Meeting Report of the ASPET-Ray Fuller Symposium: Insulin Resistance in Diabetes and Hypertension: Syndrome X and Beyond

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The second ASPET-Ray Fuller Symposium was held in London, Ontario, Canada at Spencer Hall on March 24–26, 2000. This symposium focused on three main themes: interaction of the insulin signaling pathway with the cytoskeleton, nonreceptor kinase-mediated insulin receptor (IR) signaling and the specificity of the response to insulin, and the interaction between metabolism and hemodynamics.

Domenico Accili (Columbia University, New York, NY) reminded the audience that the specificity of the insulin signaling pathway to induce glucose uptake is not well understood. The current paradigm is that the number of IR, the intracellular signaling pathway, and the machinery in place are the determinants of the biologic effect of insulin. However, dominant negative IR in adipose and skeletal muscle tissues (but not in liver) resulted only in mild glucose intolerance. These data, together with others, indicate a high degree of redundancy in the insulin signaling pathway or, at the level of the whole organism, to maintain glucose homeostasis. He reviewed the effect of combined IR and IR substrate (IRS) mutations on glucose and insulin metabolism and the risk for the development of diabetes. Currently, four IRS homologs are known. IRS-1 knockout (KO) mice are small with mild insulin resistance, IRS-2 KO exhibit impaired β cell growth and impaired insulin action in liver and skeletal muscle, IRS-3 KO appear normal, and IRS-4 KO display mild insulin resistance. Generation of double KO mice for IRS-1 or IRS-2 demonstrated an additive effect on the risk of developing diabetes. Importantly, the combined KO exhibited marked differences in insulin levels and phosphatidylinositol 3- (PI3-) kinase activity in liver and muscle. These findings indicate that the genetic background modifies the biologic response to disruptions of this system. When mice with a null IR allele who had robust insulin levels were back-crossed, quantitative trait loci analysis showed association at chromosomes 2 and 10. Because the insulin-like growth factor (IGF) receptor gene is located on chromosome 10, it is postulated that it may play a role in β cell growth to allow for robust production under conditions of impaired insulin action, preventing the occurrence of diabetes.

Michal Czech (University of Massachusetts, Amherst, MA) challenged the audience that although a number of “factoids” were known about insulin resistance, there have been few advances in the understanding of insulin resistance over the last 25 years. For example, it has only recently become clear that functional adipose tissue may be required to have normal insulin sensitivity. Furthermore, there are data suggesting that the classic insulin signaling pathway (IRS-1, PI3-kinase, Akt, etc.) may not be sufficient to explain insulin-induced glucose transporter (glut) 4 translocation.

To learn more about other pathways mediating insulin-induced glucose uptake, the genomic and proteomic approach was used. Purification of proteins in microsomal vesicles associated with glut 4 led to the discovery of a number of previously unknown substances. Not surprisingly, one observation suggested that there is cytoskeletal involvement in glut 4 trafficking. Moreover, inhibition of dynein action prevented normal localization of intracellular glut 4, and disruption of normal dynein function/structure disrupted insulin-induced glut 4 translocation. Thus, it is possible that insulin-resistant states are associated with impaired cytoskeletal microtubular function, resulting in inefficient intracellular glut 4 trafficking and translocation.

Continuing the theme of interaction between structural proteins and insulin action, Amira Klip (University of Toronto, Ontario, Canada) presented data showing that glut 4-containing vesicles associate with actin filaments before translocation to the cell membrane. Within 3 min of insulin stimulation, actin and Akt are colocalized on the membrane surface with synaptosome-associated 23-kDa protein (SNAP 23) and syntaxin-4. Shortly thereafter, glut 4 can be detected...
in this complex. Insulin causes a rapid reorganization of actin filaments below the cell surface to generate a structure that allows PI3-kinase and GLUT4-containing vesicles to associate. After this event, PI3-kinase phosphorylates lipids on the vesicles or plasma membrane, which recruit activated Akt. Moreover, the presence of activated Akt is required for GLUT4 translocation to the surface because dominant-negative mutants of Akt abrogate GLUT4 appearance at the cell surface. Dr. Klip reported that insulin-mediated glucose uptake decreases under conditions of normal GLUT4 translocation to the cell surface, indicating that insulin also regulates activity of GLUT4 transporters. Glucose transport was reduced by inhibiting p38 mitogen-activated protein (MAP)-kinase (which is activated by insulin). These data suggest that p38 MAP-kinase is involved in insulin-mediated regulation of GLUT4 activity. Because both GLUT4 translocation and activation mediate insulin-induced glucose uptake, defects in the translocation or activation pathways may lead to insulin resistance.

Research on the interaction between cytoskeletal elements and the IR signaling pathway is not only of theoretical interest but has direct relevance for insulin resistance in humans. Rob Hegele (Robarts Research Institute, London, Ontario, Canada) demonstrated this in Dunnigan-type familial partial lipodystrophy (FPLD). FPLD is a syndrome characterized by a wasting of fat on the extremities and gluteal region beginning in adolescence with acanthosis nigricans and a pseudo-Cushingoid, well masculinized body habitus. The biochemical hallmark of FPLD is insulin resistance, associated with diabetes, hyperlipidemia, hypertension, and early atherosclerosis. FPLD results from a mutation, namely R482Q, in LMNA, the gene that encodes nuclear lamins A and C. Other mutations of LMNA have been identified. Although the mechanism(s) whereby this mutation causes fat wasting/redisposition and insulin resistance is not clear, these data imply a potential role for nuclear structural proteins in the genesis of insulin resistance in humans.

The critical role of adipose tissue in glucose metabolism was further underscored by Gerald Shulman (Yale University, New Haven, CT). His group investigated lipodystrophic mice that are resistant to the effects of insulin on glucose uptake. In these mice, insulin resistance is associated with decreased PI3-kinase activity and glycogen synthesis. In addition, the effect of insulin on the liver is blunted in these animals. However, these mice exhibit significant triglyceride deposits within skeletal muscle and liver that are thought to be due to overexpression of lipoprotein lipase activity in the skeletal muscle. It is unknown how i.m. fat deposits interfere with glucose uptake. Interestingly, transplantation of adipose tissue into these lipodystrophic mice reverses the impairment in insulin action.

Insulin-stimulated glucose uptake requires activation of PI3-kinase via tyrosine kinase. However, as Jeffrey Pessin (University of Iowa, Ames, IA) pointed out, PI3-kinase activation alone is not sufficient for this to occur. His group, as well as others, demonstrated that IR stimulation also results in phosphorylation of c-Cbl, which colocalizes with flotillin and the IR in caveolae. However, it is not known whether these proteins interact directly or whether intermediaries are required. Phosphorylation of Cbl occurs only in insulin-sensitive tissues. The Cbl interacts with Cbl-associated protein (CAP), which dissociates from this complex after IR stimulation. Importantly, GLUT4 and CAP colocalize, indicating a role of Cbl/CAP in GLUT4 translocation. The mechanism whereby CAP may affect GLUT4 translocation is not understood. However, CAP contains a unique structure with three adjacent Src homology-3 (SH3) domains, which may be important for GLUT4 translocation because a CAP-SH3 mutant displayed impaired membrane GLUT4 and CAP association, and microinjection of CAP-SH3 inhibited GLUT4 translocation. Interestingly, thiazolidinediones increase expression of CAP mRNA and protein, which may explain the improvement in insulin sensitivity observed with these drugs.

Like IR, growth factor receptors couple to tyrosine kinases and regulate vascular tone. Regulation of vascular tone is mediated by nitric oxide release, arachidonate metabolites, and other less well characterized signaling pathways. Insulin enhances isoproterenol-induced vasodilation, and there is a strong relationship between insulin-mediated vasodilation and enhanced adenylate cyclase activity. Moreover, IGF and vanadate increase cyclase activity, an effect that is blocked by tyrosine kinase inhibitors. Together, these data indicate that tyrosine kinases regulate vascular tone in part by increasing adenylate cyclase activity. The signaling pathway leading to increased cyclase activity was discussed by Ross D. Feldman (Robarts Research Institute). Dr. Feldman’s group found that vanadate mediates serine/phosphoserine phosphorylation in a tyrosine kinase-dependent fashion. Inhibition of serine/threonine kinase decreased vanadate-induced cyclase activation. Moreover, the vanadate effect is attenuated by adding a dominant inactive Raff mutant, indicating that Raff plays a role in regulating cyclase activity. Eight potential target sites for serine phosphorylation were identified in the catalytic loop of adenylate cyclase. Finally, it was demonstrated that Raff kinase (p74 raff1) mediates the phosphorylation of serine residues within the C1b and C4 regions of adenylate cyclase 6. These mechanisms may be one link whereby tyrosine kinase activation regulates G protein-coupled receptor-mediated vasodilation.

Although adipose tissue participates in insulin-mediated glucose uptake, its major function is to store and release energy under the appropriate conditions, as Sheila Collins (Duke University, Durham, NC) pointed out. Adipose cell mass, and thus the potential for development of obesity/insulin resistance, depends on the balance between lipid accumulation, release, and oxidation. Lipid release, as well as energy expenditure by modulation of thermogenesis, is regulated in part by β-adrenergic receptors (ARs). In white adipose tissue, β1-ARs regulate lipolysis. In brown adipose tissue, β2-ARs not only regulate lipolysis but also increase β3-adrenergic expression, up-regulate transcription of uncoupling protein, and regulate brown adipose tissue mass. Interestingly, ob/ob mice exhibit decreased β3-AR number and near complete absence of β3-AR. Importantly, β3-agonists decrease white adipose mass, increase oxygen consumption, normalize β3-AR expression, normalize uncoupling protein levels, and increase brown adipose tissue mass. The β3-AR is constitutively coupled to both Gs and Gi, leading to activation of the cAMP and the extracellular signal-regulated kinase 1/2 (MAP kinase) cascade, respectively. In contrast to the β1- and β2-ARs, the β3-AR is not phosphorylated after stimulation and does not associate with β-arrestin, which is required for internalization and for signaling via MAP kinase of the β1-AR. In contrast to the other β-ARs, β3-AR contains proline-
rich regions in the intracellular domains, which serve as a scaffold to recruit the c-Src tyrosine kinase through its SH3 domain. These differences in signaling pathways may allow the β2-AR to escape desensitization during prolonged activation and to induce the effects observed with prolonged stimulation. If similar pathways are present in humans, elucidation of the β2-AR signaling pathway and definition of its metabolic effects might provide a mechanism for weight loss.

It is still not well understood how specificity is transferred for signal transduction by G protein-coupled receptors. The β-AR coupled to Gs undergoes phosphorylation, sequestration, and eventually down-regulation, leading to decreased responsiveness. Stephen Ferguson (Robarts Research Institute) presented data on β2-AR signaling, focusing on the signaling steps occurring during desensitization. He showed that endocytosis is an absolute requirement for both desensitization and resensitization and that β2-AR associates with β-arrestin before endocytosis. The β-arrestin interacts with a wide variety of signaling proteins, and Src translocates to the cell membrane and colocalizes with β-arrestin. Eventually, β2-AR undergoes endocytosis that is required to phosphorylate MAP-kinase. These complex and well orchestrated steps may provide the mechanism for cell specific responses to β2-AR stimulation.

Steve Shoelson (Harvard Medical School, Cambridge, MA) presented data showing that the classic insulin signaling pathway may be modulated by proinflammatory cytokines. This work evolved from the observation that the elevation of tumor necrosis factor-α (TNF-α) in response to major stress, such as burn or sepsis, induces insulin resistance in humans. However, the work with TNF-α using KO mice or TNF antibodies has been disappointing thus far. Therefore, interest has shifted to potential serine/threonine phosphorylation of the IR/IRS pathways. Phosphorylation of serine/threonine residues has been postulated to decrease insulin-mediated glucose uptake. If this is the case, reversal of serine/threonine phosphorylation should decrease insulin resistance. Proinflammatory cytokines activate IκB kinase (IKK), which in turn activates nuclear factor-κB, which is transported into the nucleus where it activates a number of genes. Salicylates inhibit IKK, thereby preventing nuclear factor-κB activation. Therefore, the investigators assessed whether aspirin (ASA) could reverse "insulin resistance" in FAO hepatoma cells induced by TNF, okadaic acid, or phorbol ester. Indeed, ASA was able to restore normal insulin response in all three cases. When 120 mg/kg ASA was fed to Zucker Fatty rats for 3 weeks, there was a decrease in glucose and insulin excursions in response to an oral glucose tolerance test. A similar effect of ASA on insulin and glucose was observed in ob/ob mice. Importantly, both ASA and salicylate also enhance insulin signaling in liver and skeletal muscle of Zucker Fatty fa/fa rats, and ASA causes a mobility shift of IRS-1. Following these observations was a review of the literature that revealed a number of case reports and a few small studies showing improved glycemic control in patients treated with high doses of ASA. Dr. Shoelson concluded that 1) IKK contributes to insulin resistance, 2) IKK inhibition mitigates against insulin resistance, and 3) IKK and associated up- and downstream signaling pathway molecules are potential targets for further research.

Todd Leff (Parke-Davis Pharmaceutical Research, Ann Arbor, MI) reviewed the current, albeit still limited, understanding of the mechanism(s) whereby thiazolidinediones improves insulin sensitivity. It is known that these compounds activate peroxisome proliferator-activated receptor (PPAR) γ and that the nonthiazolidinediones that activate these receptors also lower glucose levels in insulin-resistant subjects. However, the genes that are activated after PPAR stimulation and the precise pathways leading to the improvement of insulin sensitivity are largely unknown. It appears that different PPAR modulators recruit different coactivators (which are still unidentified) and/or recruit the coactivators to different degrees, leading to the activation of distinct subsets of genes depending on the cell type and promoter context. This idea is supported by the observation that troglitazone is a partial agonist for the AREG peroxisome proliferator response element but a full agonist for the fatty acid transport protein PPRG. Elucidation of the gene(s) responsible for the improvement in insulin sensitivity may lead to the development of more specific and potentially less toxic hypoglycemic agents.

Gerald Shulman (Yale University) demonstrated the role of sophisticated NMR techniques to elucidate mechanism(s) of insulin resistance in vivo. Using 13C NMR and 31P NMR, he found that the main defect of reduced insulin-mediated glucose uptake is at the level of glucose transport, not glucose phosphorylation or glycogen synthesis. The same NMR technology was used to elucidate the mechanism of free fatty acid (FFA)-induced insulin resistance in humans. Elevation of FFA resulted in decreased insulin-stimulated glycogen synthesis due to the decreased glucose transport. Other biochemical changes in response to FFA elevation during euglycemic hyperinsulinemia were assessed by examining muscle biopsy specimens obtained before and after FFA elevation. In this case, FFA elevation resulted in a decrease in PI3-kinase activity that is most likely secondary to IRS-1 serine phosphorylation. Because FFA induced activation of protein kinase C θ, which may be responsible for the serine phosphorylation of IRS-1, studies aimed at inhibition of kinase may provide more insight in the mechanism of FFA-induced insulin resistance.

Jean-Pierre Despres (Laval University, Quebec, Canada) presented data showing that the risk for diabetes and cardiovascular disease not only depends on how much fat is present but where it is located. Body mass index and waist/hip ratio, an indicator of central versus peripheral fat location, have a synergistic effect on the risk of developing diabetes. However, fat location appears to be a much stronger predictor for cardiovascular disease than body mass index. Dr. Despres demonstrated that insulin and apolipoprotein (apo) B levels and low density lipoprotein size provide much better risk discrimination than nontraditional risk factors. From these data, his group developed an algorithm including waist circumference and triglyceride levels to identify subjects that would benefit most from further work-up and possible intervention. Unfortunately, these risk assessment data are only valid for males because no prospective studies have been performed with females.

Alain Baron (Indiana University, Indianapolis, IN) pointed out that the major increase in cardiovascular mortality occurs long before hyperglycemia and insulin resistance are detected. Insulin resistance is associated with a number of well characterized risk factors, such as dyslipidemia or elevated blood pressure, which account for ~50% of this ele-
Elevation of FFA is one of the hallmarks of insulin resistance and type 2 diabetes. Brent Egan (Medical University of South Carolina, Charleston, SC) discussed findings relating to the effect of FFA elevation on vascular pathophysiology. Obese hypertensive subjects exhibit resistance to the FFA-lowering action of insulin with a strong correlation existing between rates of FFA turnover and diastolic blood pressure levels. This may be explained by the ability of FFA to augment α-AR-mediated vasoconstriction. Moreover, FFAs impair endothelial function, stimulate the renin-angiotensin system, and increase plasma 8-iso-prostaglandin F₂a, an index of oxidative stress. Elevated FFA also stimulates protein kinase C, reactive oxygen species, and extracellular signal-regulated kinase-dependent mitogenic and migratory responses in cultured vascular smooth muscle cells, possibly contributing to the formation of atherosclerotic plaques. The reactive oxygen species-dependent signaling pathway is synergistically enhanced by angiotensin II. Understanding the signaling mechanism(s) underlying the synergy between these pathways may lead to improved strategies for reducing the cardiovascular risk in insulin-resistant subjects.

Obesity is often accompanied by hypertension, and hypertension improves with a decrease in insulin resistance induced by weight loss, exercise, or insulin-sensitizing agents. This association between obesity, insulin resistance, and hypertension led to the hypothesis that insulin resistance and/or hyperinsulinemia causes hypertension. Michael Brands (University of Mississippi, Oxford, MS) reviewed the current data. The hypothesis was tested in different rat and dog models, but the evidence for insulin resistance itself to cause hypertension remains inconclusive. Hyperinsulinemia achieved by insulin infusion does not cause blood pressure elevation in dogs, although there is a small blood pressure elevation in rats. The blood pressure elevation in rats is associated with renovascular constriction and increased endothelin and thromboxane production. Using a model of streptozotocin-induced diabetes, Dr. Brands found that hyperglycemia (∼400 mg/dl) induced a mild increase in blood pressure (∼4 mm Hg). This blood pressure response was much more pronounced (∼30 mm Hg) after blockade of nitric oxide production, indicating that intact endothelial function protects from the effects of hyperglycemia. He concluded, therefore, that hypertension in type 2 diabetes may not be directly due to insulin resistance but is secondary to the effect of elevated glucose and enhanced by worsening endothelial dysfunction.

James Sowers (SUNY Health Center Sciences Center, Syracuse, NY) demonstrated that vascular smooth muscle tone is regulated by insulin and IGF-1. In contrast to insulin, IGF-1 is produced by vascular smooth muscle where it may act in a paracrine fashion. The IGF production in vascular smooth muscle increases in response to stretch, angiotensin II, and insulin. The IGF-1, like insulin, activates tyrosine kinase and causes the release of nitric oxide. Insulin and IGF-1 decrease the inward calcium current and modulate Na⁺-K⁺-ATPase, thus modulating the response to vasoconstrictors. Interestingly, hyperglycemia increases the inward calcium current in cardiomyocytes, an effect that is reversed by the antidiabetic agents metformin and troglitazone, suggesting that they exert vascular effects independent of their glucose-lowering action. Sulfonylureas, another class of antidiabetic drugs, do not alter calcium current in the cardiomyocytes.
Christopher Newgard (University of Texas Southwestern Medical Center, Dallas, TX) discussed genetic engineering projects for the treatment of hyperglycemia and hyperlipidemia of type 2 diabetes. His group uses adenoviral vectors to target hepatic glucose and FFA production. Adenovirus-mediated overexpression of a glycogen-targeting protein phosphatase 1 in normal rats increased hepatic glucose disposal and glycogen storage and improved glucose tolerance without changes in lipid metabolism. In contrast, glucose lowering achieved by adenovirus-mediated overexpression of glucokinase in liver is associated with elevated triglyceride and FFA levels. Exploring a potential target for lipid lowering, adenovirus-mediated overexpression of malonyl coenzyme A decarboxylase resulted in a 60% reduction in circulating FFA levels and mildly elevated glucose levels. The group now seeks to combine different adenovectors to improve both hyperglycemia and hyperlipidemia in animal models of insulin resistance and type 2 diabetes.

The presentations at this ASPET-Ray Fuller Symposium made clear that our knowledge of the complexity of glucose homeostasis and its relation to cardiovascular disease is in its infancy. More research is necessary to define the intricacies of insulin action, including the non-tyrosine kinase signaling pathways and cytoskeletal elements involved in Glut 4 trafficking. A better understanding of interactions between adipose tissue, skeletal muscle, liver, pancreas, and the cardiovascular system will lead to new approaches for the prevention of diabetes and cardiovascular disease.