1. INTRODUCTION

1.1 Diabetes mellitus (DM):

1.1.1 Definition:

Diabetes mellitus (DM)) or simply diabetes is a group of metabolic disorder of multiple etiology characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The effects of diabetes mellitus include long-term damage, dysfunction and failure of various organs. Diabetes mellitus may present with characteristic symptoms such as thirst, polyuria, blurring of vision, and weight loss. In its most severe forms, ketoacidosis or a non-ketotic hyperosmolar state may develop and lead to stupor, coma and, in absence of effective treatment, death. Often symptoms are not severe, or may be absent, and consequently hyperglycaemia sufficient to cause pathological and functional changes may be present for a long time before the diagnosis is made. Hyperglycemia, is the responsible of the development of diabetic complications. Hyperglycemia damage is produced in cells in which glucose uptake is independent of insulin, which, similarly to what happens in beta-cells, explains that the cause of the complications resides inside the cells. Prolonged exposure to high glucose levels, genetic determinants of susceptibility and accelerating factors such as hypertension and dyslipidemia participate in the development of diabetic complications. Moreover, the development and progression of damage is proportional to hyperglycemia, which makes the lowering of glucose levels the most important goal for preventing complications and treating diabetes.[1,2,3]

The main tissues affected by diabetes complications at the microvasculature levels are retina, renal glomerulus, and peripheral nerves. Diabetes is also associated with accelerated atherosclerotic disease affecting arteries that supply the heart, brain, and lower extremities. In addition, diabetic cardiomyopathy is a major diabetic complication.[4]

1.1.2 Classification of diabetes:

The classification based on etiological types and it is usually classified into 4 main categories:[5,6]

1.1.2.1 Type 1 diabetes:

Type 1 diabetes is due to an autoimmune destruction of the insulin producing pancreatic beta-cells (β - cells), which usually leads to absolute insulin deficiency. As a result, glucose cannot enter fat or muscle cells, so blood levels of glucose rise, and there is increasingly greater reliance on breakdown of fat and protein to provide an alternate source of fuel. Patients with type 1 diabetes require insulin for survival.This form was previously referred to as "insulin-dependent diabetes mellitus" (IDDM) or "juvenile diabetes".[6]

This type is further subdivided into:

Immune mediated diabetes: previously known as insulin dependent or juvenile onset diabetes, result from a cellular mediated autoimmune destruction of beta-cell of the

pancreas. Typically, it occurs in young subjects with acute onset, but may occur at any age.[7]

Idiopathic diabetes: some forms of type 1 diabetes have no known etiologies. Some of these patients have permanent insulinopenia and are prone to ketoacidosis, but have no evidence of autoimmunity.[6]

1.1.2.2 Type 2 diabetes (T2D):

This form was previously referred to as non insulin-dependent diabetes mellitus (NIDDM) or "adult-onset diabetes". It results from insulin resistance, a condition in which cells fail to use insulin properly, sometimes combined with an absolute insulin deficiency.[6] Type 2 diabetes develops when insulin secretion or insulin action fails. The impairment of insulin actions is known as insulin resistance, presented as a suppression or retard in metabolic responses of the muscle, liver and adipose tissue to insulin action. This failure is located at the signaling pathways held after insulin binding to its specific receptor. Chronic insulin resistance leads to hyperglycemia.[8]

Insulin resistance and abnormal insulin secretion are central to the development of T2D. Insulin resistance is a physiological condition where the natural hormone insulin becomes less effective at lowering blood glucose level. At least initially, and often throughout their lifetime these individuals do not need insulin treatment to survive.[6] When the beta cells cannot secrete enough insulin in response to the metabolic demand caused by insulin resistance, frank diabetes type 2 occurs. This failure in the beta cell may be due to an acquired secretory dysfunction and/or a decrease in beta-cell mass.[9] All type 2 diabetic patients have some defect in the ability of beta cells to produce or secrete insulin.[10]

1.1.2.3 Gestational diabetes mellitus (GDM):

The third main form, gestational diabetes, occurs when pregnant women without a previous diagnosis of diabetes develop a high blood glucose level. It may precede development of type 2 DM.[11]

Gestational diabetes mellitus (GDM) is a state of glucose intolerance occurring or detected for the first time during pregnancy and is often normalized after delivery, but such cases are at higher risk of developing diabetes in the future .[6]

Pregnancy is a diabetogenic factor. Etiologically, many GDM patients probably share common genetic susceptibilities with type 1 or type2 diabetes, and the deterioration of glucose tolerance is precipitated by the metabolic effect of pregnancy. During pregnancy, glucose intolerance may affect the infant and mother adversely.

1.1.2.4 Other types of diabetes due to specific causes:

Other forms of diabetes mellitus include congenital diabetes, which is due to genetic defects of insulin secretion, cystic fibrosis-related diabetes, steroid diabetes induced by high doses of glucocorticoids, and several forms of monogenic diabetes.

i. Genetic abnormalities related to cell function: These forms of diabetes are characterized by onset of hyperglycemia at early age. They are referred to as Maturity-

onset diabetes of young (MODY) and are characterized by impaired insulin secretion with minimal or no effects in insulin action.[6]

ii. Genetic abnormalities related to mechanisms of insulin action: Includes a number of insulin receptor gene abnormalities. The associated metabolic abnormalities may range from hyperinsulinemia and modest hyperglycemia to severe diabetes.[12]

iii. Disease of exocrine pancreas: Any process that diffusely injuries the pancreas can cause diabetes, including pancreatitis, trauma, infection, pancreatectomy, and pancreatic carcinoma. With exception to cancer, damage to the pancreas must be extensive for diabetes to occur. [13]

iv. Endocrinopathies: Excess secretion of several hormones as growth hormone, cortisol, glucagone and epinepherine which antagonize insulin action. The hyperglycemia typically resolves when the hormone excess is removed.[6]

v. Drug or chemical-induced diabetes: Many drugs can impair insulin secretion. These drugs may not by themselves cause diabetes but, they may precipitate diabetes in persons with insulin resistance.[14]

vi. Infections: Certain viruses have been associated with beta-cell destruction, diabetes occurs in some patients with congenital rubella.[15]

vii. Other forms of immunologically and genetically-mediated diabetes mellitus: anti-insulin receptor antibodies can cause diabetes by binding to the insulin receptor thereby reducing the binding of insulin to target tissues. However, these antibodies also can act as an insulin agonist after binding to the receptor and can thereby cause hypoglycemia. Anti-insulin receptor antibodies are occasionally found in patients with systemic lupus erythematosus and other autoimmune diseases. Many genetic syndromes are accompanied by an increased incidence of DM. These include the chromosomal abnormalities of Down's syndrome, Klinefelter's syndrome and Turner's syndrome.[6, 16]

1.1.3. Epidemiology:

The estimated worldwide prevalence of diabetes among adults was 285 million (6.4%) in 2010, and this value is predicted to rise to around 439 million (7.7%) by 2030.[17] Surprisingly, this estimates of the global prevalence of diabetes indicate that, Egypt will be ranked as the 10th country worldwide which have the highest number of people with diabetes (6.7 millions).[18] Type 2 diabetes is the predominant form and accounts for at least 90% of cases. The rise in prevalence is predicted to be much greater in developing than in developed countries (69% *vs* 20%). In developing countries people aged 40–60 years (ie, working age) are affected most, compared with those older than 60 years in developed countries. This increase in type 2 diabetes is inextricably linked to changes towards a western lifestyle (high-energy diets with reduced physical activity) in developing countries and the rise in the prevalence of overweight and obesity.[19]

1.1.4. Diagnosis of diabetes:

The clinical diagnosis of diabetes is often prompted by symptoms such as increased thirst and urine volume, recurrent infections, unexplained weight loss and, in severe cases,

drowsiness and coma; high levels of glycosuria are usually present. Single blood glucose estimation in excess of the diagnostic establishes the diagnosis in such cases .[6]

An oral glucose tolerance test (OGTT) to establish diagnostic status need only be considered if casual blood glucose values lie in the uncertain range (i.e. between the levels that establish or exclude diabetes) and fasting plasma glucose (FPG) levels are below those which establish the diagnosis of diabetes. If an OGTT is performed, it is sufficient to measure the blood glucose values while fasting and at 2 hours after a 75 gm oral glucose load. [6]

Glycosylated hemoglobin (HbA1C) is a widely used marker of chronic glycemia, reflecting average blood glucose levels over a 2- to 3-month period of time. The test plays a critical role in the management of the patient with diabetes, since it correlates well with both microvascular and, to a lesser extent, macrovascular complications and is widely used as the standard biomarker for the adequacy of glycemic management.[20] The current diagnostic criteria for diabetes are summarized in Table (1).

Table (1): The current diagnostic criteria for diabetes. (Quoted from reference 21).

A1C ≥6.5%. The test should be performed in a laboratory using a method that is NGSP certified and standardized to the DCCT assay.*
OR
FPG ≥126 mg/dl (7.0 mmol/l). Fasting is defined as no caloric intake for at least 8 h.*
OR
2-h plasma glucose ≥200 mg/dl (11.1 mmol/l) during an OGTT. The test should be performed as described by the World Health Organization, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.*
OR
In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose ≥200 mg/dl (11.1 mmol/l).

*In the absence of unequivocal hyperglycemia, criteria 1–3 should be confirmed by repeat testing.

Obesity, particularly visceral or central (as evidenced by the hip-waist ratio), is very common in T2DM (80% or more are obese). In the early stages of the disorder, glucose tolerance remains near-normal, despite insulin resistance, because the pancreatic beta cells compensate by increasing insulin output. As insulin resistance and compensatory hyperinsulinemia progress, the pancreatic islets in certain individuals are unable to sustain the hyperinsulinemic state.[22] Impaired glucose tolerance (IGT), characterized by elevations in postprandial glucose, and then develops a further decline in insulin secretion and an increase in hepatic glucose production lead to overt diabetes with fasting hyperglycemia. Ultimately, beta cell failure ensues.[22]

Patients who are not obese by traditional weight criteria may have an increased percentage of body fat distributed predominantly in the abdominal region. This form of diabetes frequently goes undiagnosed for many years because the hyperglycemia develops gradually and at earlier stages is often not severe enough for the patient to notice any of the

classic symptoms of diabetes. Nevertheless, such patients are at increased risk of developing macrovascular and microvascular complications. Whereas patients with this form of diabetes may have insulin levels that appear normal or elevated, the higher blood glucose levels in these diabetic patients would be expected to result in even higher insulin values had their β-cell function been normal. Thus, insulin secretion is defective in these patients and insufficient to compensate for insulin resistance.[23] Insulin resistance may improve with weight reduction and/or pharmacological treatment of hyperglycemia but is seldom restored to normal. The risk of developing this form of diabetes increases with age, obesity, and lack of physical activity. It occurs more frequently in women with prior GDM and in individuals with hypertension or dyslipidemia, and its frequency varies in different racial/ethnic subgroups. It is often associated with a strong genetic predisposition, more so than is the autoimmune form of type 1 diabetes. However, the genetics of this form of diabetes are complex and not clearly defined.[23]

1.1.5. Risk factors for type 2 diabetes mellitus:[22]

There are many factors affect in risk of disease:

- Family history of diabetes (i.e., parent or sibling with type 2 diabetes).
- Obesity (body mass index (BMI) 25 ≥ kg/m^2).
- Physical inactivity.
- Race/ethnicity (for example; African American, Latino, Native American, Asian American, Pacific Islander).
- Previously identified with impaired fasting glucose (IFG), IGT, or HbA1C of 5.7–6.4%.
- History of GDM or delivery of baby >4 kg (9 lb).
- Hypertension (blood pressure 140/90 mmHg).
- High density lipoprotein (HDL) - cholesterol level <35 mg/dL (0.90 mmol/L) and/or a triglyceride level >250 mg/dL (2.82 mmol/L).
- Polycystic ovary syndrome.
- History of cardiovascular disease.

1.2. Insulin secretion and actions.

1.2.1. Insulin secretion:

Insulin, the main anabolic hormone produced by the pancreas, controls the level of glucose in the blood by regulating the production and storage of glucose. In the diabetic state, the cells may stop responding to insulin or the pancreas may stop producing insulin entirely.[22]

Insulin is synthesized as preproinsulin in the ribosomes of the rough endoplasmic reticulum in the beta cells. Preproinsulin is then cleaved to proinsulin, which is transported to the Golgi apparatus where it is packaged into secretory granules located close to the cell membrane. Proinsulin is a 9 kDa peptide, containing the A and B chains of insulin (21 and 30 amino acid residues, respectively) joined by the C peptide (30–35amino acids). Insulin is synthesized from the proinsulin precursor molecule by the action of proteolytic enzymes, known as prohormone convertases (PC1 and PC2), as well as the exoprotease carboxypeptidase E. These modifications of proinsulin remove the center portion of the molecule (i.e., C-peptide), from the C- and N- terminal ends of proinsulin. The remaining polypeptides (51 amino acids in total), the B- and A- chains, are bound together by disulfide bonds.[22]

Proinsulin is cleaved into equimolar amounts of insulin and C-peptide in the secretory granules. The process of insulin secretion involves fusion of the secretory granules with the cell membrane and exocytosis of insulin, C-peptide, and proinsulin. Apart from insulin, beta cells release C-peptide, a consequence of insulin production, into the bloodstream in equimolar amounts. C-peptide helps to prevent neuropathy and other symptoms of diabetes related to vascular deterioration.[24]

Zinc is needed by over 300 enzyme systems. Some of those are involved with the metabolism of blood sugar and are so important that a lack of zinc, in and of itself, can cause type I or type II diabetes.[24]

Zinc is highly concentrated in the insulin-secreting beta cells of pancreas. Zinc can keep insulin molecules together in the beta cells. Beta cells must have zinc to function. In fact, beta cells contain their own special zinc transporter called zinc transporter 8 that enables beta cells to take up zinc. Gene alterations in this zinc transporter are now known to cause type II diabetes while type I diabetes is associated with antibodies against this zinc transporter (meaning the immune system knocks out function of beta cells so they can't produce insulin).[24]

Zinc directly influences how insulin is produced and secreted by beta cells. So the people with zinc deficiency can't store and release insulin. Furthermore, zinc is self-protecting to the beta cells. It has now been shown that zinc directly reduces the inflammatory signals that damage the beta cells, a process that leads to type I diabetes.[24]

1.2.1.1. Mechanisms of insulin secretion from beta cells:

The secretion of insulin from pancreatic beta cells is a complex process involving the integration and interaction of multiple external and internal stimuli. The primary stimulus for insulin release is the beta-cell response to changes in glucose concentration. Normally, glucose induces a biphasic pattern of insulin release.[9] First-phase insulin release occurs within the first few minutes after exposure to an elevated glucose level; this is followed by a more permanent second phase of insulin release. Of particular importance is the observation that first-phase insulin secretion is lost in patients with type 2 diabetes.[25]

A widely accepted sequence of events involved in glucose-induced insulin secretion is as follows:[25] Glucose is transported into beta cells through facilitated diffusion glucose transporter-2 (GLUT2). Glucose is metabolized via glycolysis to pyruvate and in the mitochondria to acetyl-CoA, which is then oxidized in the Krebs cycle. These actions lead

to an increased cytosolic ratio of adinosine -5-triphosphate (ATP) to adenosine diphosphate (ADP), which closes the K^+ATP channels, depolarizes the plasma membrane potential, and opens voltage-gated Ca^{2+} channels, causing influx of Ca^{2+} and the triggering of insulin-granule exocytosis. Pyruvate from glucose can also be metabolized via pyrovate carboxylazse (PC) into the anaplerosis-cataplerosis pathway. Anaplerosis refers to the processes by which Krebs cycle intermediates in the mitochondrion are replenished or increased, whereas cataplerosis refers to their egress from the mitochondrion. Changes in concentrations of cataplerosis-derived signaling molecules, including NADPH from pyruvate shuttles, and citrate-derived malonyl-CoA (Mal-CoA), can lead to augmentation of insulin secretion. Glucose interacts with non esterified fatty acid (NEFA) by promoting activity in a glycerolipid /fatty acid.(GL/FA) cycle when raised concentrations of Mal-CoA, via the anaplerosis pathway, inhibits partitioning of long-chain acyl-CoA (LC-CoA) into fatty acid (FA) oxidation, which increases the availability of the LC-CoA for esterification. Glucose also provides glycerol-3-phosphate, which is necessary for FA esterification. Glycerolipids are rapidly hydrolyzed by lipases back to NEFA and glycerol to create the GL/FA cycle process. This cycle produces lipid signaling molecules, such as diacylglycerols, that enhance glucose-stimulated insulin secretion. Amino acids, such as glutamine and leucine, also interact with the glucose metabolism pathways to increase the coupling signals produced by glucose alone. The β cell responds to other neurohormonal and metabolic extracellular signals via various plasma membrane receptors.[26]

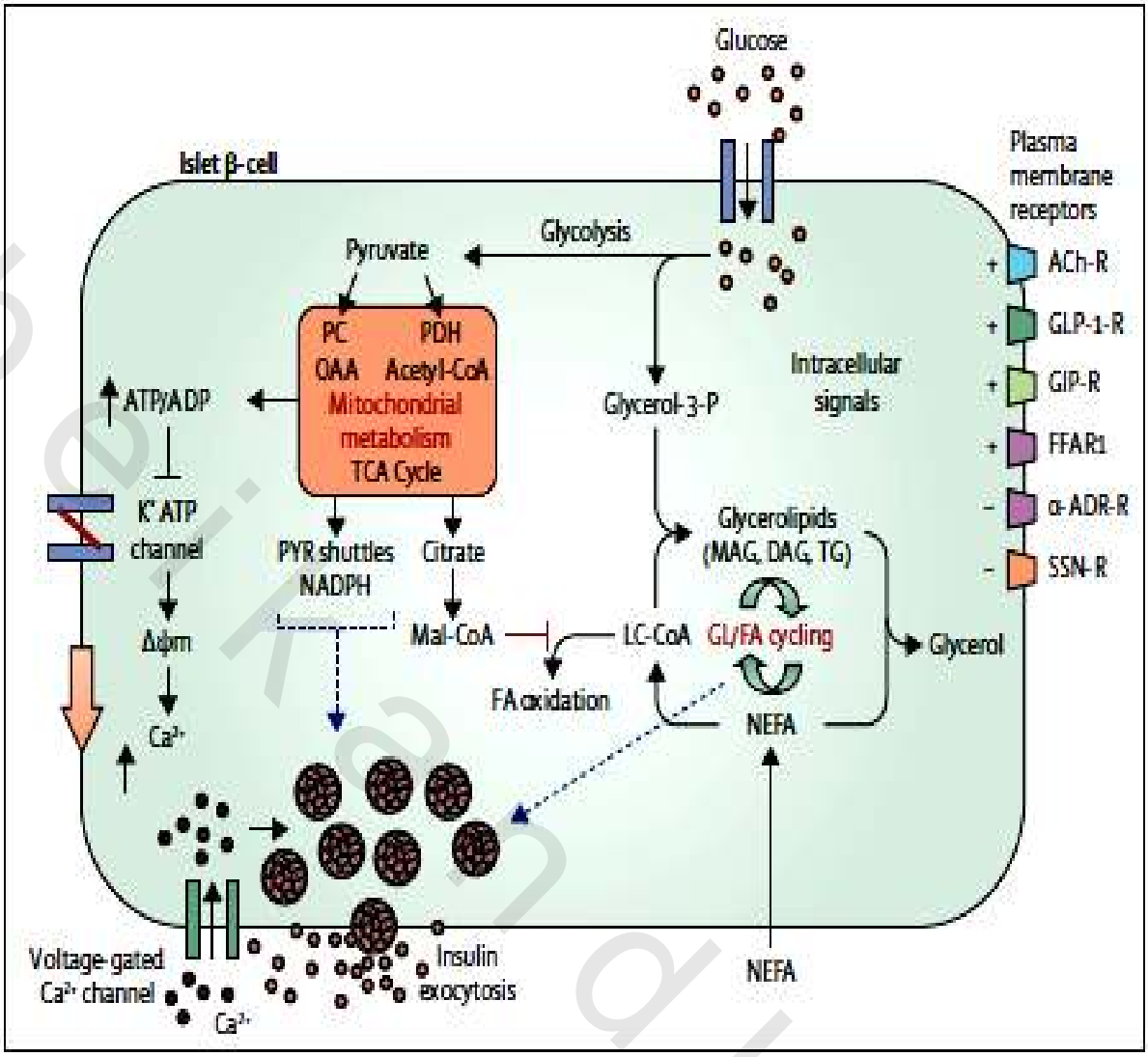


Figure (1): *Diagram of insulin secretion by Beta-cell (Quoted from reference 26). PC=pyruvate carboxylase. PDH=pyruvate dehydrogenase. Ach-R=acetylcholine receptor. GIP-R=gastric inhibitory polypeptide receptor. GLP-1-R=glucagon-like peptide-1 receptor. FFAR1=free-fatty-acid receptor-1. α-ADR-R=α2-adrenergic receptor. SSN-R=somatostatin receptor. OAA, oxaloacetate; CoA=coenzyme A. MAG, monoacylglycerides; DAG=diacylglycerides. TG=triacylglycerides. Δψm=change in plasma membrane potential. Mal-CoA=malonyl-CoA. LC-CoA=long-chain acyl-CoA. GL=glycerolipid. FA=fatty acid. NEFA=non-esterified fatty acids.*

1.2.2. Insulin Signaling:

Insulin affects a wide range of physiological processes, although it is best known for its important regulatory role in glucose homeostasis. In response to elevations in plasma glucose, insulin secretion is increased and it stimulates glucose uptake and glycogen synthesis and inhibits glycogenolysis and gluconeogenesis, thus maintaining normoglycaemia. In addition to these well-established short-term actions, insulin exerts a number of other important metabolic effects, many of which are mediated via changes in the expression of more than 100 gene.[27] For example, insulin regulates the expression of genes involved in amino acid uptake, lipid metabolism in muscle and adipose tissue[28] and in cell growth, development and survival.[29,30]

The diverse effects of insulin are mediated through a multicomponent signaling complex that is strongly conserved across a wide range of species.[31] Binding of insulin to its receptor triggers a cascade of signaling events that ultimately leads to modifications in a number of biological processes.

Proximal steps in insulin signaling, including the insulin receptor, insulin receptor substrate proteins (IRS), phosphatidylinositol 3 (PI 3) kinase, Akt/protein kinase B (Akt/PKB), and mitogen activated protein kinase (MAPK) are globally operative in multiple cell types. These proteins also serve as points of divergence or nodes in an expanding matrix of signal transduction pathways, and are highly regulated, both positively and negatively, via crosstalk with other signaling systems and modulatory pathways (Fig: 2).[32]

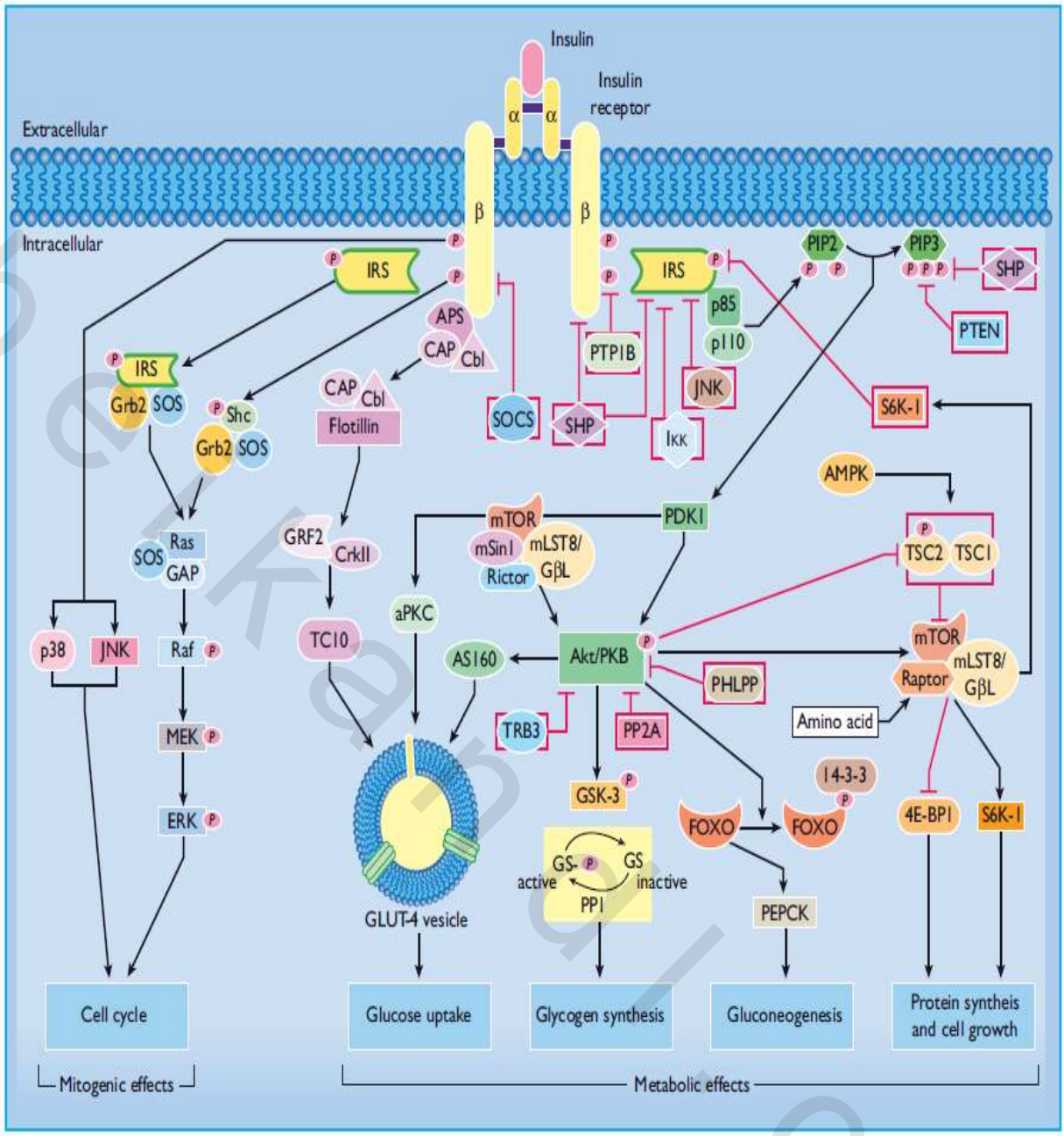


Figure (2): *A schematic illustration of insulin signaling pathways involved in both metabolic and mitogenic effects. Arrows represent an activation process; blocked arrows represent an inhibition process (Quoted from reference 32).*

1.2.2.1. Insulin Receptors.

Insulin receptors are ubiquitous in vertebrate cells although expression varies significantly between cell types, from as few as 40 receptors per cell on erythrocytes to more than 200 000 on adipocytes and hepatocytes.[33] The insulin receptor belongs to a subfamily of receptor tyrosine kinases that includes the insulin-like growth factor (IGF) receptor and the insulin receptor-related receptor (IRR). These receptors are a heterotetrameric transmembrane glycoprotein consisting of two extracellular α subunits which are linked by disulfide bridges to two transmembrane β subunits that function as allosteric enzymes in which the α subunit inhibits the tyrosine kinase activity of the β subunit. Within the intracellular portion of the beta-subunits, there are three important structural regions: a juxtamembrane (JM) region, a kinase region, and a carboxyl-terminal (CT) region (Fig:3).[34,35]

The binding of insulin to the α subunit of IR stimulates the tyrosine kinase activity intrinsic to the β subunit of the receptor. The two α subunits jointly participate in insulin binding and that the kinase domains in the two β subunits are in a juxtaposition that permits autophosphorylation of tyrosine residues, the first step of insulin receptor activation.[36,37] The kinase domain undergoes conformational change upon autophosphorylation, providing a basis for activation of the kinase and binding of downstream signaling molecules.[38,39] There are three tyrosine residues in the tyrosine kinase domain of the insulin receptor, and phosphorylation of all three is necessary to activate the tyrosine kinase activity and initiate correct signaling to effectors.[40]

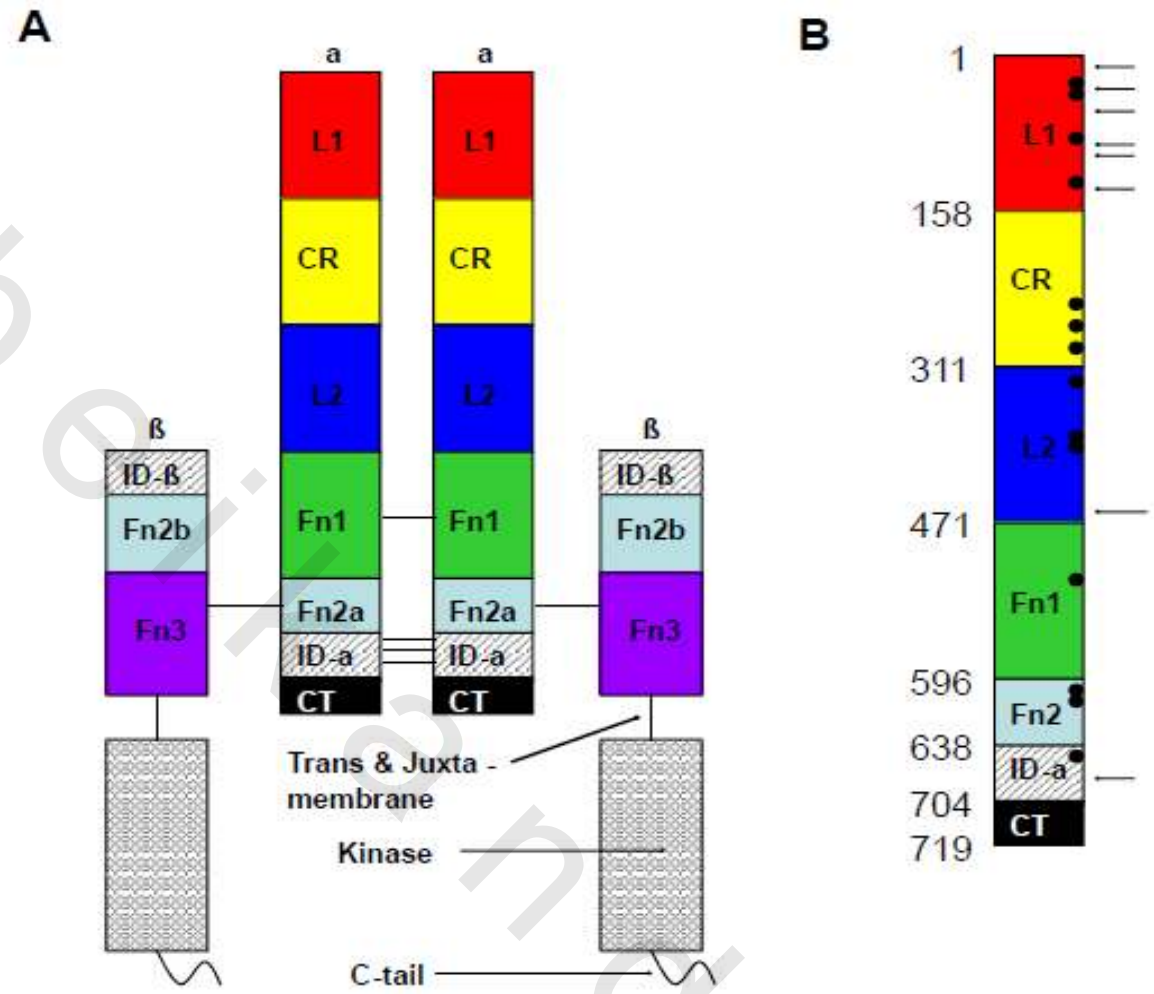


Figure (3): *(A) An overview of the organization of the insulin receptor. (B) A close up of the α-subunit of the IR. In (B) The arrows indicate insulin binding locations. The majority of these binding "hotspots" are located in the L1 region. The black spots are sites of N-glycosylation.(Quoted from reference 35).*

1.2.2.2 Insulin receptor substrate:

As described above, binding of insulin to its receptor activates tyrosine kinase, resulting in autophosphorylation of tyrosine residues on the receptor β-subunit. This in turn leads to phosphorylation of several protein substrates, primarily the insulin receptor substrate (IRS) proteins (Fig:4). These proteins have an important regulatory role, providing an interface between insulin receptors and downstream effector molecules. To date, four mammalian IRS proteins have been identified. IRS-1 is primarily involved in somatic cell growth and insulin action in muscle and adipose tissue, whereas IRS-2 plays important roles in β-cell survival/growth, insulin action in the liver, brain growth, reproduction and food intake.[41,42] IRS-3 and IRS-4 are predominantly expressed in adipose and neuroendocrine tissue, respectively [43,44], although their precise roles are still under investigation.

The four substrates belong to the IRS family: IRS-1, IRS-2, IRS-3 and IRS-4 have similar overall structure; each contains an N-terminal pleckstrin homology (PH) domain followed by an FFB domain, and a C-terminal with many tyrosine phosphorylation sites that create src-homology 2 (SH2) protein-binding sites, proline-rich regions that engage Src homology 3 (SH3) domains or WW domains (protein modules that bind proline-rich ligands) and serine-threonine-rich regions that bind other proteins.[45,46] The IRS proteins serve complimentary functions in different tissues as immediate substrates for insulin and IGF-I receptors.[47]

IRS-1 not only undergoes tyrosine phosphorylation, but it also undergoes serine/threonine phosphorylation.When a cell is stimulated by insulin, an increase in serine/threonine phosphorylation of IRS-1 occurs. The excessive serine/threonine phosphorylation of IRS-1 is one mechanism that allows tumor necrosis factor to induce a resistance to insulin. The excessive serine/threonine phosphorylation of IRS-1 has been achieved experimentally by exposing cells to high concentrations of insulin for long periods of time which decrease the ability of the insulin receptor to induce tyrosine phosphorylation of IRS-1. Consequently, this leads to a reduction in the ability of IRS-1 to interact with PI3-kinase.[48]

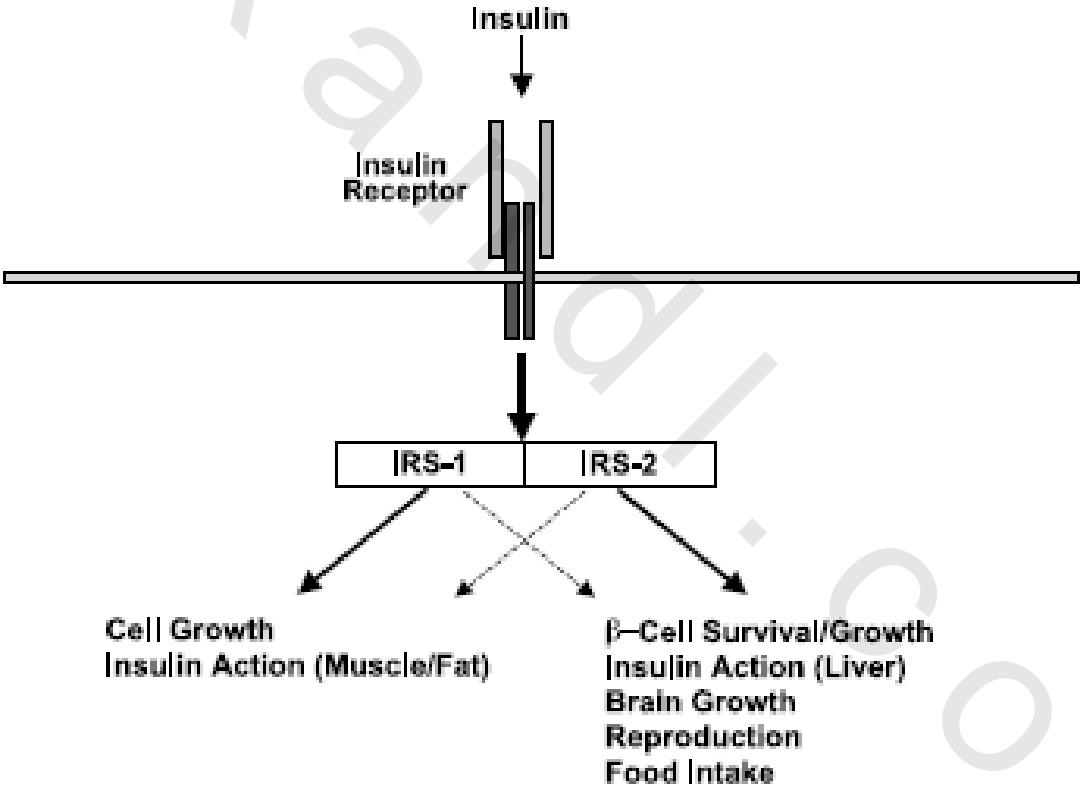


Figure (4): *Interaction between insulin and the insulin receptor substrate (IRS) proteins.(Quoted from reference 42)*

1.2.2.3 Downstream effector molecules

While the IRS proteins are early components of the insulin signaling pathway, it is the subsequent specific recruitment of multiple downstream signaling proteins that ultimately generates the unique insulin responses in various cells and tissues. During insulin stimulation, tyrosine phosphorylation sites in the IRS proteins bind specifically to the Src-homology-2 (SH2) domains in various downstream signaling molecules, including phosphatidylinositol 3-kinase (PI 3-kinase), growth factor receptor binding protein 2 (Grb-2) and SH2-containing protein-tyrosine phosphatase-2 (SHP-2). The outcome of insulin action in any cell depends on which of these effector molecules are expressed and recruited into the signaling complex and the pathways that are activated as a result.[31]

1.2.2.3.1 Phosphatidylinositol 3-kinase (PI3-kinase)

PI 3 kinase is critical for signal transduction mediating the metabolic effects of insulin, including stimulation of glucose uptake into skeletal muscle and adipose tissues. PI 3 kinase activity represents a family of related enzymes that are capable of phosphorylating the hydroxyl groups in the inositol ring of membrane – associated phosphatidylinositol (PtdIns).[49] The generation of PI(3,4,5)P 3 (PI3P) propagates the insulin signal and the downstream action of receptor tyrosine kinases (including the insulin receptor) and Ras/MAPK.[50]

Insulin - mediated activation of PI 3 kinase results in phosphorylation of the inositol ring at the 3 ′ position of phosphatidylinositol in membrane glycolipids, generating PI(3,4,5)P 3 . This leads to recruitment of certain signaling proteins with PH domains to the plasma membrane. Binding to membrane-associated phosphoinositides both activates these proteins and positions them for downstream signal transduction. 3 - Phosphoinositide - dependent protein kinase 1 (PDK1) can interact with PI(3,4,5)P 3 , and is responsible for downstream activation of Akt/PKB and aPKCs. PDK1 phosphorylates the activation loops of Akt/PKB on Thr 308 , and PKC ζ on Thr 410 , enhancing the activity of these kinases.[32]

1.2.2.3.2 Akt/ protein kinase B.

Akt/PKB is a serine-threonine kinase with multiple substrates including kinases, signaling proteins, and transcription factors. Phosphorylation and activation of Akt/PKB mediates various insulin- and growth factor - induced cellular responses, such as the stimulation of GLUT- 4 translocation to the plasma membrane, the inhibition of glycogen synthase kinase 3 (GSK- 3), induction of triglyceride synthesis via increasing the expression of sterol regulatory element - binding proteins 1c (SREBP - 1c), and the promotion of cell survival by inhibiting apoptosis..[32]

Several lines of evidence implicate a role for Akt/PKB activation in the stimulation of glucose transport and other insulin-induced biologic processes.[51] Akt/PKB activation is involved in multiple other insulin responses. One of the first substrates indentified for Akt/PKB was GSK3 β. Phosphorylation of GSK3 β decreases its activity towards glycogen synthase, which leads to increased glycogen synthesis.[52] Akt/PKB seems to stimulate glucose transport concomitant with the phosphorylation of Akt substrate of 160 kDa (AS160), a Rab - GTPase - activating protein (GAP).[53]

1.2.2.3.3 Atypical protein kinase C

Protein kinase C known as PKC is a family of protein kinase enzymes that are involved in controlling the function of other proteins through the phosphorylation of hydroxyl groups of serine and threonine amino acid residues on these proteins. PKC enzymes in turn are activated by signals such as increases in the concentration of diacylglycerol (DAG) or calcium ions (Ca^{2+}). Hence PKC enzymes play important roles in several signal transduction cascades.(54)

Protein kinase C isoforms are categorized as conventional (α , β , γ) (cPKC), novel (δ , θ , ε , η , μ) (nPKC), and atypical (ζ , λ) (aPKC) depending on their ability to be activated by calcium and DAG. cPKCs and nPKCs serve primarily as negative feedback inhibitors of the insulin receptor and IRS. The aPKCs participate in signal transduction, and like Akt/PKB, are activated by the PI 3 kinase pathway and help mediate insulin's metabolic effects.(55)

Activation of PKC ζ and PKC λ occurs proximal to Akt/PKB as a result of direct interaction with 3′phosphoinositides and/or through phosphorylation and activation by PDK1. aPKCs have been shown to have a role in insulin - stimulated glucose uptake and GLUT - 4 translocation in adipocytes and muscle.(55) Stimulation of glucose transport and translocation of GLUT - 4 vesicles by aPKC may be brought about by effects on the actin cytoskeleton, because PKC λ / ζ can impinge on Rac and actin dynamics.(56)

1.2.2.3.4. Glucose Transporter (Glut):

The high polar nature of glucose makes it impermeable to transport across the membrane and therefore specific carrier molecules on the cell membrane are required for this purpose. Movement of glucose across the membrane takes place in an energy dependent (active) as well as energy independent (passive) manner.(57) The glucose transporter proteins (Glut) have been identified in human being. The glucose transporters are 12 transmembrane proteins belong to the Major Facilitator Superfamily (MFS) of membrane transporters. The members of Glut family have been classified into different classes and isoforms based on their sequence similarity and physiological role.(58)

Out of the 14 isoforms of glucose transporters identified so far, class I glucose transporters include GLUT1, GLUT2, GLUT3, GLUT4 and GLUT14(59) were shown to have clinical importance. GLUT1 is a ubiquitously expressed protein and is the first one to be sequenced and purified.(60) GLUT2 expressed in high amount in various cells like pancreatic beta-cells, basolateral membranes of intestinal and kidney cells. As evidenced from its distribution pattern, GLUT2 plays critical role in the absorption of glucose from the intestine and also plays critical role in triggering insulin secretion in pancreatic beta-cells.(61) GLUT3 and GLUT14 are considered as much similar in its biochemical and structural aspects and are expressed in neuronal cells and testis respectively. GLUT3 has high affinity to glucose and highest turnover number ensuring efficient glucose uptake by neurons.(62) Among the various GLUTs in class I, GLUT4 is the major insulin sensitive isoform predominantly expressed in adipocytes and muscle cells.(63) The other GLUT isoforms belonging to class II and III include various fructose transporters and glucose transporters expressed in various tissues like kidney, liver, placenta, cardiac and skeletal muscle, leukocyte, blasocyte, brain, glial cells and some neurons.(59)

GLUT4 is 54 kDa protein consisting of 509 amino acids arranged as 12 transmembrane domains. The protein was discovered as distinct insulin sensitive transporter isoform.[63] Unlike GLUT1, which localizes predominantly in the plasma membrane, GLUT4 resides in the intracellular vesicles in the basal state. Intracellular amino and carboxy termini of GLUT4 have residues important for the regulation of intracellular GLUT4 trafficking.[64] FQQI motif and dileucine (LL) motif play significant role in the endocytosis of GLUT4 from the plasma membrane.[65,66] QLS motif in the transmembrane helix 7 is known to interact with glucose and plays an important role in the regulation of glucose transport (Fig:5).[67]

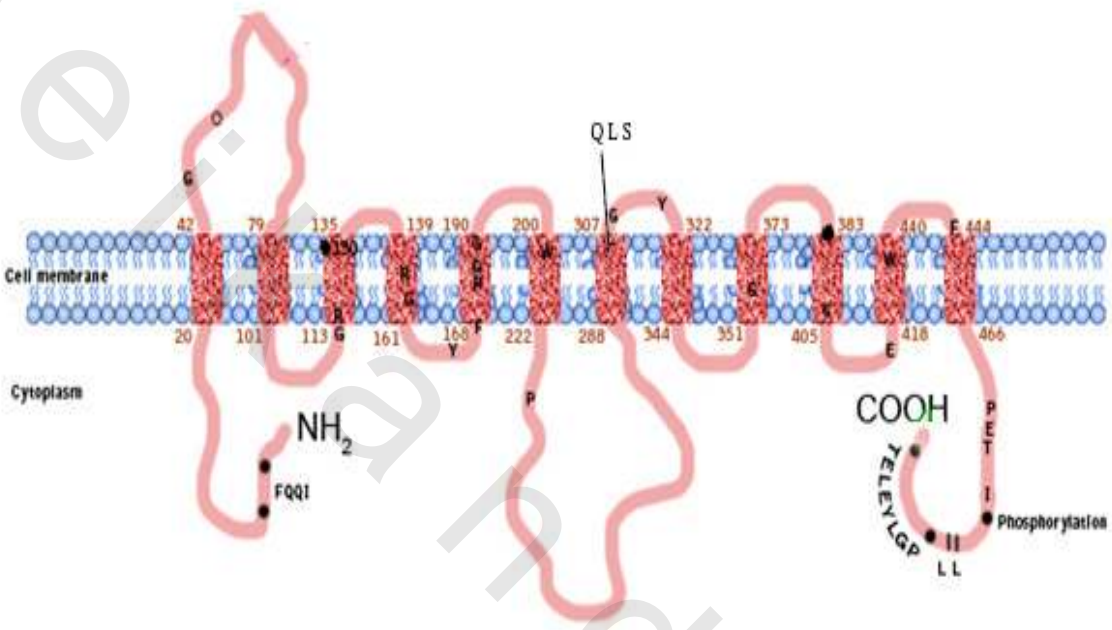


Figure (5): *GLUT4 structure: Schematic representation of the topological structure of GLUT4. The 12 transmembrane segments are shown. The important motifs in GLUT4 are marked. (Quoted from reference 67)*

As muscles and adipocytes are the major insulin sensitive tissues, a conditional depletion of GLUT4 in any of these tissues causes insulin resistance leading to diabetes. Skeletal muscle accounts for up to 75% of postprandial glucose disposal in humans. Studies have shown a possible crosstalk between adipocyte and muscles in insulin resistance, as conditional depletion of GLUT4 in muscle cells cause decreased insulin sensitivity in adipose tissue and vice-versa.

The dominant role of GLUT4 as a regulator of whole body glucose homeostasis, and acute or long term changes in the expression of GLUT4 on the surface of adipocyte and muscle cell membrane can lead to systemic changes in the glucose homeostasis (Fig:6).[68,69]

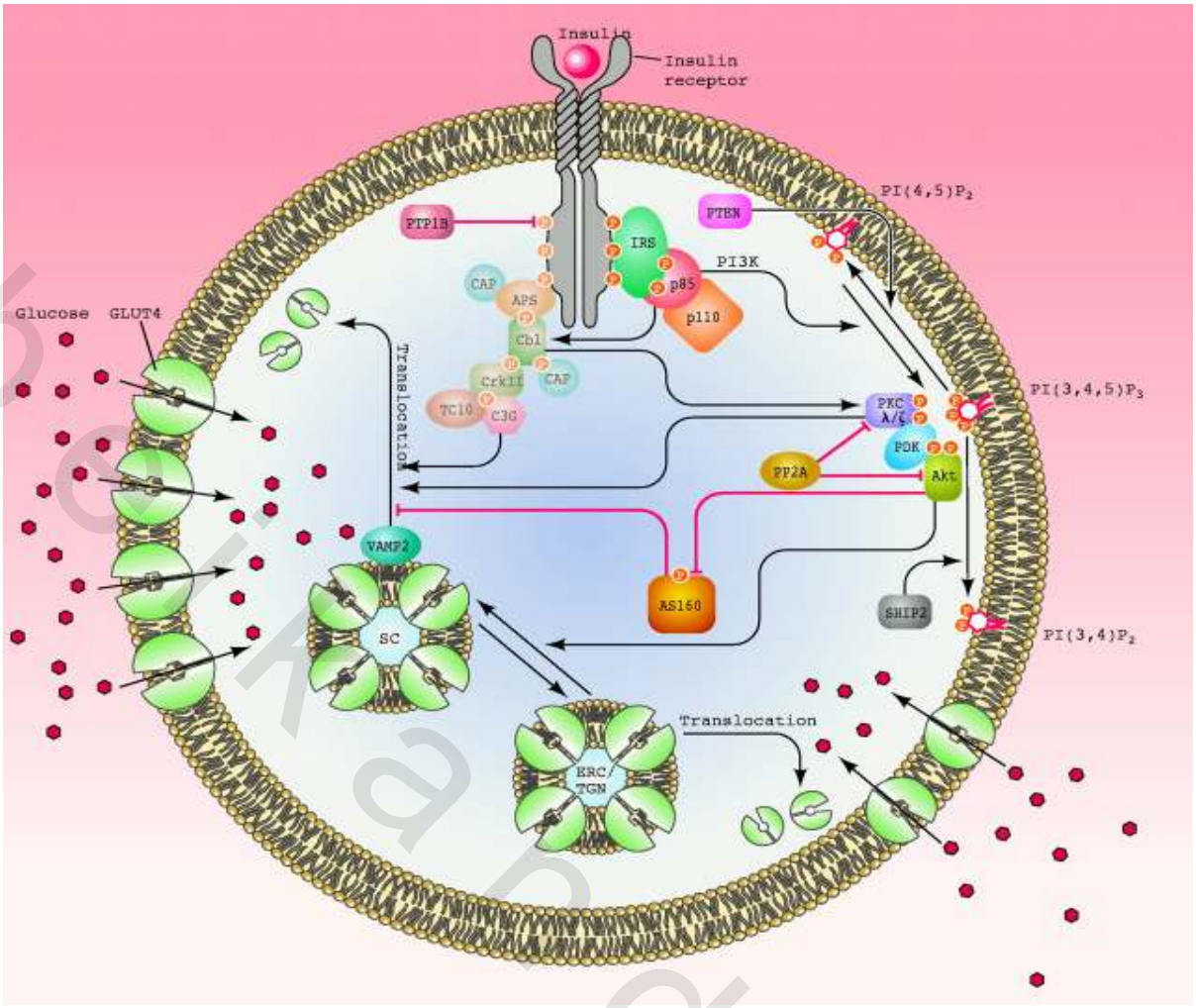


Figure (6): *The key molecular signals that are turned on and off by insulin in regulating GLUT4 traffic. (Quoted from reference 69).*

(APS, adaptor proteins associated with pleckstrin homology and SH2 domains; AS160, Akt substrate of 160 kDa; CAP, c-Cbl-associated protein; C3G, Crk SH3-binding guanine nucleotide-releasing factor; ERC, endosomal recycling compartment; IRS, insulin receptor substrate; PDK, phosphoinositide-dependent kinase; PI3K, phosphoinositide 3-kinase; PI(3,4)P2, phosphatidylinositol-3,4-bisphosphate; PI(3,4,5)P3, phosphatidylinosito l-3,4,5-trisphosphate; PI(4,5)P2, phosphatidylinositol-4,5-bisphosphate; PP2A, protein phosphatase 2A; PTB1B, protein tyrosine phosphatase 1B; PTEN, phosphatase and tensin homolog deleted on chromosome 10; SC, specialized compartment; SHIP2, SH2-containing inositol phosphatase 2; TGN, trans-Golgi network; VAMP2, vesicle-associated membrane protein 2).[69]

1.2.3. The insulin actions:

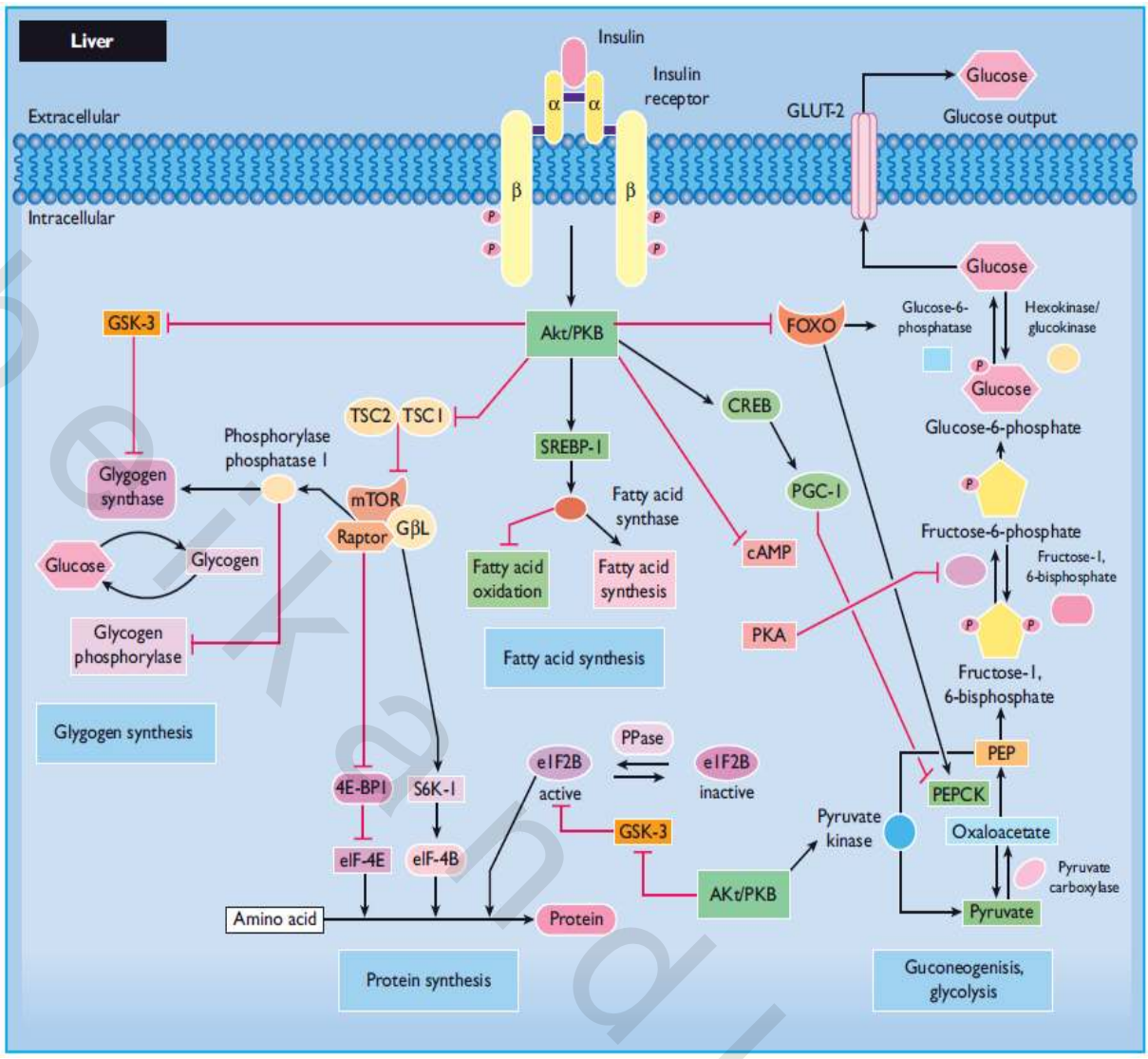
The primary targets of insulin action to maintain glucose homeostasis are skeletal muscle, liver, and adipose tissue. Once insulin secreted, insulin binds to its receptor, triggering a cascade of downstream phosphorylation events that expand the initial signal that mediates different insulin actions. The nature of these biologic actions varies dramatically from tissue to tissue, and these variations, for the most part, are not brought

about by differences in insulin signal transmission. Rather, tissue– specific insulin effects are principally explained by effector systems that are uniquely expressed in a variety of differentiated target cells. The biochemical basis of these effects is described in skeletal muscle, adipose tissue, and liver, three organs primarily responsible for fuel storage and oxidation as well as counter – regulatory metabolism.[34]

1.2.3.1. Insulin actions in Liver:

Insulin regulates hepatic metabolism through acute post-translational modifications of enzymes, such as phosphorylation, and through changes in gene expression. The stimulation of glycogen formation and regulation of gluconeogenesis by insulin are the critical determinants of hepatic glucose output. In addition, regulation of gene transcription is critical for the biologic effects of insulin on hepatic metabolism.[32]

In the liver, IRS-2, via PI3K, controls aPKC activation. In contrast, both IRS-1 and IRS-2, via PI3K, control PKB activation. The expression of sterol regulatory element - binding proteins 1c (SREBP - 1c), which transactivates many genes that are active in fat synthesis including fatty acid synthase (FAS), is largely, but not exclusively, controlled by aPKC. Increases in lipid synthesis lead to increases in the secretion of very low density lipoprotein (VLDL) triglycerides. With respect to liver handling of glucose, PKB (and, possibly, other undefined factors, but not aPKC), increases glycogen synthesis and diminishes glucose production and release. In simple obesity, insulin signaling is grossly intact in the liver. With the onset of diabetes, IRS-1 signaling to PI3K and PKB is diminished, but IRS-2 signaling to PI3K and aPKC is better or fully conserved. Thus, in hyperinsulinemic states of simple obesity and Type II diabetes, increased IRS-2 signaling to aPKC leads to increases in SREBP-1c expression, lipid synthesis, and VLDL-triglyceride secretion. In diabetes, diminished signaling to IRS-1 and PKB leads to increases in hepatic glucose output (Fig:7).[32]



Figure(7): *Summary of insulin signaling pathways in the liver that are involved in glycogen synthesis, gluconeogenesis and glycolysis, and protein synthesis, respectively. Arrows represent an activation process; blocked arrows represent an inhibition process . (Quoted from reference 32).*

1.2.3.1.1. Glycogenesis/ glycogenolysis:

Insulin exerts dramatic effects on pathways of intracellular glucose metabolism. Under conditions of insulin stimulation, the major portion of glucose uptake is stored as glycogen in humans. Insulin promotes glycogen synthesis in muscle, adipocytes, and liver by activating glycogen synthase, which adds activated glucosyl groups to growing polysaccharide chains and thus catalyzes the final step in glycogen synthesis. The regulation of glycogen synthase is complex. It involves allosteric activators, translocation of glycogen synthase to the plasma membrane in the presence of glucose metabolites and insulin, inhibition by phosphorylation on serine residues by different kinases, and activation by dephosphorylation by serine-threonine phosphatases such as protein phosphatase-1 (PP1).[70] The ability of insulin to stimulate glycogen synthase requires proximal signaling through activation of PI3 kinase and Akt/PKB. One downstream pathway that activates glycogen synthase involves Akt/PKB-mediated phosphorylation and inactivation of GSK3, which results in a reduction in net phosphorylation of glycogen synthase. The reduction in glycogen synthase phosphorylation augments its activity. GSK3 β inhibition is not insulin's only mechanism for stimulating glycogen accumulation. In adipocytes, insulin is capable of stimulating glycogen synthase even under experimental conditions when GSK3 β is either not detectable or present in very low amounts. The studies indicate the existence of additional pathways for glycogen synthase activation.[32]

1.2.3.1.2. Inhibition of gluconeogenesis and hepatic glucose output:

Hepatic glucose production is stimulated under fasting conditions by the counter-regulatory hormones glucagon, catecholamines, and glucocorticoids, which augment glucose output by promoting glycogenolysis and gluconeogenesis. During feeding and in response to exogenous insulin injections, hepatic glucose output is potently suppressed by insulin as a result of inhibition of glycogenolysis and gluconeogenesis. Gluconeogenesis is predominantly regulated through changes in gene expression for two key enzymes: phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6- phosphatase (G-6-Pase). PEPCK catalyzes one of the rate- limiting steps of gluconeogenesis, whereas G-6-Pase catalyzes the final step producing free glucose for transport out of liver via GLUT-2 glucose transporters. Gene transcription of PEPCK is tightly regulated by cAMP; counter - regulatory hormones increase cAMP and induce PEPCK, whereas both are suppressed by insulin. In addition to direct hormonal effects on hepatocytes, hepatic glucose output is modulated by the delivery of gluconeogenic substrates to the liver such as lactate, amino acids, and FFA. For example, a reduction in FFA availability contributes to suppression of hepatic glucose output by insulin through its antilipolytic action in adipocytes, and insulin minimizes counter - regulatory effects of glucagon by inhibiting its secretion from the pancreatic α cell. Increased hepatic glucose production is an important determinant of fasting hyperglycemia in diabetes, and has been given greater focus because of the potential importance of regulatory pathways controlling hepatic glucose output as targets of drug therapy.[32]

1.2.3.2. Insulin signaling in muscle:

Skeletal muscle accounts for the bulk of insulin – stimulated glucose uptake *in vivo*, and the hallmark of insulin action in this tissue is the ability to stimulate the glucose transport effector system. Under physiologic conditions, approximately two-thirds of all

glucose-6-phosphate is converted to glycogen, and one-third enters glycolysis. Of the glucose that enters the glycolytic pathway, the majority (80–90%) is converted to carbon dioxide and water, whereas the remaining 10–20% is converted to lactate. Studies have shown that the glucose oxidation is more sensitive but saturates earlier than glycogen synthesis, which has low sensitivity but high capacity. Skeletal muscle is the predominant site of glycogen synthesis.[71]

GLUT - 4 contributes minimally to glucose transport in unstimulated target cells, because > 90% of the cell content of GLUT-4 resides in intracellular membranes in the basal state. The mechanism by which insulin augments glucose transport activity is by recruiting intracellular GLUT- 4 to the plasma membrane, a rate - limiting step for insulin-stimulated glucose uptake and metabolism in peripheral target tissues. Upon dissipation of the insulin signal, deactivation of glucose transport activity is the result of a net reverse translocation of GLUT-4 transporters back into the cell interior. Thus, GLUT-4 is the major transporter mediating insulin–stimulated glucose transport activity in tissues such as skeletal and cardiac muscle and adipose tissue. In unstimulated muscle or adipose cells, a component of GLUT-4 resides in an inducible tubulo-vesicular storage compartment that includes the trans- Golgi network and endosomal vesicles located near the endofacial surface of the plasma membrane. However, another component of cellular GLUT- 4 exists in an active endocytosis–endosomal recycling pathway that cycles GLUT-4 between endosomes and the plasma membrane. The recycling pathway results in the localization of approximately 4 – 10% of GLUT-4 in the basal plasma membrane, and this steady - state distribution is the balance of rapid endocytosis and slow recycling. Insulin shifts the distribution of GLUT- 4 from intracellular pools towards the plasma membrane, both by elevating the exocytotic rate of GLUT - 4 in the recycling pathway and by recruiting GLUT - 4 from the inducible storage compartment to the cell surface. Deactivation of transport is accomplished via a slowing of the exocytotic rate and an acceleration of the endocytotic rate, as GLUT-4 is retrieved from the plasma membrane through clathrin-dependent and - independent mechanisms (Fig:8).[72,73]

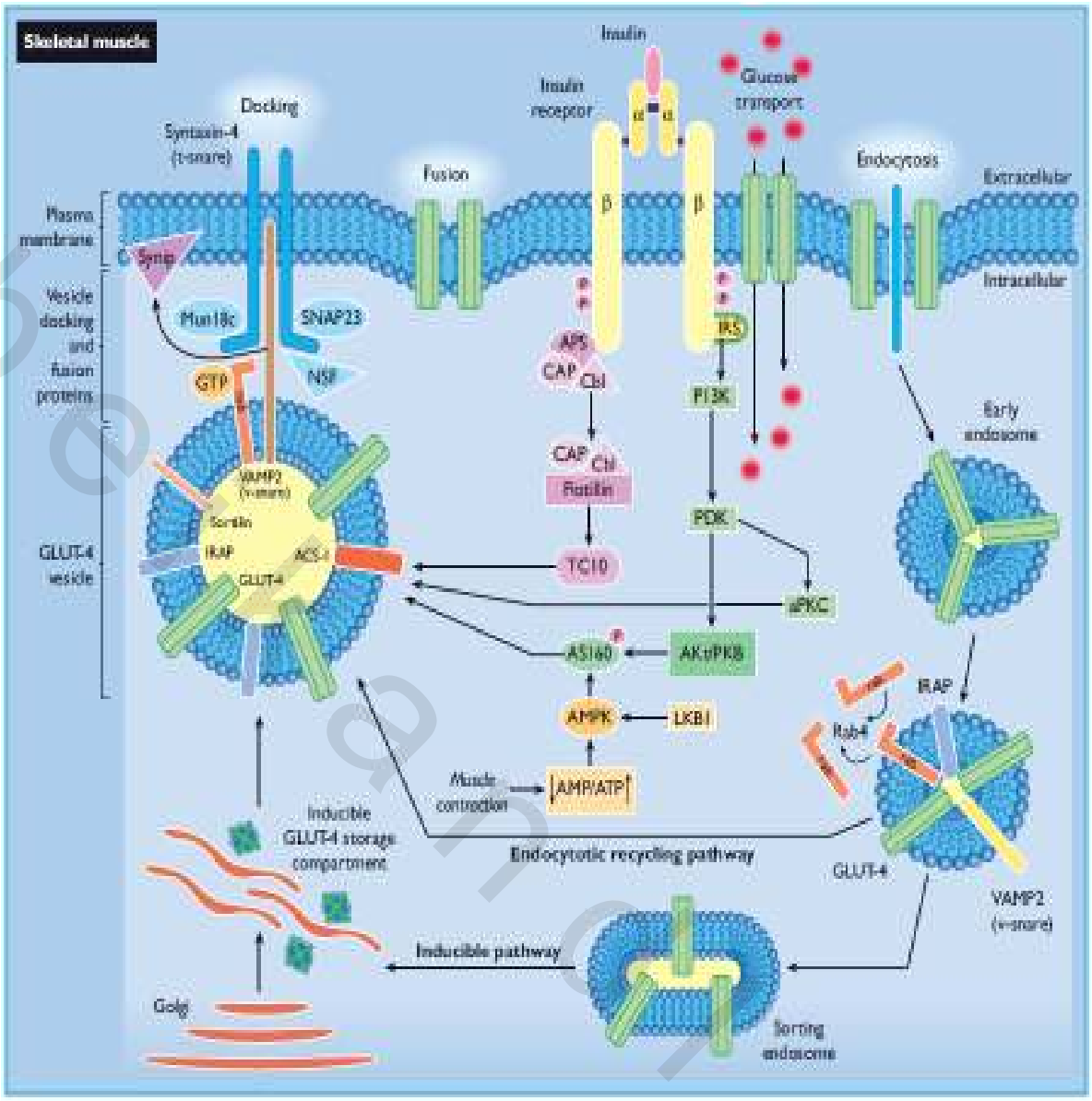


Figure (8): *An overview of insulin signaling pathways in skeletal muscle. Arrows represent an activation process.(Quoted from reference 32).*

1.2.3.3. Insulin signaling in adipocytes:

Adipose tissue is the predominant site for fuel storage as triglyceride, and effector systems responsible for the anabolic effects of insulin on lipogenesis and antilipolysis are key aspects of adipocyte biology.[32]

Regulation of glucose uptake by adenosine monophosphate kinase (AMPK) in primary adipocytes is insulin-dependent. When insulin binds to the insulin receptor (IR), it elicits a signaling cascade resulting in phosphorylation of Akt. Subsequently, Akt phosphorylates Akt substrate of 160 kDa (AS160), which under basal condition causes intracellular retention of GLUT4 -containing vesicles. Once phosphorylated, AS160 releases the brake on GLUT4 vesicles, which allows them to translocate to the plasma membrane and enhance glucose uptake. When AMPK is activated by 5-aminoimidazole-4-

carboxamide1-β-D-ribofuranoside (AICAR) in adipocytes, basal and insulin-stimulated AS160 phosphorylation is inhibited, resulting in a reduction of GLUT4 at the plasma membrane and suppression of glucose uptake in these cells (Fig:9).[74]

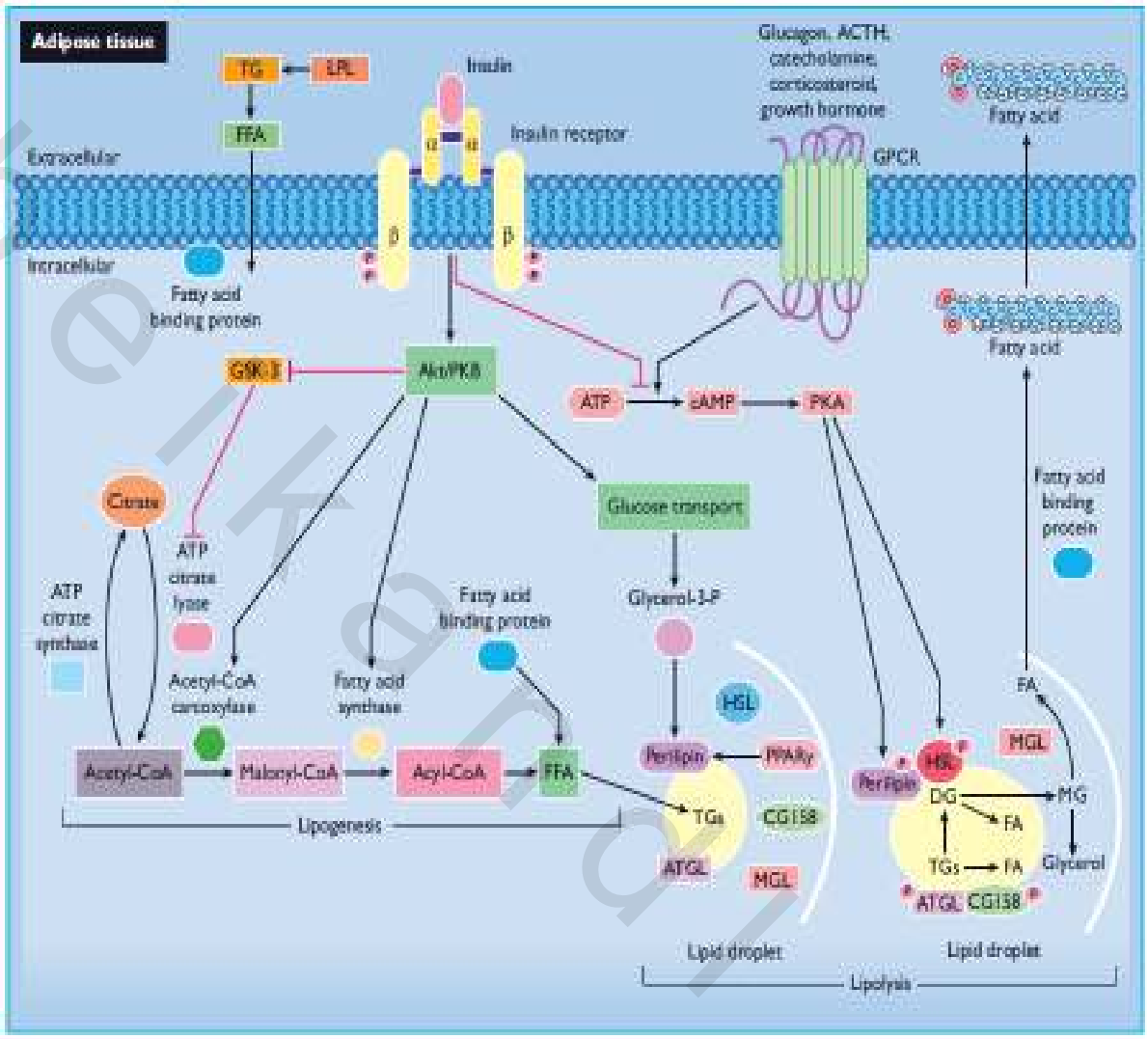


Figure (9): *Summary of insulin function involved in lipogeneis and lipolysis in adipose tissue. Arrows represent an activation process; blocked arrows represent an inhibition process*. (*Quoted from reference 32).*

1.2.3.3.1. Anabolic effects of insulin on lipogenesis

Fat accumulation in adipocytes is determined by the balance between triglyceride synthesis (fatty acid uptake and lipogenesis) and breakdown (lipolysis/fatty acid oxidation). Insulin is a critical stimulator of lipogenesis. Insulin augments availability of both glycerol and fatty acids for triglyceride synthesis by increasing the uptake of glucose in the adipose cell as well as by activating lipogenic and glycolytic enzymes. These enzymes constitute the effector system for the biologic effects of insulin on lipogenesis, and are modulated by insulin both through post- translational modifications and alteration of gene expression. Regarding post- translational effects, insulin activates Akt/PKB via phosphorylation Substrates for activated Akt/PKB include the phosphorylation and

inhibition of GSK3, and this in turn abrogates GSK3 inhibition of ATP citrate lyase; the resulting increase in ATP citrase lyase activity enhances conversion of citrate to acetyl - CoA in the cytosol. Acetyl - CoA is then available as the“ building block ” for fatty acid synthesis.(32)

Insulin also induces gene expression of two key lipogenic proteins: fatty acid synthase (FAS) and SREBP - 1. FAS is the central enzyme participating in *de novo* lipogenesis and catalyzes the conversion of malonyl - CoA and acetyl - CoA to long - chain fatty acids. Regulation of FAS activity by insulin occurs mainly at the level of gene transcription.(75)

1.2.3.3.2. Lipolysis and antilipolysis

Lipolysis in adipose tissue is tightly regulated to assure that partitioning of metabolic fuels, glucose and free fatty acids (FFA), is adapted to energy needs. During fasting, lipolysis is enhanced to make available FFAs that are the main oxidative fuel for the liver, the heart and skeletal muscle, and are metabolized by the liver to ketones that replace glucose as the principal fuel for nervous tissue. Upon feeding, lipolysis is abated and adipocytes convert to triglyceride storage. The rise (after a meal) and fall (with fasting) of insulin has a central role in this regulatory process as a result of its antilipolytic action in adipocytes. Lipolysis in normal subjects is exquisitely sensitive to inhibition by insulin, such that half-maximal suppression of lipolysis occurs at insulin concentrations well below those needed for significant stimulation of glucose uptake by skeletal muscle. Higher concentrations of insulin can reduce adipocyte release of FFA to nearly zero, although at high insulin concentrations there will still be some appearance of glycerol and FFA from the stimulatory effect of insulin on lipoprotein lipase which acts on triglycerides in circulating lipoproteins.(32) Hormones regulate lipolysis in adipocytes via coordinated action involving two major effector systems: hormone sensitive lipase (HSL) and perilipins localized to the surface of lipid droplets. Lipolytic and antilipolytic (i.e. insulin) hormones exert opposite effects on HSL and perilipins by determining cAMP

availability and protein kinase A (PKA) activity. By way of illustration, catecholamines induce lipolysis and release FFAs from adipocytes by binding to β - adrenergic receptors coupled by heterotrimeric G - proteins to adenylate cyclase, which increases production of cAMP and activates PKA. The two main targets for

PKA phosphorylation are HSL and the perilipins.(76) The ability of insulin to antagonize hormone - induced lipolysis is to a large extent accounted for by its ability to lower cAMP levels and thereby reduce PKA activity. The decrease in cAMP is mainly the result of an insulin - mediated phosphorylation and activation of phosphodiesterase 3B (PDE3B) via Akt/PKB. HSL is a key enzyme for the mobilization of triglycerides deposited in adipose tissue following its activation by cAMP/PKA - dependant phosphorylation.(77) HSL is an enzyme with three isoforms ranging from 84 to 130 kDa, yet all isoforms have three domains, a catalytic domain, a regulatory domain with several serine phosphorylation sites required for activation, and an N - terminal variable domain involved in protein – protein and protein – lipid interactions.(78) HSL in muscle can also be stimulated by adrenaline via β - adrenergic activation of PKA, or by muscle contraction via phosphorylation by PKC at least partly activated through the ERK pathway.

Perilipins are localized at the surface of the lipid droplet in adipocytes,[79] and are essential in the regulation of triglyceride deposition and mobilization. In the absence of lipolytic stimulation, perilipin inhibits lipolysis by acting as a barrier against hydrolysis of the triacylglycerol by lipases. When PKA is activated, perilipin becomes phosphorylated and translocates away from the lipid droplet, which allows HSL to hydrolyze the lipid droplet triglyceride core.[80] Insulin blocks lipolysis by inhibiting PKA - mediated phosphorylation of HSL and perilipin, thus reducing both HSL activity and its access to triglycerides in the lipid droplet. In adipocytes there are two forms of perilipin, perilipin A and perilipin B, with perilipin A present at a higher concentration. Adipocyte perilipin content has an inverse correlation with lipolytic rates and a positive correlation with plasma glycerol in humans, and is reduced in obese women. Perilipin knockout mice are lean with increased basal lipolysis and are resistant to diet - induced obesity; however, these mice develop glucose intolerance and insulin resistance more readily, probably because of elevated levels of serum FFAs.[81] In addition, perilipin has a peroxisome proliferator - activated receptor γ (PPAR γ) responsive element in its promoter region and is induced by thiazolidinedione agonists of PPAR γ.[82]

1.3. Pathogenesis of Type 2 Diabetes Mellitus.

Type 2 diabetes is a heterogeneous disorder, phenotypically, genotypically and pathogenetically. Approximately 10% of patients have a late-onset form of autoimmune diabetes which may represent a hybrid of type 1 and type 2 diabetes;[83] up to another 5% of patients have one of the autosomally dominant inherited forms of maturity – onset diabetes of youth (MODY); another 1% may have rare genetic mutations involving insulin receptors and elements of the insulin signaling pathway. The remaining 85% of patients have " garden variety " T2DM which is a polygenic disorder.[84]

The common variety of T2DM results from a combination of genetic and acquired factors which adversely affect β - cell function and tissue insulin sensitivity.[85,86] For many years it was controversial whether impaired β-cell function or tissue insulin resistance was the underlying pathogenic element. Until recently, it was generally thought that insulin resistance preceded β - cell dysfunction and was the primary factor, while β - cell dysfunction was a late phenomenon brought about by exhaustion after years of compensatory hypersecretion.[87,88,89] During the past several years, however, the accumulation of evidence from sophisticated studies examining β – cell function and tissue insulin sensitivity, both cross - sectionally and longitudinally, have outweigh the concept that impaired β-cell function is the primary underlying, probably genetic, defect.[85,90,91,92]

1.3.1 Insulin Resistance:

Insulin directs the selection of metabolic fuels for energy production and, in doing so, it is the only hormone committed to the prevention of hyperglycemi.[93] Insulin sensitivity is commonly described as the ability of insulin to lower plasma glucose levels by suppressing hepatic glucose production and stimulating glucose uptake in skeletal muscle and adipose tissue. Insulin resistance can be defined as the inability of insulin to produce its usual biologic actions at circulating concentrations that are effective in normal subjects because insulin interaction with its receptor fails to elicit downstream signaling events. Metabolically and clinically the most detrimental effects of insulin resistance are due to disruption in insulin-mediated control of glucose and lipid homeostasis in the

primary insulin-responsive tissues: liver, skeletal muscle, and adipose tissue. There is sufficient variability in normal sensitivity to insulin that there is no specific boundary at which sensitivity ends and resistance begins.[94] There is no absolute definition of hyperinsulinemia, since an insulin concentration that is raised for an individual is usually still within the wide range of normality. While hyperinsulinemia may compensate for resistance to some actions of insulin, it can result in overexpression of actions that retain normal reactivity to insulin.[95]

Insulin resistance is a characteristic feature found associated with most cases of type 2 diabetes. In addition, insulin resistance is the hallmark feature of the metabolic syndrome. The abnormal metabolism associated with obesity leads to the development of insulin resistance. The excess free fatty acids (FFAs) affect the insulin receptor-mediated signaling pathways in adipose tissue, liver, and skeletal muscle as well as the pro-inflammatory status induced by the toxic effects of excess FFAs principally in the liver and adipose tissues are cooperate to induce insulin resistance. [96]

Both adipose tissue and liver are important mediators of systemic inflammation in obesity. One model proposes that the expansion of adipose tissue that occurs in obesity results in large adipocytes that have metabolic capacities that exceed the local oxygen supply. The resultant hypoxia leads to the activation of cellular stress response pathways causing cell autonomous inflammation and the release of pro-inflammatory cytokines. As a part of the chronic inflammation adipocytes secrete chemokines such as IL-8 and macrophage chemotactic protein-1 (MCP-1) that attract pro-inflammatory macrophages into the adipose tissue. These activated adipose tissue macrophages secrete cytokines that further exacerbate the pro-inflammatory state. In the liver inflammatory processes are also activated due to the excess accumulation of fatty acids and triglycerides which is the consequence of activated stress response pathways. Within the liver, Kupffer cells (resident liver macrophages) become activated by the generation of reactive oxygen species (ROS) and induction of stress responses. These activated Kupffer cells release locally acting cytokines that, like in adipose tissue, exacerbates the pro-inflammatory environment. Within the vasculature, saturated FFAs can directly activate pro-inflammatory pathways in endothelial cells and myeloid-derived cells resulting in the induction and propagation of a systemic pro-inflammatory state.[96]

Hepatic insulin resistance is induced by the excess accumulation of FFAs. Within the hepatocyte, metabolites of the FFA re-esterification process, including long-chain acyl-CoAs and DAG, accumulate. Excess FFAs also participate in the relocation of several protein kinase C (PKC) isoforms, from the cytosol to the membrane compartment. These PKC isoforms include PKC-β2, PKC-δ, and PKC-theta (PKC-θ). DAG is a potent activator of these PKC isoforms and the membrane-associated PKCs will phosphorylate the intracellular portion of the insulin receptor on serine residues which results in impairment of insulin receptor interaction with downstream signaling proteins including IRS1 and IRS2. Loss of IRS1 and IRS2 interaction with the receptor prevents interaction with PI 3-kinase and its' subsequent activation. In addition to serine phosphorylation of the insulin receptor, various PKCs have been shown to phosphorylate IRS1 and IRS2 further impairing the ability of these insulin receptor substrates to associate with the insulin receptor and downstream effector proteins such as PI 3-Kinase.[32] The FFA-induced down-regulation of insulin signaling pathways results in activation of several kinases involved in stress responses include; Jun N-terminal kinase (JNK), inhibitor of nuclear factor kappa B

kinase beta (IKKβ), and suppressors of cytokine signaling-3 (SOCS-3). Like PKC, JNK activity is also regulated by FFAs and is an important regulator of insulin resistance. The target of JNK action is the Ser307 of IRS-1 and this phosphorylation plays an important role in the progression to hepatic insulin resistance. Activation of IKKβ (which is required for the activation of nuclear factor kappa B, NFκB) may have the most pronounced effect on inflammatory responses in the liver and adipose tissue. NFκB is the most important transcription factor activating the expression of numerous pro-inflammatory cytokine genes such as interleukin-1 (IL-1), IL-6, and tumor necrosis factor-alpha (TNF-α) each of which have been shown to be involved in promoting IR. NFκB-dependent inflammatory mediators produced in hepatocytes act to reduce insulin sensitivity and to promote liver injury (Fig:10).[96]

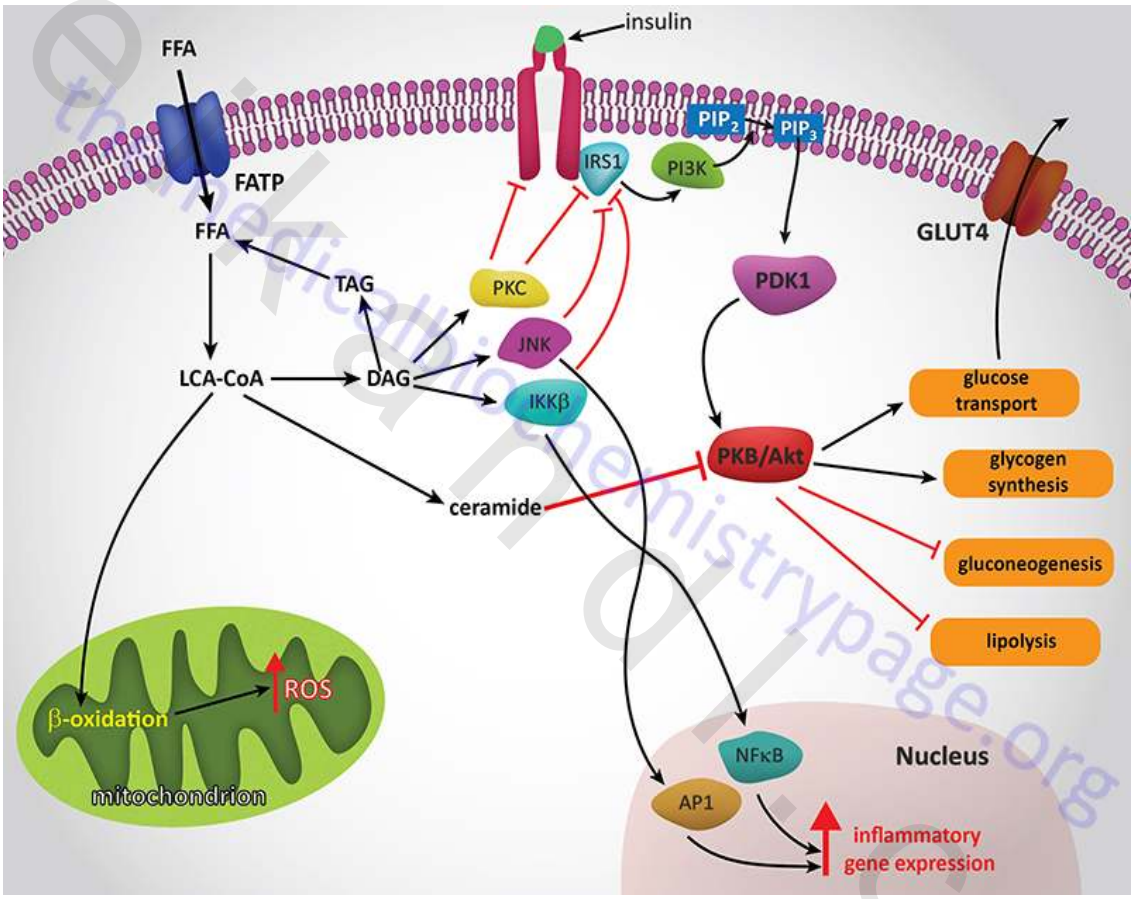


Figure (10): *Model for how excess free fatty acids (FFAs) lead to insulin resistance and enhanced inflammatory responses in cells such as liver and adipose tissue. Quoted from reference (96). Only the major pathways regulated by insulin relative to glucose and lipid homeostasis are shown. Black arrows represent positive actions and red T-lines represent inhibitory actions. JNK = Jun N-terminal kinase. PKC = protein kinase C. IKKβ = inhibitor of nuclear factor kappa B kinase beta. ROS = reactive oxygen species. PI3K = phosphatidylinositol-3 kinase. DAG = diacylglycerol. TAG = triacylglycerols. LCA-CoA = long-chain acyl-CoAs. NFκB = nuclear factor kappa B. PKB (protein kinase B) is a serine/threonine kinase also known as Akt. The role of ceramides in the development of insulin resistance is discussed in the section below*

1.4. Treatment for Type 2 Diabetes

Appropriate glycemic control is a fundamental pillar in the management of T2DM. It is required to prevent and relieve acute symptoms and complications of hyperglycemia; prevent, defer and reduce the severity of microvascular complications; and afford some benefits against macrovascular complications.[97,98] The pandemic of type 2 diabetes, along with its high human and economic costs, is showing no signs of abatement and, therefore, new approaches are urgently needed to prevent, slow the progression, and limit the consequences of this disease. Changes need to be based on knowledge of the pathophysiology and to take into account new insights from genetic and epigenetic studies

Both traditional and modern medical systems suggest the use of various combinations of drugs for the prevention and treatment of diabetes. However, beyond doubts these practices agree the importance of having a balanced life style and physical activity to prevent and cure diabetes. Cellular and molecular level studies on diabetes also points out the importance of physical activity (exercise) for diabetic patients and it is found that such activity can enhance glucose transport in peripheral tissues in an AMPK dependent pathway.[99] In addition to this, insulin sensitizers are also commonly used that makes peripheral tissues such as muscles, adipocytes and liver more sensitive to insulin action.These are much more important in the treatment of type 2 diabetes as they have the potential to counteract insulin resistant in the peripheral tissues. The common ones in this group are metformin, from the group called the biguanides and thiazolidinediones (TZDs) or glitazones including rosiglitazone and pioglitazone.[100,101] Among these metformin increases insulin sensitivity in peripheral tissues and TZD decreases insulin resistance in muscles and adipocytes through increasing the production of GLUT4.[102]

Classification of new antidiabetic agents:

Blood glucose-lowering agents may be either hypoglycemic or antihyperglycemic.[103] Both reduce hyperglycemia, but hypoglycemic agents can lower blood glucose concentrations below the euglycemic range, and therefore carry the risk of clinical hypoglycemia. Such agents include potent inhibitors of hepatic glucose output, insulin secretagogues that act at low glucose concentrations, potent insulin - mimetic drugs and agents that impair counter- regulatory mechanisms. By contrast, antihyperglycemic agents, when used as monotherapy, do not lower blood glucose into the range of overt hypoglycemia. These drugs include the inhibitors of carbohydrate digestion and intestinal glucose absorption, anti-obesity agents, weaker suppressors of hepatic glucose output or counter-regulation, mild or glucose-dependent insulin secretagogues, most insulin - sensitizing agents and modulators of lipid metabolism.[104,105]

The main classes of oral antidiabetic drugs and their principal modes of action are listed in Table **(**2**)**. The main tissues through which they exert their glucose - lowering effects are illustrated in Figure **(**11**)**, and the main cautions and contraindications associated with oral antidiabetic agents are listed in Table **(**3**)**. Although there are several different classes from which to choose, many dilemmas continue to impinge on both strategy and individualization of treatment.

Table (2): Classes of oral antidiabetic drugs and their main modes of action Quoted from reference (106).

Class with examples	Main mode of glucose-lowering	Main cellular mechanism of action
Biguanide		
Metformin	Counter insulin resistance (especially decrease hepatic glucose output)	Enhance various insulin dependent and independent actions including AMPK
Sulfonylureas		
Glimepiride, gliclazide, glyburide/ glibenclamide, glipizide	Stimulate insulin secretion (typically 6–24 hours)	Bind to SUR1 sulfonylurea receptors on pancreatic β-cells, which closes ATP-sensitive Kir6.2 potassium channels
Meglitinides		
Repaglinide, nateglinide	Stimulate insulin secretion (faster onset and shorter duration of action than sulfonylureas)	Bind to benzamido site on SUR1 receptors on pancreatic β-cells, which closes ATP-sensitive Kir6.2 potassium channels
Gliptins (DPP-4 inhibitors)		
Sitagliptin, vildagliptin, saxagliptin	Increase prandial insulin secretion	Inhibit DPP-4 enzyme, which increases plasma half-life of insulinotropic incretin hormones
Thiazolidinediones (PPAR-γ agonists)		
Pioglitazone, rosiglitazone	Increase insulin sensitivity (especially increase peripheral glucose utilization)	Activate nuclear receptor PPAR-γ mainly in adipose tissue, which affects insulin action and glucose–fatty acid cycle
α-Glucosidase inhibitors		
Acarbose, miglitol, voglibose	Slow rate of carbohydrate digestion	Competitive inhibition of intestinal α-glucosidase enzymes

AMPK, adenosine 5′-monophosphate-activated protein kinase; ATP, adenosine triphosphate; DPP-4, dipeptidyl peptidase 4; PPAR-γ, peroxisome proliferator-activated receptor γ.

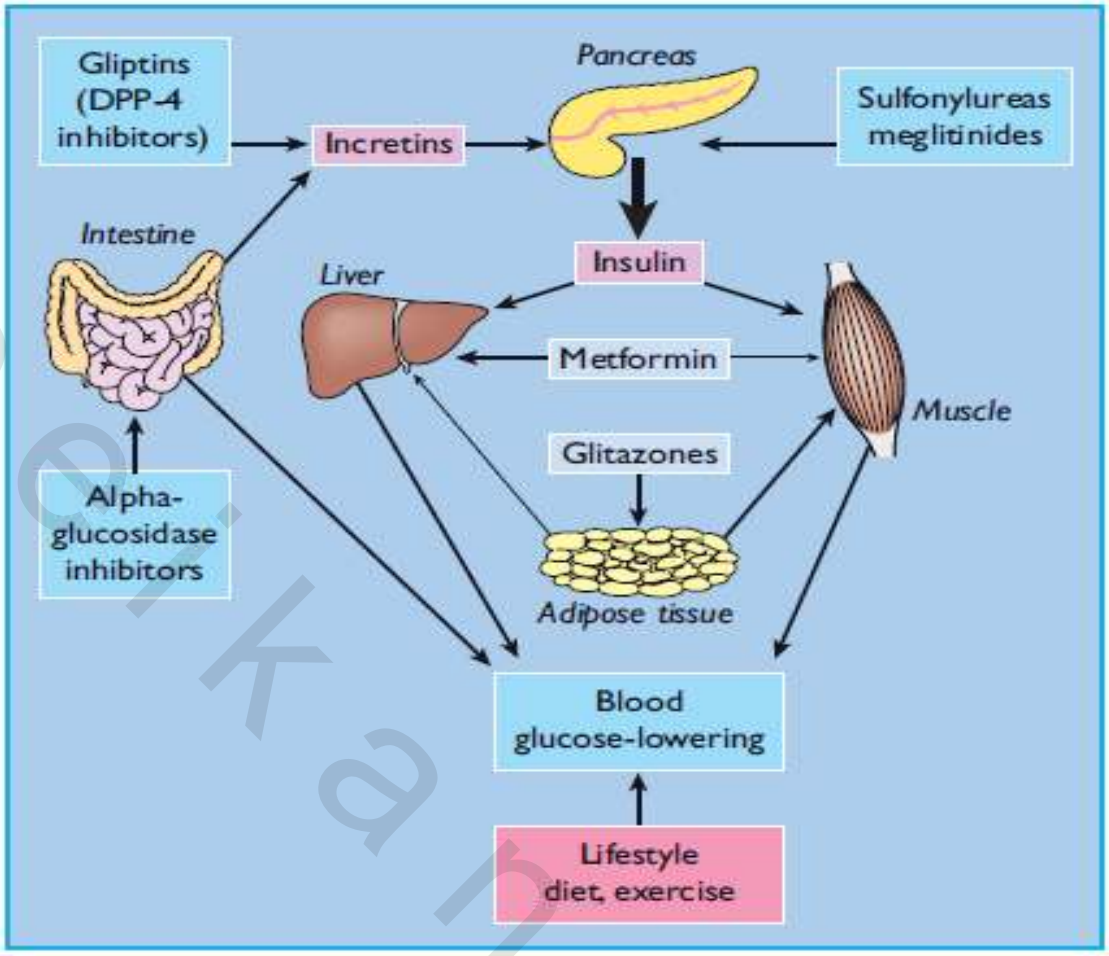


Figure (11): *Main tissues through which oral antidiabetic agents exert their glucose - lowering effects.Quoted from reference (106).*

Table (3): General features of oral antidiabetic treatments for type 2 diabetes including the main cautions and contraindications Quoted from reference (106).

	Metformin	Sulfonylureas	Meglitinides	Thiazolidinediones	Gliptins	α-Glucosidase inhibitors
HbA_{1c}	↓ 1–2%	↓ 1–2%	↓ 0.5–1.5%[h]	↓ 1–1.5%	↓ 0.5–1.5%[h]	↓ 0.5–1%[h]
Body weight	–/↓	↑	↑/–	↑	–	–
Lipids	–/+	–	–	+/–/×	–	–/+
Blood pressure	–	–	–	↓/–	–	–
Tolerability	GI[a]	Hypo[f]	Hypo[i]	Fluid[d]	–	GI[a]
Safety	LA[b]	Hypo[f]	Hypo[i]	Edema Anemia Heart failure[e] Fractures	–	–
Cautions	Renal Liver Hypoxemia[c]	Liver Renal[g]	Liver Renal[g]	CV[e]	–[j]	–

CV, cardiovascular; GI, gastrointestinal; HbA_{1c}, glycated hemoglobin (1% = ~11 mmol/mol); Hypo, hypoglycemia; LA, lactic acidosis.
↑ Increased; ↓ decreased; – neutral; + benefit; × impair.
[a] Gastrointestinal side effects.
[b] Lactic acidosis (rare).
[c] Check for adequate renal and hepatic function, avoid in conditions with heightened risk of hypoxemia.
[d] Fluid retention, anemia, increased risk of heart failure in susceptible patients.
[e] Check for pre-existing cardiovascular disease or developing signs of heart disease: controversy regarding possible early increase in myocardial infarction with rosiglitazone not confirmed in long-term prospective studies.
[f] Risk of hypoglycemia, occasionally severe.
[g] Check liver and/or renal function relevant to mode of metabolism/elimination.
[h] Mostly act to lower post-prandial hyperglycemia – lesser impact on fasting glycemia and on HbA_{1c}.
[i] Lesser risk of severe hypoglycemia than sulfonylurea.
[j] Monitoring of liver function with vildagliptin.

Glibenclamide , also known as glyburide, is an antidiabetic drug in a class of medications known as sulfonylureas, closely related to sulfa drugs. It is sold in doses of 1.25 mg, 2.5 mg and 5 mg, under the trade names Diabeta, Glynase and Micronase in the United States and Daonil, Semi-Daonmetformin.(107) As of 2003, in the United States, it was the most popular sulfonylurea.(108)

The drug works by inhibiting ATP-sensitive potassium channelsil and Euglucon in the United Kingdom and Delmide in India. It is also sold in combination with metformin under the trade names Glucovance and Glibomet. It is used in the treatment of type II diabetes. As of 2007, it is one of only two oral antidiabetics in the World Health

Organization Model List of Essential Medicines the other being.[109] in pancreatic beta cells. This inhibition causes cell membrane depolarization opening voltage-dependent calcium channel. This results in an increase in intracellular calcium in the beta cell and subsequent stimulation of insulin release. This drug is a major cause of drug induced hypoglycemia. Cholestatic jaundice is noted. Studies data suggest glibenclamide is associated with significantly higher annual mortality when combined with metformin than other insulin-secreting medications. Gilbenclamide causes cholestasis as the major side effect. [110]

1.4.1 Herbal medicines and natural products in the treatment of diabetes

The term herbal medicine is generally used to indicate various kinds of alternate medical practices that explore the use of plants and plant based formulations for the treatment of various diseases. Understanding more on these herbal medicines with respect to their active ingredients and mode of action leads to the identification of new molecules that can be used for the treatment purposes. In addition to this, understanding the mode of action of these drugs can shed light to the molecular and biochemical aspects of various diseases, helping in the identification of new targets for therapeutic purposes. Increasing number of research articles on the antidiabetic effect of phytochemicals in the recent years clearly indicate the emerging importance of this area. As diabetes is a life style related disease, alternate medicines have significant role in the treatment of diabetes. Several studies have been carried out with antidiabetic plants in *in-vivo* and *in-vitro* systems with various intentions like;

1) To establish the antidiabetic effect of plants reported in traditional medicines.

2) To understand the mode of action of antidiabetic plants.

3) To isolate and characterize the active component from the plant .

4) To elucidate molecular mechanism through which the extract and/or the active compound exert its effect both *in-vivo* and *in-vitro*. [111]

Among the various plants studied for their antidiabetic effect, *Momordica charantia* is the one which is most important as it is a commonly used food material from the time immemorial. Various studies have proven the importance and antidiabetic effect of momordica in animal models and cell based systems. It is hypothesized that the antidiabetic effect of momordica could involve a washout of glucose from the blood stream. Momordica fruit juice found to act like insulin in *in-vitro* cell based assays and proteins extracted from momordica fruit pulp was found to decrease plasma glucose concentrations in both normal and streptozotocin-induced diabetic rats and also found to induce glucose uptake in muscles and adipocytes.[112,113] Apart from these, a study showed bioactive saponins in momordica fruit extract inhibits glucose uptake across the small intestine suggests its potential as an alternative drug therapy of postprandial hyperglycaemia.[114] Various other studies are also suggesting the *in-vivo* and *in-vitro* effect of momordica fruit extract on insulin secretion and glucose uptake.[115,116] In addition to this a recent study had also shown the effect of triterpenoids isolated from bitter melon exerts its antidiabetic effect through the activation of AMPK pathway.[117]

1.4.1.1. Bitter gourd (Momordica charantia):

Bitter gourd is a popular fruit used for the treatment of diabetes and related conditions amongst the indigenous populations of Asia, South America, India and East Africa. Abundant pre-clinical studies have documented the anti-diabetic and hypoglycaemic effects of Bitter gourd through various postulated mechanisms.[118]

Bitter gourd also known as bitter melon or Momordica charantia belongs to the family Cucurbitaceae. Vernacular English names of M.Charantia include bitter gourd, bitter melon, balsam pear, bitter apple, and bitter, African, or wild cucumber. Several different names in Asia and Africa exist. The most popular is"karela," which is used both in India and in east Africa. M.charantia is an important market vegetable in southern and eastern Asia. However, they are occasionally collected from the environment as a vegetable or medicinal plant. Fruits and leaves of most wild Momordica species are consumed as vegetables, and have a similar bitter taste and almost identical medicinal uses.[119]

Bitter gourd has a higher nutritional value than other cucurbits due to its higher content of minerals (e.g iron and zinc) and vitamins (e.g., ascorbic acid).[119] The immature fruits of M. Charantia can be prepared in many ways. In addition to frying or cooking (e.g., forcurries), the fruits can be dehydrated, pickled, or canned.They are usually blanched or soaked in salt water before cooking to reduce the bitter taste. Fruits, flowers, and young shoots are also used as a flavoring.The young shoots and leaves are sometimes cooked and eaten as leafy vegetables. Bitterness is attributed to the non-toxic alkaloid momordicine..[120]

Active constituents of M. charantia include glycosides, saponins, alkaloids, fixed oils, triterpenes, proteins, and steroids.are concentrated in the fruit of the plant, which has been shown to be the part of the plant with the most pronounced hypoglycemic activity.[121] Several other phytochemicals, including cucurbitins, cucurbitacins, momorcharins, momordenol, and momordicins- identified as important for their nematocidal activity have also been isolated from bitter melon. Besides this, the plant contains a group of proteins designated MRK29 that inhibit human immunodeficiency virus (HIV), and inhibitors of trypsin, elastase, and guanylate cyclase, as well as lectins..[122]

The hypoglycemic activity of bitter gourd has been observed and documented on many occasions. Its fruits, leaves and stems have been extensively used and reported for its hypoglycemic effect. Compounds isolated from the fruits & seeds that are believed to contribute to its hypoglycemic activity include charantin (a steroidal glycoside), vicine (a glycoalkaloid) and polypeptide 'p', a plant insulin, (a 166 residue insulinomimetic peptide) and other terpenoid compounds.[115] These compounds improve blood sugar levels by increasing glucose uptake and glycogen synthesis in the liver, muscles, and fat cells. They also improve insulin release from pancreatic beta cells, and repair or promote new growth of insulin-secreting beta cells.[123,124]

Charantin extracted by alcohol, is a hypoglycemic agent composed of mixed steroids that is more potent than the drug tolbutamide, which is sometimes used in the treatment of diabetes to lower the blood sugar levels. Five compounds in bitter melon increase the activity of adenosine 5 monophosphate kinase (AMPK), an enzyme that facilitates cellular

glucose uptake and fatty acid oxidation. Hypoglycemic agents in bitter melon promotes efficient oxidation of glucose into fuel, and convertion into starch. (Glycogen or animal starch is stored in the liver and muscle cells). During glucose shortages, fats/fatty acids are used as fuel.[125,126]

Bitter gourd also contains cytotoxic (ribosome-inactivating) proteins such as momordin and momorcharin, as well as other unspecific bioactive components such as antioxidants.[127] The hypoglycemic activity of bitter gourd has been hypothesized to act via both pancreatic and extra-pancreatic mechanisms. The mechanism of action, whether it is via regulation of insulin release or altered glucose metabolism and its insulin-like effect, is still under debate.[115]

Older studies were focused on effects on plasma glucose homeostasis and hepatic glucose metabolism, while more recent studies associate the insulin-like effects with antioxidative acitivity and glucose uptake in skeletal muscle cells by GLUT4.[128]