1 –Introduction

1.1. Insulin

Insulin is the main anabolic hormone produced by the beta cells in the pancreas. It is released into the blood to stimulate glucose uptake by peripheral tissues such as the skeletal muscle. β–cells are localized in islets of Langerhans in the pancreas. The islets are the endocrine compartment of the pancreas, comprising around 2 – 3% of the total pancreatic volume. Islets are approximately spherical, with an average diameter of 100 – 200 μm, and a healthy human pancreas may contain up to a million individual islets, each having its own complex anatomy, blood supply, and innervations [(1)].

A typical mammalian islet comprises several thousand endocrine cells, including the insulin - expressing β - cells (~ 60% of adult human islet cells), glucagon- expressing α - cells (20 – 30%), somatostatin - expressing δ - cells (~ 10%), pancreatic polypeptide - expressing cells (< 5%), and ghrelin - expressing cells (~ 1%). The anatomical arrangement of islet cells varies between species. In rodents, the majority β - cell population forms a central core surrounded by a mantle of α - cells and δ - cells, but human islets show less well - defined organization with α - cells and δ - cells also being located throughout the islet [(2)].

1.1.1. Insulin biosynthesis

The ability to release insulin rapidly in response to metabolic demand, coupled with the relatively slow process of producing polypeptide hormones, means that β - cells are highly specialized for the production and storage of insulin, to the extent that insulin comprises approximately 10% (~10 pg/cell) of the total β - cell protein [(1)].

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)]. It is 1355 base pairs in length and its coding region consists of three exons: the first exon encodes the signal peptide at the N - terminus of preproinsulin, the second one encodes the B chain and part of the C (connecting) peptide, and the third exon encodes the rest of the C peptide and the A chain. Transcription and splicing to remove the sequences encoded by the introns yields a messenger RNA of 600 nucleotides, the translation of which gives rise to preproinsulin, an 11.5 - kDa polypeptide. The cellular processes and approximate timescales involved in insulin biosynthesis, processing, and storage are summarized in Figure (1)[(1)].

Preproinsulin is rapidly (< 1min) discharged into the cisternal space of the rough endoplasmic reticulum, where proteolyticenzymes immediately cleave the signal peptide, generating proinsulin. 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 – 35 amino acids). The structural conformations of proinsulin and insulin are very similar, and a major function of the C peptide is to align the disulfide bridges that link the A and B chains so that the molecule is correctly folded for cleavage (Figure 2). Proinsulin is transported in microvesicles to the Golgi apparatus, where it is packaged into membrane - bound vesicles known as secretory granules [(1)].

1-Introduction

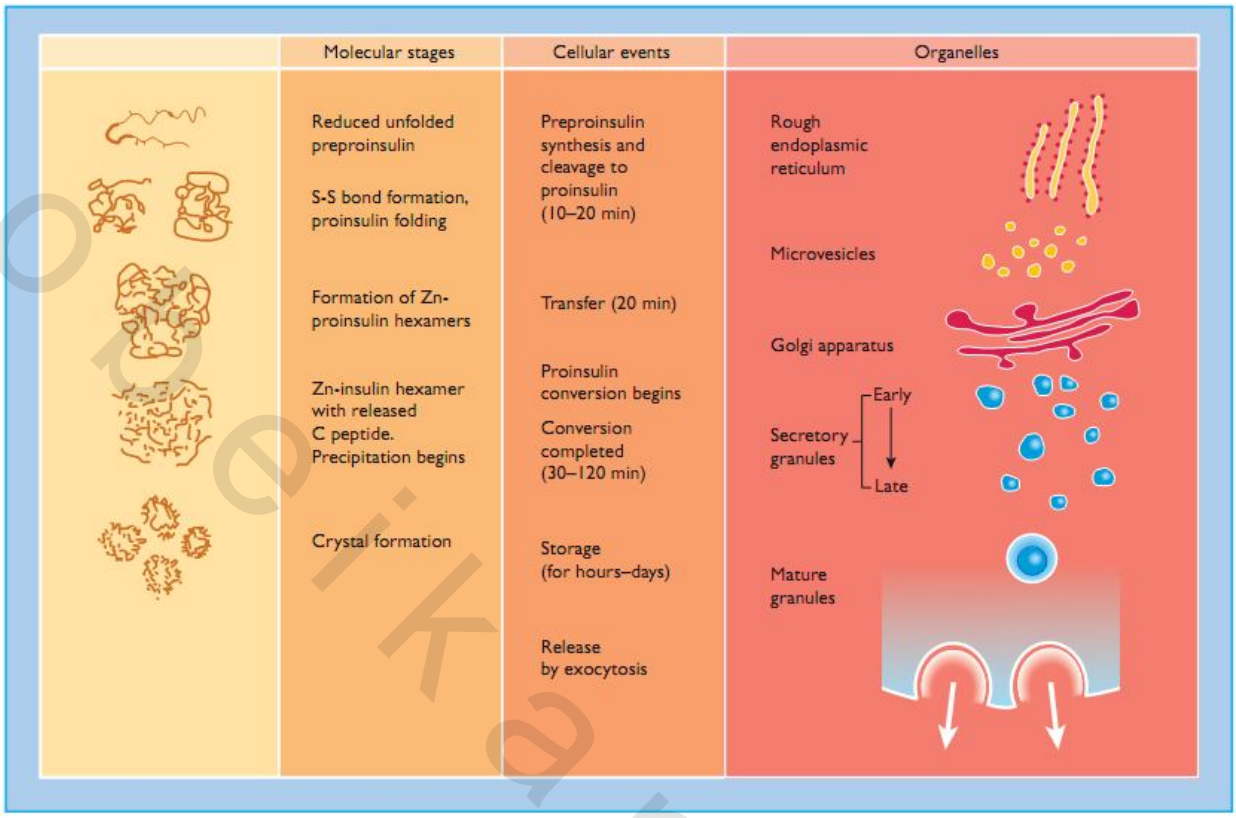

Figure (1): The intracellular pathways of (pro) insulin biosynthesis, processing, and storage. The molecular folding of the proinsulin molecule, its conversion to insulin, and the subsequent arrangement of the insulin hexamers into a regular pattern are shown at the left. The time course of the various processes, and the organelles involved are also shown. Quoted from reference (1)

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 [(5)], which remove the C peptide chain, liberating two cleavage dipeptides and finally yielding insulin (Figure 2). Insulin and C peptide are stored together in the secretory granules and are ultimately released in equimolar amounts by a process of regulated exocytosis. Under normal conditions, >95% of the secreted product is insulin (and C peptide) and < 5% is released as proinsulin. However, the secretion of incompletely processed insulin precursors (proinsulin and its "split" products; Figure 2)is increased in some patients with type 2 diabetes [(1).]

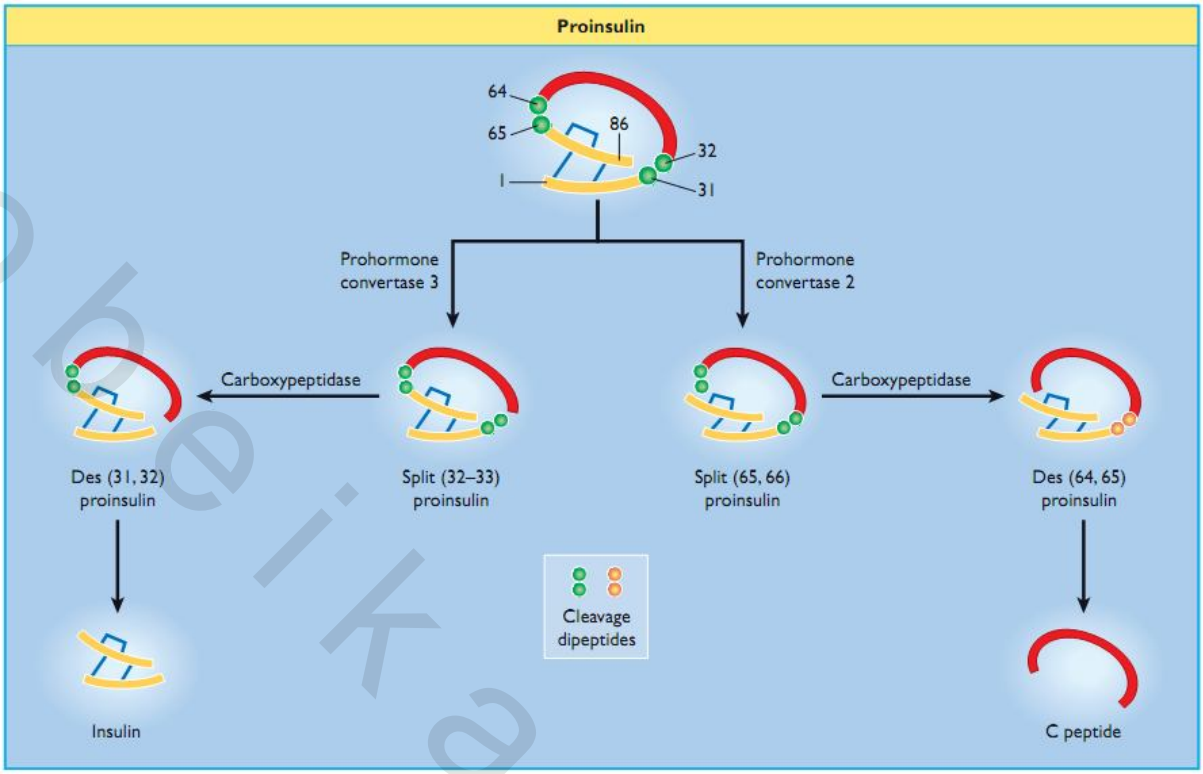

Figure (2): Insulin biosynthesis and processing. Proinsulin is cleaved on the C - terminal side of two dipeptides, namely Arg^{31} – Arg^{32} (by prohormone convertase 3) and Lys^{64} – Arg^{65} (prohormone convertase 2). The cleavage dipeptides are liberated, so yielding the "split" proinsulin products,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

1.1.2. Storage and release of insulin

Insulin stored in β–cells in insulin secretory granules. The major protein constituents of the granules are insulin and C peptide, which account for approximately 80% of granule protein[(6)], with numerous minor components, including peptides, peptide hormones and a variety of (potentially) biologically active peptides of uncertain function[(7)]. Insulin secretory granules also contain high concentrations of divalent cations, such as zinc (<20mmol/L), which is important in the crystallization and stabilization of insulin within the granule [(8)].

Insulin stored in β-cells is packed into densely clustered "granules" consisting of insoluble crystalline hexameric insulin. The concentration of insulin in these granules is roughly 40mM [(9)].

Insulin exists primarily as a monomer at low concentration (~ 10-6M) and forms dimmers at higher concentrations at neutral pH [(10, 11)]. At high concentration and in the presence of zinc ions insulin aggregates further to form hexameric complexes [(11,12)]. Hence, the monomer is the active form of insulin, while the hexamer is the storage form of insulin [(13)]

Insulin is released from secretory granules by exocytosis, a process in which the granule membrane and plasma membrane fuse together, releasing the granule contents into the interstitial space [(1)].

1.1.3. Regulation of insulin secretion

Insulin is the exclusive hormone that lowers plasma glucose concentration, and glucose homeostasis is maintained primarily as a result of regulated insulin secretion. Pancreatic β–cells recognize extracellular glucose concentration and secrete insulin as required at a given time. Glucose-stimulated insulin secretion (GSIS) is modulated by a number of factors, such as non-glucose nutrients, hormones and neural inputs (Figure 3). Thus, the intracellular network for regulation of insulin secretion is complex and multifactorial [14,15].

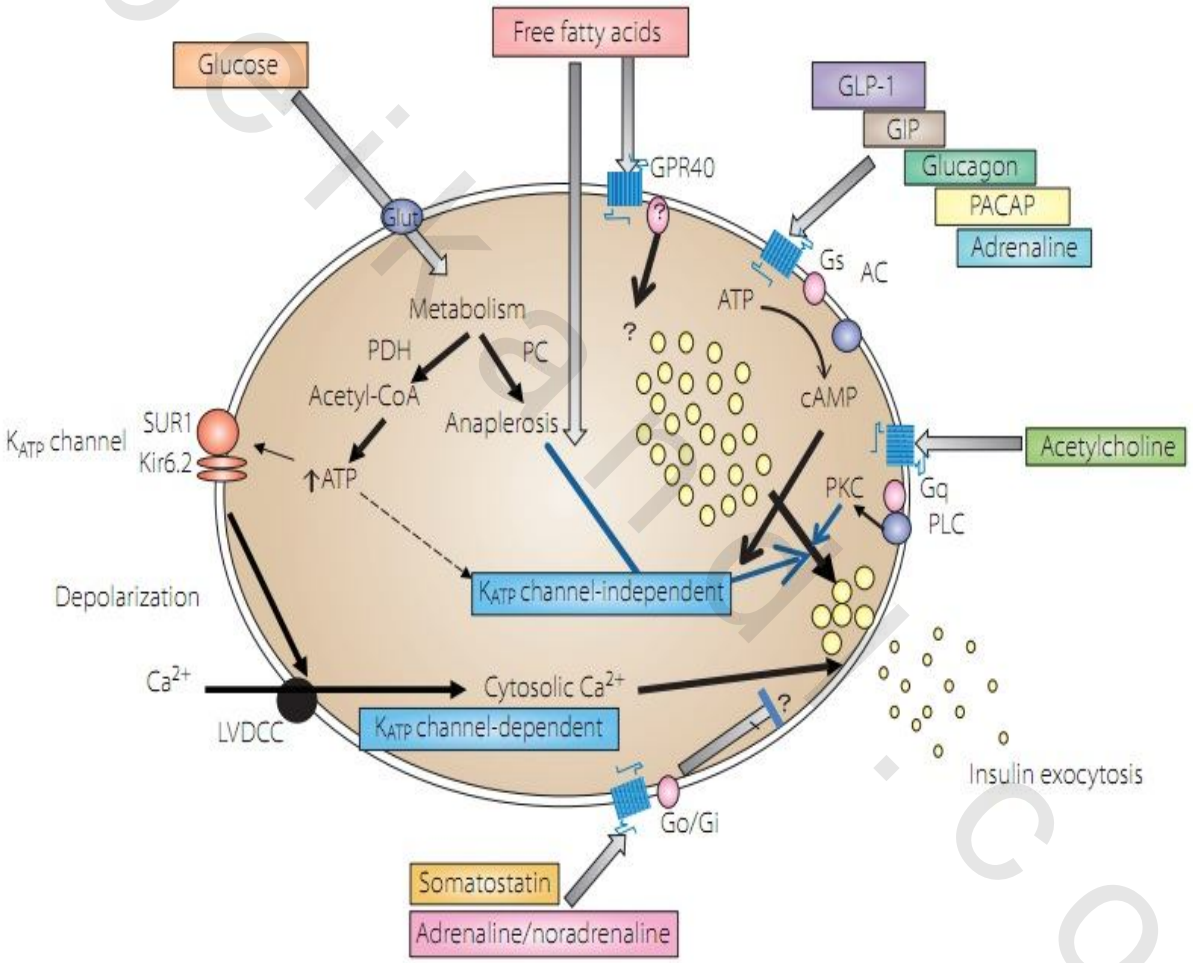

Figure (3). A proposed signaling network of insulin exocytosis in pancreatic β–cells. AC, adenylate cyclase; ATP, adenosine triphosphate; cAMP, cyclic adenosine monophosphate; GIP, glucose-dependent insulinotropic peptide; GLP-1, glucagon-like polypeptide-1; Glut, glucose transporter; KATP channel, adenosine triphosphate-sensitive K+ channel; Kir6.2, K+ channel 6.2 subunits; LVDCC, L-type voltage-dependent calcium channel; PACAP, pituitary adenylate cyclase activating peptide; PDH, pyruvate dehydrogenase; PC, pyruvate carboxylase; PKC, protein kinase C; PLC, phospholipase C; SUR1, sulfonylurea receptor 1 . Quoted from reference (15).

To ensure that circulating levels of insulin are appropriate for the prevailing metabolic status β - cells are equipped with mechanisms to detect changes in circulating nutrients, in hormone levels, and in the activity of the autonomic nervous system. Moreover, β-cells have fail-safe mechanisms for coordinating this afferent information and responding with an appropriate

secretion of insulin. The major physiologic determinant of insulin secretion in humans is the circulating concentration of glucose and other nutrients, including amino acids and fatty acids. These nutrients possess the ability to initiate an insulin secretory response, so when nutrients are being absorbed from the gastrointestinal system the β-cell detects the changes in circulating nutrients and releases insulin to enable the uptake and metabolism or storage of the nutrients by the target tissues. The consequent decrease in circulating nutrients is detected by the β-cells, which switch off insulin secretion to prevent hypoglycemia. The responses of β-cells to nutrient initiators of insulin secretion can be modified by a variety of hormones and neurotransmitters, which act to amplify, or occasionally inhibit, the nutrient - induced responses[(1)].

1.1.4. Insulin action

Insulin is exclusively synthesized in and secreted from pancreatic β-cells. It 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. 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 [(16)]. 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), phosphatidyl inositol 3 (PI_3) kinase, Akt/protein kinase B (Akt/PKB), and mitogen activated protein kinase (MAPK) are globally operative in multiple cell types (**Figure 4**) [(16)].

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[(17)]. 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[(18)].

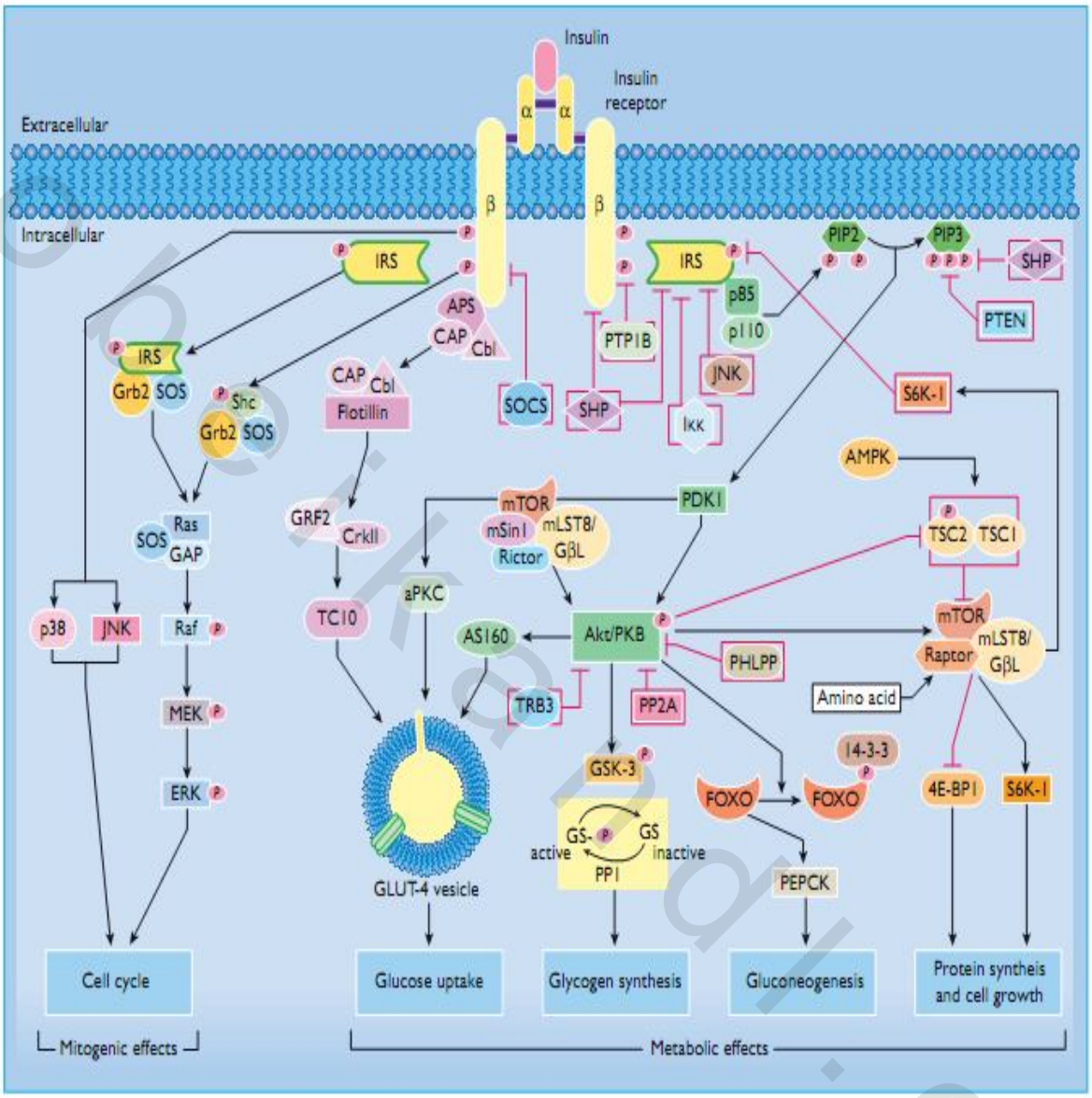

Figure (4): A schematic illustration of insulin signaling pathways involved in both metabolic and mitogenic effects. Arrows represent an activation process; blocked arrows represent an inhibition process. Quoted from reference (16)

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 binder1 (Gab1), Cas-Br-Mecotropic retroviral transforming sequence homolog (Cbl), and the various isoforms of Src-homology-2-containing protein (Shc)[(19)]. Insulin receptor substrates are 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[(20)].

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 [21]. 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)[16]. PI_3kinase plays an essential role in glucose uptake and GLUT4translocation. Inhibition of the enzyme with pharmacological inhibitors such as wortmannin completely blocks the stimulation of glucose uptake by insulin[22]. AKT/PKB is a serine/threonine kinase that is a downstream target of PI_3 kinase signaling. AKT/PKB mediates most of the PI_3 kinase-mediated metabolic actions of insulin through the phosphorylation of several substrates, including other kinases, signaling proteins and transcription factors [22].

Glycogen synthase kinase-3 (GSK3)was the first physiological target of AKT/PKB to be identified[23]. GSK3 has a key role in the regulation of many cell functions, including signaling by insulin, growth factors and nutrients, and the specification of cell fates during embryonic development. It is also implicated in the control of cell division, apoptosis and microtubule function [24].

1.1.4.1. Insulin action in adipose tissue

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 adipocytes biology. The balance between triglyceride synthesis (fatty acid uptake and lipogenesis) and breakdown (lipolysis/fatty acid oxidation) determines fat accumulation in adipocytes. 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 biological effects of insulin on lipogenesis, and are modulated by insulin through post-translational modifications and alteration of gene expression. Regarding post-translational effects, insulin activates Akt/PKB via phosphorylation[16]. 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 citrate 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. Insulin also induces gene expression of two key lipogenic proteins: fatty acid synthase (FAS) and sterol regulatory element-binding protein-1 (SREBP-1)[16].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[25]. Hormone sensitive lipase (HSL) is a key enzyme for the mobilization of triglycerides deposited in adipose tissue following its activation by cAMP/PKA-dependent phosphorylation [25] (Figure 5).

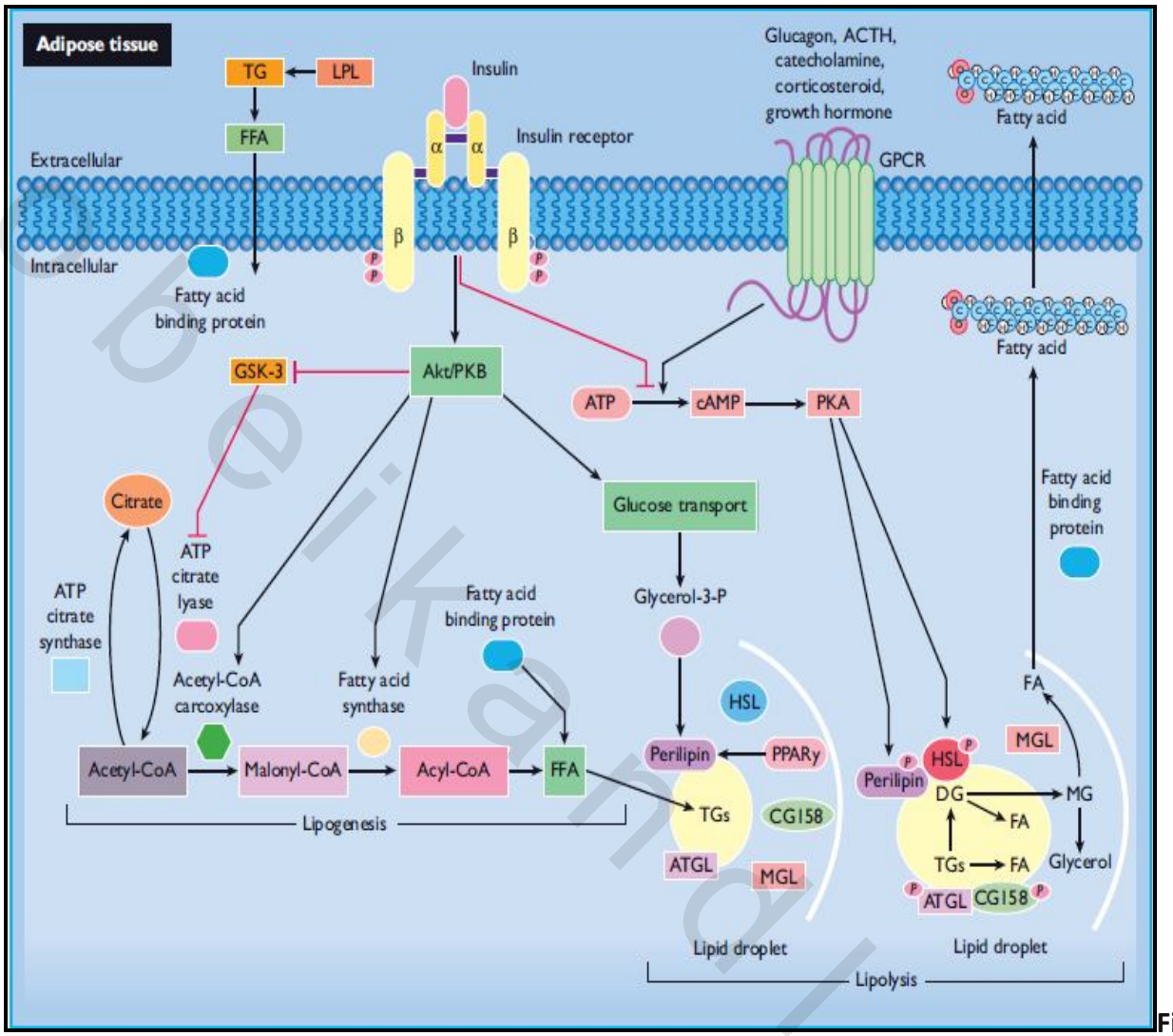

Figure (5): Summary of insulin function involved in lipogenesis and lipolysis in adipose tissue. Arrows represent an activation process; blocked arrows represent an inhibition process. Quoted from reference (16)

1.1.4.2. Insulin action 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 (**Figure 6**) [16].

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[16].

1.1.4.3. Insulin action in skeletal muscles

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. GLUT-4 is the major transporter mediating insulin-stimulated glucose transport activity in tissues such as skeletal and cardiac muscle and adipose tissue. The complete pathway linking insulin signal transduction to stimulation of the glucose transport system has not been fully elucidated. Activation of transport is critically dependent on insulin-mediated autophosphorylation of insulin receptors, tyrosine phosphorylation of IRS-1 and activation of PI_3 kinase. The production of PIP_3 by PI_3 kinase activates PDK1. At this point, PDK1 activates two separate kinase pathways that contribute to GLUT-4 translocation and stimulation of glucose transport activity, Akt2 and aPKCs (**Figure 7**) [16].

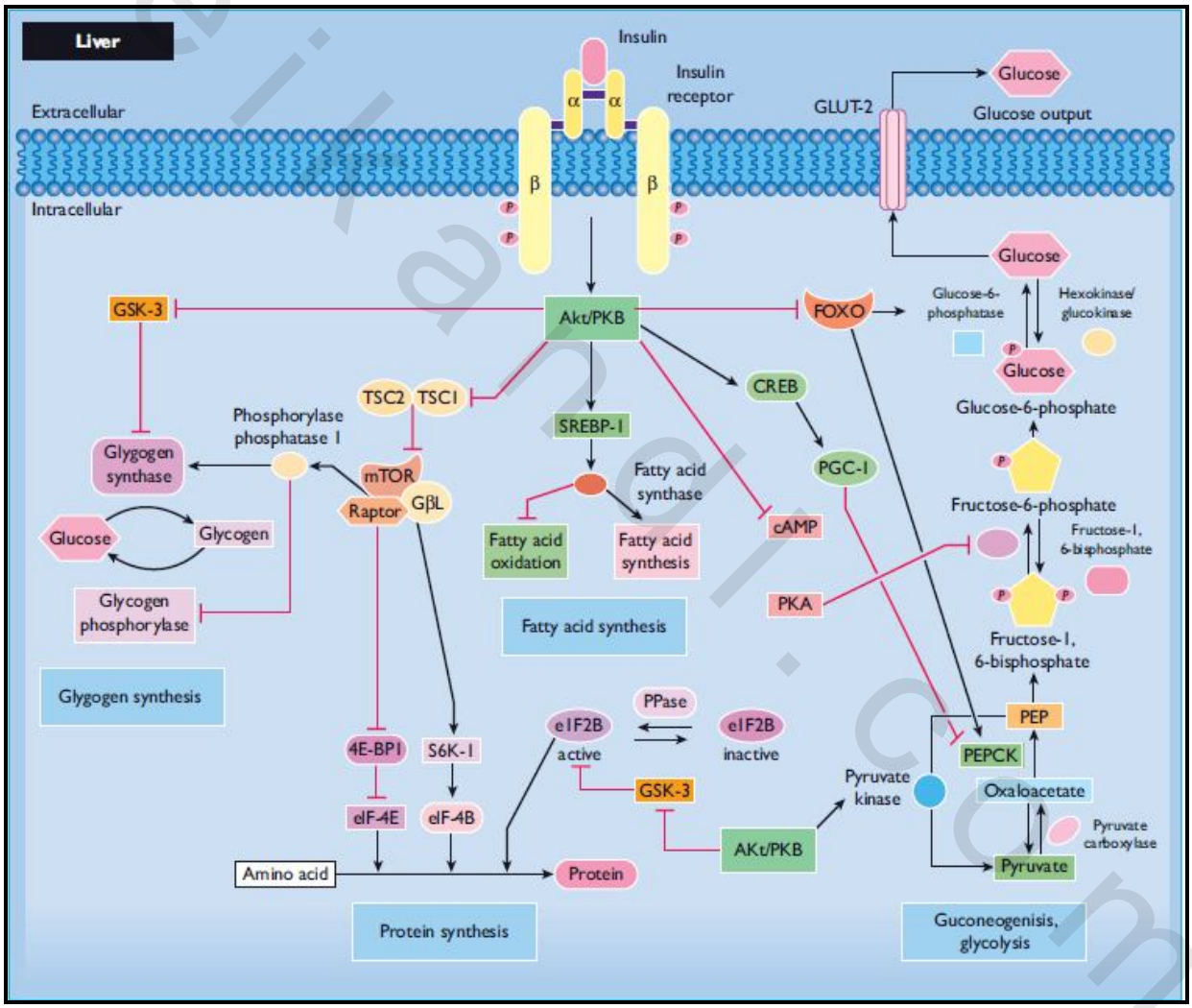

Figure (6): Summary of insulin signaling pathways in the liver that are involved in glycogen synthesis, gluconeogenesis and glycolysis, and protein synthesis, respectively. Arrows represent an activation process; blocked arrows represent an inhibition process. Quoted from reference (16)

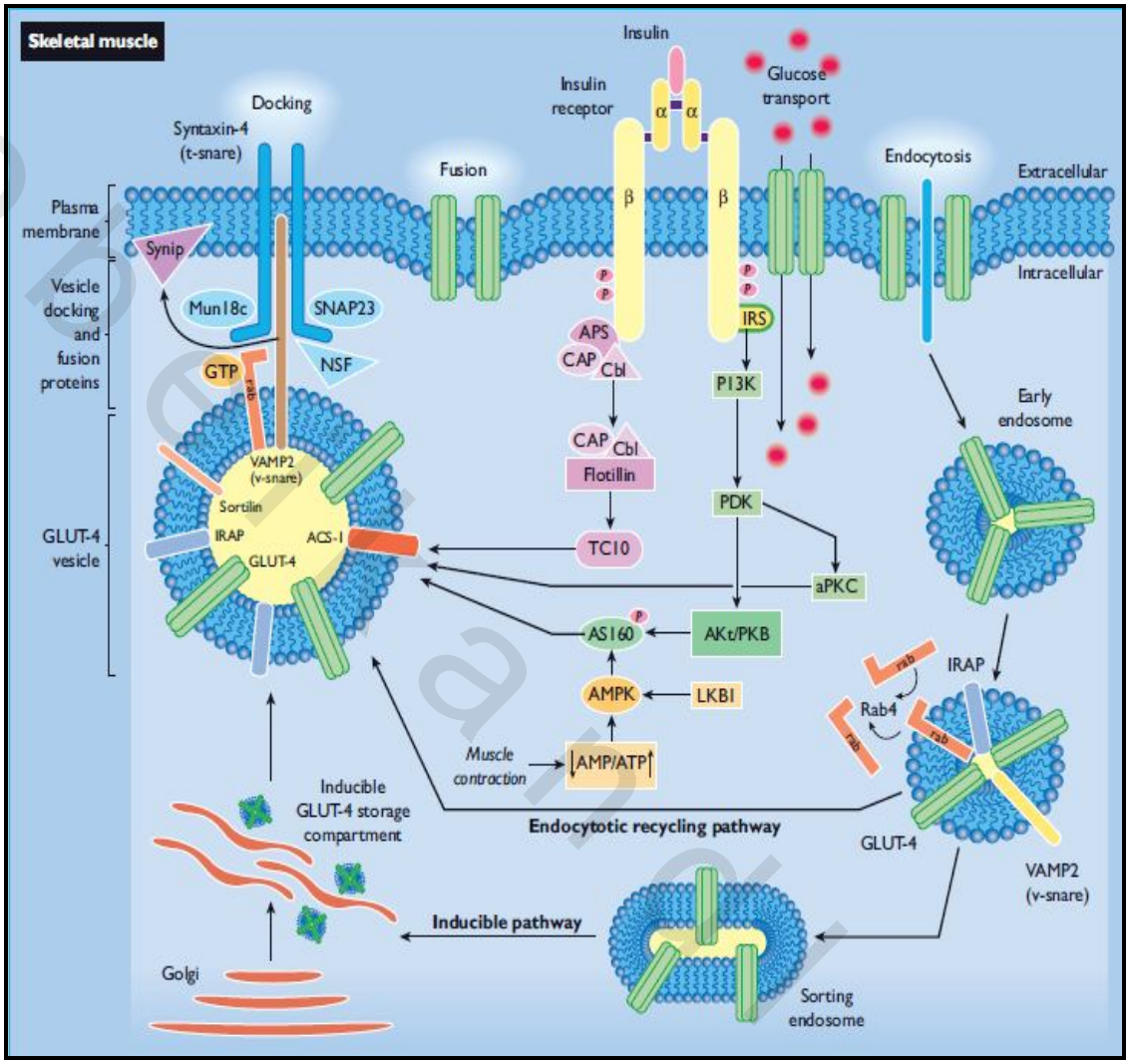

Figure (7): An overview of insulin signaling pathways in skeletal muscle. Arrows represent an activation process. Quoted from reference (16)

1.1.5. Insulin resistance

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. Insulin resistance in the context of glucose metabolism leads to impaired suppression of endogenous glucose production − under basal conditions as well as after eating (when the physiologic rise in insulin in response to glucose entry from the gut normally shuts down glucose production by the liver) − and to reduced peripheral uptake of glucose. Resistance to the ability of insulin to suppress very low density lipoprotein (VLDL) cholesterol production increases circulating serum triglycerides, while resistance in adipose tissue increases the flux of non - esterified fatty acid (NEFA) both to the liver and skeletal muscle and impairs the action of insulin on glucose metabolism in these tissues. Resistance to other actions of insulin, such as its vasodilatator and antiplatelet aggregation effects, also characterize insulin resistance in patients with type 2 diabetes mellitus (T2DM). Insulin resistance may also become more severe in patients with T2DM brought about by any factor causing insulin resistance [(26)].

There has been much debate, whether insulin resistance is the primary defect that precedes β-cell failure in the evolution of hyperglycemia in T2DM, or vice versa. There is a linear decrease in both first-phase insulin release and insulin sensitivity in individuals who progress from normal to impaired glucose tolerance. Thus, low insulin sensitivity and impaired first-phase insulin release both predict the onset of T2DM [(27)].

1.2. Diabetes Mellitus

Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels [(28)].

Several pathogenic processes are involved in the development of diabetes. These range from autoimmune destruction of the pancreatic b -cells with consequent insulin deficiency to abnormalities that result in resistance to insulin action. The basis of the abnormalities in carbohydrate, fat, and protein metabolism in diabetes is deficient action of insulin on target tissues. Deficient insulin action results from inadequate insulin secretion and/or diminished tissue responses to insulin at one or more points in the complex pathways of hormone action. Impairment of insulin secretion and defects in insulin action frequently coexist in the same patient, and it is often unclear which abnormality, if either alone, is the primary cause of the hyperglycemia [(29)].

Symptoms of marked hyperglycemia include polyuria, polydipsia, weight loss, sometimes with polyphagia, and blurred vision. Impairment of growth and susceptibility to certain infections may also accompany chronic hyperglycemia. Acute, life-threatening consequences of uncontrolled diabetes are hyperglycemia with ketoacidosis or the nonketotic hyperosmolar syndrome [(29)].

1.2.1. Classification of diabetes mellitus[28]

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 type 1 diabetes mellitus (T1DM) are severely insulin deficient and are dependent on insulin treatment for their survival. Management consists of insulin provided as a replacement hormone[30].

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[30]. 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 [31]. 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 [30].

1.2.1.3. Gestational Diabetes Mellitus (GDM)

GDM is defined as glucose intolerance occurring or first recognized during pregnancy[32]. 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 [33]. Women with gestational diabetes mellitus have a more than sevenfold increased risk of subsequently developing T2DM compared with women who experience a normoglycemic pregnancy [34].

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[35].

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 [36].

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 [37].

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 [35].

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[38].

vi. Infections: Certain viruses have been associated with beta-cell destruction, diabetes occurs in some patients with congenital rubella[39].

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 were accompanied by an increased incidence of DM. These include the chromosomal abnormalities of Down's syndrome, Klinefelter's syndrome and Turner's syndrome[35, 40].

1.2.2. Diagnosis of Diabetes

Diabetes is usually diagnosed based on plasma glucose criteria, either the fasting plasma glucose (FPG) or the 2-hours plasma glucose (2-h PG) value after a 75-g oral glucose tolerance test (OGTT) [29]. Also, the International Expert Committee added the glycated haemoglobin (HbA1C) (threshold $\geq$ 6.5%) as a third option to diagnose diabetes [41] (Table 1).

Table (1): Criteria for the diagnosis of diabetes

• 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 hours.*
OR
• Two-hour PG ≥200 mg/dL(11.1 mmol/L) during an OGTT. The test should be performed as described by the WHO, 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, result should be confirmed by repeat testing

1.2.3. Risk factors for type 2 diabetes mellitus[42]

There are many factors affecting 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.

1.2.4. 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[43]. Surprisingly, this estimate of the global prevalence of diabetes indicates that, Egypt will be ranked as the 10th country worldwide, which have the highest number of people with diabetes (6.7 millions)[44]. 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 (i.e., 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[45]. However, all of for mentioned risk factors could not completely explain the rapidly increasing global prevalence of T2DM. An important emerging concept for burdening of T2DM is "Fetal programming" or "developmental origin of diabetes" [46].

1.3. Developmental Origins of T2DM

The concept of developmental origin of diabetes proposed that mammalian fetus is completely dependent on the nutrients supplied by its mother. Disturbances in this supply can induce structural and functional changes during fetal development, with long-lasting consequences on growth and metabolism of the offspring throughout life. The intra-uterine milieu, therefore programs to a certain extent the health of an individual throughout life [47]. The altered maternal/fetal metabolism appears to be associated with a diabetogenic effect in the adult offspring even in the absence of genetic predisposition. This fetal programming of type 2 diabetes might considerably contribute to the global burden of diabetes [48,49].

In 1995 Dr. Barker wrote: "The fetal origins hypothesis states that fetal undernutrition in middle to late gestation, which leads to disproportionate fetal growth, programs later coronary heart disease"[50]. Epidemiological studies in human populations have revealed that low birth weight and reduced intra-uterine and early postnatal growth lead to the increased risk of developing adult chronic diseases including coronary heart disease, hypertension, type 2 diabetes and osteoporosis [51]. Several animal models have provided support for an early origin to the disease susceptibility. Thus, in different mammalian species, global food restriction [52], manipulation of maternal nutrition [53], uterine ligation [54] or increased maternal exposure to synthetic glucocorticoid [55] during gestation have been shown to alter postnatal growth and/or physiology into adult life.

Today, the world faces the dual burden of malnutrition that encompasses both under- and overnutrition [56]. It is estimated that maternal and child undernutrition is the underlying cause of 3.5 million deaths globally [57].

1.3.1. Hypothesis of Developmental Programming

1.3.1.1. Mismatch theory

For most organs and systems the critical period of plasticity is during intrauterine development [58]. Developmental plasticity is the ability of an organism to change its phenotype in response to changes in the environment [59]. If this change or adaptation is permanent, it is considered a "programming" change and is associated with persistent effects in structure and/or function [60].

In most cases, programming is beneficial for the health and survival of the organism. However, the problem of "mis-match" occurs when individuals developmentally adapted to one environment are exposed to another [61]. An example of this mismatch phenomenon includes people whose birth weights were towards the lower end of normal that subsequently grow up in affluent societies being at increased risk for hypertension, T2DM, and cardiovascular disease [47, 62,

[63]. The problem of mismatch is thought to be involved in the current "epidemic" of T2DM and cardiovascular disease in the young adult and middle-aged populations [(64)]. The current surge in metabolic and cardiovascular disease in India may be being fueled by a combination of undernutrition in early life and overnutrition in later life [(65)].

1.3.1.2. Thrifty Phenotype Hypothesis

Previous studies, proposes that poor fetal and early postnatal nutrition imposes mechanisms of nutritional thrift upon the growing individual. In conditions of severe intrauterine deprivation, the developing fetus may lose functional and structural units such as pancreatic β-cells, nephrons, and cardiomyocytes[(66)]. Such changes have been deemed an adaptive mechanism to ensure the survival of the fetus. Alternatively, the changes may reflect developmental malformations analogous to teratogenesis[(62)]. Interestingly, the thrifty phenotype hypothesis has been challenged by the "fetal salvage" hypothesis, which offers a different explanation for the insulin resistance seen in those affected by intrauterine growth restriction [(67)]. In this alternate explanation, it is not hypoplasia of the pancreatic β– cells that leads to impaired glucose tolerance, but rather it is that the fetus develops peripheral insulin resistance. This peripheral insulin resistance ensures that adequate amounts of glucose are delivered to essential organs such as the brain with subsequent reduced delivery to nonessential tissues such as skeletal muscle (Figure 8) [(65)].

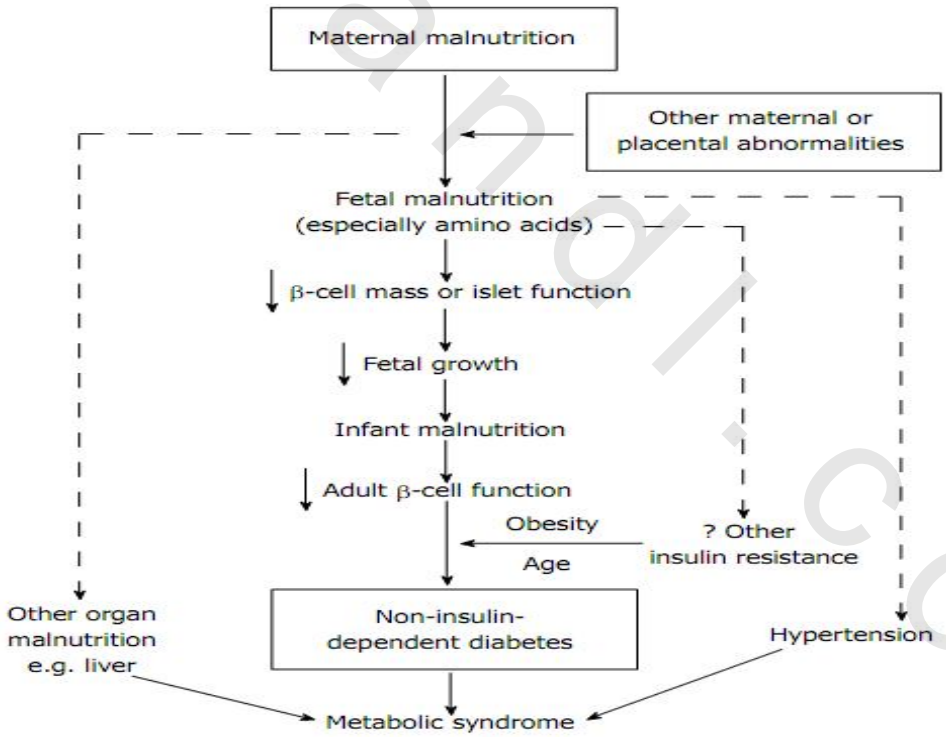

Figure (8): The thrifty phenotype hypothesis**.** Quoted from reference (65)

1.3.1.3. Catch-up growth

Catch-up growth, also known as compensatory growth, is where children return to their genetic trajectory for size after a period of growth delay or arrest. It may occur at any stage of growth but is most commonly observed in the first 2 years of life [(68)]. Studies have found that catch-up growth often results in overcompensation, whereby the organism exceeds normal weight

and often has excessive fat deposition. This rapid and excessive growth has been associated with the development of adult obesity, insulin resistance, metabolic syndrome, and type 2 diabetes [68,69].

The implication of catch-up growth is that rapidly enhancing early childhood growth by a nutrient enriched diet may cause harm overtime and that encouraging slower growth rates may actually be beneficial [65].

1.3.1.4. Oxidative Stress

Excessive reactive oxygen species can cause modulation of gene expression and/or direct damage to cell membranes and other molecules at critical developmental windows. Many believe that oxidative stress is the primary link between adverse fetal growth and later elevated risks of the metabolic syndrome, type 2 diabetes, and other disorders [69]. Smoking, hypertension/preeclampsia, inflammation/infection, obesity, and malnutrition are common causes of preterm and/or low birth weight as well as known sources of oxidative stress. Malnutrition can directly lead to a pro-oxidative state by means of creating protein and micronutrient deficiencies. Proteins provide amino acids needed for antioxidant synthesis, such as glutathione and albumin, and many micronutrients themselves are antioxidants, such as vitamins A, C, and E [69,70]. Pancreatic β–cells are particularly sensitive to reactive oxygen species because they are low in enzymatic antioxidant defense equipment [71]. It has been demonstrated that oxidative stress can blunt insulin secretion [72].With the susceptibility of pancreatic β–cells to oxidative stress; it is believed that early and ongoing exposures to oxidative insults could result in the eventual manifestations of the metabolic syndrome and related disorders [69].

1.3.1.7. Epigenetics

Epigenetics is the study of heritable changes in phenotype or gene expression that do not result from changes in the primary DNA sequence [73]. Recognized mechanisms of epigenetic regulation in mammals include DNA methylation, post-translational modification of histones, chromatin remodeling, microRNAs, and long noncoding RNAs [74]. These epigenetic regulatory mechanisms modulate chromatin structure and contribute to regulation of the major molecular processes in the nucleus including transcription, replication, repair, and RNA processing [75].

The changes in gene expression can reset the fetal homeostatic set points by manifesting changes in metabolism, hormone production, hormone sensitivity, or organ development. These epigenetic modifications remain with the genome through the child's life and have been shown to be partially passed on to the next generation [76]. Whether the fetus experiences nutritional deficiency, nutritional excess, or any other stimuli in utero, the organism can tailor its development and genetic expression to best meet the expected future environment and these responses may not become evident until later in life [77].

1.3.2. Maternal malnutrition and diabetes mellitus

The associations between maternal malnutrition, low protein diet, and T2DM have been widely studied. Typical epidemical studies from the population born during the Dutch famine period [78] or in some poor countries[79] have found that those who had been exposed to maternal malnutrition may have increased morbidity of metabolic diseases including T2DM in adult life.

The molecular mechanism(s) responsible for the maternal prenatal malnutrition programming of insulin resistance and T2DM in the offspring remain unclear [80]. The for mentioned hypothesis of programming appear to be interrelated and cooperated in the induction of diabetic phenotype in the offspring through different metabolic processes; mitochondria is the center of these processes.

Glucose and lipid metabolism are largely dependent on mitochondria to generate energy in cells. Metabolic regulation is largely dependent on mitochondria, which play an important role in energy homeostasis by metabolizing nutrients and producing ATP and heat. Imbalance between energy intake and expenditure leads to mitochondrial dysfunction, characterized by a reduced ratio of energy production (ATP production) to respiration [81].Genetic and environmental factors including exercise, diet, aging, and stress affects both mitochondrial function and insulin sensitivity [82]. These mitochondrial functions have been shown to play an important role during early embryonic development [83].

1.4 Mitochondria

Mitochondria are ubiquitous membrane-bound organelles that are a defining feature of the eukaryotic cell. Human cells have hundreds of mitochondria which are semi-autonomously functioning organelles producing cellular ATP as power plants of the cell. Each cell contains varying numbers of mitochondria depending on energetic requirements [84]. Structurally, mitochondria have four compartments: a permeable outer membrane, an ion impermeable inner membrane, the intermembrane space, and the matrix (the region inside the inner membrane) [85] (Figure 9)[86]. Mitochondria play a critical role in the regulation of both cell survival and death [87].

1.4.1 Mitochondrial functions

Mitochondria is the main player in glucose sensing and metabolism as it play a primary role in cellular energetic metabolism and homeostasis including ion homeostasis, amino acid metabolism, signal transduction and apoptosis [88]. The mitochondria are responsible for producing energy by oxidizing pyruvate through the tricarboxylic acid cycle, and lipids through b oxidation. These processes produce reducing equivalent that drive the electron transport chain enclosed in the inner membrane to produce ATP. The mitochondria are also the major site of reactive oxygen species (ROS) production, which can damage macromolecules [89]. In addition, the mitochondria play a major role in the regulation of apoptosis [90].

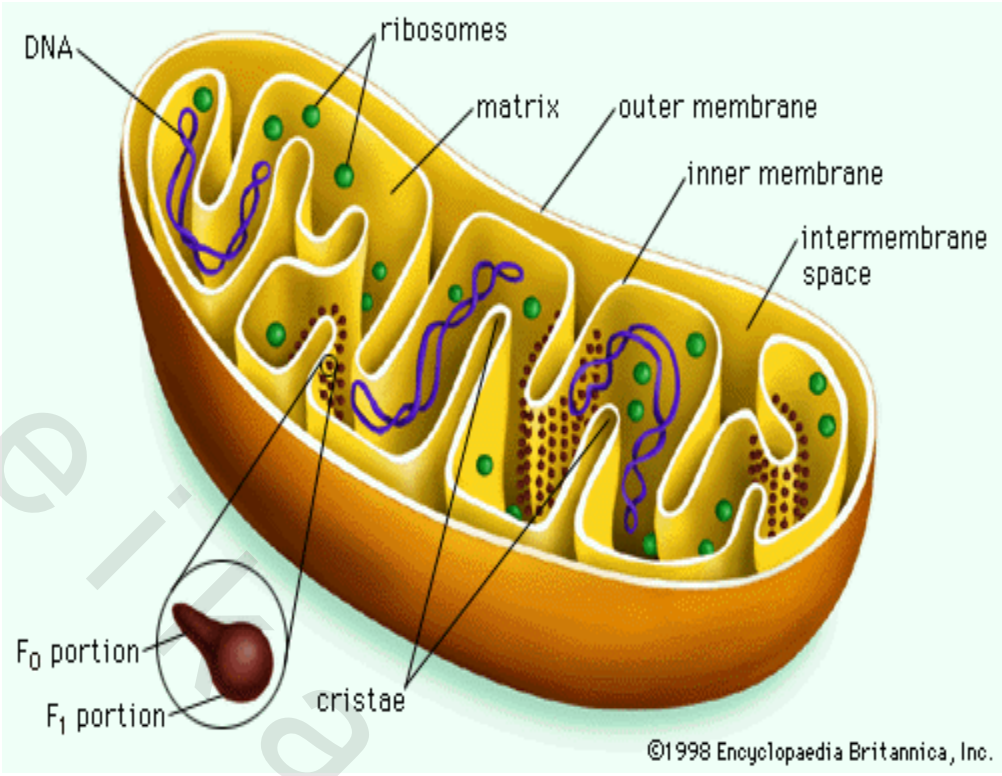

Figure (9): Mitochondrial structure. Quoted from reference (86).

1.4.1.1 Role of mitochondria in mitochondrial oxidative phosphorylation

The process of mitochondrial oxidative phosphorylation is responsible for conversion of macronutrient energy to ATP through a set of exquisitely coupled and coordinated reactions where macronutrients are oxidized (e.g., glucose, fatty acids, and amino acids), oxygen is reduced to water, and adenosine diphosphate (ADP) is phosphorylated to ATP[91] (Figure 10)[92]. The process begins when carbon substrates enter the tricarboxylic acid cycle either through acetyl CoA or anapleotric reactions. Oxidation of these substrates generates reducing equivalents in the form of reduced nicotinamide-adenine dinucleotide (NADH) and reduced flavin- adenine dinucleotide (FADH2), which provide electron flow though respiratory chain complexes I (NADH dehydrogenase) and II (succinate dehydrogenase), respectively. Electron flow through complexes I and II converges on complex III (ubiquinone–cytochromecreductase), along with electrons shuttled in from electron transferring flavoproteins (beta-oxidation), through the mobile electron carrier coenzyme Q. A second mobile electron carrier transfers electrons on to complex IV (cytochrome c oxidase) where they are finally transferred to oxygen, yielding water. A proton gradient across the inner mitochondrial membrane is generated by the action of electron transport through complexes I, III, and IV. The potential energy of this gradient is harnessed by complex V (ATP synthase) to phosphorylate ADP to ATP. Thus, the maintenance of the mitochondrial membrane potential by electron transport is critical to proper function of the organelle, and therefore, the cell[93].

The beneficial role of mitochondria in supplying high-energy phosphates generally overshadows its less favourable role in production of reactive oxygen species (ROS). Mitochondria are responsible for the majority of cellular ROS, although non-mitochondrial

sources such as cycloogygenases, NADPH oxidase, and peroxisomes also contribute a modest amount. Approximately 1–2% of oxygen consumed during physiological respiration is converted into superoxide (O2.−) when electrons prematurely leak from the electron transport chain and are aberrantly transferred to molecular oxygen. However, under specific metabolic or stress conditions, more electrons can prematurely exit the respiratory chain to further augment mitochondrial superoxide generation. Leakage occurs at complex I, complex II or complex III, although complex I and complex III are the major sites of superoxide generation within mitochondria. Superoxide from complex I and complex II is released into the matrix, whereas superoxide from complex III can be produced on either side of the inner membrane. Superoxide can then cross the outer mitochondria membrane via a voltage-dependent anion-selective channel (VDAC) or can be converted into hydrogen peroxide (H_2O_2) in the matrix by superoxide dismutase2 (SOD2) or in the intermembrane space by SOD1 . H_2O_2 can then freely cross-mitochondrial membranes (dashed arrows) or can be further detoxified by additional mitochondrial antioxidant enzymes, such as glutathione peroxidase (GPX). In the cytosol, superoxide is converted by SOD1 into H_2O_2, which is further detoxified by the peroxisomal enzyme catalase (Figure 11) [(94)].

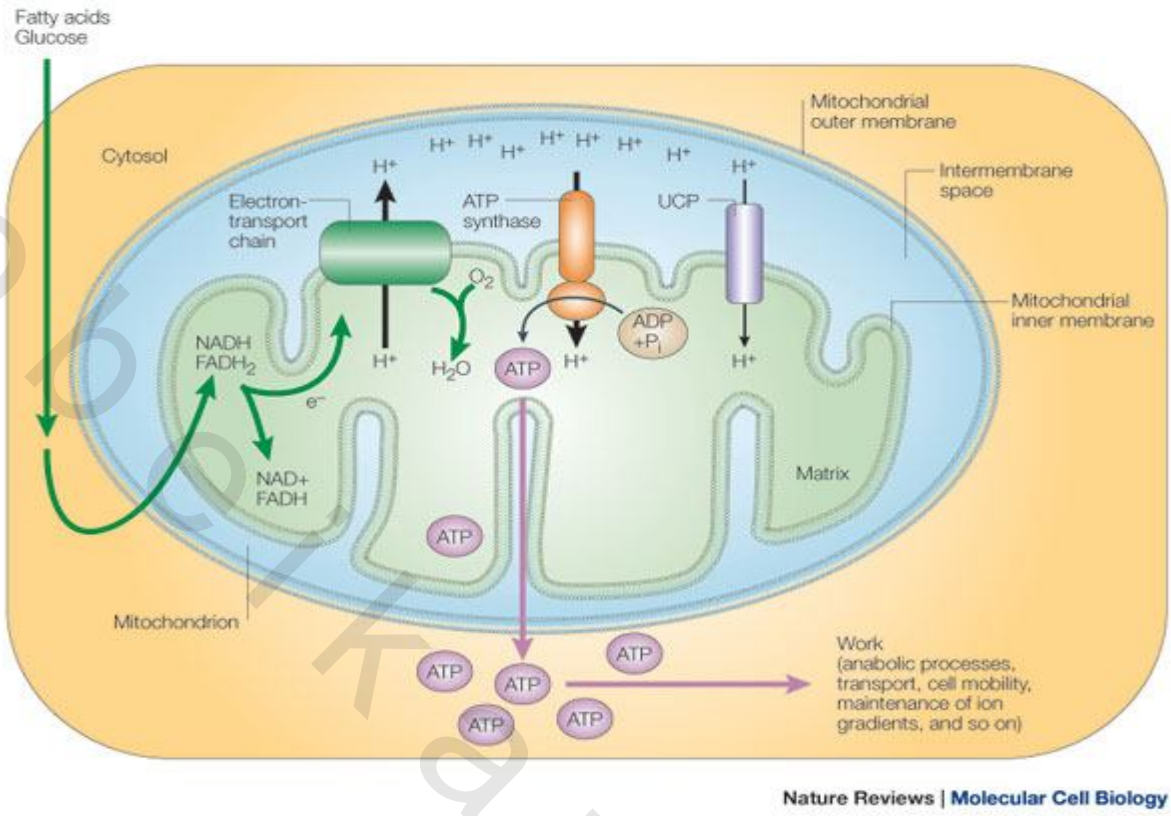

Figure (10): The cellular metabolism of substrates such as glucose and free fatty acids (green arrows in the figure) generates electrons (e-) in the form of the reduced hydrogen carriers — NADH and FADH2. NADH and FADH2 donate electrons to the electron-transport chain, which comprises protein complexes that are located in the mitochondrial inner membrane. Electrons are ultimately transported to molecular oxygen, which is reduced to water in the last step of the electron-transport chain. As electrons are transferred along the electron-transport chain, a fixed number of protons (H+) are pumped from the mitochondrial matrix into the mitochondrial intermembrane space, which establishes a proton gradient across the mitochondrial inner membrane. The energy that is conserved in this proton gradient drives the synthesis of ATP from ADP and inorganic phosphate (Pi) by ATP synthase as protons are transported back from the intermembrane space into the mitochondrial matrix. ATP is then made available to the cell for various processes that require energy. Proton leak, which, in part, is mediated by the uncoupling proteins (UCPs), uncouples the processes of electron transport/proton-gradient generation on the one hand, and ATP synthesis on the other. By dissipating the proton gradient, the energy that is derived from the oxidized substrates is released as heat. Quoted from reference (92)

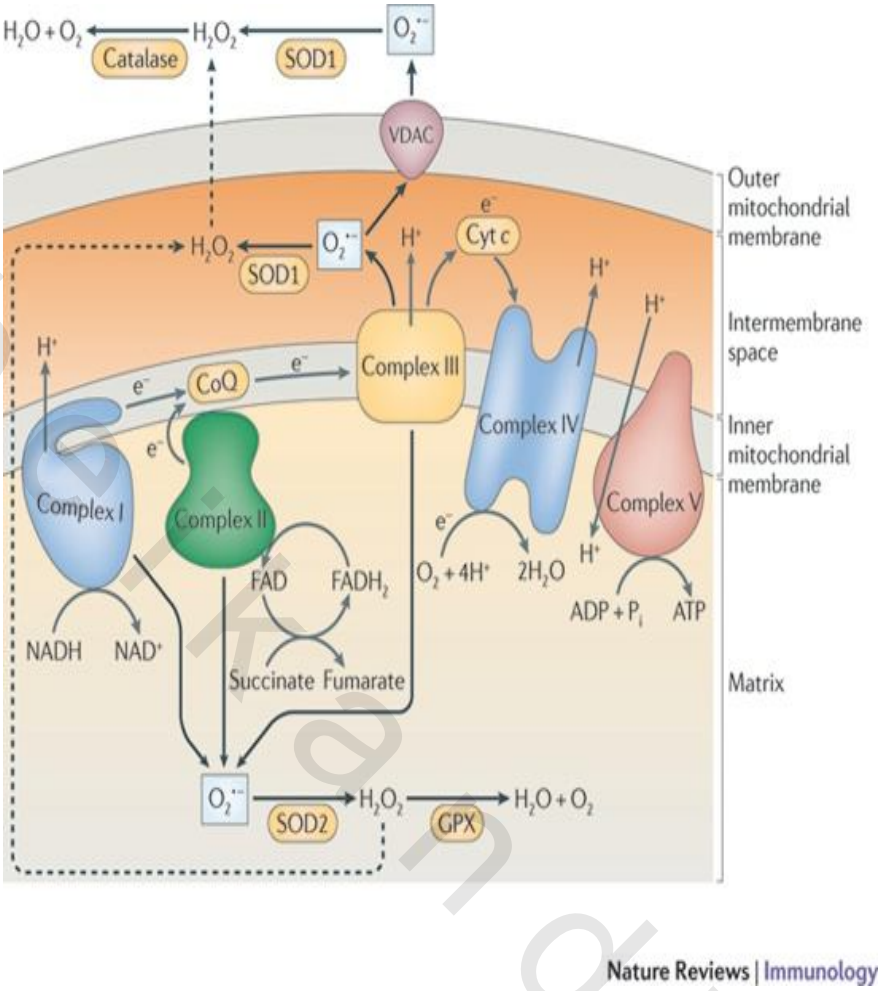

Figure (11): Mitochondrial oxidative phosphorylation is a major cellular source of reactive oxygen species (ROS). Quoted from reference (94)

1.4.1.2 Role of mitochondria in apoptosis

Programmed cell death (i.e., apoptosis) is the end-stage of the cell cycle when cellular structures are degraded by proteases such as caspases and nucleases. Apoptosis is critically important for the survival of multicellular organisms by getting rid of damaged or infected cells that may interfere with normal function. The extrinsic and intrinsic pathways represent the two major well-studied apoptotic processes [(95)].

The cell autonomous or intrinsic pathway is largely centred around and/or regulated by the mitochondria [(96)] (Figure 12) [(97)]. Cytochrome c is a mobile electron carrier that functions to shuttle electrons from complexes I and II to complex III and thus, plays a vital role in mitochondrial ATP synthesis. Ironically, a protein that is so vital to life is also a trigger for apoptosis. Under normal conditions, cytochrome c resides within the inner mitochondrial membrane, associated with cardiolipin, a membrane phospholipid [(98)] However, certain stimuli, such as DNA damage, protein damage, or perturbation of metabolic homeostasis, initiate the release of cytochrome c into the cytosol which activates caspases. Several factors, such as increased cytosolic calcium and ROS, have been proposed to weaken the interaction between cytochrome c and its cardiolipin anchor and initiate the detachment and mobilization of cytochrome c [(99)].

The outer mitochondrial membrane is ordinarily impermeable to proteins. Cytochrome c is believed to exit into the cytosol through pores that form in a process known as mitochondrial outer membrane permeabilization. The exact mechanisms of outer membrane permeabilization are still being investigated. Once in the cytosol, cytochrome c initiates the formation of apoptosomes and activation of a series of caspases that begin the process of demolishing the cell [93]

The link between mitochondria and apoptosis goes beyond caspase- dependent pathways of cellular destruction. Apoptosis-inducing factor (AIF) is another intermembrane flavoprotein that induces apoptosis when it is released into the cytosol [96]. Unlike cytochrome c release, AIF induces apoptosis through caspase-independent mechanisms. AIF translocates to the nucleus and induces DNA fragmentation and chromatin condensation [100].

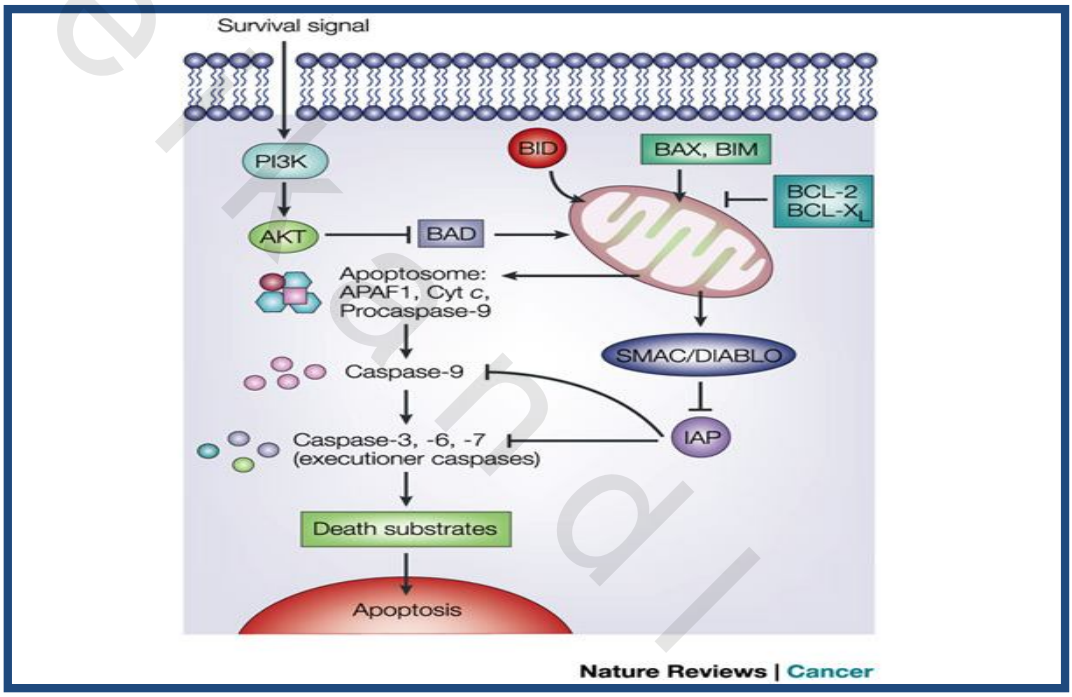

Figure (12): Apoptosis signalling through mitochondria: Chemotherapy, irradiation and other stimuli can initiate apoptosis through the mitochondrial (intrinsic) pathway. Pro-apoptotic BCL2 family proteins (BAX, BID, BAD and BIM) are important mediators of these signals. Activation of mitochondria leads to the release of cytochrome *c* (Cyt *c*) into the cytosol, where it binds apoptotic protease activating factor 1 (APAF1) to form the apoptosome. At the apoptosome, the initiator caspase-9 is activated. Apoptosis through mitochondria can be inhibited on different levels by anti-apoptotic proteins, including the anti-apoptotic BCL2 family members BCL2 and BCL-X_L and inhibitors of apoptosis proteins (IAPs), which are regulated by SMAC/DIABLO (second mitochondria-derived activator of caspase/direct IAP binding protein with low pI). Another way is through survival signals, such as growth factors and cytokines that activate the phosphatidylinositol 3-kinase (PI3K) pathway. PI3K activates AKT, which phosphorylates and inactivates the pro-apoptotic BCL2-family member BAD. Quoted from reference (97).

1.4.1.2 Role of mitochondria in insulin secretion

The homeostasis of glucose and insulin is based on the ATP generated by glucose in the β–cell [101] (Figure 13) [92]. Glucose is transported across the cellular membrane by facilitated diffusion through glucose transporters (GLUT2 in rodents; mainly GLUT1 in humans) and is retained inside the cell through its phosphorylation by glucokinase[102] Glycolysis transforms glucose to pyruvate. Pyruvate is efficiently channelled into mitochondria, where pyruvate is further broken down by the tricarboxylic acid (TCA) cycle [103].

The resultant reducing equivalents such as FADH and NADH shuttle electrons to the electron transport chain (ETC), which produces more ATP. The ratio of ATP/ADP increases as processing of the glucose increases [104]

This increase in ATP/ADP ratio causes the ATP-sensitive K^+ channels to close, causing depolarization of voltage-sensitive Ca^{2+} channels. The depolarization causes an influx of Ca^{2+} into the cytosol that triggers the exocytosis of insulin secretory vesicles produced by the Golgi complex. The processing of proinsulin to insulin within the secretory granules of the Golgi complex is dependent on critical levels of pH (3.5- 7.4) as well as ATP. Proinsulin, a precursor of insulin with little hormonal activity, is converted to insulin by the removal of the connecting C-peptide [105].

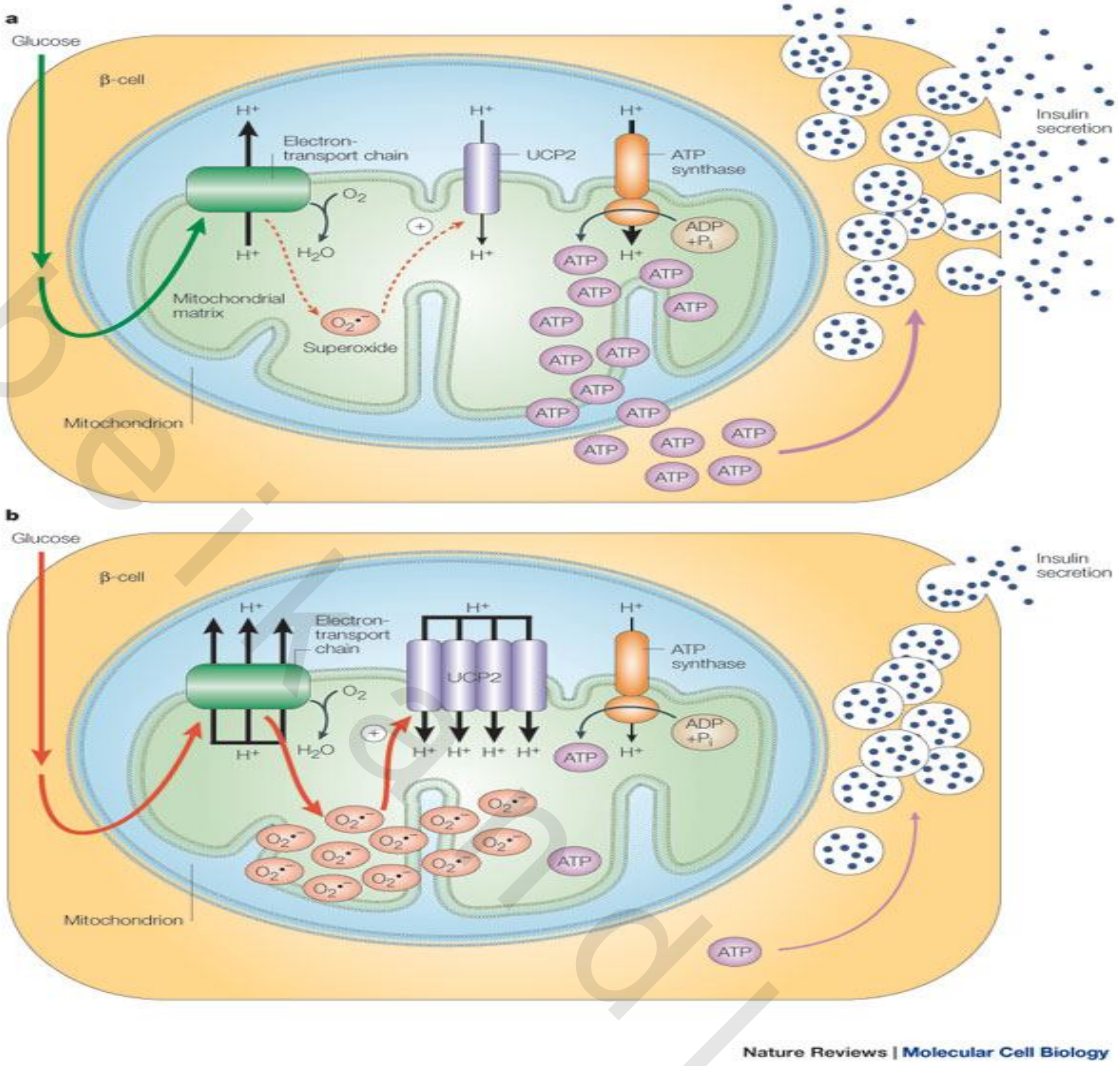

Figure (13): a |**Unperturbed glucose sensing in the pancreatic β-cell.** Pancreatic β-cells sense glucose by metabolizing it and by coupling the subsequent generation of ATP to insulin secretion. Glucose is transported into the β-cell by glucose transporter type-2 (GLUT2; not shown), and is subsequently oxidized by the pathways of glycolysis, the tricarboxylic-acid cycle and mitochondrial oxidative phosphorylation. Electrons that are derived from glucose metabolism are passed down the electron-transport chain, ultimately resulting in the generation of ATP by ATP synthase. ATP facilitates the release of insulin from the pancreatic β-cell. Uncoupling protein (UCP) 2, which can be activated by superoxide, is not usually highly expressed in β-cells and its activity are low or negligible. **b | In hyperglycaemia, the metabolism of glucose is increased.** This results in an increased donation of electrons to the electron transport chain, an increase in the amount of protons that are pumped out of the mitochondrial matrix, and the generation of a high mitochondrial membrane potential. As a consequence, large amounts of superoxide (O2●-) are produced in the mitochondrial matrix owing to an increase in 'random' single-electron transfer reactions from components of the electron-transport chain to molecular oxygen. Furthermore, UCP2 expression is increased. These independent events lead to a deleterious activation of the superoxide–UCP2 pathway, which causes decreased ATP production from glucose by ATP synthase and, subsequently, loss of glucose-stimulated insulin secretion. Quoted from reference (92).

1.4.2. Mitochondrial biogenesis

Mitochondria contain their own genome (Figure 14) [106]. Mitochondrial DNA (mtDNA) is located in the mitochondrial matrix and is present in multiple copies per mitochondrion [107,108]. It consists of a light strand, a heavy strand (rich in guanine) and a small fragment called the displacement loop or D-loop. mtDNA does not contain introns and both strands of circular mtDNA are transcribed as long primary transcripts corresponding to several genes. These primary transcripts are processed to release the individual tRNA, rRNA and mRNA[109]. Many mitochondrial genetic codons differ from nuclear codons. mtDNA is normally maternally inherited through the oocyte [110]. mtDNA present in one to several thousands of copies per cell (polyplasmy) [111].

Mammalian mitochondrial DNA (mtDNA) is a ~16.6 kilobase circular genome that consists of a regulatory control region (D-loop), 13 genes for essential catalytic proteins of the ~87 proteins in the electron transport chain (ETC), 22 tRNA's and two ribosomal RNA's that facilitate translation of the mtDNA-encoded ETC proteins in the mitochondrial matrix[112]. The remainder of the ETC proteins and ~1200–1500 of the other mitochondrial catalytic and structural proteins are imported using multi-protein translocase complexes of the outer and inner mitochondrial membranes that direct protein precursors formed outside mitochondria to their appropriate location by means of specific N-terminal mitochondrial localization sequences [113].

The mitochondrial function and biogenesis is under the control of nuclear encoded proteins; mainly mitochondrial transcription factor A (mTFA) and uncoupling proteins (UCPs).

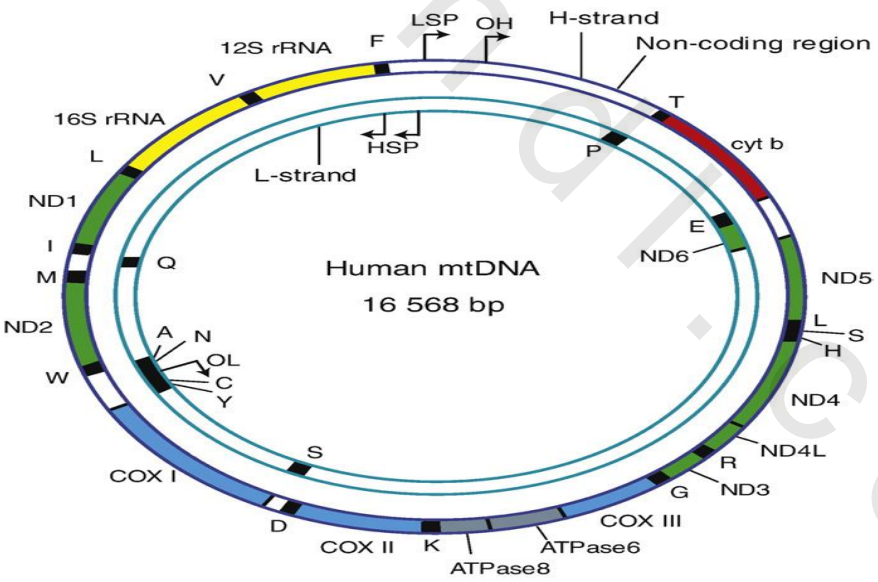

Figure (14): The human mitochondrial genome. The human mitochondrial genome consists of 16568 base pairs and contains a heavy- and (H-strand) light-strand (L-strand). Complex I NADH dehydrogenase (ND) genes are shown in green; Complex III cytochrome b (Cyt b) gene is shown in red; Complex IV cytochrome c oxidase (COX) genes are shown in light blue; Complex V ATP synthase (ATPase) genes are shown in gray. Transfer RNA genes in black and ribosomal RNA genes (rRNA) in yellow. Quoted from reference (106).

1.4.3 Mitochondrial transcription factor A (mTFA):

Mitochondrial transcription factor A(mTFA) is a single copy nuclear gene which encodes for an activator of mitochondrial transcription in mammals. The gene encoding mTFA is located on the chromosome 10 at the locus 10q21.1. mTFA gene in mammals is estimated to span about 10 kb and is structured in seven exons and six introns in rat, mouse and human (Figure 15) [(114)]. mTFA mRNA is widely distributed in mammalian tissues as well as highly conserved, short (741 base pair) and guanine cytosine (GC) poor. The gene expression of mTFA is altered depending on the cellular conditions and directly regulated by several transcription factors such as nuclear respiratory factor (NRF)-1 and NRF-2. The full-length cDNA of this nuclear gene encodes 246 amino acids of mTFA (precursor form). The N-terminal 42 amino acids function as mitochondrial targeting signal and are cleaved during mitochondrial translocation. Thus, the mature mTFA functional form is composed of 204 amino acids (25 kDa) [(115)].mTFA not only plays an important role in the maintenance of mtDNA integrity but also in mtDNA replication and transcription [(116)].

mTFA belongs to a large and diverse superfamily of high-mobility group (HMG) protein. Within this superfamily, three structurally distinct classes of HMG proteins have been defined: HMG–nucleosome-binding family (HMGN), HMG-AT-hook family (HMGA) and HMG-box family (HMGB). Mammalian HMG-box containing proteins can further be sub-divided into two major groups. The first group consists of HMGB-type non-sequence-specific DNA-binding proteins with two HMG-box domains and a long highly acidic C-tail. The second group is highly diverse and consists of mostly sequence-specific proteins having a single HMG-box and no acidic C-tails. As a HMGB protein, mTFA is unique in that it binds specific sequence preferentially, but contains two HMG-box domains. In addition to these two HMG boxes, mTFA has a linker region between the two HMG boxes and a carboxyl- terminal tail region (C-tail) composed of 27 and 25 residues, respectively [(117)].

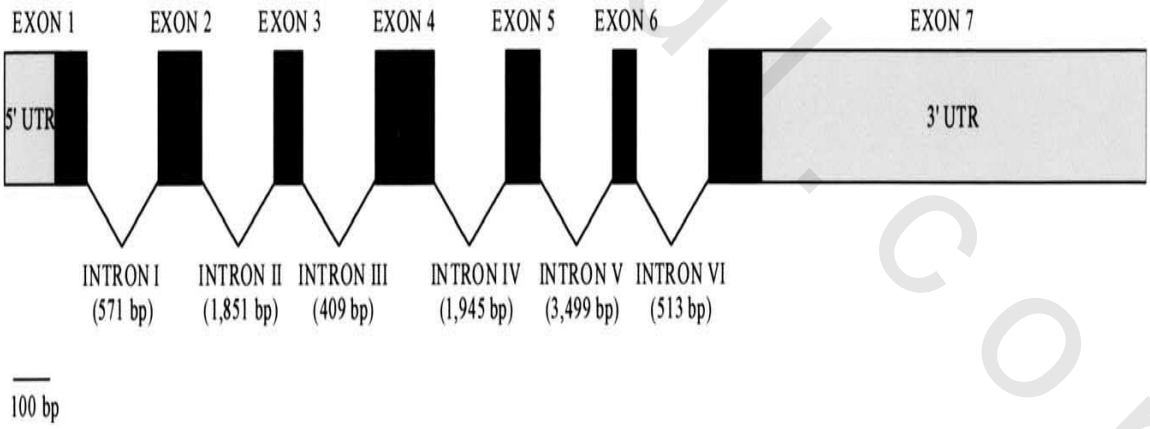

Figure (15): Gene structure of mTFA gene. The exons are boxed. The segments corresponding to the coding sequence (CDS, black boxes) and 5'- and 3'UTR (grey boxes) are reported. Intron size was determined by amplifications on human genomic DNA. Quoted from reference (114).

1.4.3.1. Functions of mitochondrial transcription factor A

1.4.3.1.1 mtDNA maintenance

mTFA binds mtDNA with an affinity compatible with the idea of the protein coating the genome, shows a strong preference for DNA with protein already bound, and is sufficient to compact and assemble DNAs into multigenomic nucleoid-like structures [(83)].

1.4.3.1.2 mtDNA transcription and replication

Transcription of mtDNA occurs following interaction between nuclear-encoded regulatory proteins and regions within the D-loop of mtDNA[(118)]. Requirements for transcription include the mitochondrial RNA polymerase, mitochondrial transcription factor A and one of the recently identified TFB1M and TFB2M[(119)]. Once initiated, transcription generates a polycistronic precursor RNA transcript, allowing coordinated transcription of all genes on the same strand. Excision of the polycistronic precursor by endonucleases produces precursor rRNAs and tRNAs, which are then processed further to allow them to translate the precursor mRNAs [(118)].

Disruption of mTFA in the pancreatic β-cell impaired insulin secretion, reduced β -cell mass, and resulted in mitochondrial diabetes, implying that the dysfunction of mTFA might implicated in the pathogenesis of type 2 diabetes[(120)].

1.4.4. Uncoupling proteins (UCPs)

Uncoupling proteins are other nuclear encoded proteins that play a pivotal role in controlling mitochondrial functions. Mitochondrial uncoupling refers to the dissociation of electron-dependent oxygen consumption to ATP generation on the respiratory chain. The most efficient way to induce mitochondrial uncoupling is to allow protons to circulate freely across the inner mitochondrial membrane, in other words to create a proton leak [(121)]. In this regard, the uncoupling proteins (UCPs) are a professional mitochondrial uncoupler dissipating the proton gradient by allowing the re-entry of protons into the mitochondrial matrix during oxidative ATP generation, resulting in the uncoupling of the respiratory chain and heat production [(122)].

The uncoupling proteins (UCPs) are a family of mitochondrial transport proteins located in the inner mitochondrial membrane. Here are five UCPs (named UCP1to 5) found in mammals [(92)]. These anion-carrier proteins transport protons (H^+) to the mitochondrial matrix and in turn dissipate the proton motive force as heat and uncouple the substrate oxidation from the production of ATP. These proteins have similarities in their structures, but different tissue distributions in mammals [(123)].

The UCPs are integral membrane proteins, each with a molecular mass of 31-34 kDa and a tripartite structure in which a region of around 100 residues is repeated three times; each repeat codes for two transmembrane segments and a long hydrophilic loop. The functional carrier unit is a homodimer [(124)].

These proteins have similarities in their structures, but different tissue distributions in mammals. UCP1 is mainly expressed in brown adipose tissue (BAT), which is responsible for thermogenesis in newborns. UCP2 is widely distributed in several tissues, including the spleen, kidney, immune system, pancreas, and central nervous system, whereas UCP3 is mainly restricted to the skeletal muscle, and UCP4 and UCP5/BMCP1 are mainly expressed in the brain. Besides the non shivering thermogenesis function of UCP1, functions of the other UCPs are still unclear. UCP2 is reported to be involved in glucose and lipid metabolism [(125)]to control immune cell activation by modulating MAPK pathways and the production of mitochondrial ROS [(126)], and a neuroprotective role is also suggested based on the regulation of mitochondria membrane potential, production of ROS, preservation of calcium homeostasis, modulation of neuronal

activity, and eventually inhibition of cellular damage [127].UCP3 is suggested to be involved in mediating energy expenditure via uncoupling, especially in fatty acid metabolism, and it seems to protect mitochondria against lipid-induced oxidative stress, which makes this protein a potential player in the development of T2DM [128]. Fewer studies focused on the physiologic roles of UCP4 and UCP5, and the protection against oxidative stress and mitochondrial dysfunction are also reported [129]. Although the physiological functions of UCPs are still not been completely elucidated, their abilities of reducing the mitochondrial ROS formation are widely accepted [130].

1.4.4.1 Uncoupling proteins 2 (UCP2)

In 1997, Fleury *et al* cloned and sequenced a gene homologous to UCP1 gene, later called UCP2. UCP2gene covers a 6.3 kb region on chromosome 11 (region 11q13), and has eight exons and seven introns (Figure 16) [131]. In humans, region 11q13 is linked to basal metabolic rate and body fat percentage. The transcriptional gene unit is constituted by two non-coding exons followed by six exons that encode the 308 amino acids of the protein. Human UCP2 share 57% amino acid-sequence identity with human UCP1, and it is 71% identical to human UCP3. In addition, the amino acid sequence of human UCP2 is 95% identical to mouse UCP2 [132].

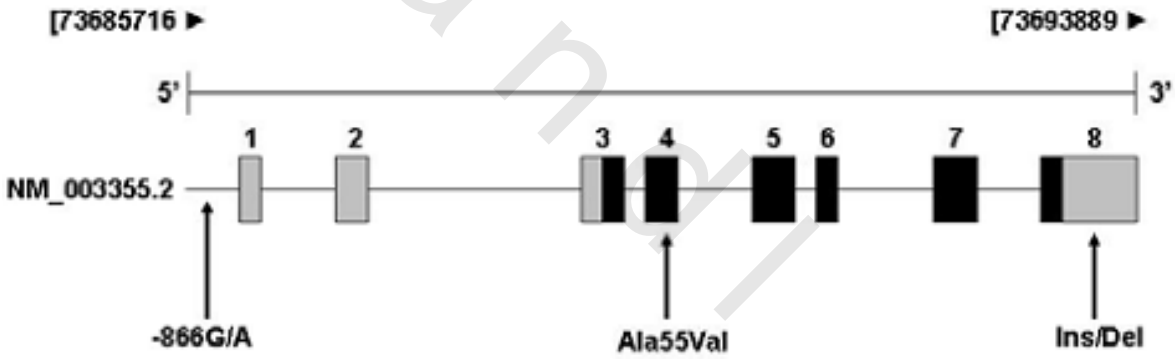

Figure (16). Mapping of UCP2 gene locus on chromosome 11 (region 11q13). The eight exons (boxes) are numbered from left to right according to the transcriptional region. The black boxes represent the coding regions, and the light gray boxes represent the non-coding region, including the 3'UTR region of exon 8. The vertical arrows show the main common polymorphisms associated with DM2 or its microvascular chronic complications. Quoted from reference (131).

UCP2 is expressed in a wide range of tissues and cell types, including brown and white adipose tissues, skeletal muscle, heart, kidneys, liver, lungs, spleen, thymus, bone marrow, macrophages, brain, gastrointestinal tract, pancreatic islets and retinal cells [133]. Although UCP2 is well expressed in many tissues at mRNA level, it would seem that UCP2 protein level is not simply proportional to mRNA concentration [134].
UCP2 exhibit singular features which distinguish it from other mitochondrial carriers, including UCP1:

1- UCP2 mRNA is found in many tissues [135] whereas UCP1, UCP3, UCP4, and BMCP1 mRNA are mainly expressed in brown adipocytes, muscle and brain, respectively [135-138]

2- UCP2 is regulated at both the transcriptional [139-141] and the translational level [142,143]. The presence of an open reading frame upstream of the one coding UCP2 exerted a constitutive inhibition of UCP2 translation. As a direct consequence of this inhibition, the protein is present in very lower abundance, less than 1% relative to UCP1 in BAT, and can only be detected in steady state in tissues with high mRNA level such as spleen, thymus, lung, pancreas, digestive tract and the immune cells. As a result, a majority of antibodies raised against the protein were not able to detect UCP2 in vivo, resulting in misleading interpretations. The inhibition of UCP2 translation can be relieved in vitro by the addition of glutamine and in vivo by fasting or an inflammatory state.
3- The half-life of the protein is unusually short, around 30 minutes [144], making UCP2 a suitable candidate for regulating rapid biological responses and regulation of its expression possess a nearly direct switch on/off regulation of UCP2 presence [145]. Although it has not been investigated thoroughly, it is commonly accepted that the half-life of other mitochondrial carriers is at least over 10hours [146].

1.4.4.1.2. Functions of UCP2

In the past few years, an accumulating set of data, suggested that the new UCPs should be considered as mitochondrial carriers rather than uncoupling proteins. The genetic loss of UCP2 leads to a faster proliferative rate associated with decreased mitochondrial fatty acid oxidation and increased glucose metabolism. The idea that UCP2 acts as a regulator of mitochondrial fatty acid oxidation is consistent with previous studies suggesting that UCP2plays a role in lipid metabolism by promoting a shift from carbohydrate to lipid metabolism during fasting or by transporting free fatty acids out of mitochondria [147]. Interestingly, the presence of glucose in the media was not only sufficient, but also required to observe such a difference, pointing out the importance of glucose metabolism in the proliferative phenotype. Other studies have shown a link between glucose metabolism and uncoupling proteins. Parton et al, have shown that UCP2 negatively regulates glucose sensing in neurons and its absence prevents obesity-induced loss of glucose sensing [148]. The flux of glutamine oxidation seems slower in the absence of UCP2, which in turn leads to the accumulation of metabolic intermediates such as aspartame and glutamate and limits the availability of reduced coenzymes for the respiratory chain. Interestingly, glutamine is also a potent enhancer of UCP2 translation [143].

Glycolytic-derived pyruvate, fatty acids and glutamine are the main mitochondrial energetic fuels providing intermediates for the TCA cycle and reduced equivalents for the respiratory chain. Interestingly the absence of UCP2 is associated with decreased fatty acid and glutamine oxidation, which provide AcetylCoA and OAA, respectively. In contrast, the oxidation of glucose is increased, providing more pyruvate to the mitochondria. Pyruvate can then be converted to either AcetylCoA via pyruvate dehydrogenase or OAA via pyruvate decarboxylase. This suggests that UCP2 could play a role as a sensor for the choice of substrates supplying the TCA cycle [146].

1.4.4.1.3. Regulation of UCP2 expression and activation.

1.4.4.1.3.1 Regulation by long-chain fatty acids

In vivo studies indicate that physiological and pathological elevation of blood long-chain fatty acids (up to 2- to 3-fold) resulting from fasting [149], high fat diet [150], suckling of newborn pups [151], sepsis [152,153], or streptozotocin-induced diabetes [154] induce up-regulation of UCP2.

Similarly, in vitro studies have shown that monounsaturated (n-9) and polyunsaturated (n-6 and n-3) fatty acids can dramatically induce up-regulation of UCP2 in 3T3-L1 preadipocytes[155], human primary cultured myotubes[156], the rat myoblast cell line L6 [157], the rat insulinoma cell line INS-1 [139], rat primary cultured adipocytes [158], and cloned bovine mammary epithelial cells[159]. These data suggest that long-chain fatty acids per se induce transcription of UCP2.

One possible candidate for transcriptional activation by long-chain fatty acids is peroxisomal proliferators-activated receptors (PPARs) that belong to a family within the lipid-activated receptor superfamily [160].

Another possible candidate is sterol response element binding proteins (SREBPs), a family of transcription factors that activate genes involved in the synthesis of cholesterols and fatty acids by binding to sterol regulatory elements (SREs) in the promoters [161].

1.4.4.1.3.2. Regulation by hormones

Various hormones have been implicated in the control of UCP2 expression. One such hormone is insulin, an anabolic hormone that controls blood glucose levels and energy homeostasis and is involved in the pathology of metabolic disorders. Pedersen et al. [162] demonstrated that in human adipose tissue, up- regulation of UCP2 occurs during a 150-min hyperinsulinaemic clamp and that in rat skeletal muscle, insulin induces up-regulation of UCP2[163]. In cultured primary rat adipocytes, insulin induces up-regulation of both UCP2 gene and protein in a glucose-concentration-dependent manner [158]. However, it has also been reported that insulin negatively regulates UCP2in mammary epithelial cells [159].

Another hormone that may influence UCP2 expression is leptin. Leptin is coded by the *ob* gene and secreted by adipose tissues [164]. It plays a crucial role in anorexigenic effects on food intake, energy expenditure, and controlling fat mass, presumably by acting on the hypothalamus or peripheral tissues via binding to cognate receptors [164-166].

Two additional hormones were identified as regulators of UCP2. One of these hormones is adiponectin, the other is ghrelin. Adiponectin is secreted by adipose tissues and controls food intake and energy expenditure [167]. Overexpression of the adiponectin receptor gene induces up-regulation of UCP2[168].

Ghrelin is secreted from specific cells in the stomach and has anorexigenic effect [169]. Ghrelin regulates the secretion of insulin, food intake and energy expenditure via the up-regulation of UCP2, in part at least, and via the AMPK signalling pathway in peripheral tissues and/or central nervous system [160].

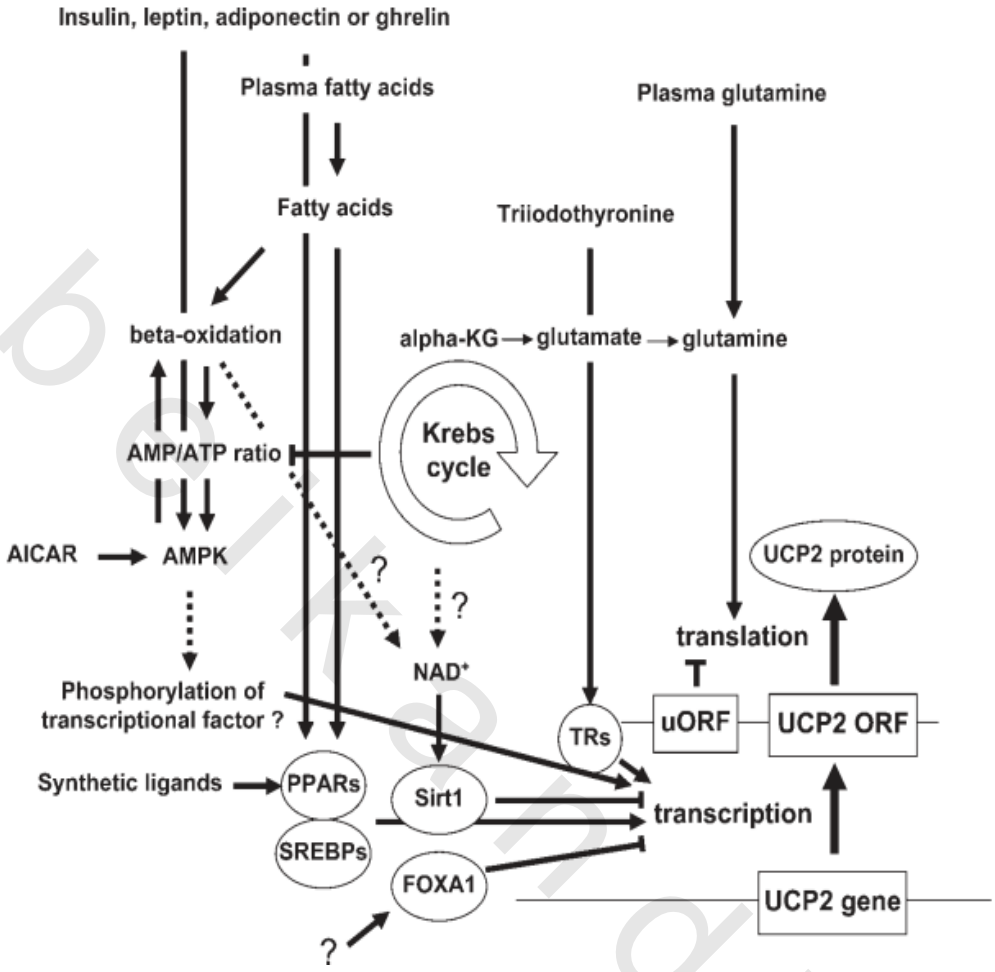

Figure (17). Schematic diagram of the regulation of UCP2 transcription and translation. Bold arrows indicate activation. Dotted arrows indicate putative activation. Barred lines indicate inhibition. Alpha-KG, AICAR, AMPK, NAD1, PARs, SREBPs, Sirt1, FOXA1, TRs, and uORF indicate alpha-ketoglutarate, 5-aminoimidazole-4-carboxamide-1-beta-D-ribofrano-side, 5'-AMP-activated protein kinase, nicotinamide adenine dinucleotide, peroxisomal proliferators-activated receptors, sterolresponse element binding proteins, silent information regulator homolog 1, forkhead box proteins A 1, thyroid hormone receptors, and upstream short-open reading frame, respectively. Quoted from reference (160)

1.4.4.1.3.3 Regulation by glutamine and the 5' short-open reading frame

Hurtaud et al. demonstrated that posttranscriptional regulation of UCP2 occurs in macrophages, colonocytes, and pancreatic beta-cells. Inclusion of glutamine in the culture medium induces translation of the UCP2 protein in a concentration-dependent manner [(143)]. Protein translation from UCP2 is inhibited in the absence of glutamine because of the existence of a short upstream open reading frame (uORF) consisting of 36 amino acids in the 5'-untranslated region [(170)]. In the presence of glutamine, the inhibitory effect of the uORF disappears [(143)]. This regulation of translation by the uORF can rapidly generate UCP2 proteins from pooled mRNA in response to cellular events such as generation of ROS and metabolic changes [(160)].