1. INTRODUCTION

1.1. Normal glucose homeostasis

Arterial plasma glucose values throughout a 24-h period average approximately 90 mg/dl, with a maximal concentration usually not exceeding 165 mg/dl such as after meal ingestion and remaining above 55 mg/dl such as after exercise or a moderate fast. Glucose in plasma comes from dietary sources or is either the result of the breakdown of glycogen in liver (glycogenolysis) or the formation of glucose in liver and kidney from other carbons compounds (precursors) such as lactate, pyruvate, amino acids, and glycerol (gluconeogenesis). In humans, glucose removed from plasma may have different fates in different tissues and under different conditions (e.g. postabsorptive vs. postprandial), but the pathways for its disposal are relatively limited. It may be immediately stored as glycogen or may undergo glycolysis, which can be non-oxidative producing pyruvate (which can be reduced to lactate or transaminated to form alanine) or oxidative through conversion to acetyl CoA which is further oxidized through the tricarboxylic acid cycle to form carbon dioxide and water. Non-oxidative glycolysis carbons undergo gluconeogenesis and the newly formed glucose is either stored as glycogen or released back into plasma.[(1)]

Although free fatty acids are the main fuel for most organs, glucose is the obligate metabolic fuel for the brain under physiologic conditions. This occurs because of low circulating concentrations of other possible alternative substrates (e.g. ketone bodies) or because of limitations of transport across the blood-brain barriers (e.g. free fatty acids). After prolonged fasting, because of an increase in their circulating concentration, ketone bodies may be used by the brain to a significant extent. Brain cannot synthesize glucose or store as glycogen more than a few minutes. Thus brain is dependent on a continuous supply of glucose from plasma.[(1)]

The maintenance of the plasma glucose concentration is a critical bodily function. Hyperglycemia is associated with long-term micro- and macrovascular complications, while hypoglycemia can lead to serious injury to the brain.[(2)] The narrow range defining normoglycemia is maintained through an intricate regulatory and counter-regulatory neuro-hormonal system. The most important factors on a moment to moment basis are the hormones (insulin, glucagon, and catecholamines), the sympathetic nervous system activity as well as the concentration of other substrates (e.g. free fatty acids). On a more prolonged time basis (hours – days), other hormones (cortisol and growth hormone), nutritional factors (e.g. diet composition), exercise and physical fitness, along with concomitant changes in the sensitivity to hormones become important.[(1)]

1.1.1. Insulin

1.1.1.1. Insulin biosynthesis

In humans, the gene encoding preproinsulin, the precursor of insulin, is located on the short arm of chromosome 11,[(3)] whereas the rat insulin I and II genes are located on chromosome 1.[(4)] Insulin is synthesized in the pancreas within the beta (β) cells of the islets of Langerhans. Insulin, one of the smallest proteins in the human body, is built from 51 amino acids. It consists of two polypeptide chains (A and B) linked by disulfide bonds. Another disulfide bond exists

within the A chain. Insulin mRNA is translated as a single sequence precursor called preproinsulin in the rough endoplasmic reticulum of β-cells. It is composed of 110 amino acids and is relatively inactive. Almost immediately, preproinsulin is being converted to proinsulin by the removal of its signal peptide.[5] Proinsulin is transported in microvesicles to the Golgi apparatus, where it is packaged into membrane-bound vesicles known as secretory granules. The conversion of proinsulin to insulin is initiated in the Golgi complex and continues within the maturing secretory granule through the sequential action of two endopeptidases (prohormone convertases 2 and 3) and carboxypeptidase H [6], which remove the C peptide chain, liberating two cleavage dipeptides and finally yielding insulin. Insulin and C peptide are stored together in the secretory granules and are ultimately released in equimolar amounts by a process of regulated exocytosis (**Figure 1**).[7]

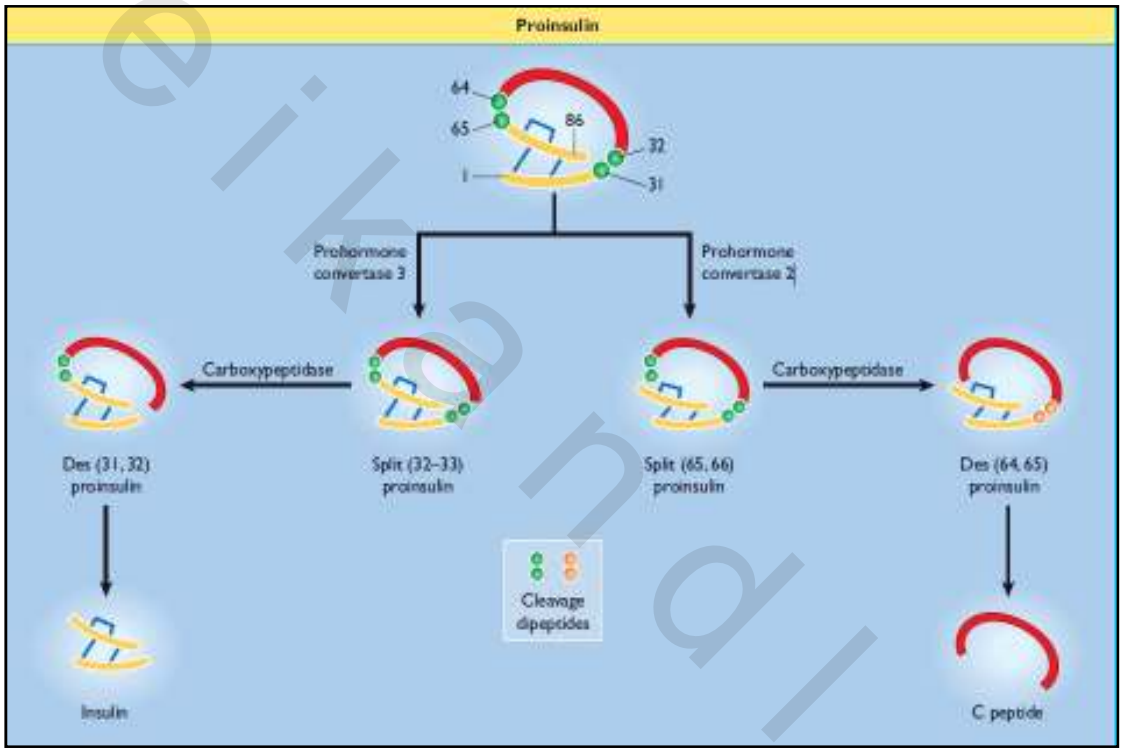

Figure (1): Insulin biosynthesis and processing. Proinsulin is cleaved on the C-terminal side of two dipeptides, namely Arg 31 – Arg 32 (by prohormone convertase 3) and Lys 64 – Arg 65 (prohormone convertase 2). The cleavage dipeptides are liberated, so yielding the "split" proinsulin products and ultimately insulin and C peptide.[7]

1.1.1.2. Insulin Secretion

The major physiologic determinant of insulin secretion in mammals is the circulating concentration of glucose and other nutrients, including amino acids and fatty acids.[7] Glucose is transported into β–cells via high capacity glucose transporters (GLUT; GLUT-2 in rodents, GLUT-1, 2 and 3 in humans), enabling rapid equilibration of extracellular and intracellular glucose concentrations. Once inside the β-cell, glucose is phosphorylated by glucokinase which acts as the "glucose sensor", coupling insulin secretion to the prevailing glucose level.[7] Metabolism of glucose increases the ratio of the concentrations of adenosine trisphosphate (ATP) to adenosine diphosphate (ADP). ATP interacts with ATP-

dependent potassium channels (K_{ATP} channels) closing Potassium channels. Potassium channel closure depolarizes the plasma membrane potential, which in turn opens L-type voltage-gated calcium channels. The cytoplasmic calcium concentration [Ca^{++}] rises and calcium activates protein kinases and interacts with the cell's secretory machinery leading to exocytosis of insulin-laden secretory vesicles, i.e., insulin secretion.[5] The β-cell K_{ATP} channel is a hetero-octamer formed from four potassium channel subunits (termed Kir6.2) and four sulfonylurea receptor subunits (SUR1).[8] The Kir6.2 subunits form the pore through which potassium ions flow and these are surrounded by the SUR1 subunits which have a regulatory role. ATP and sulfonylureas induce channel closure by binding to Kir6.2 and SUR1 subunits, respectively, while ADP activates the channels by binding to a nucleotide-binding domain on the SUR1 subunit (**Figure 2**).[7]

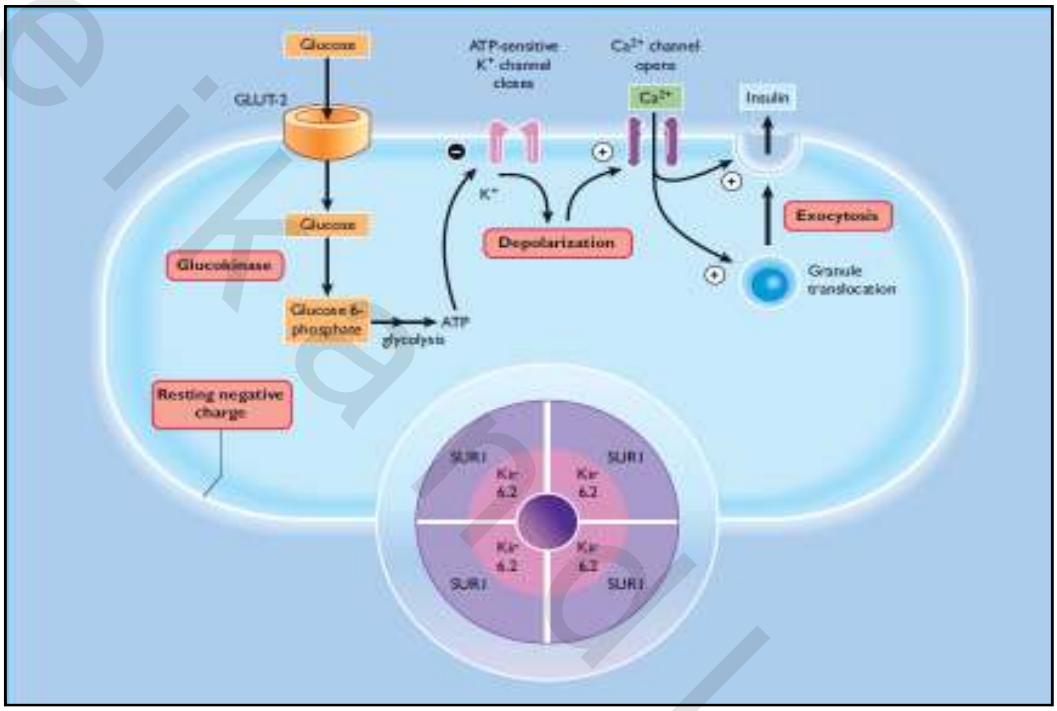

Figure (2): Intracellular mechanisms through which glucose stimulates insulin secretion. Glucose is metabolized within the β-cell to generate ATP, which closes ATP-sensitive potassium channels (K_{ATP} channels) in the cell membrane. This prevents potassium ions from leaving the cell, causing membrane depolarization, which in turn opens voltage-gated calcium channels in the membrane and allows calcium ions to enter the cell. The increase in cytosolic calcium initiates granule exocytosis. Sulfonylureas act downstream of glucose metabolism, by binding to the SUR1 of the K_{ATP} channel. GLUT, glucose transporter.[7]

The time-course of the insulin secretory response to elevated glucose is characterized by a rapidly rising but transient first phase, followed by a maintained and prolonged second phase. Most, if not all, non-nutrient modulators of insulin secretion influence the β-cell by binding to and activating specific receptors on the extracellular surface. Because of its central role in coordinating whole body fuel homeostasis, the β-cell expresses receptors for a wide range of biologically active peptides, glycoproteins and neurotransmitters.[7]

1.1.1.3. Insulin signaling

Insulin exerts a broad spectrum of anabolic effects in multiple tissues. The regulation of the whole body fuel homeostasis primarily involves insulin action in skeletal muscle, adipose tissue, and liver where insulin promotes uptake and storage of carbohydrate, fat, and amino acids, while at the same time antagonizing the catabolism of these fuel reserves. In skeletal muscle, insulin stimulates glucose transport and glucose storage as glycogen, as well as glycolysis and tricarboxylic acid cycle activity. Insulin lowers hepatic glucose output by inhibiting glycogenolysis and gluconeogenesis, and augments glycogen formation. In adipocytes, insulin promotes glucose uptake, glycerol synthesis, and triglyceride formation, while at the same time exerting an antilipolytic effect. During periods of fasting, a fall in circulating insulin combined with increased secretion of counter-regulatory hormones leads to breakdown of stored fuels and increased availability of metabolic substrates for cellular energy. In this way, alterations in insulin levels in the fed and fasting states have a key role in fuel metabolism and maintain blood glucose levels within a narrowly defined range. Insulin diminishes protein catabolism and increases translation, and also enhances cell growth, differentiation, and survival as a consequence of mitogenic and anti-apoptotic processes. Thus, the action of insulin at the level of cells and tissues affects substrate flux and coordinates the function of multiple organs as whole organisms adapt to the nutritional environment.[9,10] In mediating its pleiotropic actions, insulin binds to cell surface receptors, activates multiple signal transduction networks, and engages effector systems responsible for specific biologic functions. 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 (**Figure 3**).[10]

The insulin receptor is a heterotetrameric bifunctional complex, consisting of 2 extracellular α subunits that bind insulin and 2 transmembrane β subunits with tyrosine kinase activity. Insulin binding to the α subunit induces the transphosphorylation of one β subunit by another on specific tyrosine residues in an activation loop, resulting in the increased catalytic activity of the kinase.[11] The receptor also undergoes autophosphorylation at other tyrosine residues in the juxtamembrane regions and intracellular tail. The activated insulin receptor then phosphorylates tyrosine residues on intracellular substrates that include the insulin receptor substrate family.[12]

Following insulin binding and receptor autophosphorylation, the next committed step in signal transduction is tyrosine phosphorylation of intracellular proteins. At least 11 intracellular substrates have been identified that are rapidly phosphorylated on tyrosine residues by ligand-bound insulin receptors, including six insulin receptor substrate (IRS) proteins, Grb2-associated binder 1 (Gab1), Cas-Br-M ecotropic retroviral transforming sequence homolog (Cbl), and the various isoforms of Src-homology-2-containing protein (Shc).[13] Insulin receptor substrates are, by definition, molecules phosphorylated by the insulin receptor kinase. They are most often adaptor or scaffolding proteins which have no catalytic activity but, by means of multiple recognition domains, act to form multimolecular complexes, bringing enzymes and substrates into proximity or to the proper intracellular localization.[14]

PI_3 kinase is a key among the molecules that can associate with the IRS proteins. PI_3 kinase is a lipid kinase that phosphorylates the 3-position of the inositol ring in phosphatidylinositol. A major product is phosphatidylinositol-3',4',5'-trisphosphate (PIP_3),

an important lipid second messenger.[15] 3-Phosphoinositide-dependent protein kinase 1 (PDK1) can interact with PIP_3, and is responsible for downstream activation of Akt/PKB and atypical protein kinases C (aPKCs).[10] PI_3 kinase plays an essential role in glucose uptake and GLUT4 translocation. Inhibition of the enzyme with pharmacological inhibitors such as wortmannin completely blocks the stimulation of glucose uptake by insulin.[16] AKT/PKB is a serine/threonine kinase that is a downstream target of PI_3 kinase signalling. AKT/PKB mediates most of the PI_3 kinase-mediated metabolic actions of insulin through the phosphorylation of several substrates, including other kinases, signalling proteins and transcription factors.[16]

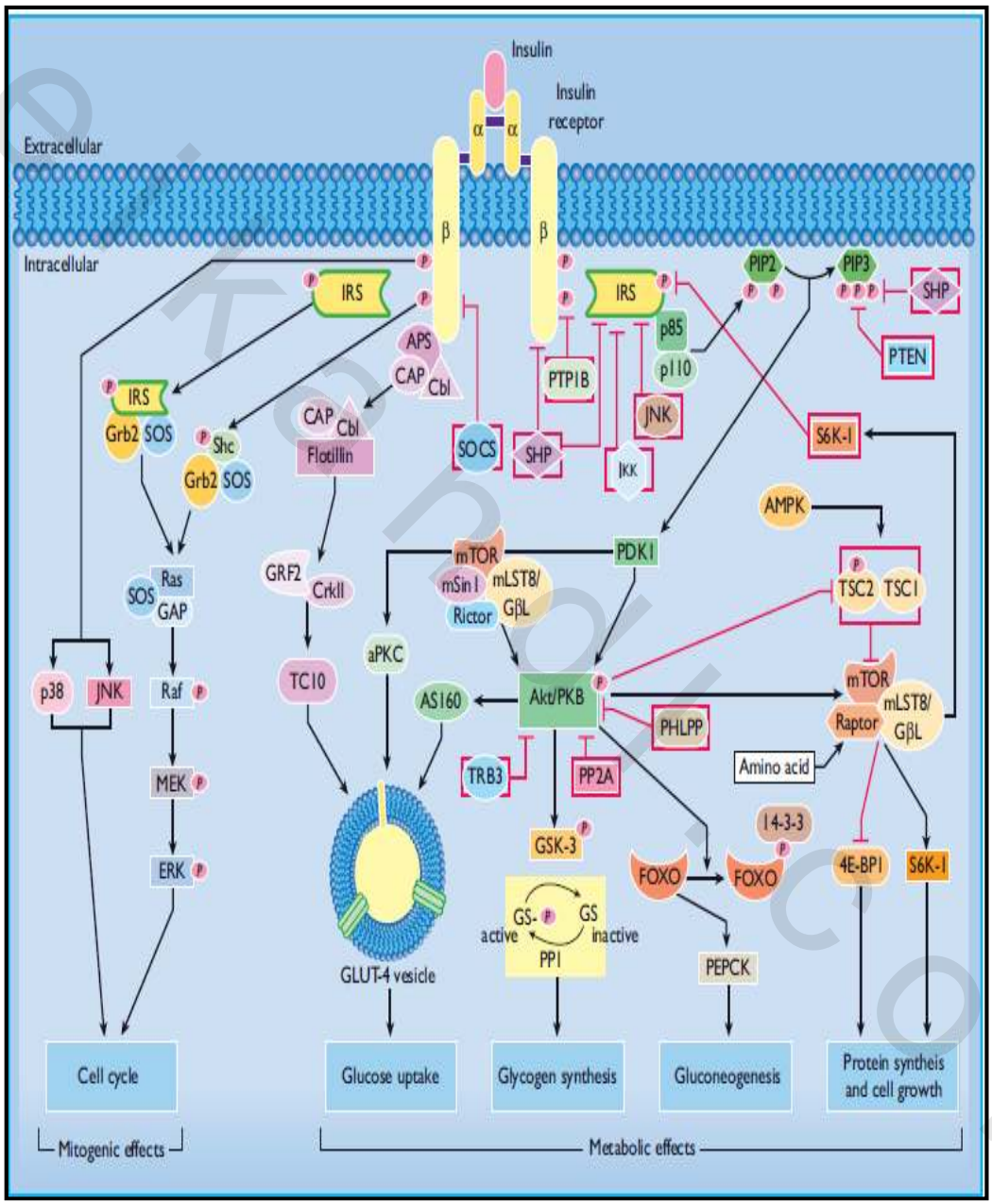

Figure (3): A schematic illustration of general insulin signaling pathways involved in both metabolic and mitogenic effects. Arrows represent an activation process; blocked arrows represent an inhibition process.[10]

1.1.1.4. 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.[12]

1.1.1.4.1 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.[9]

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 (Figure 4).[9]

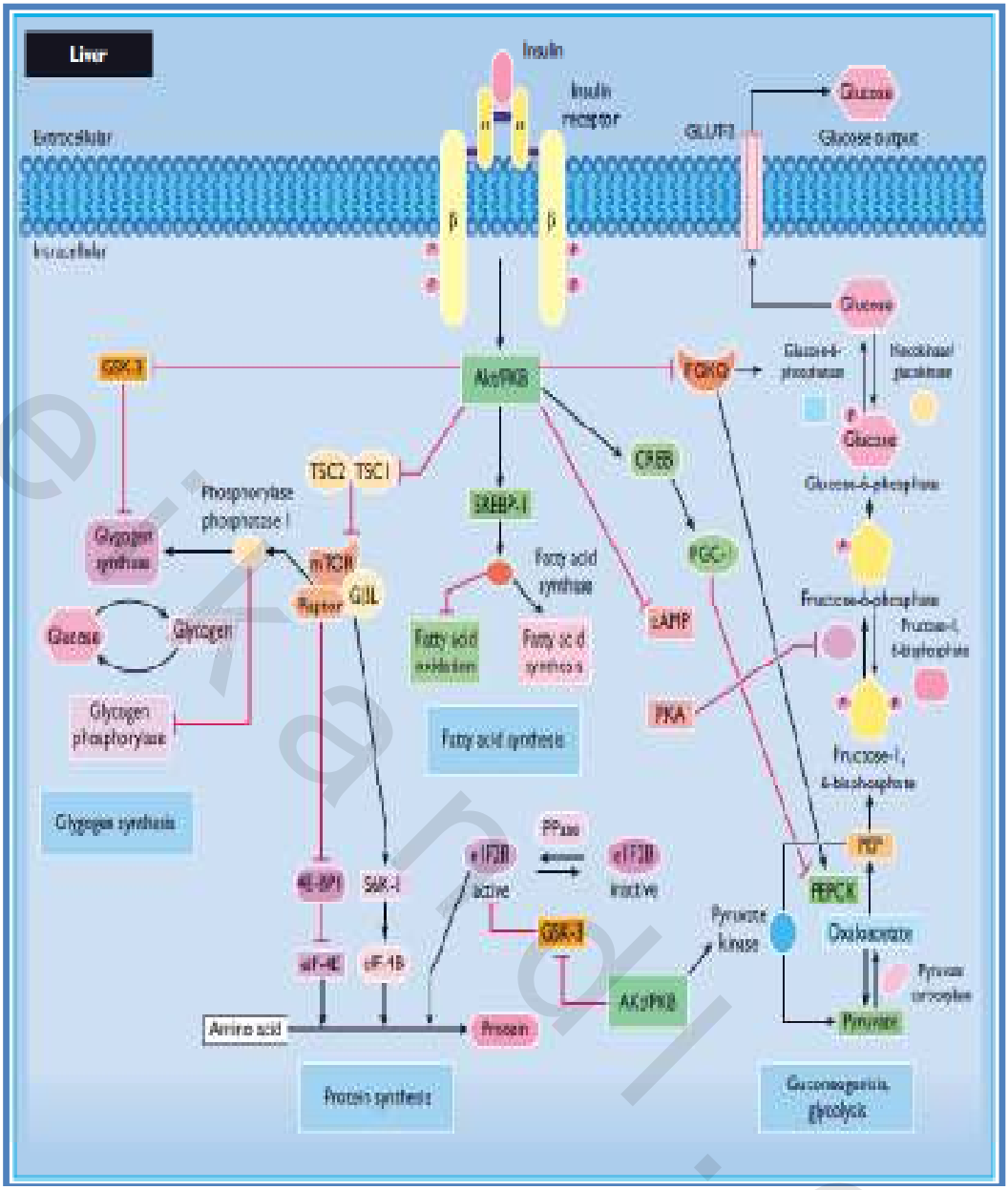

Figure (4): **Summary of insulin signaling pathways in the liver** that are involved in glycogen synthesis, gluconeogenesis and glycolysis, and protein synthesis, respectively. Arrows represent an activation process; blocked arrows represent an inhibition process. [9]

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).[17] 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 glycogen synthase kinase 3 (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.[9]

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.[9]

1.1.1.4.2 Insulin action 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.[18]

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 (Figure 5).[11]

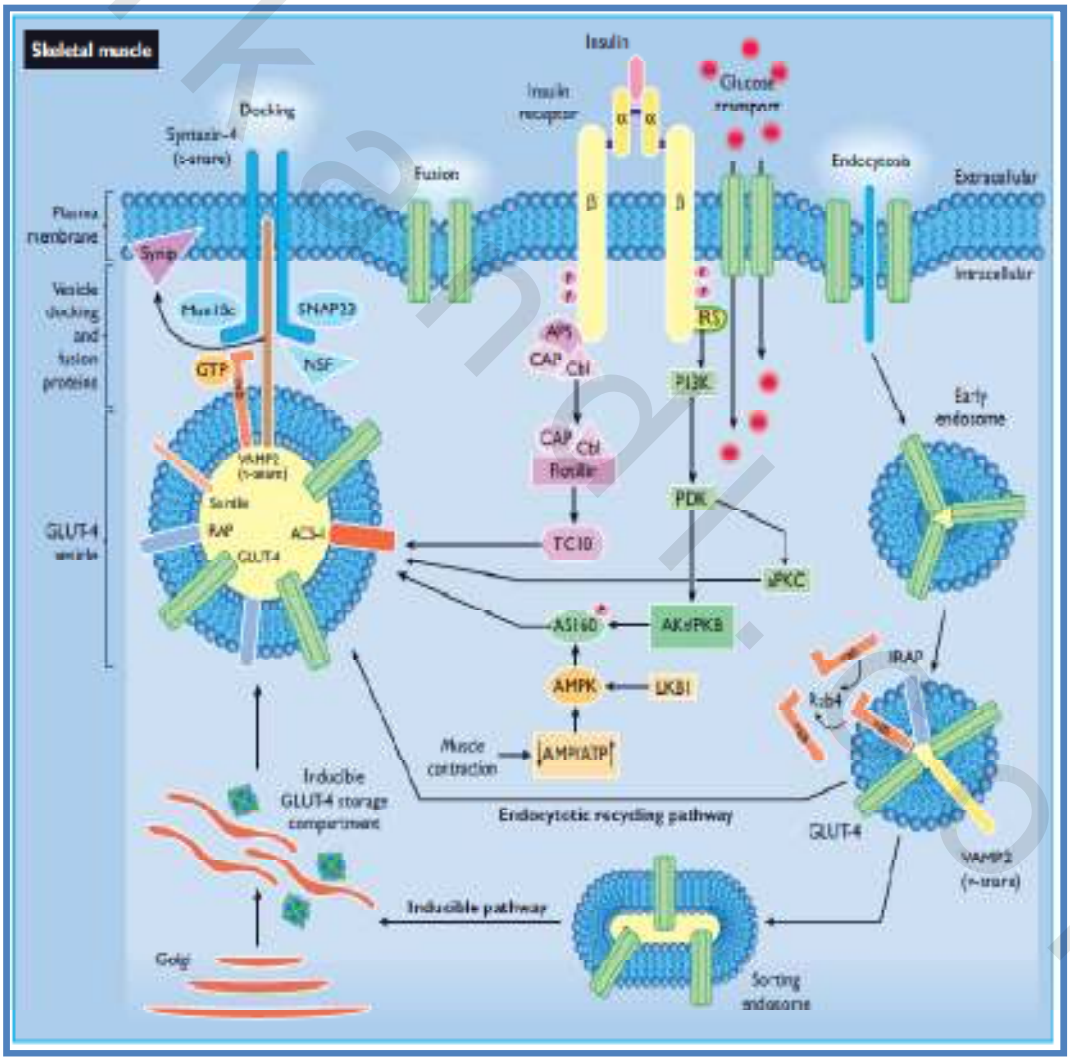

Figure (5): **An overview of insulin signaling pathways in skeletal muscle**. Arrows represent an activation process.[11]

1.1.1.4.3 Insulin action 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.[9]

Regulation of glucose uptake by adenosine monophosphate kinase (AMPK) in primary adipocytes is insulin-dependent. When insulin binds to its 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 (Figure 6).[19]

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.[9]

Insulin 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.[20]

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.[9]

The ability of insulin to antagonize hormone - induced lipolysis is to a large extent accounted for by its ability to lower the level of cyclic adenosine monophosphate (cAMP) and thereby reduce protein kinase A (PKA) activity. The decrease in cAMP is mainly the result of an insulin - mediated phosphorylation and activation of phosphodiesterase 3B (PDE3B) via Akt/PKB. Hormone-sensitive lipase (HSL) is a key enzyme for the mobilization of triglycerides deposited in adipose tissue following its activation by cAMP/PKA-dependant phosphorylation.[21] 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. Figure (6) .[9]

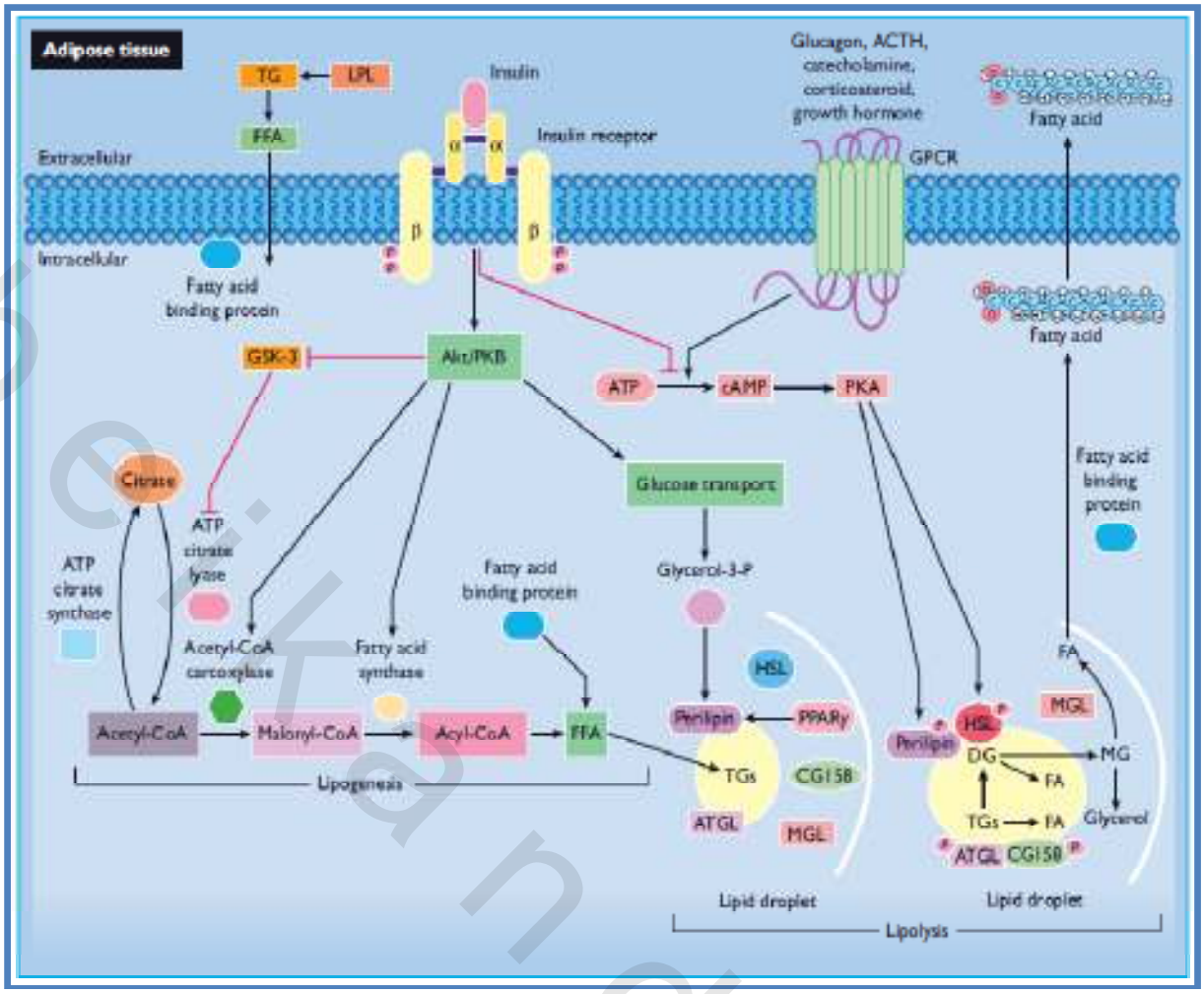

Figure (6): Summary of insulin function involved in lipogeneis and lipolysis in adipose tissue. Arrows represent an activation process; blocked arrows represent an inhibition process. [9]

1.2. Diabetes Mellitus (DM)

Diabetes mellitus is a group of diverse metabolic disorders characterized by derangement in carbohydrate, protein, and fat metabolism caused by complete or relative insufficiency of insulin secretion and/or insulin action.[22]

1.2.1. Classification of DM

1.2.1.1. Type 1 Diabetes Mellitus (T1DM)

This form of diabetes accounts for 5–10% of all cases. It results from a progressive cellular-mediated autoimmune destruction of the pancreatic β-cells that leads to complete insulin deficiency. The rate of β-cell destruction is rapid in the majority, particularly in infants and children, but may be insidious in the adults. When β-cell failure is sudden it can cause ketoacidosis, often the first manifestation of the disease. Otherwise, a more indolent onset of disease is common, with severe hyperglycemia and/or ketoacidosis found only in the presence of stress conditions or severe infections. Patients with T1DM are severely insulin deficient and are dependent on insulin treatment for their survival. Management consists of insulin provided as a replacement hormone.[23]

1.2.1.2. Type 2 Diabetes Mellitus (T2DM)

This type of diabetes consists of heterogeneous conditions responsible for approximately 90% of all individuals with diabetes. It is often associated with central or visceral obesity, as well as other cardiovascular risk factors such as hypertension, and abnormalities of lipoprotein metabolism with the characteristic dyslipidemia of elevated triglycerides (TGs) and low high-density lipoprotein (HDL) cholesterol. Type 2 diabetes is characterized by complex metabolic derangements, with two main abnormalities: insulin resistance and β-cell dysfunction.[23] Insulin resistance is defined as a failure of target organs to respond normally to the action of insulin. Insulin resistance causes incomplete suppression of hepatic glucose output and impaired insulin-mediated glucose uptake in the periphery (skeletal muscle and adipose), leading to increased insulin requirements. When increased insulin requirements are not matched by increased insulin levels, hyperglycemia develops.[24] Circulating insulin levels are higher early in the disease to compensate for insulin resistance, but eventually, insulin production becomes less sufficient and hyperglycemia develops. The capacity of insulin secretion in these patients is often enough to prevent ketosis and ketoacidosis, but still manifest during periods of severe stress or acute medical illness.[23]

1.2.1.3. Gestational Diabetes Mellitus (GDM)

GDM is defined as glucose intolerance occurring or first recognized during pregnancy.[23,25] This is distinct from women with diabetes undergoing pregnancy, who have diabetes in pregnancy rather than gestational diabetes. Plasma glucose levels, both fasting and post-prandial, are lower than normal in early pregnancy so that raised levels at this stage are almost certainly caused by previously undetected T2DM. Screening for GDM is generally undertaken at around 28 weeks. There is significant morbidity associated with GDM including intrauterine fetal death, congenital malformations, neonatal hypoglycemia, jaundice, prematurity and macrosomia. Risk factors for GDM include certain ethnic groups, those with previous GDM or abnormalities of glucose

tolerance, age, obesity and previous large babies. Women with gestational diabetes mellitus have a more than sevenfold increased risk of subsequently developing T2DM compared with women who experience a normoglycemic pregnancy.[26]

1.2.1.4. Impaired glucose tolerance (IGT) and impaired fasting glycemia (IFG)

National Diabetes Data Group (NDDG) and World Health Organization (WHO) formalized IGT as a higher than normal plasma glucose 2 hours after a glucose load but below the diagnostic cutoff for diabetes.[27] Later, both the American Diabetes Association (ADA) and WHO introduced the concept of IFG as a fasting plasma glucose above normal but below the diabetes diagnostic level.[26] Both IFG and IGT are associated with a two - to threefold increased risk of developing diabetes, while IGT is also a cardiovascular risk marker. IFG was welcomed as it could indicate an at-risk individual without the need to perform a glucose tolerance test. Collectively, IFG and IGT became known as "pre-diabetes" - a misleading term as not everyone with pre-diabetes develops diabetes, and it diminishes the importance of other risk markers such as family history. The term "intermediate hyperglycemia" is preferred by WHO.[28] IGT and IFG are more likely in older people, those who are obese, people from particular high risk ethnic groups and those with cardiovascular disease or other features of the metabolic syndrome, such as dyslipidemia, hypertension or visceral adiposity.[27]

1.2.2. Diagnosis of DM

The diagnosis of diabetes is established solely by documentation of abnormal glycemic values (**Table 1**). There are three criteria used to make a diagnosis of diabetes; elevated fasting glucose, abnormal OGTT, or symptoms of diabetes with hyperglycemia. Glucose tolerance tests are performed by providing either 75 or 100 g of glucose. This test, although more sensitive and specific than fasting glucose alone, is lengthy and cumbersome. Test should be done in the morning after an overnight fast and at least 3 days of unrestricted diet, rich in carbohydrates. The subject should remain seated and not smoking throughout the test.[23]

In the past few years, glycated hemoglobin (HbA_{1c}), a measure of average glycemia over the previous 8–12 weeks [29] has been recommended as an alternative means for the diagnosis of T2DM by ADA and the WHO. The cut-off of HbA_{1c} of $\geq$ 6.5% for the diagnosis of diabetes mellitus, as recommended by the ADA and WHO, was derived based on the association between HbA_{1c} and prevalent retinopathy.[24] However, The use of HbA_{1c} for the diagnosis of diabetes is not currently recommended due to the lack of uniformity in the assays worldwide. Therefore, there is an ongoing effort for worldwide standardization of HbA_{1c} measurement.[30]

Table (1): Criteria for the diagnosis of glucose intolerance and diabetes [27]

	Normal	Impaired fasting glucose (IFG)	Impaired glucose tolerance (IGT)	Diabetes mellitus
Fasting plasma glucose				
mg/dl	<100	100–125	–	≥126
mmol/l	<5.6	5.6–6.9	–	≥7.0
2-h post OGTT challenge				
mg/dl	<140	–	140–199	≥200
mmol/l	<7.8	–	7.8–11.0	≥11.1
HbA1c	<5.4%			>6.5

OGTT, oral glucose tolerance test

1.2.3. Risk factors for Type 2 Diabetes Mellitus

There are many reported factors for the development of T2DM which included:

A. Family history of diabetes (i.e., parent or sibling with type 2 diabetes).
B. Obesity (body mass index (BMI) 25 ≥ kg/m^2).
C. Physical inactivity.
D. Race/ethnicity (for example; African American, Latino, Native American, Asian American, Pacific Islander).
E. Previously identified with impaired fasting glucose (IFG), IGT, or HbA1C of 5.7–6.4%.
F. History of GDM or delivery of baby >4 kg (9 lb).
G. Hypertension (blood pressure 140/90 mmHg).
H. 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).
I. Polycystic ovary syndrome.
J. History of cardiovascular disease.

1.2.4. Epidemiology of DM

The global prevalence of diabetes mellitus is rapidly increasing as a result of population ageing, urbanization and associated lifestyle changes.[28] The number of people with diabetes mellitus worldwide has more than doubled over the past three decades.[31] In 2010, an estimated 285 million people worldwide had diabetes mellitus,[32] 90% of whom had T2DM.[28] The number of people globally with diabetes mellitus is projected to rise to 439 million by 2030, which represents 7.7% of the total adult population of the world aged 20 – 79 years.[32]

The major burden of diabetes mellitus is now taking place in developing rather than in developed countries. 80% of cases of diabetes mellitus worldwide live in less developed countries and areas. Among the 10 countries with the largest numbers of people predicted to have diabetes mellitus in 2030, five are in Asia (China, India, Pakistan, Indonesia and Bangladesh). In addition to Asia, the Gulf region in the Middle East and Africa are other hot spots for diabetes mellitus.[31,32]

Over the last 50 years diabetes is growing rapidly. Although some of the increase in diabetes prevalence may be due to the increasing longevity of the population, an increase in the rate of type 2 diabetes is also being observed among the young, suggesting that an active process is driving the epidemic.[31-33] The previously mentioned risk factors cannot interprate the global epidemic of T2DM. Identifying the etiology and the early detection of type 2 diabetes are keys to prevention.

During the laste years a new hypothesis emerged which stated that excessive use of antioxidants may increase the risk of T2DM. In humans, the clinical outcomes of antioxidant therapy in the treatment of T2D remain very inconclusive. A previous report even showed that use of antioxidants actually increased the incidence of all-cause death [34]. These results have cast doubt on the usefulness of antioxidant supplements, and possibly on the fundamental concept that enhancing antioxidant capacity to counteract ROS benefits T2D patients [35]. Given that ROS signaling is attenuated by antioxidants, it could not rule out the possibility that the increasing incidence of T2D over the decades could be due, at least in part, to our self-prescribed preventive measures. For T2D patients, taking antioxidant supplements may even exacerbate their diseased conditions because of the further dampening of ROS signaling by exogenous antioxidants [35]. But this hypothesis needs confirmation by experimental and clinical studies.

One of the main constituent of antioxidant supplemenations in the market is trace element selenium (Se). Selenium stands out for its unique biochemistry, its antioxidant capacity and its narrow therapeutic window. Selenocysteine, the selenium analog of cysteine, is co-translationally incorporated into 25 human selenoproteins. Glutathione peroxidases (GPx), selenoprotein P (SeP) and thioredoxin reductases are the most prominent and ubiquitously expressed selenoproteins, contributing to degradation of reactive oxygen species (ROS) and regulation of cellular redox homeostasis. [36]

An anti- diabetic impact of dietary selenium supplementation would be expected given both the long track of selenium as insulin – mimetic micronutrient and it's antioxidant capacity as constituent of ROS- detoxifying selenoenzymes, suggesting a protective role against oxidative stress – related chronic complications in the progression of diabetes. [36,37] Contrarily to those expectations, recent epidemiological and intervention studies revealed a surprising association between high plasma selenium levels and type 2 diabetes, hyperglycemia and dyslipidemia. [38,39]

1.3. Selenium:

Selenium (Se), a trace element, is the 34th element in the periodic table. It is a nonmetal and its properties are intermediate between adjacent sulfur and tellurium. (40)

Selenium acts as an antioxidant and helps protect the body against the damaging effects of free radicals. Selenium is essential for the activity of glutathione peroxidase, an enzyme that protects against reactive oxygen species and subsequent cell membrane damage. (41)

The Recommended Dietary Intake (RDI) for men is 85 µg/day and 70µg/day for women, but the update of the nutrient reference values by National Health and Medical Research Council (NHMRC) has proposed a reduction in the recommendation for selenium to 65µg/day for men and 55µg/day for women(42).

Selenium is stored in the tissues in varying density: 30% of tissue selenium is in liver, 15% in the kidney, 30% in muscle, 10% in the plasma and the remaining 15% throughout other organs(42).

Because selenium binds to mercury and is deposited in tissue in an inert complex with a 1:1 molar ratio, this selenium-mercury complex is unavailable for metabolism. When free non-mercury-bound selenium is determined in specific tissues, tissue concentrations are greatest in the kidney cortex and pituitary gland, followed by the thyroid gland, liver, spleen and cerebral cortex(43).

Selenium occurs in both organic and inorganic forms. Selenide is found frequently in the food supply among the inorganic forms (selenite, selenate or selenide) and these selenates and selenites are reduced to selenide in the liver with end products as dimethyl and trimethyl selenide(41). The organic form includes selenomethionine and selenocysteine which are found predominantly in plants and animal respectively (44).

Selenium compounds are generally very efficiently absorbed by humans and selenium absorption does not appear to be under homeostatic control . For example, absorption of the selenite from selenium is greater than 80% whereas that of selenium as selenomethionine or as selenate may be greater than 90 %.(45)

Selenium levels in blood and tissues are very much influenced by dietary selenium intake. Normal blood level varies from 0.05 to 0.34µg/ml. (46)

Selenomethionine is generally thought to be the most bioavailab form of Selenium. Selenomethionine, however, may substitute in tissue proteins for methionine and changing the methionine content of the diet will alter how much selenomethionine is used to satisfy methionine requirements(47).

Animal studies have consistently shown a beneficial effect of high selenium levels in the prevention of cancer(48).

The essential trace element selenium is believed to exert beneficial influence on human health, mainly based on the antioxidant capacity of selenoprotein such as glutathione peroxidase (GPX) and thioredoxin reductase (TrxRs) containing the 21st proteinogenic amino acid selenocysteine in their active center(36) .

Potential selenium-mediated health benefits include prevention of cardiovascular and neurodegenerative diseases, delay of aging, functioning of immune system, and prevention of certain forms of cancer[49,50]. A wide range of dietary selenium sources comprise cereals, garlic, brazil nuts, meat and fish.

Selenium alters carcinogen metabolism, inhibits tumor cell proliferation, enhances apoptosis, and suppresses tumor angiogenesis[51].

Supra Nutritional levels of selenium supplementation, exceeding that necessary to make adequate levels of glutathione peroxidase, have also been shown to increase the cytotoxic activities of natural killer cells and macrophages and up-regulate the expression of interleukin-2 receptor[52]. This immune enhancing effect has been demonstrated in human studies, where eight-week doses of 200 mcg yeast-based selenium in humans significantly increased cytotoxic lymphocyte and macrophage activity[53].

However, it has long been known that the therapeutic window of selenium is narrow, and adverse health effect may occur due to supranutritional selenium intake ever below the levels required for intoxication[54]. On this regard, an ongoing discussion on the safety of dietary Se supplementation has arisen from a coincidental and unexpected finding of the Nutritional Prevention of Cancer (NPC) trial. Participant of the trial, who received a daily dose of 200µg Se over 12 years, were more likely to develop type 2 diabetes mellitus than those assigned to placebo[38].

1.3.1. Deficient intake:

Overt Se deficiency is associated with keshan disease, a cardiomyopathy affecting mainly children and women of child-bearing age, frequently fatal[55].

1.3.2. Excessive intake:

Overt Se toxicity in humans is far less widespread than Se deficiency. Se toxicity has been studied in animals and observed in humans where signs of selenosis are hair loss, brittle, thickened and stratified nails, garlic breath and skin[56]. Chronic exposure to high levels of Se has been observed in several population in seleniferous areas of the world, such as the northern great plains of the USA, parts of Venezuela and Colombia, where the average daily intake of 4.9 mg was associated with a blood Se concentration of 3200µg/L and symptoms of selenosis[57].

1.3.3. Optimal intake:

Despite food supplies coming from diverse sources, at least in developed countries, there is evidence that in some population groups that Se intake, while not deficient, may be sub-optimal for protection against a number of adverse health conditions. [58]

Ascertaining the optimal intake of Se is not a trivial matter since it is dependent on a number of factors.

These include consideration of the mechanism by which selenium is thought to act in any particular situation, the species of Se ingested, which type of disease (or which type of cancer) is being considered, the overall nutritional adequacy of the group or population, the extent to which genomic differences between individuals or population may be relevant, and what other risk or lifestyle factors may be present within the population under consideration[58].

1.4. Selenoproteins:

In molecular biology a selenoprotein is any protein that includes a selenocysteine (Se-Cys) amino acid residue[47].

About 25 different selenocysteine-containing selenoproteins have so far been observed in human cells and tissues. Since lake of selenium deprives the cell of its ability to synthesize selenoproteins, many health effects of low selenium intake are believed to be caused by the lack of one or more specific selenoproteins[59].

Figure (7) show the classification of selenoprotein on the basis of their determined or potential function.

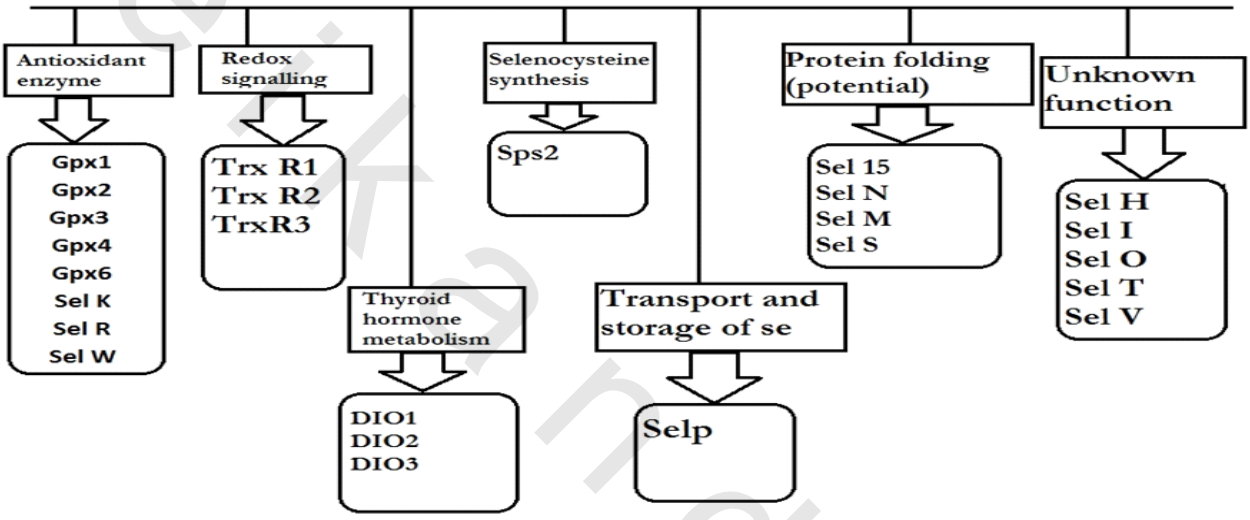

Figure (7): Human selenoprotein[60]

Where: GPX is glutathione peroxidase[60].

DIO is iodothyronine deiodinase[60].

TRX is thioredoxin reductase[60].

1.4.1. Biological function of selenoproteins:

Most of the antioxidant capacity of selenium appears to rely on ROS-degrading selenoenzymes, containing selenocysteine in their catalytic center.

In contrast to other metal ions, which are associated with their respective apoproteins as cofactors, selenium is co-translationally incorporated into selenoproteins as selenocysteine, the selenium analogue of cysteine [61].

The selenoproteome of all species investigated so far is rather small. Based on computational sequence analyses, gene for 25 human selenoproteins have been identified[62]. Nevertheless, synthesis of selenoproteins is essential for mammals, as proven by occurrence of early embryonic lethality in mice lacking the selenoprotein-$tRNA^{sec}$ gene[63] .The efficient catalysis of redox reaction by selenoenzymes is mainly based on two

biochemical properties of selenocysteine: as the selenolgroup in selenocysteine (pka≈5.2) is more acidic than the thiol group in cysteine (pka≈8.5), it is deprotonated at physiological PH values and is more reactive in nucleophilic reactions. In addition, selenoystein is morereadily oxidized than cysteine[64].

Apart from its function in the catalytic center of selenoenzymes, selenocysteine has the potential to repair oxidative damage by reducing tyrosyl radicals in proteins[65].

Intracellular concentrations of free senlenocysteine are low, and it may exert this radical scavenger activity as constituent of selenoproteins.

Selenomethionine, the selenium analogue of methionine, can also act as ROS scavenger. Free selenomethionine is rapidly oxidized by peroxynitrite to methionine selenoxide[66], which can be reduced back to selenomethionine in a non-enzymatic reaction maintained by glutathione[67], this reaction has been reported to occur in the same manner with selenomethionine residues in proteins, where selenomethionine can be incorporated non-specifically instead of methionine[68].

Therefore, selenomethionine residues in protein may provide a first line of defense against peroxynitrite and other oxidants.

Selenium is found in human and animal tissues as L-selenomethionine or L-selenocysteine. Only a small fraction of L-methionine in proteins is present as L-selenomethionine. On the other hand the incorporation of L-cystine into selenoprotein is not random. Namely, incontrast to L-selenomethionine which randomly substitutes for L-methionine, L-selenocysteine does-not randomly substitutes for L-cystine[69].

Selenoproteins perform a variety of physiological roles. Selenoprotein are made up of four selenium-dependent glutathione peroxidase (GSH PX-1,GSH PX-2,GSH PX-3 and GSH PX-4), three selenium dependent iodothyronine deiodinase, three thioredoxin reductase (selenium is at the active site of the enzyme), selenoprotein P, selenoprotein W and selenophosphate synthetase.

The glutathione peroxidases, and possibly selenoprotein P and selenoprotein W, are antioxidant protein that reduce potentially damaging reactive oxygen species (ROS), such as hydrogen peroxide and lipid hydroperoxides to harmless-products and alcohols-by coupling their reduction with oxidation of glutathione[70].

Selenium in plasma is present as selenoprotein P, the major selenoprotein in human plasma, is mainly secreted by hepatocytes, where its expression is controlled through interaction of the peroxisomal proliferatsr activated receptor-γ coactivator-1 α (PGC-1α) with FoxO1a and hepatocyte nuclear factor- 4α (HNF-4α) transcription factors[71].

SeP acts mainly as transporter protein supplying extralepatic tissues with selenium, and has been named according to its primary occurrence; P stands for plasma. Its concentration has been estimated to be 40 nM[72].

There is experimental evidance for several biological functions of SeP .The original hypothesis of SeP as a transport protein carrying Se to various extrahepatic tissues [73] .

In addition ,SeP has been shown to chelate heavy metals such as cadmium and mercury [74] , and there are several reports about its antioxidants capacity , it was shown to protect against diquat – induced oxidative damage in rats [75] , and to reduce phospholipid hydroperoxides in a cell free in vitro system[76] . Also it is found that SeP protects human plasma proteins against peroxynitrite-mediated oxidation and nitration [77], and low density lipoproteins against oxidation [78].It associated with endothelial cells, probably through its heparin properties[79,80].

1.4.2. Absorption and Bioavailability:

Absorption and thus bioavailability can be affected by the physical or chemical form of the selenium compounds or the dosing regimen. In general, the degree of selenium absorption is independent of the exposure but in so instances, absorption can be greater where selenium deficiency exists. It is thought that 55-60% of the selenium in food is absorbed following ingestion[81].

A relationship was observed between diabetes mellitus and trace elements[82]. In many cases, an alteration in the metabolism of these minerals was demonstrated[83]. Insulin action was reported to be potentiated by some trace elements like chromium, magnesium, vanadium, zinc, manganese and selenium[84]. Proposed mechanisms of enhancement of insulin action by trace elements include activation of insulin receptor sites[85], serving as cofactors or components for enzyme systems which are involved in glucose metabolism[86], increasing insulin sensitivity and acting as antioxidants for preventing tissue peroxidation[87].

1.5. Anti-diabetic and insulin-Mimetic Actions of Selenium

Diabetes mellitus is affecting over 170 million people worldwide with more than 90% of the patients suffering from type 2 diabetes[33].

The onset of type 2diabetes is hallmarked by resistance of liver, skeletal muscle and fat tissue to insulin, thereby causing dyslipidemia, hyperglycemia and a reactive increase in insulin secretion by pancreatic beta cells for compensation of the poor insulin response of major target tissues[88]. Binding of insulin to its receptor initiates the intracellular insulin signalling cascade,. Among them, the insulin receptor substrate (IRS)-2, the protein tyrosine phosphatase (PTP)-1B and the protein kinase B (serine/threonine kinase Akt) as well as the forkhead box class (Fox) Olα transcription factor and its coactivator peroxisomal proliferator-activated receptor gamma coactivator (PGC)-1 α have received particular attention in diabetes research. It is evident from in vitro and in vivo studies that dysregulated expression, localization and /or activity of one or more of those proteins may result in insulin resistance[89-93].

Early studies have been performed in isolated rat adipocytes, and found that sodium selenate stimulated glucose uptake through translocation of glucose transporters to the plasma membrane and activated serine/ therionine kinases including the p70 s6 kinase[94].

As these insulin-like actions were observed only at the very high dose of 1mM sodium selenate, an anti-diabetic application in humans appears to be difficult or impossible[37].

1.6. Adverse Effects of selenium on insulin secretion and signaling

An anti-diabetic impact of dietary selenium supplementation would be expected, given both the long track record of selenium as insulin-mimetic micronutrient and its antioxidant capacity as constituent of ROS-detoxifying selenoenzymes, suggesting a protective role against oxidative stress-related chronic complications in the progression of diabetes[37,95].

The clue to answer the pivotal question of whether and how selenium exerts advers effects on insulin-regulated metabolic pathways in humans may lie in the apparent "redox paradox" of insulin signalling, aconcept that refers to facilitated insulin action by insulin-stimulated reactive oxygen species[96]. Upon binding to its receptor at the plasma membrane of adipocytes, insulin elicits a transient burst of ROS (superoxide and H_2O_2) . Insulin activates the NAD(P)H oxidase (Nox)4 to generate superoxide, which is subsequently converted to H_2O_2 [97].

These insulin-stimulated small amounts of H_2O_2 serve as second messengers, which attenuate the activity of phosphates with redox-sensitive cysteine residues and thereby enhance the phosphorylation of components down-stream in the insulin signalling cascade[96,98]. Thus, high supra-nutritional doses of antioxidants may have the capability to impair insulin sensitivity, as it has been shown in humans administered a combination of vitamin C (1,000 mg/day) and vitamin E (400 IU/day) [99].

Inorganic and organic selenium compounds have been reported to induces expression and activity of several antioxidant seleno-proteins; the most pronounced stimulation was obtained for the selenoenzyme cytosolic GPx1, [100-102] which degrades H_2O_2 and other hydroperoxides[103]. A high GPx1 activity has been hypo-thesized to interfere with insulin signaling.. GPx1 overexprssion affected both pancreatic insulin production and insulin sensitivity of target cells; insulin resistance of liver and/or skeletal muscle was obvious from impaired insulin receptor and Akt phosphorylation[104]. In triguingly, obesity together with insulin resistance and hyperglysemia could be prevented in the GPx l-overexpressing mice by dietary restriction, whereas the chronic hyperinsulinemia persisted, even at dietary selenium deficiency[105,106]. The dysregulation of pancreatic insulin biosynthesis and secretion is the primary outcome of transgenic GPxl over-production in their experimental model[106].

Insulin-producing pancreatic beta cells are among the worst-endowed cells in terms of intrinsic enzymatic antioxidants: expression and activity of the H_2O_2 –degrading enzymes catalase and GPx1 in beta cells reach only 1% of the values in hepatocytes[107]. For this reason, beta cells are very susceptible to damage caused by hyperglycemia or proinflammatory cytokines, and overexpression of antioxidant enzymes including GPx1 has been applied to protect insulinoma cell lines and pancreatic islets from oxidative injury[108,109].

On the other hand, development of hyperinsulinemia in GPx1 over-expressing mice points to detrimental effects of high GPx1 activity on beta cell function in vivo, impairing the tight control of insulin release[104-106]. An adverse effect of high GPx1 activity on components of the insulin signaling cascade has been further substantiated by an in vitro study in MCF-7 human breast

cancer cells, where GPx1 overexpression was associated with decreased phosphorylation of p70 S6 kinase and Akt[110]. An alternative approach to increase GPx1 in a more physiological manner was done by dietary supplementation of rats with sodium selenite: the higher GPx1 activity in livers of selenium-supplemented rats was associated with increased activity of protein tyrosine phosphatase 1B (PTP-1B) [111], which antagonizes insulin-induced signaling by dephosphorylation of the insulin receptor (IR) and the IRS-I[112]. Conversely and in good agreement with the experimental models of GPx1 overexpression, knock-out of GPx1 in mice resulted in improved insulin sensitivity due to increased ROS generation, causing oxidation (inactivation) of the dual specificity protein phosphatase (phosphatase and tensin homolog) PTEN[107]. PTEN dephosphorylates the product of PI3K, phosphatidylinositol-3, 4, 5-triphosphate (PIP3). Thus counteracting insulin-induced PI3K/Akt signaling[113]. In line with elevated PI3K/Akt signaling, insulin-induced glucose uptake was increased in skeletal muscles of GPx mice, and most compelling, knock-out of GPxI protected the rodents from insulin resistance provoked by high-fat diet[107]. These results are supported by observations of increased site-specific phosphorylation of both Akt and p70 S6 kinase in transgenic mice with an overall decreased biosynthesis of selenoproteins, caused by a mutant form of selenocysteine transfer RNA ($TRNA^{[Ser]sec}$) [114].

Despite the compelling evidence from transgenic animal models of GPx1 over expression and knock-down, results from intervention studies with selenium supplements in several human populations argue against the idea that glutathione peroxidases are the only mediators of adverse effects of high dietary selenium intake under physiological conditions: plasma GPx activity in humans has been found to be saturated at selenium dietary supplement doses and total plasma selenium levels well below the values associated with increased risk for type 2 diabetes[115-117]. Human plasma contains selenium in form of the selenoenzyme GPx3, a low-molecular-weight selenium pool and most notably the selenium transporter selenoprotein P(Sep), which accounts for 50-60% of circulating selenium[69]. Compared to GPx activity, both SeP and the remaining non-selenoprotein plasma selenium pool require a higher dietary selenium intake for their optimization and saturation[115-117].

It is tempting to speculate that SeP and/or low-molecular-weight selenium compounds may affect insulin-induced signaling pathways related to carbohydrate and lipid metabolism. (Figure 8)

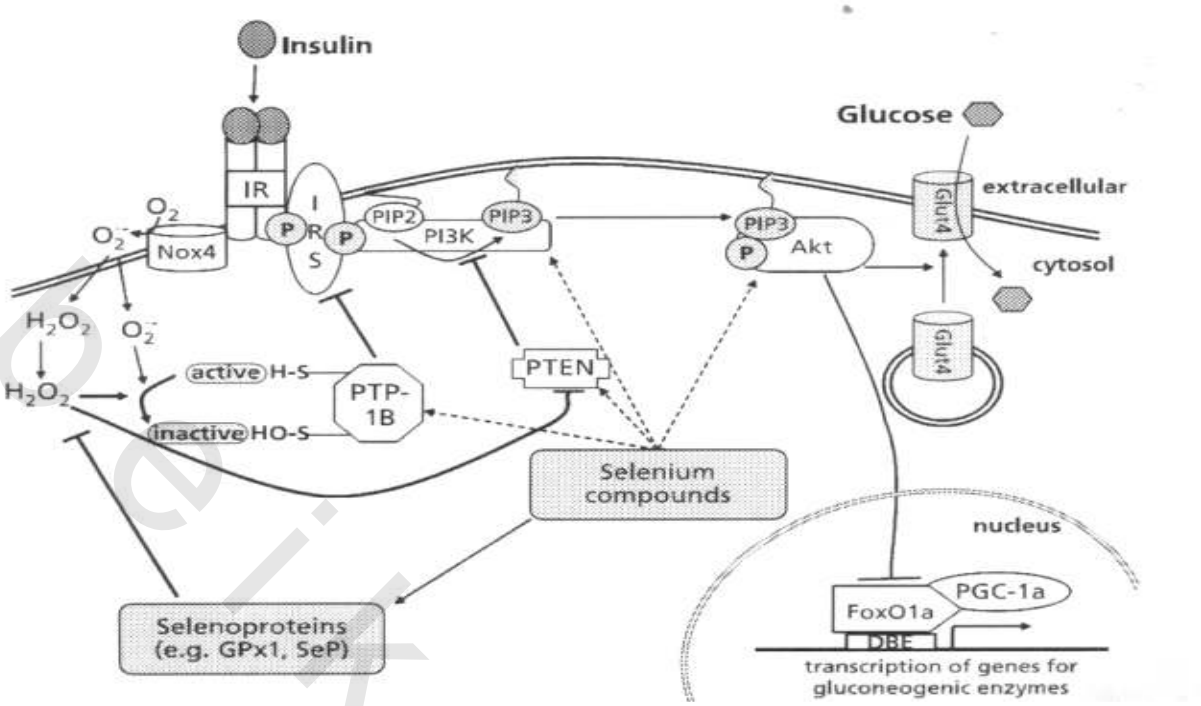

Figure (8): Scheme depicting a potential influence of selenium on components of the insulin signaling cascade. Selenoproteins and low molecular weight selenium compounds may interfere at different stages with insulin – induced signal transduction, eventually leading to dysregulation of carbohydrate metabolism.[69]

1.6.1. PGC-1α: a Molecular Switch Linking Selenium and Carbohydrate Metabolism

The epidemiological association between high plasma selenium levels and hyperglycemia might also be explained by a disturbance of selenium homeostasis as side-effect of a dysregulated carbohydrate metabolism. The major fraction of total selenium in human plasma is present as SeP, which is mainly secreted by the liver and supplies peripheral tissues with selenium[69,118]. SeP represents a suitable biomarker for selenium status, because its plasma concentration increascs in response to different dietary forms and to a wide range of doses in selenium supplementation studies[115-117]. This obvious importance of SeP for selenium homeostasis prompted us to investigate the regulation of hepatic SeP production by factors related to carbohydrate metabolism.

In the human SeP promoter, a motife was identified which consisting of a binding site for the FoxO 1α transcription factor, located in close pioximity to a binding site for hepatocyte nuclear factor 4α (HNF-4α)[71,119]. This motif is conserved in the SeP promoters of humans, rats and mice, and it mediates high-level expression of SeP in the liver as well as the hormonal regulation of hepatic SeP transcription.

Both transcription factors are co-activated by the PGC-1α, which acts as "molecular switch" in response to hormones such as insulin, glucagon and glucocorticoids, well-known for their control of hepatic glucose production and blood glucose levels[120,121]. Insulin inhibited SeP transcription via the P13K/Akt/FoxO 1α axis[119], whereas the PGC-1α-inducing glucocorticoid dexamethasone strongly enhanced SeP mRNA levels and protein secretion in cultured rat hepatocytes[71].

The complex between FoxO 1α and its coactivator PGC-1α is of crucial importance for transcriptional regulation of the gluconeogenic enzymes glucosc-6-phosphatase (G6Pase) and phospho-enolpyruvate carboxykinase (PEP-CK) [120]. The observation that the selenium transporter SeP is regulated virtually like a gluconeogenic enzyme provides a rationale for the hypothesized link between selenium and carbohydrate metabolism[71]. Moreover, PGC-1α is elevated in livers of animal diabetes models[120], and has been demonstrated to promote insulin resistance[93]. A vicious circle is observed when diabetes is not treated accurately: high glucose up-regulates expression of PGC-1α and gluconeogenic enzymes in the liver, resulting in overproduction of hepatic glucose and increased hyperglycemia[120]. Rat hepatocytes was cultivated in the presence of high glucose (25mM), and found an increase in SeP production paralleled by elevated PGC-1α mRNA levels[122]. Thus, elevated hepatic PGC-1α may trigger not only hyperglycemia, but also a disturbance in selenium homeostasis. The anti-hyperglycemic drug metformin is widely described for treatment of type 2 diabetes, because it suppresses hepatic glucose production and improves peripheral insulin sensitivity[123]. In parallel with gluconeogenesis, metformin attenuated hepatic biosynthesis and secretion of SeP in vitro, which might decrease selenium bioavailability in extrahepatic tissues and thereby impair expression and activity of selenoenzymes in vivo. This idea is supported by a study of Pavlovic et al: A two-week metformin treatment resulted in decreased GPx activity in erythrocytes of obese patients with type 2 diabetes[124].