

Review Article and Clinical Experience: A THERAPEUTIC OPTION IN THE TREATMENT OF PRE-DM, IGT, AND T2DM. (The Roles of Metformin: from Basic to Clinics)

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ABSTRACT

Minimally there are 30 hormones and biological substances secreted by Fat Cell can be summarized (Leptin, TNF α , IL-6, Resistin, Adiponectin = ACRP-30, HSL, Lipotransin, Perilipins, Aquaporins, etc). The relationship between Insulin Resistance (IR) and Adipose Tissue is exceedingly complex, and IR can be detected either in both Non-Diabetic Individuals, Pre-Diabetes (Pre-DM), or in patients with Type 2 DM (T2DM). Based on the staging of IR (Author's Classification 1997), we will be faced with 3 groups of individuals, such as: Insulin Resistant-Normoinsulinemic (Stage-I, IIIA, IIIB), Insulin Resistant-Hyperinsulinemic (IIA, IIB, IIC), and Insulin Resistant-Hypoinsulinemic Individuals (Stage-IVA, IVB); of these all will be described in this paper. According to this classification, Pre-DM may be classified as Stage-I or Stage-IIA. Such a Classification can be described more detail as follows: Stage-I (Obese People or Pre-DM or Normoinsulinemia), Stage-IIA (Non-DM or Pre-DM: Hyperinsulinemia), Stage-IIB (IGT with Hyperinsulinemia), Stage-IIC (T2DM with Hyperinsulinemia), Stage-IIIA (DM with Normoinsulinemia), Stage-IIIB (T2DM or DM-Type X-1 with moderately impaired β cell-function with Low-Normoinsulinemia), Stage-IVA (T2DM with severe impaired β cell-function: DM-Type X-2) and Stage-IVB (T2DM with very severely impaired β cell-function with subnormal plasma insulin levels: totally insulin dependent T2DM = DM-Type-X3 = LADA: Latent Autoimmune Diabetes in Adults). Fasting and Post Prandial C-peptide levels of these patients are less than 0.5 μ U/ml. Marked impairments in insulin's intracellular Signaling Cascade ($\pm$ 30%) are present in Fat Cells of T2DM, including impaired IRS-1 Gene and Glut-4 expression, impaired insulin-stimulated PI3-Kinase and PKB/Akt activities. It is proposed that IR and/or its effectors are initiated in Fat Cells and this way secondarily encompasses other target tissues for Insulin, including impaired GLUT expression in the muscles. Insulin Receptor Substrate-2 (IRS-2) is the main docking protein for PI3-kinase activation in Fat Cells in case IRS -1 is markedly reduced, such as in T2DM. The downstream signaling events for insulin are also similarly impaired. The adipose tissue does not only produce peptides which can elicit IR (TNF α , IL-6, or Resistin, etc) but also produce hormones which can improve insulin sensitivity such as Adiponectin. The circulating levels of Adiponectin are positively correlated to insulin sensitivity and negatively to BMI. Thus, it is most likely that the balance of the production of hormones from Adipose Tissue that accentuate (f.e. TNF α , IL-6, Resistin) or alleviate (Adiponectin) IR, as well as eliciting other effects, is due to several factors including Adipose Mass, Nutritional State, and Genetic background. Overstimulation of the β -Cells of pancreas due to IR (Stage-I or II, or Obesity, or Pre-DM) may cause an impaired insulin secretion. Taken together, impaired β -Cell function and IR are both responsible for the occurrence of T2DM, or may be DM-Type X-3 or LADA which are totally insulin dependent. Metformin with its 21 (9-3-9 effects) pleiotropic effects (metabolic and vasoprotective effects) is postulated to improve the receptor (IRTK) and post receptor defects of patients with Obesity, Pre-DM, or T2DM. Recent study reported that Thiazolidinediones (TZD_s) increased the expression of IRS-2 in diabetic patients having low IRS-1. Conclusions: There is link between Insulin Resistance-Obesity-Pre-DM-and T2DM, and usually such an IR is initiated in Fat Cell. Probably, the balance between TNF α , IL-6, Leptin, and Resistin in one side, with Adiponectin in the other side in Obesity, Pre-DM or patients with Stage-I or II plays a pivotal role in the development of T2DM. Hence, Metformin alone or in combination with TZD_s can be recommended as promising drugs for those patients. In addition, Metformin with its 21 pleiotropic effects (9-3-9), may have potential therapeutic benefits in the prevention of diabetic vascular complications as long as normal liver and renal functions of the patients are normal.

Keywords: Insulin resistance, obesity, pre-DM,IGT, T2DM, metformin

INTRODUCTION

Excess of visceral adipose tissue accumulation in the presence or absence of obesity, is associated with

insulin resistance, hyperinsulinemia, glucose intolerance. These metabolic abnormalities are being predictive of an increased risk of T2DM. Furthermore, it was reported that excess visceral adipose tissue accumulation was associated with a potentially atherogenic dyslipidemia which includes hypertriglyceridemia, elevated apo-B levels, an increased proportion of small, dense LDL particles, and low HDL concentrations. Then, evidence has accumulated that Insulin Resistance-Dyslipidemic Syndrome (IR-DS) of visceral obesity is also associated with alterations in haemostatic variables which

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contribute to increase the risk of atherothrombotic events in these patients (Reaven 2001).

In obesity, the enlarged fat cells oversecrete TNF α and Leptin in local circulation. The mediators are implicated as candidate mediators of obesity-associated insulin resistance. Both TNF α and Leptin have been shown to have autocrine effects and impair insulin action also in adipocytes. Resistin, as well as TNF α and Leptin, released by adipocytes, act in peripheral tissue to decrease the sensitivity of insulin action. In contrast, circulating Adiponectin levels are positively correlated in the insulin sensitivity and negatively related to BMI, FFMI=Fat-Free Mass Index, and FMI=Fat Mass Index (Schultz et al 2002).

Taken together, several factors or mediators have been postulated to be responsible for the development of peripheral insulin resistance in obesity with the Metabolic Syndrome. Based on the collection of many components of such a syndrome from many studies and reports, the author coins such a syndrome as The “Cumulative Metabolic Syndrome” (mentioned below). Metformin shows many potential therapeutic benefits for patients with the “Cumulative Metabolic Syndrome”, since this drug has pleiotropic properties (vasoprotective effects) beyond antihyperglycemic action.

The aim of this paper is to describe shortly about the link of Insulin Resistance and patients with Obesity, Pre-DM, T2DM and therapeutic option in the treatment of these patients. The promising roles of Metformin in the management of such patients will be also summarized.

OBESITY-LINKED INSULIN RESISTANCE

The Metabolic Syndrome-X or the “Cumulative Metabolic Syndrome” (the author’s term) is a cluster of metabolic components and cardiovascular risk factors which can be summarized as follows (Reaven 2001, Lebovitz 2001):

1. Insulin Resistance (with or without Glucose Intolerance)

2. Hyperinsulinemia (if no defect of pancreatic insulin secretion)
3. Abdominal (Visceral) Obesity
4. Raised Blood Pressure
5. Atherogenic Dyslipidemia:
 $\uparrow$ TG, $\uparrow$ PP Lipemia, $\downarrow$ HDL-C, $\uparrow$ Apo-B, $\uparrow$ Small Dense LDL
6. Procoagulant State:
 $\uparrow$ Fibrinogen, $\uparrow$ PAI-1, $\uparrow$ Factor VII, $\uparrow$ vWF, $\uparrow$ Adhesion Molecules
7. Hyperuricemia
8. Vascular Abnormalities:
 - $\uparrow$ Urinary Albumin Excretion
 - Endothelial Dysfunction
9. Inflammatory Markers:
 $\uparrow$ hsCRP, $\uparrow$ Cytokines (TNF α , IL-1 β , IL-6)

Insulin Resistance develops in both liver and peripheral tissues (fat and muscle) which will be used as a topic of discussion. Several mechanisms implicated in the development of Insulin Resistance in Obesity have been summarized by Reaven (2001). The enlarged fat cell oversecretes TNF α and Leptin in the local circulation. TNF α impairs insulin action by inhibiting insulin receptor signaling, possibly by increasing IRS-1 serine phosphorylation (TNF α impairs Insulin Receptor Tyrosin Kinase = IRTK), and TNF α also impairs GLUT-4 expression.

Leptin released from visceral adipocytes may inhibit insulin action in the liver by impairing insulin receptor signaling, leading to reduced down-regulation of PEPCK, the rate limiting enzyme in gluconeogenesis. Both TNF α and Leptin have been shown to have autocrine effects and to impair insulin action (thus, also in adipocytes). Resistin, as well as TNF α and Leptin, act in peripheral tissues to influence sensitivity to insulin and other cellular and metabolic process involved in the use and partitioning of substances (Steppan et al 2001,2002).

Based on clinical experiences, by the year 1997, Tjokropawiro (2001) hypothesized the link between Insulin Resistance – Obesity – Pre-DM, and T2DM, and it was termed as Clinical Classification Staging of Insulin Resistance-Linked Obesity and T2DM (Figure 1).

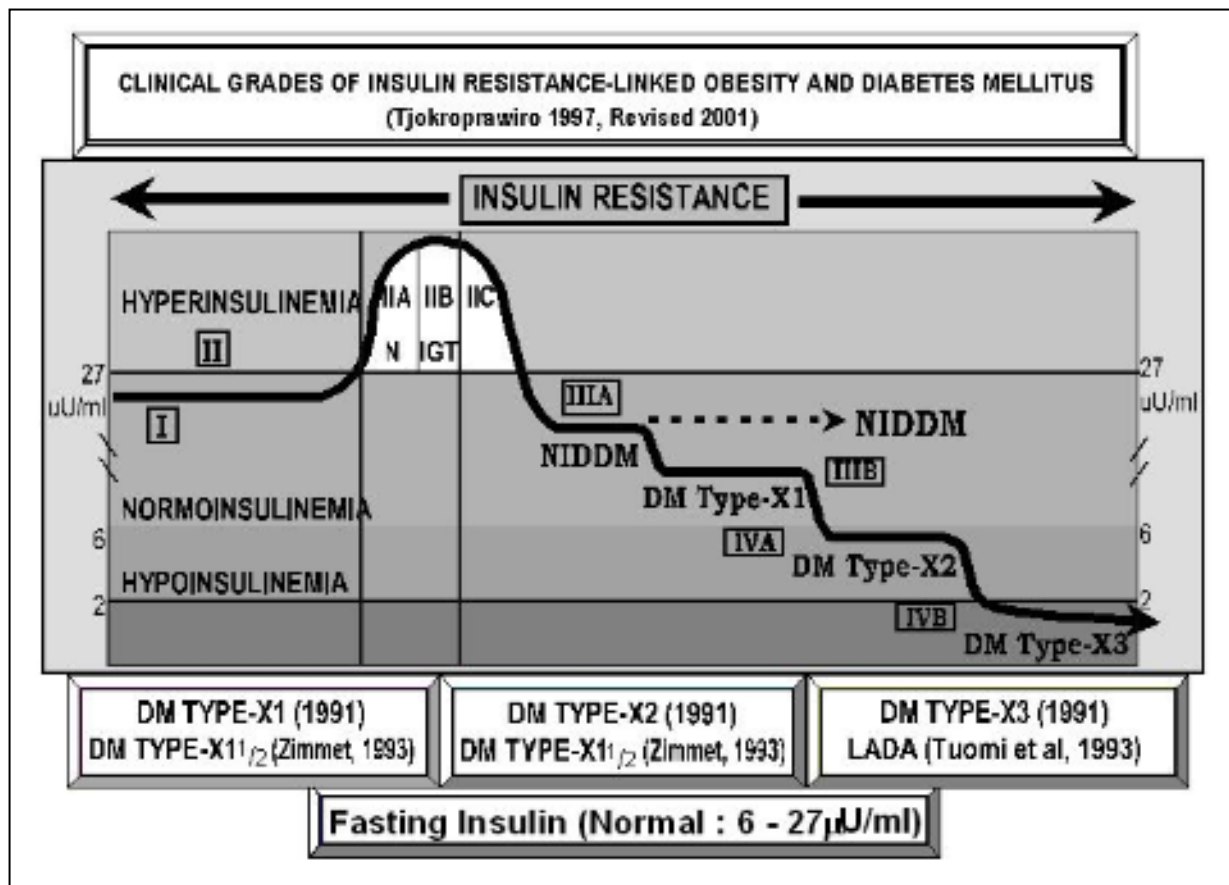


Figure 1. Clinical Staging of Insulin Resistance-linked Obesity and Diabetes Mellitus

The Classification of Insulin Resistance – Linked Obesity, Pre-DM and T2DM can be classified into 4 Stages; this classification has been already hypothesized by the author since 1997 and to be revised in the year 2001 (Tjokroprawiro 2001).

Stage-I: Normo insulin with Insulin Resistance

(Insulin Resistant, Normoinsulinemic Individuals:
Obesity, Pre-DM)

In clinical practice, the Homeostasis Model Assessment = HOMA, has been suggested as method to assess Insulin Resistance (HOMA-R) and Insulin Secretion of pancreatic B-cell (HOMA-B) from the fasting glucose and insulin concentrations (Mathews et al 1985). However, this method has not been extensively evaluated, particularly in different ethnic groups (Figure 2).

HOMA-R	=	$\frac{\text{Fasting Insulin } (\mu\text{U/ml}) \times \text{Fasting Glucose (mmol/l)}}{22.5}$
HOMA-B	=	$\frac{20 \times \text{Fasting Insulin } (\mu\text{U/ml})}{\text{Fasting Glucose (mmol/l)} - 3.5}$

Figure 2. HOMA – FORMULA (Matthews et al 1985)

Patients with Stage-I (Obesity, Pre-DM) still have normal insulin blood levels, whereas Insulin Resistance may be detected by Euglycemic Clamp or HOMA-R; Stage-I usually happens in patients with BMI ≥ 30 kg/m² or Visceral Obesity or Pre-DM.

**Stage-II: Stage-IIA, IIB, and IIC
(Insulin Resistant, Hyperinsulinemic Individuals)**

- Stage-IIA : Hyperinsulinemia with normal Glucose Tolerance, usually with BMI ≥ 30 or Visceral Obesity, or Pre-DM
- Stage-IIB : Hyperinsulinemia plus Glucose Intolerance BMI ≥ 30 or Visceral Obesity
- Stage-IIC : Hyperinsulinemia plus Type 2 Diabetes Mellitus and BMI ≥ 30 or Visceral Obesity

**Stage-III: Stage-IIIA and Stage-IIIB
(Insulin Resistant, Normoinsulinemic Individuals)**

- Stage-IIIA : Normoinsulinemia plus T2DM, and BMI is usually less than 30, Visceral Obesity is still present but less prominent
- Stage-IIIB: Low Normoinsulinemia plus Type-X₁ DM. (Type 2-DM with poor response of

β Cell to glucose stimulation, but fasting C-peptide is still > 0.8 μ U/ml)

Type X₁-DM (firstly coined by the author in 1991) is identical with Type 1½ -DM as coined by Zimmet in 1993

**Stage-IV: Stage-IVA and Stage-IVB
(Insulin Resistant, Hypoinsulinemic Individuals)**

- Stage-IVA : Hypoinsulinemia (fasting insulin < 6 μ U/ml, normal : $6 - 27$ μ U/ml plus Type X₂-DM ~ Type 1½-DM but fasting C-peptide become lower (0-6-0.8 μ U/ml)
- Stage-IVB : Hypoinsulinemia plus Type X₃-DM ~ LADA (as coined by Tuomi in 1993) and fasting C peptide < 0.6 μ U/ml. This patient (Type X₃-DM) become totally insulin dependent but more resistant to ketoacidosis compared with Type 1-DM.

The investigation of Judajana in Surabaya by the year 1995 resulted that patients with HLA-DR₃ and HLA-DR₉ are prone to develop DM-Type X-3 (LADA), whereas those with HLA-DR₅ do not. Short description of the Classification of Insulin Resistance is summarized in Table 1.

Table 1. Summarized Description of the Staging of Insulin Resistance

Obesity (BMI) - Insulin Resistance - PreDM - IGT - T2DM - DM-Type X (Clinical Experiences : 1997-2003)	
Stage	PreDM - IGT - T2DM - DM-Type X1, DM-Type-X2, DM-Type-X3
Stage I	PreDM-A : BMI*) ≥ 30 , Normoinsulinemia
Stage IIA	PreDM-B : BMI*) ≥ 30 , Hyperinsulinemia
Stage IIB	IGT : BMI*) ≥ 30 , Hyperinsulinemia
Stage IIC	T2DM : BMI*) ≥ 30 , Hyperinsulinemia, Oral Treatment
Stage IIIA (HLA-DR ₅)	T2DM : BMI*) < 30 , Normoinsulinemia, CTOL, Reversible
Stage IIIB (HLA-DR ₃ & HLA-DR ₉)	DM-Type X1 = DM-Type 1½ : BMI*) < 30 , Low Normal Insulin, Fasting C-peptide ≥ 0.8 ng/ml, Post Prandial C-peptide : Poorly Response, CTOL Partially Insulin Dependent
Stage IVA (HLA-DR ₃ & HLA-DR ₉)	DM-Type X2 = DM-Type 1½ : BMI*) < 30 , Subnormal Insulin (C-peptide 0.6-0.8 ng/ml), CTOL Partially Insulin Dependent
Stage IVB (HLA-DR ₃ & HLA-DR ₉)	DM-Type X3 = LADA : BMI*) < 30 , Fasting and 2 PP C-peptide : < 0.5 ng/ml each. Totally Insulin Dependent

Normal Values: - Fasting C-peptide: 0.8 – 4.0 ng/ml
 - 2 PP C-peptide: 1.1 – 5.0 ng/ml

LADA = Latent Autoimmune Diabetes of Adult

HLA-DR₅ = Resistant to DM-Type X; HLA-DR₃ & HLA-DR₉ = Sensitive to DM-Type X

METFORMIN AND ITS POTENTIAL THERAPEUTIC BENEFITS

Results of many recent studies are shortly summarized below (selected)

1. GLP-1 is a gastrointestinal hormone which stimulates insulin secretion and promote satiety. GLP-1 and drugs that inhibit its degradation, such as Dipeptidyl-Peptidase IV (DPP-IV)-Inhibitors, have been proposed as therapeutic tools for T2DM and Obesity (Mannucci et al 2000). In this study, Metformin is able to inhibit in vitro GLP-1 degradation by human plasma and by inhibiting DPP-IV. GLP-1 is also able to delay gastric emptying process. These actions could provide an explanation for the observed anorectic effects of Metformin, and may contribute to its hypoglycemic properties (due to GLP-1 effect as “indirect” insulin secretagogue).
2. Ruggiero – Lopez et al (2000) reported that Metformin reduced Methylglyoxal levels (Carbonyl Compound) by formation of a stalk condensation product (Triazepinone). Thus, Metformin appeared to act as an extracellular scavenger of Methylglyoxal independent of its anti hyperglycemic effect, and could thereby contribute to the prevention of chronic diabetic complications by reduction of carbonyl stress (AGE generation will be inhibited).
3. Patane et al (2000) cultured rat pancreatic islets with either high glucose or FFA concentration, and then for additional 24 hours in the presence or absence of Metformin. The results indicated that Metformin could reverse both glucose abnormalities and glucose-induced insulin release impairment consequent to islet exposure to either high glucose or FFA. These effects may contribute to the therapeutic benefits of Metformin in T2DM patients.
4. Previously it was demonstrated that Metformin increased erythrocyte insulin -stimulated IRTK (Insulin Receptor Tyrosine Kinase) activity of obese patient with normal glucose tolerance, but this increase was associated to a decrease in plasma insulin concentrations.

Nomizo et al (2000) investigated the effect of Metformin “in vitro” in the insulin receptor of NIH 3T3 Cells. The cells were maintained in Metformin supplemented with 10% FCS, and they were grown to influence in 94 mm dishes and starved in a serum free medium supplemented with 1mM glucose for 12 hours. The results showed that Metformin increased insulin – stimulated IRTK, but did not have an effect on the endogenous IRTK of receptor from NIH 3T3 Cells, after 6 and 12 hours incubation. These Metformin’s properties on IRTK may contribute to a potential therapeutic effect on Obese Diabetic patients.

Based on the results of many studies (provided with previous data), Tjokropawiro (2002) summarized 21 (9-3-9) possible potential therapeutic properties (Pleiotropic Effects) of Metformin which can be classified into 3 groups.

I. Carbohydrate Metabolism (9 Effects)

1. Decreased Intestinal Glucose Absorption
2. Decreased Fasting Blood Glucose
3. Decreased 2 h Post Prandial Blood Glucose
4. Increased Glycogenesis
5. Increased Insulin Receptor Binding
6. Increased GLUT-5 Expression in the GLUT
7. Increased Insulin-Receptor Tyrosine Kinase Activity = IRTK.
8. Inhibited GLP-1 degradation by DPP-IV and promoted satiety
9. Prevented β -cell from Gluco-or Lipotoxicity Effect

II. Lipid (3 Effects)

1. Decreased Total Cholesterol and LDL-Cholesterol
2. Decreased Triglyceride
3. Increased HDL- Cholesterol

III. Vasoprotective Effects (9Effects)

1. Decreased Insulin Levels (Increased Sensitivity)
2. Decreased Platelet Aggregation
3. Improved Erythrocyte Deformability
4. Increased Fibrinolysis (? PAI-1, ? F XIIIa, ? Fibrinogen)
5. Increased Peripheral Blood Flow
6. Decreased Capillary Permeability
7. Decreased Carbonyl Stress
8. Decreased SMC-Fibroblast Activity

The link of Obesity and Diabetes Mellitus may be schematically drawn (Figure 3).

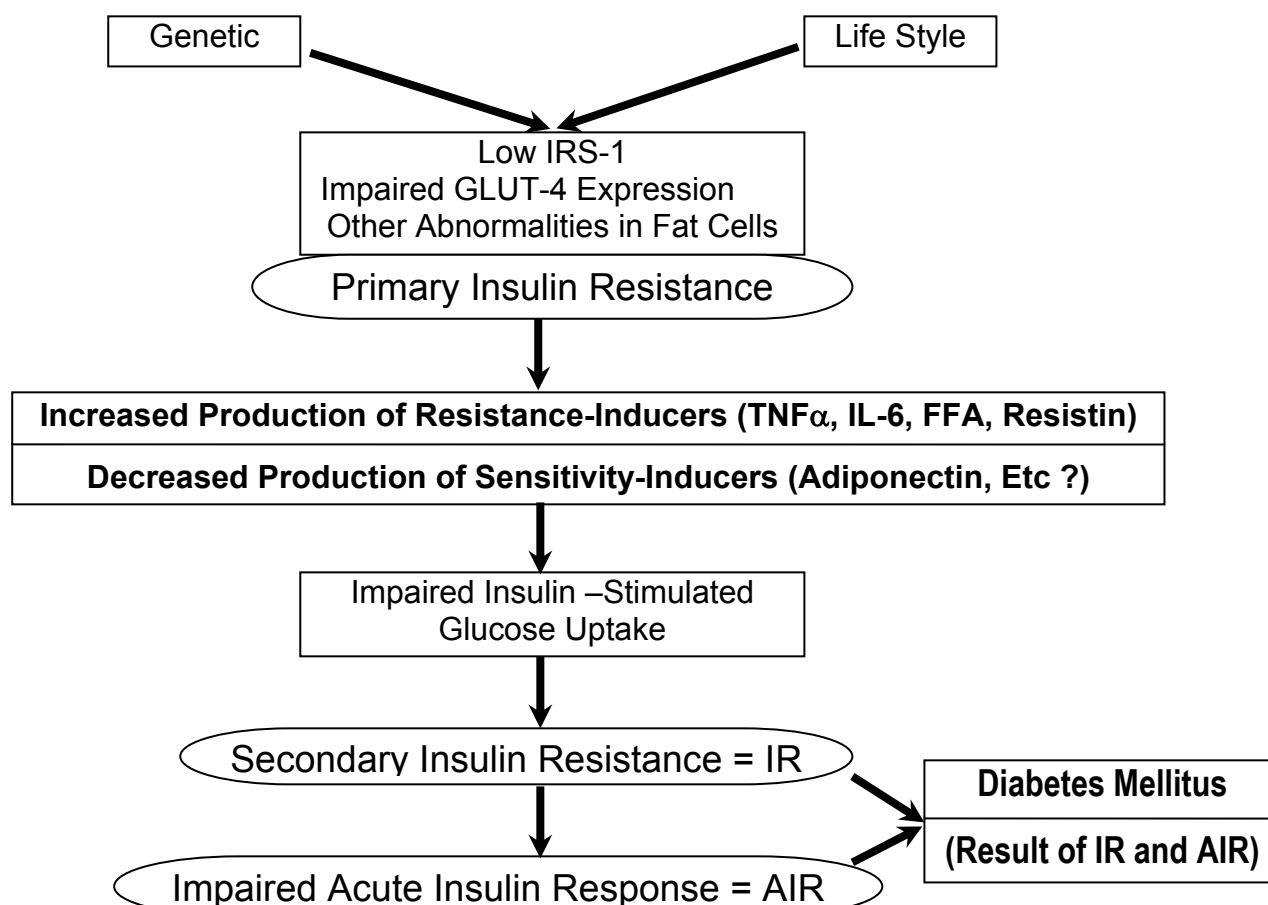


Figure 3: Hypothetical Pathway of Obesity Linked Diabetes Mellitus (Summarized: Tjokropawiro 2002)

INSULIN RESISTANCE AND IMPAIRED “AIR” IN T2DM

Type 2 Diabetes Mellitus (T2DM) is a heterogenous disorder characterized by impaired of Acute Insulin Response (AIR) or Acute Phase of Insulin Secretion of β -Cell and Insulin Resistance (IR) of peripheral tissues.

In clinical practice, the Homeostasis Model Assessment (HOMA), has been suggested as a method to assess IR (HOMA-R) and to assess insulin secretion of β -Cell (HOMA-B) from fasting Glucose and Insulin Concentration (Mathews et al 1985). However, this method has not been extensively evaluated, particularly in different ethnic group.

$\text{HOMA-R} = \frac{\text{Fasting Insulin } (\mu\text{U/ml}) \times \text{Fasting Glucose (mmol/l)}}{22.5}$
$\text{HOMA-B} = \frac{20 \times \text{Fasting Insulin } (\mu\text{U/ml})}{\text{Fasting Glucose (mmol/l)} - 3.5}$

Agents that enhance Ser/Thr phosphorylation of IRS proteins or other downstream effectors of the insulin signaling cascade play negative-regulatory roles in insulin action. Ser/Thr phosphorylation impairs insulin-stimulated Tyr phosphorylation of IRS proteins, uncouples insulin signal transduction, and has been implicated in the development of IR. Ser/Thr phosphate inhibitors such as Okadaic Acid, PDGF, Insulin or Angiotensin II, and PKC-Activators, and TNF-Activators, and other Cytokines, increase Ser/Thr Phosphorylation of IRS-1. TNF- α expression is increased in abdominal fat obesity.

Further Information: Ser/Thr phosphorylation of the IRS molecules induces IR. Ser/Thr phosphorylation of IRS proteins may serve as physiological negative feedback control mechanism or may result in IR. Decreased Tyr phosphorylation of IRS proteins and reduction in their associated PI3-K activity is observed in skeletal muscle and adipocytes both in Obesity and T2DM. Glucotoxicity and Lipotoxicity should be taken into consideration when clinicians treat T2DM patients with prolonged hyperglycemia. Glucotoxicity refers to an acquired reduction in insulin secretion caused by prolonged hyperglycemia, and improved metabolic control, however, will be achieved by Diet, Insulin therapy, Sulfonylureas, or Metformin which lead to an increase in insulin secretion.

As expressed in Lipotoxicity Hypothesis, FFA_s do not only seem to interfere with insulin action but also interfere with insulin secretion. FFA_s have been shown acutely to enhance glucose-stimulated insulin secretion, whereas chronically elevated FFA_s have been shown to inhibit insulin secretion. It has been suggested that alterations in expression of metabolic enzymes by FFA_s may account for Beta Cell insensitivity to glucose or for alterations in insulin secretion.

CONCLUSIONS

Evidence has accumulated that the Insulin Resistance – Metabolic Syndrome, or the “Cumulative Metabolic Syndrome” in Obesity is also associated with alterations in haemostatic variables which contribute to increase the risk of atherothrombotic events in these patients. All these components of the “Cumulative Metabolic Syndrome” have been summarized on the previous page. There is a link between Obesity, Pre DM and T2DM which may be initiated from the Fat Cells (Low IRS-1 and Impaired GLUT-4 expression), and then secondary Insulin Resistance and Impaired Insulin Secretion of the β -Cells of pancreas will be promoting the manifestation of Diabetes Mellitus. Pleiotropic properties of Metformin beyond blood glucose lowering (antihyperglycemic and also hypoglycemic effect via

decreased GLP-1 degradation by DPP-IV) may contribute several possible potential therapeutic benefits in the treatment of patients with Insulin Resistance.

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