

1 **Disruption of disulfide bonds as a cause of insulin resistance in D.M 2**

4 **Abstract:-**

5 **Background**

6 **Many theories have been put forward to explain insulin resistance in D.M.-2.**
7 **These theories ,Ssstill it-remainsed an enigma till date. Disruption of the**
8 **insulin- ligand-initiated receptor signal transduction is currently the cause**
9 **espoused. For insulin signal transduction to occur down-streamdownstream,**
10 **the insulin should be in tetrameric, holo-enzyme form for the conformational**
11 **changes and auto- phosphorylation steps to take place. A prerequisite to this**
12 **is, linkage of the two A sub- units and, A, B sub- units by disulfide bonds.**
13 **Without this, this, the receptor is in the A, B dimer half-enzyme form, devoid**
14 **of any binding affinity to the ligand or auto- phosphorylating activity.**

15 **Aim**

16 **To explore, disruptionexplore, disruption of the disulfide bond formation, as**
17 **a possible cause of insulin resistance-inresistance in DM 2.**

18 **Conclusions**

19 **Under normal physiological conditions of carbohydrate metabolism as a**
20 **source of energy, disulfide bond formation occurs normally. But when energy**
21 **metabolism switches to beta oxidation of fats, disruption of disulfide bond**
22 **formation of the insulin receptor occurs, leading to insulin resistance in**
23 **DM 2. The exogenously administered insulin restores the normal**
24 **carbohydrate metabolism, by reversing the conditions that resulted in switch**
25 **over to Beta oxidation, thus restoring insulin sensitivity in treated diabetics.**

26 **Key words:- Tetramer, ligand, auto phosphorylation, disulfide bond, bBeta oxidation.**

28 **Introduction:-**

Hyperglycemia, in presence the of high levels of insulin in blood, is the hall mark of insulin resistance in DM2. Despite many mechanisms suggested, the issue of insulin resistance in DM2 is yet to be resolved. While resistance observed is to endogenous insulin, the exogenously administered insulin is still effective. This suggests that the insulin in DM2 resistance is relative and reversible. The defect in Signal transduction of insulin is believed to hold the key to insulin resistance. The role played by the disruption of disulfide bond formation of the insulin receptor as a cause of Insulin resistance in DM 2 is explored.

The insulin receptor:-

The insulin receptor (IR) is a trans-membrane protein having intrinsic tyrosinase activity. It's two dimensional structure has been elucidated since long. ~~THE~~ The IR has two A and two B subunits. The A sub unit is extra-cellular and has the ligand (insulin) binding site/sites as detected by affinity labeling protocols (1,2,3). Besides it has a cystein rich region. The B sub unit has three compartmental regions - the extracellular, the transmembrane and cytosolic domains. The cytosolic Tyrosine Kinase domain has ATP binding consensus sequence and three clusters of Tyrosine residues that can be phosphorylated in response to insulin action. The three clusters are found in juxta-membrane domain residues, the "tri-tyrosine" residues in the tyrosine kinase domain and the carboxy-terminal residues. The insulin ligand binds to one A sub unit in such a way that it precludes attachment of a second ligand to the other A sub unit. And also the second A sub unit shows negative cooperability. The physiologically active form of the receptor is the tetramer which is a functional dimeric protein ~~complex~~ complex. This is the holo-enzyme which has high affinity to the insulin ligand and has the full phosphorylating activity .(4,5). The A B heteromear is devoid of full ligand affinity or has any phosphorylating activity. The potential receptor- hormone contact sites are -residues 20-120 (6), the disulfide rich region (7,8) and around the residue- 390 (9) region just to the carboxy side of the disulfide - rich region.

The disulfide bonds:-

The tetrameric form of IR is formed by the disulfide bonds between the two A sub units ~~and B and B~~ sub units. The conformational changes and auto phosphorylation that follow the docking of the insulin to the insulin receptor are dependent on the disulfide bridges. It is observed that the insulin treated receptor generated more cross linked B-B complexes than the receptor ~~with-out~~without insulin (9). Though the ligand binds to one A sub unit only, both the B units are auto phosphorylated. The disulfide bonds are formed between the cystein residues of the two A sub units (class 1 disulfide bonds) and the A B sub units (class 2 disulfide bonds). The class 1 bonds are between the cystein residues 435, 468 and 524. According to another study A-A linkage is between residues 122-270, part of cystein rich domain. The A-B linkage is between CYS 583 and CYS 199 and between residues 724-917 (10). When it is mutated to serine, no A2B2 dimers could be detected by the affinity labeling, only A2 receptors are formed. Thus the disulfide bonds are essential for auto phosphorylation of the receptor as well as for the insulin signaling transduction.

The role of protein disulfide isomerase (PDI) in disulfide bond exchange:-

The protein disulfide isomerase is an enzyme in the endoplasmic reticulum (ER). It catalyzes the formation of disulfide bonds between the cystein residues within a protein, in this case, the insulin receptor. It also acts as a chaperon catalyzing proper protein folding. PDI has 4 thioredoxine-like domains, two of which have the canonical C X C C motif. In the oxidized form it catalyzes the formation of the disulfide bonds, of the general structure R-S-S-R. It is also called S-S bond or Disulfide Bridge.

The thiol- disulfide exchange:-

In the reduced state the PDI is a dithiol. The thiol – disulfide exchange is depicted by the following equation.



The reaction proceeds through a SN 2 type mechanism. The nucleophile is the deprotonated thiol anion, which attacks the reacting sulfur of the disulfide bond making a S-S-S like transition state, with negative charge being delocalized but more abundant on attacking and leaving sulfurs.

The role of Endoplasmic oxidoreductin 4:-

Endoplasmic oxidoreductin (ERO) is a protein tightly bound to the inner membrane of ER. It transfers the preformed disulfide bonds in the endoplasmic reticulum (ER) to the insulin receptor (IR) through the PDI by way of thiol-disulfide exchange as seen above. It catalyzes the oxidation of Protein Disulfide Isomerase (PDI) by coupling the de novo disulfide bond formation to the reduction of oxygen to H_2O_2 . It oxidizes the PDI by accepting electrons from the sulfur and it-self gets reduced. Thus the SH HS thiol bond is acquired in exchange of the S-S disulfide bond to PDI. The ERO can oxidize next PDI molecule only if it loses its electrons to the Electron Transport Chain (ETC).

The reverse electron transport (RET) :-

The mitochondrial ETC consist of multi sub unit 4 complexes (Complex I - IV) which along with F₀-F₁-ATP synthase, are encoded by either mitochondrial or DNA. The complexes normally transfer electrons to the final acceptor, the molecular oxygen which is reduced by 4 electrons to water at complex IV. Premature single electron reduction of molecular oxygen, earlier in the chain forms the superoxide radical. RET is a set of reactions that allow electrons to be transported against the gradient of redox potential of electron carriers from reduced coenzyme Q to NAD⁺ instead of oxygen. The reduction of Q enzyme requires FADH₂-linked oxidizable substrates like glycerophosphate or succinate.

The redox carriers and the centres of ETC can potentially leak single electrons to oxygen and convert it to superoxide anion, a progenitor ROS. Given a moderate redox potential of the superoxide oxygen couple, ($E'_{1/2} = 0.16 \text{ V}$), the reaction of one electron reduction of oxygen is thermodynamically favorable to many oxidoreductases.

Reactive oxygen

species production (ROS):-

To maintain the oxidative state of ERO 1 activity, the transfer of disulfide bond by ERO to PDI is coupled to reduction of molecular ~~oxygen~~ inoxygen in the ETC by the electrons acquired from PDI. The reduction of oxygen is not complete since the end product is not water but H_2O_2 . Thus ERO 1 activity constitutes an important source of ER derived oxidative stress, due to production ~~of ROS~~ of ROS. ROS includes super oxide (O_2^-), Hydroxyl radical, ($OH^\cdot$) and hydrogen peroxide (~~H_2O_2~~ H_2O_2) (11). MnSOD catalyzes the dismutation of O_2^- to ~~H_2O_2~~ H_2O_2 (12), ~~and~~ And the divalent reduction form ~~H_2O_2~~ H_2O_2 . ROS production implies that there is reverse electron transport. Among the ETC complexes complex 1 and complex 3 are the major sites of super oxide production, the former generating super oxide within the mitochondrial matrix only ~~where~~ as ~~whereas~~ the later generate them in the intermembrane space also ~~(5,6)~~ (5,6). Complex ~~I~~ 4 oxidizes NADH with enzyme Q as electron acceptor coupled with proton pump generating trans-membrane potential. Complex ~~I~~ 4 can generate super-oxides in presence of NADH. Since ~~the~~ the electrons derived from sulfur are at a higher redox potential than complex ~~I~~ 4, RET occurs to reduce the NAD Both NAD reduction and Reactive Oxygen Species (ROS) production require high membrane potential provided by ATP hydrolysis.

Role of Peroxy-Redoxin (PROX) 4:-

Hydrogen Peroxide (~~H_2O_2~~ H_2O_2) formed in ERO 1 reaction is harmful, so it is removed by another protein, peroxy-redoxin (PROX 4) . These are group of enzymes present in ER which remove H_2O_2 and also form the disulfide bond. The peroxidatic cysteines in the PROX 4 , take an oxygen from ~~H_2O_2~~ H_2O_2 and form water and a $-SOH$ group which reacts with the adjacent SH group to form a disulfide bond. The disulfide bond so formed is transferred to PDI by ERO1 which by thiol-disulfide exchange forms disulfide bond between the two A and A,B sub units of the insulin receptor.

The proposed theory:-

Under the conditions of normal carbohydrate metabolism, the intermediate products of glycolysis ~~and~~ and ~~T.C.A~~ TCA enter ETC through the Complex ~~1~~ 4 ~~through~~ I through NADH and complex ~~II~~ 4 through $FADH_2$. The electrons passed by ERO 1 to complex ~~I~~ 4 of the ECT, result in RET and which generates ROS from which the disulfide bonds are generated in the ER for

ERO 1 to initiate oxidation of the reduced PDI, When the metabolism shifts to beta oxidation, the intermediate products of TCA cycle enter through complex **-2II**, no RET occurs from succinate to complex **4I**. 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 B sub units as well as consequent IRS and cascade of -auto phosphorylation downstream, is not formed. The FFA especially the LCFA, ~~inhibit RET~~**inhibit RET** from succinate to complex **4-I** thus decreasing the SUCC-dependent ROS production, in spite of increased FADH₂ production due to beta oxidation of FFA (13). This situation continues until the **B-beta** oxidation pathway of energy metabolism prevailing over the normal glycolysis is overcome. The exogenously administered insulin restores the carbohydrate metabolism in place of **B-beta** oxidation of **f**Fatty acids, by reversing the factors that lead to switch over of the energy metabolism, thus restoring the insulin sensitivity.

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