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Molecular Basis of Pathogenesis of Diabetes Mellitus Type 2- a New Perspective

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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Opinion Article

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ABSTRACT

Decreased insulin secretion due to beta cell dysfunction of the pancreas and defective utilization of insulin due to insulin resistance / Hyperinsulinemia are two important issues in the pathogenesis of DM2. There are many explanations in the literature to account for these two observed phenomena and their interrelationship. DM2 is believed to occur due to a complex interplay of environmental and Behavioural factors in genetically predisposed persons. Among the prominent theories explaining the pathogenesis of DM2, the viscera- Portal hypothesis, the Ectopic fat hypothesis and the adipose tissue as an endocrinal gland are prominent. Besides, the role played by oxidative stress, metabolic stress, mitochondrial dysfunction, endoplasmic reticulum stress, etc. are also advanced. It is felt that basic to and at the core of all the observed facts, is the shift of energy metabolism from normal glycolysis to B- oxidation of fats. Hence, how B - oxidation prevails over glycolysis is the fundamental issue to be addressed together with its interrelationships with insulin resistance, as to which is the cause and which is the effect. At the molecular level, an attempt to find answers to the above questions is made in this paper.

To this extent, the Randle fatty acid cycle (Substrate competition theory of Randle) is suitably

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modified and applied to explain the switch of Energy metabolisms in DM2 .Defective disulfide bond formation of the insulin receptor which makes it physiologically ineffective, is suggested as the cause of the insulin resistance where as the prevailing molecular mechanisms stress on post-receptor signaling defect. The cause and effect of both are discussed. This line is considered to be a departure from traditional approaches broached above and briefly outlined in this article.

Keywords: Beta cell dysfunction; insulin resistance; hyperinsulinemia; randle cycle and disulfide bonds.

1. INTRODUCTION

Both the beta cell dysfunction and insulin resistance, are two important determinants in the pathogenesis of DM2. Beta cell dysfunction [1] may be due to decreased Beta cell mass which is genetically determined or governed by acquired factors that reduce the life of the functional Beta cell mass. Among the acquired factors are glucotoxicity, Lipotoxicity or enhanced apoptosis. The later may be autoimmune or cytokine mediated. Autoimmune destruction of pancreatic β-cells may be a factor in a small subset of type 2 diabetic patients seen in about 10 % of Scandinavian patients and has been termed the syndrome of 'latent autoimmune diabetes in adults [2]. The Beta-cell dysfunction may result initially in increased insulin secretion followed by decreased secretion, as B -cell exhaustion begins to appear. Decreased insulin secretion may be due to decreased Beta cell mass the initially increased insulin secretion, ie. Hyperinsulinemia is due to the attempt of B – Cells to get over the insulin resistance. The later is manifested as unresponsiveness of the target organs like - the liver, the skeletal muscle, and the adipose tissue to the action of Insulin. Hepatic involvement causes increased gluconeogenesis, the skeletal muscle fails to take up the glucose and the adipocytes to produce increased free fatty acids (FFA).

Due to increased lipolysis, contributing to the lipotoxicity and insulin resistance the net effect is hyperglycemia which is responsible for hyperinsulinemia and insulin resistance as wall as glucotoxicity on chronic exposure chronic hyperinsulinemia inhibits both insulin secretion [3] and action and hyperglycemia can impair both the insulin secretory response to glucose. [4] as well as cellular insulin sensitivity. Sedentary lifestyle, lack of exercise and diet Excess in calories and saturated fats are contributor$_y$.The importance of diet and exercise is evident from the fact that dietary modification and exercise can lower the risk of progression from impaired glucose tolerance to type 2 diabetes and may also prevent the development of IGT in nondiabetic individuals at high risk. Obesity contributes to insulin resistance through several pathways, including an imbalance in the concentrations of hormones (e.g., increased leptin, reduced adiponectin, and increased glucagon), increased concentrations of cytokines (eg, tumour necrosis factor α, interleukin 6, suppressors of cytokine signalling (eg, suppressor of cytokine signalling inflammatory signals, and possibly retinol-binding protein 4.1 [5]. Loss of glucose sensor mechanism of the B-cells of the pancreas, loss of pulsatile nature [6] and a asynchronous nature of the insulin secretion, the loss of first phase insulin secretion [7] and decreased conversion of inulin from proinsulin [8] all suggested as causes of B – cell dysfunction's of the first phase insulin secretion, during glucose stimulation, may antedate the onset of glucose intolerance in type 2 diabetes.[8] Later in the course of the disease, the second phase release of newly synthesized insulin is impaired, an effect that can be reversed, in part at least in some patients, by restoring strict control of glycemia. This secondary phenomenon, termed desensitization or β-cell glucotoxicity, is the result of a paradoxical inhibitory effect of glucose upon insulin release and may be attributable to the accumulation of glycogen within the β-cell as a result of sustained hyperglycemia [9]. The role of amyloid whether it is primary or secondary is debatable [10]. Other candidates that have been proposed are sorbitol accumulation in the β-cell or the non-enzymatic glycation of β -cell proteins. The role of GLUT 2 which transports glucose into B-cells is suggested by the fact that experimentally, high-fat diet inhibited GLUT 2, resulting in the failure of glucose transport into the cell [11].

2. THE PORTAL-VISCERAL HYPOTHESIS

It is based on the effects of increased FFA that reach liver, through a portal vein from the gut and the central adipose tissue stores causing hepatic insulin resistance and hepatic steatosis [12].

2.1 Ectopic Fat Transport

Due to an increased influx of FFA due to increased lipolysis uninhibited by HSL due to hyperinsulinemia and insulin resistance and the predominance of glucagon action when HSL the Capacity to hold the increased influx of FFA, it is transported to organs where it is not typically stored – like hypothalamus, liver, skeletal muscle, pancreas and gut. The FFA cause inflammation in not only the adipocyte but also in the target organs just mentioned, with varied effects.

2.2 FFA Induced Inflammation and Organ Dysfunction

Inflammation set up by fat accumulation in the target organs is considered the crucial factor in the pathogenesis of DM2.Intracellular lipid accumulation occurs caused by enhanced lipolysis, adipocyte dysfunction together with an impairment in FFA oxidation in the mitochondria. The metabolites like long-chain acyl-CoA (LC-CoA), diacylglycerol (DAG), and ceramides are deleterious for the cell. These fatty acid metabolites induce sustained activation of as protein kinase C (PKC) isoforms, IKB-kinase- β and Jun N-terminal kinase, which phosphorylates insulin-recptor substrates (IRS) on serine residues. The subsequent defects in insulin signalling lead to a decrease in cellular function that depends on the cell type.

2.3 The Hypothalamus

Hthe hypothalamus is the central control point for the development of insulin resistance. Either excess calories or saturated fats (especially palmitic acid) can cause inflammation in the hypothalamus, leading to resistance to the satiety signaling of both insulin and leptin [13,14]. As a result, satiety is attenuated, and hunger is increased. Increased inflammation appears in the hypothalamus within 24 h after beginning an HFD as indicated by increases in JNK and IKK proteins as well as increased expression of TLR-4 receptors and detection of ER stress [15] induces inflammation via activation of NF-κB, which inhibits the normal hormonal signaling of leptin and insulin necessary to create satiety. Activation of JNK is often preceded by the increase in ER stress [16] this sets up a vicious cycle of increased hunger that eventually leads to the accumulation of excess calories as stored fat in the adipose tissue. It should be noted that the inflammation in the hypothalamus precedes any weight gain in the adipose tissue [17] is the interaction of the hypothalamus with the liver via signaling through the vagus nerve [18]. This may explain why any inhibition of TNFα or TLR-4 signaling in the hypothalamus also decreases glucose production in the liver. HPA axis is activated to release more cortisol thereby increasing insulin resistance [19]. On the other hand, if fatty acid being sensed is primarily oleic acid, there will be a reduction in NPY (a powerful appetite-inducing hormone) expression in the hypothalamus that promotes satiety [20].

2.4 The Heart

Impaired insulin signaling, and hence GLUT-4 involved glucose uptake premature adipocyte apoptosis and heart failure. Finally, accumulation of lipids increased DAG and ceramide levels induce a vicious cycle in which a further impairment in mitochondrial fatty acid oxidation occurs that also has negative effects on myocardial function.

2.5 Skeletal Muscle

Accumulation of intramyocellular lipids (IMCLs) is associated with insulin resistance [21,22] and T2DM. But endurance-trained athletes, who are highly insulin sensitive, also have a

high IMCLs content [23] the ratio between IMCLs and fat oxidative capacity is a better marker for insulin resistance than IMCLs alone.

2.6 The Liver

tl causes NAFLD at accumulation in the liver is associated with hepatic insulin resistance as well as with peripheral insulin resistance in skeletal muscle and adipose tissue [24,25].Hitherto, fatty acids (i.e., lipokines) and the oxidation by-products of cholesterol and poly-unsaturated fatty acids, such as the non-enzymatic oxysterols and the reactive aldehyde species, respectively, emerge as key modulators of (pre)adipocyte signaling through Wnt/β-catenin and MAPK pathways, and potential regulators of glucose homeostasis

2.7 Pancreas

Beta cells are very prone to toxicity mediated by inflammatory agents. In particular, 12-HETE derived from AA is very toxic to the beta cells. With the destruction of the beta cells by 12-HETE, the pancreas is no longer able to maintain

compensatory levels of insulin secretion to reduce blood-glucose levels, and the development of type-2 diabetes is rapid.

2.8 GIT

Fatty-acid receptors such as GPR120 and GPR40
and fatty binding proteins such as CD36 are present in the mouth and line the entire GI tract. These receptors sense fatty acid content of the diet. CD36 binds oleic acid and helps convert into oleylethanolamide (OEA) [26]. OEA activates PPARα gene transcription factor to increase satiety and also the expression of the enzyme required for fatty acid oxidation. Thus the type of fat sensed in mouth and gut provides satiety signals to the hypothalamus. The increased satiety lowers the

caloric intake and reduces the development of ER and oxidative stress thus indirectly reducing the development of insulin resistance. The hormones secreted by these cells that relate to insulin resistance include CCK (from the proximal I-cells) and GLP-1 and PYY (from the distal L-cells).CCK works in association with serotonin to suppress hunger by directly interacting with the hypothalamus via the vagus nerve) [27]. PYY and GLP-1 are the hormones released by protein and glucose respectively when sensed by the L-cells more distal in the GI tract. Both of these hormones are powerful inducers of satiety

LPS induced gram-negative bacterial endotoxemia can cause IR. Any LPS fragments that enter the blood stream are carried by chylomicrons to the lymph system where it can then interact with the TLR-4 receptors in the body to increase TNFα levels that can generate insulin resistance in a wide variety of organs.

3. INFLAMMATION IS ALSO LINKED TO THE FOLLOWING ENTITIES THAT ARE IMPLICATED IN THE PATHOGENESIS OF DM2

3.1 Metabolic Stress

Is the, catabolic response to severe injury or disease the degree of metabolic stress is correlated with the severity of the injury.

The causes: 1) in DM 2 release of catabolic hormones like glucagon and cortisol etc. 2) hyperglycemia, 3) reliance on gluconeogenesis with subsequent release of glucose.

3.2 Oxidative Stress

By reactive oxygen species (ROS) production can cause IR.

Pro-oxidants and markers for oxidative tissue damage, such as 8-hydroxy-deoxyguanine, 4-hydroxy-2-nominal (HNE) proteins, 8-epi-Prostaglandin F2α, hydroperoxides, and oxidation of DNA bases have been reported to be elevated in serum, plasma, white blood cells, and pancreas biopsies of patients with type 2 diabetes. Elevated glucose concentrations increase levels of reactive oxygen species in beta-cells, that islets have intrinsically low antioxidant enzyme defenses, that antioxidant drugs and overexpression of antioxidant enzymes protect beta-cells from glucose toxicity, and that lipotoxicity, to the extent it can be attributable to hyperlipidemia, occurs only in the context of pre-existing hyperglycemia, whereas glucose toxicity can occur in the absence of hyperlipidaemia.

3.3 Endoplasmic Stress

Hypoxia can occur when vascularisation is inadequate for the expanded adipose tissue. Also, endoplasmic reticulum (ER attic reticulum (ER) stress [15], either induced by hypoxia or nutrient excess, leads to an unfolded protein response (UPR) [16]. In the ER, proteins are translated, folded, and assessed for quality before release. With ER stress the number of misfolded proteins increases and triggers the UggPR. The UPR induces genes involved in assembling, folding, modifying, and degrading proteins to alleviate ER stress and triggers the activation of stress and inflammatory pathways and the production of cytokines and chemokines that interfere with the insulin signaling pathway. More inflammatory adipokines and cytokines than functional adipocytes. Also, stressed adipocytes attract immune cells (among which macrophages) into the stromal vascular fraction. The physiological role of infiltrating macrophages is probably debris clearing (ER stress can eventually lead to premature adipocyte apoptosis). Eventually, however, a positive feedback cycle is formed in which infiltrating macrophages recruit more immune cells, and a state of chronic inflammation is induced. Some of the cytokines and adipokines produced interfere with adipocyte differentiation, others with insulin

signaling. Some, like TNF-α and IL-6, impair adipocyte differentiation, reduce lipid accumulation, and increase adipocyte lipolysis

3.4 Glucotoxicity

Glucose itself is capable of generating reactive oxygen species (ROS) in β-cells is essential to the hypothesis that glucose-induced oxidative stress is a the Mechanism for glucose toxicity. Potential glucose-related pathways through which ROS can be formed include autoxidation, oxidative phosphorylation, glycosylation, and the Glucosamine pathways. Mitochondrial stress: Through UPR can cause IR.

3.5 Lipotoxicity

Lipotoxicity only occurs in the context of chronic hyperglycemia, consistent with the observation that most individuals with increased circulating lipid levels have normal β-cell function.

3.6 Mitochondrial Dysfunction

The role of stressed mitochondria acting through ectopic transport of FFA and cytokine/ chemokines mediated actions are already seen above.

3.7 Adipose Tissue as an Endocrine Gland

Adipose tissue produces classic cytokines (tumor necrosis factor-α, TNF-α, interleukin-6), chemokines (interleukin-8, monocyte chemoattractant protein-1, 1MCP-1), hormones (angiotensin II, adrenomedullin, atrial natriuretic peptide), growth factors, enzymes and enzyme inhibitors (visfatin, vaspin, plasminogen activator inhibitor-1) and acute phase proteins. Adipose tissue also secretes non-protein biologically active mediators including gasotransmitters (NO, CO and H.

3.8 Leptin

Leptin concerned with satiety. Nutritional regulation of leptin is mediated at least in part by insulin, as leptin decreases in response to low insulin levels and increases with feeding or in response to insulin stimulation. [28]

3.9 Adiponectin

In models of genetic and diet-induced obesity, adiponectin was shown to improve whole-body insulin sensitivity. Another role of adiponectin is to stimulate fatty acid oxidation and glucose uptake in skeletal muscle and adipose tissue; this effect is dependent on AMP-activated protein kinase (AMPK) signaling. Adiponectin is also involved in the suppression of hepatic glucose output through activation of AMPK [29].

3.10 TNF ALFA

The first adipose-derived factor suggested to represent a link between obesity, inflammation, and diabetes was TNF-α. Studies show that mRNA expression levels of TNF-α in adipose tissue in obesity are strongly implicated in the pathogenesis of insulin resistance; this is because it has been demonstrated that TNF-α can impair insulin signaling in hepatocytes and adipose tissue[30]. Other studies demonstrated that chronic treatment with TNF-α decreased insulin-stimulated glucose uptake in rat skeletal muscle, and targeted deletion of TNF-α or its receptors increased insulin sensitivity and glucose tolerance in obese rodents in some, but not all, studies.

3.11 IL 6

IL 6 has been demonstrated that IL-6 inhibits the insulin signalling pathway by up-regulating SOCS3 expression, which in turn is known to impair insulin-induced insulin receptor and IRS-1 phosphorylation in adipocytes and hepatocytes. Moreover, IL-6 can promote fatty acid oxidation and glucose uptake in skeletal muscle findings, which are also observed with the IL-6 family member ciliary neurotrophic factor (CNTF) [31]. Some studies have demonstrated that these effects require activation of AMPK-activated protein kinase but this mechanism is not understood In general, IL-6 inhibits lipase lipoprotein, induces lipolysis and increases glucose uptake.

3.12 ASP

It has an important effect on the increase of lipogenesis by the translocation of glucose transporter type 4 (GLUT4) in glycerol 3-phosphate and the activity of diacylglycerol acyltransferase (DGAT), an enzyme catalyzing the synthesis of triglycerides [32,33].

3.13 Resistin

Resistin has been implicated in the pathogenesis of diabetic complications and diabetes [34 35].

3.14 Visfatin

It was shown that visfatin's major function is related to energy metabolism and innate immunity and it is now regarded as a pro-inflammatory adipocytokine. Its properties induce activation of leukocytes and stimulate the production of TNF-α and IL-6 [36,37].

Plasminogen activator factor:

Plasma PAI-1 levels increase in proportion to visceral adiposity, raising the possibility that PAI-1 serves as the link between abdominal/central obesity and cardiovascular disease.

3.15 Angiotensin

Adipose tissue expresses all components of the renin-angiotensin-aldosterone system (RAAS), including angiotensinogen (AGT), renin, angiotensin I-converting enzyme, and angiotensin II type 1 receptor [3]. Moreover, adipose tissue angiotensinogen mRNA and protein levels are regulated by nutrition, leading to decreased levels of fasting and to increased levels with refeeding. Angiotensin II stimulates prostacyclin synthesis, adipocyte differentiation, and lipogenesis

4. THE PROPOSED NEW HYPOTHESIS OF THE MOLECULAR BASIS OF PATHOGENESIS OF DM 2

4.1 The Prelude

All the pathogenic mechanisms considered above in the literature, are but off- shoots of ills of either B-oxidation of fats or insulin resistance. The proposed new hypothesis is an attempt to get at the roots of the problem, ie, how the insulin resistance develops and switching of energy metabolism of normal glycolysis to abnormal B-Oxidation of fats occur at the molecular level.

4.2 The Insulin Resistance in DM2 – A Molecular Basis

This author earlier purposed disruption of disulfide bond formation of the insulin receptor as the cause of insulin résistance [38].

The structure, function, and signalling of the insulin receptor are extensively reviewed by Jason et al. [39].

The two alfa and two beta sub-units of the insulin receptor are bound by Alfa to Alfa and Alfa to Beta sub units by disulfide bonds forming the tetrameric form which is functional and autophosporylating (holoenzyme). The dimeric form, devoid of the disulfide bonds are of no functional activity. The important role played by the interconnecting disulfide bonds is thus evident [40].

The cysteine-rich regions of the insulin receptor are the ligand (hormone) binding sites [41]. Ligand binding to the tyrosinase rich enzyme region causes conformational changes, auto phosphorylation and signal transduction activity only in the tetrameric form.

The auto phosphorylation thus initiated, cascades down stream substrates which include insulin receptor substrate (IRS) 1- 4 and Shc (one of the MAPK pathway components). Phosphorylated IRS 1-4 mainly activate the phosphatidylinositol 3-kinase (PI3K) pathway and ShC mainly activates the Grb2/Sos (a downstream proteins in the MAPK pathway) etc.

PDI, a protein tightly bound to the inner mitochondrial membrane, through PDI, a protein attached to the inner membrane of endoplasmic reticulum (ER) transfers the preformed disulfide bonds (S-S)to cysteine-rich regions of a protein, in this case, the hormone insulin [42]. This is done by thiol-disulfide exchange mechanism which is explained below [43]. PDI also acts as a chaperon in the proper folding of the protein [44].

PDI has four thioredoxin – like domains two of which have canonical X C C X motif.

In the oxidized form it catalyzes the formation of disulfide bonds of the general structure of R-S-S-R.

R'SH+ R'SSR" ----------R'SSR' + R"SH

The reaction proceeds through a Nucleophile substitution type2 (SN2) mechanism. The nucleophile is the denominated thiol anion, which attacks the reacting sulfur of the disulfide bond making an S-S-S like transition state, with the negative charge being delocalized but more abundant on attacking and leaving sulphurs.

ERO oxidizes PDI whereby the di thiol, SH HS is oxidized to S-S disulfide bond ERO itself gets reduced by accepting the electrons from sulfur.

It catalyses the thiol-disulfide exchange by coupling oxidation of PDI to the reduction of H2O2 at complex 1 of ETC (see ROS below), The ERO while oxidizing the PDI, itself gets reduced by accepting electrons from the sulphur residues.

Unless ERO is in an oxidized state, it cannot oxidize further PDI. This it achieves by transferring electrons to the complex 1 of the ETC.

Normally the ETC complexes are arranged in increasing electronegativity. The electrons flow from lower to higher electronegativity, ie, from complex q to Q enzyme.

But since complex one becoming more electronegative due electrons discharged from Sulphur being at higher electronegativity, than Q enzyme, reverse transport (RET) of electrons from enzyme Q to complex 1 occurs. reducing NAD.

Both NAD reduction and ROS production require high membrane potential derived from ATP hydrolysis.

This incomplete cause reduction of single oxygen resulting in the production of Reactive oxygen species (ROS) like a super oxide, hydroxyl radicle, and H2O2, etc. [45]. Thus ERO by producing ROS is an important source of mitochondrial stress. Which unless removed causing cell damage.

Among the complexes of ETC, complex Q and complex e only produce these duper oxides, the former producing them in mitochondrial matrix only whereas the later in the inter- membrane space also. Membranes.

Complex 1 oxidizes NADH, the Q enzyme accepting as an electron acceptor, coupled with proton pump generating transmembrane potential.

MnSO4 catalyses the dismutation of O2 to H2O [46] .

The H2O2, unless removed immediately, will cause cell death. It is removed by another protein, called peroxy redoxin 4 (PROX 4), present in ER [47]. It takes an oxygen from H2O2 to form water an -SOH group, which reacts with an adjacent SH group to form S-S disulfide bond.

The ERO 1 transfers these disulfide bonds to PDI, which by thiol-disulfide exchange transfers them to Alfa to Alfa and Alfa to beta subunits of insulin receptor thus transforming it into tetrameric functionally active form, capable of signal transduction.

This is what happens under physiological conditions when normal glycolysis occurs.

The situation is different when energy metabolism shifts to Beta oxidation of fats as in DM2.
When the metabolism shifts to beta oxidation, as in DM2, the intermediate products of TCA cycle enter through complex -II, no RET occurs from succinate to complex I. [48]. Hence no ROS production and hence no disulfide bonds are formed which could Be transferred to the insulin receptor. The tetramer or holo-enzyme which can initiate conformational change and auto phosphorylation of the two sub units as well as consequent insulin receptor (IRS) and cascade of Auto phosphorylation down stream, do not take place. This is how insulin resistance in DM2 develops. The free fatty acids (FFA) especially the long chain free fatty acids (LCFA), inhibit RET from succinate to complex I, decreasing the succinate-Dependent ROS production, in spite of increased FADH2. Production due to β- oxidation of FFA [49]. This situation continues until the -β – oxidation pathway of energy metabolism prevailing over the normal carbohydrate based energy metabolism is Overcome. On the other hand, if carbohydrate based energy metabolism is restored, normal disulfide formation is resumed and the insulin sensitivity is restored.

5. SWITCHING OF ENERGY METABOLISM FROM GLUCOSE-BASED (GLYCOLYSIS) TO FAT BASED (BETA OXIDATION) IN DM 2 - A MOLECULAR BASIS

Readers are requested to go through this author's earlier paper titled ‘Randle hypothesis about DM 2 – spruce the basement before dusting the superstructure' [50] for full text, tables, and illustrations.

Under physiological conditions, there is a regular alternation between glycolysis in the fed state and Beta Oxidation of fats in the inter-prandial state. This happens because the glucose formed in the fed state stimulates the secretion of insulin and the same is taken up by target organs like liver and skeletal muscle (for glycogenesis) and

adipose tissue (for lipogenesis).The fall in blood glucose levels together with a decline in insulin levels, stimulates Glucagon under whose influence, the energy metabolism of the body shits to B Oxidation of fats. Under the influence Of glucagon, the glycogenolysis, and lipolysis, as well as gluconeogenesis, starts. The energy metabolism reverts back when feeding starts again under the influence of insulin to glycolysis, and the B oxidation stops under the waning influence of glucagon during the next feed, when insulin action dominates. This holds good even under conditions of prolonged fasting when Beta Oxidation predominates. But once re-feeding starts it reverts back to glycolysis. In other words, under both the above referred physiological conditions, there is a smooth transition from one energy metabolism to the other.

Randle (1963) proposed the theory of substrate competition [51] to explain the transition from glycolysis to Beta- Oxidation under physiological conditions of interpranidol and fasting states [2]. This basic model is used to explain switching of energy metabolism from glycolysis to Beta oxidation of fats in DM 2. But the analogy can't be carried too far, because, in DM2, once the energy metabolism switches to B oxidation at the onset of DM2, it is self-perpetuating, and never reverts to glycolysis unless DM2 is controlled. Further, the basic model of substrate competition as proposed by Randel (known as Randle cycle) has been suggested some modifications by this author to remove some inconsistencies and to adopt to the pathological condition of DM2 as against the physiological conditions addressed to by the original Randle cycle.

While feeding terminates the Beta oxidation state under physiological conditions, in DM2, once B oxidation predominates over glycolysis, It is self-perpetuating. This factor has to be accounted for when drawing an analogy with What Randle cycle holds good. Secondly, in prolonged fasting death occurs sooner or later, if re-feeding is not started as the fuel (fatty tissue) exhausts or due to a complication arising out of B-Oxidation / starvation (like ketoacidosis, etc.). Where as in DM2 the patient survives for decades this implies that the fuel (fat) is replenished along side its combustion. This author, therefore, proposed for the first time, with justification, that HMP shunt becomes operational in DM2, which explains how the lipogenesis is carried on, to match the ongoing fat oxidation. It also provides at least one reason for the increased gluconeogenesis in DM2. This is possible as the products of HMP shunt (G3PDH/ NADPH) can enter the lipogenic or gluconeogenic path way as the case may be.

This author further proposed with the justification that the pyruvate level is decreased rather than increased in fasting/ DM2 to satisfy the contention of Randle cycle. To this extent, this author has also proposed with justification an additional block at the level of Pyruvate Kinase enzyme (PK) in the glycolytic path way, also in addition the two blocks suggested by Randle et al. ie. at the level of enzyme phosphofructokinase (PFK) and pyruvate dehydrogenase (PDH) and accepted after experimental verification. The suggested additional block by this author (ref the article of this author for justification) sub serves two functions. Firstly, the onward glycolytic flux from PEP to pyruvate is reduced/blocked, resulting in low pyruvate levels as referred to above, and which was shown as a pre-requisite to satisfy the original contention of Randle et al for stimulating B-oxidation through reduced ACC, reduced malonyl coA and consequent stimulation of CPT Q stimulating B oxidation of fats.. Secondly, the PEPCK enzyme is stimulated diverting the glycolytic flux into gluconeogenic path way. I t may be recalled that both PK and PEPCK can not operate simultaneously as it results in a futile cycle and that inhibition of one stimulates the other enzyme.

Randle cycle in its original contention of how B-oxidation prevails over glycolysis in the interpranidol and fasting sates, is as follows. The accumulation of acetyl co A, which is the end product of B- oxidation as well as from conversion of pyruvate by PDH enzyme from glycolysis, both accumulate in the mitochondrial matrix (MM). This result in substrate competition, the substrate, oxalo acetate (OA) is essential for initiation of the first step of the citric acid cycle. Consequently the ratios of acetyl CoA / CoA and NADH/NAD increase. Randle contended that these increased ratios inhibit the PDH enzyme, blocking the glycolytic pathway and facilitating the B oxidation end product to center the citric acid cycle, ETC, the latter producing energy (ATP).

This author has shown that the same increased ratios, not only inhibit PDH but other enzymes as well like- citrate synthase, isocitrate dehydrogenase, alfa keto glutarate, citrate lyase [52,53] as well as, AMPK, the master metabolic switch itself. The inhibition of the first three

enzymes of the citric acid cycle block it for the passage of end products of not only glycolysis but also that of B- oxidation. Inhibition of the enzyme citrate lyase reduces the conversion of citrate to acetyl CoA which h is an important substrate for the enzyme ACC. The acetyl-CoA from both glycolysis as well as from B-Oxidation, since cannot proceed through citric acid cycle have to be routed through alternate routes. Also, a solution is needed for the alternative supply of energy as both major energy metabolisms are suspended briefly.

The acetyl CoA from glycolysis cannot be converted back to pyruvate nor proceed forward through the citric acid cycle. The enzyme Pyruvate carboxylase (PC) is stimulated, and the acetyl CoA is routed through GN pathway, by condensing with AA to form citrate which by citrate shuttle allows cytoplasmic OA and also forms cytoplasmic acetyl CoA. OA is converted to PEP by PEPCK and thence into GN pathway. The cytoplasmic acetyl CoA being the substrate for ACC, the other substrate being NADPH, one of the end products of HMP shunt as already seen above.

The acetyl CoA from B -oxidation enters the ketogenic pathway, forming ketone bodies like aceto acetate, ketone, etc. The ketone bodies provide an alternative source of fuel to vital organs like brain during the brief period when both energy metabolisms are temporarily blocked as seen earlier.

Now it remains to be seen how the B- oxidation is not only reestablished but is perpetuated indefinitely in DM2 at the expense of normal glycolysis as a source of energy metabolism.

It is seen above that the cytoplasmic acetyl-CoA formed as-as a result of citrate shuttle is a substrate of ACC which decides whether lipolysis or lipogenesis should occur. ACC catalyzes the conversion of cytoplasmic acetyl come together with NADPH, to form malonyl CoA enzyme. If ACC is high, malonyl CoA is high, and the enzyme CPT1 is inhibited, and so is the B oxidation. The result is lipogenesis occurs. On the other hand, If ACC is low, malonyl coA is inhibited, and the enzyme CPT 1 lis stimulated resulting in stimulation of B -oxidation of fats. I(is seen that pyruvate level is low in DM2 and hence the cytoplasmic acetyl CoA formed is is also low, as for each molecule of citrate formed, one molecule of cytoplasmic acetyl CoA is formed). Low substrate leads to low level of ACC and low activity of malonyl coA with consequent stimulation of CPT 1 and stimulation of B-oxidation. It is pertinent to recollect that the effect of concentration of the reactants is far more influencing on the rate of enzymatic reaction than any other factor that influences the same. Here again, the author differed from the original contention of Randle cycle that initiation of B – oxidation is by stimulation of AMPK. If AMPK is involved in the reestablishment of B -oxidation as contended by the Randle cycle, the PDH which is under inhibition is also stimulated and the then, the original drama of substrate competition is re-enacted. Further, in DM2 it is agreed that metformin stimulates AMPK with the consequent reduction in GN. (it may be recalled that AMPK stimulation promotes catabolic processes like B oxidation and lipolysis where as its inhibition causes anabolic processes like GN and lipogenesis). In other words, it is confirmed that in DM2, AMPK is inhibited (hence increased GN) and hence cannot be responsible for initiation of B oxidation after the stalemate of substrate competition. The novelty of this present hypothesis is that it explains the induction of B-oxidation independent of AMPK. Now the second question as to how this initiated B oxidation in place of glycolysis is perpetuated? It is known that PDK 4 which inhibits PDH is highly expressed in DM2. This keeps the PDH continuously inhibited while the B oxidation initiated continues perpetually at the expense of glycolysis. Activation of this nuclear hormone receptor promotes PDK4 expression (**10**) [54]. Because fatty acids function as endogenous PPAR-α agonists, [55,56] increased levels of free fatty acids (FFA) may promote PDK4 expression during starvation and diabetes.

Growth hormone (GH), whose function is opposite to that of insulin, stimulates PDK4 expression in the liver of wild-type mice during fasting by activating the Janus kinase/signal transducer and activator of transcription (STAT5) pathway and increasing gluconeogenesis. Metformin inhibits GH-induced PDK4 expression via the AMP-activated protein kinase/small heterodimer partner-dependent pathway that inhibits the combination of STAT5 to the PDK4 promoter [57].

Two innovative concepts are proposed by this author in his article titled molecular mechanism of metformin – a new hypothesis.

[58] which worth considering in this context, i.e., the gate control concept of DM2 pathogenesis and Warburg-like effect in DM2 to explain the

onset of GN at the expense of glycolysis which is known to be blocked in DM2, which

5.1 Gate Control Concept

Four main gates and two sub-gates are conceived in the glycolysis pathway which channelizes the glycolytic flux depending on whether the Gates are open or closed, into different modes. The four main gates are the enzymes PFK, PK, PEPCK, and PDH respectively. The two sub Gates are glycogen synthase (GS) and glucose six phosphate dehydrogenase (G-6 PDH). When the main gates, PFK, PK, and PDH are open, Glycolysis proceeds. The other main gate, PEPCK is closed and hence is the GN pathway, preventing a futile cycle. At the same time, the Sub gate GS and G-6PDH are also closed precluding glycogenesis and opening of HMP shunt. This is what happens normally under Physiological conditions or normal health. But in DM2, the three main gates, ie. PFK, PDH (as proposed by Randle under physiological Conditions) and the third gate, PK (as proposed by this author) are closed resulting in shutting off of glycolysis. Simultaneously, the fourth main gate is opened leading to the opening of GN pathway which is disturbed in DM2 and the two sub-gates GS and G-6PDH are Closed (the later is proposed by this author) leading to decreased glycogenesis and opening of HMP pathway. It was shown that mainly due to defective glucagon signaling results in shutting off the the gates were controlling the glycolysis.

5.2 The Warburg-like effect in DM2

Malignant cells have been shown to sabotage host's energy metabolism by blocking host's glycolysis and diverting the glycolytic flux to GN pathway to be utilized for producing their own energy. This is called the Warburg effect. [59] This, the malignant cells achieve by producing the expression of the M2 isoform of Pyruvate Kinase enzyme (PK M2) Which blocks the conversion of PEP to pyruvate and diverts the glycolytic flux into GN pathway. Where as the PK M1 isoform is responsible for the glycolytic flux to proceed to pyruvate from PEP. The PKM2 is expressed by the malignant cells under the anaerobic conditions resulting from stopped aerobic glycolysis and starting of anaerobic glycolysis. Normally the PKM2 is expressed in foetal embryonic tissues apart from the malignant cells. In a similar analogy, in FN2 also anaerobic glycolysis occurs, and there is no reason why PK m2 should not be expressed in DM 2 as well. In fact there are a few reports of expression of PK M2 in DM 2 also. This could explain the operation of GN in DM2, which automatically shuts the glycolysis, the twin effects that characterize DM2.

6. CONCLUSION

An attempt is made to recapitulate the various mechanisms proposed in the literature as regarding the pathogenesis of DM 2. This is hoped to serve two purposes. Firstly as already stated, they address the ills of the deranged fat metabolism and insulin resistance Secondly they fail to get at the roots at the molecular level as to how the ills are actually initiated. This paper is an attempt to address issues that may come through some new insights into the issues concerned at the molecular level.

Thus the molecular basis of basis of both the pathogenic aspects, ie, insulin resistance and the preponderance of B-oxidation over glycolysis in DM 2 are explored in this article.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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