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Beyond Type 2 Diabetes Mellitus

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Dr. Vishal Gupta's
ADVANTAGE
DIABETES, THYROID & ENDOCRINE CENTRE

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Chapter 2

Does it Boil Down to Insulin Resistance versus Beta-cell Failure, Focus of Pathophysiology and Correlation with Therapeutics?

Vishal Gupta

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CASE

A 56-year-old gentleman with a history of type 2 diabetes mellitus (T2DM) since the last 12 years presents with glycated hemoglobin (HbA1c) 8.1%. His body mass index (BMI) is 34 kg/m^2 and blood pressure is 140/90 mm Hg in the right arm. His plasma lipid profile suggests hypertriglyceridemia [total cholesterol 200 mg%, triglyceride 330 mg%, high-density lipoprotein (HDL) 31 mg%, and low-density lipoprotein (LDL) 99 mg%]. Liver enzymes suggest raised gamma-glutamyl transpeptidase (GGTP) and serum glutamic pyruvic transaminase (SGPT) 100 IU/L, international normalized ratio of 1.1, and albumin 3.8 mg%. For his diabetes, he receives 1,000 mg of metformin/day and gliclazide 320 mg/day. For risk factor control, he is on an angiotensin-receptor blocker for his blood pressure and fenofibrate 160 mg/day and atorvastatin 10 mg/day. How will you help improve his diabetes?

GOALS

- Understand all the components of the diabetic pathophysiology and learn the patient's primary contributing components.
- Use plasma insulin resistance assessment to help guide his choice of therapy.
- Approach the patient's change in glucose-lowering therapy based on the pathophysiology.

INTRODUCTION

The natural history of type 2 diabetes mellitus (T2DM) as proposed originally by Ralph DeFronzo[1] involved *insulin resistance* at level of *liver* resulting in hepatic outpouring of glucose into the hepatic venous system, *insulin resistance at peripheral tissue* (skeletal muscles) resulting in reduced glucose uptake, and *β-cell failure* resulting in a declining insulin secretion capacity. At least eight

distinct pathogenetic mechanisms have since been found to be associated with T2DM. Increased lipolysis resulting in elevated plasma free fatty acid (FFA) levels has shown to increase insulin resistance and augment β-cell failure. Abnormalities in the alpha-cell that release glucagon inappropriately have shown to further contribute to hyperglycemia in the fasting and postprandial states.[2,3] Impaired oxidative metabolism at rest and exercise, perturbed amino acid metabolism, neuronal (brain) dysregulation of appetite, and reduced renal excretion of glucose add to the hyperglycemic state.[1] In the past few decades, the standard treatment of choice for T2DM has been metformin (insulin sensitizer) and sulfonylureas (SUs) (insulin secretagogues) to address the basic pathological triad of T2DM. Metformin has been around since 1957 and has been traditionally favored as the first-line therapy of choice in the management of T2DM. Metformin monotherapy has shown to reduce glycated hemoglobin (HbA1c) by an average of 1.12%[4] and has been associated with several advantages (nonhypoglycemic/antihyperglycemic, weight loss, cardioprotective, improvement in nonalcoholic hepatosteatosis, antineoplastic, etc.). The mean failure rate of metformin has shown to be approximately 17% per year[5] suggesting additional mechanisms involved in the pathogenesis that remain unexplored. Insulin resistance seems to be central in the causation of cardiorenal complications, cancers, fatty liver disease, dementia, etc.[6,7] SUs, on the other hand, enhance β-cell secretion and have been shown to lower the HbA1c by an average of 1.51% and an average of 1.62% when added to other antidiabetes therapies.[8] Most patients on SU therapy convert to insulin over time because of secondary failure with as many as 50% requiring additional antidiabetes therapy over 3 years.[2]

PATHOPHYSIOLOGY OF DIABETES

We continue to further our understanding of diabetes until today and over the years, the several mechanisms responsible for the pathogenesis of diabetes can be summarized as follows: From the triumvirate theory to the disharmonious octet, proposed by Ralph DeFronzo, to the current understanding of the role of amylin, gut microbiota, and inflammation, each representing a separate and unique process in the development of T2DM (**Flowchart 1**).

Triumvirate Theory

DeFronzo referred to the core pathological deficiencies that result in T2DM as the triumvirate theory, which were: Decreased glucose uptake by peripheral (muscle) tissues, increased hepatic glucose production secondary to augmented gluconeogenesis, and impaired insulin secretion from declining β-cell function.[9] To understand insulin resistance, let us first understand the action of insulin.

Insulin Action (Mechanism of Insulin Action) (Fig. 1)

Insulin, an anabolic peptide hormone (51 amino acids, molecular weight 5,808 kDa), is secreted by β-cells of islets of pancreas in response to rising

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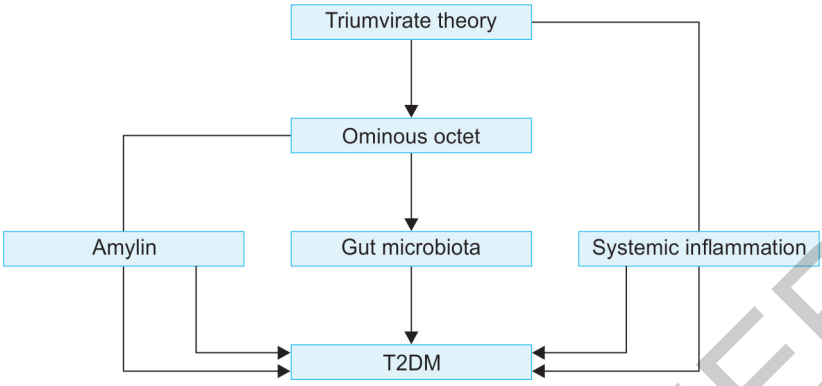

DeFronzo proposed the original triumvirate theory (beta-cell failure along with hepatic and peripheral insulin resistance) as the cause of type 2 diabetes mellitus. In 2009, he once again proposed the ominous octet and included adipocyte dysfunction/resistance, alpha-cell (glucagon excess), incretin dysfunction (loss of incretin effect), kidney (enhanced glucose reabsorption), and brain. Since then reduced amylin, altered gut microbiota and systemic inflammation have been added to the pathophysiological mechanisms.

FLOWCHART 1: The various pathogenic mechanisms involved in the development of type 2 diabetes mellitus (T2DM).

plasma levels of glucose and amino acids. It is composed of an α- and a β-chain linked together by a disulfide bridge. The pharmacologic half-life of insulin is 5–8 minutes. Insulin exerts a variety of effects on hepatocytes, myocytes, and adipocytes (white adipose tissue). It stimulates cell growth and differentiation and promotes storage of substrates in fat, liver, and muscle. It stimulates lipogenesis, lipid storage, and glycogen and protein synthesis while inhibiting fat, glycogen, and protein breakdown. It helps maintain glucose homeostasis and promotes glucose utilization in the body.[10] Insulin is secreted in response to the amount of glucose entering the β-cell.[11]

Insulin exerts its action by binding to the insulin receptor. There are two isoforms of the insulin receptor, A and B. Insulin mediates most of its effects via the B isoform. It is a heterotetrameric receptor that contains two extracellular α-subunits (bind insulin) and two transmembrane-spanning β-subunits, each of which contains a *tyrosine kinase domain*.[12] Each receptor has two insulin binding sites; however, only one insulin molecule can bind at a time. On binding of insulin (α-subunit), the β-subunit undergoes a conformational change that relieves *cis* autoinhibition which in turn activates the tyrosine kinase attached to the β-subunit by trans-auto-tri-phosphorylation of three tyrosine residues (Tyr1162, Tyr1158, and Tyr1163). This leads to further tyrosine phosphorylation of Tyr972 residue which in turn creates binding sites for signaling protein partners/docking proteins containing Src homology 2 (SH2)[13] domains or phosphotyrosine-binding (PTB) domains. Another scaffolding protein linked to the tyrosine kinase active loop is the insulin receptor substrate (IRS) family. There are six isoforms of which IRS1 (glucose metabolism) and IRS2 (de novo lipogenesis) are thought to mediate most metabolic effects of insulin. Phosphorylation of IRS helps propagate and

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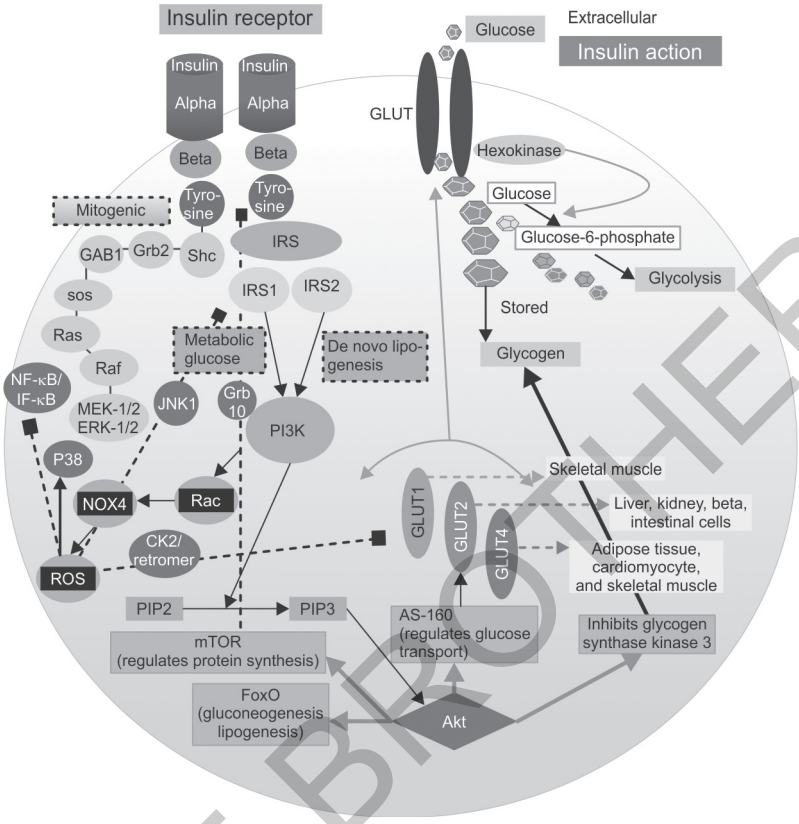

(AS160: Akt substrate of 160 kDa; CK2: casein kinase 2; ERK: extracellular signal-regulated kinase; FoxO: forkhead box-containing protein O; GAB1: Grb2-associated-binding protein 1; Grb2: growth factor receptor-bound protein 2; GLUT2: glucose transporter type 2; IF-κB: inhibitory factor-kappa B; IRS: insulin receptor substrate; JNK1: c-Jun N-terminal kinase 1; MEK: mitogen-activated protein kinase kinase; mTOR: mammalian target of rapamycin; NF-κB: nuclear factor-kappa B; NOX4: NADPH oxidase 4; PI3K: phosphatidylinositol 3-kinase; PIP2: phosphatidylinositol 4,5-bisphosphate; PIP3: phosphatidylinositol-3,4,5-triphosphate; ROS: reactive oxygen species; SOS: son of sevenless)

FIG. 1: Mechanism of insulin action. Insulin binds to insulin receptor (heterotetrameric receptor) containing two extracellular alpha and two transmembranal beta domains which are attached by *tyrosine kinase domain*. ***(For color version, see plate 1)***

amplify the insulin response.[14] The two main pathways of insulin signaling arising from the IRS protein are:

1. The *metabolic* [phosphatidylinositol 3-kinase (PI3K, a lipid kinase)/Akt (also known as PKB or protein kinase B)] pathway.
2. The *mitogenic* [Raf/Ras/MEK/MAPK (mitogen-activated protein kinase, also known as ERK or extracellular signal-regulated kinase)] pathway.

Phosphatidylinositol 3-kinase is a dimer composed of a regulatory subunit (p85), two SH2 domains, and one SH3 domain associated with p110 (catalytic subunit). In the *metabolic pathway*, PI3K gets activated after the regulatory subunit of PI3K (p85 or p55) that binds IRS1 and IRS2. This results in activation of the catalytic subunit (p110) which in turn leads to the generation of

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phosphatidylinositol-3,4,5-triphosphate (PIP3) and three isoforms of Akt/PKB by phosphoinositide-dependent protein kinases 1 and 2.[15,16] Akt, in turn, leads to activation of downstream substrates such as:

- *Mammalian target of rapamycin (mTOR)*—regulates protein synthesis.[17]
- *Glycogen synthase kinase 3 (GSK3)*—regulates glycogen synthesis by inhibiting glycogen synthase.[18]
- *Forkhead box-containing protein O (FoxO) subfamily*—regulates gluconeogenesis and adipogenesis. FoxO1 translocates to the nucleus in the absence of insulin and stimulates gluconeogenesis via expression of a key enzyme "phosphoenolpyruvate carboxykinase."[19]
- *Akt substrate of 160 kDa (AS160)*—regulates glucose transport via special glucose transporter type 4 (GLUT4).[20]

The mitogenic pathway [growth factor receptor-bound protein 2 (Grb2)-son of sevenless (SOS)-Ras-MAPK pathway] is activated independent of PI3K/Akt. Activated insulin receptor and IRS proteins possess docking sites for adaptor molecules containing SH2 domains such as Grb2 and Shc. The carboxy-terminal SH3 domain of Grb2 binds to Grb2-associated-binding protein 1 (GAB1) whereas the amino-terminal SH3 domain binds to proline-rich SOS protein. SOS is a guanine nucleotide exchange factor (GEF) for Ras which catalyzes the conversion of inactive membrane-bound Ras (Ras-GDP) to an active, GTP-bound form of Ras (Ras-GTP). Ras-GTP then stimulates downstream effectors such as the Raf (Ser/Thr kinase), which further stimulates downstream MEK1 and MEK2 that phosphorylate and activate MAPKs such as ERK1 and 2. ERK1/2 can directly stimulate cell proliferation/differentiation, regulate gene expression, and influence extranuclear events such as cytoskeletal reorganization via phosphorylation and activation of targets in cytosol and nucleus.[21]

Transport of Glucose

Glucose is a hydrophilic molecule (molecular weight 180 Da) and too big to cross the cell membrane. It requires specific carrier molecules called GLUTs to transport them intracellularly. There are at least 14 isoforms of GLUT (1–14) and unlike other isoforms, GLUT4 is entirely dependent upon insulin. *GLUT1* has a very high affinity for glucose. It is concentrated in endothelial cells of blood-tissue barriers and shuttles glucose between blood and organs via passive diffusion that have limited access to small solutes. It is the predominant transporter in skeletal muscle responsible for 3–40% of the basal uptake of glucose. *GLUT2* is present in the liver, kidney, β-cells, and intestinal cells and *GLUT4* is predominantly present in adipose tissue, cardiomyocyte, and skeletal muscle. In the skeletal muscle, glucose uptake is carried out predominantly by GLUT1 (lies on the plasma membrane) and GLUT4 (resides inside intracellular storage vesicles, being translocated to the plasma membrane by insulin action or muscle contraction). GLUT2 is a member of the SLC2A2 (solute carrier family 2 member 2) which is a membrane transporter that facilitates glucose entry down the concentration gradient.[22,23] GLUTs are linked to the high-affinity hexokinase enzyme which phosphorylates glucose to glucose-6-phosphate. Insulin-mediated glucose uptake undergoes either *glycolysis* or *glycogen synthesis*. Approximately 75% of glucose uptake form glycogen in the muscle which represents 2% of the entire muscle mass and 10% of the liver mass by

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weight. Approximately 5–60% of glucose uptake undergoes fatty acid oxidation after glycogen synthesis gets overwhelmed. The greater the glucose delivery, the greater the de novo lipogenesis. Insulin has shown to independently regulate glycolysis and glycogen synthesis.[24] Other docking proteins, such as CBL, APS, SH2B, GAB1/2, and DOCK1/2, are involved in the insulin signaling cascade.[25] For example, Src homology 2 (SH2) interacts with phosphorylated tyrosine residue (pTyr972) (part of the phosphorylated tyrosine kinase activated loop) to regulate critical regulatory functions of insulin.[26] Carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1) is another key regulator of the insulin receptor facilitating receptor internalization. Tyrosine phosphatases, particularly type 1B, can dephosphorylate the insulin receptor. NADPH oxidase 4 (NOX4)-derived hydrogen peroxide can inhibit tyrosine phosphatase, particularly type 1B activity.[27]

*Insulin resistance (**Fig. 1**)* is defined as a decrease of insulin-mediated glucose uptake in insulin-sensitive tissues and increased hepatic glucose production. When insulin secretion cannot compensate for this resistance, T2DM is the end result. Accumulation of intracellular fatty acid metabolites, such as diacylglycerol, fatty acyl-CoAs, or ceramides as occur in obesity and T2DM, can lead to phosphorylation of serine/threonine sites on insulin receptor substrates. The phosphorylated proteins fail to associate with PI3K resulting in a blunted response to insulin signal cascade causing insulin resistance.[28] In the 1980s, Reaven proposed that insulin resistance was associated with abdominal obesity. He proposed the association of insulin resistance with other cardiometabolic abnormalities [fasting hyperglycemia, elevated plasma triglycerides, low high-density lipoprotein cholesterol (HDL-C), and hypertension].[29] Insulin resistance has been associated with hypercoagulable state, endothelial dysfunction, and elevated inflammatory markers. The ARIC (Atherosclerosis Risk in Communities) study prospectively analyzed 12,606 nondiabetic participants and found that insulin resistance measured by the homeostatic model assessment of insulin resistance (HOMA-IR 1.0–2.0) was strongly associated with increased risk of heart failure.[30]

Causes of Insulin Resistance (Fig. 2)

They include the following:

- *Inflammatory mediators* such as tumor necrosis factor-α (TNF-α), monocyte chemotactic protein-1 (MCP-1), C-reactive protein (CRP), and interleukins are upregulated in insulin resistance. TNF-α has shown to impair serine phosphorylation of IRS1 and reduce GLUT4 expression.[31] Interleukin-6 (IL-6) has shown to impair phosphorylation of insulin receptor and IRS1 via induction of suppressor of cytokine signaling 3 (SOCS3), a potential inhibitor of insulin signaling.[32]
- *Adipokines* are a large family of cytokines (leptin, adiponectin, and apelin), which are generated and released by adipocytes and are involved in the pathogenesis of obesity and insulin resistance. Leptin has shown to positively correlate with body mass index (BMI), waist circumference, and insulin resistance. It has shown to impair phosphorylation of IRS1 and reduce glucose uptake.[33] Adiponectin (high-molecular-weight isoform) has shown

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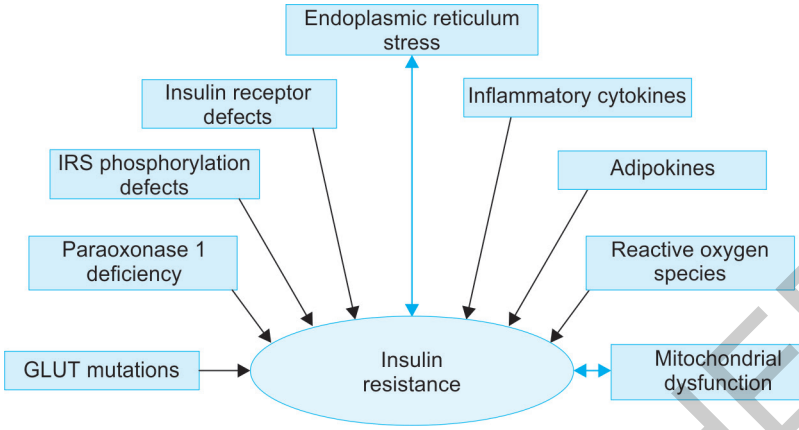

(GLUT: glucose transporter; IRS: insulin receptor substrate)

FIG. 2: Causes of insulin resistance. The blue double arrows indicate ambiguity about whether mitochondrial dysfunction and endoplasmic reticulum stress initiate or perpetuate insulin resistance.

to promote insulin sensitivity in skeletal muscle by increasing AMPK, p38 MAPK, and peroxisome proliferator-activated receptor α (PPARα)-induced fatty acid oxidation and glucose uptake via enhanced GLUT4 translocation, which is reduced in T2DM.[34] Resistin is another proinflammatory adipokine that induces secretion of TNF-α and IL-6 from various cells including pancreatic acinar cells causing insulin resistance.[35] Visfatin has shown to increase glucose transport by activating AMPK/p38, MAPK, and GLUT4 expression.[36] Fibroblast growth factor 21 (FGF21) is established as a key mediator of fat oxidation and has shown positive effects on insulin sensitivity and glucose tolerance in skeletal muscle. It has shown to increase both basal and insulin-stimulated glucose uptake. It is increased by 20% in T2DM and may represent a compensatory mechanism to maintain glucose metabolism.[37] Omentin-1 has been found to be lower in obese individuals and negatively correlate with insulin resistance and newly diagnosed T2DM patients. It is an anti-inflammatory adipokine, which suppresses TNF-α via inhibiting p38 and c-Jun N-terminal kinase (JNK) pathways. It has shown to induce Akt phosphorylation and enhance insulin-stimulated glucose uptake.[38] Secreted protein acidic and rich in cysteine (SPARC) (osteonectin), C1q tumor necrosis factor-related protein 3 (CTRP3), follistatin-like 1, preadipocyte factor 1, and chemerin are other adipokines that have shown to effect insulin sensitivity in animal studies.

- *Oxidative stress*: Oxidative stress can impair insulin signal transduction and cause adipokine dysregulation via several pathways. Reactive oxygen species (ROS), such as superoxide, hydrogen peroxide, and hydroxyl radical ions, are normally produced at low physiological levels mostly in the mitochondria and peroxisomes where they are useful, acting as signaling molecules in the maintenance of physiological functions (redox biology); however, they become damaging (oxidative stress) to cells [deoxyribonucleic acid (DNA),

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protein, or lipids] beyond these low physiological levels.[39] Normally, the cell responds to physiological increase in insulin in response to blood glucose by increasing GLUT4 expression; however, pathologically high levels of insulin signaling downregulate GLUT4 resulting in hyperglycemia, worsening hyperinsulinemia, and increased ROS generation. NOX4 is a powerful oxidizing enzyme that produces ROS in the presence of hyperinsulinemia.[40] Hyperinsulinemia results in a shift in the signaling pathway wherein PI3K phosphorylates Rac instead of phosphatidylinositol 4,5-bisphosphate (PIP2), thereby amplifying the activity of NOX4 leading to enhanced production of ROS.[41] ROS, in turn, activates casein kinase 2 which in turn activates the retromer (plays a central role in the retrieval of several different cargo proteins from the endosome to the *trans*-Golgi network) which then signals the trans-Golgi network downstream resulting in degradation of GLUT4 by the lysosomes instead of translocation to plasma membrane to carry out glucose transport.[42] ROS can activate nuclear factor-kappa B (NF-κB)/IκB (inhibitor of the NF-κB) kinases (IKK-β) and c-Jun NH2-terminal kinase pathways, which, in turn, phosphorylate IRS causing its dysregulation. NF-κB exists mainly as a heterodimer sequestered in the cytosol as an inactive complex bound to inhibitory κBs (IκBs). Phosphorylation of IκBs via IKK activation leads to further ubiquitination and proteosomic degradation resulting in free NF-κB which then translocates to the nucleus, where it binds to the κB sites in the promoter regions of target genes regulating protein expression. NF-κB causes downregulation of transcription factors necessary for differentiation and maintenance of the β-cell function such as insulin promoter factor 1 [pancreatic and duodenal homeobox 1 (PDX1)] and promotes expression of proapoptotic genes such as *CHOP*, *GADD1*, or *DDIT3* related to ER stress.[43] Oxidative stress can also reduce Akt/PKB phosphorylation and serine phosphorylation (serine 307 site) of IRS1.[44]

Defects in Serine Phosphorylation of Insulin Receptor Substrate

Insulin receptor substrate and IRS2 are polypeptides composed of >1,200 amino acids. Reduced insulin receptor phosphorylation in response to insulin has been reported in skeletal muscle of patients with T2DM. Any factor that reduces IRS1 phosphorylation or induces serine IRS1 phosphorylation at 307 site can impair insulin signal transduction.[45] Multisite serine/tyrosine phosphorylation of IRS can be stimulated by hyperinsulinemia or specific activation of the mTORC1 → ribosomal protein S6 kinase 1 (S6K1) branch of the insulin signaling cascade. Feedback S/T phosphorylation of IRS by mTORC1 and/or S6K1 leads to its disappearance during persistent insulin stimulation leading to insulin signal downregulation.[46] Grb10 is a cytoplasmic adaptor protein that decreases insulin resistance. Deletion of *Grb10* gene in animal studies has shown increased growth, enhanced insulin signaling, and improved glucose tolerance. Grb10 overexpression, on the other hand, causes impaired growth, glucose intolerance, and insulin resistance. mTOR acts via Grb10 overexpression to reduce insulin signaling.[47] Multisite S/T phosphorylation of IRS renders it inactive which along with their degradation causes desensitization of insulin signaling.[48]

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Mutations of Glucose Transporter Type 4

The intracellular insulin targeting of GLUT4 is dictated by specific insulin responsive structural motifs within cytoplasmic domains of the transporter. Mutations involving two leucine residues at the extreme C-terminus of insulin responsive motif result in aberrant targeting of GLUT4 to form large dispersed membrane vesicles that are not insulin responsive.[49] Two consecutive mutations in arginine residues (positions 333 and 334) of the GLUT4 transporter can significantly impair the function of GLUT4 and suppressed glucose transport resulting in hyperglycemia.[50]

Paraoxonase-1 Deficiency

Paraoxonase-1 deficiency has shown to reduce insulin resistance by promoting GLUT4 activity. It has been associated with increased oxidative stress, increased p38 MAPK activity, and attenuated insulin-mediated tyrosine phosphorylation of muscle IRS1 in in vitro studies.[51] A study involving 287 South Indian T2DM patients showed that paraoxonase-1 (Q192R and L55M) polymorphisms may play a crucial role in the pathogenesis and susceptibility of insulin resistance.[52]

Insulin Receptor Dysfunction

Point mutation (position 382) of the insulin receptor (valine substituted for phenylalanine) has been associated with retarded insulin signaling and increased receptor degradation.[53] Circulating polyclonal anti-insulin antibodies of the immunoglobulin G (IgG) subtype have been associated with impaired insulin receptor function.[54]

Mitochondrial Dysfunction

Understanding the Biology of Mitochondrial Function

Mitochondria are essential for maintaining cellular energy homeostasis. They play an important role in the physiological generation of ROS that helps regulate intracellular signaling, detoxification, scavenging, apoptosis, autophagy, regulation of cytoplasmic calcium, oxidation of fatty acids, and signal transduction in the innate immune response.[55,56] They are the seat of oxidative phosphorylation responsible for the generation of adenosine triphosphate (ATP) in the inner mitochondrial membrane cristae. Electrons generated from nutrient (e.g., glucose) oxidation are carried by electron donors [nicotinamide adenine dinucleotide phosphate (NADH) and flavin adenine dinucleotide (FADH2)] to the electron transport chain (complex I through V) located in the inner mitochondrial membrane which results in pumping of protons from the mitochondrial matrix to the intermembrane mitochondrial space. This creates both a pH gradient and a membrane potential across the inner membrane. Protons return to the matrix via complex V (ATP synthase) due to the gradient resulting in the generation of ATP from adenosine diphosphate (ADP) and inorganic phosphate. These protons can also leak across the inner membrane through uncoupler proteins and release energy called *nonshivering thermogenesis*, thus bypassing ATP synthesis.[57] Peroxisome

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proliferator-activated receptor γ coactivator 1 (PGC1) family of transcriptional coactivators has emerged as a key regulator of cellular and mitochondrial metabolism. It consists of three members [peroxisome proliferator-activated receptor gamma coactivator 1α (PGC1α), peroxisome proliferator-activated receptor gamma coactivator 1β (PGC1β), and peroxisome proliferator-activated receptor gamma coactivator γ (PGCγ)] which interact with transcription factors and nuclear receptors (peroxisome proliferator-activated receptor γ nuclear receptors, nuclear respiratory factor 1, and estrogen-related receptor) to exert their biological functions.[58] PGC1α (encoded by the *PPARGC1A* gene) is the most studied member of the PGC1 family which is a positive regulator of mitochondrial biogenesis and respiration, adaptive thermogenesis, gluconeogenesis, and other metabolic processes. PGC1α is highly inducible by physiological stimuli such as exercise, temperature (cold), fasting, nutrients, cellular stress, and nitric oxide. PGC1α is a key regulator of ROS-detoxifying enzymes and has shown to increase mitochondrial mass and function and minimize the impact of ROS on cell physiology.[59]

Mitochondrial fission: Many proteins belonging to the dynamin GTPase protein family, e.g., dynamin-related protein 1 (Drp1) present in the cytosol, are involved in mitochondrial turnover. Dynamin is a form of mechanical enzyme that forms large homomultimeric complexes that encircle and contracts the mitochondrion in spirals at its narrowest part until membrane fission occurs. Much of the energy for the mechanical force comes from guanosine triphosphate (GTP) hydrolysis. Dynamin gets recruited to the outer mitochondrial membrane where it interacts with proteins such as fission protein 1 (Fis1) and mitochondrial fission factor (Mff) (anchored at the outer membrane). These then further interact with cytosolic Drp1 to initiate fission.[60]

Mitochondrial fusion: It involves both outer membrane and inner membrane fusion. These processes are also dependent on dynamin-related GTPase proteins (mitofusin: Mfn1 and Mfn2). The outer membrane fusion is carried out by Mfn that has a transmembrane domain, a cytosolic domain (GTPase domain), and two coil protein-interaction domains that mediate binding of Mfn proteins to neighboring mitochondria.[61,62] Mutations in *Mfn* genes have shown loss of fusion and an increase in fragmented mitochondria. *Inner mitochondrial membrane fusion* is controlled by a dynamin-like GTPase optic atrophy 1 (OPA1) found in the intermembrane space/inner membrane. OPA1 undergoes proteolysis by presenilin-associated rhomboid-like (PARL); member of the rhomboid family of proteases, Yme1 (AAA metalloprotease found in the intermembrane space); and paraplegin (AAA protease located in the inner mitochondrial membrane) to form long and short OPA1 isoforms, which further regulate the morphology of mitochondrial cristae. Mfn1 and OPA may interact directly, and the energy for fusion of both membranes is provided by GTP hydrolysis.[63] Mitochondrial phospholipase is localized to the outer mitochondrial membrane with a catalytic domain facing the cytosol. It can hydrolyze the inner mitochondrial membrane component cardiolipin and therefore may take part in managing the lipid structure of membranes during mitochondrial fusion.[63] Mitochondria are directly connected to programmed apoptosis by releasing factors such as cytochrome *C* from their intermembrane space into the cytosol. This, in turn,

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activates a cascade of cysteine proteases (caspases) which lead to cellular degradation in an orderly fashion to kill the cell.[64] On receiving a death signal, apoptosis of the mitochondria occurs via the interaction of the apoptosis protein of the Bcl-2 family (prosurvival—Bcl-2 and Bcl-xL; prodeath—Bax and Bak). Bax along with cytochrome C translocates to mitochondria and colocalizes with Drp1 and Mfn2 at mitochondrial fission sites causing mitochondrial fragmentation.[65]

Mitochondrial Dysfunction in Brown Adipose Fat

Brown adipocytes contain many mitochondria contributing to whole-body metabolism and thermogenesis. White adipose tissue has the capability of so-called browning or beiging. Under the influence of cold exposure, white adipose tissue can undergo browning. Uncoupled mitochondria in active brown fat are specialized to produce heat by oxidizing large amounts of substrates (glucose) via the action of uncoupled protein-1 located along the inner membrane. In this manner, they can actively drain glucose from the circulation. The primary role of the uncoupled proteins in the mitochondria is to regulate the production of ROS and transport certain metabolites across the mitochondrial membrane. Uncoupled protein-2 has shown to influence glucose homeostasis by regulating intracellular events such as insulin secretion (β-cell), food intake behavior (brain), and adiponectin secretion (white adipose tissue). Uncoupled protein-3 has shown to promote fatty acid oxidation in muscle, thereby influencing glucose metabolism indirectly. Polymorphisms in genes encoding uncoupled proteins have been linked with obesity and T2DM.[66] The density of mitochondria in a cell is largely controlled by two opposing forces: (1) Fission and (2) Fusion. Through the years, mitochondrial genome has been passed horizontally from the organelle to the host nucleus allowing the cell to gain transcriptional control over mitochondrial function.[67]

Mitochondrial Dysfunction in White Adipose Fat

Besides storage, adipose tissue can also regulate systemic metabolism by secreting adipokines that regulate appetite, inflammation, insulin action, and glucose and lipid metabolism.[68] White adipose tissues can be divided into visceral adipose tissue (harmful fat) and subcutaneous adipose tissue (beneficial fat). Visceral adipose fat is associated with increased risk of T2DM, dyslipidemia, atherosclerotic cardiovascular disease, and mortality.[69,70] With increasing nutrient load, adipocyte undergoes enhanced lipid storage, glycolysis, and reduced mitochondrial biogenesis and function. Mitochondrial content and number are reduced in subcutaneous fat biopsies. Functional studies in white adipose fat, both visceral and subcutaneous, have shown decreased adipocyte oxygen consumption and ATP production[71] and reduced expression of mitochondrial oxidative phosphorylation encoding genes in abdominal fat of obese twins.

Mitochondrial Dysfunction in Liver (Hepatic Insulin Resistance)

The liver can store excess glucose as glycogen (glycogenesis), enhance systemic glucose during exercise by depolymerizing glycogen (glycogenolysis), produce glucose from amino acids and glycerol (gluconeogenesis), break down amino

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acids (deamination) to form urea, and regulate fatty acid β-oxidation. Effects of insulin on hepatocyte are to promote glycogenesis and inhibit glycogenolysis and gluconeogenesis. Obesity associated with insulin resistance can result in reduction of PGC1α expression and increased hepatic lipid accumulation. Animal studies have shown decreased activity of mitochondrial complexes C-1 to C-V in isolated mitochondria of the liver.[72] Mitochondria are the major site for degradation of fatty acids in the liver (fatty acid β-oxidation). FFA in plasma can induce hepatic mitochondrial dysfunction and cause accumulation of hepatic fatty acids, which in turn lead to hepatic insulin resistance.[73] Mitochondrial autophagy (mitophagy) is a catabolic process that can selectively remove damaged mitochondria (autophagolysosomes) in order to maintain mitochondrial function by accelerating fatty acid oxidative degradation and suppressing production of ROS by the damaged mitochondria. *Within the hepatocyte, the predominant insulin signal cascade* involves IRS1-PI3K-PDK1-Akt-GLUT2. Full activation of Akt requires phosphorylation by both PDK1 and mTORC2 at positions Thr308 and Ser473, respectively. Phosphatase and tensin homolog (PTEN) oppose the action (dephosphorylation) of PI3P. Akt then activates:

- *Glycogen synthase 2* by glucose-6-phosphate (G6P) and inhibits glycogen synthase kinase 3, thereby promoting glycogen formation.
- *mTORC1* via inhibition of tuberous sclerosis proteins, which, in turn, activates the lipogenic gene program via activation of sterol regulatory element-binding protein 1c and glucokinase, which phosphorylates glucose to G6P. G6P then activates carbohydrate-response element-binding protein, which induces lipogenesis.
- *FoxO1* inhibits glucose-6-phosphatase and phosphoenolpyruvate carboxy-kinase 1, thereby preventing glucose breakdown (glycolysis) and promoting glycogen formation.[74]

Excess consumption of high-fat diet and disordered white adipose tissue lipolysis (white fat insulin resistance) results in increased plasma FFA flux which accumulates as ectopic fat. FFA is converted to acetyl-CoA and transported to mitochondria of tissues such as liver, muscle, bone, pancreas, and heart causing lipotoxicity. FFAs degrade IκB-α which then promotes the activity of IKK-β/NF-κB leading to enhanced expression of interleukin-1β (IL-1β), TNF-α, and IL-6 in liver tissue. This then leads to retardation of the insulin cascade (*refer to this section of oxidative stress on page 23*).[75]

Obesity, insulin resistance, and T2DM have shown to alter/reduce mitochondrial number and/or function resulting in "mitochondrial dysfunction". It remains uncertain whether abnormalities in mitochondrial function are a cause, consequence, or key contributor to insulin resistance and T2DM. Moreover, data from humans and rodent model suggest that insulin resistance is often, but not always, associated with mitochondrial disruption. Studies have shown a reduced mitochondrial content in the subsarcolemmal compartment and a 3-fold reduction in muscle oxidative phosphorylation capacity in T2DM in muscle biopsy samples[76] and isolated mitochondria. Some studies showed 40% reduction in NADH:O_2 oxidoreductase activity and 50% lower cytochrome C oxidase activity, all pointing to mitochondrial dysfunction.[73] Obese insulin-resistant individuals showed a reduction in expression of genes coding for PGC1α and PGC1β. In other studies, mutations in mitochondrial fusion gene

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Beyond Type 2 Diabetes Mellitus

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- Chapter 4: Discusses elegantly the various causes of hyperglycemia in a young patient and the ideal clinical approach.
- Chapter 6: Discusses the evolution of blood pressure control as a means to reduce renal and cardiovascular outcomes. It provides clarity on the uncertainty of use of conventional blood biomarkers in evaluating renal function.
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- Chapter 8: Provides an in-depth understanding and therapeutic approach of rheumatic conditions associated with type 2 diabetes mellitus, while providing a complex interplay of factors that may heighten the risk of diabetes-related complications. It also discusses the detailed use and side effects of steroids.
- Chapter 9: Provides a detailed look into various immune dysregulatory pathways and types of infections a diabetic is at risk of with a highlight on COVID-19.
- Chapter 10: Is one of my favorites written along with my daughter. It discusses the history and evolution of sugar consumption by Indians. It provides an understanding of medical nutrition therapy in diabetes, glycemic load and glycemic index of foods, methods of cooking that impact glycemic index of foods, type of diets with their possible application to patients with type 2 diabetes mellitus, impact of diet on glycemic control, cardiorenal markers, and sleep and physical performance.
- Chapter 11: Discusses management of glycemia in special situations (renal disease, liver disease, and Ramadan).
- Chapter 12: Provides an elegant overview of risk factor control with details on role of exercise, blood pressure, and lipid management in diabetes.

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Printed in India

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