, as confirmed in rodent's studies and (5) improvement of insulin efficiency (Ahren, 2009).

Numerous drugs of DPP-IV suppression are available for use nowadays (Ahren, 2007), including sitagliptin, vildagliptin, alogliptin and saxagliptin (Karasik et al., 2008). These drugs are administered orally and are absorbed quickly via the gastrointestinal tract to exert their actions (Bergman et. al., 2007).

1.15.2.1 Sitagliptin

Sitagliptin is a powerful reversible competitive suppressor of DPP-IV and it is orally administered (see Figure 1.14), (Lotfy et al., 2011). In a previous study, STZ-induced diabetic rats under fat-enriched food were treated by sitagliptin and the results showed an elevation of the β -cells area and correction of β - to α -cells ratio. Sitagliptin also normalizes the levels of fasting and after meal blood glucose, HbA1c, blood triglycerides and free fatty acids (Mu et al., 2006). Administration of sitagliptin to T2DM patients once a day revealed marked reduction of DPP-IV action, improvement of glucose tolerance, elevation in insulin levels and reduction in glucagon secretion (Herman et al., 2006).

1.15.2.2 Vildagliptin

Vildagliptin is a DPP-IV suppressor (see Figure 1.14). Sitagliptin and vildagliptin have different inhibitory effects. Sitagliptin is a competitive inhibitor of DPP-VI, while vildagliptin and saxagliptin are DPP-IV substrate suppressors (Lotfy et al., 2011). These DPP-IV inhibitors reduce blood glucose levels in T2DM patients and they improve HbA1c levels (Rosenstock et al., 2007). Investigations on T2DM have shown that vildagliptin elevates fasting and after meal GLP-1 concentrations, increase β -cell glucose responsiveness and insulin efficiency and decreasing after meal plasma lipids. In addition, vildagliptin enhances glucose homeostasis and decreases fasting blood glucose concentrations (Garber et al., 2006). Vildagliptin also shows an elevation in

insulin release (Balas et al. 2007). Vildagliptin reduces the α -cell glucagon secretion after mealtime in glucose-dependent manner (Azuma et al., 2008). The correction in glucagon/insulin ratio correlates with increased insulin secretion that reduces blood glucose levels and with inhibition of liver glucose release via inhibition of glucagon release (Balas et al. 2007).

1.15.2.3 Saxagliptin

Saxagliptin is a DPP-IV suppressor for T2DM treatment (see Figure 1.14). Saxagliptin is a specific and reversible substrate suppressor of DPP-IV enzyme (Lotfy et al., 2011). Saxagliptin has 10-fold extra effect than both vildagliptin and sitagliptin (Kirby et al., 2008). In a previous investigation, saxagliptin was administered as single dose per day in diabetic patients and the results showed a decrease in HbA1c levels (Rosenstock, et al., 2008). As a result, saxagliptin has a significant inhibitory action on DPP-IV and it can enhance the glucose tolerance (Pratley et al., 2007). Treatment of diabetic patients with saxagliptin has shown a strong decrease in fasting and after meal, blood glucose concentrations. Treatment with saxagliptin has also revealed an increase in β -cell activities (Rosenstock, et al., 2008).

1.15.2.4 Alogliptin

Alogliptin is an oral potent and highly selective quinazolinone-based DPP-IV suppressor (see Figure 1.14) (Lotfy et al., 2011). Alogliptin has an enhancing effect on the blood glucose tolerance in patients with T2DM (Fleck et al., 2008). A single daily dose of alogliptin improves insulin secretion after glucose load in fatty rats. (Feng et al., 2007). In addition, administration of alogliptin reduces HbA1c concentrations (Takeuchi et al., 2006). Treatment by alogliptin also reduces triglycerides and enhances β -cell function in ob/ob mice (Moritoh et al., 2008).

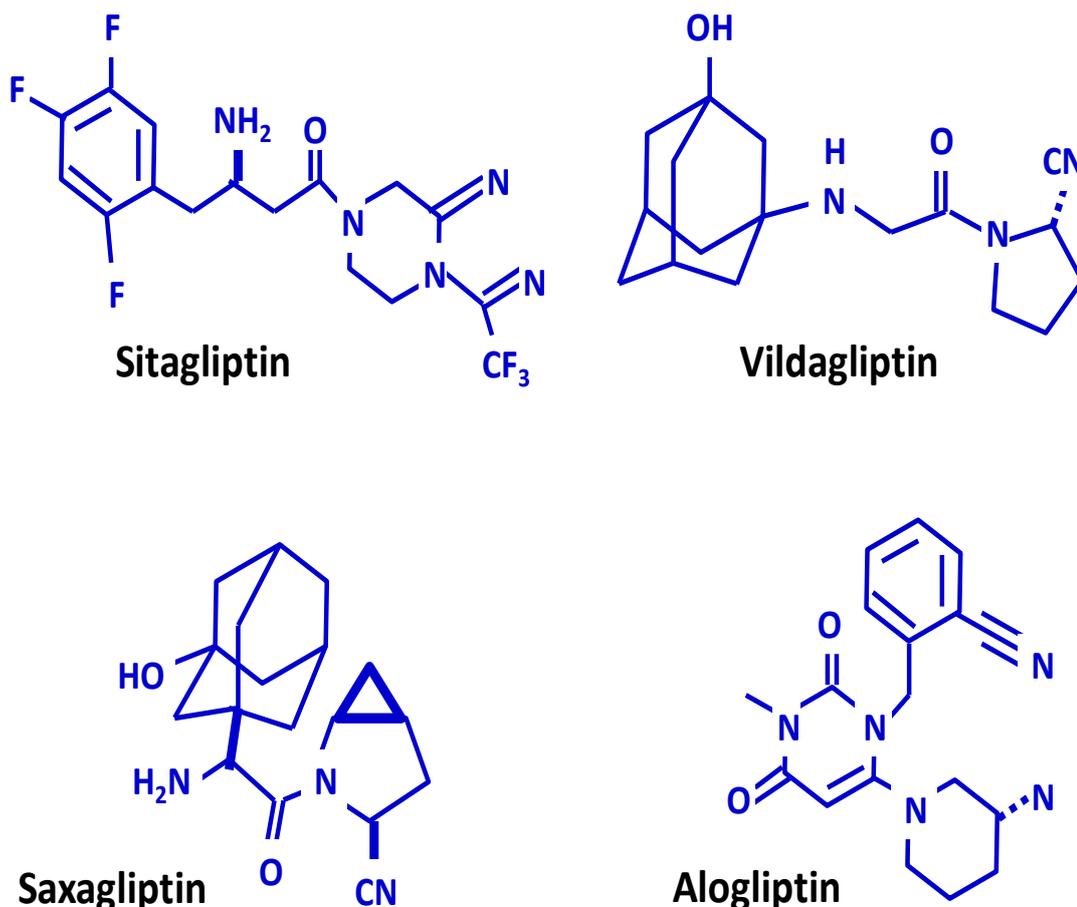


Figure 1.14: The chemical structure of sitagliptin, vildagliptin, saxagliptin and alogliptin (Modified from Thornberry and Gallwitz, 2009).

1.16 Gene therapy and GLP-1

Single injection of STZ-induced diabetic non-obese immunodeficient mice with recombinant adenoviral vector expressing GLP-1 (rAd-GLP-1), leads to a reduction and normalization of blood glucose levels during the 10 days of the study (Liu et al., 2007). GLP-1/Fc gene introduction to db/db mice has shown that the transcription of GLP-1/Fc gene improves blood glucose levels through increasing insulin release and decreasing glucagon secretion. Therefore, induction of this GLP-1 gene elevates the expression of GLP-1 with its prospective role in the treatment of DM (Kumar et al., 2007).

1.17 Working hypothesis

Newly developed incretin-like peptides may be effective in both the synthesis and the release of insulin from the endocrine pancreas in T1DM and exert their secretagogue effects on the endocrine pancreas via similar or different mechanisms.

1.17.1 Main aim

The main aim of this study was to investigate the effects of new peptides on insulin and glucagon release in age-matched control and STZ-induced diabetic rats.

1.17.2 Specific aims

1. To determine the pattern of distribution of GLP-1 and exenatide in the endocrine pancreas of normal and diabetic rats.
2. To determine the mechanism of GLP-1 and exenatide induced insulin release.
3. To investigate the subcellular localization of GLP-1 and exenatide in the endocrine pancreas of normal and diabetic rats.
4. To determine the effect GLP-1 and exenatide on liver and kidney function tests.
5. To investigate the role of GLP-1 and exenatide on distribution of endogenous antioxidants.
6. To determine whether GLP-1 and exenatide can ameliorate the impaired metabolic parameters (body weight, blood glucose, GTT and lipid profile) observed in diabetes.
7. To investigate the effects of the expression of genes related to β -cell function.
8. To analyse the data and write up the PhD thesis.

CHAPTER TWO

MATERIALS AND METHODS

2. Materials and Methods

2.1 Experimental animals

This study employed young male Wistar rats weighing approximately 250 grams (10 weeks old) at the beginning of the experimental period. The animals were obtained from the Faculty of Medicine and Health Sciences (FMHS), United Arab Emirates University, (UAEU) breeding colony and the Animal Research Group's guidelines for the care and use of laboratory animals were followed. All the rats were housed in temperature (25°C) and humidity controlled rooms and 12 hours light and dark periods. The animals were fed on a standard rat chow and tap water *ad libitum*. This project had the ethical clearance from the ethics committees from University of Central Lancashire (UCLAN) and UAEU to carry out the experiments.

2.2 Induction of experimental diabetes

Diabetes was induced in the rats by a single intraperitoneal injection (ip) of (0.3 ml) streptozotocin, (STZ) (Sigma, Poole, UK) at a dose of 60 mg/kg body weight (Adeghate, 1999). The STZ was freshly dissolved in citrate buffer (0.5 M, pH 4.5) (see appendix). Age-matched healthy control rats received the same volume (0.3 ml) of the citrate buffer only. Following five days after either STZ or citrate buffer injection, the rats were tested for diabetes by using a drop of blood from the tail end of each rat. The blood glucose estimations were made by Optium Xceed Glucometer (Abbott, Park, Illinois, USA) for each individual rat. The rats were considered diabetic if the fasting blood glucose levels were equal to or more than 280 mg/dl.

2.3 Experimental design

Following the diagnosis of diabetes, both age-matched healthy control and STZ-induced diabetic rats were divided into six groups each containing ten rats. The diabetic rats were intraperitoneally injected daily with either GLP-1 (50 nmol/kg) or exenatide (1 µg/kg) for ten weeks. GLP-1 and exenatide were purchased from Phoenix Pharmaceuticals, Burlingame, California, USA. The age-matched control rats received the vehicle for over the same period of ten weeks. The six treated groups were:

- A. Group 1, served as untreated normal healthy control.
- B. Group 2, served as untreated diabetic control.
- C. Group 3, served as normal-treated with glucagon like peptide-1 (50 nmol/kg).
- D. Group 4, served as diabetic-treated with glucagon like peptide-1 (50 nmol/kg).
- E. Group 5, served as normal-treated with exenatide (1 µg/kg).
- F. Group 6, served as diabetic-treated with exenatide (1 µg/kg).

Each group in this study contained 10 rats (n=10).

All rats were 10 weeks old at the beginning of the experiment (zero week) and were 20 weeks old at the end of the study period. All measurements were performed at the end of the experiment except the measurement of body weight and the fasting blood glucose levels, which were estimated every two weeks from zero week till week number 10.

2.4 Weight measurement

The weights of normal and diabetic rats were recorded every two weeks using a 9001 Scale (Satorius, Hertfordshire, UK). The mean \pm standard error of the mean (SEM) of the body weight for each rat within each experimental group was calculated for each two weeks of the experiment period.

2.5 Fasting blood glucose measurement

The fasting blood glucose level was measured every two weeks for each individual rat of all groups. The blood samples were drawn from the rat's tail end for blood glucose measurement. The mean \pm standard error of the mean (SEM) of the blood glucose for each experimental group was calculated every two weeks during the experiment period.

2.6 Intraperitoneal glucose tolerance test (IPGTT)

At the end of the ten weeks of the treatment, age-matched healthy control and STZ-induced diabetic rats were subjected to intraperitoneal glucose tolerance test, after an overnight fasting for 18 hours. Each rat was given an intraperitoneal glucose load of 2 g/kg body weight according to the method of Caluwaerts et al., (2007). The blood glucose measurements were made at fasting zero time (before glucose load), 30, 60, 120 and 180 minutes after the glucose load.

2.7 Biochemical analysis

Serum aspartate aminotransferase, serum alanine aminotransferase, blood urea nitrogen, serum creatinine, serum uric acid, serum cholesterol and serum triglyceride were performed in Al-Ain Hospital Clinical Laboratory using the Beckman Coulter (Synchron UniCel, DxC, 800 Synchron, Clinical System, California, USA).

2.8 Tissue collection and tissue processing

After the end of the experiments, all the rats from each group were killed humanely under general anesthesia by diethyl ether. A mid-line abdominal incision was made and the pancreas was rapidly removed. Representative pancreatic fragments were taken

from different parts of the body of the pancreas and they were used for both morphological, *in vitro* study and molecular biological studies.

2.9 Serum insulin estimation by enzyme-linked immunosorbent assay

Serum insulin level was determined by using DakoCytomation Kit, Glostrup, Denmark, which was an enzyme-linked immunosorbent assay (ELISA) method based on two monoclonal antibodies. Simultaneous incubation of sample and enzyme-labelled antibody in a microplate microwell coated with a specific anti-insulin antibody formed a complex. A simple washing step removed unbound enzyme-labelled antibody. The bound conjugate was detected by reaction with the substrate tetramethylbenzidine (TMB). The reaction was stopped by adding sulphuric acid to give a colorimetric endpoint absorbance that was measured spectrophotometrically using Biochrom Anthos 2010, Microplate Reader (Cambridge, England). The inclusion of calibrators of known insulin concentrations in the assay allowed a calibration curve to be constructed from which the level of insulin in the samples could be calculated.

2.10 Estimation of *in vitro* pancreatic insulin release

In these experiments, ten rats from each group were used. The pancreas was removed and placed in ice-cold phosphate buffer saline (PBS) (see appendix). The pancreas was trimmed free of adherent fat and connective tissue and cut into small fragments (1-2 mm³). Thereafter, the pancreatic fragments were then placed in glass tubes containing 1 ml of PBS and the tubes were weighed before and after addition of pancreatic fragments. Each tube was preincubated for 30 minutes in a shaking water bath at 37°C, in order to wash away any enzymes and hormones due to cutting of the tissues. The tissues were gassed with 5% CO₂ and 95% O₂. After the preincubation period, the PBS solution was drained and the fragments were subsequently incubated for 1 hour with 1

ml of PBS containing different concentrations of the following solutions. Before the incubation, all the tubes were weighed again to estimate the tissue net weight in each tube.

This study employed either GLP-1 or exenatide. The different concentrations employed in this study included either GLP-1 (10^{-12} , 10^{-9} and 10^{-6} M) or exenatide (10^{-12} , 10^{-9} and 10^{-6} M). In control experiments, the fragments were incubated in PBS solution alone for the same period at 37°C in a shaking water bath. During the incubation period, each vial was gassed with 95% oxygen and 5% carbon dioxide every 10 minutes. At the end of each experiment, the tissues were removed and incubation solutions were stored at -20°C for ELISA estimation of insulin concentrations. All values for insulin secretion levels were expressed as μ IU/ml/100 mg tissue.

2.11 Immunohistochemical studies

Ten rats from each group (either control or diabetic) were used for the experiments. The isolated pancreas was trimmed free of adherent fat and connective tissues and cut into small pieces (2 mm³) and fixed overnight in freshly prepared Zamboni's fixative (Zamboni and de Martino, 1967) (see appendix). The tissues were later dehydrated in graded concentrations of ethanol. The specimens were changed every 2 hours in 70% and 95% and 3 changes in absolute ethanol for 2 h. After dehydration, the specimens were cleared in xylene and subsequently embedded in paraffin wax at 55°C. Sections of 6 μ m thickness were cut on a microtome (Shandon AS325, Michigan, USA), and placed in water bath at 48°C. Thereafter, they were transferred onto prewashed microscopic slides, which were dried in an oven at 55°C for 30 minutes to enhance attachment of sections.

The slides were deparaffinized with xylene (twice, 5 minutes each) and then transferred into absolute ethanol (twice, 5 minutes each). The sections were then incubated for 30 minutes in 0.3% hydrogen peroxide solution in methanol to block endogenous peroxidase activity. The sections were hydrated in decreasing concentrations of ethanol

and brought to phosphate buffered saline (PBS) (see appendix). The slides were washed three times (5 minutes each) in PBS. After washing in PBS, the tissues were marked around with a Dako pen (DakoCytomation, Glostrup, Denmark) to prevent solutions draining away from the tissue sections. The staining procedure started by incubating the sections with blocking reagent. After 30 minutes the blocking reagent was drained off and appropriate dilution of primary antibodies and negative control reagents were applied. The sections were then incubated in primary antibodies for 1 h at room temperature. The slides were then washed (3 times, 5 minutes each) with PBS and incubated for 30 minutes with prediluted biotinylated anti-rabbit or anti-mouse IgG for 30 minutes. They were washed again in PBS (3 times, 5 minutes each) and subsequently incubated in streptavidin peroxidase conjugate for 45 minutes. After a final wash in PBS (3 times, 5 minutes each), the peroxidase activity was revealed by incubating the specimens for 3 minutes in 3,3-diaminobenzidine tetrahydrochloride containing 0.03% hydrogen peroxide in PBS. The slides were later washed for 1 minute under running tap water and counterstained with haematoxylin for 30 seconds. They were then differentiated in acid ethanol and washed for 1 minute under running tap water, dehydrated in ascending grades of ethanol and subsequently cleared in xylene for longer time to dissolve the Dako pen mark. The tissues were subsequently mounted in Cytoseal 60 (Stephens Scientific, Riverdale, New Jersey, USA). Slides were examined under Ziess Axiophot microscope (Carl Zeiss, Jena, Germany) and immunopositive cells of the tissue sections were photographed.

The number of insulin, glucagon, GLP-1, exenatide, glutathione reductase and catalase-positive cells were counted and estimated semi-quantitatively in normal and diabetic pancreatic tissues. The antibodies for insulin, glucagon, GLP-1, exenatide, glutathione reductase and catalase were purchased from commercial suppliers (see appendix). They were used at appropriate dilutions (see appendix). No specific immunostaining was observed in pancreatic tissue when primary antibodies were omitted. (The immunohistochemical staining was performed by the Labelled Streptavidin Biotin method using a Dako LSAB[®] peroxidase kit, California, USA).

2.12 Immunofluorescence studies

Isolated pancreatic tissues were retrieved, fixed and embedded in paraffin. Sections of 6 µm thickness were deparaffinized in xylene, hydrated in descending concentration of ethanol (5 minutes each) and washed 3 times in PBS for (5 minutes each). After washing in PBS, the tissue was marked around with a Dako pen to prevent solutions draining away from the tissue section. The staining procedure started by incubating the sections with blocking reagent for 30 minutes. Thereafter, the blocking reagent was drained off and appropriate dilution of primary antibodies were applied and incubated at 4°C for 24 hours. On the following day, the sections were incubated at room temperature for 1 hour. The slides were then washed 3 times in PBS (5 minutes each) and incubated with secondary antibodies conjugated with FITC or TRITC (Jackson Laboratory, West Grove, Pennsylvania, USA) for 1 hour and washed in PBS (3 times 5 minutes each). Sections were then mounted in CITI-Fluore mounting media and viewed and photographed with Ziess Axiophot Fluorescence Microscope, (Carl Zeiss, Jena, Germany).

2.13 Electron microscopic studies

The pancreas was trimmed free of adherent fat and connective tissue and cut into small pieces of 2 mm³ and fixed overnight in freshly prepared Karnovsky fixative (see appendix) (Karnovsky, 1965). Thereafter, they were washed 3 times in sodium cacodylate buffer (see appendix) and post-fixed in 1% osmium tetroxide for 1 hour. After post-fixation, they were washed 5 times in the same buffer and dehydrated. Dehydration of tissues were done in ethanol series with single change of 15 minutes in 30%, 50%, 70%, 95% and 4 changes in absolute ethanol for 15 minutes each. After the dehydration, they were cleared in propylene oxide (2 changes of 15 minutes each) and transferred to 1:1 mixture of propylene oxide and resin (see appendix) for 1 hour and later transferred to 1:2 mixtures of propylene oxide and resin. After one hour in the 1:2 mixtures, they were infiltrated with resin overnight. The samples were later embedded

in resin using moulds; polymerization of the resin block was completed in 24 hours at 55°C. The blocks were trimmed and 1µm semi-thin sections were cut with glass knives on an ultra-microtome (Reichert Ultracut 5, Massachusetts, USA) and sections were transferred onto drops of water on a microscope slide using watchmaker's forceps. The slides were stained with toluidine blue and observed under microscope to locate the islets of Langerhans. The tissue blocks were later trimmed for ultra-thin sectioning. Ultra-thin sections were cut at a thickness of 80 nm by a diamond knife. The sections were then transferred onto 300 mesh copper grid by a wire loop. The grids were dried on a filter paper and stained with uranyl acetate for 30 minutes and washed 5 times in filtrated distilled water. To further enhance the contrast, the sections were stained in lead citrate for 3 minutes and washed 3 times in filtrated distilled water. The dried grids were observed on transmission electron microscope (Philips CM10, Eindhoven, Holland) and photographed.

2.14 Immunoelectron microscopic studies

Nickel grids with sections of pancreas were immerse in 0.01 M sodium citrate buffer at 90°C for 5 minutes and cooled at room temperature in the citrate buffer for 20 minutes. Then the grids were Jet-washed with deionized water. Grids were placed in 10% H₂O₂ for 10 minutes. Washed in deionized water then immersed in 0.5 M NH₄Cl in 0.01 M PBS for 20 minutes. Grids were washed in PBS containing 1% bovine serum albumin (BSA) and 0.1% Tween-20 (washing buffer) for 5 minutes. Grids were blocked in 20% normal goat serum (NGS) diluted in washing buffer (blocking buffer) for 10 minutes and then incubated overnight at 4°C in primary antibody. After that, grids were placed in room temperature for 1 hour and washed in washing buffer for 5 minutes three times. The grids were incubated in blocking buffer for 20 minutes at room temperature and later in goat anti-mouse IgG conjugated to 20 nm gold particles (TAAB, Berkshire, UK) at room temperature for 2 hours. Grids were then washed (washing buffer) for 3 minutes five times and later incubated overnight at 4°C in secondary antibody. After that, the grids were placed in room temperature for 1 hour and washed in washing

buffer for 5 minutes three times. They were later incubated in blocking buffer for 20 minutes at room temperature. Grids were incubated in goat anti-mouse IgG conjugated to 15 nm gold particles (TAAB, Berkshire, UK) at room temperature for 2 hours. After that, the grids were washed (washing buffer) for 3 minutes (five times) and later fixed in glutaraldehyde (2.5% aqueous) for 5 minutes. Grids were then washed in deionized water for 3 minutes 5 times. Finally, the grids were placed on filter paper to dry for 1 hour and were contrasted with uranyl acetate and lead citrate for 15 minutes and 7 minutes, respectively. The dried grids were observed on transmission electron microscope (Philips CMIO, Eindhoven, Holland) and photographed.

2.15 Molecular biology studies by relative quantification assay using real-time PCR

This study investigated the effect of certain transcription factors on insulin gene expression. These included pancreatic duodenal homeobox-1, heat shock protein-70, glutathione peroxidase, glucagon, insulin receptor and glucagon-like peptide-1 receptor. Estimation the levels of these transcription factors was done using relative quantification assay by real-time polymerase chain reaction (PCR) technique. Relative quantification was used to compare expression levels of the gene expression in different tissues or in differently treated and untreated samples. Relative quantification can match the expression values of the gene expression in treated and untreated pancreatic rat tissues. Relative quantification method estimates precisely the values of gene expression in each treatment with no need for absolute determination of either gene expression copy number or even standard curves estimation.

Relative quantification assay using real-time PCR technique was used to determine whether GLP-1 and exenatide could stimulate any changes in the levels of pancreatic duodenal homeobox-1, heat shock protein-70, glutathione peroxidase, glucagon, insulin receptor and glucagon like peptide-1 receptor genes, which are implicated in the differentiation, maturation of pancreatic islet cells and their relation to insulin

secretions. The relative estimation was to find out if it could contribute to the amelioration of the signs and symptoms of DM.

2.15.1 Total RNA extraction

Total RNA was extracted from rat pancreatic tissues using RiboPure Extraction kit (Cat No. 1924, AMBION-Applied Biosystems, California, USA) according to the manufacturer`s recommendations.

2.15.2 RNA yield and quality

The concentrations and the purity of the RNA samples were determined by measuring their absorbance at 260 nm and 280 nm using the Spectrophotometer NanoDrop 1000A, Wilmington, USA.

2.15.3 cDNA synthesis

Complementary deoxyribonucleic acid (cDNA) was synthesized using high capacity cDNA reverse transcription kit with RNAase inhibitor (Applied Biosystems, California, USA).

2.15.4 Gene expression assay using real-time PCR

Gene expression assays using TaqMan labelled primers and probes (for the target genes [pancreatic duodenal homeobox-1 (Gene Bank ID: 29535), heat shock protein-70 (Gene Bank ID: 266759), glutathione peroxidase (Gene Bank ID: 24404), glucagon (Gene Bank ID: 24952), insulin receptor (Gene Bank ID: 24954) and glucagon like peptide-1 receptor (Gene Bank ID: 25051) were undertaken using relative real time PCR. The endogenous control was β -actin (Gene Bank ID: 81822). All primers and probes were

purchased from Applied Biosystems, California, USA. By using the endogenous control as an active reference, the target mRNA was normalized and quantified by measuring the differences in the amounts of total RNA added to each reaction. PCR reactions were carried out using the following cycling conditions as one cycle at 50°C for 2 minutes then 1 cycle at 95°C for 10 minutes followed by 40 cycles at 95°C for 15 second and 60°C for 1 minute. Relative quantification method was accomplished by relative real-time PCR. The technique of real-time PCR estimates directly the levels of the gene expression simultaneously during the real-time PCR amplification and finally when the experiment is completed using Applied Biosystems 7500 Real-time PCR System (California, USA).

2.15.5 Analysing the results

Data were analyzed by creating a relative quantification (RQ) study document using the sequence detection systems (SDS) software of 7500 ABI instrument (Applied Biosystems, California, USA). Relative quantification technique was employed to determine the change in expression of the target gene in treated sample relative to the untreated sample. The results are calculated as a fold-difference of the gene amplification values after their normalization to the levels of the housekeeping endogenous β -actin gene expression.

2.16 Statistical analysis

All values were calculated as mean \pm standard error of the mean (SEM). Student's *t-test* and ANOVA test were used to analyze the significance of differences between mean values and different groups were assessed using SPSS statistical analysis software. Values with ($P < 0.05$) were accepted as significant comparing control and treated samples. Values with either ($P < 0.01$) or ($P < 0.001$) were accepted as moderate or highly significant, respectively.

CHAPTER THREE

RESULTS

In vivo studies

3.1 Effect of GLP-1 and exenatide treatment on metabolic parameters of normal and diabetic rats

3.1.1 Effect of GLP-1 and exenatide treatment on body weight of normal rats

Treatment with either GLP-1 (50 nmol/kg body weight) or exenatide (1 µg/kg body weight), did not result in significant weight gain compared to control (Figure 3.1). The weight gain observed in normal rats treated with either GLP-1 or exenatide was more or less similar to that of untreated normal rats. The weight gain in exenatide treated normal rats was slightly less than in case of GLP-1 treated normal rats compared with the weight gain in untreated normal rats. All the three groups of rats gained mild weight increases over the experimental period compared to the start of experiment period at 0 week.

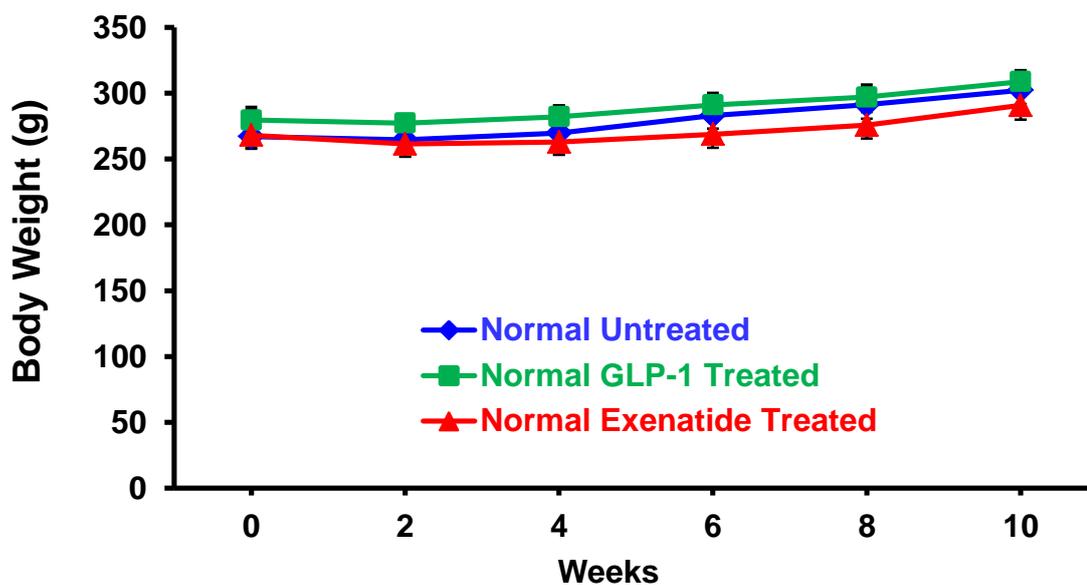


Figure 3.1: Time course effect of 10 weeks of treatment with either GLP-1 (50 nmol/kg body weight) or exenatide (1 µg/kg body weight) on body weight of normal treated rats compared to untreated normal control rats. Data are mean \pm SEM, n = 10 rats for each point. Note that there was no significant difference in the body weight between all normal groups.

3.1.2 Effect of GLP-1 and exenatide treatment on body weight of diabetic rats

Treatment with either GLP-1 (50 nmol/kg body weight) or exenatide (1 µg/kg body weight), to STZ-induced diabetic rats over the experimental period resulted in a slight but not significant weight loss compared to the weight of diabetic rats prior to treatment (time 0 week) (Figure 3.2). Untreated diabetic rats lost weight gradually from week to week until the end of the experiment. However, diabetic rats treated with either GLP-1 or exenatide had a significantly ($p < 0.05$) higher weight profile compared to untreated diabetic rats at weeks 6 and 8. Generally, the results show that the total body weight loss in either GLP-1 or exenatide treated rats was lower compared to untreated rats throughout the entire 10-week period of the study.

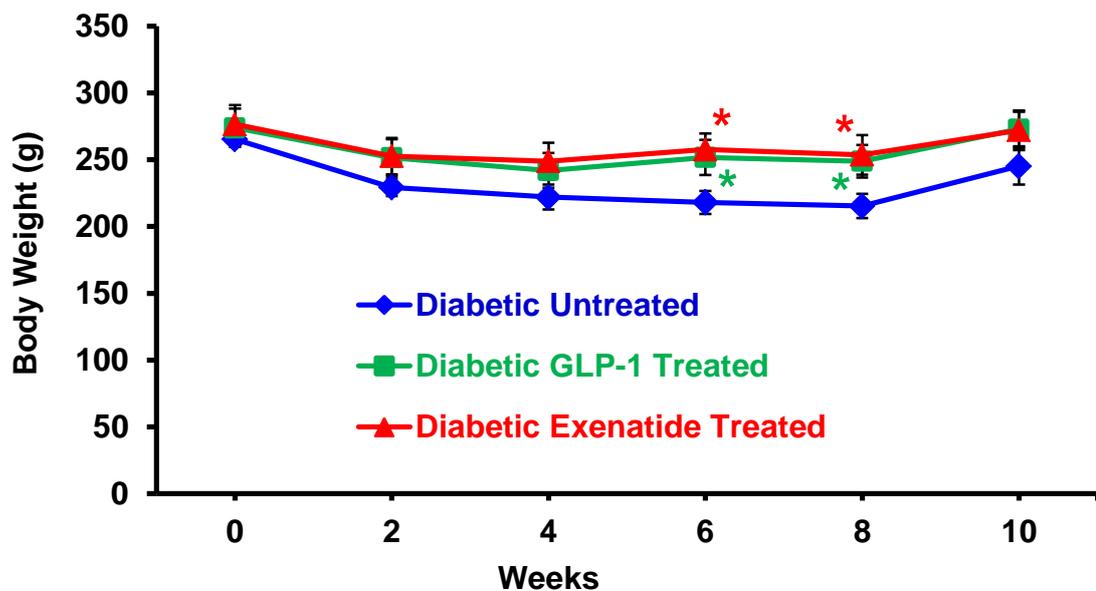


Figure 3.2: Time course effect of 10 weeks of treatment of either GLP-1 (50 nmol/kg body weight) or exenatide (1 µg/kg body weight) on body weight of diabetic treated rats compared to diabetic untreated rats. Data are mean \pm SEM, $n = 10$ rats for each point rats. * $P < 0.05$ significant increase in body weight of diabetic treated rats with either GLP-1 or exenatide compared to untreated diabetic rats at 6 and 8 weeks. Note that the higher change detected in either GLP-1 or exenatide treated diabetic rats was between weeks 6-10.

3.1.3 Effect of GLP-1 and exenatide treatment on blood glucose levels of normal rats

Figure 3.3 shows the time course for fasting blood glucose (FBG) levels of normal rats after treatment with either GLP-1 (50 nmol/kg body weight) or exenatide (1 µg/kg body weight) compared to untreated normal rats. A slight but not significant reduction in FBG was detected in normal rats at the end of the 10 weeks of the experiment period compared to the start of the experimental period in week zero mainly due to the effect of treatment with either GLP-1 or exenatide. Almost similar but not significant changes of FBG levels were detected in both normal groups treated with either GLP-1 or exenatide at the end of the 10 weeks of the experiment compared to untreated normal rats.

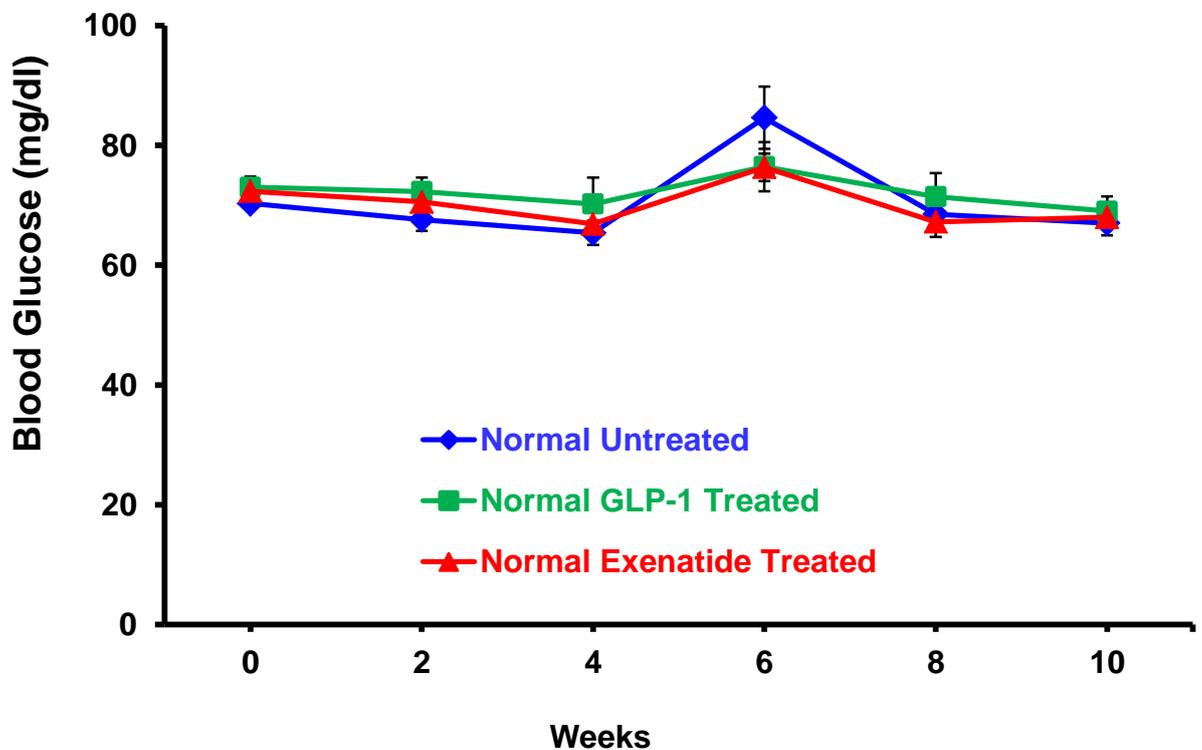


Figure 3.3: Time course effect of 10 weeks of treatment with either GLP-1 (50 nmol/kg body weight) or exenatide (1 µg/kg body weight) on blood glucose levels of normal treated rats compared to untreated normal control rats. Data are mean \pm SEM, n = 10 rats for each time point.

3.1.4 Effect of GLP-1 and exenatide treatment on blood glucose levels of diabetic rats

Figure 3.4 has shown that all the fasting blood glucose (FBG) levels of diabetic rats were markedly higher than the FBG levels of all normal rats detected in figure 3.3. The results of figure 3.4 also revealed a reduction in fasting blood glucose (FBG) levels during and at the end of the experiment period in diabetic rats treated with either GLP-1 (50 nmol/kg body weight) or exenatide (1 µg/Kg body weight) diabetic treated rats compared to untreated diabetic rats. A significant reduction was detected in diabetic rats treated with GLP-1 starting from weeks 4-8 ($p < 0.05$) to the week 10 ($p < 0.01$) compared to untreated diabetic rats. In addition, a significant reduction of FBG levels were detected in exenatide treatment diabetic rats in weeks 4 and 10 ($p < 0.05$) compared to untreated diabetic rats.

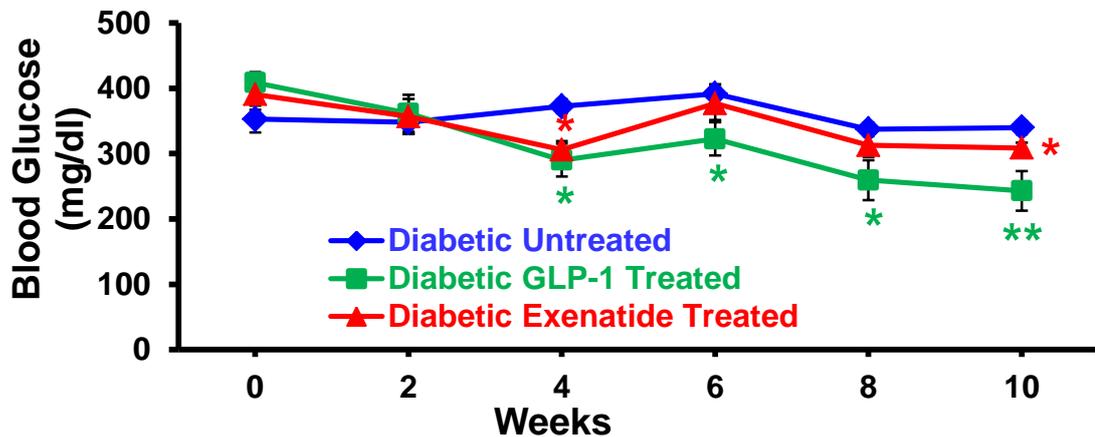


Figure 3.4: Time course effect of 10 weeks of treatment with either GLP-1 (50 nmol/kg body weight) or exenatide (1 µg/kg body weight) on blood glucose levels of diabetic rats compared to untreated diabetic control rats. Data are mean \pm SEM, $n = 10$ rats for each time point. * $P < 0.05$; and ** $P < 0.01$ significant decreases in fasting blood glucose levels of diabetic treated rats with either GLP-1 or exenatide compared to untreated diabetic rats. The results also show that the diabetic rats treated with GLP-1 showed mild significant ($p < 0.05$) reduction in FBG levels when compared to untreated diabetic rats starting from the fourth week and continued until the eighth week and the difference reached to moderate significant ($p < 0.01$) reduction detected at the tenth week. However, the results show that exenatide diabetic treated rats have mild significant ($p < 0.05$) reduction detected at weeks four and ten.

3.1.5 Intraperitoneal glucose tolerance test (IGTT) in GLP-1- and exenatide treated normal rats.

The results for the IGTT of normal rats are shown in Figure 3.5. The data show that rats treated with either GLP-1 (50 nmol/kg body weight) or exenatide (1 µg/kg body weight) have a better IGTT values compared to untreated normal rats. However, it appeared that the IGTT results of normal treated GLP-1 rats was better than that the exenatide treated normal rats. There was a significant ($p < 0.05$) decrease in blood glucose levels of rats treated with GLP-1 compared to normal, untreated rats at 30 and 120 minutes following glucose load, while, a small significant ($p < 0.05$) decrease was detected in blood glucose levels of rats treated with exenatide compared to normal untreated rats at 120 minutes following glucose load. The results also show that after 180 minutes, blood glucose level for all rats returned to almost normal level.

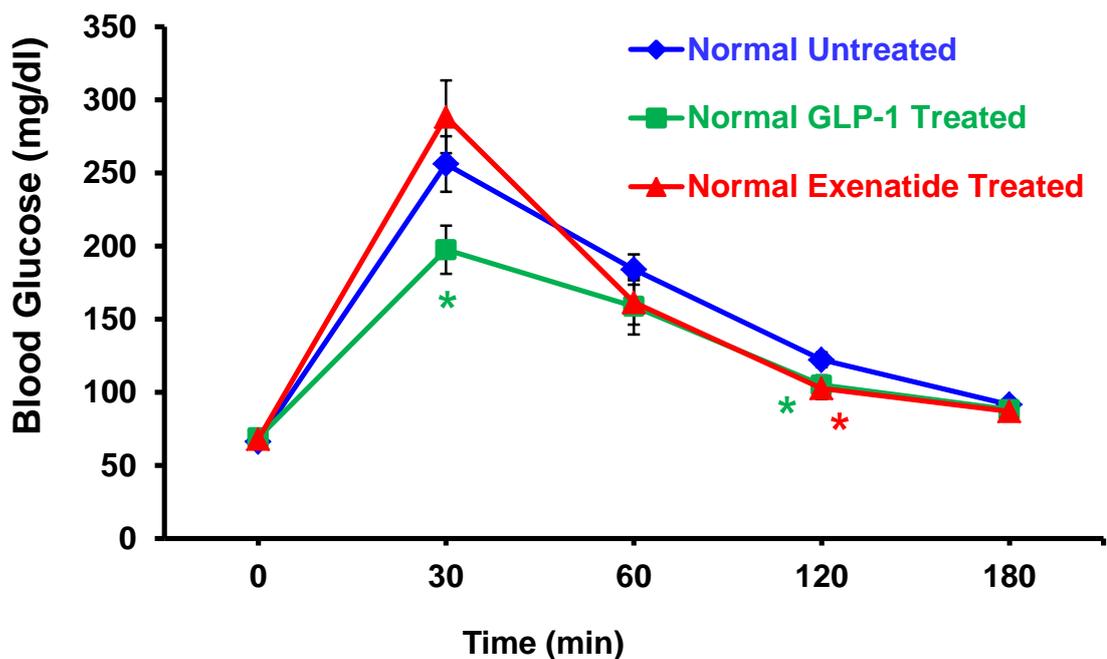


Figure 3.5: Time course effect of 10 weeks of treatment with either GLP-1 (50 nmol/kg body weight) or exenatide (1 µg/kg body weight) on IGTT of normal rats compared to untreated normal control rats. * $P < 0.05$ for glucose levels of normal treated rats compared to untreated normal control rats. Data are mean \pm SEM, $n = 10$ rats in each data point.

3.1.6 Intraperitoneal glucose tolerance test (IGTT) in GLP-1- and exenatide treated diabetic rats.

Figure 3.6 shows the time course of IGTT in diabetic rats that were treated with either GLP-1 (50 nmol/kg body weight) or exenatide (1 µg/kg body weight) compared to untreated diabetic rats. At the start of the experiments all the diabetic rats had high blood glucose level compared to normal rats (see Figure 3.5). The IGTT values of diabetic rats treated with exenatide was lower than that of the untreated diabetic rats, the difference was significant ($p < 0.05$) at both 0 minutes and 120 minutes. Moreover, rats that were treated with GLP-1 had significantly ($p < 0.05$; $p < 0.01$) lower IGTT values at 0, 30, 60, 120 and 180 minutes after the glucose load as compared to untreated diabetic rats. These results demonstrated that GLP-1 was more effective in reducing blood glucose in diabetic rats compared to exenatide.

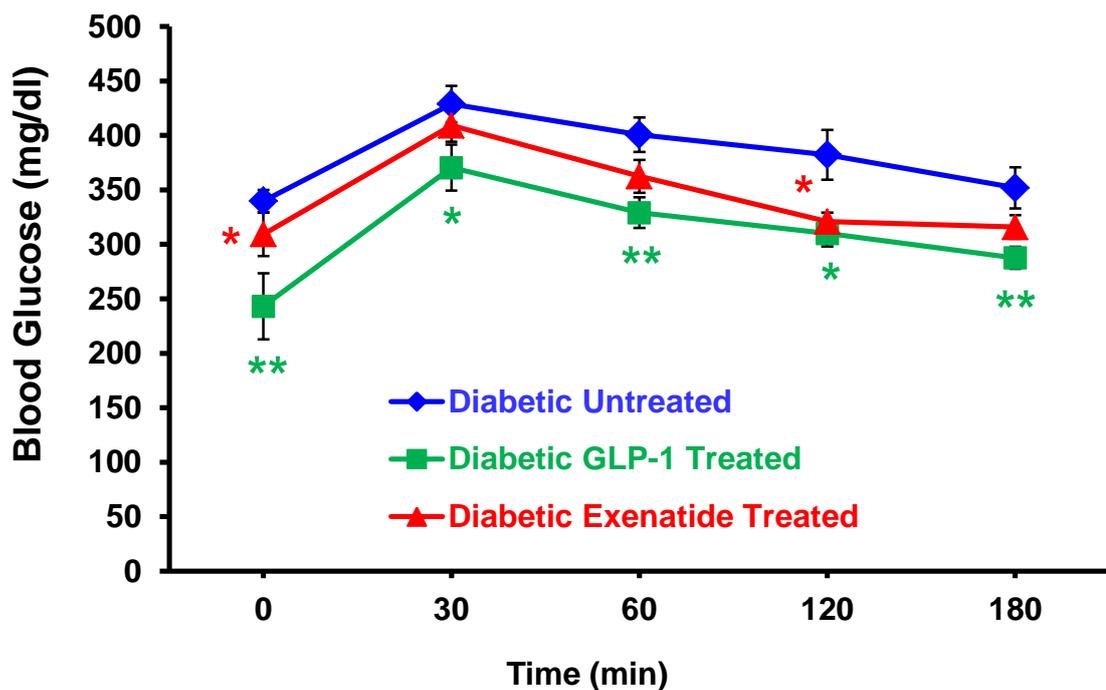


Figure 3.6: Time course effect of 10 weeks of treatment with either GLP-1 (50 nmol/kg body weight) or exenatide (1 µg/kg body weight) on IGTT of diabetic rats compared to untreated diabetic rats. * $P < 0.05$ and ** $P < 0.01$ for glucose levels of diabetic treated rats compared to untreated diabetic rats. Data are mean \pm SEM, $n = 10$ rats for each data point.

3.2 Effect of GLP-1 and exenatide treatment on biochemical parameters of normal and diabetic rats

3.2.1 Serum aspartate transferase (AST)

Figures 3.7 and 3.8, respectively show the effect of either GLP-1 (50 nmol/kg body weight) or exenatide (1 µg/kg body weight) on serum AST in normal and diabetic treated rats. Treatments of normal rats with either GLP-1 or exenatide resulted in a significant ($p < 0.001$) reduction in serum AST levels compared to untreated rats (Figure 3.7). On the other hand, diabetic rats treated with either GLP-1 or exenatide have a more pronounced and significant reduction in serum AST levels ($p < 0.001$) compared to untreated diabetic rats (Figure 3.8).

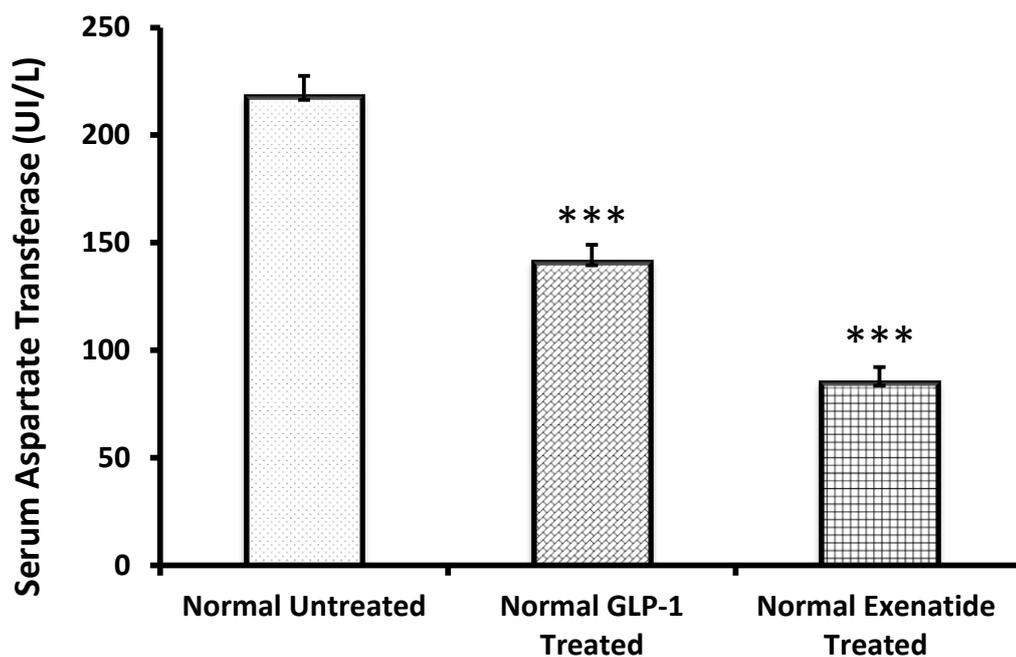


Figure 3.7: Histograms showing the effect of 10 weeks of treatment with either GLP-1 (50 nmol/kg body weight) or exenatide (1 µg/kg body weight) on serum AST levels of normal rats. *** $P < 0.001$ for normal GLP-1 and exenatide treated rats compared to untreated normal rats. Data are mean \pm SEM, $n = 10$ rats for each time point.

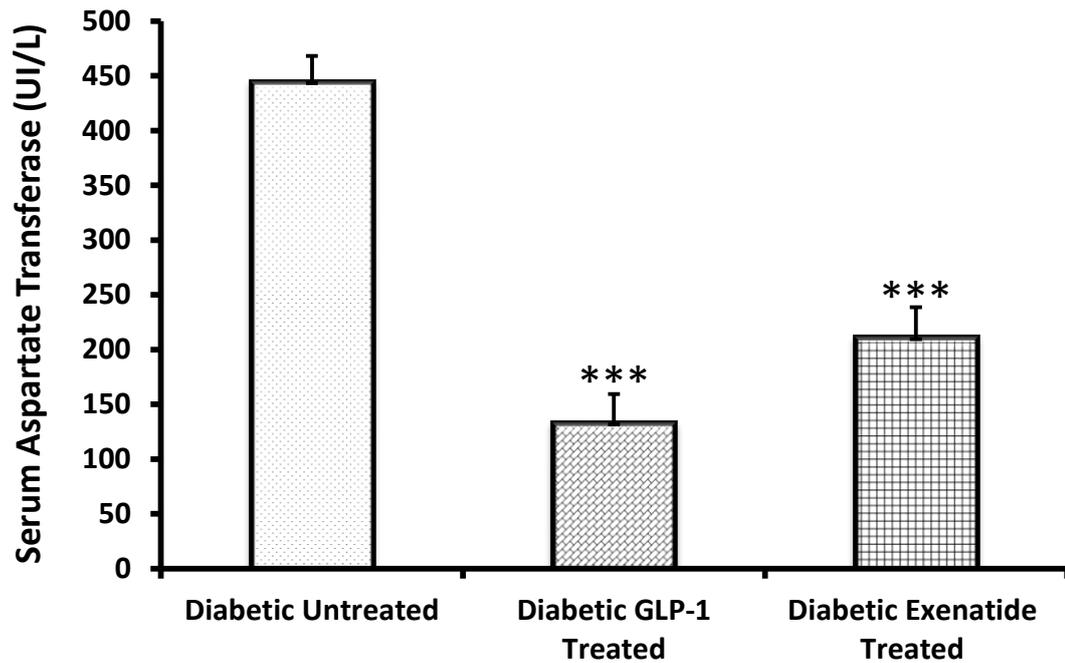


Figure 3.8: Histograms showing the effect of 10 weeks of treatment with either GLP-1 (50 nmol/kg body weight) or exenatide (1 μ g/kg body weight) on serum AST levels of diabetic rats. *** $P < 0.001$ for diabetic GLP-1 and exenatide treated rats compared to untreated diabetic rats. Data are mean \pm SEM, $n = 10$ rats for each time point.

3.2.2 Serum alanine transferase (ALT)

Figures 3.9 and 3.10 show the effect of either GLP-1 (50 nmol/kg body weight) or exenatide (1 μ g/kg body weight) on serum ALT in normal and diabetic treated rats, respectively, compared to untreated rats. Treatments of normal rats with GLP-1 resulted in a slight reduction in serum ALT levels and a significant reduction in serum ALT levels ($p < 0.01$) of normal rats treated with exenatide, compared to normal untreated rats (Figure 3.9). On the other hand, treatment of diabetic rats with either GLP-1 or exenatide resulted in a significant reduction in serum ALT levels ($p < 0.001$; $p < 0.01$), respectively, compared to diabetic untreated rats (Figure 3.10). These results also reveal that both GLP-1 and exenatide were more effective in reducing serum ALT levels in diabetic rats compared to normal treated rats.

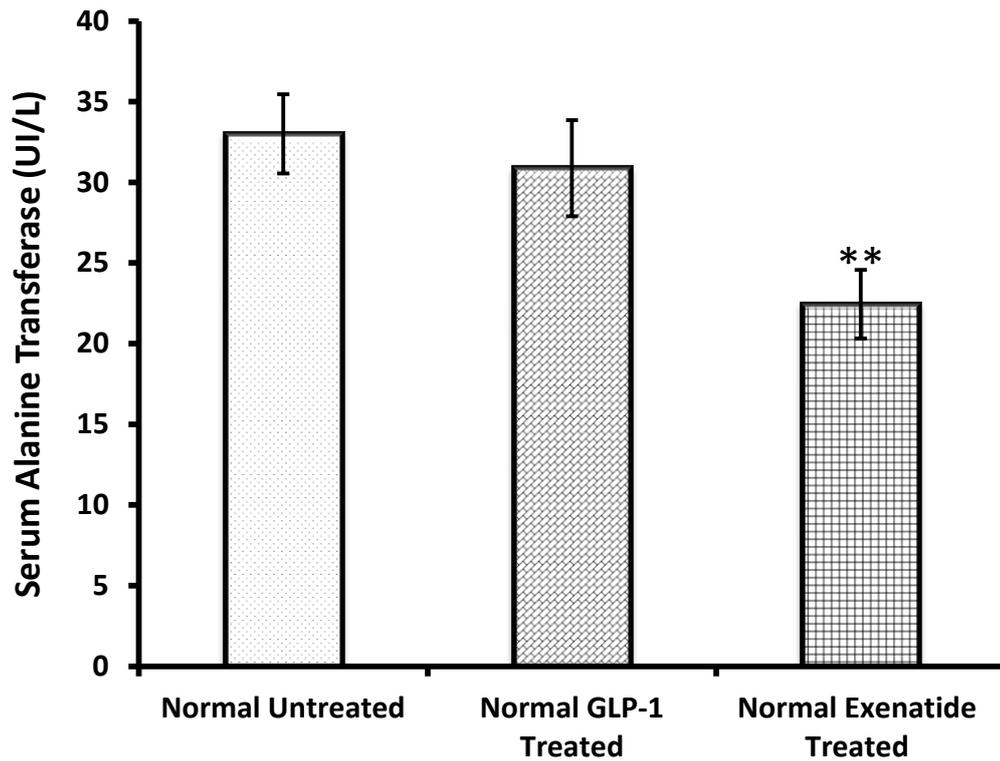


Figure 3.9: Histograms showing the effect of 10 weeks of treatment with either GLP-1 (50 nmol/kg body weight) or exenatide (1 μ g/kg body weight) on serum ALT levels of normal rats compared to normal untreated rats. ** $P < 0.01$ for exenatide treated normal rats compared to normal untreated rats. Data are mean \pm SEM, $n = 10$ rats for each group.

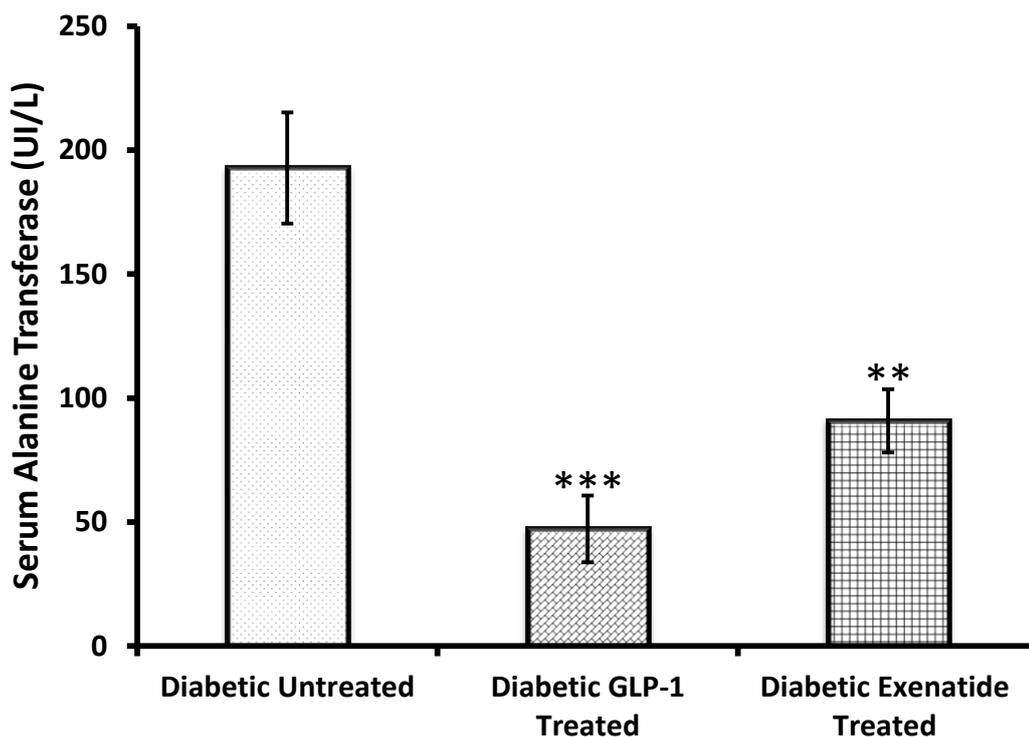


Figure 3.10: Histograms showing the effect of 10 weeks of treatment with either GLP-1 (50 nmol/kg body weight) or exenatide (1 μ g/kg body weight) on serum ALT levels of diabetic rats. ** $P < 0.01$; *** $P < 0.001$ for diabetic GLP-1 and exenatide treated rats compared to untreated diabetic rats. Data are mean \pm SEM, $n = 10$ rats for each group.

3.2.3 Blood urea nitrogen (BUN)

Figures 3.11 and 3.12 show the effect of either GLP-1 (50 nmol/kg body weight) or exenatide (1 μ g/kg body weight) on BUN levels in normal and diabetic treated rats, respectively, compared to their untreated controls. The results show that treatment of either normal or diabetic rats with either GLP-1 or exenatide resulted in only a small but not significant reduction in BUN levels compared to their respective untreated controls. However, the inhibitory effect on BUN levels in GLP-1 treated diabetic rats was significant ($p < 0.05$) compared to untreated diabetic rats. The inhibitory effect of both GLP-1 and exenatide on BUN levels was more pronounced in the diabetic rats compared to normal.

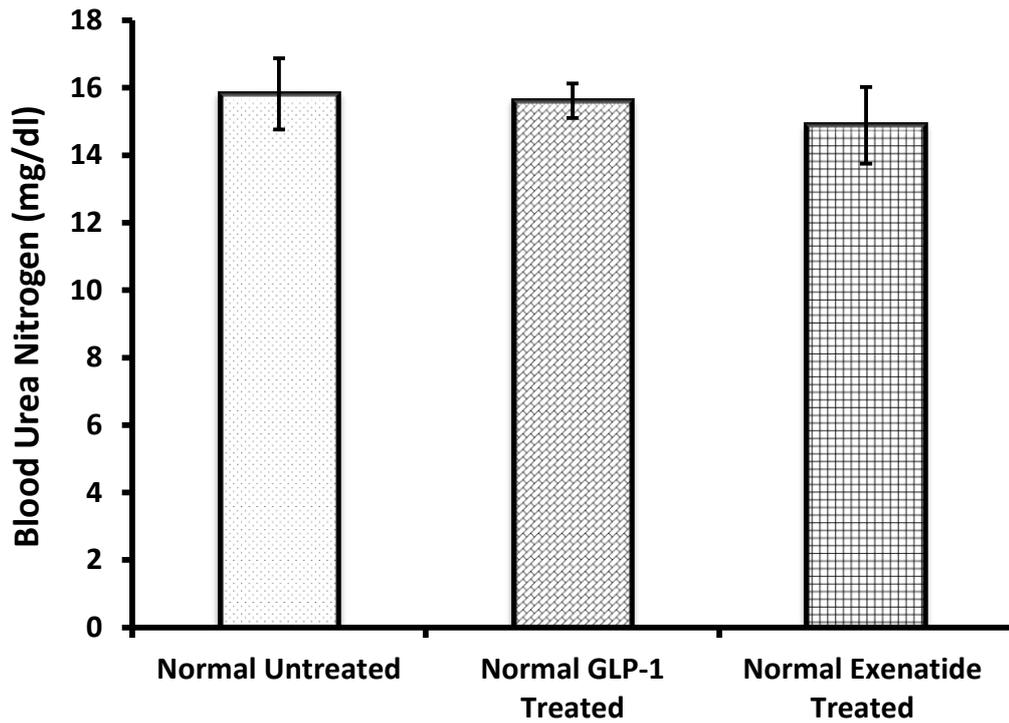


Figure 3.11: Histograms showing the effect of 10 weeks of treatment with either GLP-1 (50 nmol/kg body weight) or exenatide (1 μ g/kg body weight) on blood urea nitrogen levels of normal rats compared to normal untreated rats. Data are mean \pm SEM, n = 10 rats for each point.

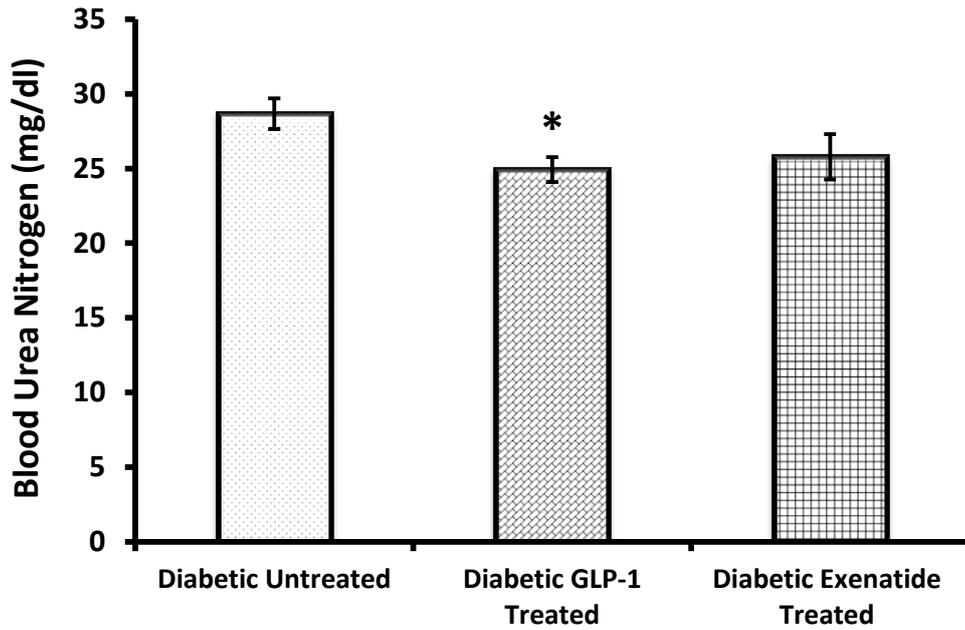


Figure 3.12: Histograms showing the effect of 10 weeks of treatment with either GLP-1 (50 nmol/kg body weight) or exenatide (1 μ g/kg body weight) on blood urea nitrogen levels of diabetic rats compared to diabetic untreated rats. Note that, the effect of GLP-1 was more prominent compared to exenatide. * $P < 0.05$ for compared to untreated diabetic rats. Data are mean \pm SEM, $n = 10$ rats for each point.

3.2.4 Serum creatinine

Figures 3.13 and 3.14 show the effect of either GLP-1 (50 nmol/kg body weight) or exenatide (1 μ g/kg body weight), on serum creatinine levels in normal and diabetic treated rats, respectively, compared to their respective controls. The results show that treatment of normal and diabetic rats with GLP-1 and exenatide resulted in small reductions in serum creatinine levels compared to their respective untreated controls. The reduction of serum creatinine levels was only significant ($p < 0.05$) in the case of normal and diabetic rats treated with exenatide compared to their respective untreated normal and diabetic controls.

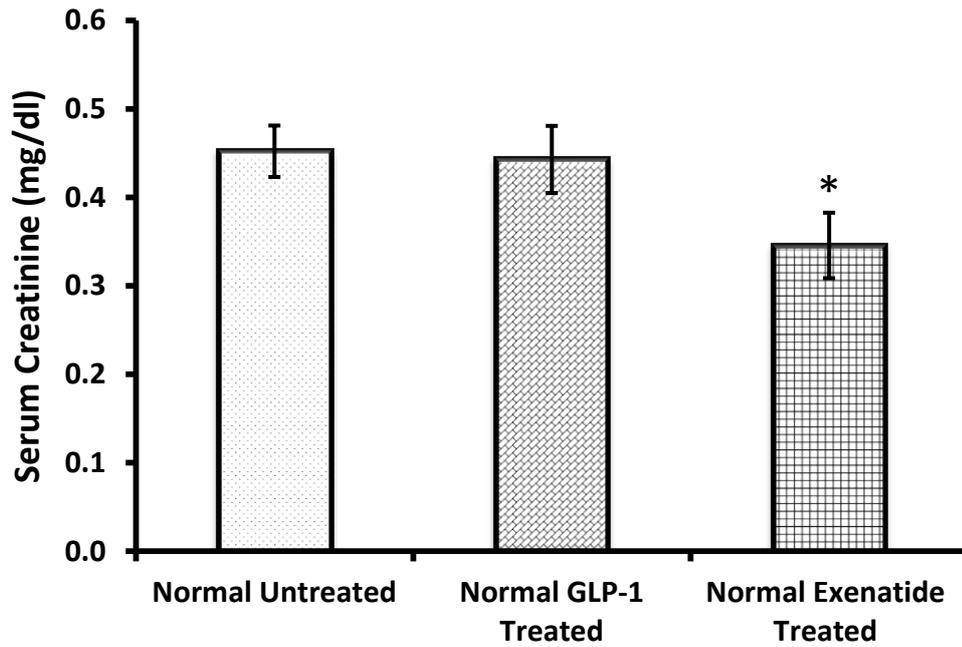


Figure 3.13: Histograms showing the effect of 10 weeks of treatment with either GLP-1 (50 nmol/kg body weight) or exenatide (1 μ g/kg body weight) on serum creatinine levels of normal rats compared to normal untreated rats. * $P < 0.05$ for exenatide treated normal rats compared to normal untreated rats. Data are mean \pm SEM, $n = 10$ rats for each point.

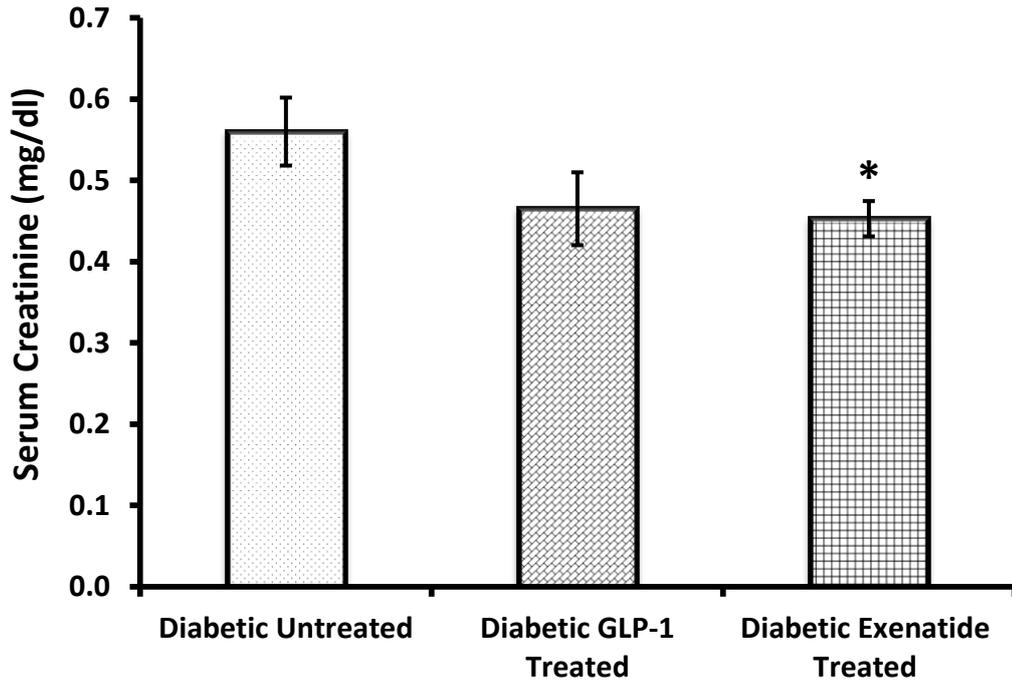


Figure 3.14: Histograms showing the effect of 10 weeks of treatment with either GLP-1 (50 nmol/kg body weight) or exenatide (1 μ g/kg body weight) on serum creatinine levels of diabetic rats compared to diabetic untreated rats. * $P < 0.05$ for exenatide treated diabetic rats compared to untreated diabetic rats. Data are mean \pm SEM, $n = 10$ rats for each point rats for each point.

3.2.5 Serum uric acid

Figures 3.15 and 3.16 show the effect of either GLP-1 (50 nmol/kg body weight) or exenatide (1 μ g/kg body weight), on serum uric acid levels in normal and diabetic treated rats, respectively, compared to the untreated control rats. The results show a small reduction in serum uric acid levels of GLP-1 treated normal rats compared to normal untreated rat. However, a significant reduction ($p < 0.05$) was obtained in exenatide treated normal rats compared to normal untreated rats. Similarly, treatment of diabetic rats with either GLP-1 or exenatide resulted in significant reduction ($p < 0.05$; $p < 0.01$) in serum uric acid levels, respectively, compared to untreated diabetic rats.

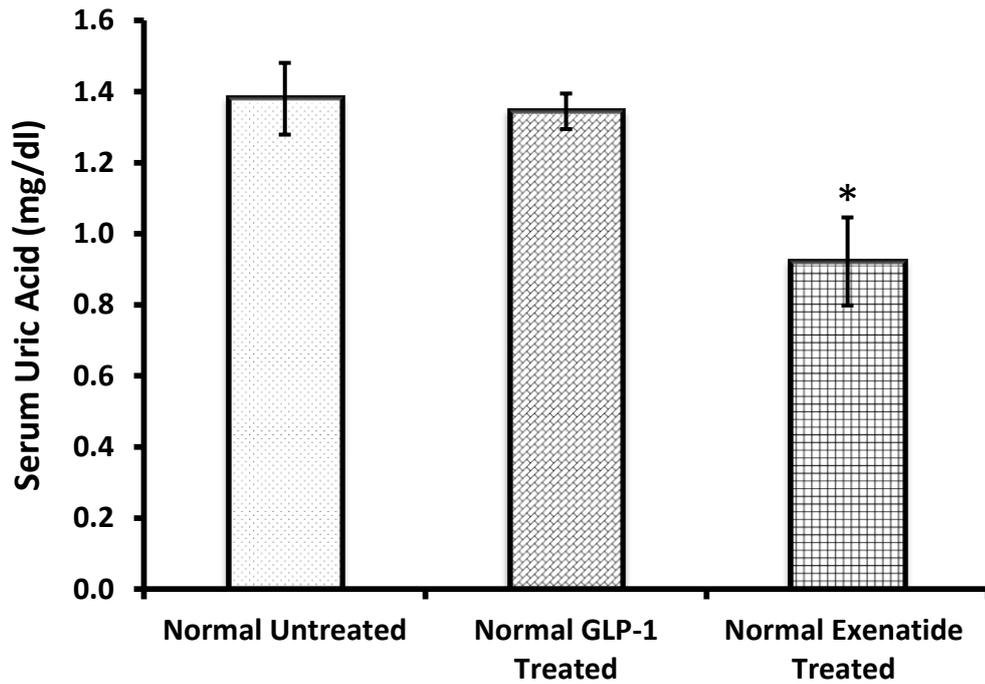


Figure 3.15: Histograms showing the effect of 10 weeks of treatment with either GLP-1 (50 nmol/kg body weight) or exenatide (1 μ g/kg body weight) on serum uric acid levels of normal rats compared to untreated normal rats. * $P < 0.05$ for exenatide treated normal rats compared to untreated normal rats. Data are mean \pm SEM, $n = 10$ rats for each point.

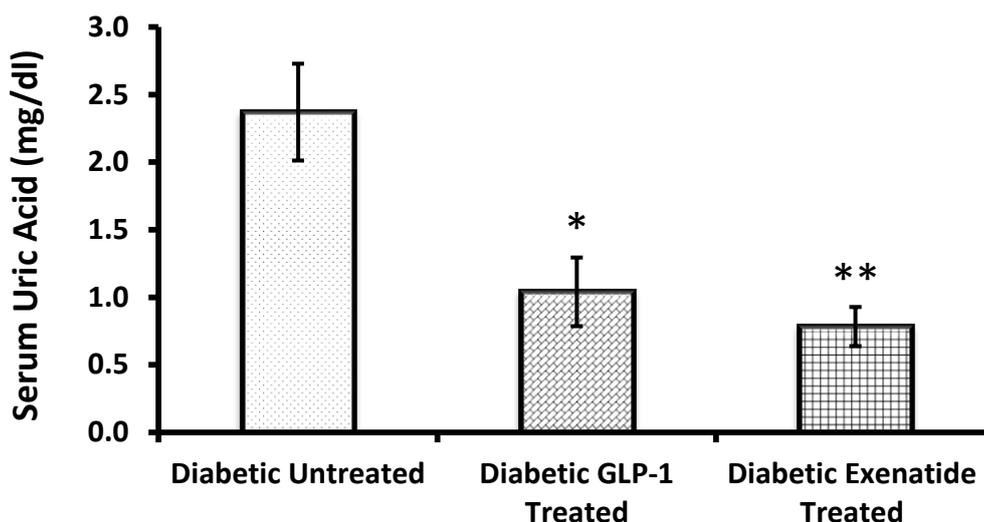


Figure 3.16: Histograms showing the effect of 10 weeks of treatment with either GLP-1 (50 nmol/kg body weight) or exenatide (1 μ g/kg body weight) on serum uric acid levels of diabetic rats compared to untreated diabetic rats. * $P < 0.05$ for GLP-1 treated diabetic and ** $P < 0.01$ for exenatide treated diabetic rats compared to untreated diabetic rats. Data are mean \pm SEM, $n = 10$ rats for each point.

3.2.6 Serum cholesterol

Figures 3.17 and 3.18 show the effect of either GLP-1 (50 nmol/kg body weight) or exenatide (1 μ g/kg body weight) on serum cholesterol levels in normal and diabetic treated rats, respectively, compared to their respective controls. The results showed no detectable effect of normal, GLP-1 treated rats on serum cholesterol levels compared to normal untreated rats. However, exenatide treated normal rats showed significant ($p < 0.05$) reduction in serum cholesterol levels compared to normal untreated rats. On the other hand, GLP-1 evoked a significant ($p < 0.05$) reduction in serum cholesterol levels diabetic rats compared to untreated diabetic rats. A small reduction in serum cholesterol levels was detected in exenatide-treated diabetic rats compared to untreated diabetic rats.

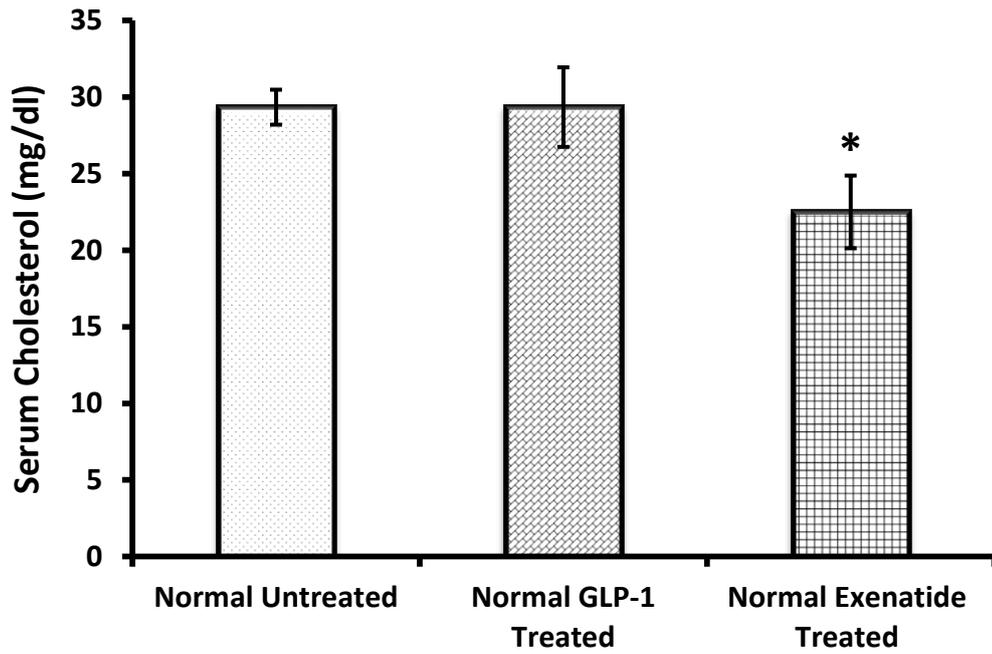


Figure 3.17: Histograms showing the effect of 10 weeks of treatment with either GLP-1 (50 nmol/kg body weight) or exenatide (1 μ g/kg body weight) on serum cholesterol levels of normal rats compared to normal untreated rats. * $P < 0.05$ for exenatide treated normal rats compared to normal untreated rats. Data are mean \pm SEM, $n = 10$ rats for each point.

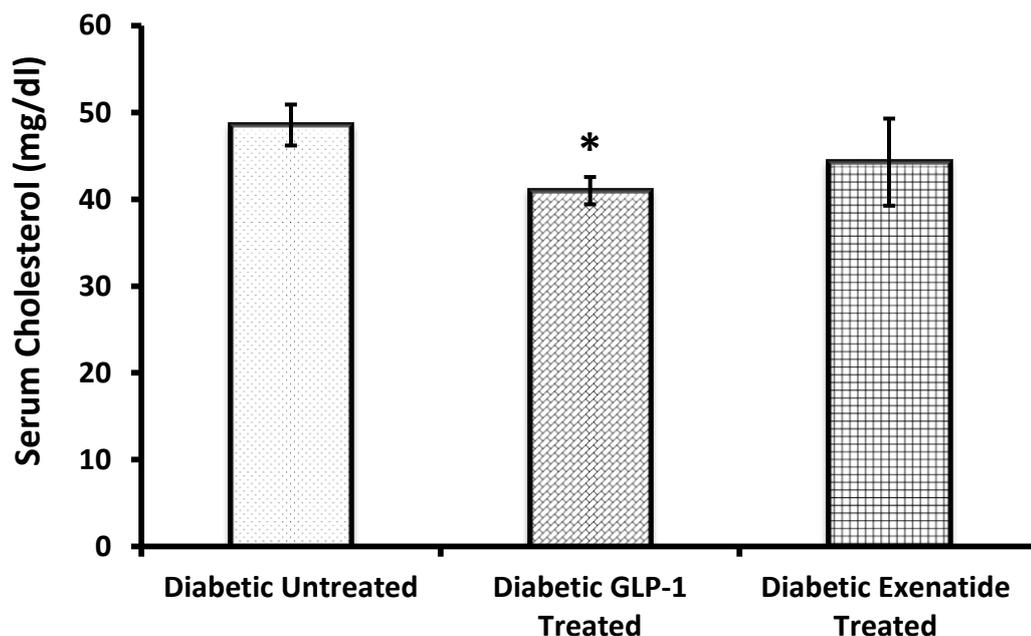


Figure 3.18: Histograms showing the effect of 10 weeks of treatment with either GLP-1 (50 nmol/kg body weight) or exenatide (1 μ g/kg body weight) on serum cholesterol levels of diabetic rats compared to diabetic untreated rats. * $P < 0.05$ for diabetic GLP-1 treated rats compared to diabetic untreated rats. Data are mean \pm SEM, $n = 10$ rats for each point.

3.2.7 Serum triglyceride

Figures 3.19 and 3.20 show the effect of either GLP-1 (50 nmol/kg body weight) or exenatide (1 μ g/kg body weight) on serum triglyceride levels in normal and diabetic treated rats, respectively, compared to their respective control. The results showed that GLP-1 reduced, but not significantly, serum triglyceride levels in GLP-1-treated normal rats compared to normal untreated rats. However, exenatide evoked more and significant ($p < 0.001$) decrease in serum triglyceride levels compared to normal untreated rats. The results also showed that treatment of diabetic rats with either GLP-1 or exenatide resulted in marked and significant reductions in serum triglyceride levels ($p < 0.001$ and $p < 0.01$), respectively, compared to diabetic untreated rats.

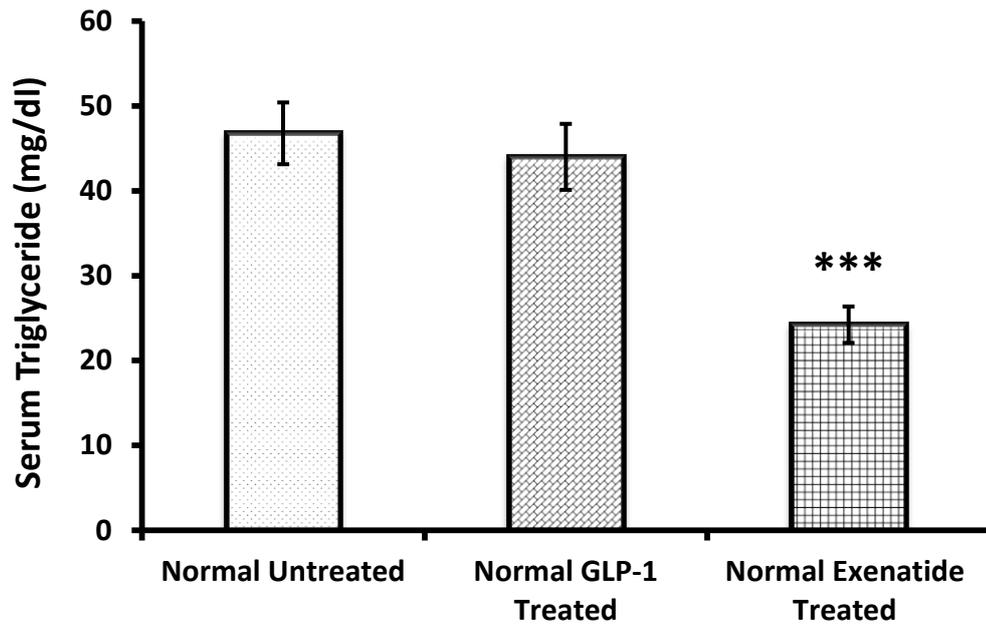


Figure 3.19: Histograms showing the effect of 10 weeks of treatment with either GLP-1 (50 nmol/kg body weight) or exenatide (1 μ g/kg body weight) on serum triglyceride levels of normal rats compared to normal untreated rats. *** $P < 0.001$ for exenatide treated normal rats compared to normal untreated rats. Data are mean \pm SEM, $n = 10$ rats for each point. Note that exenatide but not GLP-1 has evoked significant decrease in serum triglyceride levels compared to normal untreated rats.

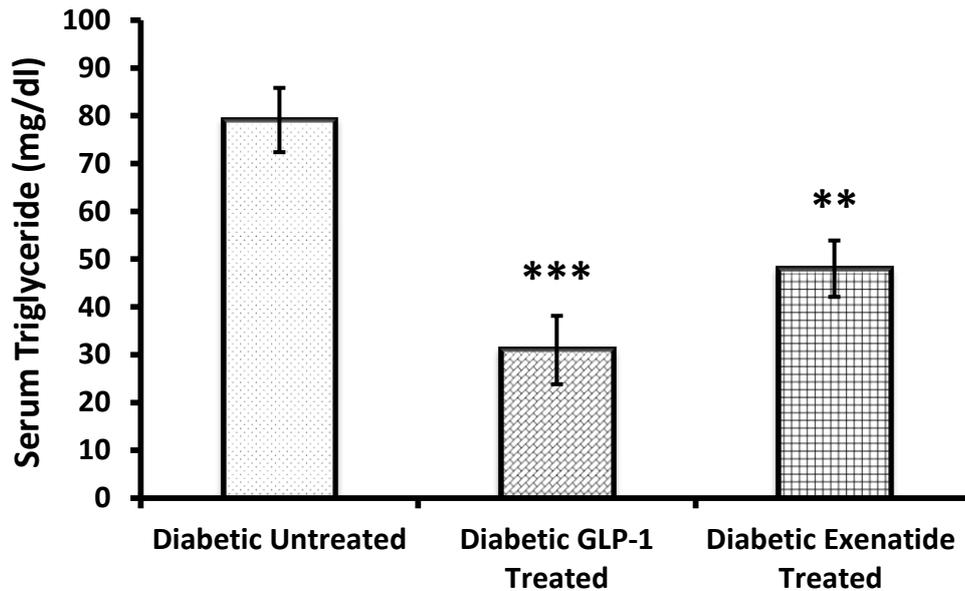


Figure 3.20: Histograms showing the effect of 10 weeks of treatment with either GLP-1 (50 nmol/kg body weight) or exenatide (1 μ g/kg body weight) on serum triglyceride levels of diabetic rats compared to untreated diabetic rats. *** $P < 0.001$ for GLP-1 and ** $P < 0.01$ for exenatide in diabetic treated rats compared to diabetic untreated rats. Data are mean \pm SEM, $n = 10$ rats for each point.

3.3 Effect of either GLP-1 or exenatide treatment on serum insulin secretion from diabetic and normal rats

Figures 3.21 and 3.22 show the effect of either GLP-1 (50 nmol/kg body weight) or exenatide (1 μ g/kg body weight) on serum insulin levels in normal and diabetic treated rats, respectively, compared to their respective controls. The results show that treatment of both normal and diabetic rats with either GLP-1 or exenatide resulted in significant increases in serum insulin levels compared to their respective untreated controls. Significant ($p < 0.05$) increase in serum insulin level was detected in normal rats treated with either GLP-1 or exenatide compared to normal untreated rats. Similarly, a moderately significant ($p < 0.01$) increase in serum insulin level was obtained in diabetic rats treated with either GLP-1 or exenatide compared to untreated diabetic rats.

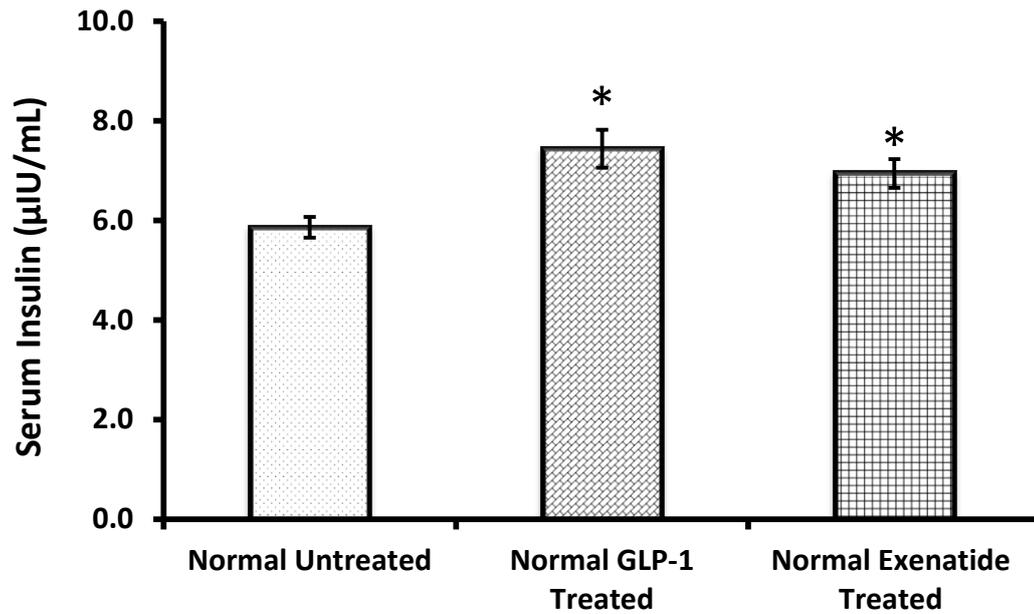


Figure 3.21: Histograms showing the effect of 10 weeks of treatment with either GLP-1 (50 nmol/kg body weight) or exenatide (1 µg/kg body weight) on serum insulin levels of normal rats compared to untreated normal rats. * $P < 0.05$ for either GLP-1 or exenatide normal treated rats compared to normal untreated rats. Data are mean \pm SEM, $n = 10$ rats for each point.

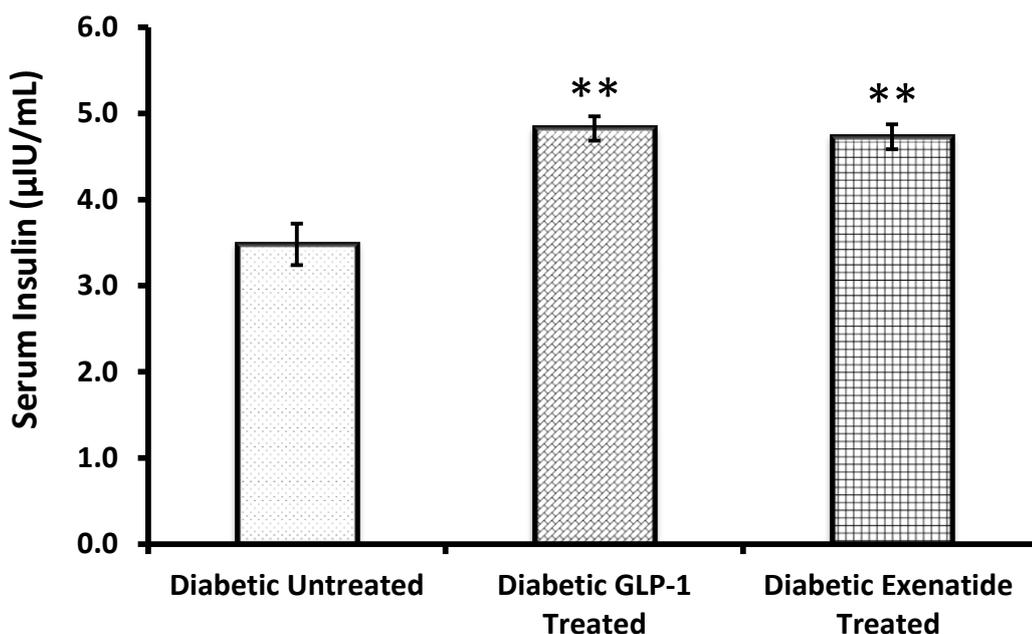


Figure 3.22: Histograms showing the effect of 10 weeks of treatment with either GLP-1 (50 nmol/kg body weight) or exenatide (1 µg/kg body weight) on serum insulin levels of diabetic rats compared to untreated diabetic rats. ** P < 0.01 for either GLP-1 or exenatide treated diabetic rats compared to untreated diabetic rats. Data are mean ± SEM, n = 10 rats for each point.

3.4 *In vitro* study of either GLP-1 or exenatide effect on insulin secretion from pancreatic tissue fragments

3.4.1 *Effect of GLP-1 on insulin secretion from normal pancreatic fragments*

The *in vitro* study shows that the basal level of insulin secretion (release) was (1.0 ± 0.04) µIU/ml/100 mg tissue from normal pancreatic fragments compared to (0.6 ± 0.03) µIU/ml/100 mg tissue from diabetic pancreatic fragments. These results reveal that the basal insulin release level estimated from normal rat pancreatic fragments is two-fold of that retrieved from pancreatic fragments of diabetic rats. Figure 3.23 shows insulin release after incubation of normal pancreatic tissue fragments with different concentrations (10⁻¹², 10⁻⁹ or 10⁻⁶ M) of GLP-1. The results show that 10⁻¹² or 10⁻⁹ M of

GLP-1 can evoke a significant ($p < 0.01$) and dose dependent increase in insulin secretion from normal pancreatic fragments compared to the basal. The maximal significant elevation ($p < 0.001$) of insulin secretion from normal pancreatic fragments was obtained with 10^{-6} M of GLP-1 compared to basal insulin secretion.

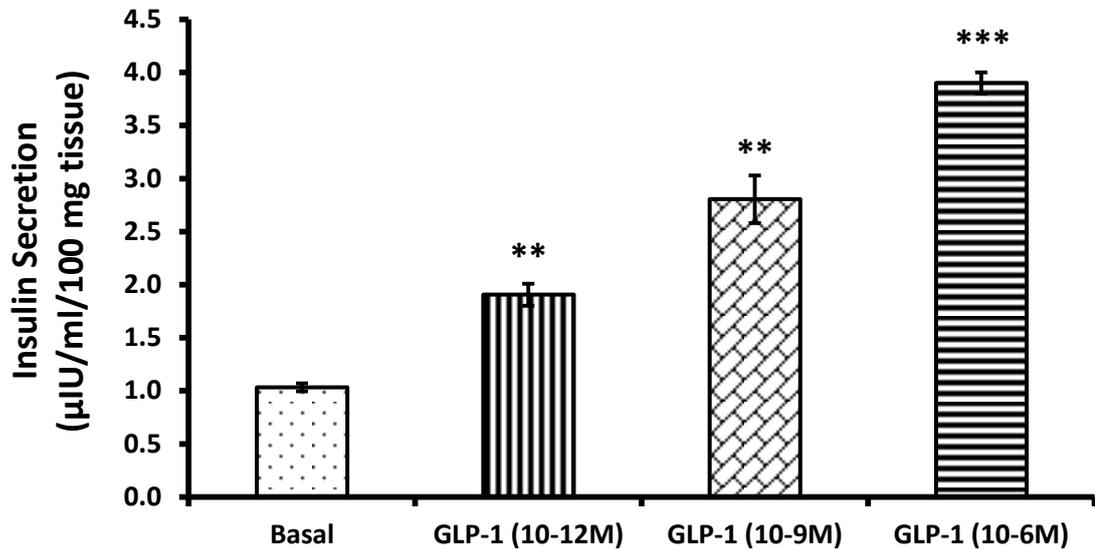


Figure 3.23: Histograms showing in vitro insulin secretion levels from pancreatic tissues of normal rats incubated with different concentrations (10^{-12} , 10^{-9} or 10^{-6} M) of GLP-1. ** $P < 0.01$ and *** $P < 0.001$ showing significant increase in insulin secretion levels at concentrations (10^{-12} , 10^{-9} and 10^{-6} M) of GLP-1 compared to basal insulin secretion. Data are mean \pm SEM, $n = 10$ rats for each point.

3.4.2 Effect of GLP-1 on insulin secretion from pancreatic fragments of diabetic rats

Figure 3.24 shows insulin release after incubation of pancreatic tissue fragments of diabetic rats with different concentrations (10^{-12} , 10^{-9} or 10^{-6} M) of GLP-1. The results show that 10^{-12} M of GLP-1 can evoke a significant ($p < 0.05$) increase in the secretion of insulin from diabetic rat tissue compared basal. GLP-1 at concentrations of 10^{-9} and 10^{-6} M evoked highly significant ($P < 0.001$) increases in insulin release compared to basal. These effects were dose-dependent with maximal secretion occurring with 10^{-6} M of GLP-1.

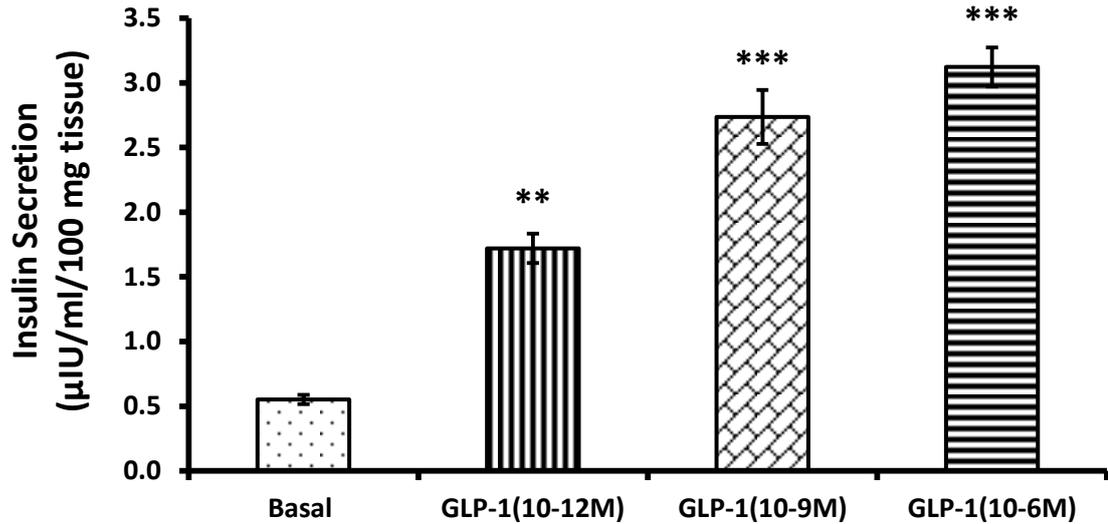


Figure 3.24: Histograms showing in vitro insulin secretion levels from pancreatic tissues of diabetic rat incubated with different concentrations (10^{-12} , 10^{-9} or 10^{-6} M) of GLP-1 compared to the basal. ** $P < 0.01$ and *** $P < 0.001$ showing significant increase in insulin secretion at 10^{-12} , 10^{-9} and 10^{-6} M of GLP-1 compared to basal insulin secretion. Data are mean \pm SEM, $n = 10$ rats for each point.

3.4.3 Effect of exenatide on insulin secretion from pancreatic tissue fragments of normal rats untreated tissue

Figure 3.25 shows insulin release after incubation of pancreatic tissue fragments of normal rats with different concentrations (10^{-12} , 10^{-9} or 10^{-6} M) of exenatide. The result is compared to that of basal secretion. The results show that exenatide can evoke a markedly significant ($p < 0.001$) increase in insulin secretion from pancreatic fragments of normal rats at all concentrations (10^{-12} , 10^{-9} and 10^{-6} M) of exenatide compared to basal. The maximal effect was obtained with 10^{-6} M of exenatide.

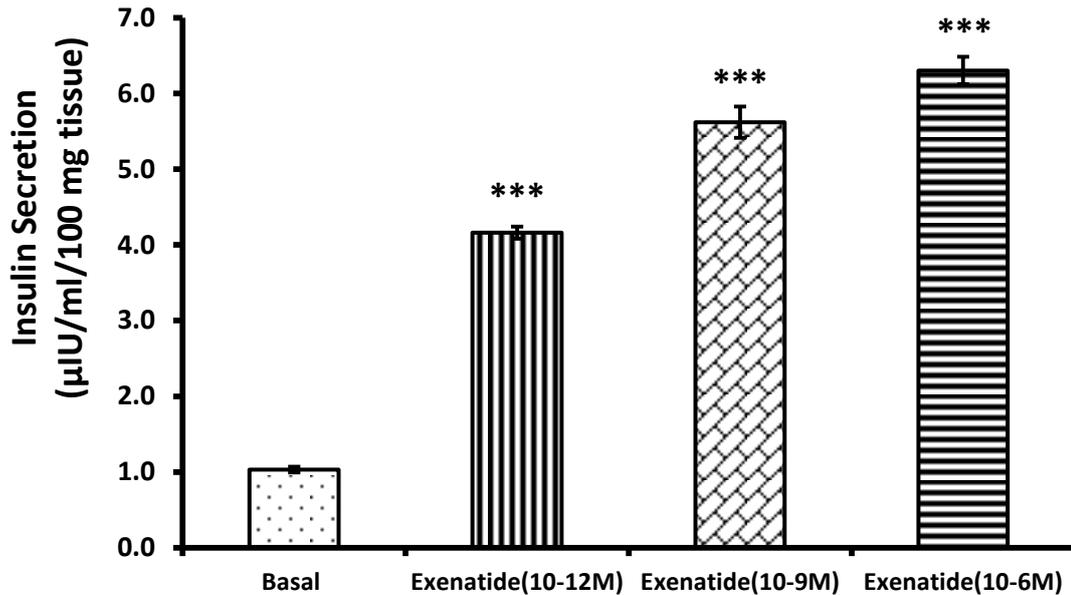


Figure 3.25: Histograms showing in vitro insulin secretion levels from pancreatic tissues of normal rats incubated with different concentrations (10^{-12} , 10^{-9} or 10^{-6} M) of exenatide. *** $P < 0.001$ showing significant increase in insulin secretion levels at all concentrations (10^{-12} , 10^{-9} and 10^{-6} M) of exenatide compared to basal insulin secretion. Data are mean \pm SEM, $n = 10$ rats for each point.

3.4.4 Effect of exenatide on insulin secretion from pancreatic fragments of diabetic rats

Figure 3.26 shows insulin release after incubation of pancreatic tissue fragments of diabetic rats with different concentrations (10^{-12} , 10^{-9} or 10^{-6} M) of exenatide. The result is compared to that of basal secretion. The results show that exenatide can evoke markedly significant increase ($p < 0.001$) in insulin secretion from pancreatic fragments of diabetic rats at all concentrations (10^{-12} , 10^{-9} and 10^{-6} M) of exenatide compared to basal insulin secretion. The maximal effect was obtained with 10^{-6} M of exenatide. Data are mean \pm SEM, $n = 10$ rats for each point.

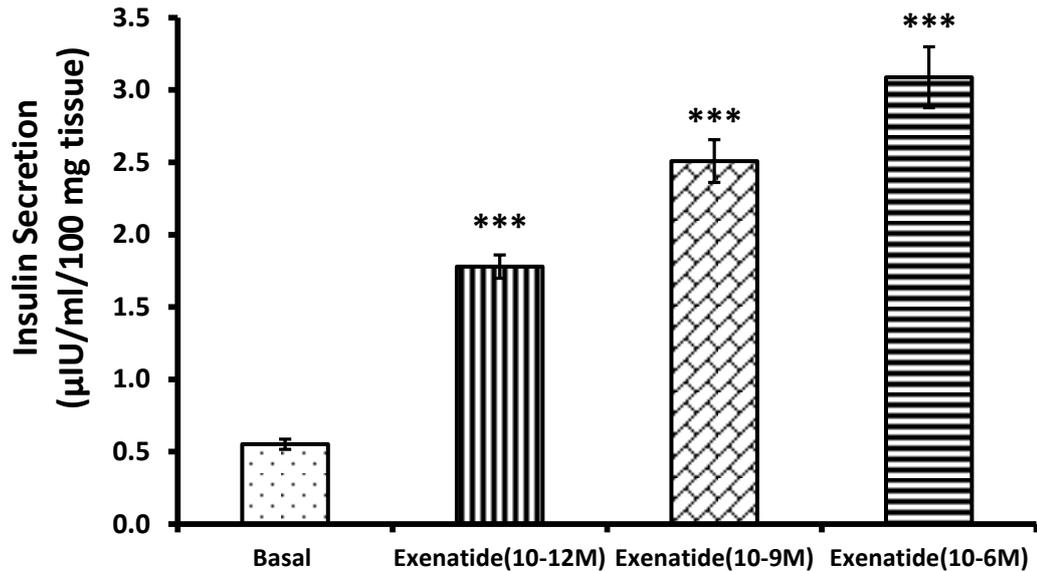


Figure 3.26: Histograms showing in vitro insulin secretion levels from pancreatic tissues of diabetic rats incubated with different concentrations (10^{-12} , 10^{-9} or 10^{-6} M) of exenatide compared to the basal. *** $P < 0.001$ showing significant increase in insulin secretion at all concentrations (10^{-12} , 10^{-9} and 10^{-6} M) of exenatide compared to basal insulin secretion. Data are mean \pm SEM, $n = 10$ rats for each point.

3.5 Effect of GLP-1 and exenatide treatment on the distribution of insulin, glucagon, GLP-1, exenatide, catalase and glutathione reductase immunoreactive cells in the pancreas of normal and diabetic rats

3.5.1 Insulin immunoreactive cells in the pancreas of normal and diabetic rats

Figures 3.27, 3.28, 3.29 and 3.30 show the effect of either GLP-1 (50 nmol/kg body weight) or exenatide (1 µg/kg body weight) on the pattern of distribution of insulin immunoreactive cells in normal and diabetic rats. Figures 3.27 and 3.28 show the percentages of insulin-positive cells in normal and diabetic rats, respectively, following treatment with either GLP-1 or exenatide. Similarly, figures 3.29 and 3.30 show

micrographs of insulin immunoreactive cells of normal and diabetic rats following treatment with either GLP-1 or exenatide, respectively. The results show that the insulin-positive cells in the pancreatic islets of normal rats are distributed mainly in the region position compared to the peripheral portions. In contrast, the distribution of insulin-positive cells in diabetic rats were much fewer compared to the islets of normal pancreas. Treatments of normal and diabetic rats with either GLP-1 or exenatide can result in small significant ($p < 0.05$) increases in the percentage of insulin-positive cells in normal treated rats. Similarly, diabetic rats treated with either GLP-1 or exenatide show marked significant increases in insulin-positive cells ($p < 0.001$) and ($p < 0.01$), respectively, compared to diabetic untreated rats. GLP-1 has (3-fold) higher effect than exenatide on increasing the percentage of insulin immunopositive cells in diabetic rats. The results also show that islets from normal treated rats contain more insulin immunopositive cells compared to both treated and untreated diabetic rats.

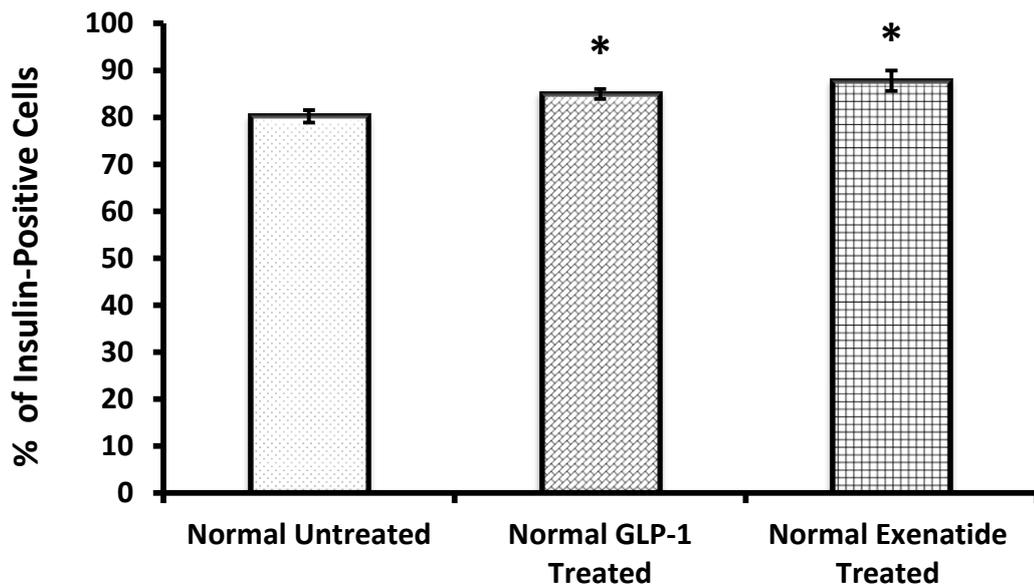


Figure 3.27: Histograms showing the effect of 10 weeks of treatment with either GLP-1 (50 nmol/kg body weight) or exenatide (1 μ g/kg body weight) on the percentage of insulin-positive cells in pancreatic islets of normal rats. The data show significant increases * $P < 0.05$ in both GLP-1 and exenatide treated rats compared to normal untreated rats. Data are mean \pm SEM, $n = 10$ islet/10 rats for each point.

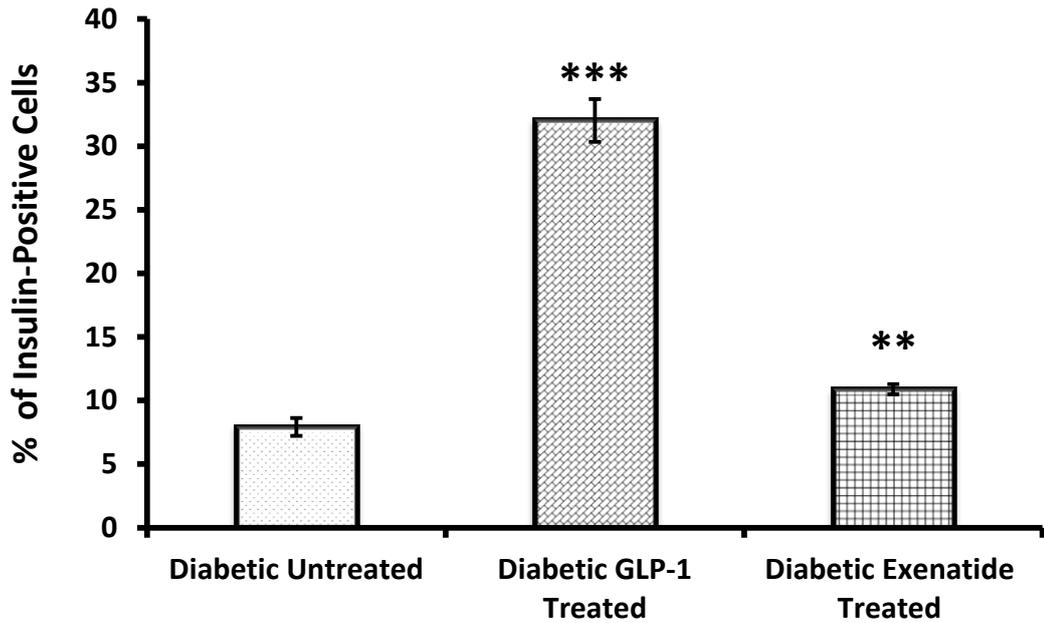


Figure 3.28: Histograms showing the effect of 10 weeks of treatment with either GLP-1 (50 nmol/kg body weight) or exenatide (1 μ g/kg body weight) on the percentage distribution of insulin-positive cells in pancreatic islets of diabetic rats. The data show significant increases. *** $P < 0.001$ and ** $P < 0.01$ in both GLP-1 and exenatide treated diabetic rats, respectively compared to untreated diabetic rats. GLP-1 was about 3-fold more effective than exenatide in increasing the number of insulin immunopositive cells in the treated diabetic rat pancreas. Data are mean \pm SEM, $n = 10$ islets/10 rats for each point.

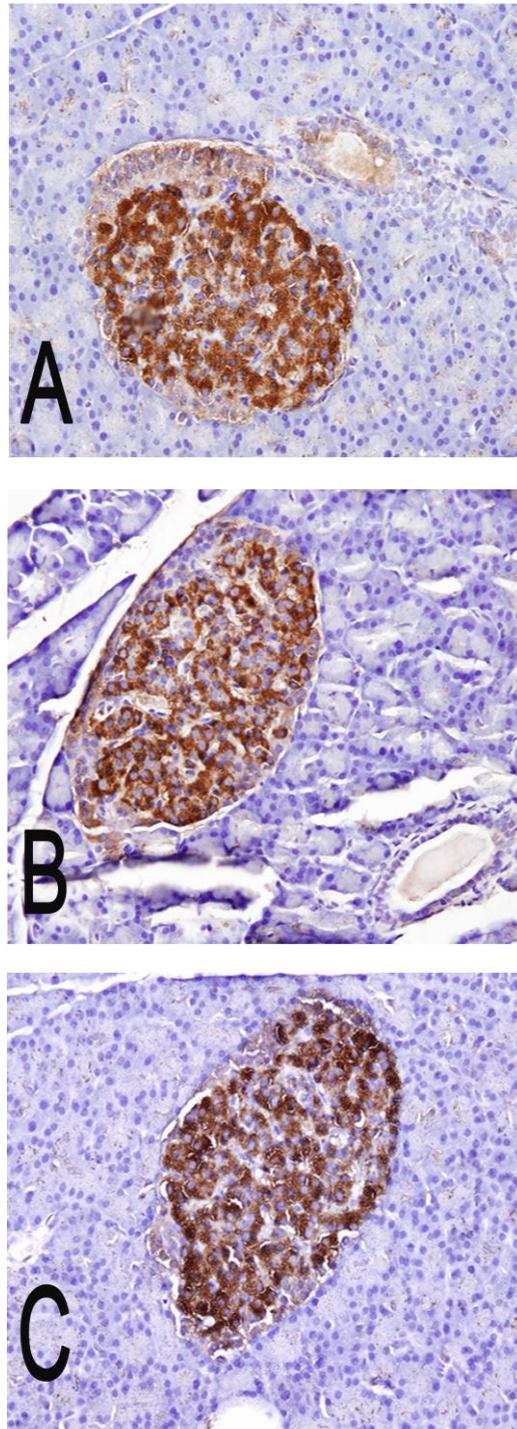


Figure 3.29: Micrographs showing the distribution of insulin-positive cells in the pancreatic islet of normal rats. The micrographs are typical of similar micrographs taken from 10 different rats in each group. (A) Insulin-positive cells in normal untreated rat pancreas, (B) insulin-positive cells in normal GLP-1-treated rats and (C) insulin-positive cells in normal exenatide-treated rats. Magnification: X 400.

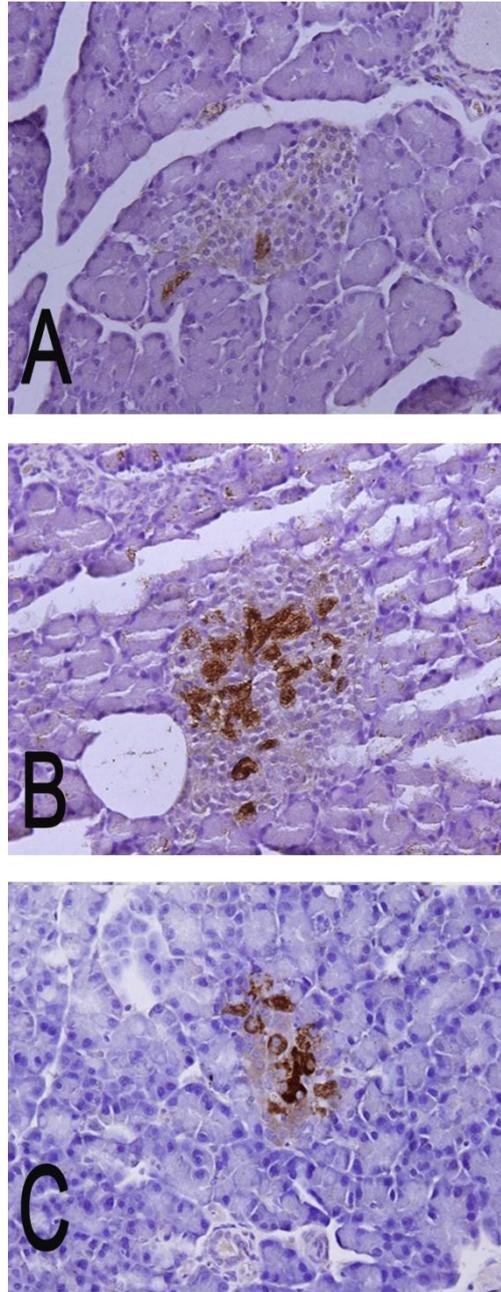


Figure 3.30: Micrographs showing the distribution of insulin-positive cells in the pancreatic islet of diabetic rats. The micrographs are typical of similar micrographs taken from 10 different rats in each group. (A) Insulin-positive cells in untreated diabetic rat pancreas, (B) insulin-positive cells in GLP-1-treated diabetic rats and (C) insulin-positive cells in exenatide-treated diabetic rats. Magnification: X 400. Note the decreased distribution of insulin-positive cells in the islets of diabetic rats compared to islets of normal control rats in both treated and untreated conditions (see Figure 3.33).

3.5.2 Glucagon immunoreactive cells in the pancreas of normal and diabetic rats

Figures 3.31, 3.32, 3.33 and 3.34 show the effect of either GLP-1 (50 nmol/kg body weight) or exenatide (1 µg/kg body weight), on the distribution of glucagon immunoreactive cells in islets of normal and diabetic rats. The percentages of glucagon-positive cells of treated and untreated normal and diabetic rats are shown in figures 3.31 and 3.32, respectively. In addition, figures 3.33 and 3.34 show the micrographs of normal and diabetic rats. The results showed that glucagon-positive cells were the second most commonly occurring cells in the normal endocrine pancreas and were located mainly in the peripheral part of the islet of Langerhans. Solitary glucagon-positive cells were found scattered throughout normal islets. In the diabetic pancreas, the number of glucagon immunoreactive cells increased with abnormal pattern of distribution when compared to the normal islets. Moreover, glucagon-positive cells were located in both the peripheral and the central regions of the diabetic rat islets. There was a marked significant increase in the number of glucagon-positive cells in the diabetic rat pancreas compared to normal rats. Both GLP-1 and exenatide-treated normal rats showed significant ($p < 0.05$; $p < 0.01$) reduction in the percentage of glucagon-positive cells, respectively, compared to untreated normal rats. In addition, GLP-1 and exenatide treated diabetic rats showed significant ($p < 0.001$) reduction in the percentage of glucagon-positive cells compared to untreated diabetic rats.

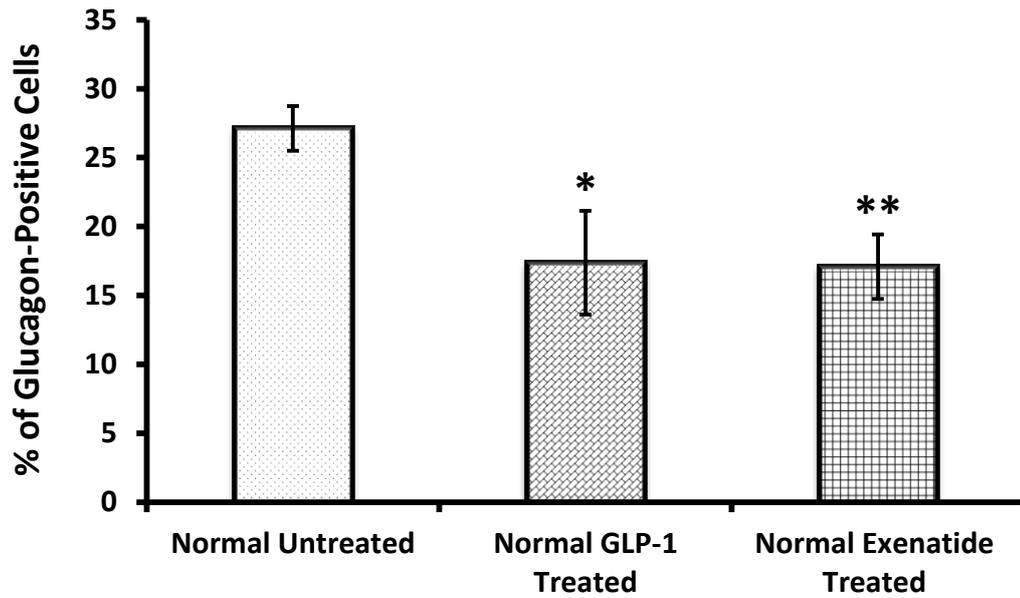


Figure 3.31: Histograms showing the effect of 10 weeks of treatment with either GLP-1 (50 nmol/kg body weight) or exenatide (1 μ g/kg body weight) on the percentage of glucagon-positive cells in pancreatic islets of normal rats. * $P < 0.05$ and ** $P < 0.01$ for treated rats compared to untreated rats. Data are mean \pm SEM, $n = 10$ islets/10 rats for each point.

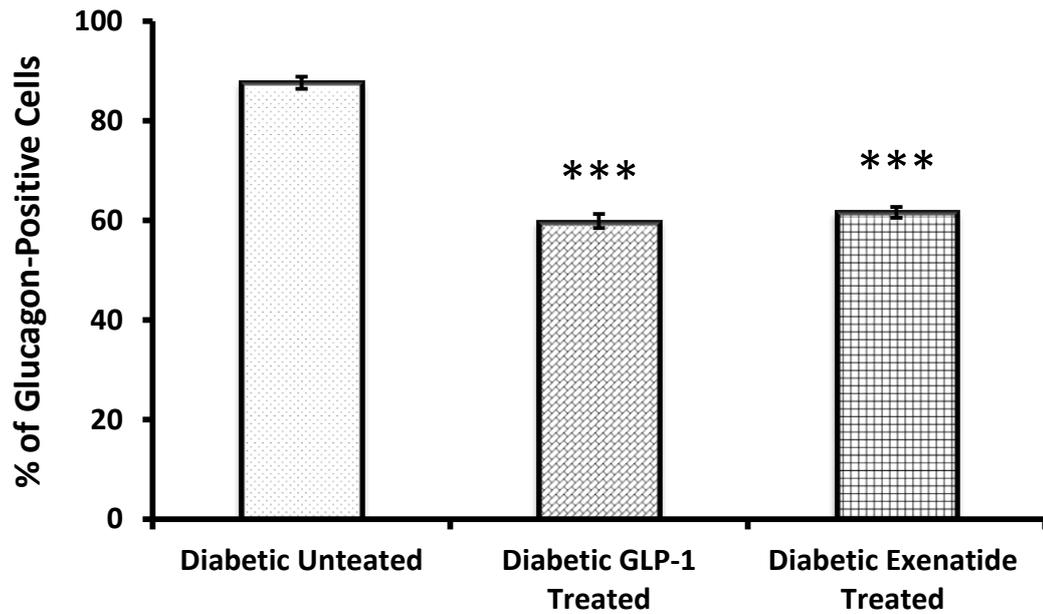


Figure 3.32: Histograms showing the effect of 10 weeks of treatment with either GLP-1 (50 nmol/kg body weight) or exenatide (1 μ g/kg body weight) on the percentage of glucagon-positive cells in pancreatic islets of diabetic rats. *** $P < 0.001$ for treated rats compared to untreated diabetic rats. Note the significant reductions in glucagon-positive cells in diabetic rats following either GLP-1 or exenatide treatment compared to untreated rats. Data are mean \pm SEM, $n = 10$ islets/10 rats for each point.

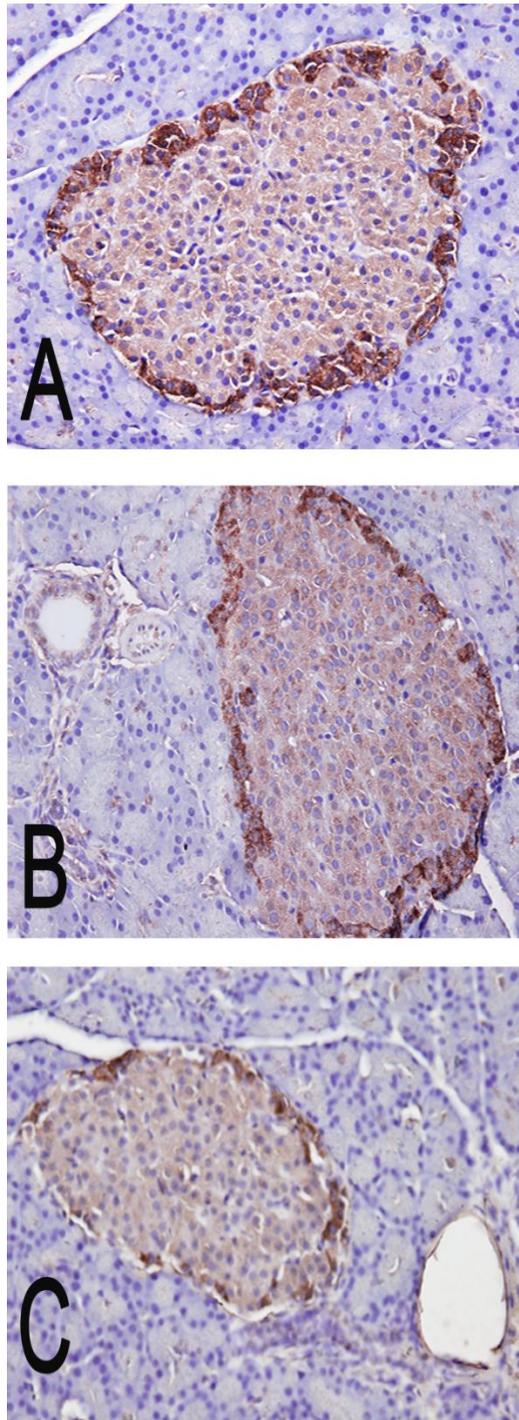


Figure 3.33: Micrographs showing the distribution of glucagon-positive cells in the pancreatic islet of normal rats. The micrographs are typical of similar micrographs taken from 10 different rats in each group. (A) Glucagon-positive cells in untreated normal rat pancreas, (B) glucagon-positive cells in normal GLP-1-treated rats and (C) glucagon-positive cells in normal exenatide-treated rats. Magnification: X 400.

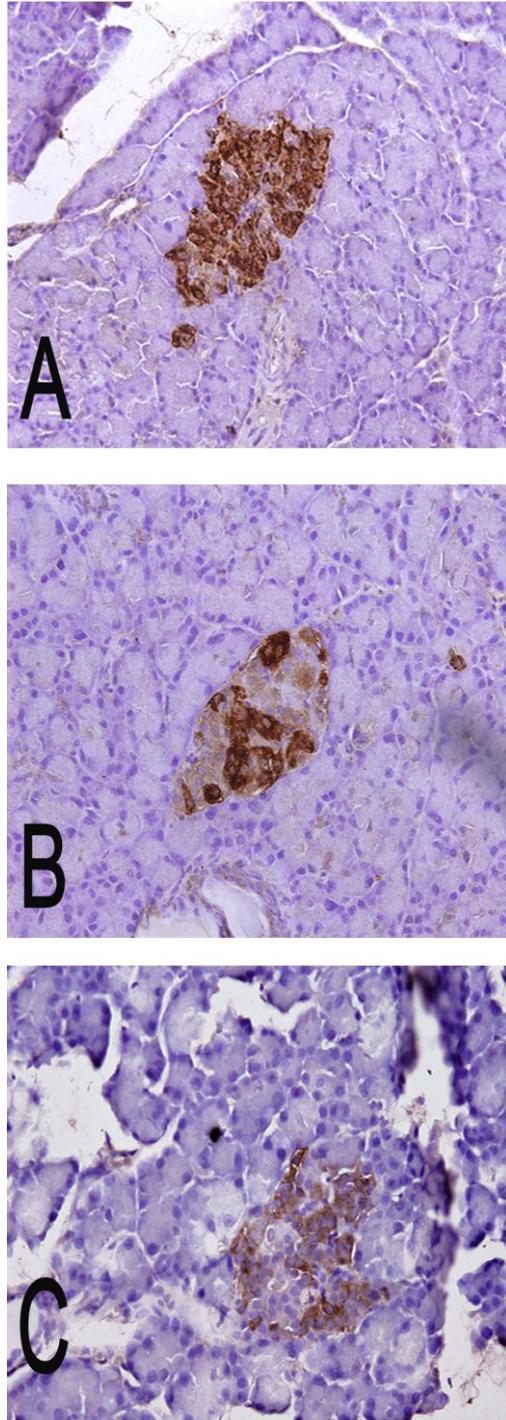


Figure 3.34: Micrographs showing the distribution of glucagon-positive cells in the pancreatic islet of diabetic rats. The micrographs are typical of similar micrographs taken from 10 different rats in each group. (A) Glucagon-positive cells in untreated diabetic rat pancreas, (B) glucagon-positive cells in GLP-1-treated diabetic rats and (C) glucagon-positive cells in exenatide-treated diabetic rats. Magnification: X 400.

3.5.3 GLP-1 immunoreactive cells in the pancreas of normal and diabetic rats

Figures 3.35, 3.36, 3.37 and 3.38 show the effect of either GLP-1 (50 nmol/kg body weight) or exenatide (1 µg/kg body weight), on percentage distribution of GLP-1 immunoreactive cells in normal and diabetic rats. The percentages of GLP-1-positive cells are shown in figures 3.35 and 3.36 while the micrographs are shown in figures 3.41 and 3.42 for treated and untreated normal and diabetic rats, respectively. The results presented in figures 3.37 and 3.38 show that treatment of normal and diabetic rats with either GLP-1 or exenatide caused significant ($P < 0.01$) increases in the percentage of GLP-1-positive cells in treated normal and diabetic rats compared to untreated rats. In relation to the morphology, the results show that GLP-1-positive cells are more prominent in the peripheral edges of the islets. The pancreatic islets of diabetic rats contain more GLP-1-positive cells than the pancreatic islets of normal rats (see figures 3.37 and 3.38).

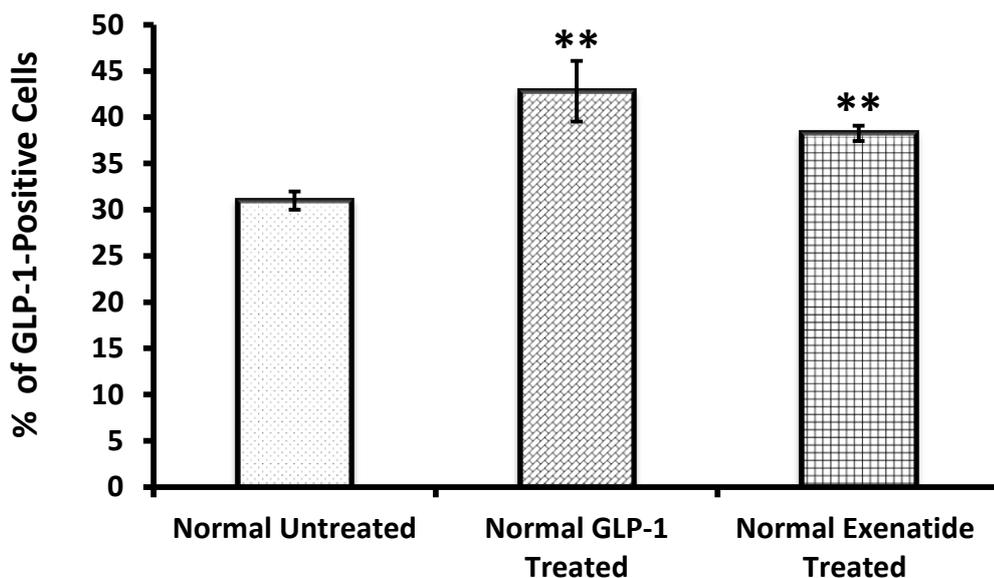


Figure 3.35: Histograms showing the effect of 10 weeks of treatment with either GLP-1 (50 nmol/kg body weight) or exenatide (1 µg/kg body weight) on the percentage of GLP-1-positive cells in pancreatic islets of normal rats. ** $P < 0.01$ for treated normal rats compared to normal untreated rats. Data are mean \pm SEM, $n = 10$ islets/10 rats for each point.

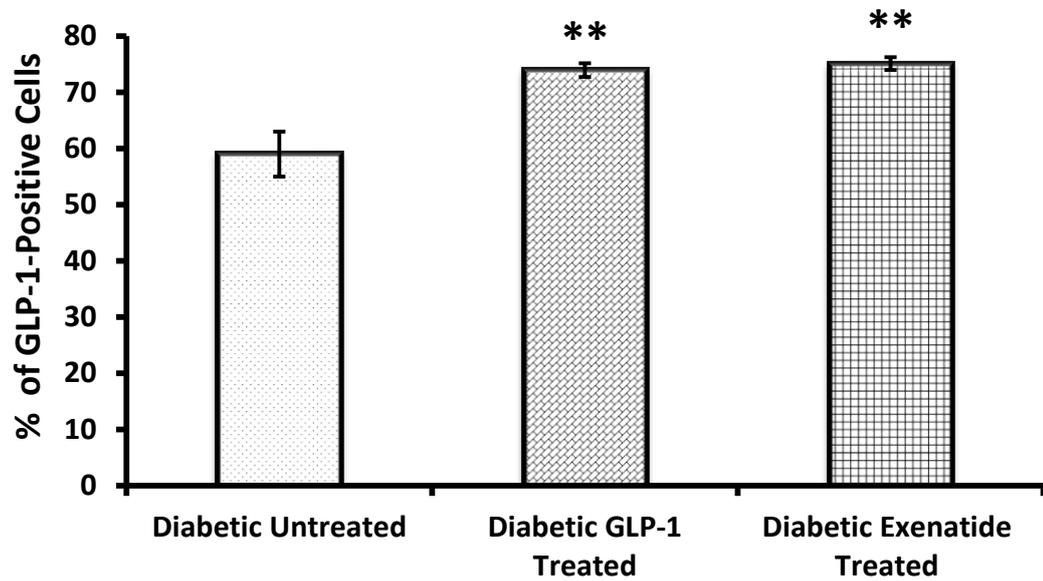


Figure 3.36: Histograms showing the effect of 10 weeks of treatment with either GLP-1 (50 nmol/kg body weight) or exenatide (1 μ g/kg body weight) on the percentage of GLP-1-positive cells in pancreatic islets of diabetic rats. ** $P < 0.01$ for treated diabetic rats compared to diabetic untreated rats. Data are mean \pm SEM, $n = 10$ islets/10 rats for each point.

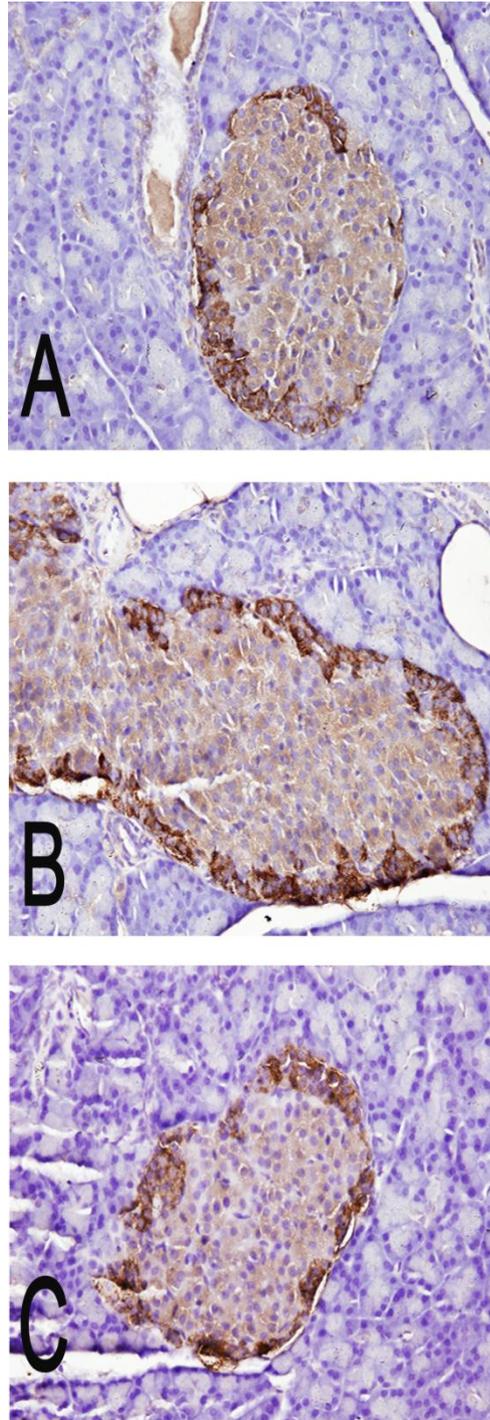


Figure 3.37: Micrographs showing the distribution of GLP-1-positive cells in the pancreatic islet of normal rats. The micrographs are typical of similar micrographs taken from 10 different rats in each group. (A) GLP-1-positive cells in normal untreated rat pancreas, (B) GLP-1-positive cells in normal GLP-1-treated rats and (C) GLP-1-positive cells in normal exenatide-treated rats. Magnification: X 400.

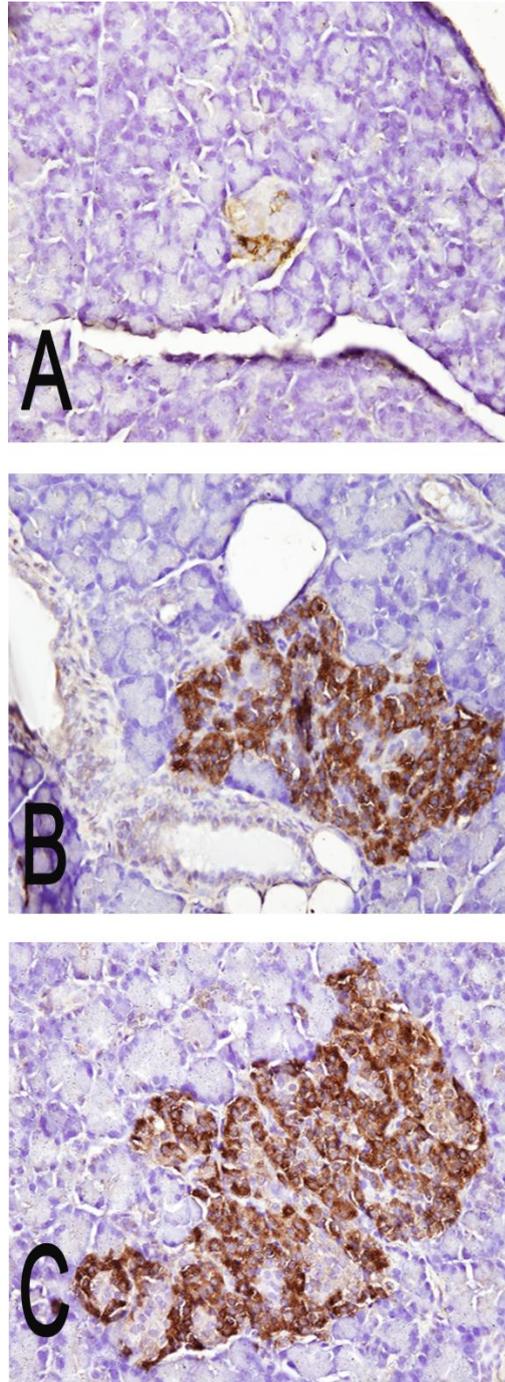


Figure 3.38: Micrographs showing the distribution of GLP-1-positive cells in the pancreatic islet of diabetic rats. The micrographs are typical of similar micrographs taken from 10 different rats in each group. (A) GLP-1-positive cells in diabetic untreated rat pancreas, (B) GLP-1-positive cells in diabetic GLP-1-treated rats and (C) GLP-1-positive cells in diabetic exenatide-treated rats. Magnification: X 400.

3.5.4 Exenatide immunoreactive cells in the pancreas of normal and diabetic rats

Figures 3.39, 3.40, 3.41 and 3.42 show the effect of either GLP-1 (50 nmol/kg body weight) or exenatide (1 µg/kg body weight), on the percentage distribution of exenatide-positive cells in normal and diabetic rats. The percentage changes in exenatide immunoreactive-positive cells are shown in figures 3.39 and 3.40 while the micrographs of exenatide immunoreactive cells for normal and diabetic rats, respectively, are shown in figures 3.41 and 3.42. The results show that in normal rats, neither GLP-1 nor exenatide can evoke any detectable changes in the percentage of exenatide-positive cells compared to untreated normal rats. However, in diabetic rats, either GLP-1 or exenatide produces significant ($p < 0.05$ and $p < 0.01$) increase, respectively, in the percentage of exenatide immunoreactive cells compared to untreated diabetic rats. The results of this study also show that islet from untreated and treated diabetic rats contain more exenatide immunoreactive cells compared to pancreatic islets from untreated normal rats. The results from the micrographs show that the exenatide immunoreactive cells were more pronounced in the peripheral parts of the pancreatic islets in normal treated rats. On the other hand, in the islets of diabetic rats, the exenatide immunoreactive cells were localized randomly in the islets of both untreated and treated diabetic rats.

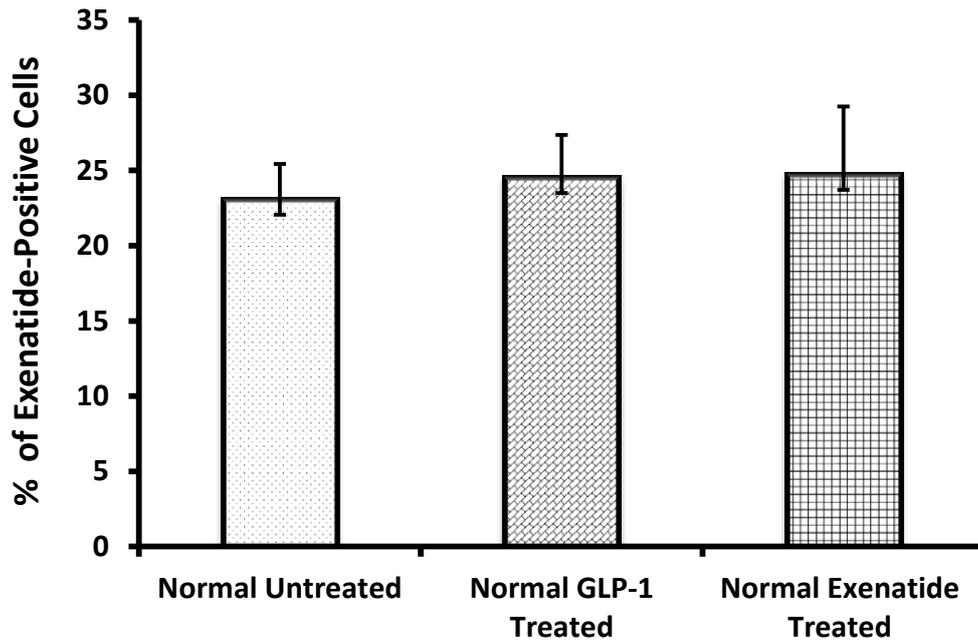


Figure 3.39: Histograms showing the effect of 10 weeks of treatment with either GLP-1 (50 nmol/kg body weight) or exenatide (1 μ g/kg body weight) on the percentage of exenatide immunoreactive cells in pancreatic islets of normal rats. Note that there was no significant change in the exenatide-positive cells when comparing treated with untreated normal rats. Data are mean \pm SEM, n = 10 islets/10 rats for each point.

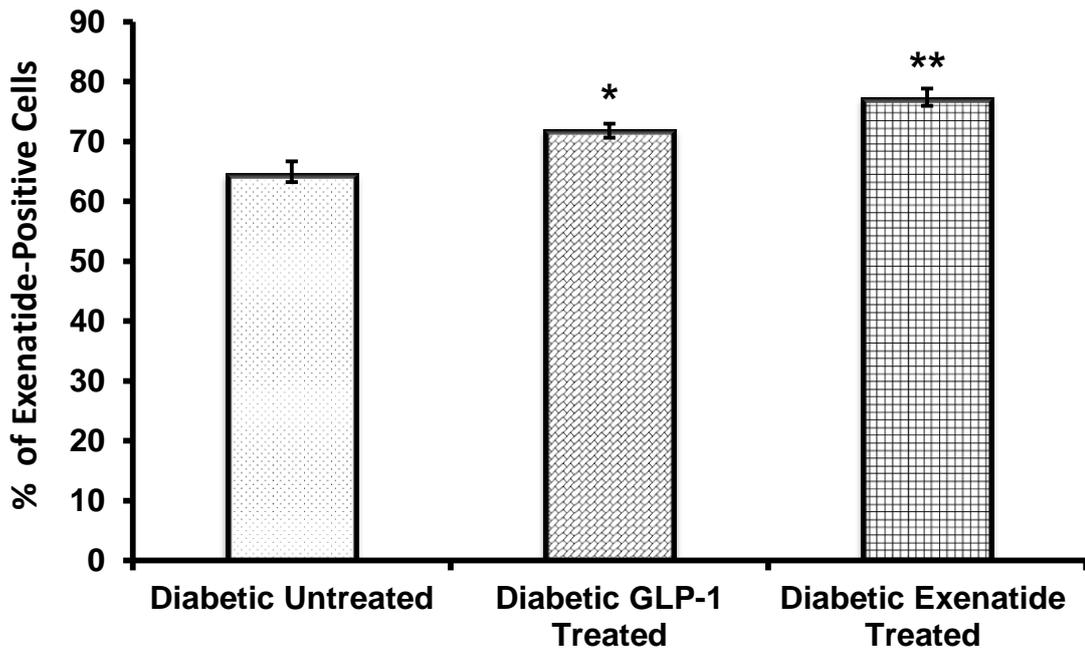


Figure 3.40: Histograms showing the effect of 10 weeks of treatment with either GLP-1 (50 nmol/kg body weight) or exenatide (1 µg/kg body weight) on the percentage of exenatide-positive cells in pancreatic islets of diabetic rats. * $P < 0.05$ and ** $P < 0.01$ for both GLP-1 and exenatide treated rats compared to untreated diabetic rats. Note that the islets of diabetic rats have more exenatide immunoreactive cells compared to the pancreatic islets of normal rats in both treated and untreated conditions. Data are mean \pm SEM, $n = 10$ islets/10 rats for each point.

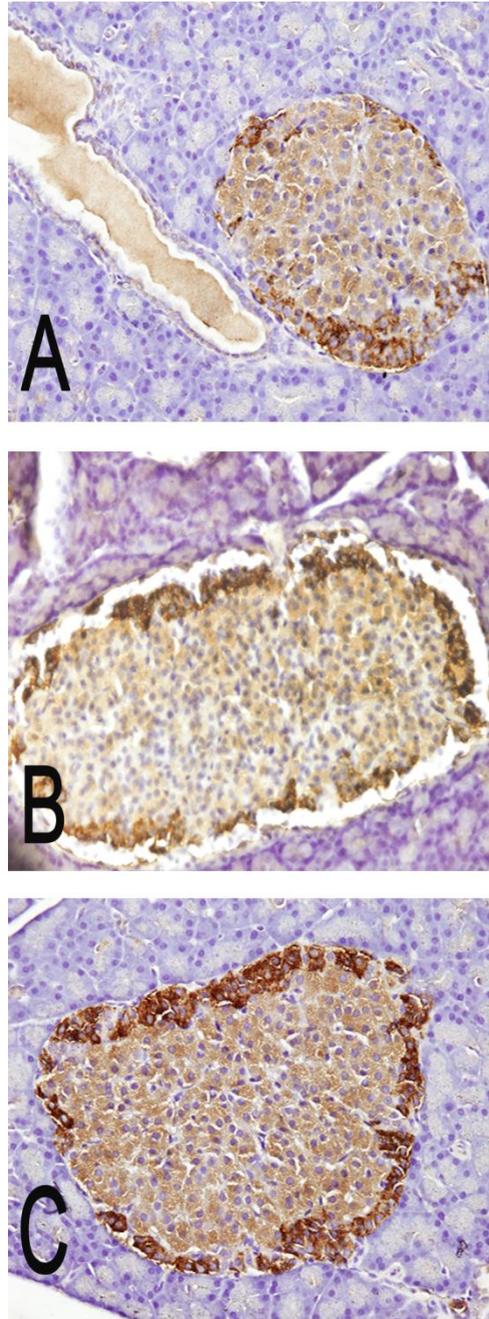


Figure 3.41: Micrographs showing the distribution of exenatide-positive cells in the pancreatic islet of normal rats. The micrographs are typical of different experiments taken from 10 rats in each group. (A) Exenatide-positive cells in untreated diabetic rat pancreas, (B) exenatide-positive cells in GLP-1-treated diabetic rats and (C) exenatide-positive cells in exenatide-treated diabetic rats. Magnification: X 400. Note that the exenatide immunoreactive cells are more pronounced in the peripheral parts of the islets.

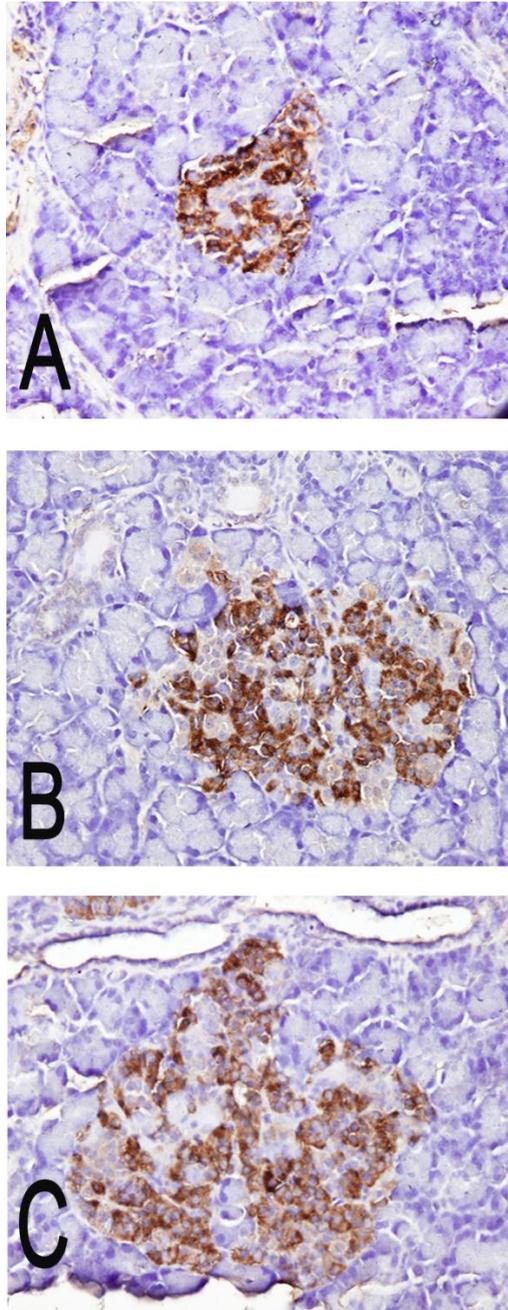


Figure 3.42: Micrographs showing the distribution of exenatide-positive cells in the pancreatic islet of diabetic rats. Micrographs are typical of 10 such different experiments taken from 10 rats. (A) Exenatide-positive cells in untreated diabetic rat pancreas, (B) exenatide-positive cells in GLP-1-treated diabetic rats and (C) exenatide-positive cells in exenatide-treated diabetic rats. Magnification: X 400. Note that the exenatide immunoreactive cells are localised all over the pancreatic islets of the diabetic rats.

3.5.5 Catalase immunoreactive cells in the pancreas of normal and diabetic rats

Figures 3.43, 3.44, 3.45 and 3.46 show the effect of either GLP-1 (50 nmol/kg body weight) or exenatide (1 µg/kg body weight), on catalase immunoreactive cells in normal and diabetic rats. The percentage of immunoreactive catalase-positive cells are shown in figures 3.43 and 3.44 while the micrographs of immunoreactive catalase-positive cells are shown in figures 3.45 and 3.46 for normal and diabetic rats. The results for normal rats show that neither GLP-1 nor exenatide had any significant effect on the number of catalase-positive cells. However, diabetic rats treated with either GLP-1 or exenatide showed significant ($p < 0.001$) increases in the percentage of catalase-positive cells compared to untreated diabetic rats. The results presented in figures 3.43 and 3.44 show that the islets of normal rats contain more immunoreactive catalase-positive cells compared to the islet of diabetic rats in both treated and untreated conditions. Moreover, in the islets of treated and untreated normal rats, the number of immunoreactive catalase-positive cells was more prominent at the periphery of the islets. In contrast, in the islets of the diabetic pancreas, the immunoreactive catalase-positive cells were distributed randomly in the islets of both untreated and treated diabetic rats.

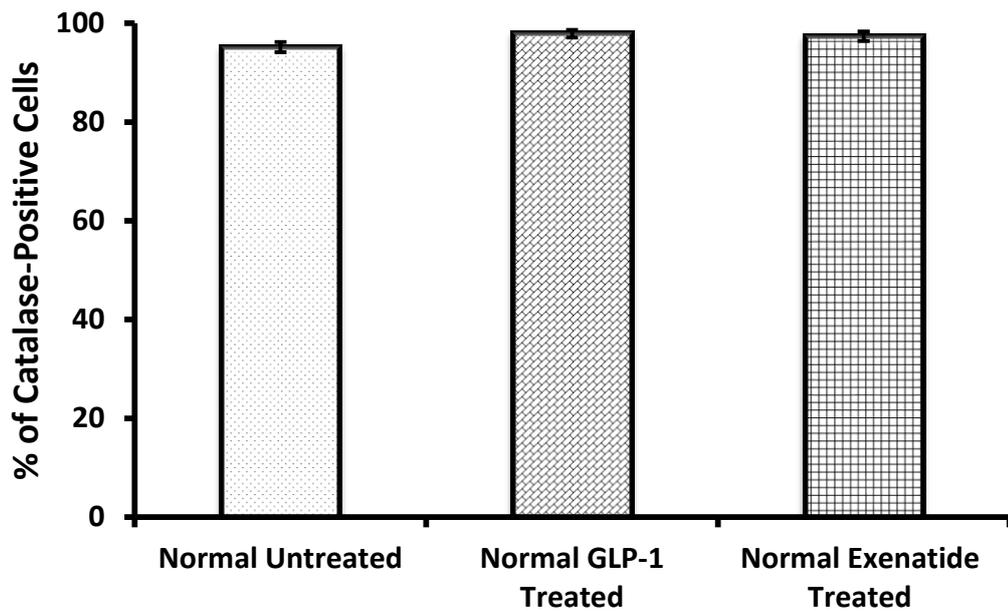


Figure 3.43: Histograms showing the effect of 10 weeks of treatment with either GLP-1 (50 nmol/kg body weight) or exenatide (1 μ g/kg body weight) on the percentage of catalase-positive cells in pancreatic islets of normal rats. Note that both GLP-1 and exenatide had no significant effect on the percentage of catalase-positive cells in normal treated rats compared to normal untreated rats. Data are mean \pm SEM, n = 10 islets/10 rats for each point.

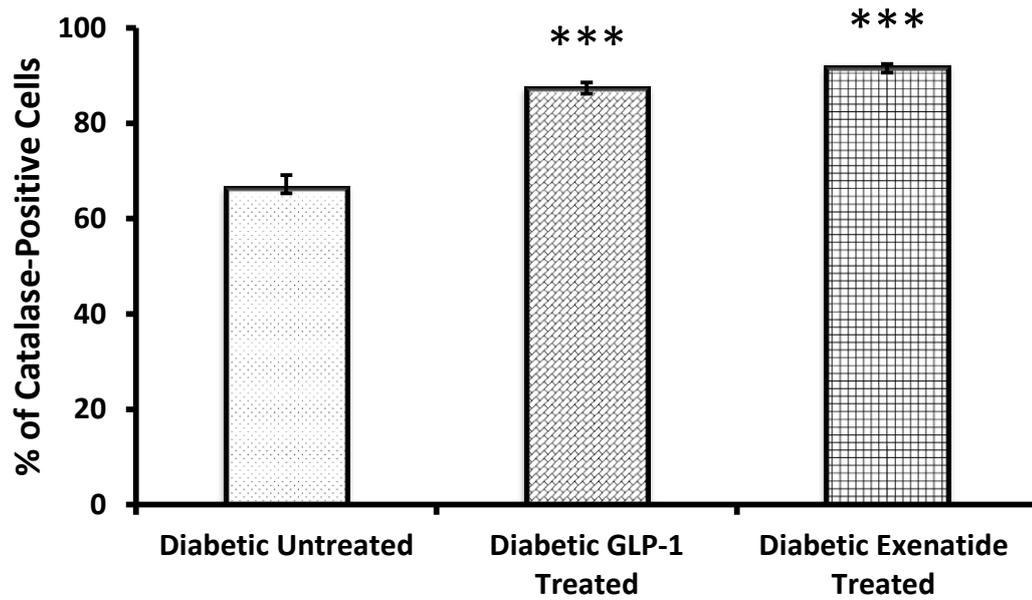


Figure 3.44: Histograms showing the effect of 10 weeks of treatment with either GLP-1 (50 nmol/kg body weight) or exenatide (1 μ g/kg body weight) on the percentage of catalase-positive cells in pancreatic islets of diabetic rats. *** $P < 0.001$ for GLP-1 and exenatide treated rats compared to untreated diabetic rats. Data are mean \pm SEM. Note that both GLP-1 and exenatide can evoke significant increases in the percentage of catalase-positive cells in diabetic treated rats compared to diabetic untreated rats. $n = 10$ islets/10 rats for each point.

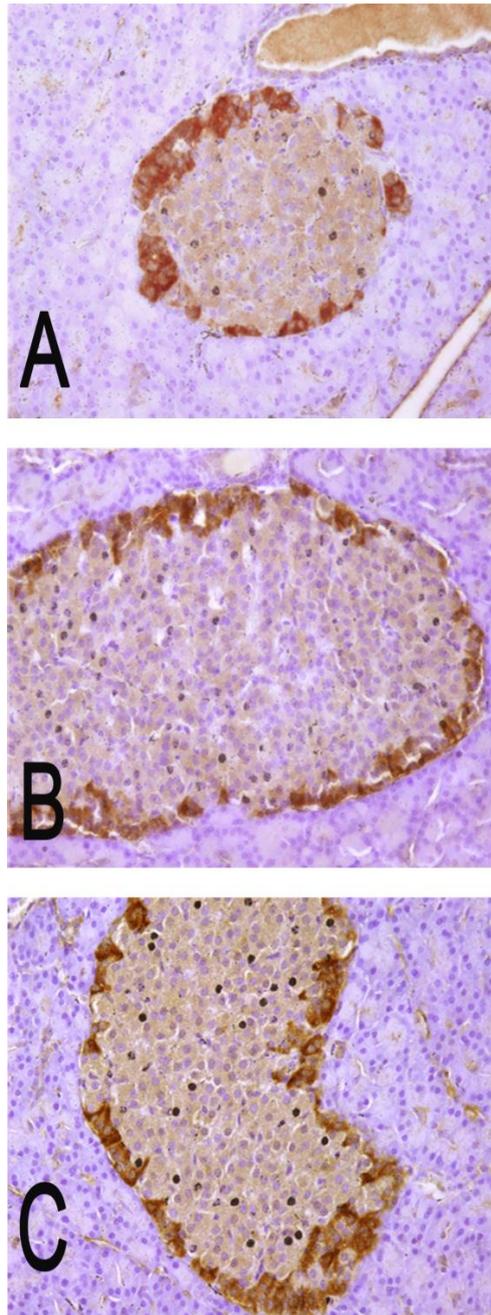


Figure 3.45: Micrographs showing the distribution of catalase-positive cells in the pancreatic islet of normal rats. The micrographs are typical of similar micrographs taken from 10 different rats in each group. (A) Catalase-positive cells in normal untreated rat pancreas, (B) catalase-positive cells in normal GLP-1-treated rats and (C) catalase-positive cells in normal exenatide-treated rats; Magnification: X 400. Note the increase in catalase-positive cells in B and C compared to A and these cells are more prominent at the periphery of the islets.

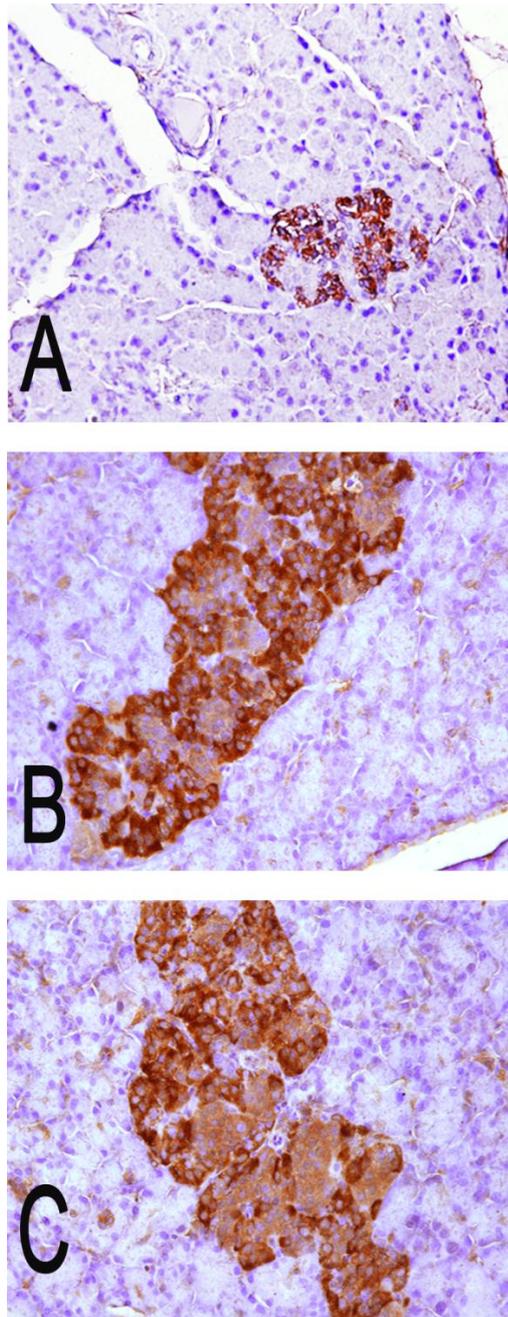


Figure 3.46: Micrographs showing the distribution of catalase-positive cells in the pancreatic islet of diabetic rats. The micrographs are typical of similar micrographs taken from 10 different rats in each group. (A) Catalase-positive cells in untreated diabetic rat pancreas, (B) catalase-positive cells in GLP-1-treated diabetic rats and (C) catalase-positive cells in exenatide-treated diabetic rats. Magnification: X 400. Note the increase in catalase-positive cells in B and C compared to A and these cells are distributed randomly throughout the islets.

3.5.6 Glutathione reductase immunoreactive cells in the pancreas of normal and diabetic rats

Figures 3.47, 3.48, 3.49 and 3.50 show the effect of either GLP-1 (50 nmol/kg body weight) or exenatide (1 µg/kg body weight), on glutathione reductase (GSH) immunoreactive cells in normal and diabetic rats. The percentage of changes in glutathione reductase-positive cells are shown in figures 3.47 and 3.48 for normal and diabetic rats while the micrographs are shown in figures 3.49 and 3.50, respectively. Both GLP-1 and exenatide treated normal rats showed no significant increase in the percentage of glutathione reductase-positive cells compared to untreated control rats. In contrast, in the islets of diabetic rat treated with either GLP-1 or exenatide, there were significant ($P < 0.001$ and $P < 0.01$) increases, respectively, in the number of GSH immunoreactive cells compared to normal untreated rats. The results also show that pancreatic islets of normal rats contain more GSH immunoreactive cells compared to pancreatic islets of diabetic rats in both treated and untreated conditions. The results presented in figures 3.49 and 3.50 also show that GSH immunoreactive cells were distributed more prominently at the peripheral parts of the islets of normal rats. In contrast, in the pancreatic islets of diabetic rats, GSH immunoreactive cells were distributed randomly.

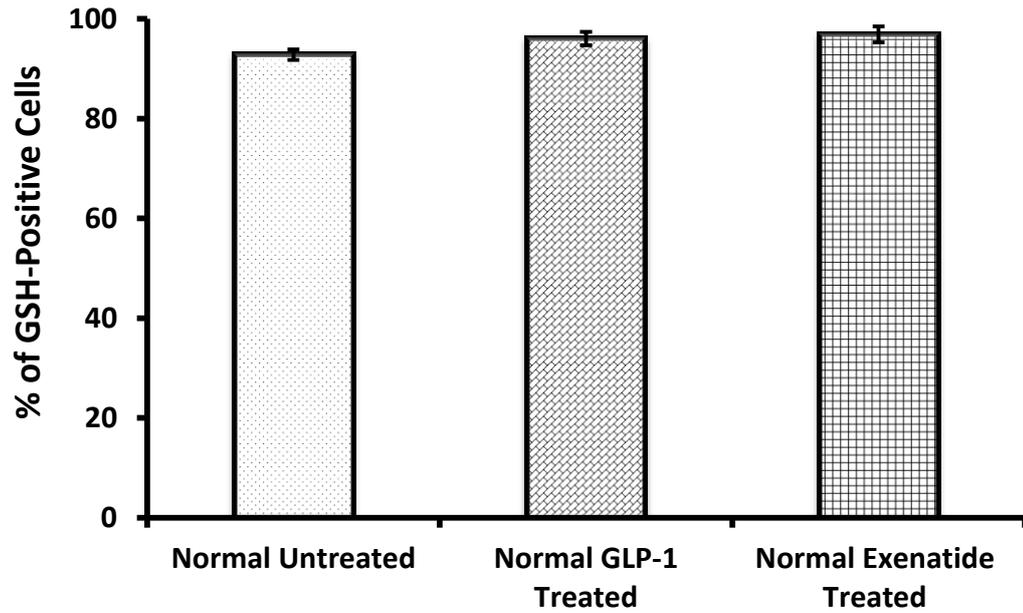


Figure 3.47: Histograms showing the effect of 10 weeks of treatment with either GLP-1 (50 nmol/kg body weight) or exenatide (1 μ g/kg body weight) on percentage of glutathione reductase (GSH)-positive cells in pancreatic islets of normal rats. Note the small increases in the percentage of GSH cells in GLP-1 and exenatide treated normal rats compared to normal untreated rats. Data are mean \pm SEM, n = 10 islets/10 rats for each point.

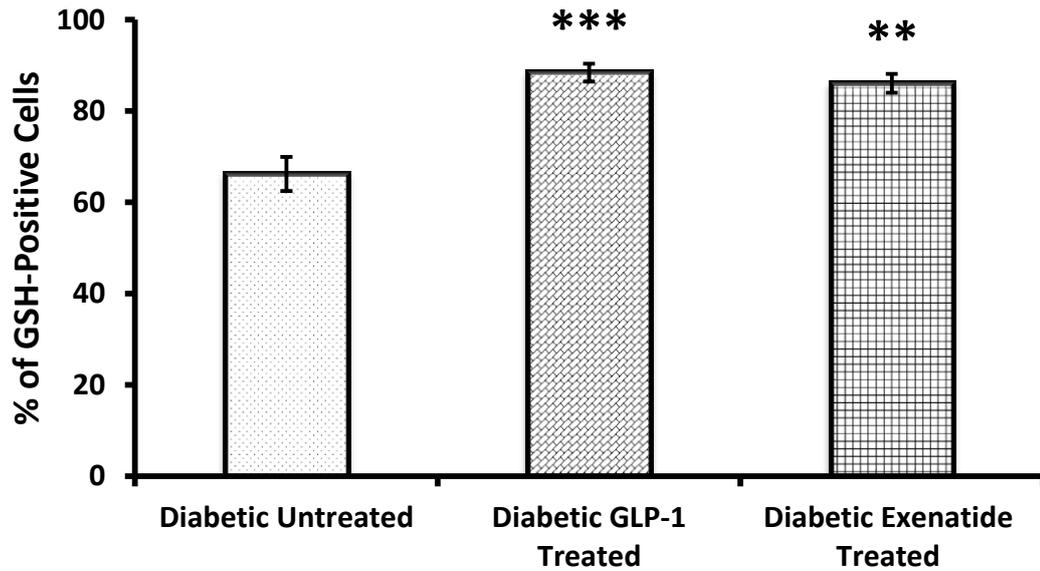


Figure 3.48: Histograms showing the effect of 10 weeks of treatment with either GLP-1 (50 nmol/kg body weight) or exenatide (1 μ g/kg body weight) on percentage of glutathione reductase (GSH)-positive cells in pancreatic islets of diabetic rats. *** $P < 0.001$ and ** $P < 0.01$ for treated diabetic rats with both GLP-1 and exenatide, respectively, compared to diabetic untreated rats. Data are mean \pm SEM, $n = 10$ islets/10 rats for each point.

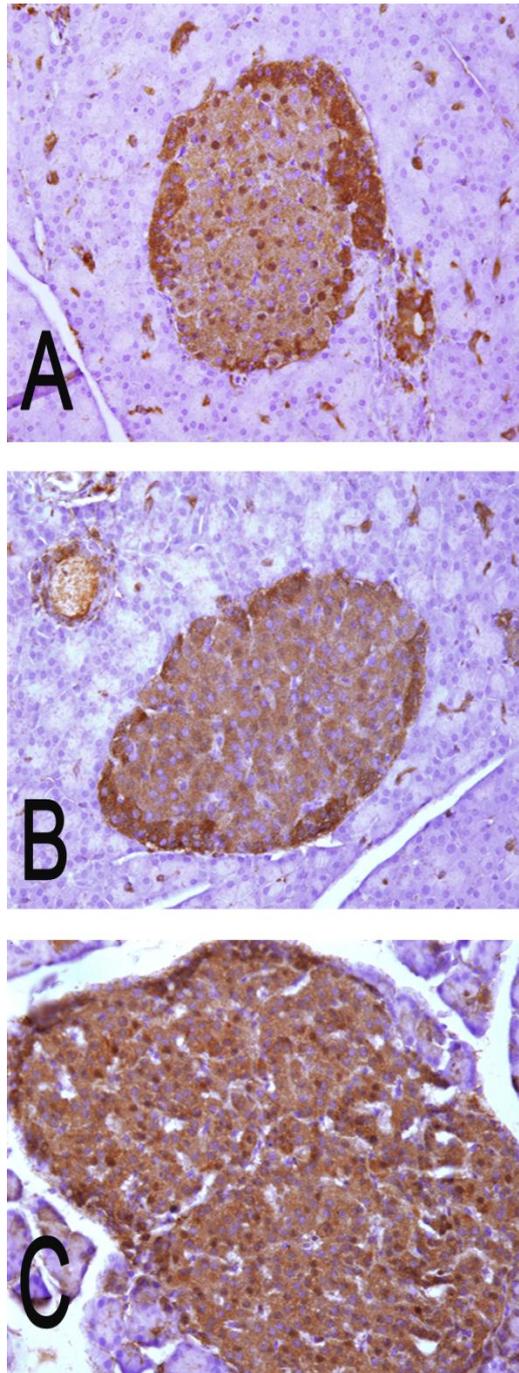


Figure 3.49: Micrographs showing the distribution of glutathione reductase (GSH)-positive cells in the pancreatic islet of normal rats. The micrographs are typical of similar micrographs taken from 10 different rats in each group. (A) GSH-positive cells in untreated normal rat pancreas, (B) GSH-positive cells in normal, GLP-1-treated rats and (C) GSH-positive cells in normal, exenatide-treated rats; Magnification: X 400. Note the distribution and increases in GSH-positive cells in B and C compared to A.

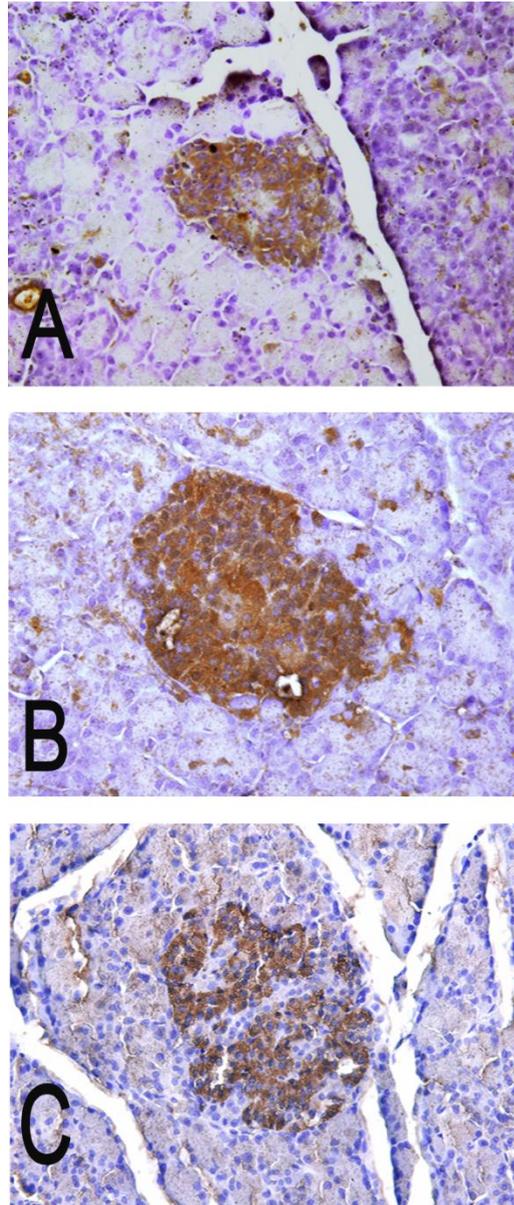


Figure 3.50: Micrographs showing the distribution of glutathione reductase (GSH)-positive cells in the pancreatic islet of diabetic rats. The micrographs are typical of similar micrographs taken from 10 different rats in each group. (A) GSH-positive cells in untreated diabetic rat pancreas, (B) GSH-positive cells in GLP-1-treated diabetic rats and (C) GSH-positive cells in exenatide-treated diabetic rats. Magnification: X 400. Note the distributions and increases in GSH-positive cells in B and C compared to A. GSH immunoreactive cells seem to be distributed randomly all over the islets in the diabetes rats compared to the islet normal rat, where they were localised more prominent at the periphery.

3.6 Co-localization of insulin and GLP-1 cells in the pancreatic islet of normal and diabetic rats

3.6.1 The distribution of insulin and GLP-1 cells in the pancreatic islet of normal rats

Figure 3.51 shows the immunofluorescence double labelling method for the co-localization of insulin (green) cells (A) and GLP-1 (red) cells (B) and (C) shows the merging of the two cell types in normal rats in (1) untreated normal rat, (2) GLP-1 treated normal rat and (3) exenatide treated normal rat. This figure shows that GLP-1 is randomly localized in the islet cells with both insulin and glucagon. The islets of untreated normal rats contained fewer either insulin-positive cells in (A1) or GLP-1-positive cells (B1) compared to treated rats. The number of insulin cells increased in normal rats treated with either GLP-1 (A2) or exenatide (A3). A similar increase in GLP-1 stained cells was detected in normal rats treated with either GLP-1 (B2) or exenatide (B3).

3.6.2 The distribution of insulin with GLP-1 cells in the pancreatic islet of diabetic rats

Figure 3.52 shows the immunofluorescence double labelling method for the co-localization of insulin (green) cells (A) with GLP-1 (red) cells (B) and (C) the merging of the two cell types in diabetic rats in (1) untreated diabetic rat (2) GLP-1 treated diabetic rat (3) exenatide treated diabetic rat. This figure shows that GLP-1 is randomly co-localized with insulin and glucagon in pancreatic islets. The islets of untreated diabetic rats contained fewer either insulin-positive cells in (A1) or GLP-1-positive cells in (B1) compared to treated rats. The number of insulin cells increased in diabetic rats treated with either GLP-1 (A2) or exenatide (A3). Similar increase in GLP-1 stained cells was detected in diabetic rats treated with either GLP-1 (B2) or exenatide (B3).

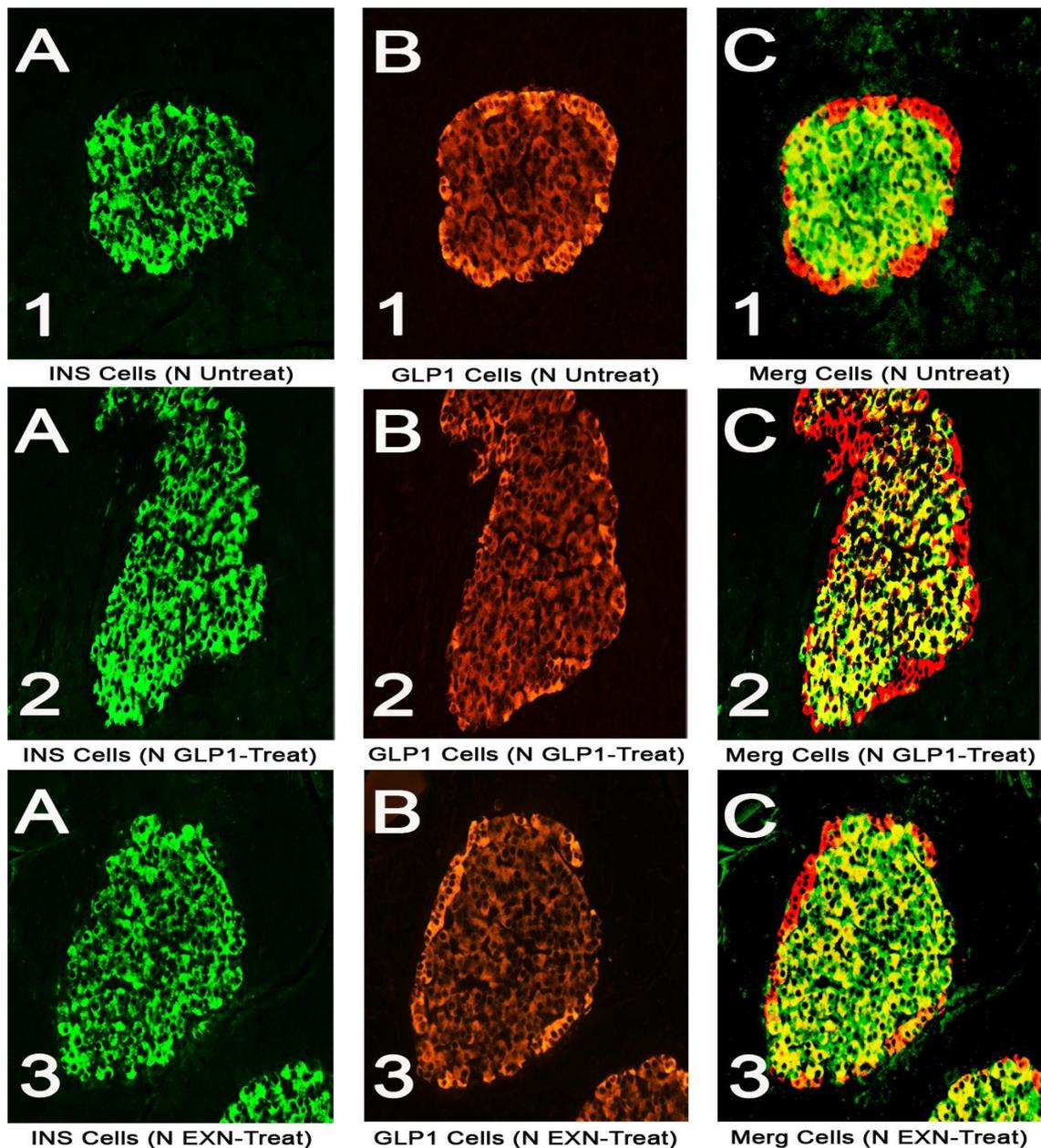


Figure 3.51: Micrographs of immunofluorescence double labelling showing the distribution of (A) insulin cells (green) and (B) glucagon like peptide-1 cells (red) in the pancreatic islet of normal rats while (C) shows the merging of the two cell types. The micrographs are typical of 10 different experiments taken from 10 different rats in each group. Magnification: X 400. N (normal rats), insulin (INS), glucagon like peptide-1 (GLP-1), exenatide (EXN), merged cells (Merg), untreated normal rat (1), GLP-1 treated normal rats (2) and exenatide normal treated rats (3).

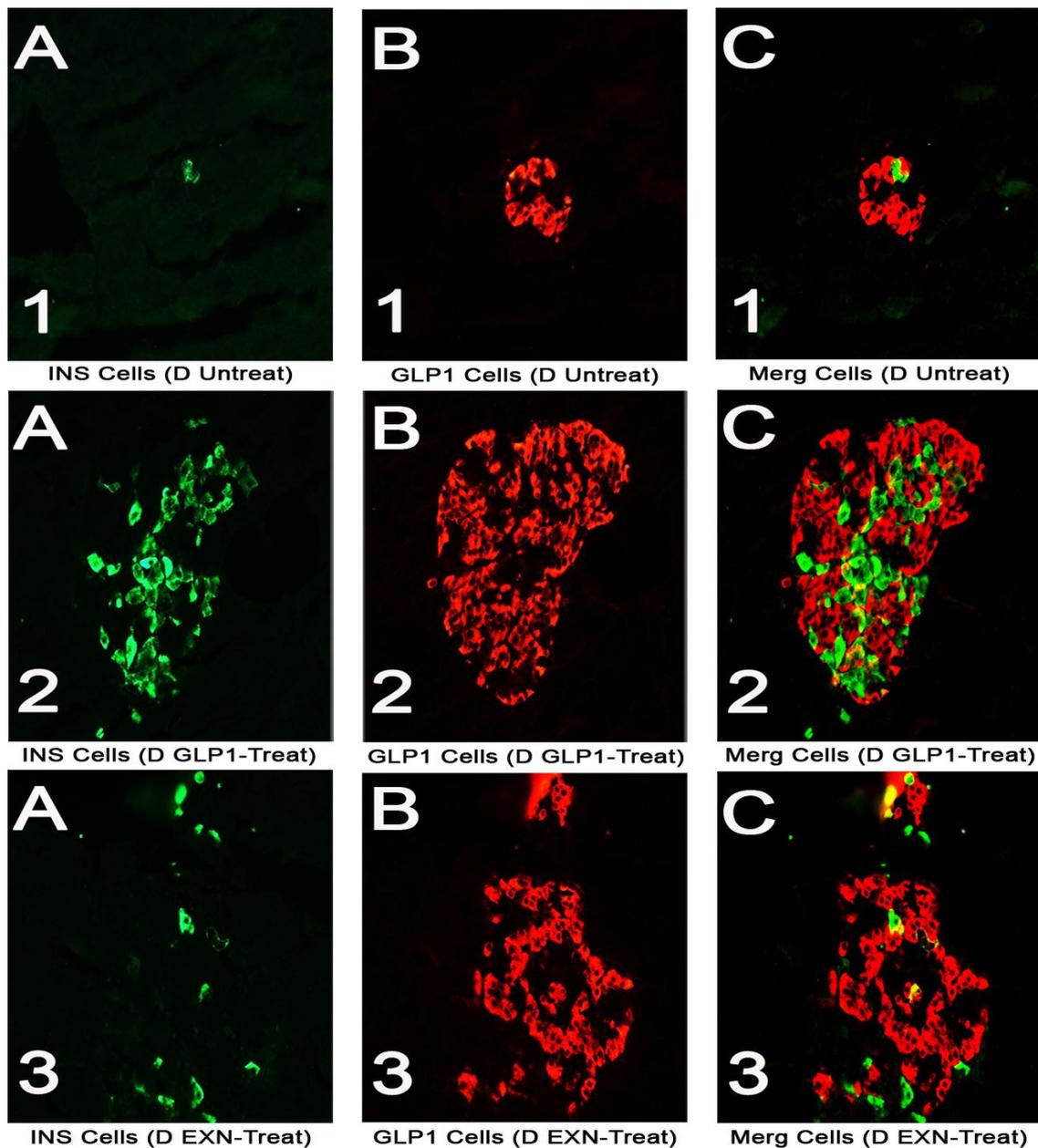


Figure 3.52: Micrographs of immunofluorescence double labelling showing the distribution of (A) insulin cells (green) and (B) glucagon like peptide-1 cells (red) in the pancreatic islet of diabetic rats while (C) shows the merging of the two cell types. The micrographs are typical of 10 different experiments taken from 10 different rats in each group. Magnification: X 400. Diabetic rats (D), insulin (INS), glucagon like peptide-1 (GLP-1), exenatide (EXN), merged cells (Merg), untreated diabetic rat (1), GLP-1 treated diabetic rats (2) and exenatide diabetic treated rats (3).

3.6.3 The distribution of insulin with exenatide cells in the pancreatic islet of normal rats

Figure 3.53 shows the immunofluorescence double labelling method for the co-localization of insulin (green) cells (A) and exenatide (red) cells (B) and (C) the merging of the two cell types in normal rats in (1) untreated normal rat (2) GLP-1 treated normal rat (3) exenatide treated normal rat. This figure shows that exenatide is randomly co-localized with both insulin and glucagon in pancreatic islets. The islets of untreated normal rats contained fewer either insulin-positive cells in (A1) or exenatide-positive cells (B1) compared to treated rats. The number of insulin cells increased in normal rats treated with either GLP-1 (A2) or exenatide (A3). Similar increase in exenatide stained cells was detected in normal rats treated with either GLP-1 (B2) or exenatide (B3).

3.6.4 The distribution of insulin with exenatide cells in the pancreatic islet of diabetic rats

Figure 3.54 shows the immunofluorescence double labelling method for the co-localization of insulin (green) cells (A) and exenatide (red) cells (B) and (C) the merging of the two cell types in diabetic rats in (1) untreated diabetic rat (2) GLP-1 treated diabetic rat (3) exenatide treated diabetic rat. This figure shows that exenatide is randomly co-localized with insulin islet cells. The islets of untreated diabetic rats contained fewer either insulin-positive cells in (A1) or exenatide-positive cells in (B1) compared to treated rats. The number of insulin cells increased in diabetic rats treated with either GLP-1 (A2) or exenatide (A3). Similar increase in exenatide stained cells was detected in diabetic rats treated with either GLP-1 (B2) or exenatide (B3).

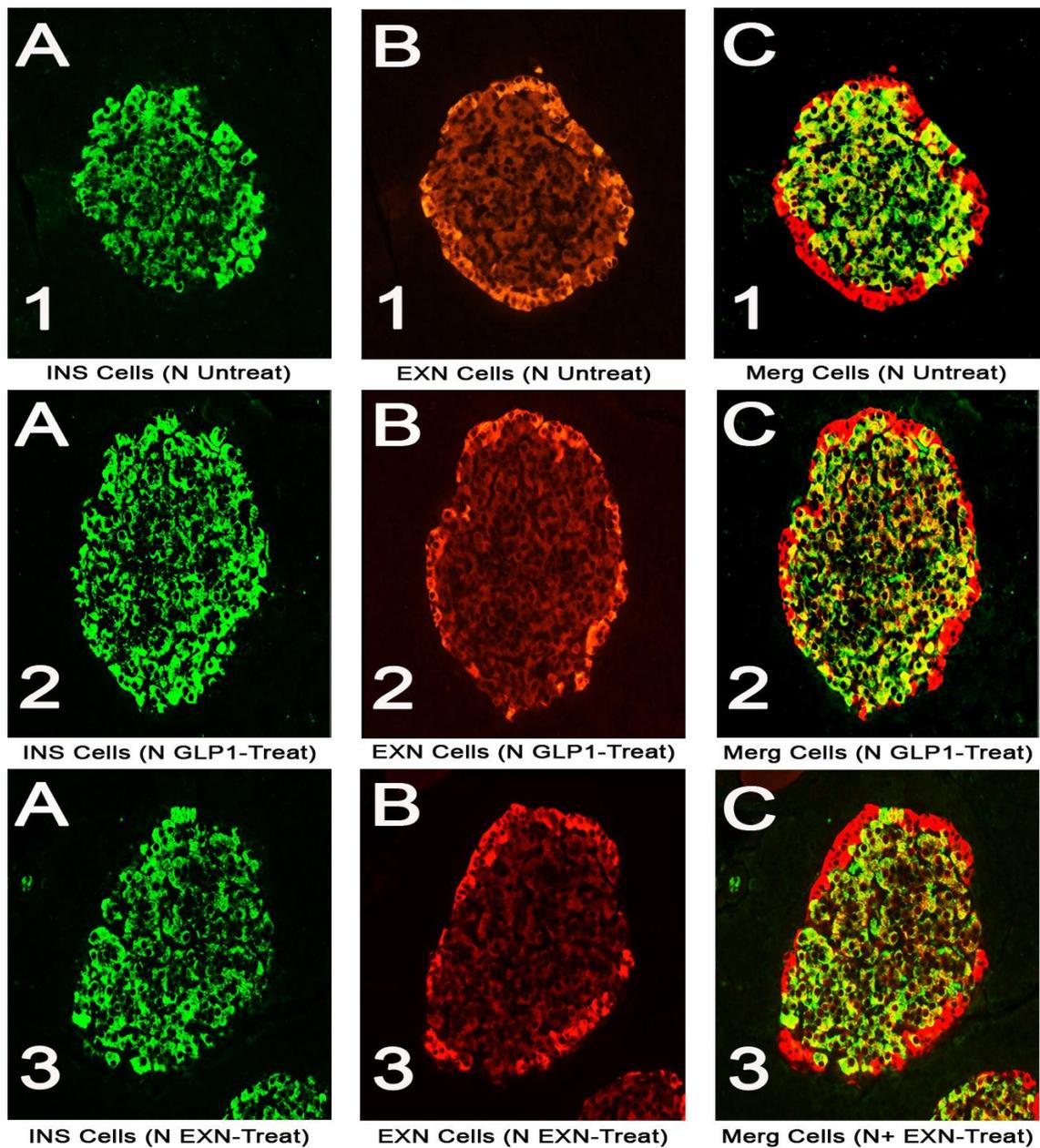


Figure 3.53: Micrographs of immunofluorescence double labelling showing the distribution of (A) insulin cells (green) and (B) exenatide cells (red) in the pancreatic islet of normal rats while (C) shows the merging of the two cell types. The micrographs are typical of similar micrographs taken from 10 different rats in each group. Magnification: X 400. Normal rats (N), insulin (INS), glucagon like peptide-1 (GLP-1), exenatide (EXN), merged cells (Merg), untreated normal rat (1), GLP-1 treated normal rats (2) and exenatide normal treated rats (3).

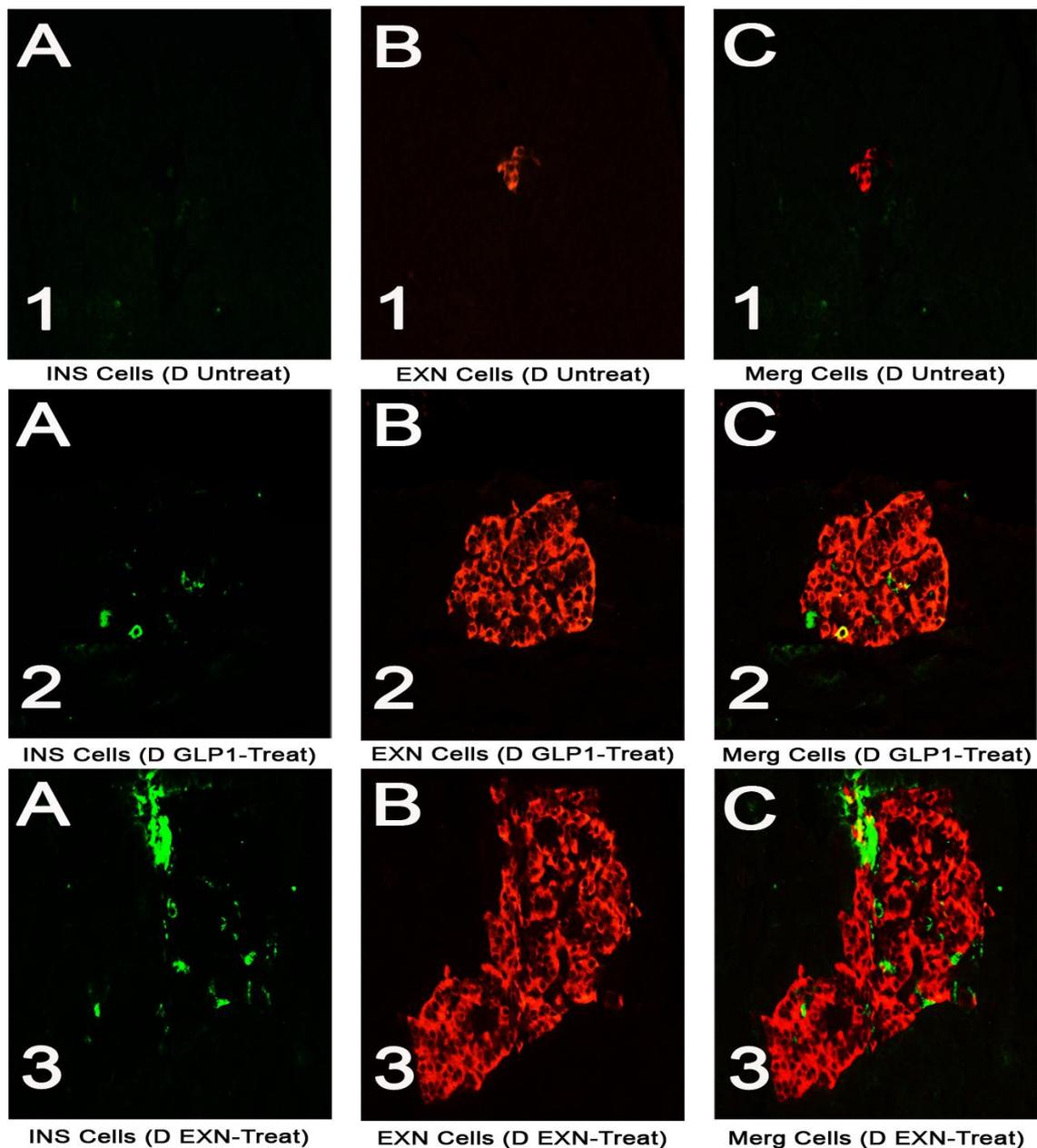


Figure 3.54: Micrographs of immunofluorescence double labelling showing the distribution of (A) insulin cells (green) and (B) exenatide cells (red) in the pancreatic islet of diabetic rats while (C) shows the merging of the two cell types. The micrographs are typical of similar micrographs taken from 10 different rats in each group. Magnification: X 400. Diabetic rats (D), insulin (INS), glucagon like peptide-1 (GLP-1), exenatide (EXN), merged cells (Merg), untreated diabetic rat (1), GLP-1 treated diabetic rats (2) and exenatide diabetic treated rats (3).

3.6.5 The distribution of glucagon with GLP-1 cells in the pancreatic islet of normal rats

Figure 3.55 shows the immunofluorescence double labelling method for the co-localization of glucagon (green) cells (A) GLP-1 (red) cells (B) and (C) the merging of the two cell types in normal rats in (1) untreated normal rat (2) GLP-1 treated normal rat (3) exenatide treated normal rat. This figure shows that GLP-1 is randomly co-localized with both insulin and glucagon in pancreatic islets. The number of glucagon-positive cells decreased in normal rats treated with either GLP-1 (A2) or exenatide (A3). An increase in GLP-1 stained cells was detected in normal rats treated with either GLP-1 (B2) or exenatide (B3).

3.6.6 The distribution of glucagon with GLP-1 cells in the pancreatic islet of diabetic rats

Figure 3.56 shows the immunofluorescence double labelling method for the co-localization of glucagon (green) cells (A) and GLP-1 (red) cells (B) and (C) the merging of the two cell types in diabetic rats in (1) untreated diabetic rat (2) GLP-1 treated diabetic rat (3) exenatide treated diabetic rat. This figure shows that GLP-1 is randomly co-localized with both insulin and glucagon in pancreatic islets. The number of glucagon cells decreased in diabetic rats treated with either GLP-1 (A2) or exenatide (A3). An increase in GLP-1 stained cells was detected in diabetic rats treated with either GLP-1 (B2) or exenatide (B3).

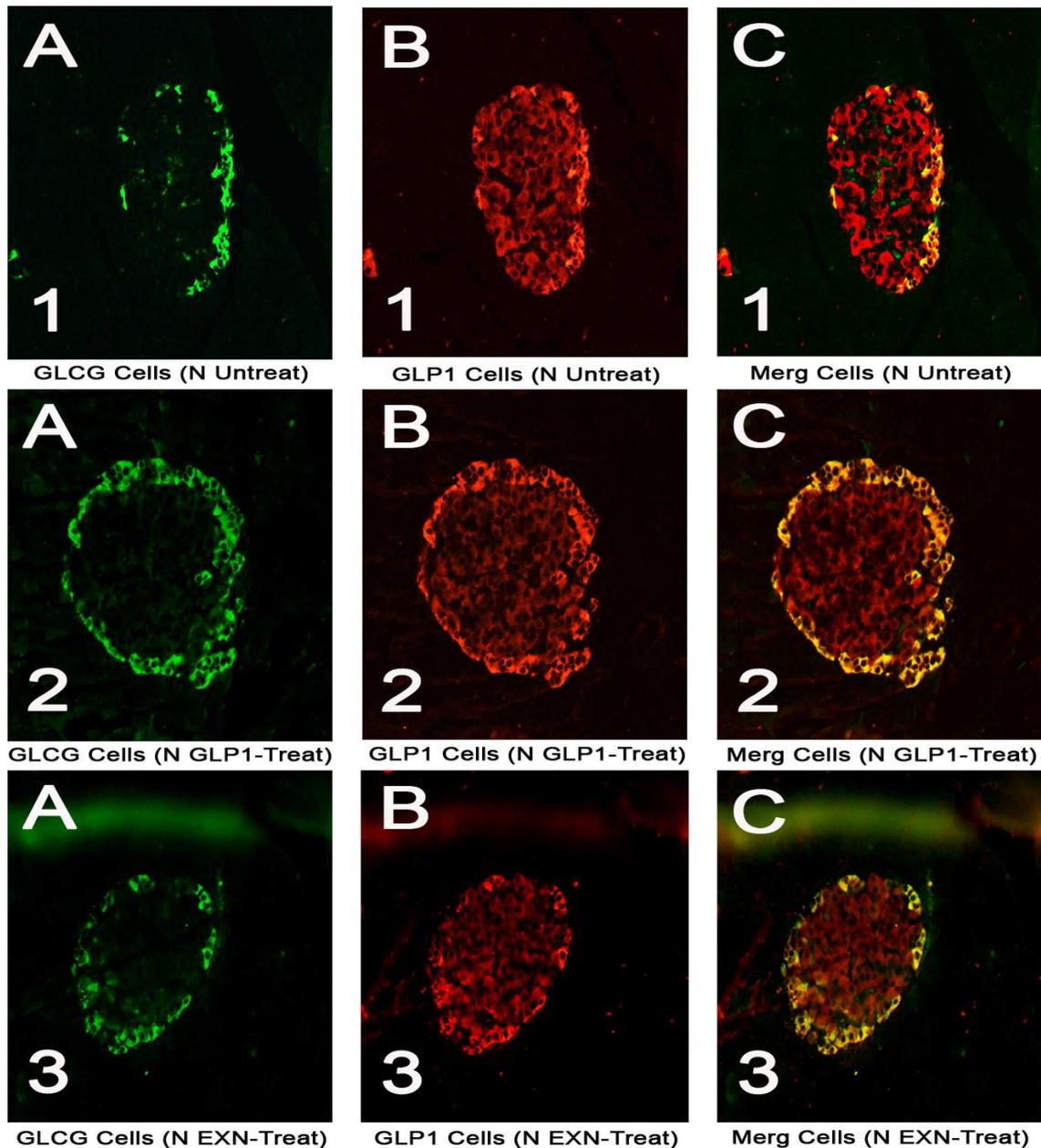


Figure 3.55: Micrographs of immunofluorescence double labelling showing the distribution of (A) glucagon cells (green) and (B) glucagon like peptide-1 cells (red) in the pancreatic islet of normal rats while (C) shows the merging of the two cell types. The micrographs are typical of similar micrographs taken from 10 different rats in each group. Magnification: X 400. Normal rats (N), glucagon (GLCG), glucagon like peptide-1 (GLP-1), exenatide (EXN), merged cells (Merg), untreated normal rat (1), GLP-1 treated normal rats (2) and exenatide normal treated rats (3).

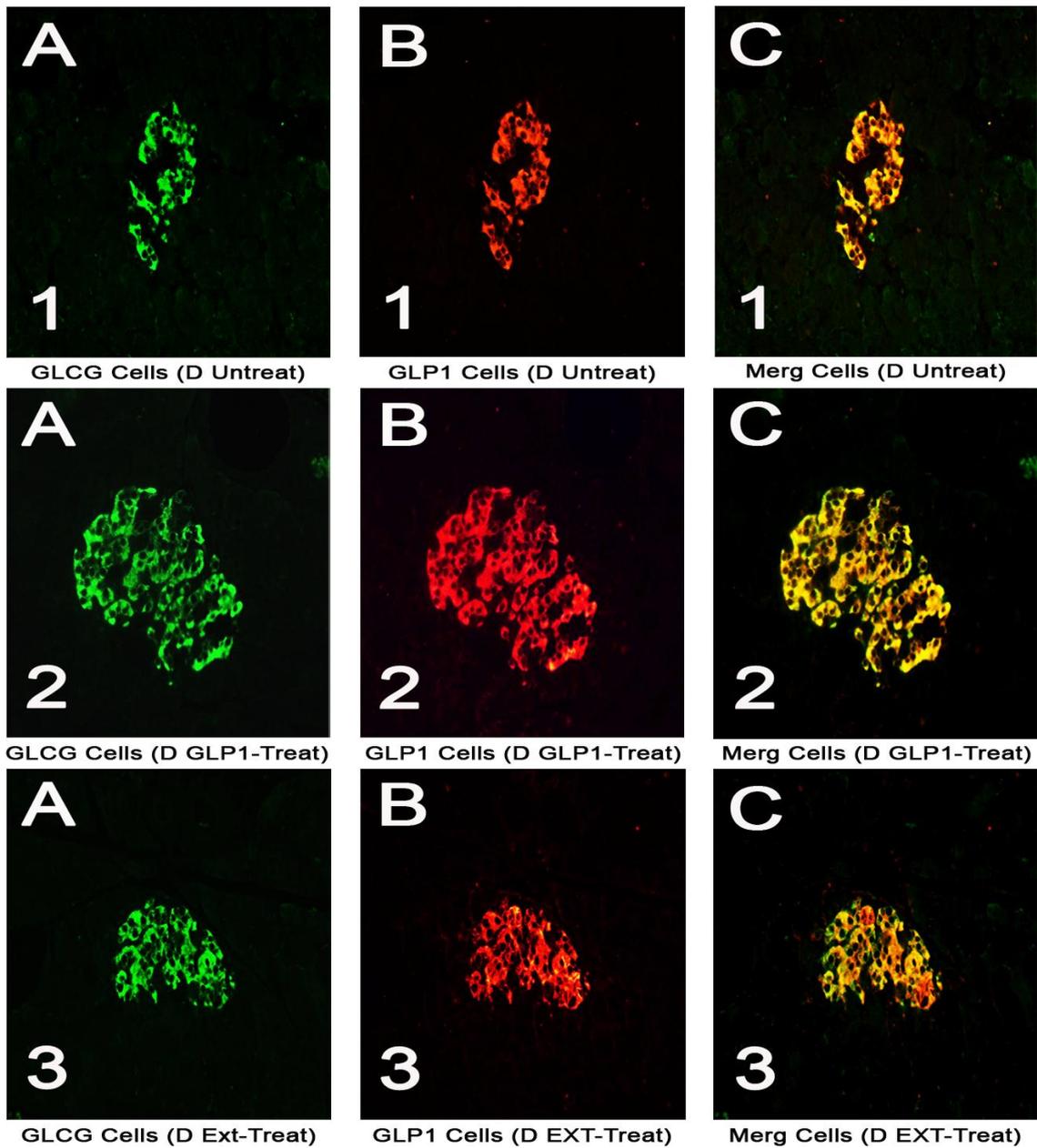


Figure 3.56: Micrographs of immunofluorescence double labelling showing the distribution of (A) glucagon cells (green) and (B) glucagon like peptide-1 cells (red) in the pancreatic islet of diabetic rats while (C) shows the merging of the two cell types. The micrographs are typical of similar micrographs taken from 10 different rats in each group. Magnification: X 400. Diabetic rats (D), glucagon (GLCG), glucagon like peptide-1 (GLP-1), exenatide (EXN), merged cells (Merg), untreated diabetic rat (1), GLP-1 treated diabetic rats (2) and exenatide diabetic treated rats (3).

3.6.7 The distribution of glucagon with exenatide cells in the pancreatic islet of normal rats

Figure 3.57 shows the immunofluorescence double labelling method for the co-localization of glucagon (green) cells (A) and exenatide (red) cells (B) and (C) the merging of the two cell types in normal rats in (1) untreated normal rat (2) GLP-1 treated normal rat (3) exenatide treated normal rat. This figure shows that exenatide is co-localized with both insulin and glucagon in pancreatic islets. The number of glucagon cells decreased in normal rats treated with either GLP-1 (A2) or exenatide (A3). An increase of exenatide stained cells was detected in normal rats treated with either GLP-1 (B2) or exenatide (B3).

3.6.8 The distribution of glucagon with exenatide cells in the pancreatic islet of diabetic rats

Figure 3.58 shows the immunofluorescence double labelling method for the co-localization of glucagon (green) cells (A) and exenatide (red) cells (B) and (C) the merging of the two cell types in diabetic rats in (1) untreated diabetic rat (2) GLP-1 treated diabetic rat (3) exenatide treated diabetic rat. This figure shows that exenatide randomly co-localized with both insulin and glucagon in pancreatic islets. The number of glucagon cells decreased in diabetic rats treated with either GLP-1 (A2) or exenatide (A3). An increase in exenatide stained cells was detected in diabetic rats treated with either GLP-1 (B2) or exenatide (B3).

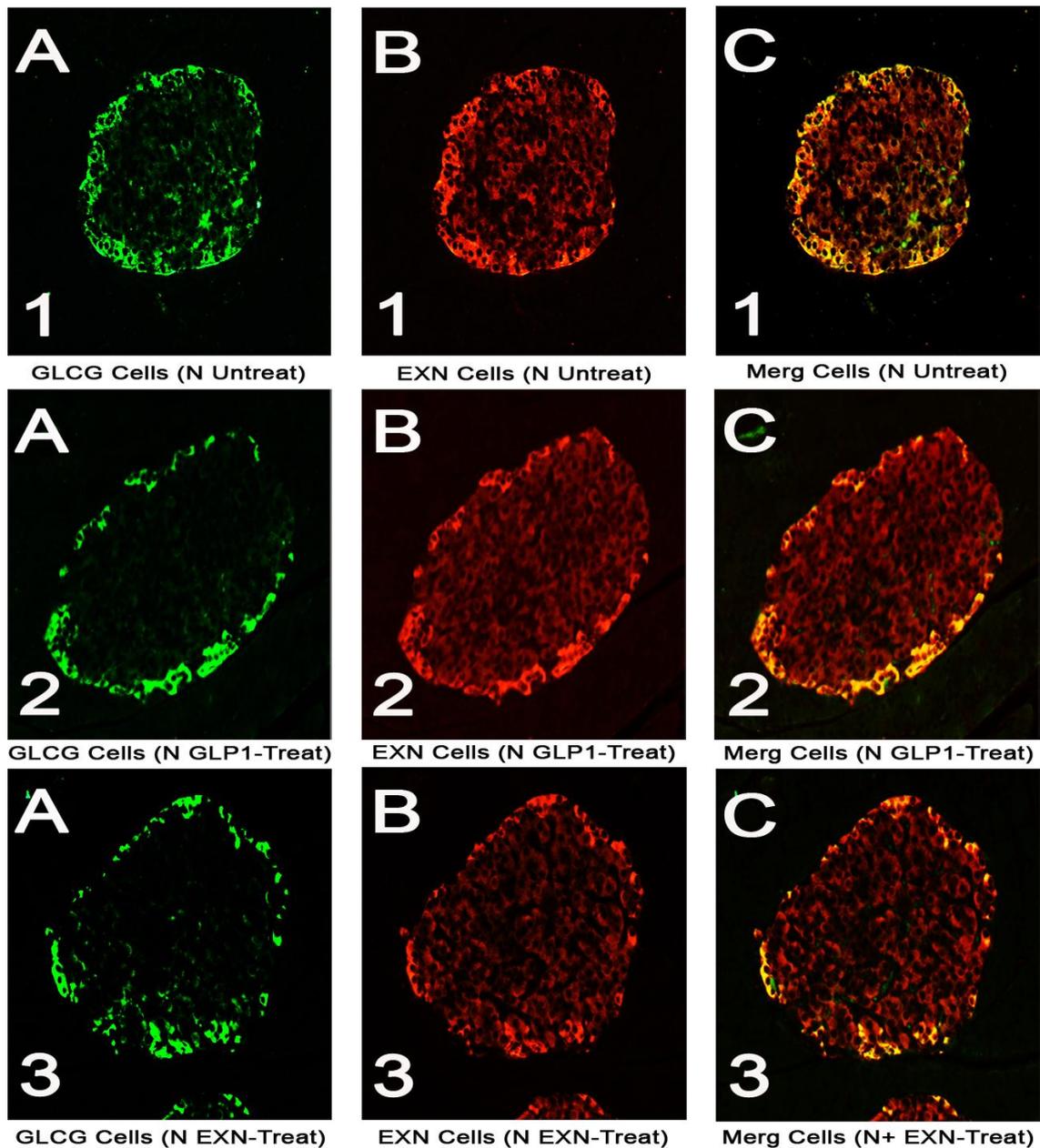


Figure 3.57: Micrographs of immunofluorescence double labelling showing the distribution of (A) glucagon cells (green) and (B) exenatide cells (red) in the pancreatic islet of normal rats while (C) shows the merging of the two cell types. The micrographs are typical of similar micrographs taken from 10 different rats in each group. Magnification: X 400. Normal rats (N), glucagon (GLCG), glucagon like peptide-1 (GLP-1), exenatide (EXN), merged cells (Merg), untreated normal rat (1), GLP-1 treated normal rats (2) and exenatide normal treated rats (3).

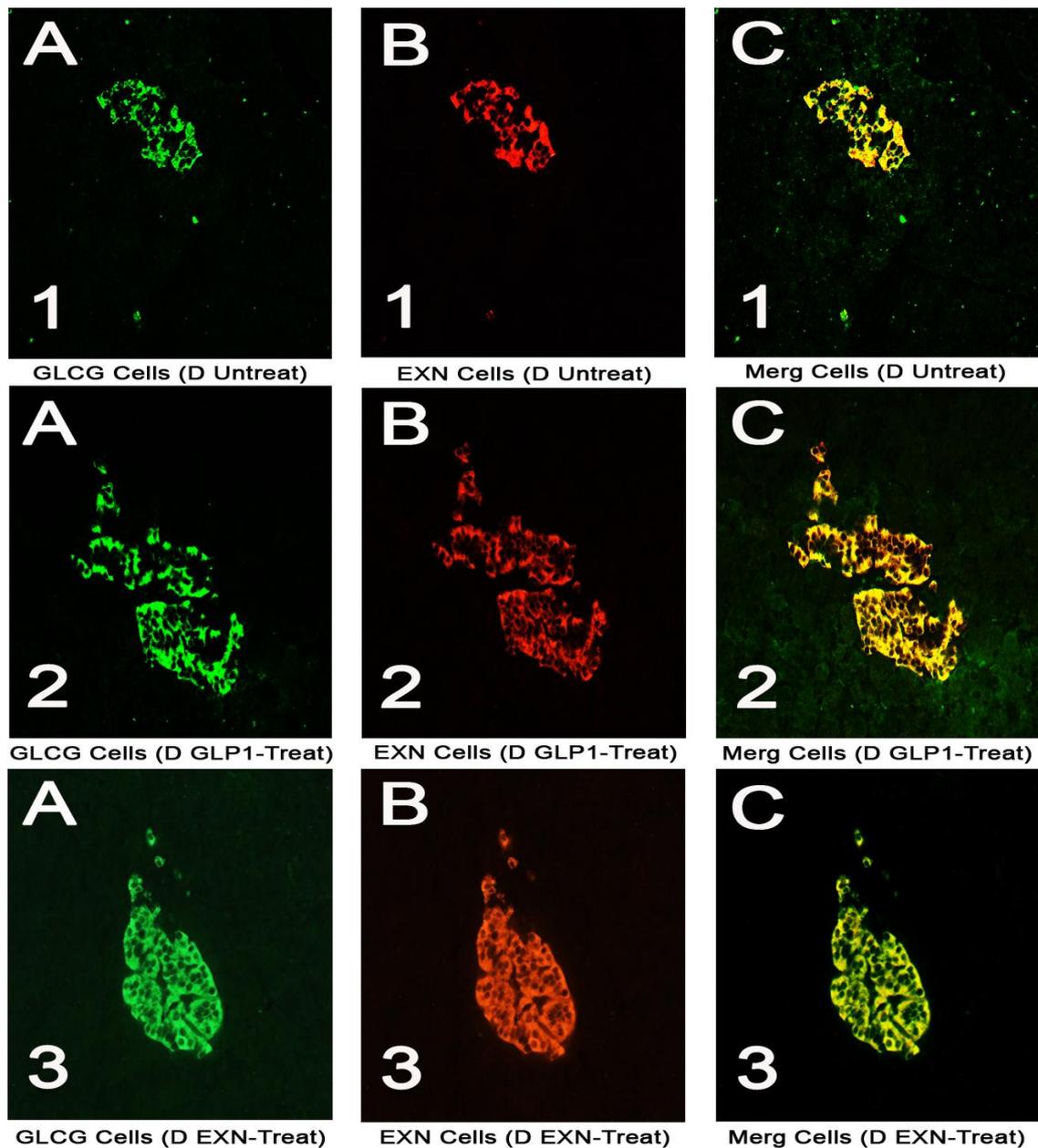


Figure 3.58: Micrographs of immunofluorescence double labelling showing the distribution of (A) glucagon cells (green) and (B) exenatide cells (red) in the pancreatic islet of diabetic rats while (C) shows the merging of the two cell types. The micrographs are typical of similar micrographs taken from 10 different rats in each group. Magnification: X 400. Diabetic rats (D), glucagon (GLGC), glucagon like peptide-1 (GLP-1), exenatide (EXN), merged cells (Merg), untreated diabetic rat (1), GLP-1 treated diabetic rats (2) and exenatide diabetic treated rats (3).

3.6.9 The distribution of insulin with catalase in the pancreatic islet of normal rats

Figure 3.59 shows the immunofluorescence double labelling method for the co-localization of insulin (green) cells (A) and catalase (red) cells (B) and (C) the merging of the two cell types in normal rats in (1) untreated normal rat (2) GLP-1 treated normal rat (3) exenatide treated normal rat. This figure shows that catalase is co-localized with both insulin and glucagon in pancreatic islets. The islets of untreated normal rats contained fewer either insulin-positive cells in (A1) or catalase-positive cells in (B1) compared to treated rats. The number of insulin cells increased in normal rats treated with either GLP-1 (A2) or exenatide (A3). Similar increase in catalase stained cells was detected in normal rats treated with either GLP-1 (B2) or exenatide (B3).

3.6.10 The distribution of insulin with catalase in the pancreatic islet of diabetic rats

Figure 3.60 shows the immunofluorescence double labelling for the co-localization of pancreatic insulin (green) cells (A) and catalase (red) cells (B) and (C) the merging of the two cell types in diabetic rats in (1) untreated diabetic rat (2) GLP-1 treated diabetic rat (3) exenatide treated diabetic rat. This figure shows that catalase is randomly co-localized with both insulin and glucagon in pancreatic islets. The islets of untreated diabetic rats contained fewer either insulin-positive cells in (A1) or catalase-positive cells in (B1) compared to treated rats. The number of insulin immunoreactive cells increased in diabetic rats after treatment with either GLP-1 (A2) or exenatide (A3). Similar increase in catalase stained cells was detected in diabetic rats treated with either GLP-1 (B2) or exenatide (B3).

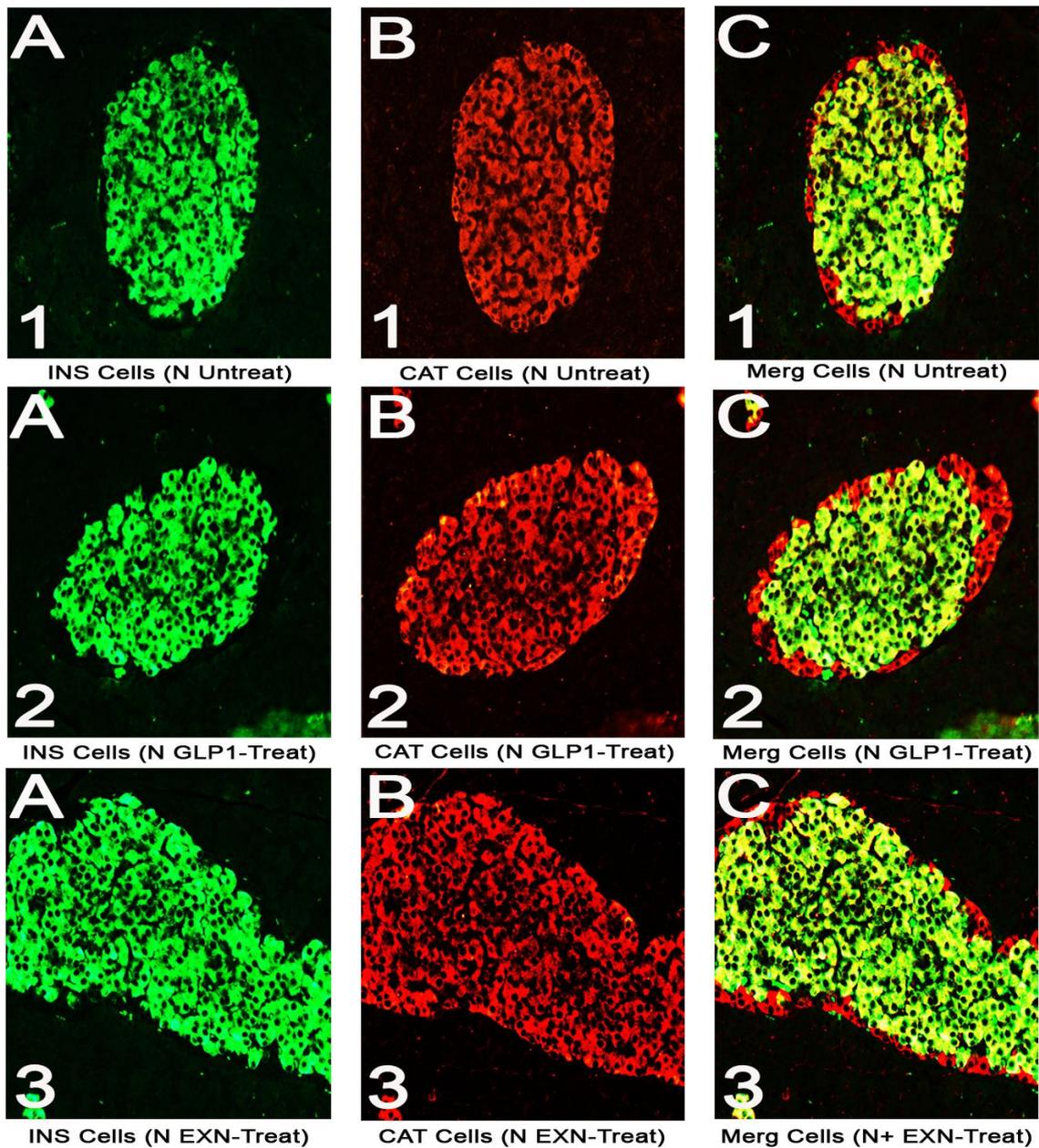


Figure 3.59: Micrographs of immunofluorescence double labelling showing the distribution of (A) insulin cells (green) and (B) catalase cells (red) in the pancreatic islet of normal rats while (C) shows the merging of the two cell types. The micrographs are typical of similar micrographs taken from 10 different rats in each group. Magnification: X 400. Normal rats (N), insulin (INS), catalase (CAT), glucagon like peptide-1 (GLP-1), exenatide (EXN), merged cells (Merg), untreated normal rat (1), GLP-1 treated normal rats (2) and exenatide normal treated rats (3).

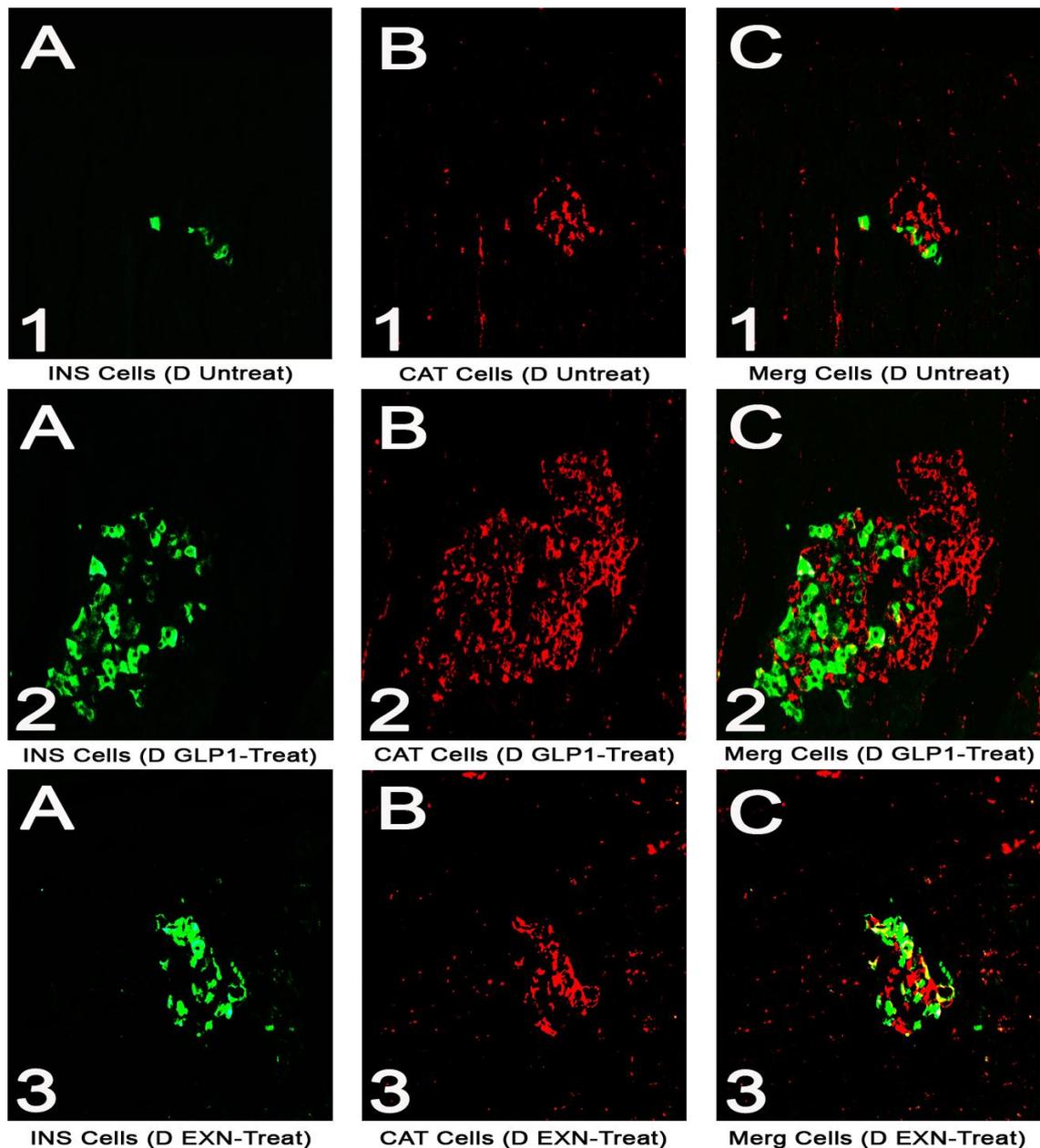


Figure 3.60: Micrographs of immunofluorescence double labelling showing the distribution of (A) insulin cells (green) and (B) catalase cells (red) in the pancreatic islet of diabetic rats while (C) shows the merging of the two cell types. The micrographs are typical of similar micrographs taken from 10 different rats in each group. Magnification: X 400. Diabetic rats (D), insulin (INS), catalase (CAT), glucagon like peptide-1 (GLP-1), exenatide (EXN), merged cells (Merg), untreated diabetic rat (1), GLP-1 treated diabetic rats (2) and exenatide diabetic treated rats (3).

3.6.11 The distribution of insulin with glutathione reductase in the pancreatic islet of normal rats

Figure 3.61 shows the immunofluorescence double labelling images for the co-localization of insulin (green) cells (A) and glutathione reductase (red) cells (B) and (C) the merging of the two cell types in normal rats in (1) untreated normal rat (2) GLP-1 treated normal rat (3) exenatide treated normal rat. This figure shows that glutathione reductase is co-localized with both insulin and glucagon in pancreatic islets. The islets of untreated normal rats contained fewer either insulin-positive cells in (A1) or catalase-positive cells in (B1) compared to treated rats. The number of insulin-positive cells increased in normal rats treated with either GLP-1 (A2) or exenatide (A3). Similar increase in glutathione reductase stained cells was detected in normal rats treated with either GLP-1 (B2) or exenatide (B3).

3.6.12 The distribution of insulin with glutathione reductase in the pancreatic islet of diabetic rats

Figure 3.62 shows the immunofluorescence double labelling images for the co-localization of insulin (green) (A) and glutathione reductase (red) (B) and (C) the merging of the two cell types in diabetic rats in (1) untreated diabetic rat (2) GLP-1 treated diabetic rat (3) exenatide treated diabetic rat. This figure shows that glutathione reductase is randomly co-localized with both insulin and glucagon in pancreatic islets. The islets of untreated diabetic rats contained fewer either insulin-positive cells in (A1) or catalase-positive cells in (B1) compared to treated rats. The number of insulin immunoreactive cells increased in diabetic rats treated with either GLP-1 (A2) or exenatide (A3). Similar increase in glutathione reductase stained cells were detected in diabetic rats treated with either GLP-1 (B2) or exenatide (B3).

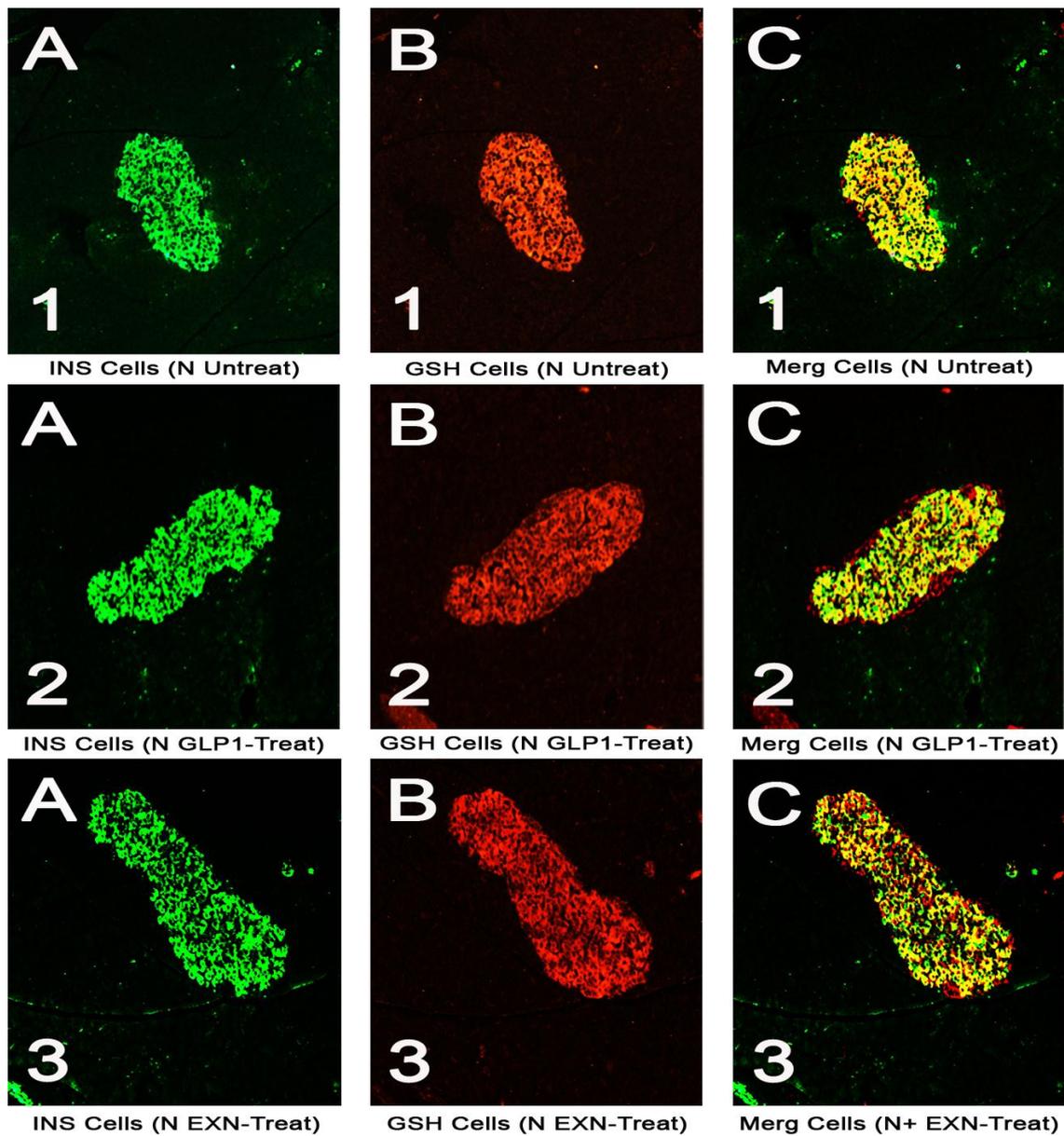


Figure 3.61: Micrographs of immunofluorescence double labelling showing the distribution of (A) insulin cells (green) and (B) glutathione reductase cells (red) in the pancreatic islet of normal rats while (C) shows the merging of the two cell types. The micrographs are typical of similar micrographs taken from 10 different rats in each group. Magnification: X 400. Normal rats (N), insulin (INS), glutathione reductase (GSH), glucagon like peptide-1 (GLP-1), exenatide (EXN), merged cells (Merg), untreated normal rat (1), GLP-1 treated normal rats (2) and exenatide normal treated rats (3).

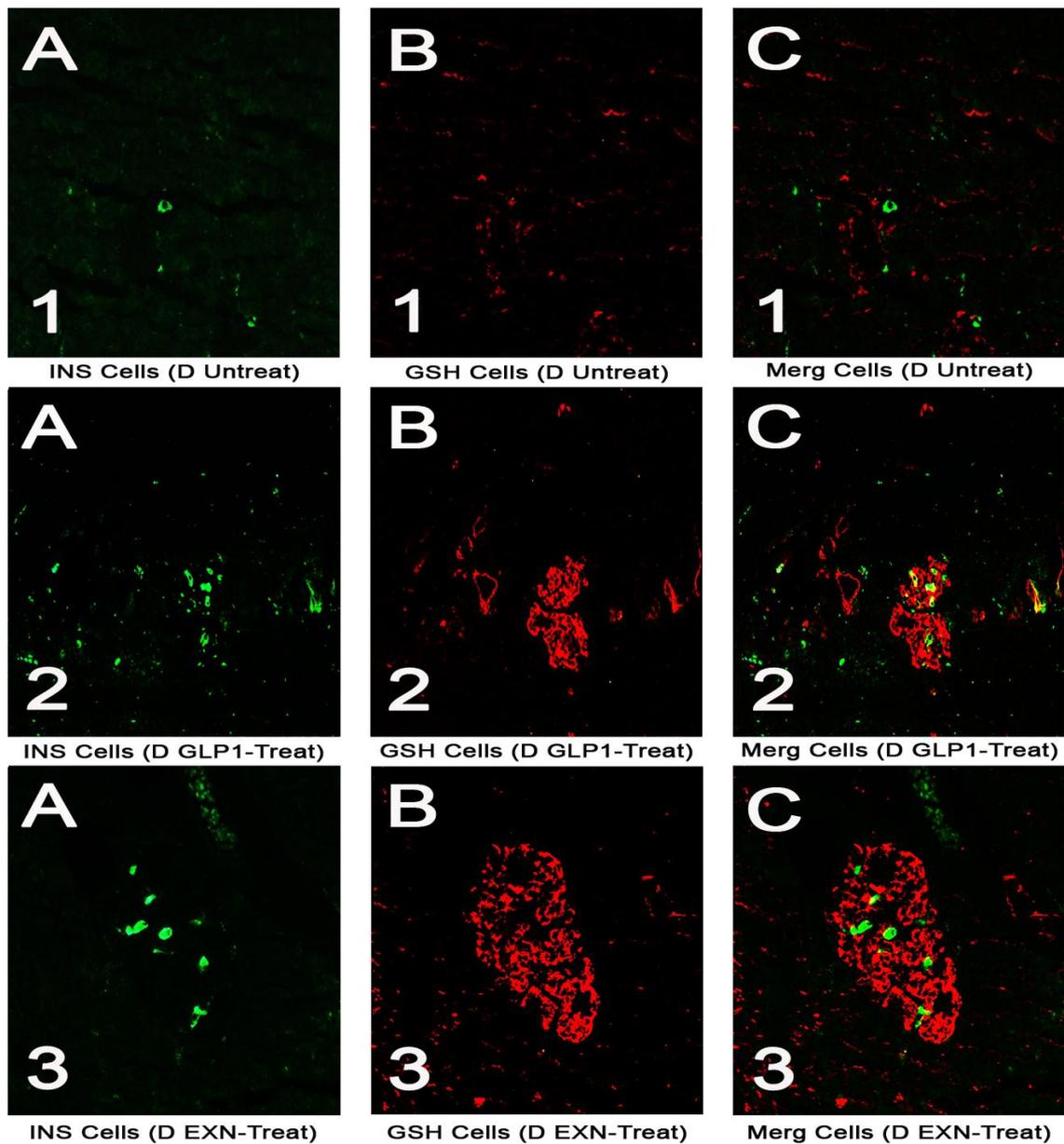


Figure 3.62: Micrographs of immunofluorescence double labelling showing the distribution of (A) insulin cells (green) and (B) glutathione reductase cells (red) in the pancreatic islet of diabetic rats while (C) shows the merging of the two cell types. The micrographs are typical of similar micrographs taken from 10 different rats in each group. Magnification: X 400. Diabetic rats (D), insulin (INS), glutathione reductase (GSH), glucagon like peptide-1 (GLP-1), exenatide (EXN), merged cells (Merg), untreated diabetic rat (1), GLP-1 treated diabetic rats (2) and exenatide diabetic treated rats (3).

3.6.13 The distribution of glucagon with catalase in the pancreatic islet of normal rats

Figure 3.63 shows the immunofluorescence double labelling for the co-localization of glucagon (green) cells (A) and catalase (red) cells (B) and (C) the merging of the two cell types in normal rats in (1) untreated normal rat (2) GLP-1 treated normal rat (3) exenatide treated normal rat. This figure shows that catalase is co-localized with both insulin and glucagon in pancreatic islet cells. Many cells contain either glucagon (A1) or catalase (B1) in normal untreated rats. The number of glucagon cells seemed to decrease in normal rats treated with either GLP-1 (A2) or exenatide (A3). An increase in catalase stained cells was detected in normal rats treated with either GLP-1 (B2) or exenatide (B3).

3.6.14 The distribution of glucagon with catalase in the pancreatic islet of diabetic rats

Figure 3.64 shows the immunofluorescence double labelling images for the co-localization of glucagon (green) cells (A) or catalase (red) cells (B) and (C) the merging of the two cell types in diabetic rats in (1) untreated diabetic rat (2) GLP-1 treated diabetic rat (3) exenatide treated diabetic rat. This figure shows that catalase is randomly co-localized with insulin and glucagon in the pancreatic islets. Fewer cells contain either glucagon (A1) or catalase (B1) in untreated diabetic rats. The number of glucagon cells seemed to decrease in diabetic rats treated with either GLP-1 (A2) or exenatide (A3). An increase in catalase stained cells was detected in diabetic rats treated with either GLP-1 (B2) or exenatide (B3).

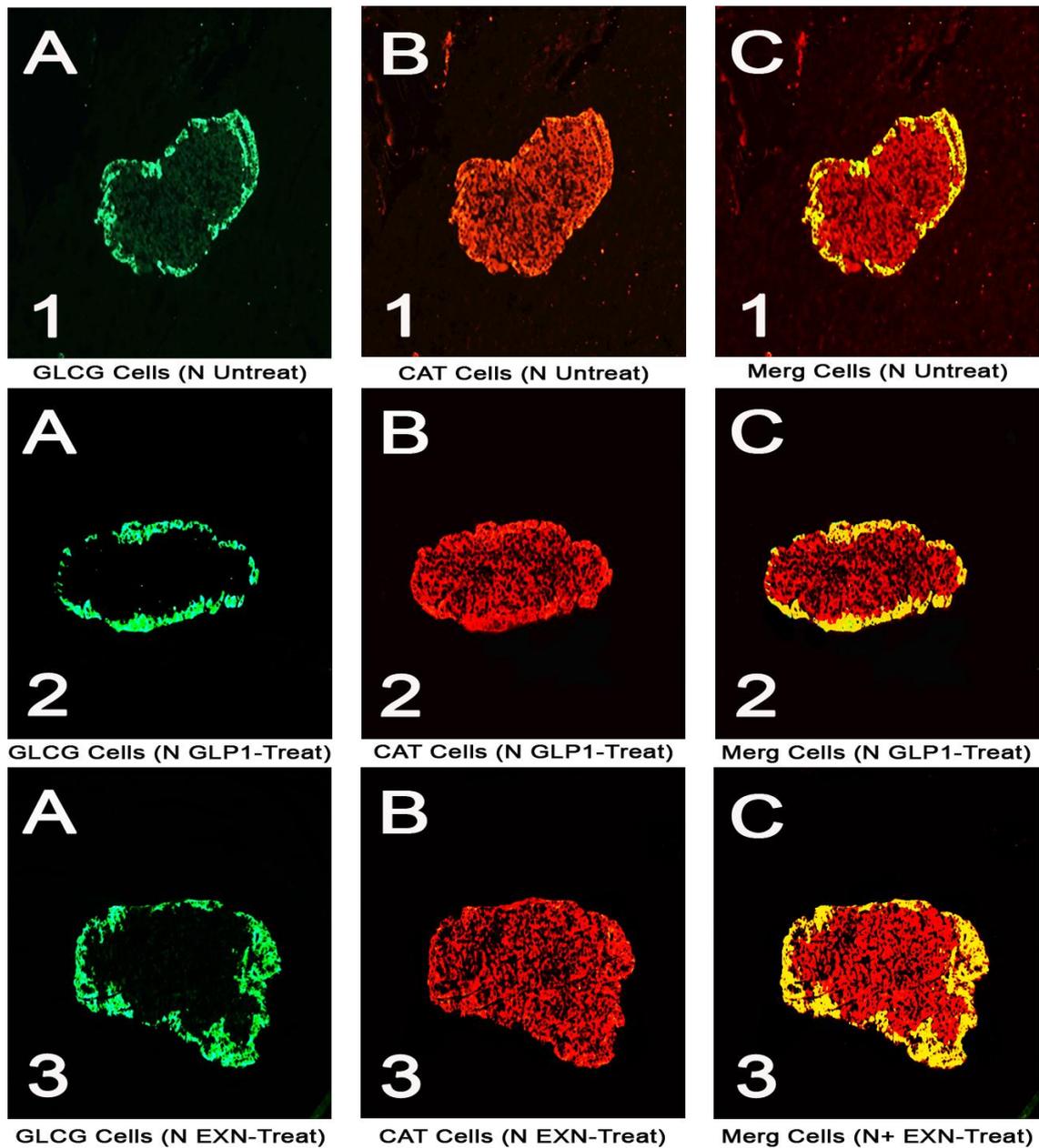


Figure 3.63: Micrographs of immunofluorescence double labelling showing the distribution of (A) glucagon cells (green) and (B) catalase cells in the pancreatic islet of normal rats while (C) shows the merging of the two cell types. The micrographs are typical of similar micrographs taken from 10 different rats in each group. Magnification: X 400. Normal rats (N), glucagon (GLCG), catalase (CAT), glucagon like peptide-1 (GLP-1), exenatide (EXN), merged cells (Merg), untreated normal rat (1), GLP-1 treated normal rats (2) and exenatide normal treated rats (3).

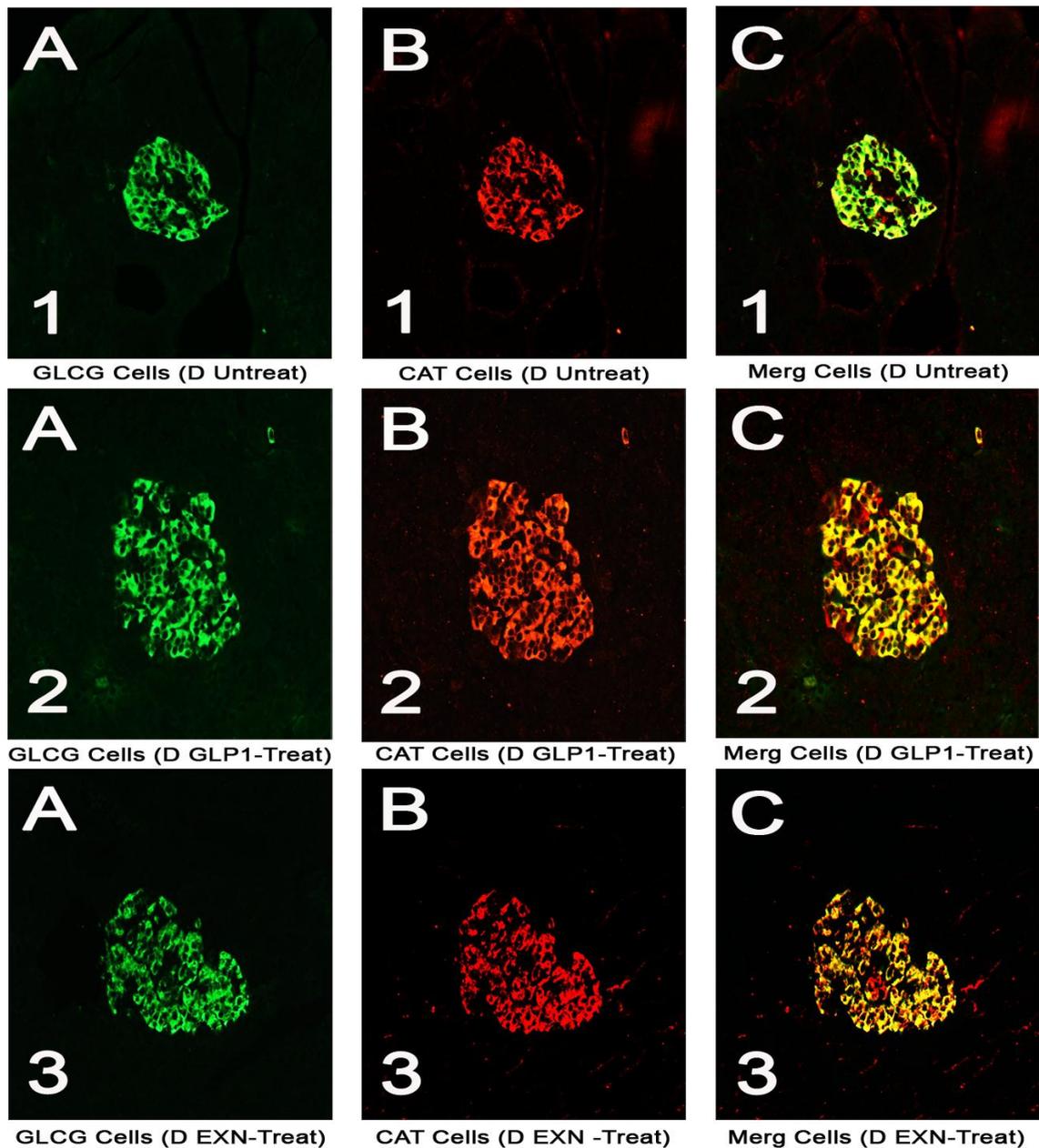


Figure 3.64: Micrographs of immunofluorescence double labelling showing the distribution of (A) glucagon cells (green) and (B) catalase cells (red) in the pancreatic islet of diabetic rats while (C) shows the merging of the two cell types. The micrographs are typical of similar micrographs taken from 10 different rats in each group. Magnification: X 400. Diabetic rats (D), glucagon (GLCG), catalase (CAT), glucagon like peptide-1 (GLP-1), exenatide (EXN), merged cells (Merg), untreated diabetic rat (1), GLP-1 treated diabetic rats (2) and exenatide diabetic treated rats (3).

3.6.15 The distribution of glucagon with glutathione reductase in the pancreatic islet of normal rats

Figure 3.65 shows the immunofluorescence double labelling images for the co-localization of glucagon (green) cells (A) and glutathione reductase (red) cells (B) and (C) the merging of the two cell types in normal rats in (1) untreated normal rat (2) GLP-1 treated normal rat (3) exenatide treated normal rat. This figure shows that glutathione reductase is co-localized with both insulin and glucagon in pancreatic islets. Some cells contain either glucagon (A1) or catalase (B1) in untreated normal rats. The number of glucagon-positive cells seemed to decrease in normal rats treated with either GLP-1 (A2) or exenatide (A3). An increase in glutathione reductase stained cells was detected in normal rats treated with either GLP-1 (B2) or exenatide (B3).

3.6.16 The distribution of glucagon with glutathione reductase in the pancreatic islet of diabetic rats

Figure 3.66 shows the immunofluorescence double labelling images for the co-localization of glucagon (green) cells (A) and glutathione reductase (red) cells (B) and (C) the merging of the two cell types in diabetic rats in (1) untreated diabetic rat (2) GLP-1 treated diabetic rat (3) exenatide treated diabetic rat. This figure shows that glutathione reductase is randomly co-localized with both insulin and glucagon in pancreatic islets. Few cells contain either glucagon (A1) or catalase (B1) in untreated diabetic rats. The number of glucagon cells seemed to decrease in diabetic rats treated with either GLP-1 (A2) or exenatide (A3). However, an increase in glutathione reductase stained cells was also detected in diabetic rats treated with either GLP-1 (B2) or exenatide (B3).

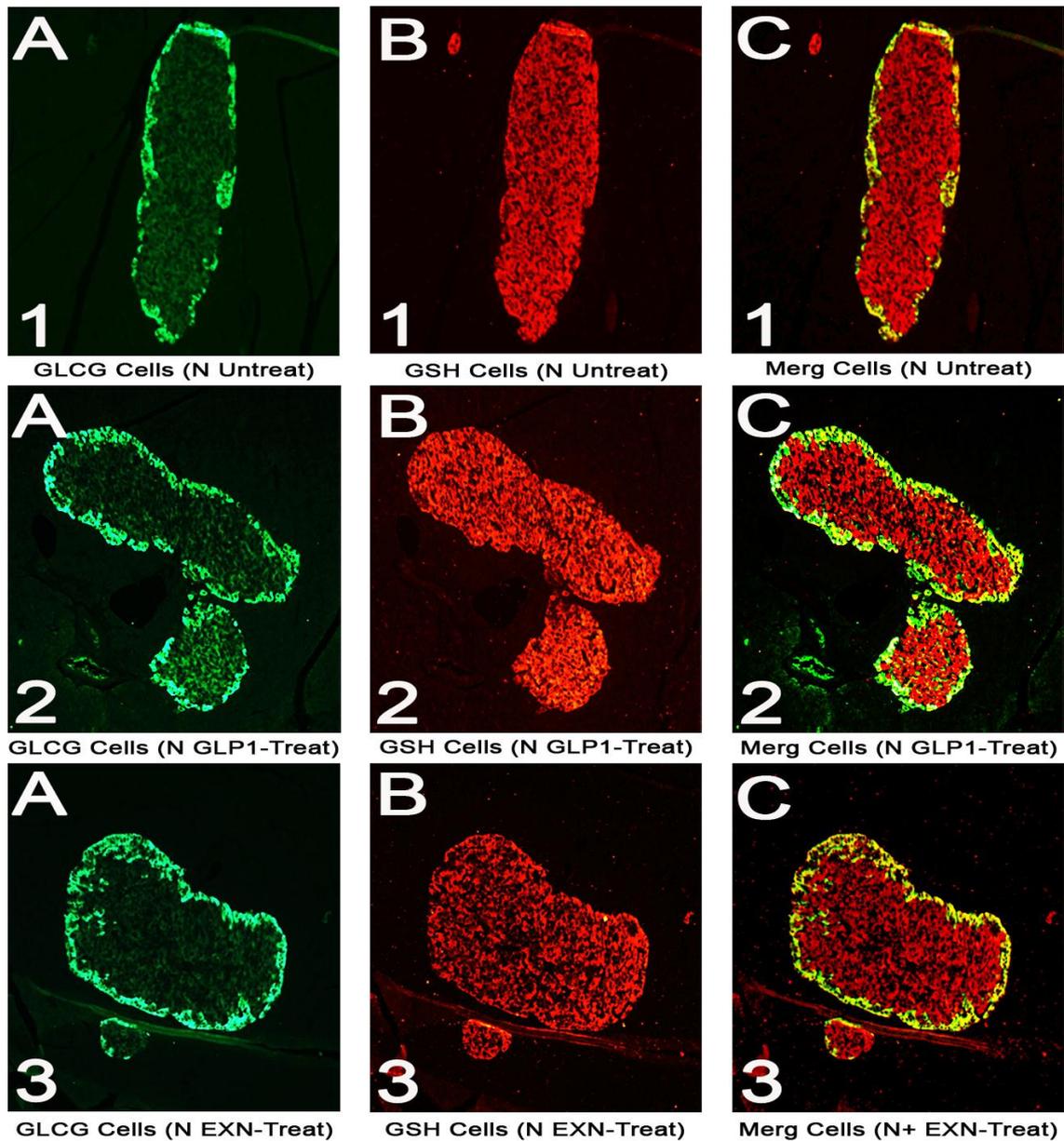


Figure 3.65: Micrographs of immunofluorescence double labelling showing the distribution of (A) glucagon cells (green) and (B) glutathione reductase cells (red) in the pancreatic islet of normal rats while (C) shows the merging of the two cell types. The micrographs are typical of similar micrographs taken from 10 different rats in each group. Magnification: X 400. Normal rats (N), glucagon (GLCG), glutathione reductase (GSH), glucagon like peptide-1 (GLP-1), exenatide (EXN), merged cells (Merg), untreated normal rat (1), GLP-1 treated normal rats (2) and exenatide normal treated rats (3).

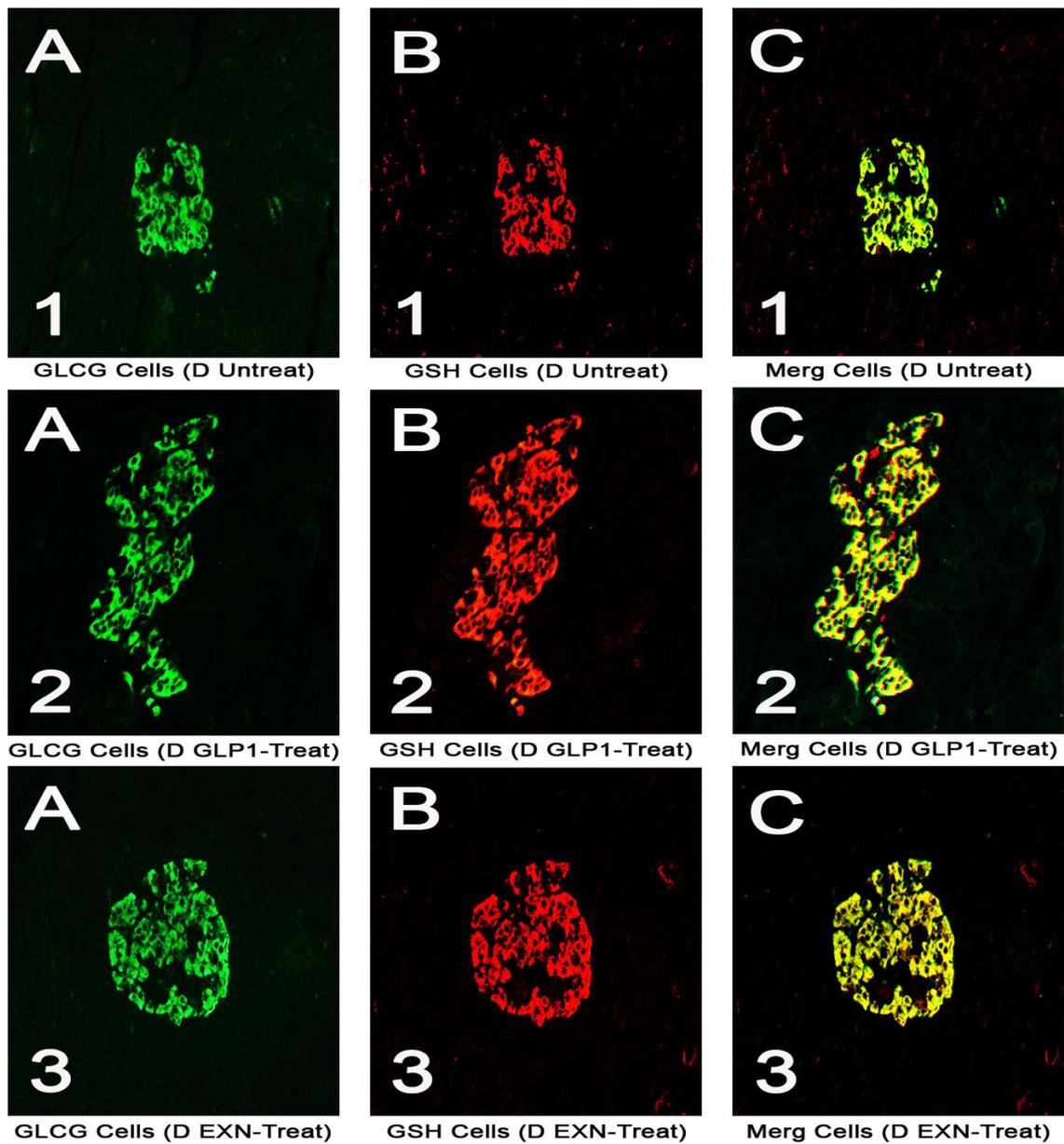


Figure 3.66: Micrographs of immunofluorescence double labelling showing the distribution of (A) glucagon cells (green) and (B) glutathione reductase cells (red) in the pancreatic islet of diabetic rats while (C) shows the merging of the two cell types. The micrographs are typical of similar micrographs taken from 10 different rats in each group. Magnification: X 400. Diabetic rats (D), glucagon (GLCG), glutathione reductase (GSH), glucagon like peptide-1 (GLP-1), exenatide (EXN), merged cells (Merg), untreated diabetic rat (1), GLP-1 treated diabetic rats (2) and exenatide diabetic treated rats (3).

3.7 Electron microscopic study

3.7.1 Electron microscopy of islet β -cells of untreated normal and diabetic rats

Figure 3.67 shows electron micrographs of β -cells in the pancreas of normal (A) and diabetic (B) rats. The results reveal that the β -cells are the most numerous cell type in the endocrine pancreas. They have secretory granules with a diameter of 400 nm. These insulin secretory granules are oval to round in shape with a dense core. The dense core of these granules is surrounded by relatively a large halo area. Other cytoplasmic organelles including mitochondria, rough and smooth endoplasmic reticulum and Golgi apparatus were dispersed between the secretory granules. The ultrastructure of pancreatic β -cells in the diabetic pancreas was displayed by loss of nuclear envelope, mitochondrial vacuolization, swelling and dilatation of the endoplasmic reticulum. It was noticed that the secretory granules were scanty and they seem to be scattered between a large numbers of empty vesicles and moreover, they are significantly fewer than those found in normal rat pancreas.

3.7.2 Electron microscopy of islet β -cells in normal and diabetic rats treated with GLP-1

Figure 3.68 shows electron micrographs of β -cells in the pancreas of GLP-1 treated normal rats (A). These β -cells show many intact and abundant secretory granules. In contrast, the β -cells of diabetic rats (B) treated with GLP-1 show few secretory granules. From this figure, it can be noted that, treatment of normal and diabetic rats with GLP-1 increased the number of insulin secretory granules and GLP-1 was more effective in increasing the number of insulin secretory granules in the pancreas of normal rats compared to diabetic rats.

3.7.3 Electron microscopy of the islet β -cells of diabetic and normal rats treated with exenatide

Figure 3.69 shows the electron micrographs of β -cells in the pancreas of exenatide treated normal rats (A) and (B) exenatide treated diabetic rats. The results show that

many intact and abundant secretory granules were found in β -cells of exenatide treated normal rats. In contrast, in diabetic rats treated with exenatide, there were fewer secretory granules. From this figure, it can be noted that, treatment of normal and diabetic rats with exenatide resulted in an increase in the number of insulin secretory granules. However, this increase was much more pronounced in the β -cells of exenatide treated normal rats compared to islet cells of diabetic rats treated with exenatide.

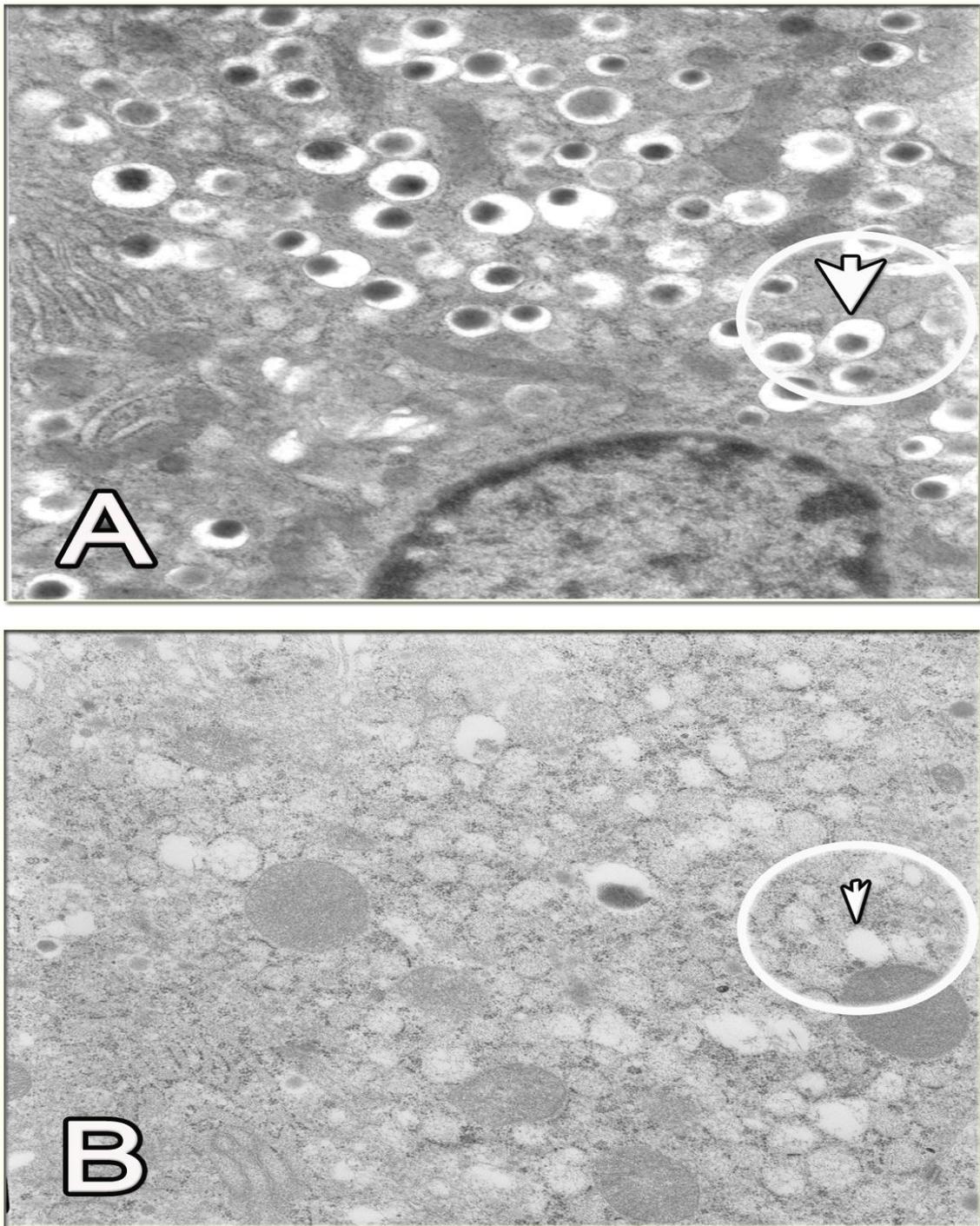


Figure 3.67: Electron micrographs showing (A) β -cell of normal untreated rats with intact secretory granules (large arrow) and (B) β -cell of untreated diabetic rat with an empty secretory granule (small arrow). The electron micrographs are typical of 10 such different experiments taken from 10 different rats in each group. Magnification: X 14,000.

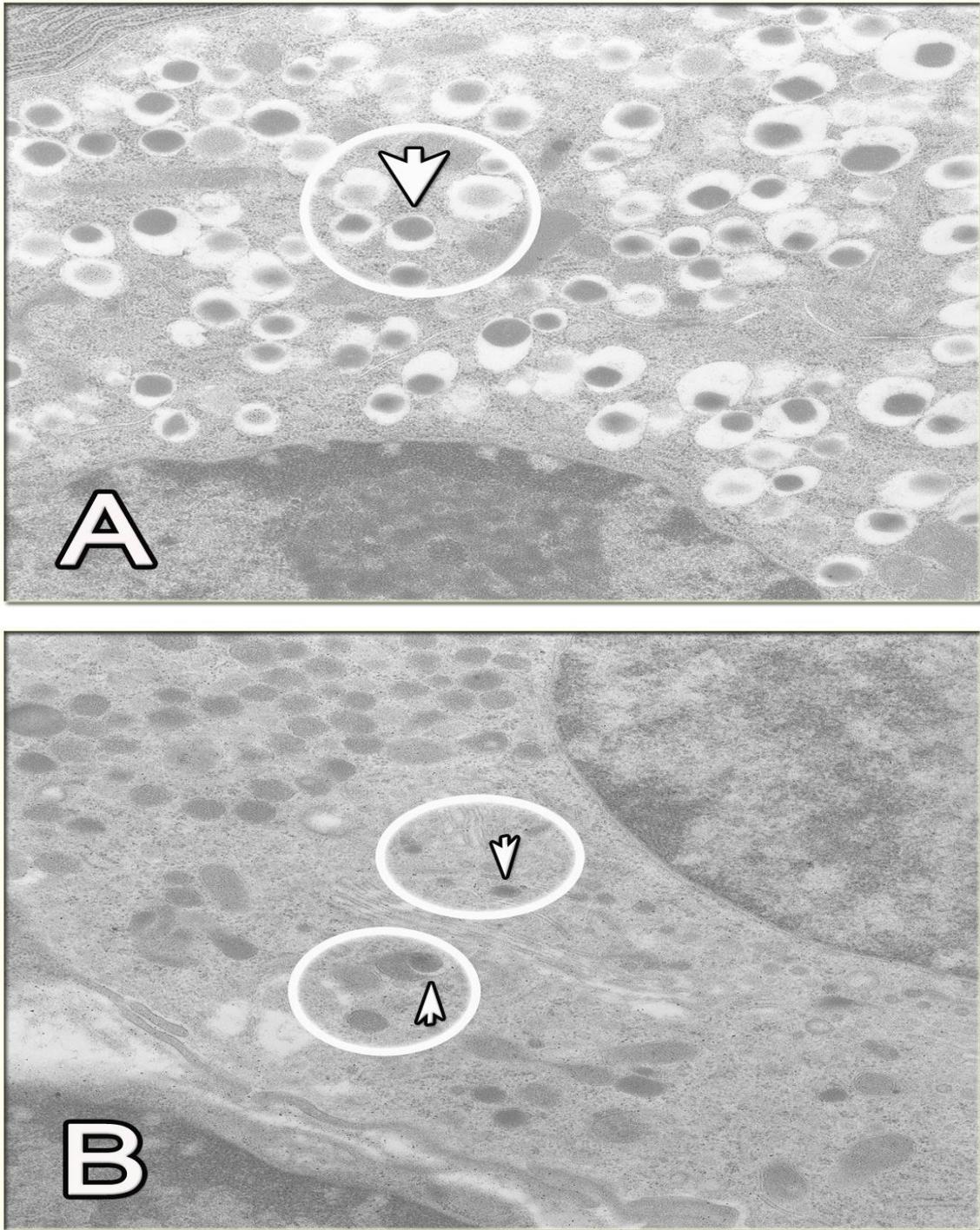


Figure 3.68: Electron micrographs showing (A) β -cell of GLP-1 treated normal rats with intact secretory granule (large arrow) and (B) β -cell of GLP-1 treated diabetic rats with some secretory granules (small arrow). The electron micrographs are typical of 10 such different experiments taken from 10 different rats in each group. Magnification: X 14,000.

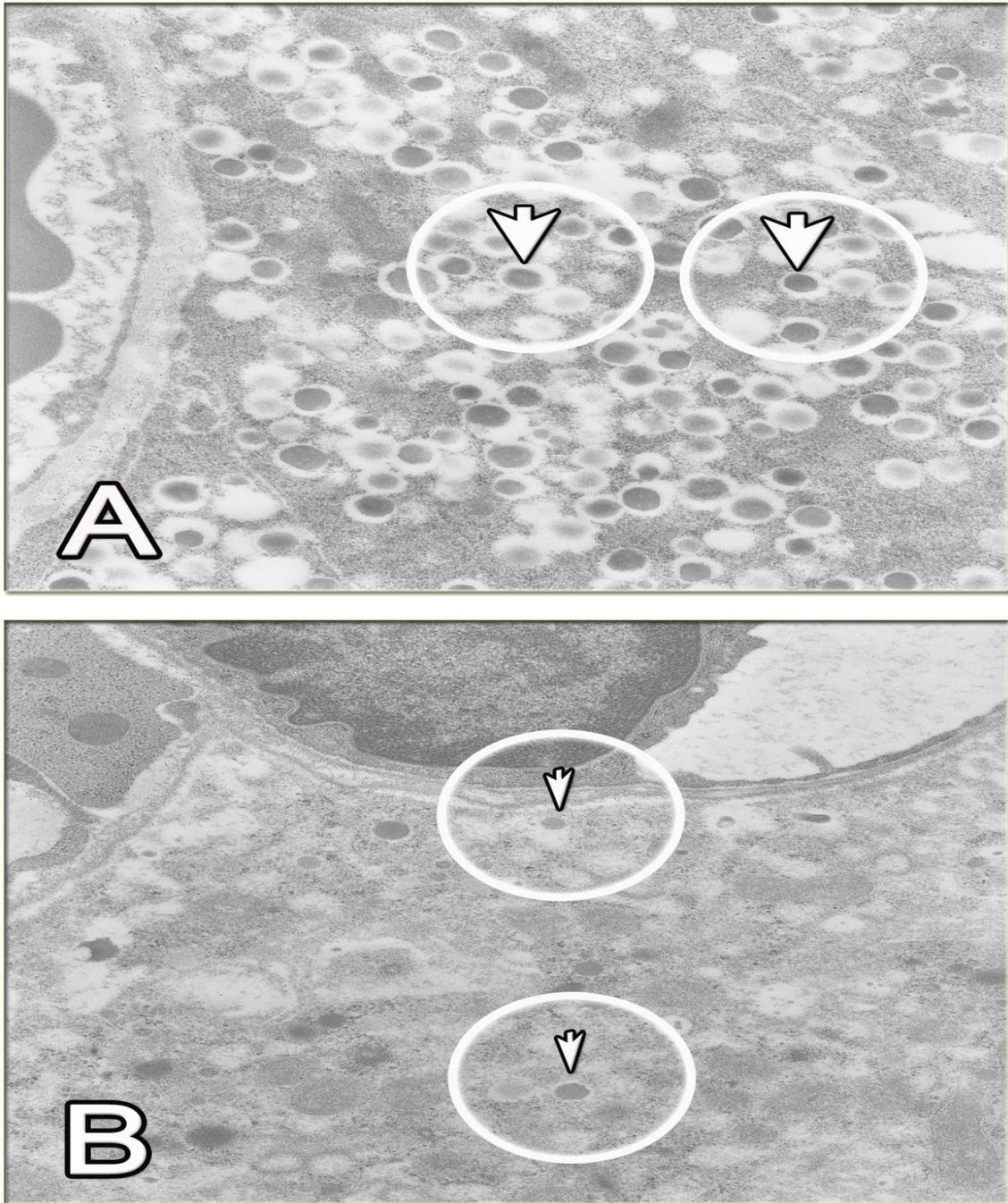


Figure 3.69: Electron micrographs showing (A) β -cell of exenatide treated normal rats with a large number of intact secretory granules after exenatide treatment (large arrows) and (B) β -cell of exenatide treated diabetic rats with some secretory granules (small arrows) after exenatide treatment. The electron micrographs are typical of 10 such different experiments taken from 10 different rats in each group. Magnification: X 14,000.

3.7.4 Immunogold double labelling of insulin and GLP-1 in β -cells of pancreatic islets of normal and diabetic rats

Figure 3.70 shows immunoelectron microscopy of insulin and GLP-1 in pancreatic β -cells of normal (A) and diabetic (B) rats. The number of insulin secretory granules in normal rats was much greater than in diabetic rats. In addition, normal and diabetic rats showed insulin secretory granules (20 nm gold particles) with GLP-1 (15 nm gold particles). This indicates that insulin and GLP-1 are co-localized in pancreatic β -cells.

3.7.5 Immunogold double labelling of insulin and exenatide in β -cells of pancreatic islets of normal and diabetic rats

Figure 3.71 shows immunoelectron microscopy of insulin and exenatide in pancreatic β -cells of normal (A) and diabetic (B) rats. The number of insulin secretory granules in normal rats was much greater than in diabetic rats. Normal and diabetic rats also showed insulin secretory granules (20 nm gold particles) with exenatide (15 nm gold particles). This indicates that insulin and exenatide are co-localized pancreatic β -cells.

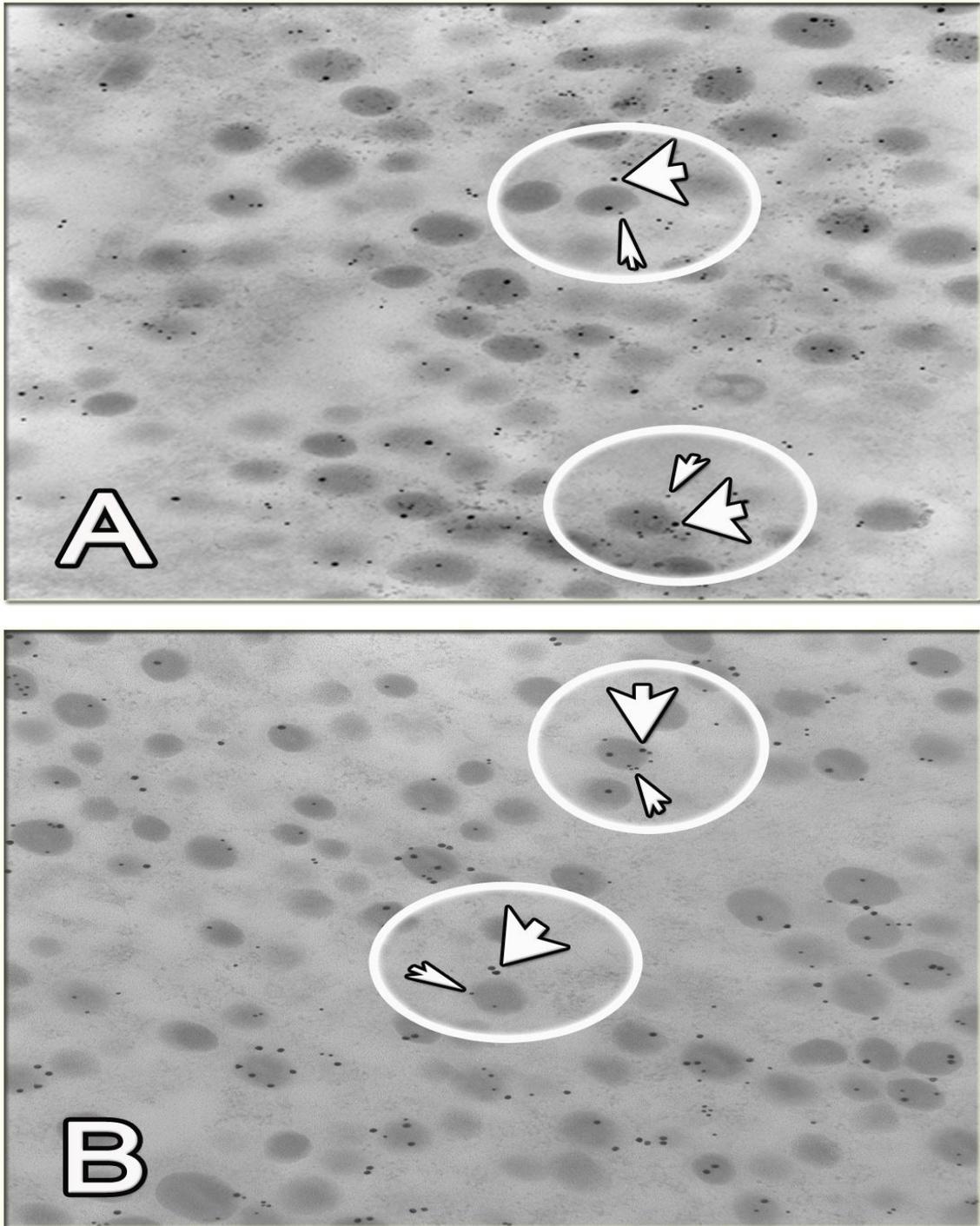


Figure 3.70: Electron micrographs showing immunogold labelling of insulin secretory granules in β -cells of (A) normal and (B) diabetic rats. Large arrows in (A) and (B) show insulin granules (20 nm gold particles) with GLP-1 (15 nm gold particles) small arrows. The electron micrographs are typical of 10 such different experiments taken from 10 different rats in each group. Magnification: X 27,500.

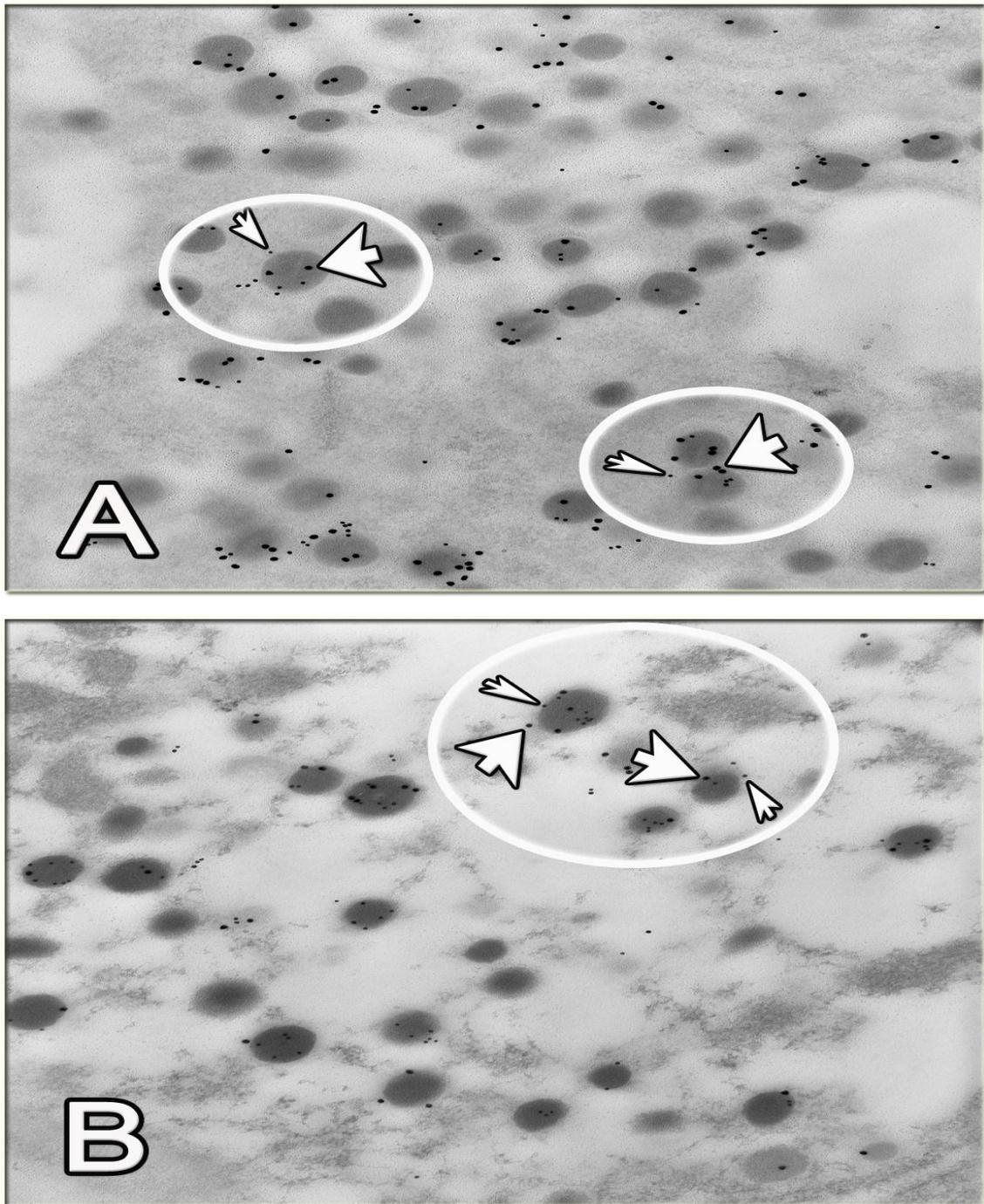


Figure 3.71: Electron micrographs showing immunogold labelling of β -cells in (A) normal and (B) diabetic rats. Large arrows in (A) and (B) show insulin granules (20 nm gold particles) with exenatide (15 nm gold particles) small arrows. The electron micrographs are typical of 10 such different experiments taken from 10 different rats in each group. Magnification: X 27,500.

3.7.6 Immunogold double labelling of glucagon and GLP-1 in α -cells of pancreatic islets of normal and diabetic rats

Figure 3.72 shows immunogold double labelling of glucagon and GLP-1 in α -cells of normal (A) and diabetic (B) rats. The results show that the number of glucagon secretory granules in normal rats was much less than that of diabetic rats. The data also show that the pancreatic α -cells of normal and diabetic rats have glucagon secretory granules (20 nm gold particles) with GLP-1 (15 nm gold particles). This indicates that glucagon and GLP-1 are co-localized in pancreatic α -cell.

3.7.7 Immunogold double labelling of glucagon and exenatide in α -cells of pancreatic islets of normal and diabetic rats

Figure 3.73 shows immunogold double labelling of glucagon and exenatide in α -cells of pancreatic islets of normal (A) and diabetic (B) rats. The number of glucagon secretory granules in normal rats was much less than diabetic rats. The results also reveal that normal and diabetic rats have glucagon secretory granules (20 nm gold particles) with exenatide (15 nm gold particles). This indicates that glucagon and exenatide are co-localized in pancreatic α -cells.

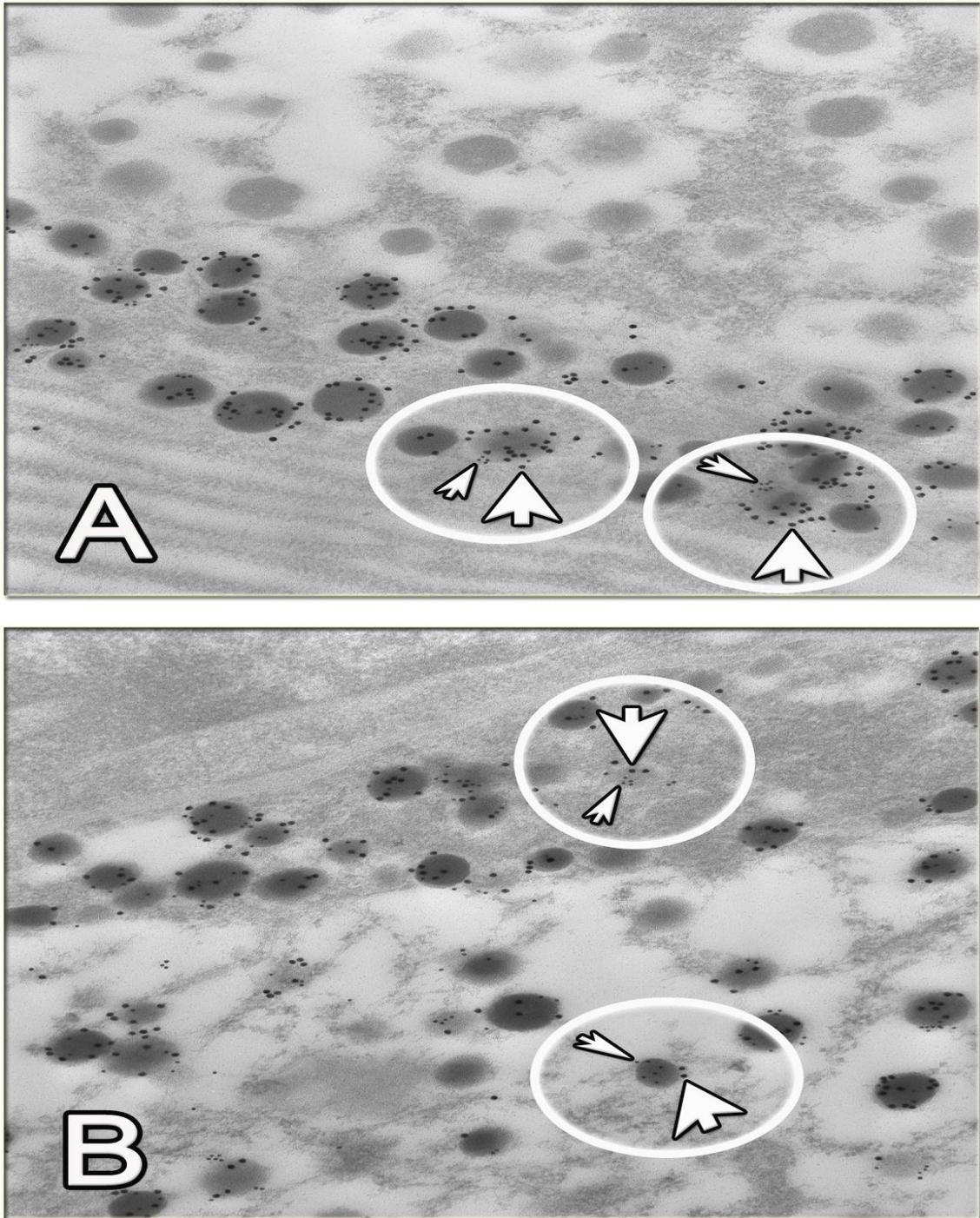


Figure 3.72: Electron micrographs showing immunogold labelling of α -cells of pancreatic islets in (A) normal and (B) diabetic rats. Large arrows in (A) and (B) show glucagon granules (20 nm gold particles) with GLP-1 (15 nm gold particles) small arrows. The electron micrographs are typical of 10 such different experiments taken from 10 different rats in each group. Magnification: X 27,500.

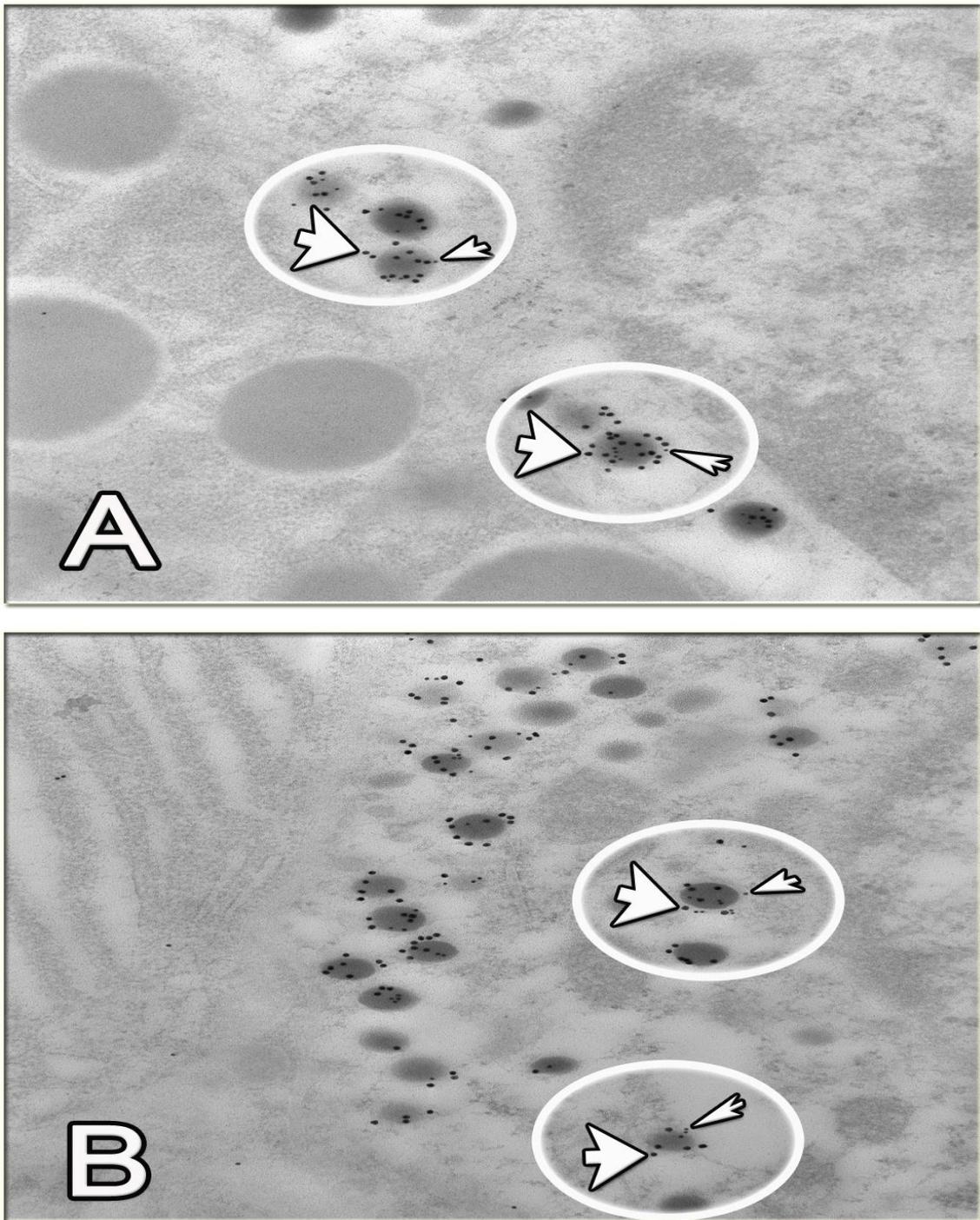


Figure 3.73: Electron micrographs showing immunogold labelling of α -cells of pancreatic islets in (A) normal and (B) diabetic rats. Large arrows in (A) and (B) show glucagon granules (20 nm gold particles) with exenatide (15 nm gold particles) small arrows. The electron micrographs are typical of 10 such different experiments taken from 10 different rats in each group. Magnification: X 27,500.

3.8 The effect of treatment with either GLP-1 or exenatide on gene expression in normal and diabetic rats.

3.8.1 The effect of treatment with either GLP-1 or exenatide on pancreatic duodenal homeobox-1 (PDX-1) gene expression in normal and diabetic rats.

Figure 3.74 shows the pancreatic duodenal homeobox-1 mRNA levels in normal rats treated with either GLP-1 (50 nmol/kg body weight) or exenatide (1 µg/kg body weight) compared to normal untreated rats, measured by relative real-time PCR and normalized to β-actin expression, the reference housekeeping gene. The results show that, there was a significant ($p < 0.05$) increase in the levels of pancreatic duodenal homeobox-1 (PDX-1) mRNA in normal rats treated with either GLP-1 or exenatide compared to normal untreated rats.

Figure 3.75 shows the pancreatic duodenal homeobox-1 mRNA levels in diabetic rats treated with either GLP-1 or exenatide compared to untreated diabetic rats, measured by relative real-time PCR and normalized to β-actin expression, the reference housekeeping gene. The results show that, there was a small but significant ($p < 0.05$) increase in the levels of PDX-1 mRNA in diabetic rats treated with GLP-1 compared to diabetic untreated rats. Similarly, there was a marked and highly significant ($p < 0.001$) increase in the levels of PDX-1 mRNA in diabetic rats treated with exenatide compared to diabetic untreated rats.

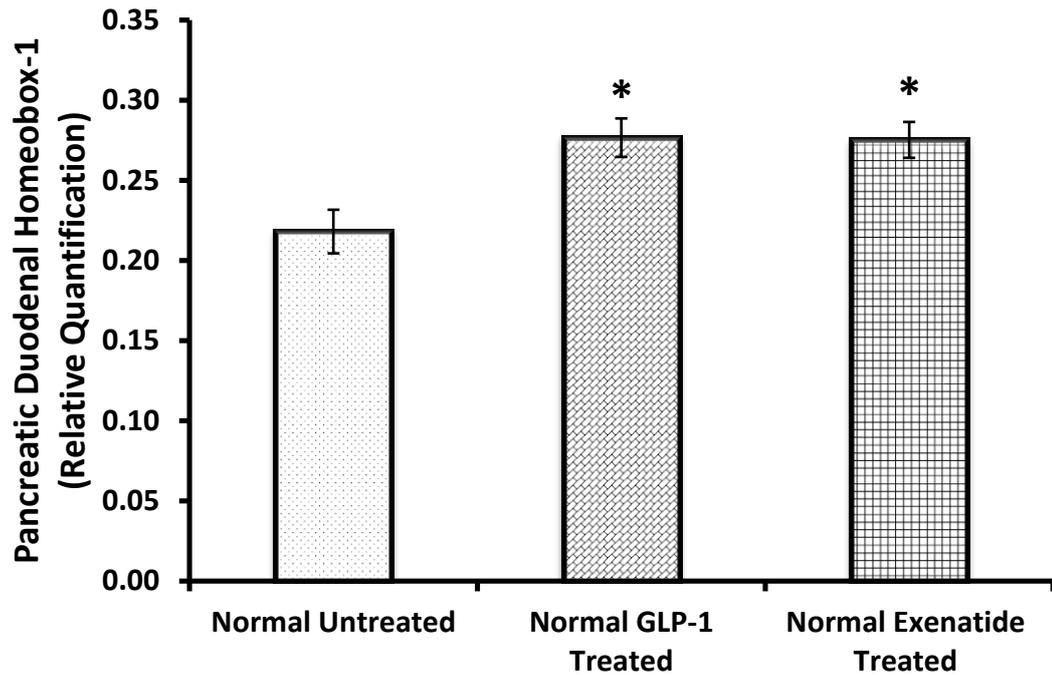


Figure 3.74: Histograms showing the effect of 10 weeks of treatment with either GLP-1 (50 nmol/kg body weight) or exenatide (1 μ g/kg body weight) on mRNA expression of pancreatic duodenal homeobox-1 in treated normal rats compared to normal untreated rats. The level of mRNA expression was measured by relative real-time PCR following normalization for β -actin mRNA in each sample. * $P < 0.05$ for both GLP-1 and exenatide treated normal rats compared to normal untreated rats. Note that the significant increase in gene expression after either GLP-1 or exenatide treatment. Data are mean \pm SEM, $n = 10$ rats for each point.

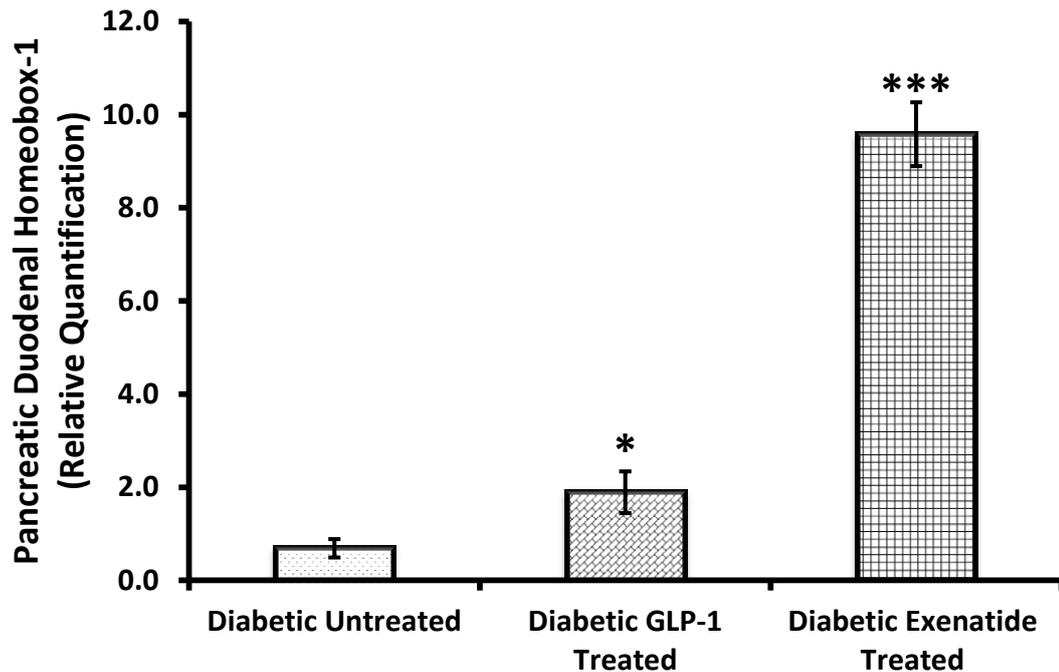


Figure 3.75: Histograms showing the effect of 10 weeks of treatment with either GLP-1 (50 nmol/kg body weight) or exenatide (1 μ g/kg body weight) on mRNA expression of pancreatic duodenal homeobox-1 in treated diabetic rats compared to untreated diabetic rats. Level of mRNA expression was measured by relative real-time PCR following normalization for β -actin mRNA in each sample. * $P < 0.05$ and *** $P < 0.001$ for both GLP-1 and exenatide, respectively, treated diabetic rats compared to untreated diabetic rats. Note that exenatide elicited stronger effect compared to GLP-1 action. Data are mean \pm SEM, $n = 10$ rats for each point.

3.8.2 The effect of treatment with either GLP-1 or exenatide on heat shock protein-70 (HSP-70) gene expression in normal and diabetic rats.

Figure 3.76 shows the heat shock protein-70 mRNA levels in normal rats treated with either GLP-1 or exenatide compared to normal untreated rats measured by relative real-time PCR and normalized to β -actin expression, the reference housekeeping gene. The results show that, there was a significant ($p < 0.05$) increase in the levels of heat shock protein-70 mRNA in normal rats treated with either GLP-1 or exenatide compared to normal untreated rats.

Figure 3.77 shows the levels of heat shock protein-70 mRNA in diabetic rats treated with either GLP-1 or exenatide compared to diabetic untreated rats measured by relative real-time PCR and normalized to β -actin expression, the reference housekeeping gene. The results show that there was a significant ($P < 0.05$; $P < 0.01$) increase in heat shock protein-70 mRNA levels in diabetic rats treated with either GLP-1 or exenatide, respectively, compared to untreated diabetic rats. The effect of exenatide was much more pronounced compared to that of GLP-1.

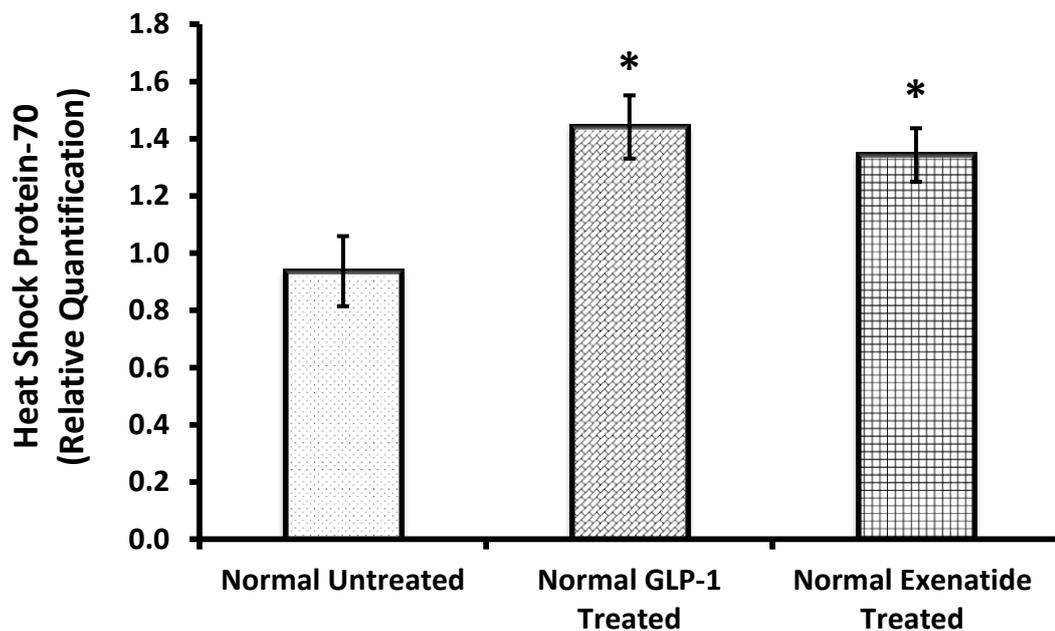


Figure 3.76: Histograms showing the effect of 10 weeks of treatment with either GLP-1 (50 nmol/kg body weight) or exenatide (1 μ g/kg body weight) on levels of mRNA expression of heat shock protein-70 in treated normal rats compared to untreated normal rats. Level of mRNA expression was measured by relative real-time PCR following normalization for β -actin mRNA in each sample. * $P < 0.05$ for both GLP-1 and exenatide, treated normal rats compared to normal, untreated rats. Note that both GLP-1 and exenatide can evoke significant increases in HSP-70 gene expression in the pancreas of normal treated rats compared to untreated normal rats. Data are mean \pm SEM, $n = 10$ rats for each point.

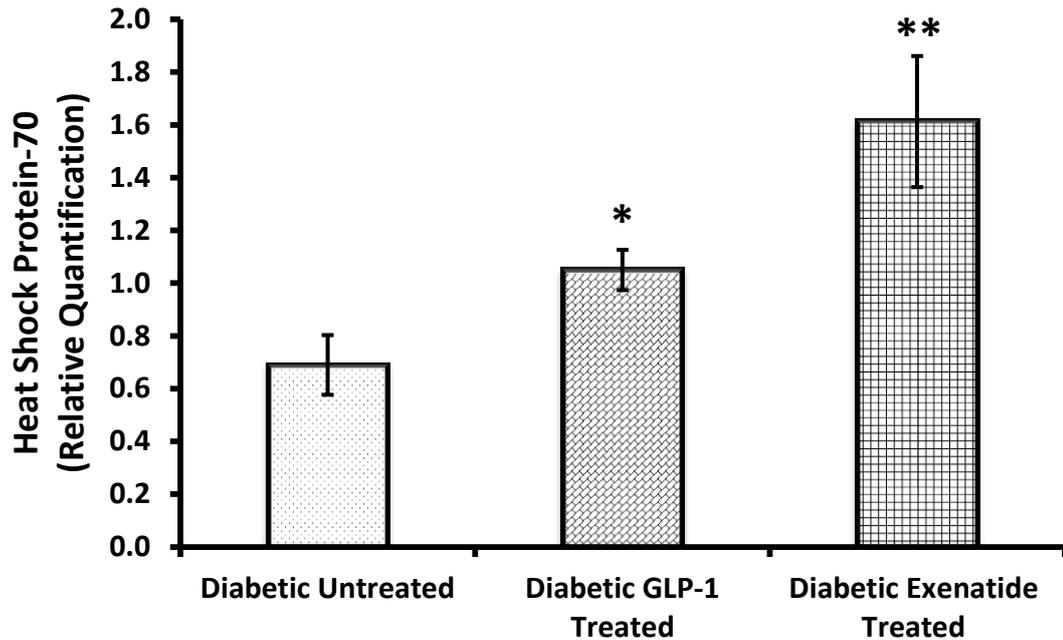


Figure 3.77: Histograms showing the effect of 10 weeks of treatment with either GLP-1 (50 nmol/kg body weight) or exenatide (1 μ g/kg body weight) on levels of mRNA expression of heat shock protein-70 in treated diabetic rats compared to untreated diabetic rats. Level of mRNA expression was measured by relative real-time PCR following normalization for β -actin mRNA in each sample. * $P < 0.05$ and ** $P < 0.01$ for both GLP-1 and exenatide, respectively, treated diabetic rats compared to untreated diabetic rats. Note that exenatide was more effective in enhancing HSP-70 mRNA expression compared to GLP-1. Data are mean \pm SEM, $n = 10$ rats for each point.

3.8.3 *The effect of treatment with either GLP-1 or exenatide on glutathione peroxidase (GPx) gene expression in normal and diabetic rats.*

Figure 3.78 shows the levels of glutathione peroxidase mRNA in normal rats treated with either GLP-1 or exenatide compared to untreated normal rats measured by relative real-time PCR and normalized to β -actin expression, the reference housekeeping gene. The results show that both GLP-1 and exenatide can, respectively, evoke significant ($p < 0.05$; $p < 0.01$) increases in glutathione peroxidase mRNA expression in the pancreas of normal rats compared to untreated normal rats. The effect of GLP-1 was more

pronounced compared to exenatide on glutathione peroxidase mRNA expression in treated normal rats.

Figure 3.79 shows the levels of glutathione peroxidase mRNA expression in diabetic rats treated with either GLP-1 or exenatide compared to untreated diabetic rats measured by relative real-time PCR and normalized to β -actin expression, the reference housekeeping gene. The results show a significant ($p < 0.01$) increase in the levels of glutathione peroxidase mRNA levels in diabetic rats treated with either GLP-1 or exenatide compared to untreated diabetic rats.

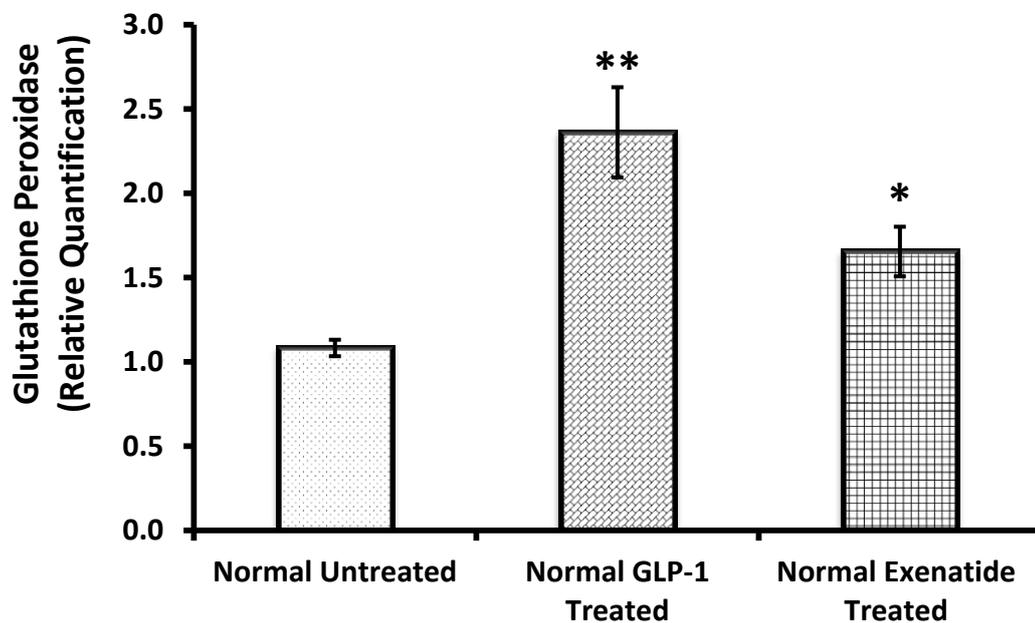


Figure 3.78: Histograms showing the effect of 10 weeks of treatment with either GLP-1 (50 nmol/kg body weight) or exenatide (1 μ g/kg body weight) on the levels of mRNA expression of glutathione peroxidase in normal treated rats compared to normal untreated rats. Level of mRNA expression was measured by relative real-time PCR following normalization for β -actin mRNA in each sample. ** $P < 0.01$ for GLP-1 and * $P < 0.05$ for exenatide treated normal rats compared to normal untreated rats. Note that the increase in gene expression after GLP-1 treatment compared to exenatide. Data are mean \pm SEM, $n = 10$ rats for each point.

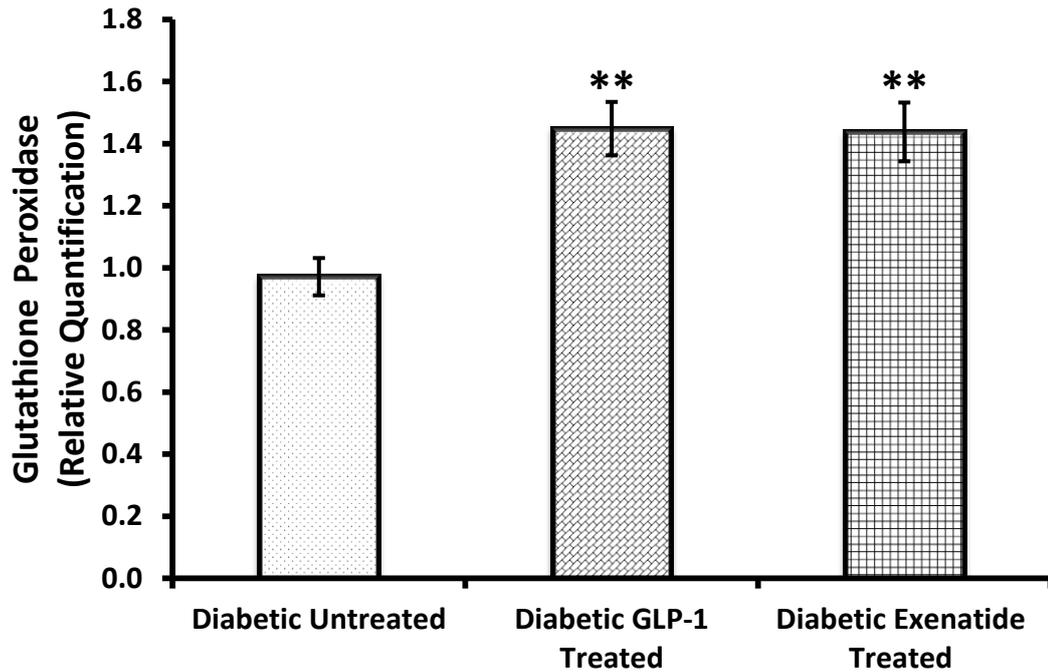


Figure 3.79: Histograms showing the effect of 10 weeks of treatment with either GLP-1 (50 nmol/kg body weight) or exenatide (1 µg/kg body weight) on the levels of mRNA expression of glutathione peroxidase in treated diabetic rats compared to untreated diabetic rats. Level of mRNA expression was measured by relative real-time PCR following normalization for β-actin mRNA in each sample. ** P < 0.01 for both GLP-1 and exenatide, treated diabetic rats compared to untreated diabetic rats. Data are mean ± SEM, n = 10 rats for each point.

3.8.4 The effect of treatment with either GLP-1 or exenatide on glucagon gene expression in normal and diabetic rats.

Figure 3.80 shows the levels of glucagon mRNA in normal rats treated with either GLP-1 or exenatide compared to normal untreated rats measured by relative real-time PCR and normalized to β-actin expression, the reference housekeeping gene. The results show that there was a significant (p < 0.01; p < 0.001) decrease for GLP-1 exenatide, respectively, in the levels of glucagon mRNA expression in normal treated rats compared to normal untreated rats.

Figure 3.81 shows the levels of glucagon mRNA in diabetic rats treated with either GLP-1 or exenatide compared to diabetic untreated rats measured by relative real-time PCR and normalized to β -actin expression, the reference housekeeping gene. The results show that there was a significant ($p < 0.001$; $p < 0.05$) decrease for GLP-1 exenatide, respectively, in the levels of glucagon mRNA in diabetic treated rats compared to diabetic untreated rats. However, the reduction in glucagon mRNA levels in diabetic rats treated with GLP-1 was more pronounced than exenatide treatment.

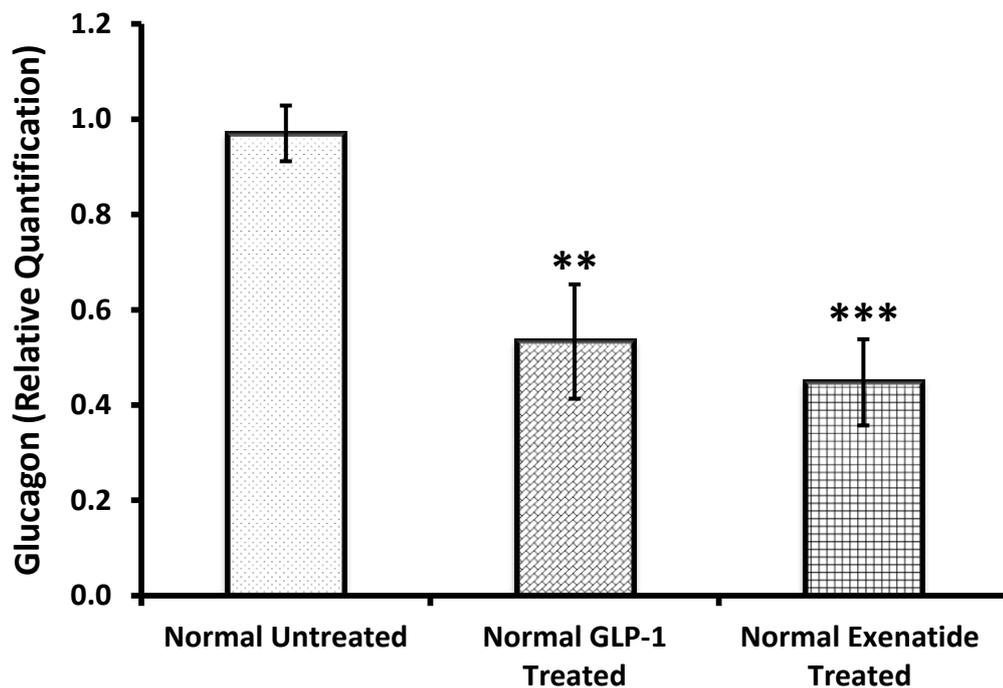


Figure 3.80: Histograms showing the effect of 10 weeks of treatment with either GLP-1 (50 nmol/kg body weight) or exenatide (1 μ g/kg body weight) on the levels of mRNA expression of glucagon in normal treated rats compared to normal untreated rats. Level of mRNA expression was measured by relative real-time PCR following normalization for β -actin mRNA in each sample. ** $P < 0.01$ for GLP-1 and *** $P < 0.001$ for exenatide treated normal rats compared to normal untreated rats. Data are mean \pm SEM, $n = 10$ rats for each point.

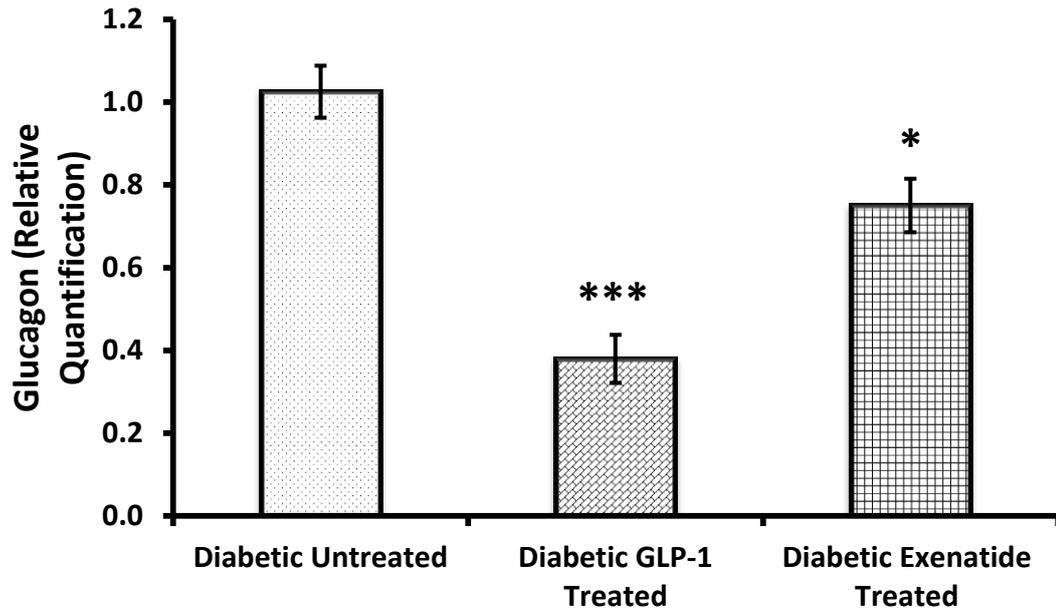


Figure 3.81: Histograms showing the effect of 10 weeks of treatment with either GLP-1 (50 nmol/kg body weight) or exenatide (1 μ g/kg body weight) on the levels of mRNA expression of glucagon in treated diabetic rats compared to untreated diabetic rats. Level of mRNA expression was measured by relative real-time PCR following normalization for β -actin mRNA in each sample. *** $P < 0.001$ for GLP-1 and * $P < 0.05$ for exenatide treated diabetic rats compared to untreated diabetic rats. Note that GLP-1 treatment was more effective in inhibiting glucagon mRNA expression compared to exenatide. Data are mean \pm SEM, $n = 10$ rats for each point.

3.8.5 The effect of treatment with either GLP-1 or exenatide on insulin receptor (IR) gene expression in normal and diabetic rats.

Figure 3.82 shows the levels of insulin receptor mRNA in normal rats treated with either GLP-1 or exenatide compared to normal untreated rats as measured by relative real-time PCR and normalized to β -actin expression, the reference housekeeping gene. The results show that there were increases in insulin receptor mRNA levels in normal rats treated with either GLP-1 or exenatide compared to untreated normal rats. However, there was a significant ($p < 0.05$) increase in insulin receptor mRNA expression for exenatide treated rats compared to untreated normal rats.

Figure 3.83 shows the levels of insulin receptor mRNA in diabetic rats treated with either GLP-1 or exenatide compared to diabetic untreated rats as measured by relative real-time PCR and normalized to β -actin expression, the reference housekeeping gene. There was a significant ($p < 0.01$) increase in insulin receptor mRNA levels in diabetic rats treated with either GLP-1 or exenatide compared to untreated diabetic rats. Both GLP-1 and exenatide seem to exert similar elevation in insulin receptor mRNA expression in treated diabetic rats.

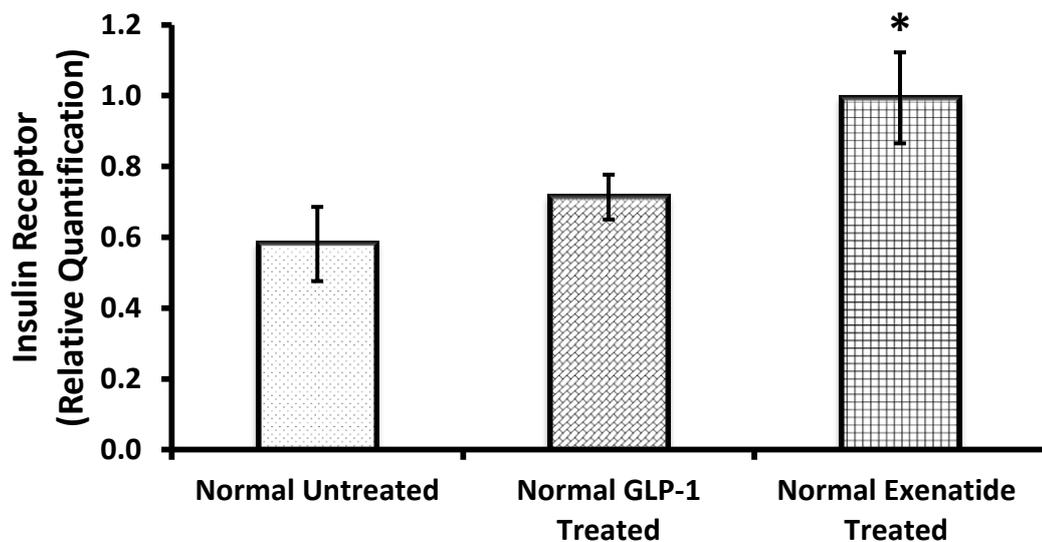


Figure 3.82: Histograms showing the effect of 10 weeks of treatment with either GLP-1 (50 nmol/kg body weight) or exenatide (1 μ g/kg body weight) on levels of mRNA expression of insulin receptor in normal treated rats compared to normal untreated rats. Level of mRNA expression was measured by relative real-time PCR following normalization for β -actin mRNA in each sample. * $P < 0.05$ for exenatide treated normal rats compared to normal untreated rats. GLP-1 was able to cause a small but not significant increase in insulin receptor mRNA. In contrast, exenatide induced significant expression of insulin receptor mRNA compared to normal untreated rats. Data are mean \pm SEM, $n = 10$ rats for each point.

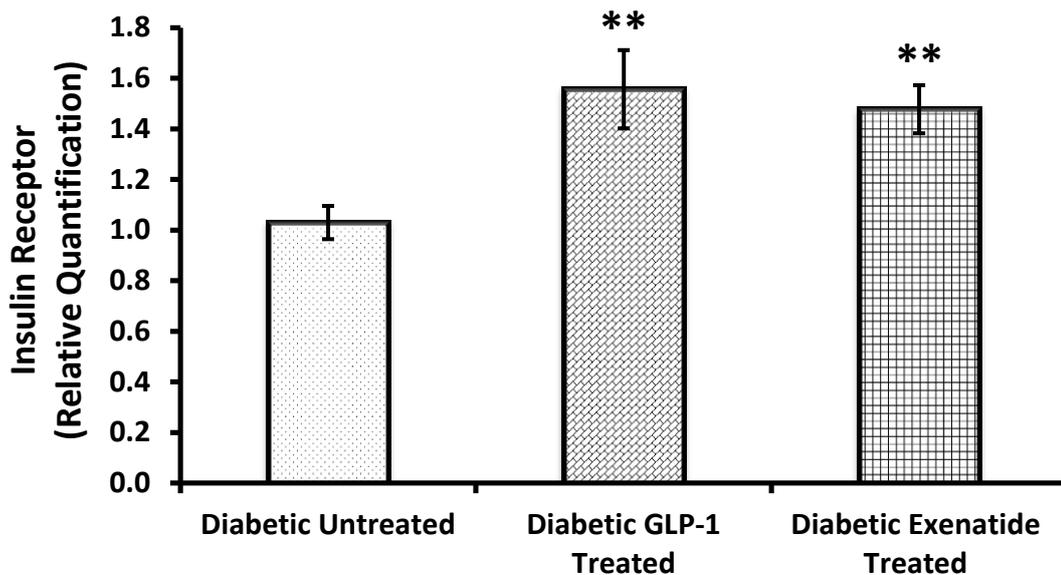


Figure 3.83: Histograms showing the effect of 10 weeks of treatment with either GLP-1 (50 nmol/kg body weight) or exenatide (1 μ g/kg body weight) on levels of mRNA expression of insulin receptor in treated diabetic rats compared to untreated diabetic rats. Level of mRNA expression was measured by relative real-time PCR following normalization for β -actin mRNA in each sample. ** $P < 0.01$ for both GLP-1 and exenatide treated diabetic rats compared to untreated diabetic rats. Note that both GLP-1 and exenatide produced more or less similar effect on insulin receptor mRNA expression. Data are mean \pm SEM, $n = 10$ rats for each point.

3.8.6 The effect of treatment with either GLP-1 or exenatide on glucagon like peptide-1 receptor (GLP-1R) gene expression in normal and diabetic rats.

Figure 3.84 shows the levels of GLP-1R mRNA in normal rats treated with either GLP-1 or exenatide compared to normal untreated rats measured by relative real-time PCR and normalized to β -actin expression, the reference housekeeping gene. The results show that there was a little but not significant increase in GLP-1R mRNA levels in normal rats treated with GLP-1 compared to normal untreated rats. However, in normal rats treated with exenatide there was a significant ($p < 0.05$) increase in GLP-1R mRNA expression compared to normal untreated rats.

Figure 3.85 shows the levels of GLP-1R mRNA in diabetic rats treated with either GLP-1 or exenatide compared to diabetic untreated rats measured by relative real-time PCR and normalized to β -actin expression, the reference housekeeping gene. The results reveal a significant increase ($p < 0.001$) in glucagon like peptide-1 receptor mRNA levels in GLP-1 and exenatide treated diabetic rats compared to untreated diabetic rats. The effect of exenatide treatment was more prominent than that of GLP-1.

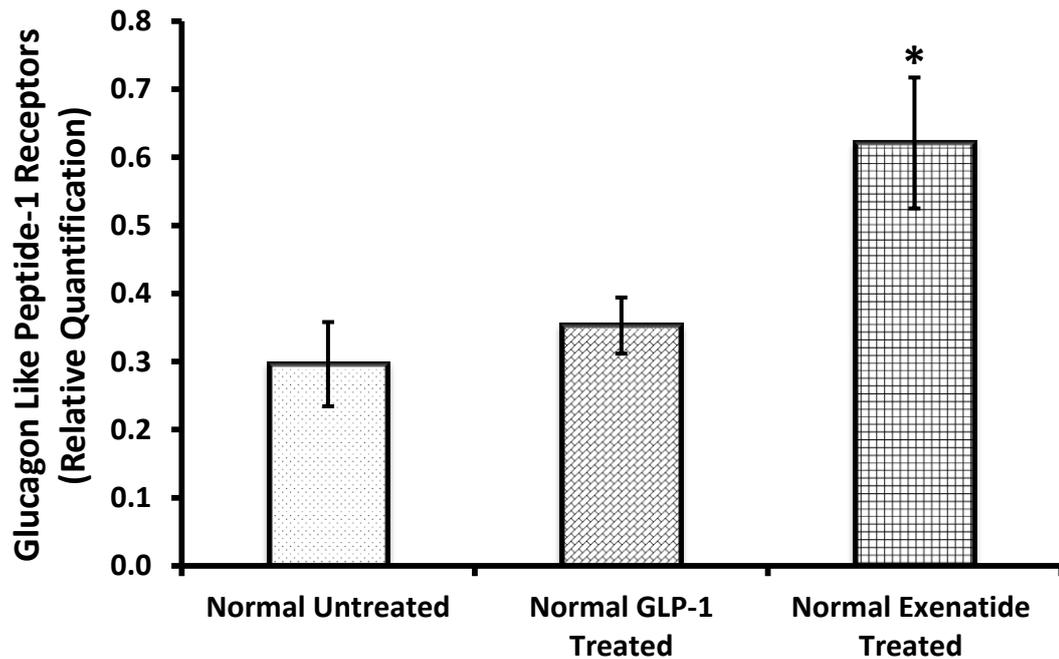


Figure 3.84: Histograms showing the effect of 10 weeks of treatment with either GLP-1 (50 nmol/kg body weight) or exenatide (1 μ g/kg body weight) on levels of mRNA expression of GLP-1 receptor in normal treated rats compared to normal untreated rats. Level of mRNA expression was measured by relative real-time PCR following normalization for β -actin mRNA in each sample. Note that the increase in GLP-1 receptor mRNA expression after GLP-1 treatment was not significant compared to the normal untreated rats. In contrast, exenatide evoked significant ($p < 0.05$) increase in glucagon like peptide-1 receptor mRNA expression compared to normal untreated rats. Data are mean \pm SEM, $n = 10$ rats for each point.

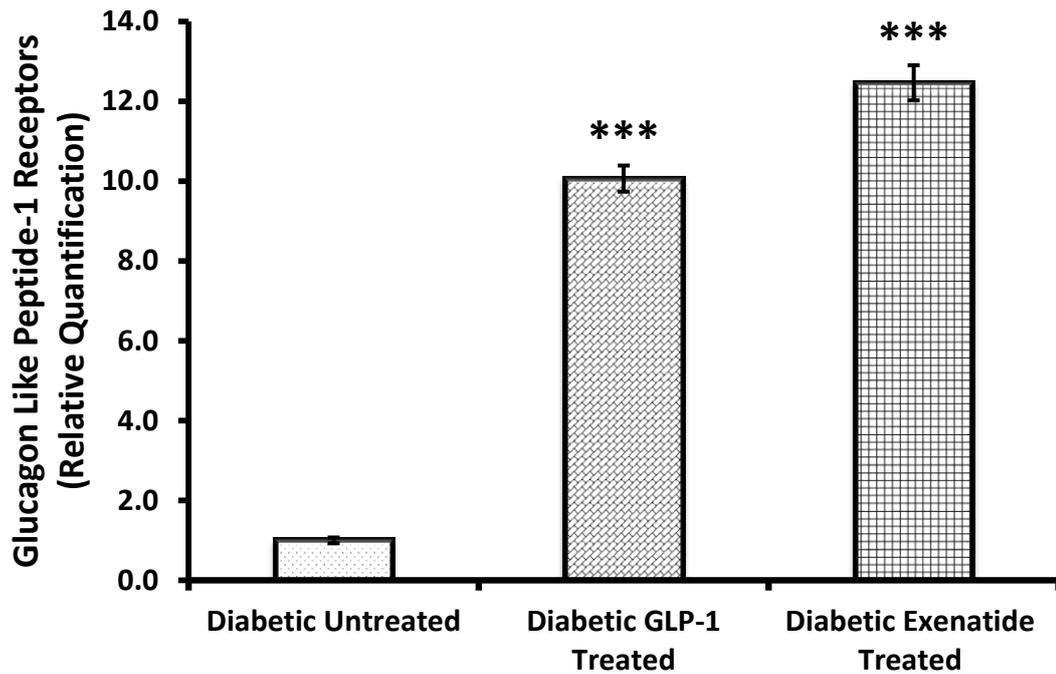


Figure 3.85: Histograms showing the effect of 10 weeks of treatment with either GLP-1 (50 nmol/kg body weight) or exenatide (1 μ g/kg body weight) on levels of mRNA expression of GLP-1 receptors in treated diabetic rats compared to untreated diabetic rats. Level of GLP-1 receptor mRNA expression was measured by relative real-time PCR following normalization for β -actin mRNA in each sample. *** $P < 0.001$ for GLP-1 and exenatide treated diabetic rats compared to diabetic untreated rats. Note that exenatide treatment was more effective than that of GLP-1 in increasing glucagon like peptide-1 receptor mRNA expression. Data are mean \pm SEM, $n = 10$ rats for each point.

CHAPTER FOUR

DISCUSSION

The present *in vivo* and *in vitro* studies were designed to investigate the beneficial effects of the two incretins, GLP-1 and its agonist, exenatide on the STZ-induced diabetic pancreas compared to age-matched controls. In both conditions, two control untreated groups of rats (normal and diabetic) did not receive either GLP-1 or exenatide.

DM is a major metabolic disorder currently affecting more than 225 million people globally. The disease is highly prevalent globally and in the United Arab Emirates (UAE) in particular. A prevalence study on adults in the UAE, in which fasting blood glucose levels were above normal, showed that the incidence of DM was higher than 6% (El Mugamer et al., 1995). A more recent survey suggests a much higher prevalence of about 20-40% of the urban adult population in the UAE (Malik et al., 2005).

Many T2DM complications are implicated with prolonged elevated blood glucose levels, pancreatic β -cell failure and insulin resistance (Bouwens and Rooman, 2005). Most of the available anti-diabetic drugs are involved in improvement of glucose tolerance, insulin secretion, insulin resistance and reducing diabetic complications. Recent hypoglycaemic drugs focus on inducing insulin secretion in a glucose-dependent way (Farret et al., 2005).

Presently, there is an increased demand for the development of new and novel antidiabetic drugs, which are capable not only in reducing blood glucose level in the body but also in inducing insulin secretion from the endocrine pancreas. Moreover, the drugs must also improve the β -cell functions, induce its proliferation and suppress its apoptosis. The discussion of this study will cover the beneficial effects of either GLP-1 or exenatide in diabetic conditions compared to control.

4.1 Effect of GLP-1 and exenatide treatment on metabolic parameters of normal and diabetic rats

***In vivo* study**

4.1.1 Body weight

The results of this study have demonstrated that there was no significant body weight change in all normal and diabetic rats treated with either GLP-1 or exenatide. However, the results showed a small but not significant gain in body weight of normal treated rats, which was most likely due to normal body growth and ageing during the 10 weeks of the experiment period. All groups of were under the same conditions of open access to food without any restrictions and this was a constant factor for all experimental rats. Although, GLP-1 and exenatide suppress gastric emptying and appetite, they have the ability to induce insulin release and therefore improve the well-being of the animals (Meier et al., 2002). The secreted insulin will help in glucose absorption and therefore in the weight of the animal (Patel et al., 2006a). However, the effect of ageing on the overall weight cannot be excluded. The results of the present study are in agreement with previous reports in which a prolonged intracerebroventricular treatment of GLP-1 leads to body weight gain that may be induced via GLP-1 receptors in the central nervous system (CNS) (Goke et al., 1995; Davis et al., 1998).

On the other hand, the results of the present study showed a non significant body weight loss in diabetic rats treated with either GLP-1 or exenatide. This is in agreement with other studies that have reported reductions in body weight after treatment of diabetic models and patients with either GLP-1 or exenatide (Bergenstal et al., 2010). These authors suggested that GLP-1 and exenatide initiate their effects by reducing gastric emptying, appetite and enhancing satiety (Bloomgarden, 2004; Kaushal et al., 2006; Murphy and Bloom, 2006; Mudaliar and Henry, 2010). The difference between the observations of the present study and others in the literature may be due to the methodology and dosage used. A relatively lower dose of 1 µg/kg/day of exenatide was used in the present study while a larger dose of 10 µg/kg/day of exenatide was used in

other study (Nachnani et al., 2010). This larger dose is 10-fold higher and may play a role in the magnitude of the effects caused by exenatide. In addition, the variations may be due to the 10-week period of treatment in the present study compared to other investigation with longer treatment time (3 months to 3 years) (Klonoff et al., 2008). Additionally, other previous studies also documented that on the average, the reduction in body weight was either moderate or insignificant, while in others it was significant. The explanation for these differences may depend on the dose and duration of the study. It is suffice to say that significant body weight reduction is associated with higher doses and longer period of treatment of incretins (Moretto et al., 2008; Marre et al., 2008).

4.1.2 Blood glucose level

This study revealed no pronounced differences in fasting blood glucose levels between the normal rats treated with either GLP-1 or exenatide compared to normal untreated rats. On the other hand, the present study detected a significant reduction in fasting blood glucose levels in diabetic rats treated with either GLP-1 or exenatide. This reduction of fasting blood glucose level was consistent with other reports (Bosi et al., 2008; Buse et al., 2009). Both GLP-1 and exenatide have insulinotropic actions since they can stimulate insulin secretion in glucose-dependent way. Moreover, they can exert their beneficial effects by increasing plasma insulin concentrations, leading to a reduction and improvement in blood glucose levels (Patel et al., 2006a). Glucose homeostasis is under continuous antagonistic actions of both insulin and glucagon. GLP-1 reduces glucagon secretion from pancreatic α -cells, probably through inhibitory binding effect of GLP-1 to its receptors expressed in pancreatic α -cell (Heller et al., 1997). Alternatively, GLP-1-induced glucagon inhibition may be an indirect way via binding to δ -cell receptor and stimulation of somatostatin release, which suppresses glucagon secretion from α -cells in a paracrine mode of action (de Heer et al., 2008). In addition, GLP-1 plays a significant role in stimulation of β -cell to secrete insulin that also inhibits glucagon release from α -cell (Creutzfeldt et al., 1996). Oral glucose and other food components can enhance GLP-1 release from the intestinal mucosal L-cells, which directly influence insulin secretion from pancreatic β -cells in a glucose-

dependent response and inhibit glucagon secretion from α -cells. These actions, besides the delay in gastric emptying, feel of stomach fullness and reduction of appetite will lead to hypoglycaemic effect following GLP-1 administration (Vilsbøll and Holst, 2004; Mudaliar and Henry, 2009). The small influence of GLP-1 on glucose homeostasis might be minor in normal subjects and this may explain the result of the present study that showed no significant changes in blood glucose levels in normal rats treated with either GLP-1 or exenatide compared to normal control rats (Nauck et al., 1997).

4.1.3 Intraperitoneal glucose tolerance test (IGTT)

This study showed that treatment with either GLP-1 or exenatide improved glucose tolerance in both normal and diabetic rats. In all normal and diabetic rats treated with either GLP-1 or exenatide there were significant reductions in blood glucose levels at different periods of the glucose tolerance test. The improvement in glucose tolerance was more prominent in diabetic rats treated with GLP-1. All rats were 10 weeks old at the beginning of the experimental period (zero week) and were aged 20 weeks after the 10-week study period. There was a difference in the fasting blood glucose levels within diabetic and normal groups at the beginning of the study at 0 week and at the end of the study period after 10 weeks. This difference was probably due to the effect of ageing and also to the beneficial effect of 10 week-treatment period with either GLP-1 or exenatide (See figures 3.3 and 3.5 for normal rats and figures 3.4 and 3.6 for diabetic rats). The beneficial effect of either GLP-1 or exenatide established in the present study may be due to the insulinotropic effect of these incretins. This result is consistent with those detected in other studies, which reported the effectiveness of GLP-1 and exenatide in improving blood glucose levels in treated rats. GLP-1 maintains glucose homeostasis via enhancement of insulin release and suppression of glucagon secretion in response to blood glucose levels without inducing hypoglycaemia (Schwartz et al., 2008; Verspohl, 2009). The inhibition of glucagon secretion is under the GLP-1 effect via stimulation of both insulin secretion from β -cells and somatostatin from δ -cells of the pancreas. Both are involved in suppressing glucagon release from α -cells. In addition, the stimulatory effect of GLP-1 on insulin secretion also lowers blood glucose

levels after glucose load in glucose tolerance test. At the same time, GLP-1 stimulates insulin biosynthesis in glucose-dependent way. Therefore, GLP-1 seems to stimulate insulin secretion at both early and late phases following its treatment (Doyle and Egan, 2007).

4.2 Effect of GLP-1 and exenatide treatment on biochemical parameters of normal and diabetic rats

4.2.1 Liver function tests

In the present study, the plasma levels of AST increased markedly after the onset of diabetes. In addition, in both groups of normal and diabetic rats treated with either GLP-1 or exenatide, the levels of AST were reduced significantly. On the other hand, in this study, the AL

T levels were reduced in normal and diabetic rats after treatment with either GLP-1 or exenatide. The reduction was significant in all groups except in normal rats treated with GLP-1. However, the effect of GLP-1 in lowering the ALT levels was much more pronounced in diabetic-treated rats than in exenatide-treated diabetic rats. These reductions of both AST and ALT indicate the protective action of either GLP-1 or exenatide in improving liver functions especially in diabetic rats and these results are in agreement with a previous study (Vaghasiya et al., 2010).

Liver enzymes, including AST and ALT, are considered as markers that reflect liver functions including hepatocellular integration and protein synthesis in the liver. Elevation in the levels of these two enzymes is an important indicator of hepatocellular damage since they play vital roles in gluconeogenesis. AST is found in several body organs such as the liver, brain, kidney, lung and pancreas and it is present in both the cell cytoplasm and mitochondria. However, ALT is mainly a cytosolic enzyme which is found in large quantities in hepatocytes (Opara, 2002).

In a previous report and in agreement with the present study, the plasma level of ALT was increased after the onset of diabetes (Aragon and Younossi, 2010). This indicates

that diabetes mellitus can cause liver damage if not treated. Hepatocellular damage leads to a release of these two liver enzymes into blood circulation. STZ-induced diabetic rats are mainly characterized by elevation in the plasma AST and ALT due to the toxic effect of STZ on hepatocytes. As indicated in the STZ-induced diabetic rats in the present study, treatment with either GLP-1 or exenatide can lead to significant reductions in the levels of these two enzymes. These protective effects are consistent with the results of a previous study in which T2DM patients with impaired elevated liver enzymes were treated with exenatide for 3 years showing improvement in the ALT levels (Klonoff et al., 2008). In addition, elevation in the liver enzymes, ALT and AST were mainly associated with excessive lipid peroxidation and oxidative stress in diabetic rats. These toxic markers were principally due to excessive production of reactive oxygen species. Treatment with either GLP-1 or exenatide improves liver functions (Bulchandani et al., 2009), probably due to an increase in the liver uptake of glucose which reduces the glucotoxic effect of prolonged hyperglycaemia in diabetic rats (Arnés et al., 2009).

Furthermore, increased serum ALT and AST levels in obese mice confirm the strong relationship between obesity and liver steatosis. Reduction of liver fat content and liver cells injury were detected after exenatide treatment of either ob/ob obese mice or T2DM patients (Ding et al., 2006; Klonoff et al., 2008; He et al., 2010).

Exenatide-treated rats have revealed an improvement of liver functions via elevation in the activities of antioxidant enzymes, superoxide dismutase and catalase with reduction in nuclear oxidative stress (Bose et al., 2005; Bose et al., 2007). In agreement with these previous studies, the results of the present study have shown similar significant increases in both catalase and glutathione reductase-positive cells in pancreatic islets of either normal and diabetic rats treated with either GLP-1 or exenatide. This observation suggests that either GLP-1 or exenatide-treated rats have increased levels of these protective antioxidant enzymes, which explains the lowering effect on AST and ALT. Suppression of oxidative stress by exenatide treatment may lead to a protective effect on the liver. The protective effect of exenatide in the reduction biomarkers of hepatic injury, AST and ALT may be due to the elevation of antioxidant enzyme activities (Vaghasiya et al., 2010; Mudaliar and Henry, 2010; Sathyanarayana et al., 2011).

4.2.2 Kidney functions tests

Levels of blood urea nitrogen, serum creatinine and uric acid contents are pathophysiological markers used in the evaluation of kidney function (Zhu et al., 2005). The results of the present study have shown that STZ-induced diabetes could elevate the levels of blood urea nitrogen, serum uric acid and to slight degree, serum creatinine levels compared to normal, untreated control rats. The present study also shows that treatment of diabetic and normal rats with either GLP-1 or exenatide can lead to a general reduction in the levels of blood urea nitrogen, serum creatinine and serum uric acid. The reduction of blood urea nitrogen levels was significant in diabetic rats treated with GLP-1. In addition, the reduction of serum creatinine was also significant in normal and diabetic rats treated with exenatide and non-significant in GLP-1 treated normal and diabetic rats. These differences may be due to the fact that GLP-1 has a very short half-life of about 2 minutes because of fast degradation by DPP-IV enzyme. In contrast, exenatide is a long-acting agonist and DPP-IV resistant drug (Perry and Greig, 2003). This may make exenatide more effective in reducing serum creatinine levels. In addition, the reduction of serum uric acid was significant in normal and diabetic rats treated with exenatide and diabetic rats treated with GLP-1.

These reductions in three kidney function markers including blood urea nitrogen, serum creatinine and uric acids indicate the beneficial effects of either GLP-1 or exenatide on the kidney functions in normal and diabetic rats. The results of the present study have shown a better improvement in kidney functions compared to a previous study, which demonstrated no significant difference in kidney markers between db/db mice treated with exenatide and control group (Park et al., 2007). In another study, the exenatide agonist, AC3174 and GLP-1 treatment in Dahl salt-sensitive rats revealed reduction of renal injury and improvement in renal function (Liu et al., 2010a). The beneficial effect of GLP-1 and exenatide on renal functions may be due to a reduction in blood hypertension, which is normally associated with diabetes via elevation of circulating angiotensin, enhancement of sodium and water excretion in urine or through the reduction of corticosterone-induced hypertension (Kohara et al., 1993; Hirata et al., 2009; Timmers et al., 2009). In addition, GLP-1 and exenatide may prevent the formation of renal glomerulosclerosis, tubular necrosis, interstitial fibrosis, leading to

improved renal morphology (Yu et al., 2003). All of these effects of GLP-1 and exenatide may lead to enhancement of the overall kidney function markers, including blood urea nitrogen, serum creatinine and serum uric acid, as confirmed in the present study.

In contrary to the beneficial results on renal function in the present study, a previous study done on four diabetic patients (age 52-73 years) treated with exenatide and diuretic drugs has shown a deterioration of renal function due to renal failure. As such, exenatide treatment was curtailed. This adverse effect of exenatide was observed in those patients taking both diuretic drugs beside the exenatide. In turn, this case was associated with a synergistic action of both drugs and a severe reduction of body fluids that cause renal failure (Weise et al., 2009). However, the study of Weise and co-workers had a limited number of patients, four, and they were mainly over age. As such, this referred study could not be considered as a reliable study.

4.2.3 Lipid profile

The present study has shown that GLP-1 and exenatide can evoke marked reductions in serum cholesterol and triglyceride levels in both normal and diabetic rats. The reduction of serum cholesterol and triglyceride was significant in normal rats treated with exenatide and in diabetic rats treated with GLP-1. In addition, the reduction was also significant in serum cholesterol and triglyceride in diabetic rats treated with either GLP-1 or exenatide except in diabetic rats treated with exenatide where the reduction in serum cholesterol was not significant. The reduction in serum cholesterol and triglyceride under the influence of either GLP-1 or exenatide in both normal control and diabetic rats was consistent with previous studies (Blonde et al., 2006; Klonoff et al., 2008; Mudaliar and Henry, 2010). All normal and diabetic groups in the present study have free access to food and water. This means that the food ingestion was considered as a constant factor for all rats and had no specific influence on serum triglyceride on certain group of rats. The reduction in serum triglyceride levels in rats treated with either GLP-1 or exenatide may be due to an increase in the consumption of triglyceride in body cells rather than their storage. A recent study has reported that GLP-1 and

exenatide treatment can inhibit lipolysis and decrease the release of triglyceride in plasma resulting in reduced levels of serum triglyceride and total cholesterol. The mechanism of this suppression may be due to the delay in gastric emptying that causes a slowing in food reaching the intestine from the stomach (Nuche-Berenguer, et al., 2011). This observation is in agreement with the reduction of serum cholesterol and triglyceride discerned in the present study. In another previous study, GLP-1 and exenatide treatments lead to a suppression of postprandial increase in plasma triglyceride and non-esterified fatty acid in normal and T2DM subjects (Cervera et al., 2008). This reduction of triglyceride after a meal may be due to the suppression of the action of lipase, inhibition of intestinal apolipoproteins synthesis, reduction of lymph flow in intestinal cells and an inhibition of lipid absorption (Qin et al., 2005; Hsieh et al., 2009).

T2DM revealed an elevation in hepatic fat levels, decreased plasma adiponectin, and hepatic insulin resistance (Bajaj et al., 2004). Reduced plasma adiponectin concentrations may be related to peripheral insulin resistance, elevated fasting plasma triglyceride and diminished plasma high-density lipoprotein (HDL) cholesterol level (Yu et al., 2002). In a previous study, exenatide treatment elevates plasma adiponectin concentrations and suppresses the fat content in rodent liver (Li et al., 2008). The action of exenatide in reducing the food intake may play an essential role in decreasing the fat content in rodent liver. Exenatide enhancement of adiponectin can lead to stimulation of cAMP-activated protein kinase, improvement of mitochondrial fat oxidation and reduction of lipogenesis in the liver (Ding et al., 2006). Moreover, GLP-1 treatment can also cause marked suppression in liver lipogenesis and enhancement of fatty acid oxidation (Yamauchi et al., 2002). The results of these previous studies confirm the lowering action of either GLP-1 or exenatide treatments on the serum cholesterol and triglyceride levels confirmed the data obtained in the present study.

4.3 Effect of GLP-1 and exenatide treatment on serum insulin in normal and diabetic rats

The present study has shown a pronounced reduction in levels of serum insulin in untreated diabetic rats compared to normal rats. Treatment of both normal and diabetic rats with either GLP-1 or exenatide showed a significant elevation in serum insulin levels. These results suggest that both GLP-1 and exenatide may improve glucose tolerance via enhancement of insulin secretion in glucose-dependent manner only when the blood glucose level was above the normal range. This finding is consistent with previous studies, which showed an elevation of serum insulin in healthy cats treated with exenatide (Gilora et al., 2011). An increase in serum insulin was also detected after GLP-1 treatment either in healthy humans or in diabetic animal models (Edwards et al., 1998; Henquin, 2000).

The insulinotropic action of GLP-1 is maintained via potentiation of β -cell function and an increase of β -cell mass by enhancement of β -cell neogenesis and proliferation. In addition, GLP-1 is associated with the activation of insulin gene expression and it also stimulates many steps during insulin production and release from β -cells (Holz and Chepurny, 2003; Dungan and Buse, 2005). In addition, one of the indirect actions of GLP-1 in stimulating insulin secretion may be through inhibition of glucagon release as the main insulin antagonistic hormone. Moreover, GLP-1 may also suppress β -cell apoptosis, especially in diabetic rats as demonstrated in previous studies (Arulmozhi and Portha, 2006; Nayak et al., 2010). GLP-1 prevents the apoptosis of pancreatic β -cell by inhibiting caspase-3 enzyme activities, an enzyme involved mainly in the last steps of β -cell death. In addition, GLP-1-treated islets revealed an elevation in the concentrations of antiapoptotic survival protein (Bcl-2). At the same time, GLP-1 treatment can also improve the number of insulin-immunopositive cells with elevation of insulin content and enhancement of insulin release in response to glucose level. The decrease in β -cells apoptosis in GLP-1-treated diabetic rats may be due to the ability of GLP-1 to preserve normal morphology of islet cells (Farilla et al., 2003).

In vitro studies

4.4 The physiological roles of GLP-1 and exenatide on insulin secretion (release) from the pancreatic tissue fragments of normal and diabetic rats

The results in the present study have shown that diabetic pancreatic fragments produce about 50% less insulin compared to the normal healthy pancreas. Similar results have also been reported in several previous studies, which employed STZ-induced diabetic rats (Noguchi, 2007; Spellman, 2007). Moreover, the present study has also demonstrated that either GLP-1 or exenatide can evoke dose-dependent increases in insulin secretion from both normal and diabetic pancreatic fragments compared to basal secretion. The maximum effect was obtained with 10^{-6} M concentration of either GLP-1 or exenatide. Together, these results clearly confirm the beneficial use of either GLP-1 or exenatide in inducing insulin secretion from pancreatic fragments confirming their secretagogue effect. These results are in agreement with previous studies (Irwin et al., 2010). The possible explanation of either GLP-1 or exenatide in inducing a lower level of insulin secretion from pancreatic fragments of diabetic rats compared to normal rats is probably due to the destruction of transduction signals associated with calcium release in diabetic pancreatic rats (Adeghate and Ponery, 2002). The results of the present study confirm those of a previous study, in which, exenatide treatment potentiates the normalization of insulin release under glucose stimulation in T2DM patients (Quddusi et al., 2003). Exenatide is believed to exert its effect by binding to its GLP-1 receptors that induce proinsulin production and substituting the released insulin secretory granules with stimulation of new insulin granules synthesis. The stimulation of insulin release via exenatide in T2DM patients was about 50% of that estimated in normal volunteers as reported in a previous study (Fehse et al., 2005). This could be related to the reduced number of β -cells in diabetic rats as confirmed in the present study. It is well known that GLP-1 stimulates insulin release from β -cell via its binding to GLP-1 receptor generating such second messenger as cAMP that provokes protein kinase A and guanine nucleotide exchange factor cascades. In turn, these induce the secretion of insulin from its granules in β -cell into the blood (Song et al., 2011).

The results of the present study have shown that there was a marked reduction in the β -cell number in diabetic pancreatic fragments. Surprisingly, the β -cells were still able to secrete insulin in a concentration-dependent manner to either GLP-1 or exenatide stimulation. One possible explanation for the effect of either GLP-1 or exenatide to induce the secretion of insulin from pancreatic fragments, although there was a reduction in β -cells number, is probably due to their ability to enhance β -cell functions. The insulintropic action of GLP-1 and exenatide seen in the present study is consistent with a previous study employing aged rats. In this study, the aged rats have age-dependent reduction in β -cells number, reduced function and moreover diminished glucose homeostasis. These aged rats restored the normoglycaemia after GLP-1 treatment in accordance with the results in the present study (Farilla et al., 2003).

The *in vitro* study on β -cells revealed that GLP-1 treatment can lead to an elevation in insulin release with improvement in glucose tolerance (Hui et al., 2003). In T2DM patients with reduced β -cells mass, GLP-1 treatment can restore normal fasting blood glucose levels with enhancement of insulin release (Kim and Egan, 2008). Exenatide treatment of isolated pancreatic rat islets showed an activation of the glucose-induced insulin release (Doggrell, 2007). In db/db diabetic mice treated with exenatide, pancreatic insulin level was 38% more than that in untreated diabetic db/db (Wang and Brubaker, 2002). This action of GLP-1 in improving insulin sensitivity and stimulation of insulin release may be via the activation of the expression of insulin gene (Sinclair and Drucker, 2005). In another previous study, it was shown that a culture of human islet β -cell line treated with GLP-1 has revealed a conservation of the normal islet cell structure, incensement of cell survival, enhancement of the glucose-stimulated insulin release and elevation in insulin cytosolic granules. Therefore, GLP-1 seems to maintain β -cells structure, function and decreases in β -cells death (Farilla et al., 2003).

4.5 Effect of GLP-1 and exenatide treatment on the distribution pattern of pancreatic islet cells in normal and diabetic rats

4.5.1 Distribution of insulin immunoreactive cells in normal and diabetic rats

The results presented in this study have demonstrated significant changes in the pattern of distribution of insulin-positive cells in the pancreas of diabetic rats when compared to normal control. Insulin-positive cells were located mainly in the central area of the pancreatic islet and to a certain extent in the peripheral parts of the islets in normal pancreas. In contrast, the diabetic pancreas had less insulin-positive cells in both the central and peripheral parts. This observation on the pattern of distribution of insulin producing β -cells is similar to other previous report (Ahmed et al., 1998). The number of insulin-positive cells was higher in normal pancreas compared to STZ-induced diabetic rats and this decrease was due to the destruction of pancreatic beta cells. From the present study, it was obvious that STZ does not destroy all the β -cells in the islets of diabetic rat pancreas. It is possible that some cells were partially destroyed and with time, they survived and subsequently recover. A previous study has also reported that STZ does not completely destroy all the pancreatic beta cells after STZ treatment (Adeghate and Parvez, 2000). The present study has also shown that treatment of rats with either GLP-1 or exenatide can lead to a significant increase in insulin immunoreactive β -cells in both diabetic and normal healthy rats. GLP-1 had been previously shown to exert beneficial effects on β -cell mass and function (Buteau et al., 2004). GLP-1 exerts its action by the enhancement of glucose-induced insulin release, improvement of β -cell quantity, insulin gene expression and suppression of β -cell death. These actions lead to the control of insulin release via preserving normal action and β -cell mass. The action of exenatide as GLP-1 agonist on pancreatic cells is mainly similar to GLP-1 due to the fact that exenatide can exert its action via binding to GLP-1 receptor (Drucker, 2006).

A previous study, using a pancreatic necropsy for T2DM patients, has demonstrated a decrease in the number and action of β -cells. The decline of β -cells number is related to

elevated β -cell death in diabetes. The causes of increased rate of either β -cells death or dysfunction were related to increased toxic elements including chronic hyperglycaemia, elevated lipids concentration, induced cell inflammation and increased level of amyloid secretion from pancreatic islet cells (Muio and Newgard, 2008). In another study, elevation in β -cell death and reduction in β -cell growth can lead to a decrease in β -cell number in T2DM. This may be due to destructive glucolipotoxic effect on β -cell functions as this was associated with reduced insulin release, inhibition of insulin gene expression and increase in β -cell apoptosis (Poitout and Robertson, 2008).

In diabetes mellitus, β -cells are under persistent oxidative stress because of elevated concentrations of reactive oxygen species due to prolonged hyperglycaemia, leading to impaired β -cell function (Robertson, 2004). At the same time, the levels of harmful lipid derived products increase and thus, disrupting β -cells function (Poitout and Robertson, 2002). All of these β -cell toxins propagate an endoplasmic reticulum stress and eventually failure, all of which are hallmarks of T2DM. In turn, these are associated with insulin resistance and hyperinsulinaemia. The condition of endoplasmic reticulum failure is due to the fact that it loses its normal function of protein folding that cause β -cell death at the end (Fonseca et al., 2007).

In the beginning of T2DM before the progressive deterioration of the disease, the normal action and number of β -cells may be restored by incretin treatment because GLP-1 and exenatide enhance β -cells proliferation, neogenesis and decrease β -cells apoptosis. These effects lead to an increase in the number of β -cells, a vital action of incretins (Wajchenberg, 2007). GLP-1 and exenatide are also able to enhance insulin release and elevate both β -cells mass and proliferation (Buteau et al., 2004).

Pancreatic β -cell number is controlled by a preservation of the equilibrium between β -cell differentiation and apoptosis. In addition, β -cell size variation is also associated with controlling the β -cell number (Bonner-Weir, 2001). In rodents, β -cells mass propagated under GLP-1 treatment through increasing β -cells neogenesis, proliferation and reduction of apoptosis (Hanley et al. 2008).

Previous *in vitro* studies have shown that exenatide treatment enhances the proliferation of pancreatic islet tumour cells and progenitor cells toward insulin-secreting β -cells

(Abraham et al., 2002). In addition, the proliferative effect of exenatide treatment on β -cells may, indirectly, via reduction of the elevated blood glucose levels in diabetes leading to increased β -cells mass (Dungan and Buse, 2005).

The actions of GLP-1 on β -cells may lead to prolongation of its life span with enhancing increase in β -cells and finally, insulin release. These beneficial effects of GLP-1 may therefore be due to its effect on improving β -cells mass. The authors have shown that GLP-1-receptor double knockout mice have a reduced β -cells mass (Hansotia and Drucker, 2005). Additionally, in another study, GLP-1 G-protein (α_s) subunit receptor deletion in β -cell of mice causes prominent reduction in β -cell volume, number and growth with elevation of β -cell death rate (Xie et al., 2007).

The results of previous studies have clearly shown a beneficial effect of either GLP-1 or its agonist exenatide in increasing β -cells mass and reducing their apoptosis leading to a significant increase in the number of insulin immunoreactive cells observed in the present study.

A different *in vitro* study employing human pancreatic islet, GLP-1 treatment was shown to abolish gluco-lipotoxicity. This beneficial effect of GLP-1 may be due to enhancement of antiapoptotic proteins (Bcl-2, IAP-2) gene expression (Buteau et al., 2004). Furthermore, the mechanism of action of GLP-1 may be related to the stimulation of the transcription factor (NF-B) and protein kinase B. The action of GLP-1 on β -cells mass may also be through cAMP and protein kinase A-dependent pathways (Karaca et al., 2009). This mode of action protects the β -cells through inhibiting mitochondrial dysfunction, increasing the expression of β -cell protective genes and suppression of β -cell apoptosis (Hausenloy and Yellon, 2011).

4.5.2 Distribution of glucagon immunoreactive cells in normal and diabetic rats

The results of the present study have also shown a pronounced disturbance in the number and arrangement of glucagon-positive, α -cells in the pancreas of diabetic rats in comparison with normal control rats. In the normal pancreas, glucagon-positive α -cells

were located mainly on the peripheral areas of the islets and this is similar to previous report (Ahmed, 2002). In STZ-induced diabetic pancreas, the number and the way of organization of glucagon-positive α -cells altered prominently when compared to normal controls. Glucagon-positive α -cells were found both in the peripheral and central regions of the islets of diabetic rats (Ahmed et al., 1998). This may be due to the fact that the number of glucagon-positive α -cells increased greatly in such a way that makes the peripheral region of pancreatic islets too small for them to occupy or some glucagon-positive cells may migrate into the central area of the islet to replace the diminishing β -cells. Aberrations in the quantity and action of the β - and α -cells can lead to a reduction in insulin secretion and increased glucagon release that are an added value for worsening the complications of DM (Irwin et al., 2009). However, treatment of normal and diabetic rats with either GLP-1 or exenatide as demonstrated in the present study has resulted in a significant reduction in the number of immunoreactive glucagon α -cells in pancreatic islets. This beneficial effect of GLP-1 and exenatide may be due to their essential action in lowering the harmful effect of prolonged elevated blood glucose levels via inhibiting the glucagon release in a glucose-dependent way from pancreatic α -cell (Schirra et al., 2006).

A previous investigation had reported that GLP-1 and exenatide can suppress the release of glucagon and enhance α -cell glucose sensing in T2DM patients (Dunning et al., 2005). Another previous study had also reported the ability of GLP-1 in the suppression of α -cells (Hare, 2010). In another study, it was shown that treatment of STZ-induced diabetic rats with chlorella, an antioxidant agent, can result in a significant reduction of α -cells number with increase in β -cells count (Amin et al., 2011). These results are consistent with the suppression of α -cells and elevation β -cells in the current study. According to these results, it is tempting to suggest that GLP-1 not only decreased the mass of α -cells, but also improves the function of the existing β -cells thereby improving glucose tolerance.

The mechanism of action of GLP-1 and exenatide treatments in readjusting the disturbed numbers of both β - and α -cells in STZ-induced diabetic rats may be explained via the enhancement of the antioxidant effects of the both GLP-1 and exenatide. It was demonstrated in this study that both GLP-1 and exenatide can increase the number of

both catalase and glutathione reductase-positive cells in pancreatic islets of diabetic rats. This observation is consistent with the finding of a previous study in which STZ-induced diabetic rats were treated with the antioxidant product such as chlorella leading to an elevation of β -cells and reduction in α -cells mass. These effects were associated with increase in the levels of the antioxidant enzymes catalase and glutathione reductase (Amin et al., 2011). Therefore, enhancing the antioxidant protective system may cause a reduction of the oxidative stress and gluco-lipotoxicity, resulting in less destruction of pancreatic islet cells in DM (Robertson, 2004). Through this beneficial antioxidant effect of GLP-1 and exenatide treatments, the increase in the number of β -cells and reduction in α -cells number may be explained in the present study. The results of the present study are supported by those of a previous study in which genetically engineered mice with a reduced β -cell were treated with insulin. The mice showed regeneration of β -cells, which developed from α -cells precursors (Thorel et al., 2010). This result confirms the data of the present study, which shows a reduction in α -cells coupled with an increase in β -cells under the insulinotropic action of GLP-1. Therefore, α -cells may act as a potential source for β -cells following GLP-1 treatment. In turn, this will lead to elevation in insulin secretion.

4.5.3 Distribution of GLP-1 and exenatide immunoreactive cells in normal and diabetic rats

The immunohistological figures of the present study have shown that the percentage of either GLP-1- or exenatide-positive cells were increased in pancreatic islets in normal and diabetic rats treated with either GLP-1 or exenatide. This elevation was significant in all the groups of rats except for exenatide-positive cells in normal rats treated with either GLP-1 or exenatide. In addition, the distribution of the immunoreactive GLP-1- and exenatide-positive cells were seen at the periphery of pancreatic islets of normal rats while their location were randomly distributed in diabetic rats. They were associated with both β - and α -cells, probably, due to the distortion of islet cells organization pattern in diabetic rats (Adeghate and Parvez, 2000). These results are mainly related to the beneficial effects of GLP-1 and exenatide in increasing the numbers of GLP-1- and exenatide-positive cells with relative increases in β -cells as

detected in the present study. The results of the present study have also shown that the GLP-1- and exenatide-positive cells in pancreatic islets of diabetic rats treated with either GLP-1 or exenatide were distributed randomly in the islets due to the disruption of the organization of all cells in the diabetic islets (Ahmed, 2002).

In the present study, the higher number of GLP-1-positive cells observed in diabetic rats compared to control can be explained as follows, (1): GLP-1 and glucagon are derived from the same proglucagon gene. It has been shown that proglucagon gene is processed differently according to tissue specificity. The processing action is activated via prohormone convertase (PC) enzymes, which post-translates the proglucagon to glucagon by PC2 in pancreatic α -cells or to GLP-1 by PC1/3 in intestinal L-cells (Sinclair and Drucker, 2005). In addition, glucagon-positive cells are higher in diabetic rats compared to control. Since GLP-1 and glucagon are derived from the same gene, it is imperative that the levels of the two peptides will be high (Ahmed et al., 1998; Adeghate and Ponery 2003), (2): High glucose concentrations induce PC1 expression, which processes proglucagon to produce GLP-1 from α -cells of islets. The mechanism of α -cells induced production of GLP-1 is related mainly to TGR5 receptor stimulation pathway (Whalley et al., 2011). Similar findings were also detected in the α -cell line (α TC1-6). Incubation of these cells in high glucose concentration resulted in GLP-1 formation and reduced glucagon release from α -TC1-6 cells (Whalley et al., 2011). In another study, transplanting encapsulated α -PC1/3-expressing cells in mice showed induction of GLP-1 formation, which in turn improves glucose homeostasis and initiate β -cell propagation (Wideman et al., 2007). A similar result was detected in db/db diabetic mice of α -PC1/3-expressing cells that revealed enhanced glucose reduction via increased proglucagon processing to GLP-1 (Wideman et al., 2009). Furthermore, induction of isolated α -cell of mouse islets by adenovirus-mediated PC1/3 expression causes elevation of GLP-1 secretion. In turn, GLP-1 enhances glucose tolerance and activates β -cells function within the islets (Wideman et al., 2006). The conversion of adult pancreatic α -cells to β -cells was also confirmed in another study, which showed that β -cell restoration after insulin treatment in diphtheria-toxin-induced β -cell destruction (Thorel et al., 2010), (3): GLP-1- or exenatide-positive cells can be expressed in both β - and α -cells of the diabetic islets under specific circumstances

mentioned in the previous studies. In turn, this condition induces both β - and α -cells to increase GLP-1 production, which is significantly more in diabetic pancreatic islet cells compared to normal rats (Wideman et al., 2006).

4.5.4 Distribution of catalase and glutathione reductase immunoreactive cells in normal and diabetic rats treated with either GLP-1 or exenatide

In the present study, the treatment of normal and diabetic rats with either GLP-1 or exenatide leads to an increase in catalase and glutathione reductase immunoreactive cells. This increase in both catalase and glutathione reductase-positive cells was not significant in pancreatic islets of normal treated rats. However, there was a significant and marked increase in both catalase and glutathione reductase-positive cells in pancreatic islets of diabetic treated rats.

STZ is a powerful reactive oxygen species (ROS) producer that causes the formation of nitric oxide (NO). It has been reported that β -cell dysfunction with impaired insulin synthesis and release are caused by ROS produced after STZ uptake by β -cells through GLUT-2 (Thulesen et al., 1997). STZ-induced diabetes in rats caused an elevation of blood glucose levels that worsen during the oxidative stress, leading to major chronic complications of DM (Vincent et al., 2005). The ROS are considered as metabolic products of exaggerated blood glucose oxidation and glycation, which disturb the β -cells antioxidant defence mechanisms (Bonfont-Rousselot et al., 2004; D'Souza et al., 2009).

Consistent with the results in this study, a previous study on untreated diabetic rats has shown a significant concentration of oxidative stress (Vaghasiya et al., 2010a). Untreated diabetes causes depletion of glutathione, superoxide dismutase, catalase and glutathione peroxidase. Exenatide ameliorates the effect of oxidative stress by increasing the levels of antioxidant enzymes (Vaghasiya et al., 2010a).

Similar results were achieved in another previous study in which STZ-induced diabetic mice were treated with exenatide. The results showed a reduction of blood glucose

levels, enhancement of insulin secretion, suppression of β -cells death and marked elevation in pancreatic antioxidant enzymes, catalase, glutathione peroxidase, and superoxide dismutase. GLP-1 and its agonist, exenatide treatment may exert their antioxidant protective effects through the nerve growth factor/p75 neurotrophin receptor (NGF/p75NTR) system which evokes PKA and nuclear factor kappa-B (NF- κ B) that induce the expression of antioxidant enzymes and sustain β -cells survival (Gezginci-Oktayoglu and Bolkent, 2009).

In agreement with these studies, the increases in catalase and glutathione reductase-positive cells observed in the present study can be explained by the ability of either GLP-1 or exenatide to increase the expressions of catalase and glutathione reductase in pancreatic islet cells of diabetic rats.

4.6 Immunofluorescent double labelling study in pancreatic islet cells of normal and diabetic rats treated with either GLP-1 or exenatide

In the present study, the immunofluorescent double labelling examination has shown an increase in the number and distribution of insulin secreting β -cells and reduction in glucagon secreting α -cells in normal and diabetic rats treated with either GLP-1 or exenatide. In addition, the immunofluorescent double labelling in the present study has shown that the intra-islet random co-localization of either GLP-1 or exenatide with both insulin and glucagon in β -cells and α -cells, respectively. There was not much overlap between insulin and GLP-1 in diabetic rats treated with either GLP-1 or exenatide because GLP-1 is more related to glucagon rather than insulin. The increase in the number (staining) of insulin is due to treatment with either GLP-1 or exenatide. It has been shown that either GLP-1 or exenatide can induce neogenesis and proliferation of pancreatic β -cell (Tornehave et al., 2008; Dungan and Buse, 2005). In normal and diabetic rats treated with either GLP-1 or exenatide there was an increased co-localization GLP-1 and exenatide with insulin in pancreatic β -cells. These findings of co-localization of either GLP-1 or exenatide with insulin in β -cells may indicate the

beneficial interaction of either GLP-1 or exenatide in insulin production and secretion as indicated in a previous study (Tornehave et al., 2008). In addition, association of either GLP-1 or exenatide with insulin in β -cells can lead to enhanced action of β -cell function, stimulated insulin secretion, improved glucose tolerance, initiated β -cell differentiation and reduced β -cell death (Garber, 2011).

However, in the present study, the number of glucagon-positive cells increases significantly after the onset of diabetes (Ahmed et al., 1998; Adeghate and Ponery, 2003). Since GLP-1 is closely related to glucagon, it is imperative that the number of either GLP-1 or exenatide stained cells will increase during the onset of diabetes. The co-localization of either GLP-1 or exenatide with glucagon in α -cells may indicate that pancreatic α -cells may be stimulated by GLP-1 via binding of GLP-1 to its receptors on α -cells. This in turn leads to suppression of glucagon secretion thereby playing an important role in reduction of postprandial glucose excursions as reported in a previous study (Holst, 2007). In addition, the co-localization of GLP-1 and exenatide with glucagon in α -cells may enhance α -cell glucose sensing in patients with T2DM who improve their blood glucose homeostasis (Dunning et al., 2005). At the same time, GLP-1 may lead to α -cells re-programming to insulin producing β -cell (Thorel et al., 2011). A recent investigation showed that mature α -cells produce small quantity of GLP-1. In addition, results from diabetic rodents have demonstrated an elevated level of GLP-1 secretion from α -cells with increased proglucagon and prohormone convertase 1/3 expression via elevated plasma levels of interleukin-6. This in turn, promotes GLP-1 release from pancreatic α -cells and GLP-1 enhances insulin release from β -cells and improves the overall glucose homeostasis. Therefore, interleukin-6 may be considered as a link among insulin-sensitive tissues and pancreatic islet cell via GLP-1 (Ellingsgaard et al., 2011). As revealed from these data and confirmed in the present study, the co-localization of either GLP-1 or exenatide within α -cells may play a beneficial effects of GLP-1 on both β - and α -cells in decreasing diabetic complications (Ellingsgaard et al., 2011). Additionally, the findings of the present study are in agreement with a previous related study in which the expression of the GLP-1 receptor mRNA and its protein in α -cells was reported in both rat islets α -cells and in α -

cell lines (Heller et al., 1997). Therefore, GLP-1 may have pronounced actions on isolated α -cells (Gromada et al., 1998).

In addition, the present immunofluorescent double labelling study has shown the co-localization of either catalase or GSH with both insulin and glucagon in β - and α -cells, respectively in normal and diabetic rats treated with either GLP-1 or exenatide. This co-localization may explain the pronounced protective antioxidant action of catalase and GSH on increasing insulin secretion and decreasing glucagon secretion. This beneficial action of catalase and GSH may act through reduction of the harmful effects of reactive oxygen species and chronic gluco-lipotoxicity conditions, which are associated mainly with diabetes (Figueroa-Quevedo and Agil, 2011). GLP-1 and exenatide treatment increases antioxidant enzymes, which in turn protect against β -cell injury in diabetes leading to a reduction in toxic lipid peroxidation and reactive oxygen species. The co-localization of catalase or GSH antioxidant enzymes with insulin in diabetic β -cell under the GLP-1 and exenatide treatment can lead to improvement in β -cell functions by suppressing β -cell apoptosis via activation of PKA and PI-3K/PKB pathways and reduction of caspase-3 apoptotic action (Vaghasiya et al., 2009). The results of these previous studies explain the beneficial action of the co-localization of either catalase or GSH within β -cells, which was detected in the present study.

Like the β -cells, the immunofluorescent double labelling figures of the present study have also demonstrated the co-localization of either catalase or GSH glucagon in α -cells of normal and diabetic rats treated with either GLP-1 or exenatide. GLP-1 induces its effect in a glucose-dependent way via both insulin release and glucagon inhibition by activating GLP-1 receptors in either β - or α -cells or both (D'Alessio and Vahl, 2004). The blood glucose level is normally under continuous control of both insulin and glucagon. In T2DM, the normal action of glucose response in both β - and α -cells is abolished. This is mainly associated with reduction of insulin secretion and elevated glucagon release that lead to a condition of chronic hyperglycaemia. The loss of glucose sensitivity on both β - and α -cells not only leads to cell function failure but also lead to a structural abnormalities of both cells. The beneficial effect of GLP-1 in restoring the normal sensitivity of β - and α -cells to glucose stimulation in turn may lead to improved glucose tolerance (Göke, 2008, Vaghasiya et al., 2010).

4.7 Electron microscopic study of pancreatic islet cells of normal and diabetic rats treated with either GLP-1 or exenatide

In the present study, the ultrastructural findings markedly support the immunohistochemical and immunofluorescent data. The β - and α -cells of diabetic pancreas differ from that of the normal pancreas in the quantity of secretory granules, which are markedly less in diabetic pancreas compared to normal pancreas. The electron microscopy used in this study has shown also that treatment of normal and diabetic rats with either GLP-1 or exenatide can increase the number of insulin secretory granules in treated rats (Persaud and Jones, 2008).

These findings are in agreement with a previous study in which transplanted pancreatic β -cells were treated with exenatide in mice (Toyoda et al., 2008). These authors showed that the number of insulin secretory granules was markedly elevated in transplanted exenatide treated β -cells. The study also demonstrated an improvement in the action of GLP-1R signalling in restoring normal blood glucose levels in diabetic mice. The quantity and mass area of the pancreatic islet grafts were pronouncedly increased after exenatide treatment with reduction of islet cell death due to enhancement of GLP-1R signalling (Toyoda et al., 2008).

The results of other studies also showed that GLP-1 can activate glucose-stimulated insulin release (Lupia and Prato 2008). In another study, the effects of GLP-1 and GLP-1R agonists were shown to promote insulin gene expression, insulin synthesis, β -cell differentiation, islet cells proliferation, suppression of islet cell death and also growth of β -cell mass (Wajchenberg, 2007). Murine model of diabetes is associated with the development of endoplasmic reticulum (ER) stress in β -cells and treatment with exenatide significantly reduced the biochemical markers of islet ER stress thereby improving cell organelles structure and survival in a PKA-dependent way (Yusta et al., 2006).

In the present study, electron microscopic examination of β -cells in diabetic islet showed a pronounced cellular organelle deformation, decreased secretory granules,

shrinking of the nucleus, destruction of nuclear envelope, hypertrophied Golgi apparatus and swelling of both mitochondrial and rough endoplasmic reticulum. These β -cell structural abnormalities can lead to suppression of its functions, practically, insulin expression and release. The findings of the present study are in agreement with a previous study (Bolkent et al., 2000).

GLP-1 and exenatide treated diabetic rats showed that β -cells ultrastructure was slightly near to normal. In addition, the treated-diabetic rat β -cells showed conservation of cells number and mass with an increase in insulin secretory granules, which verified a reduction of blood glucose concentrations detected in the present study. The normal architecture of β -cells was due to the beneficial effect of either GLP-1 or exenatide on insulin secretion and improvement of diabetic cellular abnormalities like degranulated secretory vesicles, swollen mitochondria and endoplasmic reticulum stress. These results are consistent with those obtained from a previous study (Zhou et al., 2009).

The deficient antioxidant protective system in diabetic rats may lead to destructive effects on β -cells at the level of cellular structure (Degirmenci et al., 2005). The inhibition of antioxidant enzymes activity in diabetic rats may be due to excessive production of harmful free oxygen species and glucotoxicity of chronic hyperglycaemia (Teixeira-Lemos et al., 2011). The levels of both catalase and GSH have shown a marked reduction in these protective antioxidant enzymes in pancreatic islet cells of STZ-induced diabetic rats (Sathishsekar and Subramanian, 2005; Mahmood et al., 2003). In the present study, treatment of diabetic rats with either GLP-1 or exenatide leads to elevation of catalase and GSH levels in diabetic rat islets. This beneficial effect of catalase and GSH on pancreatic isles may help in reducing the production of toxic oxygen radicals, lipotoxic products and other xenobiotic elements, which can lead to a destruction of β -cells structure (Ilhan et al., 2001; Arulselvan and Subramanian, 2007). In addition, catalase and GSH stimulate β -cells regeneration and proliferation via cAMP-activated protein kinase that induces insulin production and release in β -cells (Richards et al., 2005), that may lead to returning the architecture of the β -cells near normal as proved via electron-microscopy figures in the present study under the effect of either GLP-1 and exenatide treatment.

On the other hand, the electron microscopy figures in the present study have also shown a co-localization of insulin with either GLP-1 or exenatide within β -cells in immunogold double labelling study. Insulin and GLP-1 co-localize in pancreatic β -cells. This is more conspicuous in normal rat pancreas. Similarly, the electron microscopy figures have also shown co-localization of glucagon with either GLP-1 or exenatide within pancreatic α -cells in immunogold double labelling in the present study. The absence of staining on some granules may be due to several reasons, namely, (1): The concentration of the antibody and incubation time may not be sufficient, (2): The unstained granules may be different, because it has been that pancreatic endocrine cells contain heterogeneous granules as some cells will contain more C-peptide, the precursor of insulin, (3): The plane of section may be outside of the antigen-antibody complex.

The co-localization of insulin with either GLP-1 or exenatide in the pancreatic β -cells may be due to the fact that GLP-1 is also co-synthesized in β -cells in a way similar to many other peptides including γ -aminobutyric acid (GABA) and galanin, which are produced regularly in β -cells (Adeghate and Ponery, 2003). This observation may indicate an autocrine action of GLP-1 and exenatide on insulin secretion (Wang et al., 2002). Suppression of the PDX-1 gene of mice β -cells showed a reduction in normal insulin expression and an elevation in glucagon expression (Wang et al., 2002). Similar results were detected in PDX-1 gene knockout and in the expression of brain-4 gene in INSr- β -cell line leading to inhibition of insulin expression and stimulation of glucagon expression (Wang et al., 2004). The possibility of this INSr- β -cell line to secrete insulin or glucagon under the effect of stimulation or inhibition of PDX-1 may explain the co-localization of insulin granules with either GLP-1 or exenatide in β -cells in immunogold double labelling in β -cell electron microscopic figures shown in the present study. The processing of proglucagon to glucagon and further processing to GLP-1 occur in α -TC1-6-cell line. Incubation of α -TC1-6-cell line in high glucose concentration resulted in GLP-1 formation and reduced glucagon release (Whalley et al., 2011). This may induce a similar mechanism of GLP-1 production in β -cells under specific condition such as brain-4 gene stimulation. In addition, the co-localization of glucagon with either GLP-1 or exenatide in α -cell as shown by double labelling

immunogold electron microscopic figures in this study, clearly support immunohistochemical and immunofluorescent results.

The co-localizations of either insulin or glucagon with either GLP-1 or exenatide may also explain the critical role of GLP-1 and exenatide in enhancing insulin secretion from β -cells (Kim and Egan, 2008) and the inhibition of glucagon release from α -cells (Quesada et al., 2008), as confirmed by electron microscopy in the present study.

4.8 Gene expression in the pancreas of normal and diabetic rats treated with GLP-1 and exenatide

Relative real-time PCR is one of several modern molecular biology techniques, which can help in exploring advanced physiological actions in depth at the gene expression levels. Estimating the levels of gene transcription factors was done in the present study using relative quantification assay by real-time PCR technique. In this study, relative quantification was used to compare the levels of gene expression of treated rats relative to untreated rats after their normalization by the housekeeping β -actin gene expression using relative real-time PCR. Relative quantification provided accurate comparison between the gene expression in each group in a direct way and without the use of standard curves (Dall'Oglio et al., 2010). The cause of diabetes mellitus is multifactorial and includes factors such as environmental, physical body activities and gene-related heredity. For the genetic cause of diabetes, it was decided to investigate the changes in the gene expression for a number of transcription factors (mRNA) that are associated with insulin gene expression and insulin secretion. These genes include pancreatic duodenal homeobox-1, heat shock protein-70, glutathione peroxidase, glucagon, insulin receptor and glucagon like peptide-1 receptor in the pancreatic tissues of both normal and diabetic rats following treatment with either GLP-1 or exenatide. These genes are very important for the control of glucose homeostasis, β -cell functions and related to the development of diabetes (Barry et al., 2002).

The results presented in this study have shown that both GLP-1 and exenatide can increase all of the above mRNAs, except for glucagon mRNA expression, which was

reduced in both normal and diabetic rats. In the majority of cases, there were significant increases in most of the treated rats.

4.8.1 Pancreatic duodenal homeobox-1 (PDX-1) gene expression in normal and diabetic rats treated with either GLP-1 or exenatide

The results shown in the present study have indicated that either GLP-1 or exenatide treatment can lead to significant elevation in the gene expression of pancreatic duodenal homeobox-1 (PDX-1) in the pancreas of both normal and diabetic rats. These enhancements in the gene expression of PDX-1 correlated well with the increased levels of insulin secretion obtained in the *in vivo* and *in vitro* studies in the present project in agreement with previous study (Shu et al., 2011).

In addition, the increase in the gene expression for PDX-1 obtained in diabetic rats treated with exenatide was much more pronounced compared to diabetic rats, which were treated with GLP-1, and also normal rats treated with either GLP-1 or exenatide. This observation is in agreement with previous studies, which have shown that rats treated with exenatide showed stronger and more extended enhancing effect on insulin secretion compared to GLP-1 treatment through the activation of PDX-1 expression (Brissova et al., 2002; Doggrell, 2007). Furthermore, the result of PDX-1 gene expression in the present study was much higher in diabetic rats than in normal rats. This may be due to the fact that diabetic rats exhibited marked reduction in β -cells number and function compared to normal rats. This difference revealed an exaggerated demand in diabetic rats for more functional β -cells than in normal rats. In addition, PDX-1 gene is one of the essential genes that can stimulate the development of the pancreas as a whole and also it enhances insulin gene expression, increases β cells proliferation, neogenesis and also it inhibits β cell apoptosis. This explanation was in agreement with those of Perfeitti et al., (2000), in which PDX-1 protein concentrations was reported to be elevated 4-fold in pancreatic tissues of normal rats treated with GLP-1 compared to untreated normal control rats. While in another related study on C57B16 genetically engineered diabetic rats treated with GLP-1, an increase of about 5-fold of PDX-1 protein was reported in pancreatic tissues in GLP-1 treated diabetic

rats compared to untreated diabetic control (Stoffers et al., 2000). According to the two previous studies, the elevation of PDX-1 expression in GLP-1 treated diabetic rats was obviously more than in GLP-1 treated normal rats and these results were consistent with the results in the present study.

Several pancreatic transcription factors are associated with the development of the pancreas and β -cell differentiation (Cerf, 2006). PDX-1 is considered as one of the homeodomain (HD)-containing protein gene family that are essentially involved in the development of the pancreas and the preservation of the normal action of β -cell (Kaneto et al., 2007). In a previous study, a critical role of the PDX-1 transcription factor on the action of GLP-1 receptor in mice β -cells was reported. This study showed that exenatide can markedly improve the growth, insulin mRNA expression and insulin secretion of pancreatic β -cell in addition to inhibiting cell death. However, exenatide could not enhance insulin release in β -cell PDX-1 knockout mice (Li et al., 2005). In a similar study, increasing GLP-1 receptor signalling might enhance insulin gene expression (Fontes et al., 2010). Exenatide may also improve diabetic β -cell transcription factor PDX-1 via reduction of the glucolipototoxicity down regulatory adverse effects (McCarty, 2007).

Many other studies have shown that GLP-1 may enhance the expression of insulin gene, insulin secretion and several other involved genes with insulin action including glucokinase and GLUT-2 in a glucose-dependent manner. These effects of GLP-1 are mediated mainly by enhancing the PDX-1 transcription (Wang et al., 2001; Kemp and Habener, 2001; Movassat et al., 2002; Alarcon et al., 2006; Lamont and Drucker, 2008). A β -cell line cultured in elevated glucose concentration revealed a decrease in the expression of PDX-1. In addition, a suppression of GLUT-2 gene transcription was also detected in diabetic animal. Therefore, these results postulate that the GLUT-2 expression could be PDX-1-dependent (Hart et al., 2000).

A previous study has earlier shown that the expression of murine GLUT-2 cell line was activated via binding of PDX-1 to GLUT-2 receptor (Waeber et. al., 1996). Another study has revealed that GLP-1 can elevate the expression of PDX-1, GLUT-2,

glucokinase and insulin mRNAs through the PDX-1-dependent and PI-3K activation cascades (Buteau et al., 1999).

Another earlier study has demonstrated a reduction in the transcription of β -cells insulin related genes and GLUT-2 in rats after 4 weeks of about 90% pancreatectomy (Laybutt et al., 2002). The results of this mentioned study have shown a diminished glucose-stimulated insulin release and reduced ATP production with impaired carbohydrate and lipid metabolism (Laybutt et al., 2002). A similar *in vitro* study has shown that prolonged increased concentrations of fatty acids, hyperglycaemia and reduction in both insulin secretion and insulin gene transcription could lead to β -cell apoptosis (Poitout and Robertson, 2008). All of these adverse effects of chronic gluco-lipotoxic action may be attributed to the suppression of the PDX-1 transcription factor (Hagman et al., 2005).

In another previous study, increased neogenesis of pancreatic β -cells under GLP-1 treatment via enhancement of PDX-1 and insulin genes transcription was shown to increase β -cells mass and it also stimulated both insulin synthesis and secretion. The action of GLP-1 on PDX-1 expression was mainly specific for β -cell insulin gene translation. The GLP-1 exerts its action through binding to its receptor and promotes GLP-1R expression cascade (Yue et al., 2006). The action of GLP-1 and exenatide on β -cell differentiation in response to PDX-1 activation may be through stimulation of protein kinase B and a cAMP-dependent mechanism (Drucker, 2003a; Holz, 2004).

4.8.2 Effect of GLP-1 and exenatide treatment on heat shock protein 70 (HSP-70) gene expression in normal and diabetic rats

The results of the present study have shown a significant increase in the expression of the anti-apoptotic gene, heat shock protein 70 (HSP-70) in normal and diabetic rats treated with either GLP-1 or exenatide.

HSP-70 is one of cell stress-induced proteins via molecular chaperone control of protein synthesis, formation of protein polypeptides, folding, removal of misfolded proteins, and reposition of recently produced proteins (Kim et al., 2007a). In addition,

HSP-70 extends cell life span and suppresses cell death (Mokhtari et al., 2009). The main function of HSP-70 under cell stress is to minimize cell apoptosis via maintenance of normal cell protein structure and elimination of injured proteins for demolition (Muranyi et al., 2005). Consistent with the result of the present study, elevated blood glucose levels in diabetic rats seem to reduce the HSP-70 expression due to decreased insulin secretion, which has been shown to stimulate HSP-70 expression (Chen et al., 2006).

The concentrations of antioxidant protective enzyme mRNA are mainly related to their functions (Tiedge et al., 1997). The β -cell antioxidant is a fragile protective system, which can be easily disturbed by excessive formation of either reactive oxygen or nitrogen species (Hong et al., 2004). This in turn can cause impaired effects including marked production of oxidative stress elements such as lipid peroxidation, proteins oxidation, disruption of transduction signal cascades and DNA injures. All of these destructive effects add markedly on the disturbance of β -cell function leading to β -cell apoptosis in both T1DM and T2DM (Hayden and Tyagi, 2002). Dysfunction and death of β -cells can cause overproduction of inflammatory elements in T1DM and excessive formation of toxic chronic hyperglycaemia and hyperlipidaemia in T2DM. These are probably due to increased toxic oxygen species of superoxide radicals, hydrogen peroxide, hydroxyl radicals and peroxynitrite (Vincent et al., 2004; Li et al., 2008). In addition, elevation of the level of reactive oxygen species in the mitochondria of pancreatic β -cell can lead to impairment of the nutrient-induced insulin release (Lenzen, 2008).

All of these previous studies clearly documented that the destructive effects of elevated oxidative stress can be reduced by enhancement of the antioxidant protective system. Moreover, the results of the present study have shown an elevation of the antioxidant enzymes in pancreatic islet cells of diabetic rats treated with either GLP-1 or exenatide. The elevation of the antioxidant protective enzymes can reduce the adverse complications in diabetic rats in agreement with previous study (Vaghasiya et al., 2010a). GLP-1 and its agonist, exenatide can exert their protective effects via enhancement of HSP-70 action as confirmed in a previous related study (Kanitkara and Bhonde, 2008). In this related study, the pretreatment of pancreatic islets graft with

antioxidant agent such as curcumin for transplantation can preserve the grafted β -cells from damage. This antioxidant pretreatment increases cell recovery after the transplantation. Curcumin seemed to exert its protective effect mainly through the elevation of HSP-70 leading to reduction of the harmful action of the oxidative stress and enhancement of glucose-induced insulin secretion (Kanitkara and Bhonde, 2008).

In DM, elevated HSP-70 levels can lead to improvement of glucose tolerance and insulin resistance (Kavanagh et al., 2011). GLP-1 and exenatide treatment can also increase HSP-70 levels as detected in the present study. HSP-70 may exert its action via normalizing protein homeostasis in β -cell by inhibiting misfolding protein signalling pathways. This HSP-70 mode of action may promote insulin production, inhibit β -cells apoptosis and enhance β -cell propagation (Hutt et al., 2009).

4.8.3 Effect of GLP-1 and exenatide treatment on glutathione peroxidase (GPx) gene expression in normal and diabetic rats

The results of the present study have shown a significant increase in the expression of antioxidant gene, glutathione peroxidase (GPx), in normal and diabetic rats treated with either GLP-1 or exenatide.

The results obtained in the present study in the diabetic rats are consistent with previous studies, which demonstrated a marked reduction in gene expression of GPx when compared to healthy normal control rats (Otsuka et al., 2002; Wentzel et al., 2008). In another previous study, the level of either gene expression of antioxidant enzymes or oxidative stress was estimated in diabetic STZ-induced diabetic mice. The expression of GPx and superoxide dismutase were diminished due to disturbance of the antioxidant enzyme protective system under the effect of reduced insulin secretion and increased blood glucose levels in diabetic mice (Fujita et al., 2005).

Another study has shown that GPx antioxidant enzyme level was elevated in diabetic rats treated with such antioxidants agents as vitamin E and C, or in combination with insulin. This treatment improved the function and the protein synthesis of GPx leading to recovery from diabetic complications (Sindhu et al., 2004). The elevation of GPx

levels in this study after treatment with insulin and antioxidant agents may explain the elevation of GPx gene expression level in the present study after either GLP-1 or exenatide treatment. This shows that these incretins have insulintropic and antioxidant effects (Timmers et al., 2009).

Several clinical studies have shown a prolonged increase in oxidative stress in T2DM patients. The increased production of GPx protects pancreatic β -cells against the destructive effects of oxidative stress (Monnier et al., 2006; Robertson, 2010). Other studies have also shown that induction of GPx carried on adenovirus into a defective human pancreatic β -cell line can lead to enhancement of the GPx expression against the harmful action of oxidative stress. The beneficial effect of GPx does not only target oxidative stress, but it also causes a reduction in glucose toxicity by improving glucose tolerance. In this context, it is able to preserve β -cell morphology and its functions (Robertson and Harmon, 2007).

GLP-1 and exenatide treatment can lead to a reduction in the level of the oxidative stress by reducing lipid peroxidation and myeloperoxidase, agents that can induce cellular injuries in diabetic rats (Altunoluk et al., 2006). In addition, GLP-1 and exenatide may exert their effects on β -cells through the activation of PI-3K/PKB (a protective cellular pathway) and suppression of caspase-3 apoptotic activity (Vaghasiya et al., 2010).

4.8.4 Effect of GLP-1 and exenatide treatment on glucagon gene expression in normal and diabetic rats

The results of the present study have shown a significant reduction in the expression of glucagon gene in both normal and diabetic rats treated with either GLP-1 or exenatide.

The release of glucagon from pancreatic α -cells is stimulated via several hormones and neurotransmitters. However, GLP-1 inhibits glucagon release (Ahren, 2009). In T2DM, chronic elevated blood glucose levels can lead to increased glucagon concentrations and enlarged α -cell mass relative to β -cell mass with direct elevation of serum glucagon/insulin ratio. This condition is reversed with GLP-1 treatment (Ahren, 2009).

In diabetic patients, the marked increase in glucagon release is mainly associated with lack of insulin inhibition. However, in healthy individuals food intake induces insulin secretion, which in turn inhibits glucagon release from α -cell of the pancreas (Strowski et al., 2006).

Another previous study also showed that insulin could decrease glucagon mRNA level via suppression of glucagon gene transcription and reduction of glucagon release (Kaneko et al., 1999). GLP-1 enhances the release of either insulin or somatostatin and reduces glucagon release. GLP-1 inhibits glucagon release in either healthy or diabetic individuals through the paracrine action of elevated intra-islet secretion of both insulin and somatostatin (Fehmann et al., 1995). GLP-1 also exerts its direct inhibitory action on glucagon secretion from pancreatic α -cells through GLP-1 receptors found on α -cells (Heller et al., 1997). In T2DM, fasting hyperglucagonaemia is present and elevated glucagon response to food ingestion can lead to additional increase in blood glucose level (Toft-Nielsen et al., 2001). Although the β -cell mass is markedly reduced in T1DM, GLP-1 is still capable of reducing the levels of fasting blood glucose, probably via the suppression of glucagon secretion (Creutzfeldt et al., 1996). Insulin is an inhibitor of glucagon secretion, but the pronounced inhibitory effect of GLP-1 in T1DM is without the involvement of β -cells, suggesting that it may be inhibiting glucagon secretion from α -cells (Creutzfeldt et al., 1996). The inhibitory effect of GLP-1 on glucagon secretion is seen only at glucose levels either at or above fasting levels. This is important because it implies that treatment with GLP-1 does not lead to hypoglycaemia (Holst, 2007), as it tends to correct only abnormal rise in blood glucose level. A previous study on mice with a glucagon receptor double knockout was shown to be associated with suppression of prohormone convertase-2, which is essential for the transformation of proglucagon to active glucagon. This condition can lead to an increase in GLP-1 production from mice pancreatic α -cells (Kedees et al., 2009).

Other studies have also shown that STZ-treatment in rats can result in enhancement of prohormone converting enzymes, PC1 and PC2 in pancreatic α -cells with increased levels of glucagon. In addition, these results also showed an increase in GLP-1 production from these α -cells. This increased production of GLP-1 stimulates insulin secretion from β -cells in pancreatic islets (Nie et al., 2000). Therefore, activation of

PC1 and PC2 enzymes may initiate the processing of glucagon gene to GLP-1 production in STZ-induced rats. This in turn may explain the reduction of glucagon gene expression, and elevation of the number of GLP-1-positive cells, increased insulin levels and better glucose homeostasis as observed in the present study.

4.8.5 Effect of GLP-1 and exenatide treatment on insulin receptor (IR) gene expression in normal and diabetic rats

The results of the present study have shown a significant increase in the expression of insulin receptor gene (IR) in normal and diabetic rats treated with exenatide. However, the increase in the expression of IR gene was not significant in normal rats treated with GLP-1. IR is a type of tyrosine kinase receptor and it is a vital element for pancreatic β -cell growth, normal β -cell action and extended β -cell life. Signalling via IR in the β -cell regulates insulin production and secretion (Kitamura et al., 2003). IR signalling also has an important role in conserving the β -cell responsiveness for ingested food (Fisher and White, 2004; Shamji et al., 2003). Reduction of two receptors, IR and insulin-like growth factor-I receptor (IGF-IR) in addition to their associated substrates, can disturb the normal action of β -cell. These findings demonstrate the importance of insulin signalling in insulin release and the increase in β -cell mass-induced by insulin resistance. In consequence, enhancement of IR expression in β -cells could be considered as a valuable treatment for diabetes in general and restoring β -cells functions (Kitamura et al., 2003; Nandi et al., 2004; Rhodes and White, 2002). The results of the present study are in agreement with studies that showed that the expression of IR was markedly induced in β -cells after exenatide treatment (Park et al., 2006). Nutrition and exercise have also been reported to induce IR expression (Belfiore et al., 2009).

The failure of insulin signalling, referred to as insulin resistance, is one of the major causes of T2DM. Double deletion of IR in β -cells of animal models can lead to a reduction in insulin production in response to β -cell glucose-stimulus (Srinivasan and Ramarao, 2007). Moreover, a deficient IR can markedly reduce the influx of glucose into β -cells (Escribano et al., 2009).

A genetically engineered diabetic mice with IR deletion in pancreatic β -cells, showed a reduction in insulin release with elevated blood glucose levels after food consumption and this is probably due to failure of β -cell for glucose sensing (Kulkarni et al. 1999). In addition, these mice revealed markedly decreased expression levels of both GLUT-2 and glucokinase. Thus, suppression in glucose uptake by β -cell may be associated with a reduction in GLUT-2 level (Otani, et. al., 2004).

Insulin resistance influences the structure and the normal action of β -cells. Insulin releases via β -cells after glucose stimulation influences insulin gene expression in an autocrine way (Leibiger et al., 2001). Disturbance of IR gene in mice can cause ketoacidosis. In addition, IR expression was reduced in islets isolated from humans with T2DM (Gunton et al., 2005). A decrease in IR expression in β -cells can cause glucose intolerance, which is accompanied by a decrease in islet volume (Kulkarni et al., 1999). Small reduction in IR expression in human islets completely suppressed glucose-stimulated insulin gene expression, suggesting that insulin regulates its own gene expression in human β -cells. Defects in this regulation may explain the association between metabolism dysfunction and T2DM (Muller et al., 2006).

A previous study has demonstrated that there are two IRs isoforms IR-A and IR-B. Both of them seem to initiate dissimilar downstream mechanisms in pancreatic β -cells. IR-A activates insulin gene expression while IR-B stimulates glucokinase via various types of phosphatidylinositol-3 kinase (PI-3K) (Leibiger et al. 2001).

4.8.6 Effect of GLP-1 and exenatide treatment on GLP-1 receptor (GLP-1R) gene expression in normal and diabetic rats

The results of the present study have shown an increase in the expression level of GLP-1 receptor gene (GLP-1R) which was significant in normal and diabetic rats treated with either GLP-1 or exenatide except in normal rats treated with GLP-1.

GLP-1R is expressed on the plasma membrane of β - and α -cells in the pancreas and many other body tissues including gastrointestinal tract, heart, lung, kidney, peripheral (vagus) and central nervous systems (Salehi et al., 2008). GLP-1 can cross the blood

brain barrier by simple diffusion and thus contributes to the regulation of the activities of the satiety and appetite centres in the brain (Kastin, et. al., 2002). Exenatide stimulates GLP-1R in a similar way to the native GLP-1 and it is considered as a GLP-1R agonist (Drucker, 2003a). GLP-1R mRNA was detected in the β -cells of pancreatic tissues of mice and rats (Tornehave et al., 2008).

Binding of GLP-1 to its GLP-1R on β -cell initiates a signal transduction cascade leading to improved β -cell function including elevation of insulin gene expression, release of insulin, propagation of many other regulatory genes which are involved in the proteins synthesis, increases of β -cell differentiation and reduction of the rate of β -cell death (Holst and Deacon, 2006; Druker, 2005). It was shown in a previous study that treatment of pigs with exenatide inhibits cardiac cell apoptosis via reduction of caspase-3 expression (Timmers et al., 2009).

Many other studies have shown a similar beneficial effect of GLP-1 and exenatide treatment in the improvement of the expression of GLP-1R (Scrocchi et al., 1996; Dungan and Buse, 2005). In an *in vitro* study, GLP-1R was shown to enhance signalling in pancreatic islets grafted into STZ-induced diabetic mice after treatment with exenatide. This in turn could lead to enhancement of the antioxidant protective system, normalization of both the blood glucose concentrations and the structure of grafted cells (Toyoda et al., 2008). Furthermore, GLP-1R knockout mice showed an elevated fasting blood glucose level, which are associated with glucose intolerance and decreased insulin release (Preitner et al., 2004; Dungan and Buse, 2005).

Islets of GLP-1R^{-/-} mice is associated with a significant reduction in islet size (Burcelin et al., 2001). This is confirmed by the fact that GLP-1R knockout mice showed a reduction in pancreatic islet cells number and size with aberrations of cells arrangement (Ling et al., 2001). Earlier studies have shown that exenatide treatment causes an increase in β -cell mass and moreover, it can also enhance its action via initiation of islet cell differentiation and suppression of cell death. Ablation of GLP-1R, results in the reduction of the compensatory growth observed after partial pancreatectomy (De Leon et al., 2003). Increase in GLP-1R expression can lead to

improvement of β -cell sensitivity to blood glucose levels (Montrose-Rafizadeh et al., 1997).

GLP-1R signalling plays a crucial role in controlling β -cell differentiation and extending β -cell life span. The exenatide probably acts via GLP-1R as confirmed in GLP-1R^{-/-} mice treated with exenatide (Deleon et al., 2003). No islet cell proliferation was observed after exenatide treatment.

Moreover, treatment with either GLP-1 (Bulotta et al., 2002), or exenatide (Stoffers et al., 2000), of pancreatic ductal cells can lead to cell differentiation into β -cells compared to GLP-1R^{-/-} mice, where this effect is abolished. As documented from these studies, GLP-1R is expressed on pancreatic ductal cells and thus could be involved in the differentiation of new β -cells. GLP-1 and exenatide may thus be exerting their beneficial effects by increasing the expression GLP-1R, as confirmed in the present study.

Exenatide binds to the human pancreatic GLP-1R and initiates glucoregulatory effects (Parkes et al., 2001) and induces insulin secretion only under elevated blood glucose levels. This stimulating effect of exenatide on insulin gene expression and transcription is responsible for a continuous and a prolonged supply of insulin. After the glucose level has returned to normal level, the GLP-1 no longer binds to GLP-1R, thus preventing further insulin release and thus avoiding unwanted hypoglycaemia (Knop et al., 2009). This mechanism of GLP-1 action gives GLP-1 treatment a unique effect over the other traditional insulin secretagogue drugs.

GLP-1 treatment has been shown to increase β -cells regeneration in STZ-model of diabetes. In addition, suppression of GLP-1R can prevent β -cells regeneration (Thyssen et al., 2006). In addition, in human pancreatic progenitor cells, GLP-1 binds to its receptor and propagates cell signalling that initiates cell proliferation (Suen et al., 2008). The cascade of the GLP-1 receptor signalling is regulated via cAMP and PI-3K dependent pathways leading to enhancement of β -cells proliferation (Yew et al., 2005; Salehi et al., 2008).

4.9 Mode of action(s) of GLP-1 and its agonist exenatide

The schematic model in figure 4.1 summarizes the main signalling cascades of action of either GLP-1 or its receptor agonist, exenatide, after binding to GLP-1R on pancreatic β -cell. GLP-1 binds to a specific receptor, GLP-1R, a seven-transmembrane domain, G-protein-coupled receptor on pancreatic beta cell (Girard, 2008). This binding process initiates the $G_{\alpha s}$ -receptor subunit of GLP-1R leading to acute insulin release. It also induces chronic expression of insulin gene and many other related genes involved with insulin gene expression, augment pancreatic cell proliferation and inhibits cell beta cell apoptosis (Liu and Habener, 2008). These cascades are regulated in a glucose-dependent manner and they are only called into play when the blood glucose level is increased. At high, abnormal glucose levels, GLP-1 binds to GLP-1R and in turn stimulates the expression of GLUT-2 transporter and glucokinase that ultimately increase β -cell uptake of glucose via GLUT-2 receptor (Kim and Egan, 2008a). Increased cytosolic glucose concentration leads to stimulation of glucokinase, a β -cell glucose sensor, leading to enhancement of glycolysis and increased production of pyruvate, resulting in the production of more ATPs via the Krebs's cycle (Levine and Puzio-Kuter, 2010). Increased ATP/ADP ratio causes the closure of K_{ATP} channels, propagation of action potential, depolarization of the plasmalemma, leading to opening of the L-type voltage-dependant Ca^{2+} (VDC) channels that increases the Ca^{2+} influx into the cytoplasm. Elevated cytosolic Ca^{2+} binds to insulin-containing secretory granules that initiate aggregation, fusion, exocytosis and finally insulin secretion (Holst, 2007). At the same time, increased ATP level activates the $G_{\alpha s}$ -receptor subunit of the GLP-1R to stimulate adenylate cyclase (AC) that mediate the conversion of ATP to cyclic AMP (cAMP). In turn, cAMP activates protein kinase A (PKA) (Wang et al., 2001a) and exchange protein (Holz, 2006). Simultaneously, after ATP generation, both PKA and phosphatidylinositol-3 kinase (PI-3K) take part in the closure and activation of K_{ATP} channel depolarization and delay the K^+ voltage channel (K_v) repolarization. The two actions enhance the propagation of membrane action potential (MacDonald et al., 2003). The spreading of action potential through the pancreatic β -cell plasma membrane opens the VDC channels leading to increased influx of Ca^{2+} into the cytoplasm (Kang et al., 2003). In addition, activation of PKA and Epac leads to an

increase in the release of intracellular Ca^{2+} from endoplasmic reticulum (ER) stores via activation of inositol trisphosphate receptor (IP_3R) activated by PKA (Taylor et al., 2004). Elevation of cytosolic Ca^{2+} in turn promotes exocytosis leading to the secretion of insulin (Light et al., 2002). Increased cytoplasmic Ca^{2+} will also stimulate calcineurin, which is responsible for the translocation of nuclear factor of activated T cells (NFAT) into the nucleus. NFAT protein binds to insulin gene and initiates insulin transcription (Heit et al., 2006).

Furthermore, PKA and Epac activate docking and union of insulin-containing vesicles during exocytosis of insulin (Kwan et al., 2007). PKA activation via cAMP can also cause a downstream activation and translocation of PDX-1 into the nucleus. At this point, PDX-1 initiates insulin gene expression resulting in increased β -cell proliferation and mitosis (Wang et al., 2005; Babu et al., 2007). PKA also stimulates cAMP response element binding protein (CREB) in the nucleus, which results in increased IRS-2 gene transcription. IRS-2 is translocated from the nucleus to the cytoplasm and help in the activation of phosphatidylinositol-3 kinase (PI-3K) (Kemp and Habener, 2001).

Moreover, sustained GLP-1 binding to its GLP-1R leads to the activation of $G_{\beta\gamma}$ -receptor subunit of GLP-1R, which in turn activates directly the PI-3K or indirectly via activation of cytoplasmic tyrosine kinase (cSrc) that enhances β -cellulin, which stimulates the epidermal growth factor receptor (EGFR) on β -cell membrane. EGFR is a stimulator of PI-3K (Buteau et al., 2003). Downstream activation of PI-3K and protein kinase B (PKB) and protein kinase C- ζ (PKC- ζ) pathways regulate β -cell mitosis and proliferation. PKB is also involved in the prevention of β -cell death by suppression of the apoptotic factor, caspase-3 and forkhead box O1 (FoxO1). In turn, FoxO1 is deactivated by nuclear exclusion (Wang and Brubaker, 2002). Consequently, PKB prevents the inhibitory effects of both caspase-3 and FoxO1 on PDX-1 that promotes β -proliferation (Buteau et al., 2004). Elevation in ATP production and activation of PKB will both lead to stimulation of a mammalian target of rapamycin (mTOR) activity, which is involved in increasing β -cell mitosis (Kwon et al., 2004).

In conclusion, GLP-1 binding to its GLP-1R activates a large variety of signalling cascades which eventually lead to increased transcription of insulin and its secretion, β -

cell mitosis, enhancement of β -cell proliferation, suppression of β -cell apoptosis and improved β -cell functions (Salehi et al., 2008).

The major pathways numbered 1-19 in Figure 4.1 is summarized as follows:

1. Binding of GLP-1 or exenatide to GLP-1R.
2. Glucose uptake by GLUT-2 and activation of glycolysis leading to production of pyruvate and ATP.
3. Entrance of pyruvate into mitochondria activates the Krebs's cycle and more ATPs are release into the cytoplasm leading to increase in cytosolic ATP and ATP/ADP ratio.
4. ATP elevation induces closure of K_{ATP} channel leading to membrane depolarization leading to opening of VDC channels and influx of Ca^{2+} .
5. $G\alpha_s$, the subunit of GLP-1R, activates the conversion of the ATP into cAMP via adenylate cyclase.
6. cAMP activates PKA and Epac, leading to Ca^{2+} release from the ER.
7. PKA and PI-3K both induce membrane action potential via closure and depolarization of K_{ATP} channel leading to a delayed repolarization of Kv channel eventually resulting in the opening of VDC channels and influx of Ca^{2+} .
8. Elevated levels of cytosolic Ca^{2+} fuses with insulin vesicles and initiates exocytosis of insulin granules with the help of PKA and Epac.
9. Exocytosis of insulin-containing granules.
10. The expression of insulin gene and other β -cell regulatory genes are also activated through stimulation NFAT in the nucleus which is stimulated by calcineurin after elevation of cytosolic Ca^{2+} .
11. PKA activates the translocation of PDX-1 into the nucleus to initiate the expression of insulin gene and other related regulatory genes. At the same time, PKA stimulates CREB in the nucleus to initiate IRS-2 gene transcription, which will in turn induce insulin gene expression via PDX-1 stimulation.

12. IRS-2 is translocated from the nucleus to the cytoplasm and share in the activation of PI-3K.
13. The G $\beta\gamma$ subunit of GLP-1R stimulates PI-3K either directly or indirectly by activation the binding of β -cellulin to EGFR via cSrc. Then EGFR stimulates PI-3K.
14. PI-3K activates both PKB and PKC- ζ pathways.
15. The downstream activation of both PKB and PKC- ζ initiate the expression of insulin gene and other β -cell regulatory genes through stimulation PDX-1.
16. PKB also inhibits β -cell apoptosis through suppression of caspase-3 and FoxO1.
17. PDX-1 and NFAT stimulate insulin and other regulatory genes leading to more insulin synthesis and increased β -cell proliferation.
18. Both ATP and PKB stimulate mTOR, which induces β -cell mitosis.
19. In conclusion, GLP-1 binding to its GLP-1R leads to elevation of insulin gene expression, insulin synthesis and insulin secretion. In addition, activation of GLP-1R improves the function, neogenesis, proliferation of pancreatic β -cell.

Abbreviations used in Figure 4.1

Abbreviations	Meanings
AC	Adenylate cyclase
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
Ca ²⁺	Calcium ion
cAMP	Cyclic adenosine monophosphate
CREB	cAMP response element binding protein
cSrc	Cytoplasmic tyrosine kinase
EGF receptor	Epidermal growth factor receptor
Epac	Exchange protein activated by cAMP
ER	Endoplasmic reticulum
FoxO1	Factor forkhead box O1
GLUT-2	Glucose transporter-2
IP ₃ R	Inositol trisphosphate receptor
IRS-2	Insulin receptor substrate-2
K _{ATP} channel	Potassium adenosine triphosphate channel
K _v channel	Potassium voltage channel
mTOR	Mammalian target of rapamycin
NFAT	Nuclear factor of activated T cells
PDX-1	Pancreatic duodenal homeobox-1
PI-3K	Phosphatidylinositol-3 kinase
PKA	Protein kinase A
PKB	Protein kinase B
PKC δ	Protein kinase C- δ
RyR	Ryanodine receptor
VDC channel	L-type voltage dependant calcium channel

4.10 Conclusions

From this study, it can be concluded that the treatment of normal and diabetic rats with either GLP-1 or exenatide can result in marked improvements in the body weight and glucose tolerance as well as concurrent decreases in blood glucose levels. Similarly, both GLP-1 and exenatide can significantly improve the biochemical parameters of both normal and diabetic rats. Both incretins improve the status of liver enzymes, kidney functions and lipid profile, which were deranged in STZ-induced diabetic rats. Both GLP-1 and exenatide can stimulate the release of insulin from isolated pancreatic fragments in a dose-dependent manner in age-matched healthy control and diabetic rats. The number of insulin immunopositive cells was significantly reduced in STZ-induced diabetic rat pancreas and treatment with either GLP-1 or exenatide significantly increased insulin-positive cells in both diabetic and normal rat pancreas. Similarly, significant improvement and increase in immunopositive cells of either catalase or glutathione reductase as antioxidant protective markers were detected in the pancreas of diabetic rats. Treatment with either GLP-1 or exenatide decreased the number of glucagon immunopositive cells in STZ-induced diabetic rats. Similar results were also seen in electron micrograph microscopy studies. Pancreatic β -cells of both normal and diabetic rats treated with either GLP-1 or exenatide have increased secretory granules compared to controls. At the molecular level, this study shows that treatment of normal and diabetic rats with either GLP-1 or exenatide can enhance insulin gene expression through significant increases in mRNA levels of many transcription factors, which have positive impacts on insulin gene biosynthesis and its secretion. The results obtained in this study have enhanced the knowledge and understanding of the possible cellular and molecular mechanisms of the hypoglycaemic effect of either GLP-1 or exenatide (see Figure 4.1). They may provide additional impact to the arsenal of drugs to treat diabetes mellitus, not only as hypoglycaemic agents, but also as protective agents for increasing and maintenance of β -cell integrity and the proliferation of new β -cells. GLP-1 improves glucose-stimulated insulin secretion, restores glucose competence in glucose-resistant β -cells and stimulates insulin gene expression and biosynthesis (see Figure 4.1). Furthermore, GLP-1 acts as a growth factor by promoting β -cell proliferation, survival and neogenesis. Therefore, GLP-1 addresses both the defect in insulin

secretion and the decline in β -cell mass that contribute to the deterioration of β -cell function in the aetiology of diabetes.

4.11 Scope for future work

More studies would provide further insights into new ways to treat or even cure diabetes, one of the most endemic chronic diseases that is rapidly increasing at present. New studies have to be done to discover promising and novel peptides that could cure and stop the progress or even delay the onset and appearance of diabetic complications. These investigations would employ a diverse range of scientific techniques including confocal microscopy, establishment and maintenance of cell and tissue culture and molecular biological techniques. These techniques can be used in a number of possible studies to support the following:

- (A) Investigating the actions of the new peptides by focusing on their roles on the stimulation of insulin gene expression in collaboration with different related genes sharing in glycaemic homeostasis.
- (B) Investigating the mechanism(s) of actions of these new peptides starting from their binding to different cell receptors until the synthesis and secretion of insulin from pancreatic β -cells.
- (C) Investigating the role of the oxidative stress and antioxidant defense systems in the preservation of pancreatic beta cell mass.
- (D) Imaging of apoptotic and proliferation of β -cell signals using confocal and electron microscopic studies.
- (E) An *in vitro* investigation of the effects of new peptides on insulin secretion, cell proliferation and cell mass growth through β -cell line culture technique.
- (F) An investigation into the use of stem cells in the treatment of diabetes mellitus.

(G) An investigation into the use of β -cell transplantation in the treatment of diabetes mellitus.

(H) An investigation into the use of gene therapy to replace the defect in insulin gene in diabetic rats.

In general, there are a number of new studies, which can help to control and to treat DM, a disease which has affected more than 250 million people worldwide and which is spreading rapidly, almost beyond control.

CHAPTER FIVE

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APPENDIX

1. Phosphate buffer saline (pH 7.4)

1. Distilled water 800 ml.
2. Sodium chloride 8 g.
3. Potassium chloride 0.2 g.
4. Sodium dihydrogen phosphate 1.44 g.
5. Potassium dihydrogen phosphate 0.24 g.
6. Add distilled water to a total volume of 1 liter.
7. Adjust the pH to 7.4

2. Citrate buffer (pH 4.5)

1. 0.1 M Sodium citrate solution 220 ml
2. 0.1 M Citric acid solution 280 ml
3. Distilled water 500 ml
4. Adjust the pH to 4.5

3. Karnovsky's fixative

1. Formaldehyde 40% 80 ml.
2. Glutaraldehyde 25% 160 ml.
3. Sodium cacodylate buffer 360 ml.
4. Distilled water 400 ml.

4. Resin (Total = 51.25 gm)

1. Agar 24 g.
2. DDSA 16 g.
3. MNA 10 g.
4. DMP-30 1.25 g.

5. Sodium cacodylate buffer (pH 7.4)

1. 80 g of paraformaldehyde dissolved in 696 ml of distilled water.
2. Paraformaldehyde 696 ml.
3. Picric acid (aqueous) 300 ml.
4. 0.2 M Phosphate buffer 1000 ml.

6. Antibodies

Number	Antibody	Source	Dilution	Manufacturer
1	Anti-Insulin	Rabbit	1:1000	Dako, Copenhagen, Denmark
2	Anti-Glucagon	Rabbit	1:1000	Dako, Copenhagen, Denmark
3	Anti-GLP-1	Rabbit	1:1000	Phoenix Pharmaceuticals, Burlingame, California, USA
4	Anti-Exenatide	Rabbit	1:1000	Phoenix Pharmaceuticals, Burlingame, California, USA
5	Anti-GSH	Rabbit	1:100	Sigma-Aldrich, Missouri, USA
6	Anti-Catalase	Rabbit	1:200	Sigma-Aldrich, Missouri, USA

PUBLICATIONS

Full-length papers

1. **Lotfy, M.**, Singh, J., Kalász, H., Tekes, K. and Adeghate, E. (2011). Medicinal Chemistry and Applications of Incretins and DPP-4 Inhibitors in the Treatment of Type 2 Diabetes Mellitus. *The Open medicinal Chemistry Journal* **5(2)**, 82-92.
2. Amin, A., **Lotfy, M.**, Mahmoud-Ghoneim, D., Adeghate, E., Al-Akhras, M., Al-Saadi M.A., Al-Rahmoun, S. and Hameed, R. (2011). Pancreas-protective effects of chlorella in STZ-induced diabetic animal model: insights into the mechanism. *Journal of Diabetes Mellitus* **1(3)**, 36-45.
3. El Keblawy, A., Al-Shamsi, N., Lotfy, M., Al Shaik, N.S. and Al-Yammahi, A. Impact of Crop Residues on Seed Germination of Native Desert Plants Grown as Weeds. *Weed Biology and Management*, (In Press).
4. Amr, A., **Lotfy, M.**, Shafiullah, M. and Adeghate, E. (2006). The Protective effect of Tribulus Terrestris in Diabetes. *Annals of New York Academy of Sciences*. **1084**, 391-401.
5. El-Agouza I.M.; Doss S.H.; **Lotfy M.** (1997). The Effect of Some Amino Acids on Human Sperm Motility and Their Possible use as a Local Contraceptives. *Egypt. Journal of Zoology*, **29**, 255-272.

Abstracts

1. **Lotfy, M.**, Singh, J. and Adeghate, E. (2011). The beneficial effect of GLP-1 treatment in type 1 diabetic rats. *Proceedings of The Physiological Society Conference*, July 11-14, University of Oxford, UK, 23, PC222.
2. **Lotfy, M.**, Singh, J. and Adeghate, E. (2010). Protective and Antioxidant Effects of GLP-1 and Exenatide on Rat Pancreatic Beta Cells. The 10th Annual Faculty Graduate School Conference, June 30, Uclan, Preston, UK.
3. El-Keblawy, A., and **Lotfy, M.** (2009). Crop Residues with Allelopathic Capability: Toward Searching for Natural Herbicides. The 10th Annual UAE University Research Conference, April 13-16, Al Ain - United Arab Emirates. UAEU, Al-Ain, United Arab Emirates.
4. **Lotfy, M.**, Singh, J. and Adeghate, E. (2008). Pattern of distribution of glucagon-like peptide-1 and exenatide in pancreas of normal and diabetic rats. The 9th Annual Faculty Graduate School Conference, June 18, Uclan, Preston, UK.
5. Adeghate, E., **Lotfy, M.** and Rashed, H. (2008). Effect of Glucagon-like Peptide-1 and Exenatide on Insulin Release in Diabetic Rats. The 9th Annual UAE University Research Conference. April 21-23, UAEU, Al-Ain, United Arab Emirates.
6. Amr, A., **Lotfy, M.** and Adeghate, E. (2006). The Protective effect of Tribulus Terrestris in Diabetes. International Conference on Recent Advances in Diabetes Mellitus and its Complications. 6-9 March, Al Ain, United Arab Emirates.

**MEETINGS ATTENDED
AND COLLABORATIVE
WORK**

1. Participation in "The First Faculty of Science Forum on Teaching and Learning" October 13, 2011, AL Ain, UAE University.
2. Participation in "Abu Dhabi Genomics and Systems Biology Meeting", New York University Abu Dhabi Institute and NYU Center for Genomics and Systems Biology. 2010.
3. Participation in workshop on "Affymetrix Gene Expression Micro-Arrays" during Arab Health, held by Gulf Scientific Corporation, Dubai. UAE. 2010.
4. Attendance of seminar on "Life Beyond DNA " by Dr. Stefan Dimitrov. (Gernoble Institute, France). 2010.
5. Attendance of seminar on "The Oncogene Aurora Kinase Family " by Dr. Stefan Dimitrov. (Gernoble Institute, France). 2010.
6. Attendance of seminar on "Histone Variant Nucleosomes: From Structure To Epigenetic Function " by Dr. Stefan Dimitrov. (Gernoble Institute, France). 2010.
7. Attendance of seminar on "Critical role of chromatin acetylation in genome expression and maintenance" By Prof. Jacques Côté, Université Laval, Canada, Faculty of medicine and Health Science, UAE University, 2010.
8. Participation in "14th Annual workshop on diabetes mellitus and its complications" April 4, 2009, presented by "Diabetes and cardiovascular research group, FMHS, UAE University, Al Ain diabetes research group, and Emirates diabetes society. April 4, 2009.
9. Participation in Research Project funded by the Research Affairs of the United Arab Emirates University, in collaboration with Professor. Ernest Adeghate, "The effect of incretin on gene expression in pancreatic islet cells in diabetes mellitus". 2008-2009
10. Participation in workshop on "ADInstruments for Physiology" Faculty of Medicine and Health Sciences, at UAEU. 2009.

11. Attendance of seminar on "Protein Complexes that Modify Chromatin for Transcription" by: Professor Jerry L. Workman, Stowers Institute for Medical Research, Kansas City, Missouri, USA, Faculty of medicine and Health Science, UAE University. 2008.
12. Participation in workshop "Discussion Forum on Diabetes" Organized by: Professor Ernest Adeghate, Faculty of medicine and Health Science, UAE University. 2007.
13. Participation in Research Project funded by the Research Affairs of the United Arab Emirates University, in collaboration with Professor. Ernest Adeghate "Effects of GLP-1 and Exenatide on Insulin Release in Diabetic Rats". 2006-2007.
14. Participation in Research Project funded by the Research Affairs of the United Arab Emirates University, in collaboration with Dr. Amr Amin and Professor Ernest Adeghate, "Examining the Anti-Diabetic Protective Effects of a Selected Local Herb in Mammals". 2004-2005.