MINIREVIEW

Insulin Resistance: Cellular and Clinical Concepts

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Insulin resistance is defined as a clinical state in which a normal or elevated insulin level produces an attenuated biologic response. Specifically, the biologic response most studied is insulin-stimulated glucose disposal, yet the precise cellular mechanism responsible is not yet known. However, the presence of insulin resistance is observed many years before the onset of clinical hyperglycemia and the diagnosis of Type 2 diabetes. Insulin resistance at this stage appears to be significantly associated with a clustering of cardiovascular risk factors predisposing the individual to accelerated cardiovascular disease. An overview of insulin resistance and the associated clinical insulin resistant state will be discussed. [E.B.M. 2001, Vol 226:13–26]

Key words: insulin; type 2 diabetes; atherosclerosis; insulin resistance; metabolic syndrome

Insulin resistance is a key pathogenic parameter observed in the natural history of Type 2 diabetes (1, 2). Development of insulin resistance results in compensatory hyperinsulinemia, a state that is maintained until pancreatic secretory defects occur. However, once β-cell dysfunction occurs, inability to compensate for the increased insulin resistance results in hyperglycemia, and the diagnosis of Type 2 diabetes is made on clinical grounds. As such, many would argue that insulin resistance may be the initial lesion leading to (and appears to be predictive of) Type 2 diabetes (3–5). Intensive research efforts have been aimed at identifying the cellular mechanisms responsible for insulin resistance and designing pharmacologic therapies to alleviate the attenuation in insulin action. This review will focus on current concepts in understanding the cellular defects contributing to insulin resistance, describe methods to clinically assess this parameter, and discuss risk factors associated with this clinical state. An overview of management options will also be presented.

The concept of insulin resistance originated well over 50 years ago, and a brief historical overview was recently provided by Hunter and Garvey (6). Specifically, early clinical observations noted with the advent of insulin therapy for treatment of diabetes suggested that there were two groups of diabetic patients, roughly divided by their response to the hypoglycemic effects of exogenously administered insulin (6). These two groups may now be argued to correspond to the current definitions of Type 1 and Type 2 diabetes. The term insulin resistance continued to evolve to describe diabetic patients whose clinical treatment required markedly elevated insulin dosing (≥200 units of insulin a day). This elevated exogenous insulin demand was often associated with antibodies induced by the insulin preparations available at the time (i.e., bovine and porcine insulin) (6). With the advent in the 1960s of the radioimmunoassay for insulin (which distinguished Type 1 diabetic patients with absolute insulin deficiency from Type 2 diabetic patients who were found to have relatively normal or elevated insulin levels), it became readily apparent that a cohort of individuals existed with normal or near-normal glucose levels but elevated insulin levels. The use of sophisticated *in vivo* techniques, which were widely used in clinical research studies in the 1970s and 1980s to assess glucose disposal, added greatly to the understanding. Specifically, these metabolic studies demonstrated conclusively that insulin resistance was due to impaired insulin action in insulin-sensitive peripheral tissues such as fat, muscle, and liver (1). These studies also defined insulin resistance as a postreceptor defect, referring to abnormalities in the insulin

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signaling cascade after stimulation of the insulin receptor. The observations from these studies provide the most accepted and current-day definition of insulin resistance as "a clinical state in which a normal or elevated insulin level produces an impaired biological response" (6).

Insulin is a growth factor and, by definition, would be expected to elicit myriad biological responses; the response could be a metabolic process (changes in carbohydrate, lipid, or protein metabolism) or a mitogenic process (alterations in growth, differentiation, DNA synthesis, or regulation of gene transcription) (6). Therefore, insulin resistance could apply to any of these pleiotrophic effects of insulin. The term insulin resistance, however, is classically applied to insulin's ability to stimulate glucose uptake in insulin-sensitive peripheral tissues (i.e., fat and muscle) since this is the biological response most directly relevant to the clinical manifestations (e.g., hyperinsulinemia and impaired glucose tolerance). Further, insulin resistance, although generally referring to the glucose-insulin relationship, should not be confused with the clinical concept of the insulin resistance syndrome, which applies to additional biological actions of insulin, including its effects on lipid and protein metabolism, endothelial function, and gene expression (6–10). Indeed, the insulin resistance syndrome consists of a cluster of disorders and biochemical abnormalities and has been given the name Syndrome X or the deadly quartet (7–10). The associated clinical and laboratory abnormalities that represent this syndrome consist of Type 2 diabetes mellitus, central obesity, dyslipidemia (increased triglycerides, decreased HDL, and increased small dense LDL), hypertension, increased prothrombotic and antifibrinolytic factors (i.e., hypercoagulability), and a predilection for heart disease (Fig. 1). Furthermore, there are a number of other conditions associated with insulin resistance that refer to specific clinical presentations (such as polycystic ovarian syndrome, pregnancy, or glucocorticoid therapy) that may include some or none of the features of the insulin resistance syndrome or Syndrome X (6).

Insulin Resistance in the Natural History of Type 2 Diabetes

Studies in families and populations with a high incidence of Type 2 diabetes have shown that reduced insulin-dependent glucose transport is frequently found in nondiabetic relatives and offspring of patients with Type 2 diabetes (5). The presence of this abnormality in these people suggests that insulin resistance may be a primary factor in the development of Type 2 diabetes and the early development of accelerated atherosclerosis. As such, current concepts in the development of Type 2 diabetes indicate that glucose and fasting insulin levels may be normal for many years before the development of the disease. In the presence of obesity and a family history of diabetes, insulin resistance typically is present, and the individual will increase insulin secretion, particularly after meals, to compensate for the insulin resistance (1–4). Euglycemia is therefore maintained as long as the individual continues this compensatory hyperinsulinemia to overcome the resistance (1–4). As recently reviewed from the Consensus Development Conference on Insulin Resistance, plasma insulin levels, whether measured fasting or postprandially, appear to be predictive for development of Type 2 diabetes, and this risk appears independent of obesity or waist circumference (5). In addition, this risk is particularly strong for individuals with a

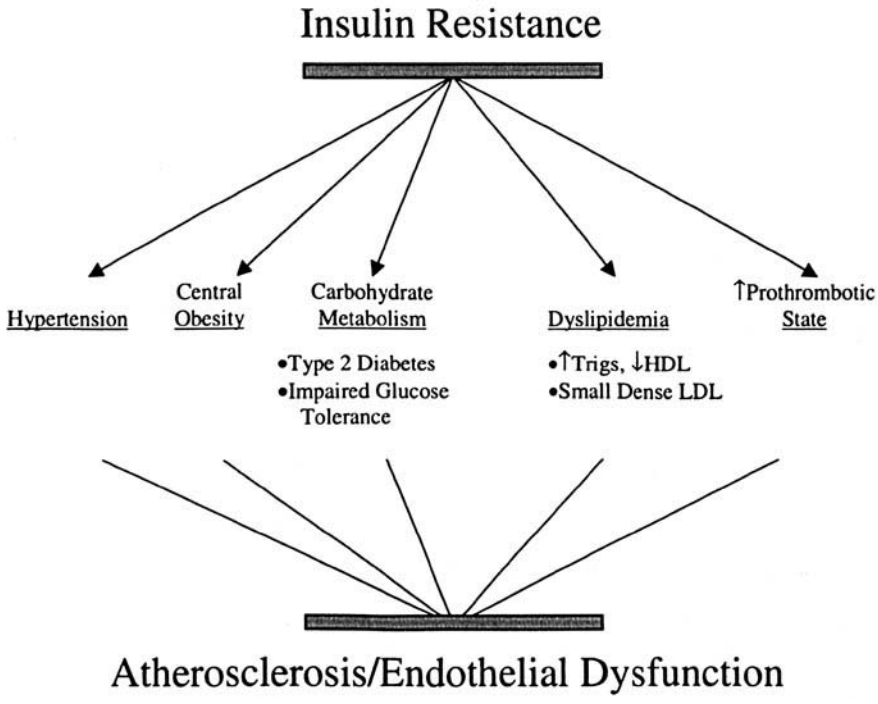

Figure 1. Clinical and laboratory abnormalities associated with the insulin resistance syndrome.

known family history of Type 2 diabetes. However, as β-cell dysfunction becomes apparent, leading to a relative decrease in insulin, the individual is unable to compensate for the insulin resistance (3). Increased hepatic gluconeogenesis may occur, and fasting blood glucose begins to rise such that the clinician now makes the diagnosis of Type 2 diabetes mellitus. This period in the patient's life associated with insulin resistance and impaired glucose tolerance is felt to represent the prediabetic phase, as insulin resistance appears highly predictive of development of Type 2 diabetes (2–5). Indeed, it is at this stage in the natural history of Type 2 diabetes where prevention trials are currently addressing the need to reduce the insulin resistance, by both pharmacologic and nonpharmacologic means, in the hope that Type 2 diabetes can be prevented or delayed (11). It is also at this stage where the clustering of clinical risk factors (e.g., Syndrome X, Cardiovascular Dysmetabolic Syndrome, "deadly quartet") is observed (7–10). Therefore, we now recognize that insulin resistance may be present many years before the diagnosis of Type 2 diabetes is made and is associated with a clustering of risk factors that predisposes a patient to accelerated atherosclerosis (7–10, 12–18). A schematic representing insulin resistance and compensatory hyperinsulinemia, associated risk factors, and when a diagnosis of Type 2 diabetes is likely to be made is outlined in Figure 2 (13).

Cellular Events of Insulin Action

Understanding the cellular mechanism(s) that contribute to insulin resistance would be important in identifying its genetic basis and would allow both the development of effective therapies and optimal use of current therapies. As stated, the aspect of insulin resistance that has been the most well described is the inefficient glucose uptake and utilization in response to insulin stimulation in insulin-sensitive tissues (1, 5). Under *in vivo* conditions, this is represented by a reduction in the insulin-stimulated storage of glucose as glycogen in both muscle and liver (1, 5). It has been described that the primary mechanism in muscle appears to be a block in the glucose transport and phosphorylation step, and both genetic and environmental factors appear to induce this defect (5). In order to have an understanding of the potential cellular abnormalities that predispose an individual to insulin resistance, a brief overview of the cellular factors regulating insulin action will be presented.

Molecular Events of Insulin Action. Insulin action in insulin-sensitive peripheral tissues (e.g., fat or muscle) begins with specific binding to high-affinity receptors on the plasma membrane of the target tissue (Fig. 3) (19, 20). The insulin receptor is a large transmembrane protein consisting of α- and β-subunits. Insulin initiates its cellular effects by binding to the α-subunit of its receptor

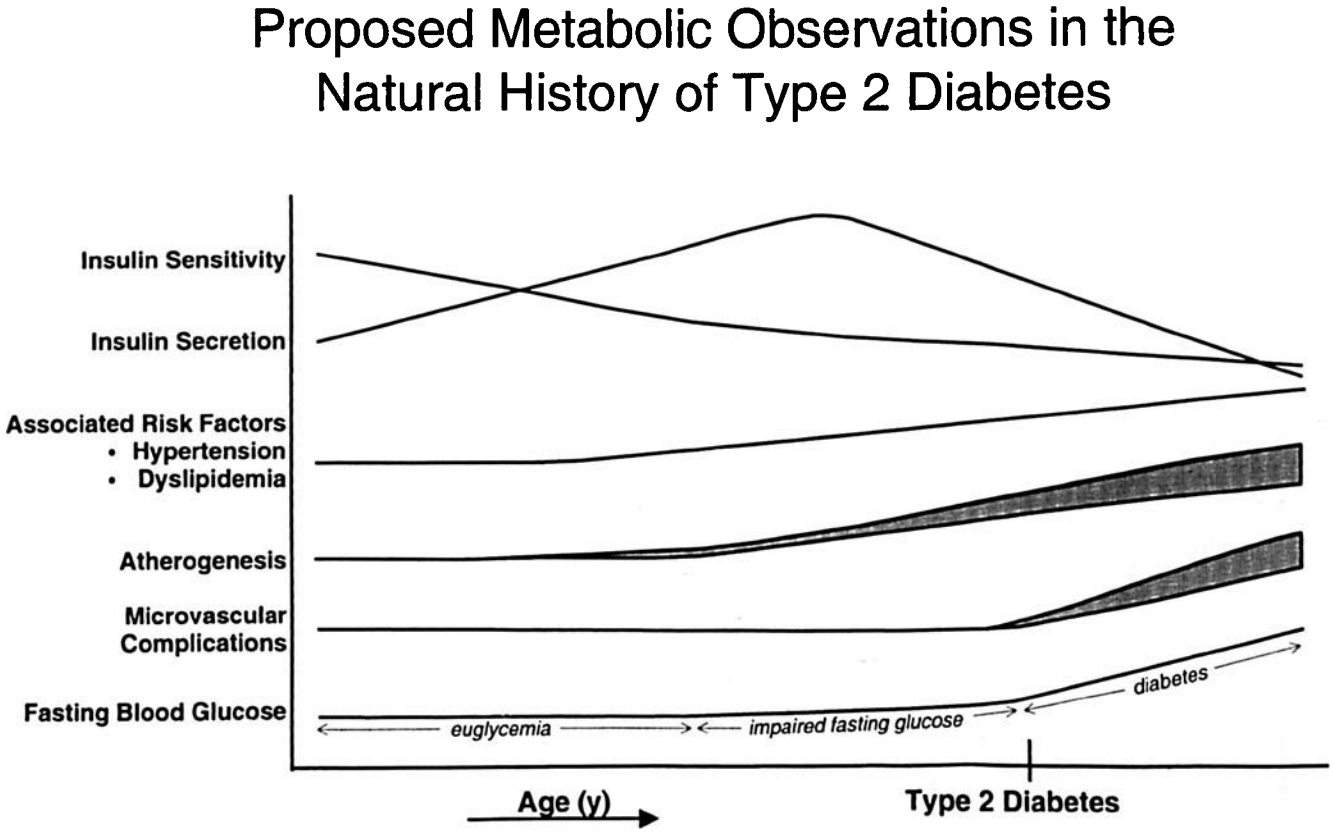

Figure 2. Schematic representing clinical and laboratory findings in the natural history of Type 2 diabetes.

(whose structure establishes the specificity for insulin binding) and thus leads to the autophosphorylation of specific tyrosine residues of the β-subunit (19, 20). The β-subunit possesses tyrosine kinase activity, and this process enhances the tyrosine kinase activity of the receptor toward other protein substrates. Considerable evidence demonstrates that activation of insulin receptor kinase plays an essential role for many, if not all, of the biological effects of insulin (19–22). Furthermore, the insulin receptor tyrosine kinase plays a major role in signal transduction distal to the receptor as activation results in tyrosine phosphorylation of insulin receptor substrates (IRSs), including IRS-1, IRS-2, IRS-3, IRS-4, Gab-1, and SHC (19, 20, 23–27). The IRS proteins are cytoplasmic proteins with multiple tyrosine phosphorylation sites that, following insulin stimulation, serve as docking sites for cytosolic substrates that contain specific recognition domains, termed SH2 domains (Fig. 3) (28–30). These structural domains on the IRS proteins provide an extensive potential for interaction with downstream signaling molecules *via* the multiple phosphorylation motifs, including p85α/β, p50, Grb-2, SHP-2, and Nck (19, 20, 2330). As described, the divergence of insulin signaling pathways within the cell may reside at the level of the IRS docking proteins; therefore, the IRS proteins have been referred to as the metabolic switches of the cell.

The explanation provided for insulin's effect on glucose uptake is less well defined but involves the enzyme phosphatidylinositide-3 kinase (PI-3 kinase). Insulin stimulation increases the amount of PI-3 kinase associated with IRS, and its activity is activated directly by docking (19, 20, 24, 25, 29). Specifically, binding of IRSs to the regulatory subunit of phosphatidylinositol-3-OH kinase (PI-3 kinase) at Src homology 2 domains results in activation of PI-3 kinase, which appears necessary for insulin action on glucose transport (31–34), glycogen synthesis (35), protein synthesis (36), antilipolysis (32), and gene expression (37). Furthermore, PI-3 kinase activation is responsible, in part, for Glut-4 translocation from intracellular vesicles to the plasma membrane after insulin stimulation (32, 38, 39). As activation of PI-3 kinase appears to be of crucial importance

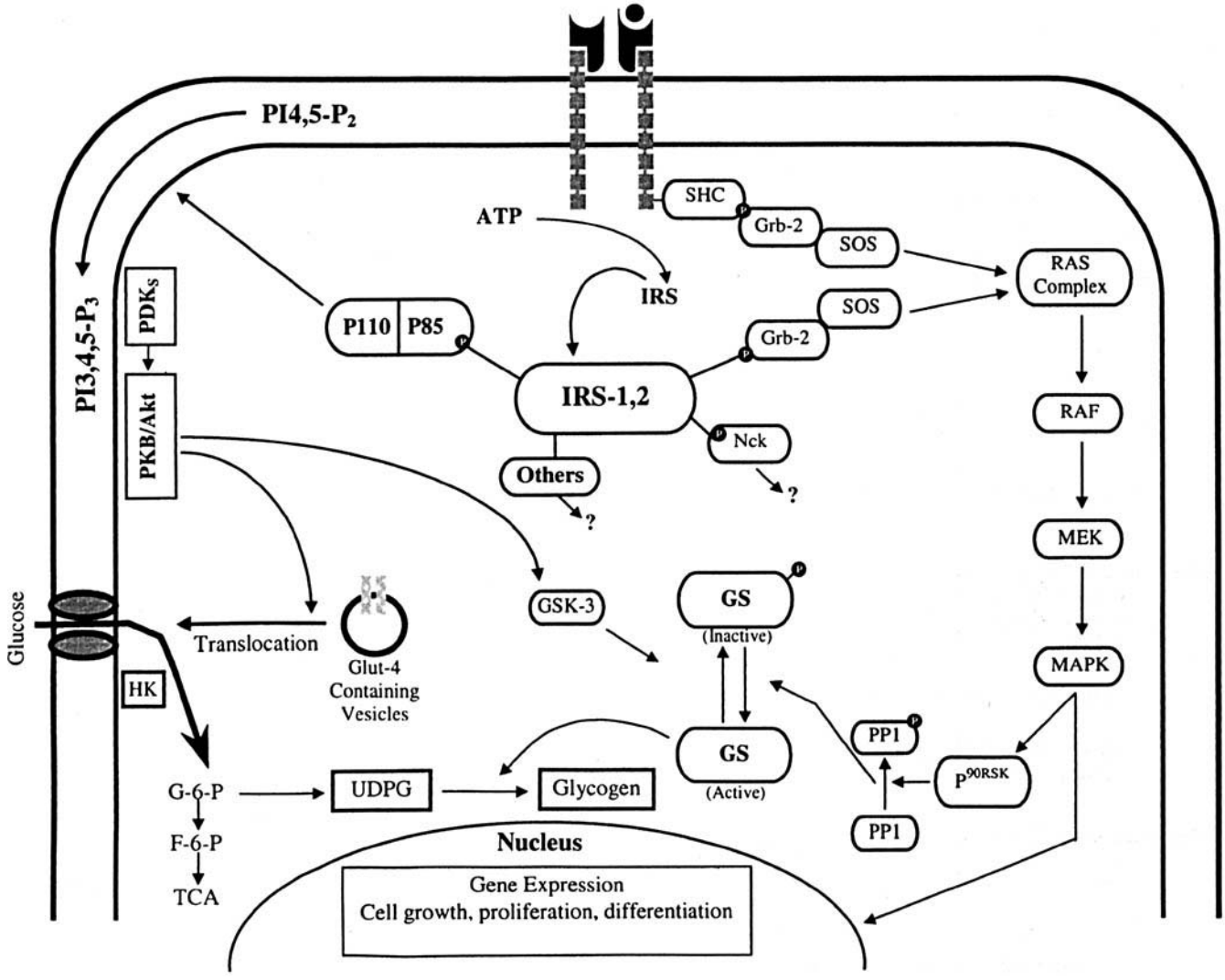

Figure 3. Schematic representing proposed signals in the insulin signaling cascade.

for Glut-4 translocation and glycogen synthase activation (two cellular parameters clearly affected by insulin resistance), the study of upstream intracellular signals (e.g., IRS phosphorylation, PI-3 kinase activity) that regulate glucose uptake and glycogen synthesis would provide a cellular basis for understanding insulin resistance. Activation of PI-3 kinase appears to be critical for transducing the metabolic effects of insulin, as inhibition of PI-3 kinase activation blocks insulin's ability to stimulate glucose transport. However, other growth factor receptors have been shown to activate PI-3 kinase to the same extent as the insulin receptor, but they do not stimulate glucose transport. Therefore, it appears that although PI-3 kinase is necessary for the action of insulin, it is not sufficient in and of itself to account for the glucose uptake process. The additional factors that are responsible for the stimulation of glucose transport are currently unknown. In summary, current evidence suggests that IRS proteins, in their phosphorylated form, may regulate insulin signaling by acting as a docking site by binding to and regulating intracellular enzymes containing SH2 domains that allow the insulin signal to diverge throughout the target cell (19, 20, 24, 25, 28–30).

Insulin-Stimulated Glucose Transport. After the generation of the second messengers for insulin action, glucose transport into the cell is activated. This effect of insulin is brought about by the translocation of a large pool of glucose transporters from an intracellular pool to the plasma membrane (40). Two distinct molecular families of glucose transporters have been cloned, and they consist of at least five homologous transmembrane proteins (Glut-1, -2, -3, -4, and -5) encoded by distinct genes. These glut proteins have distinct specificities, kinetic properties, and tissue distribution that define their clinical role (40). Two major glut proteins (Glut-1 and -4) have been identified in skeletal muscle; Glut-1 may be involved primarily in basal glucose uptake, whereas the major insulin-responsive glucose transporter isoform is termed Glut-4 and is predominantly expressed in insulin target tissues such as skeletal and cardiac muscle and adipose tissue. In normal muscle cells, Glut-4 is recycled between the plasma membrane and intracellular storage pools; thus, it differs from other transporters in that 90% of it is sequestered intracellularly in the absence of insulin (40). With insulin stimulation, the equilibrium of this recycling process is altered to favor translocation (regulated movement) of Glut-4 from intracellular stores to the plasma membrane and transverse tubules in the muscle, resulting in a rise in the maximal velocity of glucose transport into the cell (40). Therefore, impaired glucose transport may contribute greatly to the reduced glycogen synthesis observed in insulin resistance (41, 42).

Insulin stimulates Glut-4 translocation by first binding to the receptor and activating tyrosine kinase phosphorylation at the intracellular portion of the receptor. As discussed above, in both adipocyte and muscle, subsequent activation of PI-3 kinase by IRS phosphorylation appears to be a necessary step for insulin action on glucose transport (31–34), glycogen synthesis (35), and Glut-4 translocation (32, 38–40, 43). Studies have demonstrated that activation of PI-3 kinase is necessary for insulin-stimulated glucose uptake in rat adipocytes (32, 44), 3T3-L1 adipocytes (31, 45–47), L6 muscle cells (48), and rat skeletal muscle (49). In addition, studies have suggested that PI-3 kinase is required for glucose transporter translocation, as specific inhibitors of PI-3 kinase inhibited insulin-stimulated glucose uptake in 3T3-L1 adipocytes (45) and a P85 mutant lacking the binding site for P110 inhibited insulin-stimulated glucose uptake in CHO cells (34). However, the downstream pathway by which PI-3 kinase activation results in Glut-4 translocation remains unknown. A candidate molecule that has received recent interest is the serine/threonine kinase Akt, also known as protein kinase B, or Rac (50, 51). Evidence that PI-3 kinase is an upstream regulator of Akt comes from studies demonstrating that wortmannin, dominant-negative PI-3 kinase mutants, and growth factor point mutations prevent the activation of Akt (50–53), and constitutively active mutants of PI-3 kinase are sufficient to stimulate Akt in cells (54, 55).

Signaling Pathways Regulating Glycogen Synthesis. The rate-limiting step in glycogen synthesis is conversion of UDP-glucose to glycogen by glycogen synthase (43). Glycogen synthase is regulated by both allosteric and phosphorylation-dephosphorylation mechanisms (43, 56, 57) and has been found to be serine-phosphorylated on multiple sites. Insulin stimulation results in dephosphorylation of several of these sites, and dephosphorylation activates the enzyme and results in increased glycogen synthesis (57). Furthermore, there is evidence in support of insulin stimulation regulating glycogen synthase activity by protein phosphatase-1 (PP1) activation and glycogen synthase kinase-3 (GSK-3) inhibition (57, 58). Recent studies have questioned that insulin signaling to GS is mediated exclusively through the ras-MAP kinase transduction, but have indicated the presence of yet another parallel pathway likely involving PI-3 kinase.

It is unclear how PI-3 kinase activation relays the insulin signal to activation of GS, but it may be secondary to the interaction of the lipid products of PI-3 kinase with the serine/threonine kinase protein kinase B (PKB)/Akt (50, 51). Thus, the lipid products of PI-3 kinase appear to play a role in Akt activation. Specifically, the PI-3,4-P_3 lipid products of PI-3 kinase activate and recruit PtdIns 3,4,5-triphosphate-dependent protein kinase 1, which phosphorylates Akt (Fig. 3) (50, 51). Support of this mechanism is found in studies whereby inhibitors of PI-3 kinase prevent activation of PKB/Akt (59). Akt has been shown to mediate the effects of PI-3 kinase on cellular events such as apoptosis (60) and protein synthesis (61, 62). Also, it is thought to mediate the phosphorylation and inactivation of GSK-3 by insulin (63). Specific inhibition of PI-3 kinase in rat L6 cells by wortmannin, which also decreases Glut-4 translocation and activation, prevented the inactivation of insulin on GSK-3 and the activation of $p90^{RSK}$, $p70^{S6K}$, and the

MAP-kinases (64). The activation of protein kinase B (PKB) is prevented by blocking PI-3 kinase (63). These results demonstrate a link between PI-3 kinase and PKB to the insulin-dependent glycogen synthesis.

In summary, the insulin signaling to muscle glycogen synthesis appears to be mediated through two complementary pathways. One is through PI-3 kinase and PKB with inhibition of GSK-3 and thereby activation of glycogen synthase. Another pathway involves dephosphorylation (activation) of glycogen synthase through activation of PP1. However, the most interesting signal protein is probably PI-3 kinase, which, when associated with the IRS proteins, seems to be deleterious in Type 2 diabetes for Glut-4 translocation and activation of glycogen synthesis. The reduction in glycogen synthesis observed in insulin-resistant states may be due to diminished intracellular insulin signaling.

Defining the Cellular Lesion

The cellular abnormality accounting for clinical insulin resistance theoretically could involve any one of the multiple steps of the insulin-signaling cascade as described above, as alterations in insulin production, insulin binding, or intracellular signaling all have the potential to induce an insulin-resistant state (6). For example, an abnormal β-cell product, secondary to a mutation in the gene coding for the insulin molecule, is associated with an attenuated biological effect. These clinical conditions have been referred to as mutant insulin syndromes, whereby single amino acid substitutions in regions of the molecule that interact with the insulin receptor with reduced affinity ultimately result in an impaired biological action (6). An example of an acquired defect associated with insulin resistance is anti-insulin antibodies. In this state, antibodies directed against the insulin molecule can complex with insulin and reduce the amount available to target insulin receptors (6). Fortunately, high titers of insulin antibodies are now rare due to the common use of recombinant human insulin. Such examples as cited above are referred to as prereceptor causes of insulin resistance since these defects occur prior to or at the binding of insulin to the receptor. The insulin resistance most commonly observed clinically is referred to as a postreceptor defect because insulin signaling and/or effective glucose transport after insulin binding (i.e., intracellular events) is attenuated.

Insulin resistance is also frequently observed in clinical conditions associated with overproduction of counterregulatory hormones such as cortisol, epinephrine, and growth hormone (6). Specifically, acromegaly, Cushings Syndrome, and pheochromocytoma, on clinical grounds, are associated with attenuated insulin action and may present with impaired carbohydrate metabolism. A number of other human diseases and conditions characterized by insulin resistance have been described, as recently reviewed by Hunter and Garvey (6); these are listed in Table I.

As understood from a clinical perspective, the aspect of insulin resistance most studied is defective insulin-mediated glucose uptake and utilization (1, 41–43). In patients, this defect is manifested by a reduction in glycogen synthesis in muscle and liver. With the observation that the rate-limiting role in cellular glucose metabolism is the plasma membrane transport, Glut-4 defects have the potential to result in insulin resistance at the level of the glucose transport effector system. A decrease in gene expression (i.e., protein content), diminished functional capacity, or impaired translocation of Glut-4 to the plasma membrane are defects that may explain the diminished transport. However, this defect in glucose transport cannot be explained by a reduction in the total number of glucose transporter units, and studies have not defined whether the intrinsic activity of the glucose transporter is impaired or whether there is a defect in translocation (5).

Whether defects in intracellular signaling are the cause for the resistance has been argued to be very likely, but a specific defect in any one signaling pathway to explain insulin resistance has not been observed (5). It has been described that a critical threshold level of IRS activity is necessary to stimulate PI-3 kinase maximally, and that IRS proteins play a major role in insulin-stimulated glucose up-

Table I. Human Diseases and Conditions Characterized by Insulin Resistance

Insulin resistance may be primary	Insulin resistance may be secondary	Insulin resistance associated with genetic syndromes
Type 2 diabetes mellitus	Obesity	Progeroid syndromes (e.g., Werner's syndrome)
Insulin resistance syndrome (Syndrome X)	Type I diabetes mellitus	Cytogenetic disorders (Down's, Turner's, and Klinefelter's)
Gestational diabetes mellitus	Type B severe insulin resistance	Ataxia telangiectasia
Type A severe insulin resistance	Hyperlipidemias	Muscular dystrophies
Lipoatrophic diabetes	Pregnancy	Friedreich's ataxia
Leprechaunism	Acute illness and stress	Alstrom syndrome
Rabson-Mendenhall syndrome	Cushing's disease and syndrome	Laurence-Moon-Biedl syndrome
Hypertension	Pheochromocytoma	Pseudo-Refsum's syndrome
Atherosclerotic cardiovascular disease	Acromegaly	Other rare hereditary neuromuscular disorders
	Hyperthyroidism	
	Liver cirrhosis	
	Renal failure	

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take (19, 25, 30). But precise and specific intracellular defects to account for the majority of cases of insulin resistance are not yet described. However, it is highly likely that the molecular basis of insulin resistance is polygenic, and the relative contribution of any one signaling defect varies greatly among individuals (5). It is further suggested that the additive effects of several mild alterations of signal transduction are needed to induce insulin resistance.

The studies outlined above have provided valuable information regarding the proposed mechanism by which insulin exerts its effect. Yet the understanding of specific defects of *in vivo* signaling processes that contribute to insulin resistance in humans is not currently known, and this remains an area of very active investigation.

Measurement and Assessment of Insulin Resistance

A variety of procedures have been developed to detect the presence of clinical insulin resistance (65, 66). The most studied and specific measure is a technique called the euglycemic hyperinsulinemic clamp. A second, less invasive, method is the frequently sampled intravenous glucose tolerance test (FSIVGTT) or the so-called minimal model. The third, and simplest from a clinical perspective, is the fasting insulin level. Studies that have used any or all of these techniques have demonstrated that there is a wide range of insulin sensitivity in normal individuals, and these values overlap with similar values in Type 2 diabetics. Therefore, it is very difficult to distinguish between nondiabetic and diabetic individuals on the basis of insulin resistance (5).

The most widely accepted research gold standard is the euglycemic hyperinsulinemic clamp technique (65, 66). In this procedure, exogenous insulin is infused to maintain a constant plasma insulin level above fasting whereas glucose is infused at varying rates to keep glucose within a fixed range. The amount of glucose that is infused over time (*M*-value) is an index of insulin action on glucose metabolism. As described, the more glucose that has to be infused per unit time to maintain the fixed glucose level, the more sensitive the patient is to insulin. With this procedure, the insulin-resistant patient requires much less glucose to maintain the basal level of glucose. However, this technique has a number of limitations, primarily in the procedure's complexity and expense. Due to the rapid feedback needed from multiple glucose checks during the procedure, these procedures generally require a well-staffed clinical research setting (65, 66). Therefore, these procedures are unrealistic for clinical practice or large population–based studies and limit the use of clamp studies to research laboratories.

The FSIVGTT is a method that is less invasive and more practical than the clamp and one that can be applied to larger populations (65, 66). With this procedure, glucose is injected as a bolus, and both glucose and insulin levels are assessed frequently from an indwelling catheter over the next several hours. The results are entered in a computer model that generates a value that is an index of insulin sensitivity, termed S_I units. This measure of insulin resistance has been shown to correlate well with the euglycemic hyperinsulinemic clamp in nondiabetic subjects, but its accuracy deteriorates in diabetics because the immediate plasma insulin response to the glucose challenge, a major determinant for this analysis, is diminished. This problem has been addressed in diabetic patients by giving exogenous insulin or a secretagogue (i.e., tolbutamide) during the early parts of testing.

The homeostasis model assessment (HOMA) of insulin sensitivity is another procedure that has received interest (67, 68). This parameter was proposed ≈10 years ago as a simple, inexpensive alternative to more sophisticated techniques and derives an estimate of insulin sensitivity from the mathematical modeling of fasting plasma glucose and insulin concentrations. Specifically, an estimate of insulin resistance by HOMA score is calculated with the formula: [fasting serum insulin (μU/ml) × fasting plasma glucose (m*M*)]/22.5 (68). The HOMA method has been shown to correlate strongly to glucose disposal methods as assessed by clamp studies (67).

An additional surrogate marker of insulin resistance has been proposed as the total integrated insulin response to a 75-gram oral glucose challenge. This marker was recently found to be the best surrogate marker of insulin resistance, accounting for over two-thirds in the variability in insulin-mediated glucose disposal in 490 healthy, nondiabetic volunteers (69).

However, from a clinical perspective, the most practical way of assessing insulin resistance is the measurement of plasma insulin levels (5). This is suggested to be done in the overnight fasting condition, since in the postprandial state glucose levels are changing rapidly, and the variable levels of glucose confound the simultaneous measure of insulin. There is a significant correlation between fasting insulin levels and insulin action as measured by the clamp technique. In addition, it is generally true that very high plasma insulin values in the setting of normal glucose tolerance are very likely to reflect insulin resistance, and high insulin levels are a predictor of the development of diabetes. The value of a fasting insulin is limited by the fact that again there is considerable overlap between insulin-resistant and normal subjects, and another major limitation is the lack of standardization of the insulin assay procedure. However, if the assay for insulin were reliable, it would be useful to detect the insulin resistance early, before clinical disease appears (5).

The Insulin-Resistant Clinical State

The Cardiovascular Dysmetabolic Syndrome or Syndrome X represents a clustering of risk factors associated with insulin resistance. Cause and effect are difficult to establish, and significant interaction exists between multiple risk factors (7–10).

Obesity. That obesity is associated with chronic dis-

eases such as Type 2 diabetes, coronary heart disease, and dyslipidemia is well recognized by all clinicians, yet the underlying mechanisms are not well defined. However, the evidence is strong that insulin resistance contributes greatly to the pathophysiology of these observed metabolic abnormalities and their associated morbidity (70). Insulin resistance is frequently observed in obese subjects and has been established as an independent risk factor for the development of both Type 2 diabetes and coronary artery disease (70–73). Although it is established that hyperinsulinemia, insulin resistance, and other obesity-related metabolic abnormalities are significantly associated with overall accumulation of fat in the body, there is now substantial evidence that the specific distribution of fat is important. Excessive accumulation of fat in the upper body's so-called truncal region, or central obesity, is a better predictor of morbidity than excess fat in the lower body, the so-called lower body segment obesity (70, 72, 73). These types of body composition have been clinically separated based on a waist-to-hip circumference ratio, and individuals are referred to as having apple- or pear-shaped bodies. Vague (74) was first to report on this type of body composition over 40 years ago and noted that the incidence of metabolic complications among equally obese subjects varied depending on their physique. Morbidity was shown to be higher in android-type obesity than in gynoid-type obesity. This heterogeneity is supported by several studies suggesting regional differences in adipose tissue metabolism (75–77). This heterogeneity of fat distribution has led investigators to accept the concept of morbid regional adiposity (i.e., that accumulation of fat in certain adipose tissue regions appears to be more deleterious than accumulation of fat in other adipose tissue regions). The hypothesis that has been put forward is that mesenteric adipose tissues constitute the morbid areas of the body, and accumulation of fat in these regions has major implications for metabolism and particularly for insulin sensitivity (70, 72, 73).

If specific abdominal fat depots appear to have clinical relevance, then a precise measure assessing quantity of these fat depots is needed. Sonography has been used for the evaluation of intra-abdominal tissue (78, 79) but has not been as widely used in clinical research settings compared with magnetic resonance imaging (MRI) and computer tomography (CT) scans. However, both CT and MRI allow direct visualization of internal adipose tissue compartments, and both MRI and CT scans have been tested and validated in human subjects for assessment of intra-abdominal fat stores (80). Studies that have used MRI and CT scans have demonstrated a very significant relationship between intra-abdominal fat and insulin resistance (81, 82). In particular, it was observed in studies evaluating the insulin resistance of aging that insulin resistance related more to the visceral fat depot than to the subcutaneous fat depot (82). Additional studies have evaluated adipose tissue distribution in other areas, such as thigh skeletal muscle, and have shown significant correlation with insulin sensitivity (83). Thus, it is well established that obesity, in particular central obesity, appears to be the depot most associated with insulin resistance.

Lipid Abnormalities. Unfavorable changes in lipoproteins, in part, may help explain the increased risk for cardiovascular disease observed with insulin-resistant states (84–89). The major quantitative change associated with the insulin resistance syndrome is an elevation in triglyceride-rich lipoproteins, often accompanied by a decreased HDL cholesterol level (86). Thus, dyslipidemia (by its association with insulin resistance) may precede the diagnosis of Type 2 diabetes. Although LDL cholesterol levels may be comparable to those seen in the general population, LDL compositional differences may make these particles more atherogenic (87, 89). Specifically, hyperinsulinemia has been shown to be associated significantly with both quantitative changes (e.g., increased triglycerides, high Apo B, low Apo A1 levels) in the lipoproteins and also qualitative changes (e.g., low LDL cholesterol/Apo B and low HDL cholesterol/low Apo A1) (86–90). It is further established that insulin levels appear to not be associated with the absolute concentration of the LDL cholesterol, but are associated with the relative decrease in the small dense LDL particles termed LDL subclass pattern B. Insulin resistance has also been associated with this preponderance of small dense LDL particles (84, 85). It is the small dense LDL particle that has been suggested to be the more atherogenic LDL. Studies have suggested that insulin sensitizers (e.g., thiazolidinediones) may favorably improve LDL size. Although it has been shown that the ratio of LDL to HDL cholesterol may not change with treatment with insulin sensitizers, the qualitative properties of LDL may change with their use: large (buoyant) LDL is increased and small dense LDL is decreased (91). Whether the compositional change in LDL is indeed secondary to improvement in insulin resistance or secondary to other characteristics of insulin sensitizers (e.g., antioxidant effect) is an area of great debate because there appears to be no relationship between the effect of glitazones on lipoproteins and on insulin sensitivity.

Endothelial Function. The vascular endothelium has received considerable research attention based on its primary role to modulate the underlying blood vessel tone by producing a number of vasoconstrictors and vasodilators (92–94). Agents that preferentially dilate the vascular wall include nitric oxide (NO), prostacyclin, bradykinin, and endothelium-derived hyperpolarization factor. Agents that have been found to constrict blood vessel tone include endothelin, superoxide anion, endothelium-derived constricting factor, locally produced angiotensin II, and thromboxane. These agents have been described not only to control and regulate arterial tone, but also to affect other parameters that contribute to development of atherosclerosis (92–94). Factors such as platelet adhesion, aggregation, and thrombogenicity of the blood have been postulated to play a role. Therefore, if endothelial damage results in more production of vasoconstrictors and less of vasodilators, particu-

larly NO, circulating platelets may aggregate in these particular areas, releasing cytokines and growth factors and may initiate the inflammatory reaction. After the initial inflammatory reaction, LDL cholesterol uptake is taken up into the vessel wall (*via* a direct mechanism or possibly in the form of foam cells—lipid-laden macrophages) and may result in the formation of a fatty streak. Ultimately, vascular smooth muscle cells participate in the process by migrating into the intima, proliferating, and increasing their production of extracellular matrix proteins. The summation of these processes results in the formation of organized atherosclerotic plaque (92–94). Therefore, from the above discussion, one can appreciate that the endothelium has great potential to participate in cell proliferation contributing to the development and progression of atherosclerosis.

It is now well described that endothelial dysfunction may be secondary to insulin resistance and hyperinsulinemia, in addition to other components of the Cardiovascular Dysmetabolic Syndrome. Hyperlipidemia, hyperglycemia, hypertension, smoking, and homocysteine have all been reported to damage the endothelium. The resulting endothelial dysfunction leads to an imbalance in the endothelial production of the vasoconstrictors versus the vasodilators. Studies have evaluated pharmacologic and nonpharmacologic regimens in treatment of the endothelial dysfunction. In particular, a study evaluated the role of an insulin sensitizer in individuals who were felt to be impaired-glucose-tolerant and insulin-resistant and who had attenuated brachial artery vasoactivity (95). After 2 months of therapy with an insulin sensitizer, vasoactivity was shown to improve and appeared to normalize after 4 months (95). Although this demonstrates that pharmacologic treatment of insulin resistance may have favorable effects on endothelial dysfunction, this should not imply that insulin resistance is the sole factor in the development of endothelial dysfunction. As stated above, lipids, glucose, hypertension, and smoking have all been shown to damage the endothelium, and studies that have treated these particular components have also shown favorable effects on endothelial dysfunction.

Atherosclerosis. Although it is still unclear whether insulin itself is a pathogenic factor in the development of atherosclerosis, it is clear from epidemiologic studies that insulin levels are strongly associated with coronary artery disease. Several large-scale prospective trials have clearly shown that insulin levels correlate with coronary artery disease in multivariate analyses (13–18). A prospective study of men in Quebec found that fasting insulin levels were indeed associated with ischemic heart disease after adjustment for coexisting factors such as hypertension, medications, family history, and lipid levels (15). In the MR-FIT (Multiple Risk Factor Intervention Trial) it was demonstrated that fasting insulin levels were a risk factor for coronary artery disease only in men with a certain lipid phenotype (apolipoprotein E3/2 phenotype) (96). However, in the Caerphilly Prospective Study the effect of insulin levels on heart disease event rates appeared to be present only in the setting of hypertriglyceridemia (97). Therefore, the possibility exists that hyperinsulinemia is a risk factor only in certain ethnic groups or in patients with certain risk factor abnormalities. Another explanation is that it may simply be a marker for insulin resistance (93).

Despite the conflicting data with insulin levels, insulin resistance appears to be a better correlate with coronary artery disease (93). Although it is argued that the number of patients studied to date with direct measurement of insulin action is small, many of these studies have shown a relationship between insulin resistance and specific measures of atherosclerosis such as arterial lesion size. In particular, the Insulin Resistance and Atherosclerosis Study (IRAS) measured insulin resistance in three groups of patients: Hispanics, non-Hispanic whites, and African Americans (71). Insulin resistance was found to correlate with carotid intimal medial wall thickness in non-Hispanic whites after adjustment for factors such as smoking, lipids, hypertension, medications, and gender (71). This suggested that insulin resistance had an independent effect on the development of atherosclerosis. However, in African-Americans, there appeared to be no detectable relationship between insulin resistance and the carotid intimal wall thickness. Another report from the same group of investigators demonstrated an association between insulin resistance and definite coronary artery disease, even after adjusting for demographics, hypertension, smoking, and dyslipidemia (98).

It is important to note from the effects of the IRAS, that over 50% of the subjects in the study were women (and most of these women were postmenopausal), therefore providing substantial evidence that insulin resistance and coronary artery disease are indeed related in women (71). Taken together, the observations as outlined above indicate that a more precise measure of insulin action is critical for investigating and defining the relationship between insulin resistance and coronary artery disease (93).

Hypertension. The relationship between hypertension and insulin resistance has been well observed, but correlation between blood pressure and plasma insulin levels has been demonstrated to be inconsistent and relatively weak. Specifically, there appears to be little scientific evidence that chronic hyperinsulinemia causes blood pressure elevations in humans (99). This has been shown in animal and human studies in which both acute and chronic hyperinsulinemia lasting for several weeks did not cause a hypertensive shift of pressure natriuesis or increased arterial pressure (100, 101). The insulin infusions that were observed to raise concentrations of insulin levels to those comparable to levels found in obesity tended to reduce arterial pressure by inducing peripheral vasodilation (100, 101). Insulin has also been found not to potentiate the blood pressure or kidney effects of other vasoactive substances, such as norepinephrine or angiotensin II (100, 101). Furthermore, in obese subjects who are resistant to the metabolic and vasodilator effects of insulin, elevated insulin did not appear

to increase arterial pressure (102). Therefore, multiple clinical research studies strongly suggest that hyperinsulinemia does not explain the increased renal tubular NaCl reabsorption, shifts of pressure natriuesis, or the hypertension associated with obesity in both animals and humans (99).

In contrast to the above, chronic elevated insulin levels have been observed to cause significant elevations in arterial pressure in rodent studies. This is felt to be mediated through interactions with the RAS and thromboxane (99). Studies have suggested that inhibition of thromboxane synthesis or ACE inhibition did indeed abolish the insulin-induced rise in arterial pressure in rodents (103, 104). Additional evidence is provided in studies that demonstrate that blockade of endothelial-derived NO synthesis appears to enhance insulin-induced hypertension in rodents (105). It is unclear whether these findings in rodents are relevant to the hypertension noted in obese humans, but summation of the currently available studies does suggest that chronic elevated insulin levels cannot account for obesity-induced increases in blood pressure. Therefore, the very close correlation between hyperinsulinemia and hypertension in obese subjects is felt to be caused by the fact that obesity itself not only elevates arterial pressure but induces peripheral insulin resistance in hyperinsulinemia through parallel but independent mechanisms (99).

The question that remains is the mechanism by which obesity contributes to hypertension. A recent review by Hall *et al.* (99) outlines a summary of mechanisms by which obesity may cause hypertension and glomerulosclerosis by activation of the renin-angiotensin and sympathetic nervous systems, including metabolic abnormalities and compression of the renal medulla. A summary of these mechanisms is outlined in Figure 4 (99).

Prothrombotic Activity. An additional mechanism put forth to explain the accelerated atherosclerosis observed with insulin resistance and Type 2 diabetes is a hypercoagulable state. The body's fibrinolytic system normally limits vascular thrombosis and appears responsible for dissolution of thrombi after vascular repair has occurred. However, a disturbance of the fibrinolytic system favors the development of vascular damage and the final occlusion event in the progress of coronary heart disease (106–111).

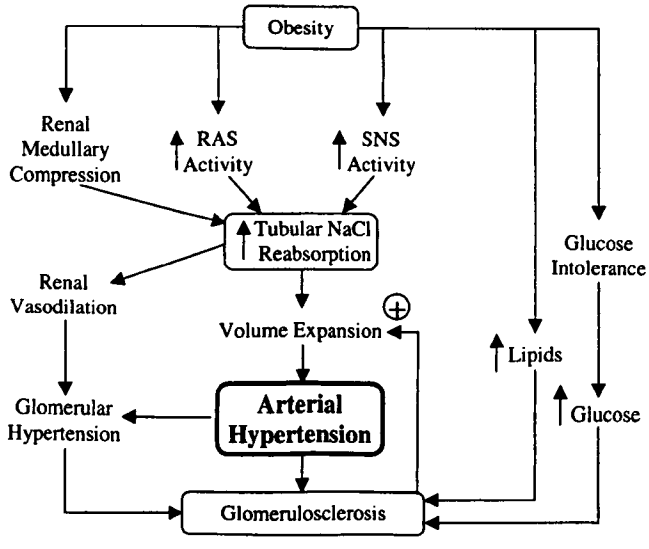

Figure 4. Schematic outlining postulated mechanisms by which obesity contributes to hypertension (adapted from Ref. 99).

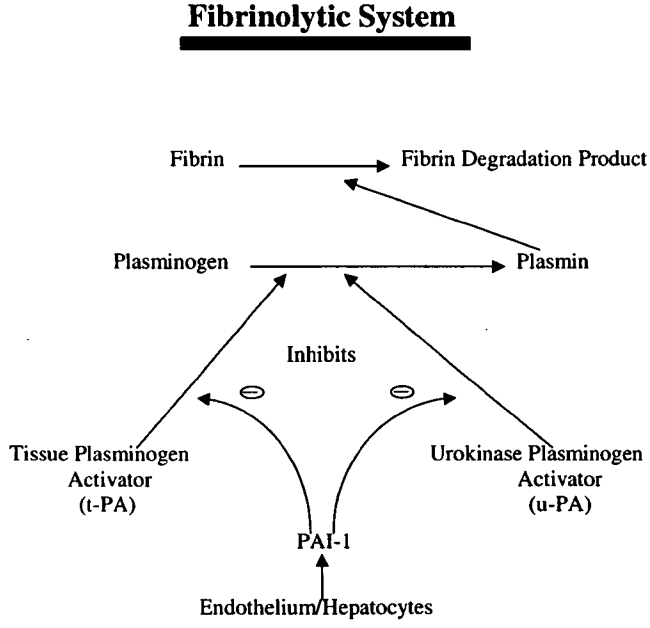

Figure 5. Schematic demonstrating components of the fibrinolytic system.

A balance normally exists between plasminogen activators and inhibitors, and diminished fibrinolysis secondary to elevated concentrations of plasminogen activator inhibitors may help to explain the exacerbation and persistence of thrombosis observed in acute events. A diminished release of tissue plasminogen activator (t-PA) and increased levels of PAI-1 (Fig. 5) both may contribute to impaired fibrinolysis (106–110). PAI-1, a major regulator of the fibrinolytic system, is a serine protease inhibitor and binds to and inhibits t-PA and u-PA (urokinase plasminogen activator). Sources of PAI-1 include hepatocytes, endothelial cells, adipocytes, and smooth muscle cells. PAI-1 is also present in the alpha granules of platelets.

Elevated PAI-1 activity or reduced t-PA resulting in defective fibrinolysis may predispose individuals to sequela from thrombotic events and contribute to the development and progression of atherosclerosis (106–112). PAI-1 appears to modulate vessel wall proteolysis, and increased production of PAI-1 has been demonstrated in components of the atherosclerotic plaque and the vessel wall (109). Diminished vessel wall proteolysis may predispose to accumulation of extracellular matrix. Further, cell migration is dependent on cell surface expression of u-PA. Thus, overexpression of PAI-1 in the vessel wall may limit migration of smooth muscle cells into the neointima. This limitation of migration may predispose to the development of a thin cap overlying the lipid core, a feature associated with increased

risk of evolution of vulnerable plaque rupture, when acute events trigger proteolysis (110, 111).

The fibrinolytic variables (PAI-1 and t-PA antigen) are strongly associated with components of the insulin resistance syndrome in cross-sectional studies (113, 114). Furthermore, the observed association between insulin resistance and PAI-1 or t-PA antigen levels has also been confirmed in intervention studies aimed at reducing insulin resistance (111). The improvement in insulin resistance is paralleled by improvement of the metabolic abnormalities altering the concentrations of these moieties. Among those subjects who manifest insulin resistance and components of the syndrome (i.e., excess body weight, increased waist-hip ratio, hypertension, and elevated lipids), treatment of the condition is associated with a decrease in PAI-1 and improvement of the fibrinolytic activity in the majority of these studies.

Clinical Considerations in the Treatment of Insulin Resistance

That insulin resistance is associated with increased morbidity and mortality is convincing. But whether improvement of insulin resistance will prevent mortality and morbidity resulting from the insulin-resistant state has not been established. This is further complicated by the fact that a clinically practical and reliable test for insulin resistance or a way to measure clinical resistance serially with less invasive techniques for large-scale studies is not well established (5). However, it is well established that there are a number of interventions that do reduce insulin resistance. These interventions include a calorie-restricted diet, weight reduction, exercise, and pharmacologic intervention with agents such as metformin and glitazones (5). Most clinicians will readily agree that a calorie-restricted diet will markedly improve insulin resistance. For those patients who do comply, insulin resistance is significantly reduced within a few days of instituting the diet, and this reduction is observed even before significant weight loss has occurred. Clinically, this is reflected by either an improvement in glycemic control or a marked decrease in the need for exogenous insulin or higher doses of oral antidiabetic medications to maintain glycemic control. It has also been firmly established that weight reduction over a longer time frame continues to improve insulin sensitivity. Should the patient not be able to lose weight, avoiding additional weight gain may provide the most efficient means of preventing insulin resistance and worsening morbidity (5). The most controversial element in nutrition is whether distribution among carbohydrates and the various fats is a critical parameter. Whereas there is a prevailing school of thought that total calorie intake is the critical parameter, others would argue that distribution is the key. Unfortunately, comparison trials evaluating such diets have not been done (5).

Insulin sensitivity is significantly improved with exercise, as vigorous exercise has been demonstrated to reduce resistance, even in elderly patients. Unfortunately, the effect on insulin resistance is known to diminish quickly (within 3–5 days) after stopping the exercise. However, long-term exercise would result in little weight reduction unless caloric intake is also part of the regimen.

Pharmacologic treatment of insulin resistance is an area of great debate. Over the last several years there have been two specific pharmacological approaches available to reduce insulin resistance. A class of compounds called biguanides, as represented by the agent metformin, has a predominant effect to diminish hepatic glucose production and has modest effects on skeletal muscle insulin resistance. On the other hand, a class of drugs referred to as thiazolidinediones, represented by agents such as troglitazone, rosiglitazone, and pioglitazone, are considered true insulin sensitizers by enhancing insulin-stimulated glucose disposal in muscle. Although both classes of drugs are currently available in the United States for treatment of the Type 2 diabetic condition, neither class is approved to treat insulin resistance in the absence of the Type 2 diabetic state.

Both classes of drugs have been postulated to be beneficial in either delaying or preventing the progression to Type 2 diabetes. In particular, the National Institute of Health's study termed the Diabetes Prevention Program is designed to determine if any treatment (nutrition, exercise, pharmacologic treatment) is effective in the primary prevention of Type 2 diabetes in people who have been diagnosed with impaired glucose tolerance (11). As originally designed, there was to be a control group that employed intensive lifestyle changes and was designed to effect an ≈7% reduction in body weight through caloric restriction and exercise. The second and third groups were to consist of pharmacologic treatments to reduce insulin resistance, mainly metformin and troglitazone. The troglitazone arm was dropped from the study due to an adverse event involving the liver. Because of the hepatic concern, troglitazone was recently (March 2000) removed from the market.

It is not currently recommended that a patient receive pharmacologic treatment for insulin resistance before the diagnosis is established for Type 2 diabetes. Depending on the outcome of the current prevention trials, this may be a recommendation in the near future. However, until the results of the prevention trials are known, a nonpharmacologic approach is probably the most reasonable option the clinician can offer to the patient in hopes of reducing insulin resistance and preventing the development of Type 2 diabetes. Candidates for such therapy include those who are overweight (particularly with central obesity) and those who have a strong family history of diabetes or gestational diabetes, a condition termed impaired fasting glucose, or other clinical symptoms associated with insulin resistance (e.g., hypertension, dyslipidemia).

Summary

This review has summarized the current thinking regarding insulin resistance. As now believed, insulin resistance is very much part of the natural history of Type 2

diabetes and may be present many years before the clinical diagnosis. The cellular abnormalities that contribute to insulin resistance are not clearly defined, yet it is well established that cardiovascular risk factors are strongly related to insulin resistance. Whether specific treatment of insulin resistance will delay or prevent development of Type 2 diabetes and whether a concomitant decrease in cardiovascular disease will be observed are questions currently left unanswered.

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