Insulin resistance

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Insulin resistance is best defined as an inappropriately high level of plasma insulin required to maintain metabolic homeostasis. Historically, this definition has tended to be applied to glucose homeostasis, since the methods used (fasting insulin levels, estimation of the steady-state plasma glucose levels with constant rates of glucose-insulin infusion, the euglycaemic-hyperinsulinaemic clamp, and the minimal model of Bergman) have measured insulin stimulated glucose disposal from plasma. However, advances in our knowledge of insulin action have required the expansion of the definition to include all insulin sensitive processes at both the whole body and molecular level.

Some notable advances in the area of insulin resistance have been made over the past few years. The concept that insulin resistance may be involved in the aetiology of a variety of diseases, including non-insulin-dependent diabetes mellitus (NIDDM), coronary heart disease, and hypertension continues to attract considerable attention and support. There has been a re-emergence of the hypothesis that derangements in lipid metabolism are a driving force in the pathogenesis of insulin resistance. At the molecular level, cellular factors have been identified that can markedly influence insulin action either

directly or indirectly, notably Rad, (PC)-1, tumor necrosis factor (TNF) α and peroxisome proliferator activated receptor (PPAR) γ , while increased glucose flux through the hexosamine pathway has been shown to induce insulin resistance in skeletal muscle. Finally, thiazolidinediones have emerged as clinically effective insulin sensitizing agents, giving hope that they will be a potent therapeutic tool in the treatment of insulin resistance.

Peter Bennett: Epidemiology of insulin resistance

Although insulin resistance is not observed in well-controlled insulin-dependent diabetes (IDDM), it is demonstrable when metabolic control is poor. In contrast, there is general agreement that insulin resistance is a primary feature of NIDDM. Insulin resistance is associated with, and may be involved in the aetiology of, several other conditions that include obesity, hypertension, dyslipidaemia – particularly hypertriglyceridaemia and low HDL cholesterol levels, coronary artery disease, hyperuricaemia and the polycystic ovarian syndrome. Several of these diseases were considered by Reaven in 1988 to represent a syndrome “syndrome X”. The relative importance of insulin resistance in the aetiology of each of them is unclear, since the components of the syndrome and their associated diseases are complex traits, each with a multifactorial aetiology. A fundamental question is to determine whether the appearance of insulin resistance (and hyperinsulinaemia) precedes the onset of the disease state and furthermore predicts its future development. For NIDDM there is uniform agreement that this is the case and that in some populations, perhaps all, insulin resistance represents a major underlying factor in the development of the disease. For coronary artery disease (CAD) the predictive ability of insulin resistance is less clear, but

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several prospective studies do show that CAD is predicted by fasting insulin levels. Less clear is whether or not insulin resistance predicts the development of hypertension. In cross-sectional analysis the correlation between insulin resistance and blood pressure varies appreciably in different ethnic groups from almost zero to quite high values (r 0.7–0.8). It can be stated, however, that elevated blood pressure does not predict the development of insulin resistance.

Although obesity was not included in the original cluster of diseases described by Reaven, it is associated with the insulin resistant state, and it is the one condition that also appears to predict the development of insulin resistance. Additionally, it is the factor that is most commonly associated with the other diseases of syndrome X. Therefore, most investigators now include obesity as a component of the “insulin resistance syndrome” or “metabolic syndrome”. Analysis of the influence of obesity on insulin resistance has suggested that even moderate degrees of overweight, particularly when associated with a central weight distribution (android pattern) is strongly predictive of the development of insulin resistance.

Obesity, and even moderate overweight, is associated with perturbed lipid metabolism and elevated plasma non-esterified fatty acids (NEFA). Elevations in NEFA have also been linked to NIDDM, dyslipidaemia (particularly associated with excessive production of VLDL cholesterol and triglycerides), and poorly controlled IDDM, all conditions that are associated with insulin resistance. Limited prospective data suggest that fasting elevations in NEFA may be linked with development of impaired glucose tolerance, and the progression to NIDDM in Caucasians and Pima Indian populations. Increased fat cell size, associated with increased lipolytic turnover, also predicted progression to NIDDM in Pima Indians. Evidence from the session on NEFA and beta-cell function, provided data to suggest that inappropriately increased NEFA are not only a manifestation of insulin resistance at the fat cell level, but may also be a primary stimulatory force to beta-cell hypersecretion of insulin. If true in humans, this suggests the novel possibility that the hyperinsulinaemia contributes to insulin resistance and/or that the insulin resistance is a protective mechanism against inappropriate hypoglycaemia.

Underlying many of the above observations is the question of what role genetic and environmental factors play in the development of insulin resistance. Fasting insulin and glucose disposal rates show quite strong familial aggregation, and the distributions of “M” values (glucose disposal rates measured in hyperinsulinaemic-euglycaemic clamps) show trimodal frequency distributions in Pima Indians and in lean Caucasians, suggesting the possibility of determination by a major co-dominant gene. Fasting insulin levels also show higher degrees of concordance in

monozygotic twins than in dizygotic twins, again supporting an important hereditary component.

Searches for specific mutations that lead to insulin resistance have been conducted by several groups. Polymorphisms in the protein phosphatase 1 (PP 1) and in the fatty acid binding protein 2 have been shown to be associated with insulin resistance in different populations. Never the less, none of these can be considered a major gene which determines the variation in insulin resistance at the population level. The question remains as to whether there are major genes involved in the development of insulin resistance or whether the trait is polygenic.

Environmental factors that influence insulin resistance and hyperinsulinaemia are numerous, but diet (both excess caloric intake, and the percent saturated fatty acid content), physical activity, smoking and certain drugs such as glucocorticoids and nicotinic acid all influence the level of insulin resistance. Furthermore, insulin resistance is ameliorated by improved metabolic control, weight loss, physical training, and more acutely by reduction of caloric intake. A striking example of environmental influence of insulin resistance is that similar ethnic groups living in different environments show different degrees of insulin resistance and fasting insulin levels. A predominant environmental role for the development of insulin resistance is also predicted from the hypothesis formulated by Barker and Hales. This states that syndrome X associated diseases are the result of nutritional deprivation in utero. The essential observation on which the hypothesis is based is that each of these conditions, at least in certain populations is more prevalent in individuals of low birth weight. Alternative explanations for this data are possible. For example, it could be theorized that low birth weight babies who are genetically susceptible to insulin resistance have a selective advantage for survival in the perinatal period, but develop insulin resistance under the influence of a western lifestyle when they become adults.

Gerald Shulman: New insights into the pathogenesis of insulin resistance using nuclear magnetic resonance (NMR) spectroscopy

Sophisticated metabolic studies using tracer kinetics and non-invasive NMR spectroscopy were employed to elucidate possible mechanisms of insulin resistance in NIDDM subjects and in normal glucose tolerant, but insulin resistant, subjects with a family history of NIDDM. These studies addressed the potential role of impaired skeletal muscle glucose transport/phosphorylation, glycogen synthesis, and deranged lipid metabolism in the development of insulin resistance.

Studies in both groups clearly showed that rates of skeletal muscle glucose disposal are dramatically decreased during hyperinsulinaemic glucose clamps

compared to normal control subjects. Quantitatively the major defect is in non-oxidative glucose metabolism, which can be accounted for by decreased muscle glycogen synthesis. Glucose oxidation is also decreased but this is a minor component of total glucose disposal. The increases in muscle glucose 6-phosphate (G6P) levels were significantly below those of normal insulin sensitive control subjects suggesting that glucose transport and/or phosphorylation is defective in insulin resistant subjects.

Clamp studies that were performed after physical training, an established manoeuvre to improve insulin sensitivity, resulted in similar increases in G6P levels in insulin resistant and normal subjects. Insulin stimulated glucose disposal rates were increased by 40% in trained insulin resistant individuals compared to untrained insulin resistant control subjects; however, it remained below that of sedentary normal control subjects. These data suggest that the defect in muscle glucose uptake and/or phosphorylation can be corrected by physical activity, but that there is an additional defect in muscle glycogen synthesis that contributes to the defect in non-oxidative glucose disposal in insulin resistant individuals.

Deranged lipid metabolism, leading to inappropriately elevated plasma NEFA levels that may in turn lead to impairments in tissue glucose uptake (glucose-NEFA cycle) is one possible mechanism involved in the pathogenesis of insulin resistance. Indeed, fasting NEFA was the best predictor of insulin sensitivity in young non-obese Caucasians with normal glucose tolerance but genetically at risk for NIDDM. In the classic formulation by Randle of the glucose-fatty acid cycle, excess availability of NEFA results in an increased energy charge of the cell and via feedback mechanisms (particularly increased G6P, a powerful allosteric inhibitor of hexokinase) results in impaired glucose transport/phosphorylation and glucose oxidation.

In normal insulin sensitive humans, the effect on muscle glucose metabolism of experimental elevations of NEFA through simultaneous infusion of lipid emulsions and heparin have yielded contradictory results. A consistent effect has been demonstrated only after prolonged lipid infusion (more than 3–4 h). In data presented here, experimental elevations of plasma NEFA initially resulted in decreased glucose oxidation, but normal rates of glycogen synthesis. The expected increase in G6P was not observed, suggesting that decreased glucose oxidation resulted from an inhibition of pyruvate dehydrogenase, the entry point of glucose into the tricarboxylic acid cycle. At longer time points G6P levels actually decreased in the insulin resistant group compared to control subjects and this was followed by a decrease in muscle glycogen synthesis. These results imply a direct inhibitory effect of NEFA on glucose transport and/or phosphorylation, and in addition, possibly a direct effect on

glycogen synthesis. These effects do not appear to require elevated G6P levels. However, it is possible that G6P levels initially increase, consistent with the original Randle hypothesis, and then decrease consistent with a direct effect of NEFA to inhibit glucose transport/phosphorylation. The availability of higher strength magnetic fields will improve the time resolution for detection of changes in metabolites such as G6P and free glucose in future experiments.

Hannele Yki-Jarvinen: Insulin resistance, blood flow, and the hexosamine pathway

Other mechanisms may also be responsible for insulin resistance observed in NIDDM and IDDM diabetic subjects. Two current hypotheses that have received much scrutiny are potentially defective insulin stimulated blood flow (resulting in flow-limited glucose extraction), and the effects of increased flux through the hexosamine biosynthetic pathway in skeletal muscle.

In normal insulin sensitive subjects during hyperinsulinaemic glucose clamp studies, limb blood flow increases several-fold associated with increased tissue glucose disposal. Studies where blood flow is increased independent of insulin action, such as with the vasodilator bradykinin, do not result in increased glucose uptake. When NIDDM subjects were compared to normal control subjects during hyperinsulinaemic glucose clamp studies, no difference in limb blood flow as (measured directly with radioactive water tracers) was detected despite a marked difference in glucose uptake. Similar results were obtained when insulin resistance was induced with a 24 h hyperglycaemic clamp in normal subjects. It was concluded that limitations in total limb blood flow cannot account for decreases in glucose disposal.

Although NIDDM subjects are felt to be maximally insulin resistant, recent data clearly show that a further defect in insulin stimulated glucose disposal occurs as glycaemic control worsens. This further impairment in insulin action correlates well with indices of glycaemia such as glycated haemoglobin and mean plasma glucose concentrations during oral glucose tolerance testing. In vitro and in vivo studies suggest that increased glucose flux through the glucosamine pathway may induce insulin resistance in skeletal muscle. Diabetic patients have increased tissue glucose uptake basally compared to normal control subjects, and normal uptake postprandially, due to the mass action effect of glucose. When glucose flux is artificially elevated, insulin stimulated glucose disposal is impaired associated with increases in flux through the glucosamine pathway. The impairment can be reproduced by administering glucosamine to non-diabetic rats but cannot be further exacerbated in already insulin resistant diabetic rats. When the

rate limiting enzyme for hexosamine production (glucosamine fructose amido transferase – GFAT) is increased in transgenic mice, this produces insulin resistance despite normal plasma glucose levels, implying that over-activity of this pathway may contribute to insulin resistance in the absence of hyperglycaemia.

In humans, GFAT activity is demonstrable in muscle and correlates significantly with glucose disposal rates during hyperinsulinaemic glucose clamp studies and also the chronic level of glycaemia as measured by glycated haemoglobin in both NIDDM and IDDM subjects [?true for both]. Potential mechanisms by which overproduction of glucosamine could induce insulin resistance were discussed. Increased flux into hexosamines provides increased substrate for intracellular O-linked glycosylation. This in turn may adversely affect the activity of enzymes and transporters essential to tissue glucose uptake and storage as glycogen. Alternatively, such post-translational modifications may affect post insulin receptor signalling pathways. No direct data, however, demonstrating these effects are available at present. The enzyme responsible for protein O-linked glycosylation, UDP-N-acetyl-glucosamine transferase, is present in human muscle tissue, and therefore this mechanism is possible, but as yet unproven. It was speculated that activation of the hexosamine pathway, and the induction of muscle insulin resistance, is an adaptive mechanism which protects normally insulin sensitive tissues against excessive glucose flux.

Kenneth Polonsky: Treatment of diabetes and insulin resistance with thiazolidinediones

The thiazolidinediones (TZDs) are a new group of anti-diabetic chemical compounds that increase insulin sensitivity in insulin responsive tissues. The treatment of insulin resistance with TZDs, specifically troglitazone, in NIDDM subjects receiving over 30 U insulin/day and with HbA_{1c} over 8.5% was recently approved by the United States Food and Drug Administration (FDA). FDA approval was based on results from two 6-month double-blind clinical trials in insulin-treated NIDDM patients. These studies demonstrated that daily administration of troglitazone (200–600 mg) led to significant decreases in insulin requirements, plasma HbA_{1c} levels, and fasting plasma glucose. To date, little or no deleterious side effects of troglitazone have been observed. There were small increases (3–11%) in plasma LDL, a less than 1% fall in haemoglobin, and no change in body mass index (BMI). One potential risk, that of increased frequency of hypoglycaemic episodes due to over-insulinization during TZD treatment, can be minimized by closely matching decreases in fasting blood glucose levels to decreases in insulin dosage.

Two questions that are now being aggressively addressed are the mode and site of action of the TZDs. In vitro studies have demonstrated that TZDs bind to the peroxisome proliferator activated receptor gamma (PPAR γ). PPAR γ , a member of a large family of nuclear receptors that serve as transcription factors, plays a critical role in adipogenesis. Activation of PPAR γ leads to the formation of a heterodimeric complex with (RXR), that in turn activates the expression of specific genes involved in differentiation. The mechanism by which PPAR γ activation increases insulin sensitivity is unknown, but it is assumed to require the activation of specific genes, the products of which, in turn, may affect insulin signalling. This possibility is discussed in the section on the molecular mechanism of insulin action. Although the predominant site of PPAR γ expression is adipose tissue, it is also expressed at low levels in other tissues, including skeletal muscle and liver, raising the possibility that the site of TZD action may not be restricted to adipose tissue. Although it appears that a high dose (600 mg) of troglitazone may reduce hepatic glucose output, the liver does not appear to be the major site of action of the TZDs. The increases in whole body insulin sensitivity induced by troglitazone administration suggests either direct or indirect effects on skeletal muscle, since skeletal muscle is the major insulin sensitive tissue by mass in the body. In animal models of insulin resistance improved glucose stimulated insulin secretion is observed after TZD administration, suggesting improved beta-cell function. Further evidence for TZD effects on the beta cell, be they direct or indirect, come from recent clinical studies in obese subjects with impaired glucose tolerance and with clear-cut defects in beta-cell function. Improved beta-cell responsiveness to glucose was demonstrated after a regimen of troglitazone treatment. This occurred despite substantial decreases in the net insulin response to an oral glucose tolerance test (suggesting increases in peripheral insulin action). There were also significant improvements in the ability of the beta cell to sense and respond to changes in the plasma glucose level. The mechanisms responsible for these effects, and for TZD effects in other tissues, are currently not known but could include direct troglitazone effects on the beta cell, decreases in insulin resistance/relief of hyperinsulinaemia, and/or a reduction in circulating NEFA.

Bruce Spiegelman: TNF α and the molecular basis of insulin resistance

The molecular basis of insulin resistance has seen substantial advances over the last few years. The identification of the hexosamine pathway in skeletal muscle as a potential player in the induction of skeletal muscle insulin is discussed above. Rad, a small

GTPase related to Ras, and PC-1, a membrane glycoprotein have both been implicated in inhibition of insulin action, but will not be discussed further. In skeletal muscle and adipocytes, it has been demonstrated the cytokine TNF α can induce insulin resistance in adipocytes, potentially by inhibiting insulin signalling. More recently, an involvement of PPAR γ in the regulation of insulin action has been postulated.

TNF α is over expressed in the adipose tissue of a number of animal models of obesity (the ob, db, tubby, and KKA^y mice and the fa and MSG rats) and in obese insulin resistant humans. TNF α inhibits insulin stimulated glucose transport in adipose tissue, and in the diabetic *fa/fa* Zucker rat neutralization of plasma TNF α by soluble TNF receptor administration results in substantial reductions in plasma insulin (fivefold), glucose (25%), and NEFA (40%). Additionally, insulin receptor autophosphorylation and insulin receptor substrate (IRS)-1 phosphorylation are increased in fat and muscle, events that are known to increase insulin signalling. In insulin resistant human subjects studies are ongoing that are attempting to neutralize circulating TNF α using anti-TNF α antibodies or soluble TNF receptor. However, since it is likely that TNF α can work in an autocrine or paracrine fashion, neutralization of circulating TNF α may not be sufficient for observing improvements in insulin action. Recent studies have demonstrated that complete knockout of the TNF α gene in transgenic mice results in a phenotype (obesity in the absence of insulin resistance) that suggests a larger role for TNF α than has been suggested in previous neutralization studies. Further studies are required in both animal models and humans to determine the potential role of TNF α in the pathogenesis of insulin resistance.

Recent observations suggest that TNF α induces insulin resistance in adipocytes by suppression of insulin signalling. The binding of insulin to the insulin receptor normally initiates a complex signal cascade that begins with tyrosine autophosphorylation of the insulin receptor and IRS-1. Binding of TNF α to the p55TNF receptor down-regulates tyrosine phosphorylation of both the insulin receptor and IRS-1. Additionally, IRS-1 from TNF α treated cells is an inhibitor of the insulin receptor. The exact mechanism of this inhibition remains to be determined, but may involve a serine phosphorylation event on IRS-1 that would inhibit IRS-1 function, thus inhibiting insulin signalling.

A potentially important insight into both the mechanism of TNF α induced insulin resistance and the mechanism of TZD induced increases in insulin action is the demonstration that pioglitazone treatment reduces TNF α mRNA in diabetic rats and can overcome TNF α induced insulin resistance. The mechanism involved in the latter process may involve a blocking of TNF α -induced phosphorylation

of IRS-1. The target of pioglitazone action is PPAR γ , an orphan receptor expressed predominantly in adipocytes that plays a central role in adipogenesis, probably via activation of expression of a number of genes involved in the regulation of carbohydrate and lipid metabolism. The activation of gene expression requires that PPAR γ bind specific DNA elements (ARE6 and ARE7) in the fat specific aP2 enhancer. Other chemical activators of PPAR γ and RXR, the heterodimeric partner of PPAR γ , also block TNF α induced insulin resistance, suggesting that alterations in gene expression and possibly a modulation of a phosphatase or kinase involved in TNF α signalling may be involved in blocking TNF α action. Interestingly, another target of TNF α action, the activation of nuclear factor κ B, is not blocked by pioglitazone, suggesting that the effect on TNF α signalling is specific for the insulin pathway.

Future directions and recommendations

- The involvement of insulin resistance in the aetiology of diseases of the metabolic syndrome requires further study. Additionally, more data are needed on the epidemiology of lipid abnormalities, particularly NEFA, fat cell size and perinatal measures of adiposity, as they relate to the onset of insulin resistance and the risk for development of IDDM and NIDDM. A practical step in this regard would be the development of a simple, rapid, accurate and inexpensive device for monitoring blood NEFA, such as is now performed with capillary finger stick glucose monitoring.
- Increased development and utilization of safe and non-invasive real time methodologies for the study of insulin-mediated metabolism in human subjects. Examples would include stable isotope and positron emission tomography studies of substrate kinetics, and also magnetic resonance spectroscopy of tissue carbohydrate and lipid fluxes, and tissue lipid content.
- More studies that integrate alterations in metabolic processes with the molecular actions of insulin (signal transduction and insulin regulated gene expression). Examples would include the investigation of the effects of changes in the levels/activation of critical enzymes and/or insulin signal molecules on insulin stimulated metabolic fluxes and overall metabolic homeostasis.
- Continued efforts to understand the mechanism(s) and major sites of action of the thiazolidinediones. These studies should not be restricted to adipocytes, but should also include muscle, liver and the beta cell. At the molecular level, the mechanism by which the binding of TZDs to PPAR γ leads to decreases in insulin resistance remains a critical unanswered question. The identification of the natural ligand(s) of PPAR γ would offer new insights into the regulation

of PPAR γ activation, and possibly would serve as a pharmacologic target for disease intervention.

- The exact role of the hexosamine pathway in the development and/or the exacerbation of skeletal muscle insulin resistance remains unclear. Inhibitors of the hexosamine pathway would be an important tool in answering this question. Additionally, more basic studies that address the regulation of the hexosamine pathway, and identification of any O-linked

glycosylation events that may play a role in the development of insulin resistance, are required.

- The role of TNF α in human insulin resistant states needs to be determined. This may require that clinical trials achieve effective local neutralization of TNF α , since the major mode of TNF α action may be auto-crine or paracrine. Practically, this will require the development of assays that are sufficiently sensitive to monitor neutralization at the tissue level.