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Insulin Resistance
Pathological Basis and Clinical Significance

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Prologue : In their classical landmark paper describing the radioimmunoassay of insulin, Solomon Berson and Rosalyn Yalow defined insulin resistance as a *"state of a cell, tissue, system or body in which greater than normal amounts of insulin are required to elicit a quantitatively normal response"*(1). David Kipins, in a chapter on insulin physiology, in the monumental monograph : Methods in Investigative and Diagnostic Endocrinology' edited by Berson and Yalow and published prior to the demise of Berson, clearly states(2) :

'Skeletal and cardiac muscle, adipose tissue and the liver are the principal insulin responsive tissues of the body. Exposure of these tissues to physiological levels of insulin *in vivo* results in prompt and readily detectable changes in various parameters of carbohydrate, lipid, and protein metabolism. Peripheral nerve, ciliary muscle, cartilage, fibroblasts, circulating lymphocytes and granulocytes, and the arterial wall have also been reported to respond to the hormone, but the physiological relevance of these observations with respect of either total body metabolic homeostasis or individual tissue viability remains to be elucidated — ——. It is well recognized that the insulin sensitivity of target tissues is influenced by a variety of metabolic states; insulin resistance is characteristically associated with obesity, trauma, pregnancy, acromegaly and hyperadrenocorticism.'

This descriptive narrative provides the essential context to understand the effects of differential insulin sensitivity (and resistance) in various insulin responsive tissues and organs, besides the possibility of differential sensitivity with regard to the effects on carbolydrate, lipid, and protein metabolism. It also alludes to the effects on arterial wall, and circulating elements such as lymphocytes, granulocytes and platelets.

Measurement of Insulin Sensitivity : The most frequently used method of measuring insulin sensitivity is by Euglycemic clamp technique(3). In all

research laboratories, use of this technique is considered essential to demonstrate impaired sensitivity i.e. insulin resistance. The technique is highly reproducible, although time consuming, requiring skill and expertise.

The other computer program based techniques include FSIVGTT (Frequently sampled intravenous glucose tolerance test) (4) and CIGMA (Continuous infusion of glucose with model assessment) (5). The values obtained by using these techniques generally correlate well with the results obtained with the euglycemic clamp technique.

A number of techniques have also been described using standard oral glucose tolerance tests (OGTT) wherein 75 gm. glucose load is given orally after a 10-16 hour fast (WHO, 1980)(6). Blood samples are collected at 30-minute intervals following the administration of glucose load. The test duration may be 2 hours (WHO, 1980) or may be extended to 3 or 4 hours. Glucose and insulin are measured in each sample. The data is analysed using various indices, some of which depend on $AUC_{glucose}$ (area under glucose curve) and $AUC_{insulin}$ (area under insulin curve)(7). Other investigators have used I_{mean} (mean plasma insulin during OGTT; mlU/L) and G_{mean} (mean glucose concentration during OGTT (mg/dl).

Mathematical models have been used in clinical practice wherein only fasting glucose concentration, and fasting insulin concentration have been used to calculate insulin sensitivity. For example, Raynaud Index uses the formula $R1 = 40/1_0$ where 1_0 is the fasting insulin level (μU/ml)(8). Likewise, fasting glucose to insulin ratio (FGIR) has been recommended using the formula :

$$FGIR = G_0/1_0$$ (G_0 = fasting glucose, mg/dl; 1_0 = fasting insulin, μU/ml)

The major problem with a single sample-based calculation of insulin sensitivity is due to the fact that insulin secretion occurs in an oscillatory manner, thereby rendering single value-based measurement amenable to possible error. Although it has been suggested that collection of three samples at intervals of five minutes (and using pooled sample for subsequent measurements) may minimize the error, it must be remembered that 'oscillations' or 'pulses' of insulin secretion occur at two different periodicities : (i) rapid small amplitude oscillations which occur every 10-15 minutes and are superimposed on (ii) slower, larger amplitude ultradian oscillations with periods ranging from 80-150 minutes(9).

In a general mathematical model which incorporates β-cell kinetics and a gastrointestinal absorption term for glucose insulin feedback, and comprises of a set of four nonlinear, coupled ordinary differential equations(10), numerical simulations showed that glucose and insulin levels oscillate before reaching a steady state. Interestingly, glucose oscillations are seen

to lead the insulin oscillations. In our study we observed that the time period of oscillations is about 90 minutes and the glucose peak precedes insulin peak by about 06 minutes(10).

Homeostasis Model Assessment (HOMA): Insulin resistance (and sensitivity as measured by this method) is one of the most commonly used parameters in clinical metabolic research and is based on a model of insulin-glucose interaction, with measurement of fasting glucose (G_0 : mmol/L) and fasting insulin (1_0 : μIU/ml).

$$IR_{HOMA} = \frac{1_0 \times G_0}{22.5}$$

In the mathematical model proposed by us(11) and referred to in the preceding paragraph, it was further shown that in obese controls with normal glucose tolerance, there was a four-fold increase in β-cell function, an increased peripheral resistance to insulin action, and an increased initial rate of gastro-intestinal absorption as compared to non-obese controls. In contrast, in both obese and non-obese subjects with type 2 diabetes mellitus (T2DM), the changes appear to occur as a result of decreased β-cell capacity and function and an increased peripheral resistance to insulin action(12).

Insulin Resistance : metabolic basis and clinical significance : The preceding mini-review makes it abundantly clear that although the term is frequently used to indicate impaired insulin-stimulated glucose disposal as generally measured with the hyperinsulinemic euglycemic clamp technique, it must be clearly understood that insulin resistance may occur at the level of one or more of the insulin sensitive tissues such as the skeletal muscle, liver, and the adipose tissue(13). Thus not only muscle glucose uptake but also inhibition of adipose tissue lipolysis and suppression of hepatic glucose production, all regulated by insulin, must be considered both individually and jointly for a comprehensive understanding of insulin action and insulin resistance. The subject is recently reviewed by us(14). The account that follows draws upon our published review.

There is a paucity of data examining the insulin dose response characteristics of stimulation of glucose uptake (muscle) and suppression of glucose production (liver) in normals and in subjects with T2 DM. A well designed study(15) showed in the normal subjects a significant shift to the right of the dose-response curve for glucose uptake with EC_{50} of 58 μU/ml. In contrast, the corresponding EC_{50} for suppression of glucose production was approximately one-half of this i.e. 26 μU/ml. Thus, with low physiological increments in plasma insulin, the liver is the primary determinant of whole body glucose homeostasis. In subjects with T2DM, there was a marked shift of a similar magnitude of both these curves to the right, with EC_{50} for glucose uptake of 118 μU/ml. and EC_{50} for glucose production of 66 μU/ml.

In contrast to EC_{50} for glucose uptake and glucose production, data on whole

body lipolysis during stepwise hyperinsulinemic-euglycemic clamp studies show that EC_{50} for suppression of lipolysis in normal subjects is much lower and ranges between 7 and 16 μU/ml(16,17). Thus, suppression of lipolysis seems to be the most sensitive of insulin actions, with the dose response curve of adipose tissue distinctly to the left of the corresponding curves for glucose production and glucose uptake. It is also obvious from these studies that inhibition of lipolysis can be achieved at a fasting insulin concentration which is well within the normal range, and suppression of glucose production can also be achieved within the physiological range of postparandial insulin concentration. Thus, while the term Insulin Resistance Syndrome (IRS) may provide a conceptual framework for diverse cardiometabolic risk factors grouped together as Metabolic Syndrome (MS), in depth study of insulin action and resistance at different sites (muscle, liver, adipocyte) is required to compare and contrast the metabolic defects in each of the clinical disorders including obesity, T2 DM, Coronary Heart Disease (CHD), Hypertenion, Polycystic Ovaries Syndrome (PCOS) and Non-Alcoholic Fatty Liver Disease (NAFLD), and finally to investigate their relationship, if any, with high and/or low birth weights.

The need of such studies becomes imperative in the light of information recently made available as a result of disruption of insulin receptor gene in specific target tissues. Mice without insulin receptors in skeletal muscle do not develop diabetes, suggesting that insulin sensitivity (and glucose uptake) in skeletal muscle might not always be a primary cause of diabetes(18). However, these mice develop increased fat mass, elevated serum triglycerides, and high levels of circulating FFA, suggesting that insulin resistance in the muscle contributes significantly to altered fat metabolism in these animals. Likewise, mice lacking hepatic insulin receptors also fail to develop diabetes, as compensatory hyperinsulinemia apparently maintains fasting normoglycaemia (19).

In summary, resistance to insulin may be viewed in the context of multisystem insulin resistance, and the clinical sequalae must depend not only on the system(s) exhibiting resistance in a more severe form, but also on the compensatory mechanism(s), such as β-cell function, operating to respond to the challenge posed by insulin resistance.

Free Fatty Acids and Insulin Resistance: The plasma FFA concentration is determined by: (i) the rate of FFA production (lipolysis) in the adipose tissue; and (ii) the rate of uptake from plasma either for oxidation or for reesterification to triglycerides. FFA from the visceral adipose tissue drain directly into the portal vein and reach the liver. The rate of lipolytic activity is higher in the abdominal visceral adipocytes(20). Subjects with central (visceral) adiposity have higher levels of FFA in portal vein, in addition to the daylong elevation of FFA in the peripheral

plasma. Chronically elevated plasma FFA can lead to insulin resistance in muscle and liver(21,22). The thesis that abnormalities of FFA metabolism may be involved in the etiology of T2 DM (then called maturity-onset diabetes mellitus) was first propounded by Randle et al in 1963 who suggested a fundamental role of glucose-fatty acid cycle in the regulation of energy balance. Based on their studies on rat cardiac muscle in vitro, it was concluded that the rate of fat oxidation increased relative to carbohydrate oxidation in response to elevated FFA concentration(23). It was suggested that in the fasting state with low basal insulin, there was an increase in the oxidation of fatty acids by muscle (with reciprocal reduction of glucose uptake). Opposite was the case in the fed-state where enhanced insulin secretion increases glucose uptake by muscle, in addition to inhibiting lipolysis in the adipose tissue. Concluding their classical paper in the Lancet, Randle et al stated(23) : 'We propose that the interactions between glucose and fatty-acid metabolism in muscle and adipose tissue take the form of a cycle, the glucose fatty-acid cycle, which is fundamental to control of blood glucose and fatty acid concentrations and insulin sensitivity'. Nearly three decades later, these observations were confirmed in healthy human skeletal muscle wherein a decrease in carbohydrate oxidation in association with an increase in fat oxidation was demonstrated following 1-hour of lipid infusion, under euglycaemic – hyperinsulinemic clamp(24). Although the precise molecular basis of the relationship between circulating FFA levels and insulin resistance in the muscle remains uncertain, it is recognized that chronically elevated FFA levels may also impair insulin secretion from the β – cells (lipotoxicity)(25), in addition to enhancing hepatic glucose output(26). The latter may be due to a combination of increased availability of FFA for gluconeogenesis, and decreased sensitivity (resistance) to the action of insulin at the hepatocyte.

Even though the conceptual framework of Randle's hypothesis remains intact, more recent studies have shown that FFAs and their metabolites also inhibit insulin signaling, and glucose transport, in addition to affecting the activities of several enzymes involved in glucose metabolism(27). Following a 5-h infusion of lipid in healthy volunteers, a significant decrease in intracellular glucose was demonstrated, supporting the hypothesis that elevated FFA induce insulin resistance principally at the level of glucose transport(28). This could be either due to a direct effect on glucose transporter GLUT 4, or mediated through an indirect effect by modifying upstream signaling events, notably at the level of phosphatidylinositol 3-kinase (P1 3-kinase). That the latter may be the case was demonstrated by the fact that increase in P 1 3-kinase activity in response to insulin stimulation was nearly completely abolished in human volunteers given lipid infusion(29). Additional studies

show that high FFA concentration may effect several upstream proteins in the signaling pathway, including IRS-1 and protein kinase C theta (PKC θ)(30). It has been proposed that elevated FFA may activate PKC θ, with the resulting decrease in IRS-1 tyrosine phosphorylation, a suppression of P1 3-kinase activity, reduced GLUT translocation, and cultiminating in a reduction of glucose transport(31). Such lipid infusions have, therefore, been shown to closely resemble the effects of chronically elevated FFA levels such as inhibition of glucose transport and phosphorylation, glycogen synthase and pyruvate dehydrogenase activity, and insulin signaling through the IRS-1, PKC θ, and P1 3-Kinase pathways.

Additional evidence for the role of intracellular lipid in mediating insulin resistance has been obtained from transgenic mice with muscle-specific and liver-specific overexpression of lipoprotein lipase (LPL). Muscle-LPL-overexpressing mice had a threefold increase in muscle triglyceride content and were insulin resistant due to (i) decrease in insulin-stimulated glucose transport and (ii) a reduced insulin activation of IRS-1 associated P1 3-kinase activity. In contrast, liver-LPL-overexpressing mice had a two-fold increase in liver triglyceride content. These mice were insulin resistant primarily due to the impaired ability of insulin to suppress endogenous glucose production(32). These defects in insulin signaling (and action) were associated with increases in intracellular fat metabolites (i.e. diacylglycerol, fatty acylcoenzyme A). Thus there is a causal relationship between intracellular accumulation of fat metabolites and insulin resistance mediated through changes in insulin signaling pathway.

In a recent study aimed at investigating the molecular mechanism (s) underlying the biochemical basis of insulin resistance due to the effect of elevated FFA on the muscle, a triglyceride emulsion was infused in healthy subjects for 48 hours, followed by muscle biopsis (vastus lateralis muscle), microarray analysis, quantitative real time PCR, and immunoblots. After lipid infusion, extracellular matrix genes and connective tissue growth factor were significantly over expressed. In contrast, nuclear encoded mitochondrial genes and PGC-1α (peroxisome proliferator activated receptor γ – coactivator –1α) expression were decreased(33). As PGC-1α is the transcriptional coactivator that initiates the expression of several genes coding for mitochondrial proteins, a decrease in PGC-1 expression may result in decreased expression of a number of metabolic and nuclear encoded mitochondrial genes involved in electron transport and oxidative phosphorylation. Indeed, a decreased expression of nuclear encoded mitochondrial genes, accompanied by a decreased expression of PGC-1α, has been demonstrated in insulin resistant subjects(34,35).

Notwithstanding the effect on nuclear encoded mitochondrial genes, a marked increase in expression of extracellular matrix-related genes following lipid infusion was of considerable interest(33). Such a pattern characterizes inflammatory

response leading to extracellular matrix remodelling and fibrosis. Thus chronic elevation of FFA may result in inflammation-associated extracellular matrix changes in the skeletal muscle. Such fibrotic inflammatory responses are mediated by the Connective tissue growth factor (CTGF), also termed CCN2, a $38KD_a$ protein belonging to the CNN family(36). Fig. 1 projects a conceptual model of the molecular basis of insulin resistance(14). Metabolic activity in the adipocytes, through changes in the circulating levels of adipokines and elevated levels of FFA may result in a decreased expression of PGC-1α and several nuclear encoded mitochondrial genes, thereby reducing oxidative phosphorylation, in addition to producing defects in glucose transport and insulin signaling pathways. The resultant insulin resistant state in turn increases lipolysis, further increasing the levels of circulating FFA. Adipokines, such as resistin, lead to increase in hepatic fat, causing insulin

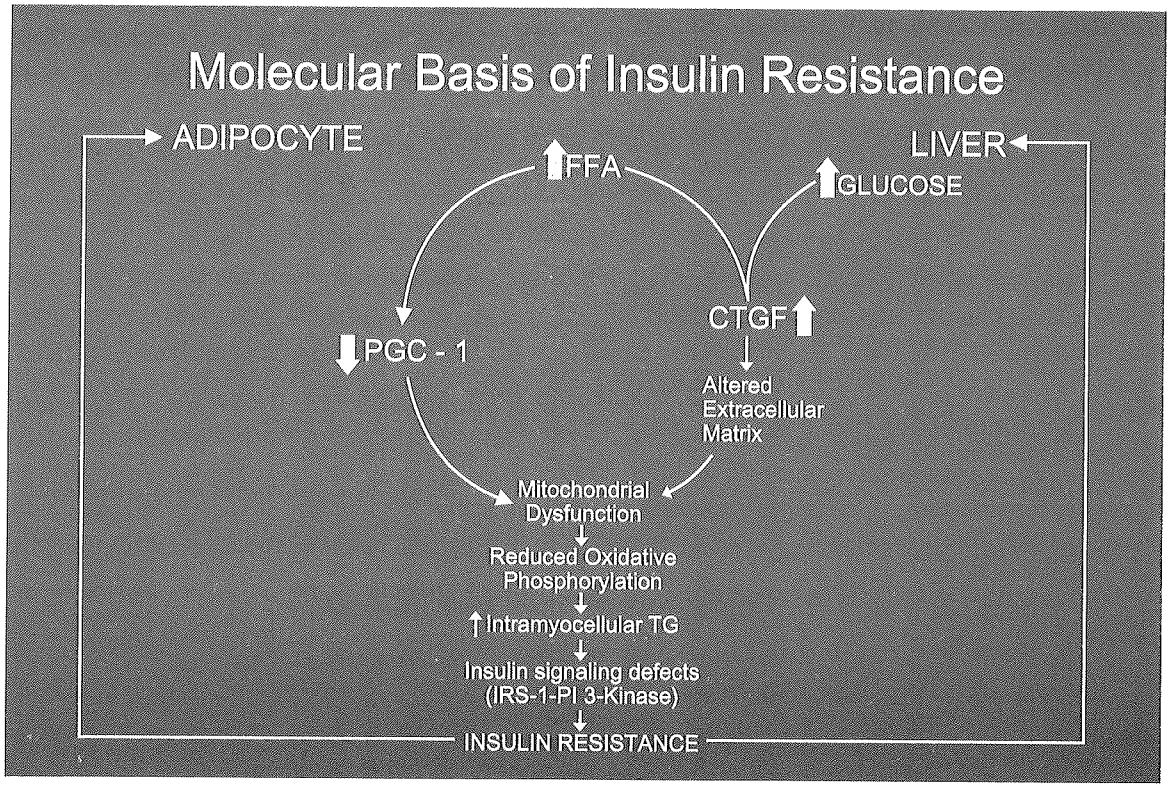

Fig. 1 : Decreased expression of nuclear encoded mitochondrial genes and increased expression of extracellular matrix - related genes may contribute to the molecular basis of insulin resistance (for explanation, see text). From Bajaj and Bajaj (14) with permission of Editor and Publisher

PGC - 1α : peroxisome proliferator activated receptor γ - coactivator - 1α; CTGF : connective tissue growth factor; FFA : free fatty acids.

resistance in the liver, and increased hepatic glucose output. In contrast, adiponectin reduces hepatic fat content. Increased levels of glucose and FFA enhance the expression of CTGF, leading to altered cellular matrix. It is obvious that new therapeutic targets aimed at increasing PGC-1α activity and reducing CTGF activity bear therapeutic potential.

There is evidence that CTGF mediates fibrotic changes at multiple sites i.e. atheromatous plaques(37); mesangium in the glomerulus(38); myocardium following ischemic injury(39) and activated hepatic stellate cells(40). As angiotensin II (acting through angiotensin receptor 1) increases the expression of CTGF, the current use of specific angiotensin receptor blockers in the prevention and management of diabetic nephropathy seems to be most rational. Of major interest is the observation that liver biopsis from nondiabetics and T2DM patients with non-alcoholic steatohepatitis (NASH) showed enhanced expression of CTGF that correlated with the degree of fibrosis(40). As the CTGF expression is increased in the liver from Zucker obese rats in association with lipid abnormalities and fatty liver in this animal model of

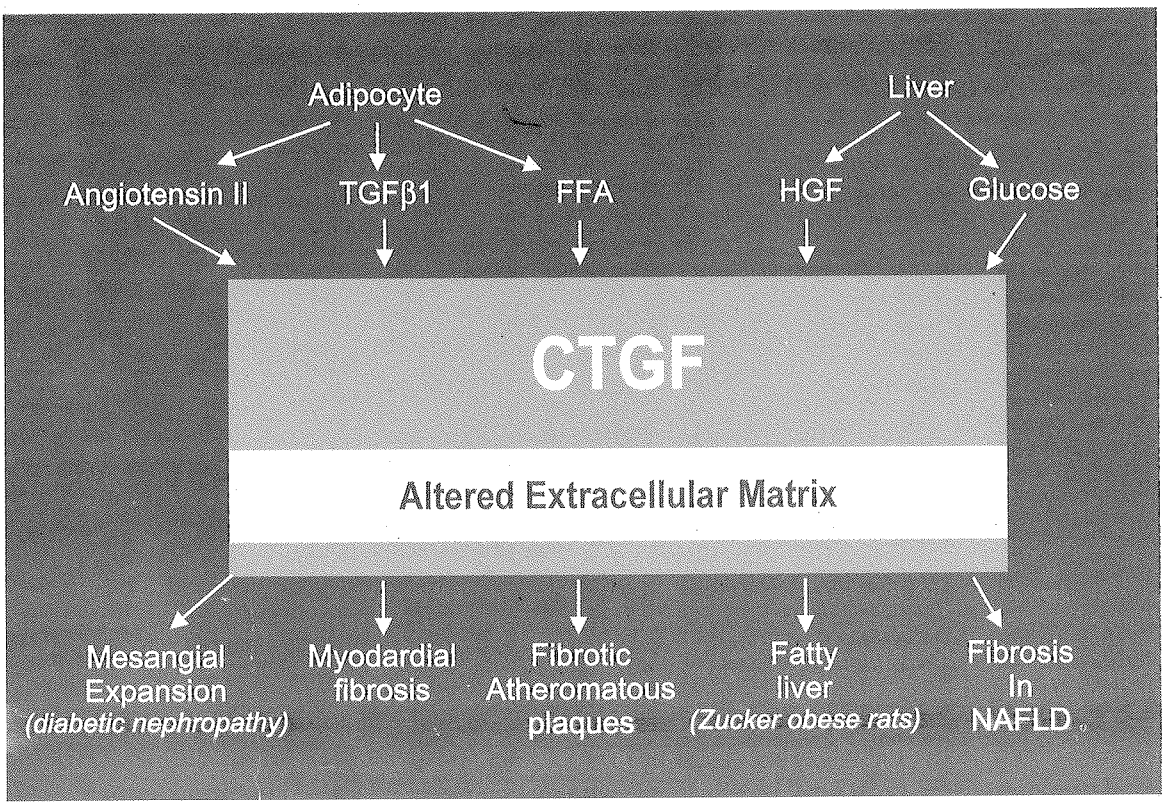

Fig. 2 : Enhanced expression of connective tissue growth factor and its possible consequences. From Bajaj and Bajaj (14) with permission of Editor and Publisher.

insulin resistance, it may be a rational molecular target for future drug development. Indeed, an angiotensin II type 1 receptor antagonist, olmesartan medoxomil, has been shown to improve experimental liver fibrosis by suppression of proliferation and collagen synthesis in hepatic stellate cells(41). A **unifying** model of several diseases included in the Metabolic Syndrome, and their complications, is shown in Fig. 2 which may also portend possible molecular sites for future drug development(14).

Adiponectin and Insulin resistance : Hypoadiponectinemia characterizes T2DM, a well recognized insulin-resistant state. Recent studies have provided evidence that increased hepatic fat content is an important determinant of hepatic insulin resistance in type 2 diabetic patients(42). Thiazolidinediones have been shown to reduce hepatic fat content and improve hepatic insulin sensitivity in patients with T2DM(42). Thiazolidinediones initiate their action by binding PPARγ, primarily located on adipocytes(43), and thereby increasing plasma adiponectin levels. Indirect evidence suggests that adiponectin might mediate some of the insulin-sensitizing effects of PPARγ agonists.

The first-ever clinical study aimed at investigating the effect of long term (14 weeks) administration of 45 mg. pioglitazone daily in subjects with T2DM resulted in a three-fold increase in plasma adiponectin which correlated inversely with endogenous (hepatic) glucose production. There was also a significant inverse correlation of plasma adiponectin with hepatic fat content. Higher the plasma adiponectin levels, lower the hepatic fat content. Thus the increase in plasma adiponectin following pioglitazone therapy is strongly associated with a decrease in hepatic fat content and enhanced hepatic and peripheral insulin sensitivity(44).

Put together with a similar study(45), there is unequivocal evidence that pioglitazone (thiozolidinedione) treatment of subjects with T2DM ***increases*** plasma adiponectin and ***decreases*** plasma resistin levels, resulting in a decrease in hepatic fat content and a reduction in hepatic glucose production. Indicators of net therapeutic benefit included a decrease in fasting plasma glucose as well as a lowering of the HbA1c and serum triglyceride levels(44).

In addition to its metabolic effects, adiponectin has also been shown to modulate endothelial inflammatory response through TNF-α-induced expression of endothelial adhesion molecules(46). *In vitro* studies in human aortic endothelial cells have shown that human recombinant adiponectin not only suppresses endothelial expression of adhesion molecules but also decreases the proliferation of vascular smooth muscle cells, and reduces lipid accumulation in macrophages, thereby modulating transformation of macrophages to foam cells(47).

Two clinical studies published recently provide interesting data linking the metabolic and anti-inflammatory roles of adiponectin. In a study of 77 subjects who had diabetes or were at high risk to develop diabetes, there was a significant negative correlation between circulating levels of adiponectin and C-reactive protein (CRP), plasminogen activator inhibitor-1 (PAI-1), and tissue plasminogen activator (tPA)(48). These negative associations remained significant after adjusting for gender and BMI. This study reinforces earlier observation regarding the protective role of adiponectin against inflammation and endothelial dysfunction, and provides evidence of its negative association with tPA, which is known to play a role in impaired fibrinolysis. A similar study in women with prior gestational diabetes mellitus (pGDM) who are known to be at higher risk of developing T2DM and associated cardiovascular complications, showed that plasma adiponectin was significantly lower in pGDM as compared to women with normal glucose tolerance during pregnancy. The differences remained statistically significant even after adjustment for body fat mass. Equally significant were the differences in the levels of PAI-1 and ultrasensitive CRP which were higher in the pGDM group. It was concluded that lower plasma adiponectin concentrations characterize women with previous GDM independently of the prevailing glucose tolerance, insulin sensitivity or the degree of obesity and are associated with subclinical inflammation and atherogenic parameters(49).

Thus, the role of adiponectin as a mediator of insulin resistance and an integrator of metabolic and inflammatory signals underlying obesity, T2DM, and coronary heart disease has assumed considerable significance, both in terms of its potential as a part of preventive strategies, and also as a prototype molecule for the development of new analogues and related compounds aimed at therapeutic intervention. Further, development of PPAR-γ agonists which increase endogenous adiponectin may be equally promising and rewarding.

Pathophysiological basis of metabolic syndrome : The foregoing narrative clearly delineates a constellation of risk factors of metabolic origin which have a close association with increased risk of T2DM, hypertension and atherosclerotic cardiovascular disease (CVD). Although there is a considerable agreement on the role and place of various cardiometabolic risk factors, the terminology of metabolic syndrome has raised intense debate(50). Nevertheless, the recognised risk factors include derangements of lipid metabolism (atherogenic dyslipidemia) comprising an increased levels of apoliprotein B, triglycerides, small LDL particles, and low levels of high density lipoproteins (HDL). Also present may be disturbances of

carbohydrate metabolism such as impaired fasting glucose, impaired glucose tolerance, or diabetes mellitus. A prothrombotic state comprising of an increase in procoagulant factors such as fibrinogen and factor VII, alongwith increased anti-fibrinolytic factors such as PAI-1, and endothelial dysfunction are important constituents. An associated proinflammatory state characterized by elevation of circulating cytokines i.e. TNF-α, resistin and IL-6 in addition to an increase in acute phase reactant (CRP) is generally observed. A significant reduction in circulating adiponectin is a contributory factor. The most important clincopathological basis is obesity, especially trunkal obesity or visceral adiposity, and the key molecular link is insulin resistance at various levels, and in different compartments *(vide supra)*.

The causes of the 'metabolic syndrome' are complex and as suggested above, involve hormonal and metabolic factors. Genetic susceptibility is described and gene-environment (lifestyle) interactions are well recognized. Although obesity, especially central adiposity remains a key clinical link, its definition based on ethnicity-specific cut-off values of waist circumference has been delineated only recently (Table(51)). A combination of accurately measured waist circumference, along with BMI {Wt. (kg) / Ht. (in meters)^2} may be more informative. It is however suggested that a single accurate measurement of waist circumference if culturally permissible, should be the preferred choice.

Ethnicity-specific values for waist circumference (51) :

Country/ ethnic group		Waist circumference (cm) (as measure of central obesity)
Europids	Male	≥ 94
	Female	≥ 80
South Asians	Male	≥ 90
	Female	≥ 80
Chinese	Male	≥ 90
	Female	≥ 80
Japanese	Male	≥ 90
	Female	≥ 80

While recommending these cut-off points for waist circumference, it is emphasized that 'these are pragmatic cut-off points and better data are required to link them to risk. It is also mentioned that ethnicity should be the basis for classification, not the country of residence' (51).

Epilogue : While the purists may continue to argue about the diagnostic criteria (WHO(52) or IDF(53) or NCEP : ATP III(54)) and the cell biologists may continue their efforts at localizing molecular lesion (s) underlying insulin resistance and deranged adipocyte biology, there is enough information already available to launch rationally sound public health measures at the *individual, community* and *national* levels(50). Time to act was yesterday : to-day may already be too late!

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