Chemistry and Biochemistry of Type 2 Diabetes

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1. Introduction

Today, we know that a primary metabolic action of insulin is to facilitate the postprandial disposition of glucose via its actions on three key target tissues: suppression of glucose output from the liver and stimulation of glucose uptake and metabolism in skeletal muscle and adipose tissue. Defects in insulin secretion and insulin action on its target tissues manifest clinically as diabetes, syndrome X, and insulin resistance. The discovery of insulin by Banting and colleagues in the early 1920s stands as one of the greatest scientific achievements of the 20th century. Few scientific endeavors have such profound "(lab) bench to bedside" implications. Prior to this discovery the prospects for patients afflicted with diabetes were grim. Few scientific discoveries are as rich in history as the identification of insulin as the glucose-lowering hormone. Insulin is perhaps the second most-decorated molecule in biology behind cholesterol in that work on insulin (directly or indirectly) has yielded six Nobel Prizes. Despite these striking accomplishments and accolades, there is

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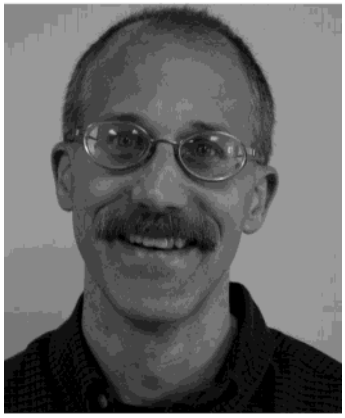
Stuart Ross obtained his Ph.D. degree from the University of Wisconsin—Madison in 1994 and did his NIH-funded postdoctoral studies at Dartmouth Medical School. He has a long-standing interest in protein trafficking and mechanisms of signal transduction. His efforts have focused largely on the mechanism of insulin-stimulated glucose transport by characterizing the insulin-responsive aminopeptidase (IRAP), a marker protein for Glut4 vesicles, and on identifying all of the protein components of Glut4-containing vesicles. Since 1998, Dr. Ross has had tenures at Pharmacia and then Pfizer, where he has led type 2 diabetes drug discovery teams with specific emphasis on the hexosamine biosynthetic pathway and the insulin signaling pathway. In addition, he worked on the identification/validation of novel diabetes drug targets. He is currently Associate Director in Research Molecular Biology at the Institutes of Pharmaceutical Discovery in Branford, CT.

Minghan Wang received his Ph.D. in biochemistry and molecular biology from the Medical College of Ohio in 1994. After spending one year as a postdoctoral fellow at the University of Michigan, he continued his postdoctoral work at Parke-Davis Pharmaceutical Research Division of Warner-Lambert Co. in Ann Arbor, MI. He joined the Department of Molecular Biology at Parke-Davis as a Senior Scientist in 1998, working as Project Team Leader in the Cardiovascular Molecular Sciences group after the Pfizer acquisition in 2000 and later working as Research Advisor and Project Leader in the Cardiovascular and Metabolic Diseases Department of Pharmacia Corp. in St. Louis, MO. He currently holds a position of Research Scientist III/Project Leader in the Metabolic Disorders group at Amgen in Thousand Oaks, CA. His research interests include cardiovascular and metabolic diseases, including dyslipidemia, atherosclerosis, insulin resistance, and obesity, and fundamental approaches for small molecular drug discovery in these areas.



Eric Gulve received his A.B. in chemistry from Occidental College (Los Angeles) and his Ph.D. in physiology and biophysics from Harvard University (1987). He moved to St. Louis, MO, to work with Dr. John Holloszy in the Department of Internal Medicine at Washington University School of Medicine as a postdoctoral fellow and later as an Instructor and Research Assistant Professor. With Dr. Holloszy he studied the regulation of skeletal muscle glucose transport. The laboratory focused on characterizing the mechanisms by which muscle contractile activity and insulin regulate glucose transport and metabolism, the differential responses of muscle to acute and chronic exercise, the interactions between contractile activity and insulin action, and the effects of development and aging on muscle glucose transport. The Holloszy laboratory was a stimulating environment in which animal research findings often sparked studies in human subjects. Dr. Gulve helped to train a number of postdoctoral fellows in the group. In 1994 he joined the Cardiovascular Diseases Research group at G. D. Searle/Monsanto to help initiate a program in diabetes research and led Monsanto's first diabetes project. He held positions of increasing responsibility at G. D. Searle (which later merged with Pharmacia-Upjohn to form Pharmacia), eventually assuming the position of Associate Director of Cardiovascular and Metabolic Diseases. He has contributed to efforts in diabetes, dyslipidemia, and heart failure. Currently he is turning his attention also to thrombosis and hypertension research after the acquisition of Pharmacia by Pfizer.

much that remains to be discovered in how insulin exerts its effects. While more than 80 years have passed since the discovery of insulin, the molecular actions of insulin have only begun to be elucidated over the past 20 years with the cloning of the insulin receptor, the advent of transgenic technology, and the development of phosphotyrosine-specific antibodies. The ultimate unifying theme among diabetes researchers today is to uncover novel targets for which to develop improved therapeutic modalities. The importance of these endeavors is underscored by the impending world diabetes epidemic with numbers expected to reach ~250 million worldwide or ~5% of the world's population by the year 2030.[1]

2. Pathophysiology and Mechanisms of Diabetes

Diabetes mellitus is a chronic metabolic disease resulting from insulin deficiency. There are roughly 35 million diabetics in the seven major world markets with only about half being diagnosed, and these numbers are expected to double by 2030.[1] There are two forms of diabetes mellitus: type 1, or juvenile diabetes or insulin-dependent diabetes mellitus (IDDM), and type 2, or adult-onset diabetes or non-insulin-dependent diabetes mellitus (NIDDM). Type 1 diabetes patients have an absolute insulin insufficiency due to the immunological destruction of pancreatic β cells that synthesize and secrete insulin. Of the estimated 16 million diabetics in the United States, <10% have type 1 diabetes. Type 2 diabetes is more complex in etiology and is characterized by a relative insulin deficiency, reduced insulin action, and insulin resistance of glucose transport in skeletal muscle and adipose tissue.

Type 2 diabetes is typically a polygenic disease that results from a complex interplay between genetic predisposition and environmental factors such as diet, degree of physical activity, and age.[2,3] The manifestation of frank type 2 diabetes is typically a continuum of insulin resistance culminating in the failure of augmented insulin secretion to compensate for insulin resistance. Initially, in type 2 diabetes, insulin-stimulated glucose transport in skeletal muscle is impaired. As compensation, pancreatic β cells display augmented secretion of insulin, resulting in hyperinsulinemia. Peripheral insulin resistance, in combination with impairment in the early phase of insulin secretion, results in hyperglycemia. In end-stage type 2 diabetes, changes in insulin signaling, such as insulin's inability to inhibit hepatic gluconeogenesis, are accompanied by a deterioration of pancreatic β cell function and β cell "exhaustion". In essence, the progression to full-blown type 2 diabetes ensues when the β cell hypersecretion of insulin fails to compensate for insulin resistance. These patients require one to several daily insulin injections for proper glycemic control. In rarer circumstances, type 2 diabetes can occur primarily as a result of β cell aberrations as illustrated in Maturity Onset Diabetes of the Young (MODY) patients, who have mutations in genes for glucokinase or a variety of transcription factors. Despite intensive research, the identification of a set of unifying type 2 "diabetogenes" has thus far remained elusive. The importance of endeavoring to uncover such genes is further underscored by the additional (i.e., on top of insulin resistance) chronic maladies associated with diabetes. Diabetes mellitus often results in long-term microvascular, neurological, and macrovascular complications including retinopathy, nephropathy, neuropathy, and increased risk of cardiovascular disease. Diabetes is the leading cause of blindness, lower limb amputations, and renal failure in the United States. The healthcare cost of diabetes is high, with the total estimated cost in the United States exceeding $100 billion. Although two main biological abnormalities (insulin action and insulin secretion) are associated with type 2 diabetes, this review will focus on the insulin action aspect of type 2 diabetes.

3. Insulin Signal Transduction and Regulation of Glucose Uptake

3.1. Introduction

A primary metabolic effect of insulin is to stimulate the uptake of circulating glucose into muscle and adipose tissue. Under most physiological conditions, glucose transport is thought to be rate-limiting for glucose uptake and metabolism by skeletal muscle and adipose tissue.[4,5] Thus, the regulation of the glucose transport process by insulin is a critical factor in glucose homeostasis. Conceptually, insulin-mediated glucose transport could occur by either stimulating the activity of existing cell surface glucose transport proteins or by translocation of an intracellular transporter to the cell surface in response to insulin. Compelling evidence for the translocation hypothesis came in 1980 with the publication of two landmark papers.[6,7] We now know insulin-stimulated glucose uptake is mediated by the muscle- and fat-specific, insulin-regulatable glucose transporter isotype 4 (Glut4) as depicted in Figure 1. First identified in 1988[8] and cloned in 1989 by several independent groups,[9−12] Glut4 has garnered the lion's share of interest in the glucose transport field because of its unique cell biology of translocation to the plasma membrane in response to insulin or muscle contraction. This simple-looking process has captivated the attention of thousands of researchers because it is defective in diabetes mellitus, regardless of the type. In either case, the insulin-stimulated translocation of Glut4 to the cell surface of muscle and fat tissue is attenuated. Therefore, as a first step toward identifying therapeutic intervention points, much work has focused on identifying and characterizing the molecular machinery of insulin signaling with the ultimate aim of identifying rational therapeutic intervention points. Below we shall summarize what is currently known about how insulin signaling mediates the translocation of Glut4-containing vesicles to the plasma membrane. Furthermore, significant advances have been reported with regard to the modulation of insulin signaling via tyrosine phosphatases and serine−threonine phosphorylation of the insulin receptor and IRS molecules by I kappa kinase 2 (IKK2), TNFα, and protein kinase C (PKC). These mechanisms are covered in the later sections dealing with animal models and emerging drug targets. It is important to note that in type 2 diabetes, the ability of muscle contractions to stimulate glucose

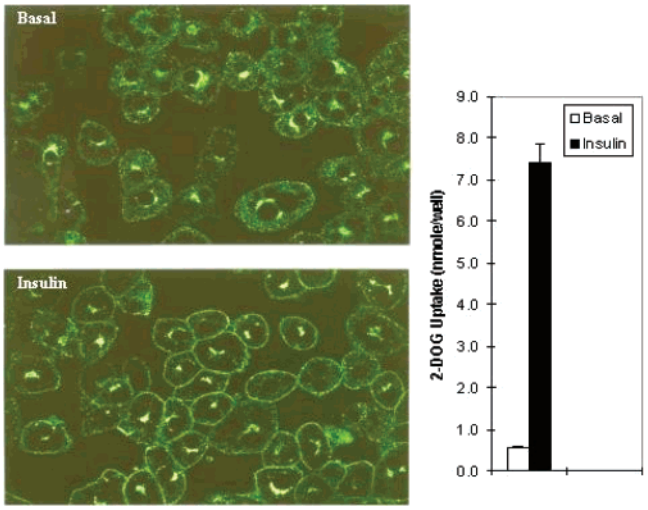


Figure 1. Insulin stimulation of Glut4 translocation and glucose transport in murine 3T3-L1 adipocytes. Cells were stimulated with 160 nM insulin for 15 min or left in the basal state, fixed in paraformaldehyde, permeabilized with the detergent saponin, and reacted with antibodies against the C-terminal 19 amino acid peptide of the glucose transporter Glut4 followed by detection with fluorescently labeled secondary antibody and visualization with confocal microscopy. (A, upper left) Glut4 is detected in a perinuclear and punctate distribution in the basal state. Following insulin treatment Glut4 redistributes to the cell surface as indicated by the ring around the cell periphery. (B, lower left) Glut4 translocation is accompanied by a concomitant increase glucose uptake. Insulin stimulates a large-fold increase in glucose uptake (in the example shown ~13.5-fold) relative to basal.

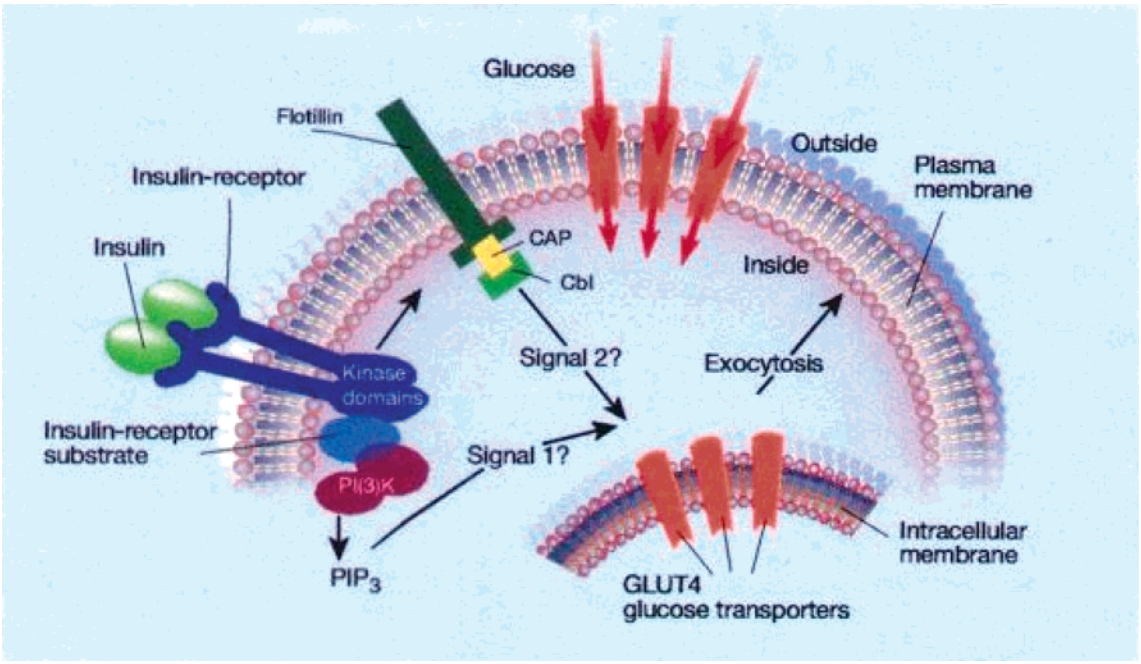


Figure 2. Insulin signal transduction pathways linked to glucose transport in fat and muscle. Insulin binding to the α subunit of the insulin receptor on the extracellular surface elicits autophosphorylation of select intracellular tyrosine residues of the β subunit, which in turn phosphorylates and activates various effector proteins collectively known as insulin-receptor substrates. These proteins serve as docking/effector sites for a number of SH2-containing proteins that mediate insulin signaling. Phosphatidylinositol 3′-kinase is one such SH2-containing protein that is activated in response to insulin and is necessary for translocation of vesicle containing Glut4 from an intracellular site to the plasma membrane to facilitate glucose uptake. Recently, a second pathway of insulin signaling emanating from specific locales on the plasma membrane has also been thought to play a role in the translocation of Glut4 storage vesicles. This pathway involves the insulin-stimulated recruitment of a flotillin-c-Cbl−CAP (C-Cbl associated protein). The convergence of these two pathways, if any, and their coordinated Glut4 translocation/glucose transport remains to be determined. [Reprinted with permission from Czech et al. *Nature* (http://www.nature.com) **2000**, *47*, 147−148. Copyright 2000 Nature Publishing Group.]

transport is maintained, providing the basis for exercise as a therapy and another potential avenue for drug development.

3.2. Insulin Receptor (IR)

The insulin receptor is a member of the receptor tyrosine kinase family forming a heterotetramer of two α subunits and two β subunits (Figure 2), and it shares similarity with the insulin-like growth factor-1 receptor (IGF-1R) and the insulin-related receptor (IRR). Under basal conditions, the α subunit serves as an allosteric inhibitor of the β subunit. Insulin stimulation relieves the repression of the β subunit, thereby eliciting its tyrosine kinase activity through a conformational change that leads to transphosphorylation of specific β-subunit tyrosine residues.[13] This in turn leads to tyrosine kinase activity toward substrate proteins (discussed below). Unlike other tyrosine kinases (e.g., PDGF-R, EGF-R) that recruit signaling proteins to their intracellular cytoplasmic tail, the insulin receptor propagates insulin signaling via tyrosine phosphorylation of substrate proteins that form a scaffold for a myriad of signaling events.

Perhaps the strongest evidence for the insulin receptor mediating insulin signal transduction comes from the identification of humans harboring mutations in the insulin receptor gene that lead to diabetes with a broad spectrum of cell biological defects. For example, one patient has been identified as containing a deletion in the tyrosine kinase domain of the receptor that manifested as normal insulin binding yet attenuated activity toward substrate proteins.[14] There are three tyrosine residues within the β subunit that have been identified as critical in mediating insulin signaling. These are Tyr-1146, -1162, and -1163, with Tyr-1146 perhaps being most critical in that mutation of this residue results in an ∼80% reduction in tyrosine autophosphorylation and a failure to detect endogenous substrates.[15] Some evidence suggests that insulin, IGF-1, and insulin-related receptors can form functional hybrids with each other and that an inhibitory mutation in one receptor monomer could inhibit the activity of the other.[16] Whether this phenomenon occurs in vivo is unknown.

3.3. Insulin Receptor Substrates (IRSs)

With the identification of the insulin receptor as a tyrosine kinase, the search for candidate substrate proteins was greatly aided with the advent of phosphotyrosine-specific antibodies. The first and best-characterized substrate, aptly named insulin receptor substrate-1 (IRS-1),[17−19] is a 160 kDa docking/effector protein. Phosphorylation of numerous tyrosine residues in IRS-1 is elicited upon insulin stimulation. Two major consensus sequences, Y(p)XXM and Y(p)MXM, where X is any amino acid, predominate as the sites of tyrosine phosphorylation. When phosphorylated, these sites in turn serve as docking sites for a variety of proteins harboring src homology 2 (SH2) domains. There are presently nine insulin receptor substrate proteins (IRS1−4, Dok, Gab-1,

Cbl, APS, and isoforms of Shc), although the name is somewhat of a misnomer because tyrosine phosphorylation of some of these proteins can be elicited by numerous extracellular stimuli. This review will focus on insulin action on some of these proteins. In response to insulin, IRS proteins recruit other proteins (e.g., PI3K, Nck, Grb2, and CrkII), thereby forming a multifunctional signaling center from which to emanate insulin action. Indeed, these IRSs serve as the first major rotary of insulin action. Many of the SH2-containing proteins that bind to IRSs act as adapter molecules (e.g., the p85 subunit of PI3K and Grb2), whereas others themselves carry out enzymatic functions (e.g., SHP2, a tyrosine phosphatase).

Despite significant degrees of amino acid homology among the IRS proteins, experiments with both transgenic animals and cultured cells imply their functions are complementary in nature rather than redundant. For example, ablation of IRS-1 in mice manifests as insulin resistance in peripheral tissues, impaired glucose tolerance, and pre- and postnatal growth retardation.[20,21] Similarly, IRS-2 knockout mice are also characterized as having peripheral insulin resistance. In contrast to the IRS-1 (-/-) mice, however, IRS-2 (-/-) animals also demonstrate hepatic insulin resistance and diminished β cell mass, culminating in frank type 2 diabetes,[22] whereas knockouts of IRS-3 and IRS-4 display apparently normal growth and glucose homeostasis.[23,24]

Differential tissue expression, intracellular localization, and/or functional activity of the IRS isoforms may explain their different phenotypes of knockout mice. For example, embryonic and 3T3 fibroblasts derived from IRS-1 (-/-) mice show lowered IGF-1-induced DNA synthesis activity[25] and also fail to differentiate into brown adipocytes in culture.[26] Brown adipocytes from IRS-2 (-/-) display attenuated insulin-stimulated glucose uptake relative to controls.[27] Presently, the functions of IRS-3 and IRS-4 are unknown, but evidence via overexpression in primary mouse fibroblasts suggests they may act as negative regulators of IRS-1 and IRS-2 signaling.[28]

Emerging data indicate that serine phosphorylation of IRS-1 attenuates IRS-1 signaling activity via prevention of tyrosine phosphorylation. The precise identification of all the candidate regulatory phosphoserine sites within IRS-1 and the kinases that phosphorylate them is under intense investigation. Enhanced serine kinase activity toward exogenous glutathione *S*-transferase fusion proteins of IRS-1[29] has been observed in homogenates of cells rendered insulin resistant by chronic insulin treatment or in extracts of muscle and liver from obese JCR:LA-cp rats when these preparations were mixed with various IRS-1 fragments. Recently, Ser^{307} has been implicated as a potentially critical residue as a candidate regulatory phosphorylation site, whereby phosphorylation of this site prevents insulin-elicited tyrosine phosphorylation of IRS-1. Conceivably, any kinase that could phosphorylate IRS-1 at this site (and perhaps any of the other ~70 potential consensus Ser/Thr phosphorylation sites within IRS-1) could elicit insulin resistance if its activity were dysregulated in insulin-resistant or type 2 diabetic individuals. Recent data suggest the c-Jun NH_2-terminal kinase isotype 1 as a mediator of insulin resistance. In Chinese hamster ovary cells endogenous JNK1 associates with IRS-1; anisomycin, a JNK1-activating compound, stimulates the kinase activity of IRS-1-bound JNK1, thereby eliciting phosphorylation of Ser^{307} with concomitant inhibition of IRS-1 tyrosine phosphorylation.[30] Indeed, a role for dysregulated JNK1 in mediating obesity and insulin resistance has come to light with characterization of JNK1 knockout mice. The absence of JNK1 resulted in decreased adiposity coupled with enhanced insulin signaling. That is, insulin induced a greater extent of tyrosine phosphorylation of the IR and IRS-1 in JNK (-/-) mice versus control. Furthermore, whereas obese mice showed an increase in IRS-1 Ser^{307} phosphorylation relative to lean controls, there was no evidence of Ser^{307} phosphorylation in either lean or obese JNK (-/-) mice.[31]

3.4. Phosphatidylinositol 3-Kinase (PI3K)

Class 1A phosphatidylinositol 3′-kinase comprises two protein subunits designated p85 and p110 with molecular weights of 85 and 110 kDa, respectively. Upon insulin treatment, the p85 subunit associates via its SH2 domain with tyrosine-phosphorylated IRS proteins. This interaction activates the catalytic p110 subunit to phosphorylate the 3-position of phosphatidylinositol 4-phosphate and phosphatidylinositol 4,5-bisphosphate to form phosphatidylinositol 3,4-bisphosphate and phosphatidylinositol 3,4,5-trisphosphate, respectively.

Numerous studies have defined a central role for the activation of PI3K in mediating Glut4 vesicle translocation and increased glucose transport in response to insulin. Treatment of rat and 3T3-L1 adipocytes with inhibitors of PI3K, such as the fungal metabolite wortmannin[32] or a compound developed by Eli Lilly and Co., LY-294002,[33] or expression of dominant interfering mutants of PI3K,[34−36] overexpressed proteins that swamp out their respective wild-type protein function, completely blocks insulin-stimulated Glut4 translocation and glucose transport. Consistent with these data, introduction by microinjection of a neutralizing antibody to the p110 subunit of PI3K into 3T3-L1 adipocytes completely abolished Glut4 translocation to the cell surface as assessed by the plasma lawn assay (whereby sonication of adherent cells leaves behind plasma membrane "sheets" that are reacted with an antibody to the C terminus of Glut4).[37] Consistent with the data in adipocytes, treatment of rat skeletal muscle with wortmannin completely blocked both the production of 3′-phospholipids and increased hexose uptake in response to insulin.[32,38] In skeletal muscle, glucose uptake can also be activated by muscle contractile activity. Wortmannin did not inhibit glucose transport stimulated by contractions or hypoxia (a condition that mimics contraction-induced glucose uptake), suggesting a divergence in upstream signaling events in the insulin and contraction signaling pathways.[38] Both pathways culminate in Glut4 translocation to the plasma membrane and increased glucose uptake.

The ability of muscle contractions to stimulated glucose uptake through this PI3K-independent pathway is preserved in diabetes, which accounts for part of the beneficial effects of exercise in diabetics.

Whereas inhibition of PI3K by one of the above methods inhibits insulin-stimulated glucose transport, several converse experiments that introduced wild-type or a constitutively active PI3K into cells promote glucose transport. Overexpression of a wild-type PI3K tagged with a Glut2 carboxy-terminal epitope[39] or a constitutively active mutant of PI3K in 3T3-L1 adipocytes promoted glucose transport activity and Glut4 vesicle translocation comparable to the maximal effect induced by insulin.[40]

Interestingly, growth factors such as platelet-derived growth factor (PDGF) as well as certain cytokines activate PI3K to a similar extent as insulin, but they do not stimulate Glut4 translocation or other metabolic effects of insulin.[41−43] Current thinking suggests the exquisite sensitivity to insulin is a matter of intracellular location, where insulin-stimulated PI3K kinase activity is localized to an intracellular "low-density" microsomal compartment at or near the Glut4 storage vesicle versus PDGF-stimulated PI3K activity localizing to the plasma membrane.[44,45] Whether IRS-1-associated PI3K associates with Glut4 vesicles is still debatable. It is possible that only a "spark" of signal initiation is needed, and thus the full complement of signaling molecules need not be present at the Glut4 storage vesicle. One report suggested 100 nM insulin treatment for 3.5 min elicited the association of IRS-1 bound to PI3K to Glut4 containing vesicles in both isolated rat and 3T3-L1 adipocytes.[46] In contrast, other investigators failed to detect either IRS-1 or PI3K in Glut4-containing vesicles after a 10 min insulin challenge.[47,48] It is possible that an association of PI3K with Glut4 vesicles, if any, is rapid and/or transient. Arguing against a direct association of PI3K with Glut4-containing vesicles were studies in which a constitutively active PI3K specifically targeted to Glut4 vesicles was without effect on Glut4 translocation.[49] A gap in understanding still exists in the link between the coupling of insulin-stimulated PI3K activation and Glut4 vesicle translocation.

3.5. Signaling Downstream of PI3K

Several pathways downstream of PI3K are activated by growth factor stimulation. The "atypical" λ and ζ isoforms of protein kinase C, so-called because they are not activated by either diacylglycerol or phorbol ester, are activated in vitro in the presence of PI3K products[50,51] or PDGF or EGF stimulation.[52] In addition, either or both PKCλ and PKCζ have been implicated in insulin-stimulated glucose transport in 3T3-L1 adipocytes,[53,54] L6 myotubes,[55] rat skeletal muscle,[56] and rat[53] and human[57] adipocytes. Interestingly, following activation by insulin, PKCλ and ζ may be targeted to Glut4-containing vesicles.[58] A third isoform, PKCδ, a novel but not atypical isoform, was shown to participate in insulin-elicited glucose transport in rat skeletal muscle.[59] However, some recent data have appeared that do not support a role of the atypical PKC isoforms in mediating glucose transport. Robust overexpression of PKCλ, PKCζ, or PKCδ wild-type or dominant negative isoforms in 3T3-L1 adipocytes did not alter basal or insulin-stimulated glucose transport.[60] As is true for other controversial aspects of insulin action, the role of atypical PKCs in mediating insulin-stimulated glucose transport requires further clarification.

Akt, also known as protein kinase B, is activated by insulin in a variety of cell types through phosphorylation of its serine and threonine residues.[61] PI3K inhibitors block this activation.[62,63] When a myristoylated, constitutively active form of Akt1 that targets it to the plasma membrane was expressed in 3T3-L1 adipocytes, it promoted Glut4 translocation in an insulin-independent manner, albeit with slower kinetics than insulin.[64] Three isoforms of Akt exist, so the question as to which form or forms are involved in mediating glucose transport remains open. Recently, ablation of Akt2 in mice yielded animals that were insulin resistant as characterized by elevated blood glucose and insulin concentrations, defective suppression of hepatic gluconeogenesis, and modestly decreased insulin-stimulated glucose uptake in muscle.[65] Because the disruption of Akt2 signaling had effects on glucose homeostasis in liver and to a lesser extent muscle, this confounds the relative importance of Akt2 in Glut4 translocation/glucose uptake, which is muscle (and adipose) specific. It is possible, and remains open to experimental assessment, that another isoform of Akt compensated for the loss of Akt2, although Akt1 knockout mice show normal glucose homeostasis.[66]

The importance of intracellular localization of Akt to Glut4-containing vesicles has been investigated. Although a myristoylated, plasma membrane resident, constitutively active Akt1 could elicit Glut4 translocation in 3T3-L1 adipocytes, the majority of endogenous Akt1 resides in the cytosol. In rat adipocytes, Akt2 is localized largely to membranous compartments, including Glut4 vesicles, in the basal state, and insulin elicits an increase in Akt2 abundance in the Glut4 vesicles.[67] After insulin treatment, Akt2 may phosphorylate resident target proteins within the vesicle,[68] which somehow sparks the release of the Glut4 storage vesicle for translocation to the cell surface. Because Glut4 is distributed in many membranous compartments, including what appears to be a bona fide storage vesicle (see below), whether Akt2 associates with Glut4 on endosomally derived vesicles or storage vesicles is still an open question. Some evidence for the latter possibility comes from studies in which microinjection of a dominant negative Akt, targeted to Glut4 vesicles via fusion with the N terminus of Glut4, prevented the insulin-elicited translocation of IRAP (see below), a Glut4 vesicle marker protein.[69] Recently, two groups have identified putative Akt substrate proteins using an immunoprecipitation method with commercial antibodies raised against the minimal Akt consensus sequence phosphorylation site RXRXXS/T.[70] One study identified a novel adipocyte protein termed AS160, for Akt substrate with a molecular weight of 160 kDa, after a short stimulation with insulin.[71] This protein contains a Rab-GAP (GTPase activating

protein) domain and PTB (phosphotyrosine binding) domains. Although not localized on Glut4 vesicles, this protein could possibly link insulin signaling with translocation of Glut4 vesicles because the rab family of proteins are key mediators in membrane trafficking.[72] Another study using the same antibody identified ATP citrate lyase as an Akt substrate in rat adipocytes.[73] Whether either or both of these proteins are mediators of Glut4 vesicle translocation remains to be determined.

3.6. Sorting through Glut4-Containing Vesicles

A family of facilitative membrane-bound transporter proteins mediates glucose entry into cells. Insulin causes the increased uptake of glucose primarily into skeletal muscle and fat via the Glut4 isoform. This process provides intracellular glucose for several processes such as ATP, NADPH, ribose phosphate, hexosamine, and glycogen production. The fundamental importance of fully characterizing Glut4-containing vesicles is underscored by the fact that a primary defect in poorly controlled type 2 diabetes is the failure of insulin to signal proper glucose uptake by the recruitment of Glut4 to the cell surface of muscle and adipose tissue (Figure 1), and although this process is not a primary defect in type 1 diabetics, a secondary state of insulin resistance develops in type 1 diabetics with poorly controlled blood glucose levels.[74] Furthermore, different defects manifest in muscle and adipose tissues because type 2 diabetics appear to contain the full complement of Glut4 in muscle with concomitant, markedly reduced Glut4 levels in adipose, but, interestingly, both tissues appear to be defective in Glut4 recruitment to the plasma membrane. The importance of Glut4 is underscored by experiments with knockout and overexpression in mice. Whereas whole animal knockout of Glut4 was without effect on glucose homeostasis,[75] presumably due to partial compensation by other transporters, the animals were somewhat insulin resistant. Adipose- or muscle-specific ablation of Glut4 manifests as severe insulin resistance.[76,77] Furthermore, transgenic overexpression of Glut4 in skeletal muscle increases muscle glucose transport and metabolism,[78,79] ameliorates insulin resistance, and lowers plasma glucose in diabetic db/db mice.[80] Therefore, pharmacological modulation of Glut4 abundance and/or presence at the cell surface with a small molecule instead of injected insulin would be an attractive diabetes treatment option.

Therefore, although it is now well established that insulin rapidly and markedly stimulates glucose uptake by eliciting the translocation of intracellular Glut4-containing vesicles to the cell surface, there are nonetheless several fundamental questions to which answers remain elusive. What is the nature of the signal emanated from the insulin receptor that causes translocation? What, if anything, intrinsic to, or associated with, Glut4 vesicles receives insulin's signal? What is the nature of the Glut4 compartment? What other proteins reside in Glut4 vesicles, and does insulin elicit their translocation so as to modify cell surface functions? Several laboratories have undertaken the identification of Glut4 vesicle proteins. In principle, such proteins could be involved in the biogenesis, docking, and fusion of the vesicles with the plasma membrane, in signal reception from the insulin signaling pathway, in altering other cell surface functions in response to insulin, and in protein secretion of intravesicular cargo proteins, thereby broadening insulin's spectrum of action. Understanding the composition and function of Glut4 vesicle proteins could enable the development of novel treatment modalities for type 2 diabetes mellitus wherein a primary defect is insulin's inability to recruit Glut4 to plasma membrane of fat and muscle tissue for postprandial circulating glucose disposal. A translocation-specific approach would not, however, address the underlying hepatic insulin resistance that also accompanies type 2 diabetes.

Isolation of Glut4 vesicles typically involves anti-Glut4 antibodies coupled to a solid support that is mixed with low-density microsomes from either rat or 3T3-L1 adipocytes. Adhered vesicles are then washed and solubilized in nonionic detergent and their contents visualized by staining. Although numerous proteins have been identified as resident to Glut4 vesicles,[81−87] so far only one protein, the insulin responsive aminopeptidase (IRAP), has emerged as having localization and trafficking characteristics virtually identical to those of Glut4. Initially, a protein of 165 kDa was first identified in subcellular fractions and shown to be translocated to the plasma membrane in response to insulin.[88,89] Subsequently, IRAP was cloned,[90] identified in Glut4 vesicle preparations,[91] and shown to translocate to the cell surface with kinetics and characteristics similar to those of Glut4.[84] That is, like Glut4, insulin stimulation of IRAP translocation leads to an ~8-fold increase in cell surface IRAP that is completely inhibited by wortmannin. Furthermore, isolation of IRAP vesicles with anti-IRAP antibodies quantitatively depletes Glut4 vesicles and yields the same protein profiles as isolated Glut4 vesicles with anti-Glut4 antibodies, suggesting the two proteins are completely colocalized.[84] Thus, besides Glut4 itself, IRAP serves as an excellent marker for Glut4-containing vesicles and, like Glut4, insulin-elicited IRAP translocation to the cell surface is attenuated in type 2 diabetics.[92] Presently, the precise function of IRAP is unknown, although one hypothesis is that IRAP acts on circulating peptide hormones. For example, IRAP was shown to cleave angiotensin III, angiotensin IV, and Lys-bradykinin with half-saturation constants between 20 and 600 nM.[93] In addition, insulin stimulation of rat or 3T3-L1 adipocytes led to an ~3-fold stimulation of vasopressin cleavage. These data suggest a heretofore unknown role of insulin in enhancing cleavage of extracellular substrate via the translocation of IRAP. Consistent with the previous work, IRAP has recently been identified as a receptor for angiotensin IV.[94] IRAP is also translocated by contraction, suggesting it may be specific for Glut4 function but not insulin signaling.

Insulin has a general effect on membrane recycling and elicits a modest ~2-fold increase in the abundance of several proteins at the cell surface. One such protein is the transferrin receptor, the canonical

marker protein for the endosomal system.[95] Glut4 resides intracellularly in a heterogeneous population of vesicles that are either endosomal in nature or true Glut4 storage vesicles. This was elegantly demonstrated in studies utilizing transferrin (Tf) labeled with horseradish peroxidase (HRP). Treatment of 3T3-L1 adipocytes with Tf-HRP followed by the addition of hydrogen peroxide and diaminobenzidene causes any vesicles in contact with Tf-HRP to become highly cross-linked and easily removed from other intracellular vesicles by size exclusion centrifugation. Tf-HRP ablation of endosomally derived vesicles led to a 30−40% decrease in the intracellular Glut4 pool,[96] whereas the remaining complement of Glut4-containing vesicles were still fully competent to translocate to the plasma membrane in response to insulin treatment.[97] Therefore, endosomally localized Glut4 does not encompass the main insulin-sensitive storage vesicle for Glut4. Rather, there is clearly something unique about skeletal muscle and fat tissue that targets a major portion of Glut4 to intracellular storage sites. Indeed, Glut4 expressed ectopically in numerous other cell types localizes intracellularly, but it does not translocate to the cell surface in response to an insulin challenge.[98−100]

An intriguing possibility to explain the uniqueness of the muscle and adipose Glut4 storage vesicle is the presence of one or more retention proteins that hold the vesicles in stasis until insulin signals their translocation. Initial findings made in skeletal muscle demonstrated greater Glut4 immunoreactivity after insulin treatment when probing with antibody specific for the carboxy terminus was performed.[101] Subsequent studies showed that introduction of a synthetic Glut4 C-terminal peptide elicited an immediate 4.5-fold increase in plasma membrane Glut4 concomitant with a 3-fold increase in glucose uptake.[102] Similar findings were also reported in 3T3-L1 adipocytes via the introduction of a GST-fusion protein containing the IRAP cytoplasmic domain.[103] Collectively, these studies suggest the existence of an intracellular retention protein and that disruption of the interaction of this factor with Glut4 storage vesicles elicits translocation and transport. This interaction may prove to be an important therapeutic intervention point. However, although the search for such a factor has ensued for several years via myriad approaches (e.g., co-immunoprecipitation, glutathione *S*-transferase pulldowns, and yeast two-hybrid screening) identifying a number of proteins, there is as yet no consensus on a definitive protein for retention of Glut4 storage vesicles.

3.7. c-Cbl/CAP Signaling Pathway

There is a general acceptance that PI3K activation is necessary for Glut4 translocation and glucose transport, but multiple lines of evidence suggest that stimulation of PI3K is not solely sufficient to mediate the process. One or more additional pathways may be required. For example, other growth factors, such as PDGF, activate PI3K to a similar extent as insulin yet are without effect on glucose transport.[104] Furthermore, introduction into 3T3-L1 adipocytes of membrane-permeant forms of PIP_3, a product of the PI3K reaction, had no effect in increasing basal glucose transport, whereas pretreatment of adipocytes with the PI3K inhibitor wortmannin followed by addition of the PIP_3 analogue in combination with insulin did elicit maximal glucose transport.[105] These data are consistent with the presence of a second PI3K-independent pathway for stimulating glucose transport.

Recently, potential upstream mediators of this pathway have been described. Insulin stimulates the tyrosine phosphorylation of the Cbl proto-oncogene[106] that associates with the c-Cbl associating protein (CAP)[107] and flotillin in caveolae and/or lipid rafts, known plasma membrane microdomains. Caveolae are present in many cell types, are thought to be involved in the coordination of signaling events, and, like CAP and flotillin, proliferate dramatically during adipogenesis. Upon phosphorylation, the c-Cbl−CAP complex is recruited to lipid rafts, where it forms a ternary complex with flotillin. This process is blocked in adipocytes via expression of a dominant-interfering mutant of CAP.[108] In addition, CrkII associates through its SH2 domain with phosphorylated c-Cbl. CrkII is also in a constitutive complex with the guanine exchange protein C3G, such that when it comes into close proximity with the plasma membrane, it causes GDP exchange for GTP on the G protein TC10, thereby activating it.[109] Lipid raft localization of TC10 appears to be involved in its activation by insulin,[110] and this somehow initiates a second signaling pathway leading to Glut4 translocation. Recently, a TC10 interacting protein, CIP4.2 (Cdc42-interacting protein 4/2), was identified in a yeast two-hybrid screen. Insulin causes the recruitment of CIP4/2 to the plasma membrane, where it interacts with TC10, and overexpression of TC10 in adipocytes prevents this translocation event. Finally, expression of mutant forms of CIP4/2 appeared to inhibit Glut4 translocation.[111] Presently, the signaling events between TC10-CIP4/2 and the Glut4 storage vesicle are being delineated, and whether a convergence exists with the PI3K-dependent pathway remains to be determined.

4. Other Insulin Signaling Effects

Certain tissues such as the brain rely heavily on glucose as an energy source. Because food intake is intermittent, the liver supplies glucose for peripheral tissue utilization between meals, maintaining circulating glucose concentrations of ~6 mM (110 mg/dL). The liver generates glucose in two ways: release of glucose from glycogen stores (glycogenolysis) and generation of glucose de novo from smaller precursors (gluconeogenesis). The relative contributions of glycogenolysis and gluconeogenesis to the overall hepatic glucose production vary depending on physiological conditions (e.g., length of time since the last meal) and are influenced by hormones and substrate availability.

Carbohydrate intake triggers secretion of insulin from pancreatic β cells. In addition to stimulating glucose uptake into skeletal muscle and adipose tissue, insulin inhibits the hepatic output of glucose. In type 2 diabetes, the ability of insulin to suppress

hepatic glucose production is impaired (hepatic insulin resistance), and hepatic oversupply of glucose contributes to the hyperglycemic state.[2,3]

4.1. Gluconeogenesis

Gluconeogenesis provides glucose for extrahepatic tissues via synthesis from smaller three-carbon precursors such as glycerol, lactate, alanine, and pyruvate. Expression of key gluconeogenic enzymes is restricted primarily to the liver and, to a lesser extent, the kidney. The onus of this important metabolic responsibility falls chiefly on the liver and to a lesser extent the kidneys. While insulin triggers the uptake of glucose and the synthesis of glycogen (discussed below), it concomitantly inhibits gluconeogenesis and glycogenolysis. This type of regulation makes sense in that futile cycles of glucose utilization with simultaneous production are avoided. Insulin directly affects liver gluconeogenesis by inhibiting the transcription and consequently the production of key gluconeogenic enzymes, namely, phosphoenolpyruvate carboxykinase (PEPCK), the rate-limiting enzyme of gluconeogenesis, fructose-1,6-bisphosphatase (F1,6Bpase), and glucose-6-phosphatase (G6Pase), thereby dampening hepatic glucose output. Simultaneously, insulin elicits the transcription of key glycolytic enzymes such as glucokinase and pyruvate kinase, thereby ensuring glucose oxidation. The transcription factors that mediate the effects of insulin have been elusive, but recent data point to roles for certain wortmannin-inhibitable forkhead transcription factor family members that are activated via Akt phosphorylation in response to insulin[112] and also the peroxisome proliferator-activated receptor gamma (PPARγ) coactivator known as PGC-1.[113]

4.2. Glycogen Metabolism

Insulin also orchestrates the coupling of glucose uptake to glycogen synthesis. Incoming glucose is immediately phosphorylated by either hexokinase or glucokinase to glucose-6-phosphate, a major metabolic crossroad metabolite. A portion of glucose-6-phosphate is earmarked for cytosolic glycogen production via the enzyme glycogen synthase. Most of the action in glycogen synthesis occurs at the burgeoning cytosolic glycogen particle. Insulin stimulates the production of glycogen, the storage form of glucose, through activation of protein phosphatase 1 (PP1), which dephosphorylates and thereby activates glycogen synthase. Interestingly, only a subset of the total intracellular PP1 is affected by insulin and targeted to the glycogen particle via intrinsic glycogen-targeting domains within the protein. In addition, insulin inhibits the activities of protein kinase A (PKA) and glycogen synthase kinase-3 (GSK-3), thereby preventing the phosphorylation and inactivation of glycogen synthase. As is the case for glucose transport, inhibitors of both PI3K and Akt prevent insulin-stimulated glycogen accumulation. Although in skeletal muscle and adipose, inhibition of glycogen synthesis with PI3K and Akt inhibitors may be accounted for, at least in part, by the inhibition of Glut4 translocation/glucose uptake, thereby depleting substrate.

4.3. Other Insulin-Stimulated Signaling Pathways

As has been shown for other growth factors, insulin stimulates GTP for GDP exchange on Ras, thereby triggering a kinase signaling cascade consisting of Raf, the mitogen-activated protein kinase kinase (MAPKK), and mitogen-activated protein kinase (MAPK). MAPK appears to translocate to the nucleus where it phosphorylates and activates transcription factors leading to cellular proliferation or differentiation.[114] The elevation of Ras-GTP appears to result from stimulation of the guanine nucleotide release protein Sos, which is bound to either tyrosine-phosphorylated IRS-1 and/or the adaptor protein Grb2 bound to tyrosine-phosphorylated Shc.[115] SHP2, a tyrosine phosphatase involved in the activation of Ras, may also participate.[116] Blockade of this pathway in 3T3-L1 adipocytes intervening upstream or downstream via introduction of dominant negative Ras[117] or with inhibitors of MAPKK[118] prevents transcriptional effects associated with the MAPK pathway but is generally thought to be without effect (with some exceptions REF KLIP) on the metabolic actions of insulin (e.g., glucose uptake, glycogen synthesis). In addition, stimulation of 3T3-L1 or rat adipocytes with epidermal growth factor (EGF) activates the MAPK pathway (along with PI3K) to the same extent and with a similar time course as insulin but was without effect on glucose transport.[119−121] Interestingly, data from some diabetic patients suggest that MAPK pathway activation is not attenuated despite insulin resistance with respect to glucose uptake. This finding suggests that pathological mitogenic effects may ensue in patients displaying chronic hyperinsulinemia, possibly mediating some of the pathophysiological complications associated with diabetes.[122] Thus, the broad nature of insulin resistance may be restricted to the metabolic effects of insulin.

Insulin also has important roles in the regulation of fat and protein metabolism, but these effects are outside the scope of this review.

5. Animal Models of Insulin Resistance and Type 2 Diabetes

Rodents are commonly used for type 2 diabetes studies. Most antidiabeteic agents have been developed on the basis of primary efficacy in rodent models. Genetic models of insulin resistance or hyperglycemia are frequently used for studies of diabetic phenotypes. Hyperinsulinemia and hyperglycemia are often observed in these models, with possible additional phenotypes such as obesity and dyslipidemia. Animal models that are induced by environmental factors such as dietary treatment are often used for studies of certain aspects of insulin resistance. These include fructose-, high-fat-diet-, and glucosamine-induced insulin resistance. Most of these models do not develop frank diabetes but have certain aspects of the diabetic phenotype, allowing investigators to dissect in detail the biological events that are

associated with or responsible for the onset of the disease. Only models for type 2 diabetes and insulin resistance will be discussed in this section.

5.1. Genetic Models of Insulin Resistance

Type 2 diabetes involves complex interactions of metabolic and genetic defects. To understand the roles of individual genes in the pathogenesis of the disease, transgenic and knockout animals have been used to dissect gene functions in the context of either normal or environmentally challenged backgrounds. Transgenic technology is employed to alter the sites that control expression levels of functional genes, to introduce a foreign gene into an animal to change the expression of other genes, or to replace genes with variants.[123] The study of animals generated with these approaches increase the understanding of the functional roles of genes directly involved in, or that modulate, signaling pathways regulating glucose metabolism. In addition, the functions of the introduced genes and their involvement in the origination of the pathogenesis of type 2 diabetes can be revealed. Questions such as the mechanism of glucose-stimulated insulin secretion, the control of hepatic glucose production, the functional roles of genes in mediating insulin action and in vivo energy expenditure, and the development of obesity can be partially or fully addressed by studies with these models.[123]

One of the generic flaws of the transgenic technology is that the gene overexpression or underexpression may not be physiologically relevant and it is hard to draw definitive conclusions on gene functions from the phenotypic information gained from transgenic animals. Knockout technology offers a different perspective in that it links gene deficiency or deficiencies to one or more of the NIDDM phenotypes.[124] From these findings, one can identify and further determine if a particular gene is a possible inducer of insulin resistance and type 2 diabetes, assuming phenotypes are expected to reflect the physiological function of the deleted gene. Homozygous knockout allows the study of the physiological function of a single gene, whereas heterozygous knockout helps to examine the gene dosage effect.[124] By crossing animals of single knockouts, one can create multiple knockouts in the same strain to investigate the interplay of multiple genes in disease formation. Conditional knockout makes it possible to study mutations that would be lethal when made using the conventional approach.[124] Most knockout experiments have been focused on the mediators of insulin signaling to assess how they account for the development of overt diabetes.[124] Many genetically altered mouse models display little or no phenotype until animals are stressed by environmental perturbations such as a high-fat diet, which can induce or substantially accentuate a phenotype.

The roles of several genes important for insulin signaling and glucose production or utilization have been studied in knockout animals, including the insulin receptor (IR), the insulin receptor substrates (IRS-1−4), PI 3-kinase, glucose transporter 4 (Glut4), and PPARγ[124]. Global IR knockout led to severe diabetes,[124] although muscle-specific IR knockout led to metabolic syndrome, a disease with multiple phenotypes including insulin resistance.[125] These latter animals had a 95% reduction in IR content and early signaling events. They had elevated fat mass, serum triglycerides (TG), and free fatty acids (FFA), but their blood glucose, serum insulin, and glucose tolerance were normal. Surprisingly, only the β-cell-specific IR knockout results in mild diabetes.[124] On the basis of the fact that NIDDM is a polygenic disease with insulin resistance in muscle, fat, and liver in conjunction with β-cell failure, heterozygous knockouts for IR and IRS-1 in the same animal[126] have been made. Mice with global double-heterozygous alleles have 50% reduction in both proteins, yet 5−50-fold elevated plasma insulin levels, suggesting synergism of the two defects. At 4−6 months, 40% of the animals become overtly diabetic.[126] This NIDDM model arises in an age-dependent manner from the interaction of two genetic defects. Animals with heterozygous null mutations in IR, IRS-1, and IRS-2 were generated by intercrossing mice heterozygous for each mutation.[126] Tissue-specific insulin resistance was analyzed to identify the roles of the three genes in insulin signaling and the development of disease.[126] Severe hepatic insulin resistance was observed in the double knockouts IR/IRS-1 and IR/IRS-2.[126] Both of the double knockouts (IR/IRS-1 and IR/IRS-2) developed insulin resistance in skeletal muscle, but it was more severe in the IR/IRS-1 animals,[126] suggesting the more important role of IRS-1 in insulin signaling in skeletal muscle. Only 20% of the above double knockouts developed diabetes, whereas 40% of the triple knockouts (IR/IRS-1/IRS-2) did,[126] suggesting decreased functions of all three genes can result in the development of diabetes. This study further implicates the polygenic and genetically heterozygous interactions for diabetes.[127] Accumulated evidence from transgenic and knockout animals has helped to define the importance of insulin resistance in liver, pancreatic β cells, and brain, in addition to skeletal muscle.[128] Furthermore, the differential influence of genetic backgrounds on the phenotypes can be dissected using knockout animals.[129] By crossing animals harboring the same null allele but with different severity of phenotypes, investigators can map and identify the loci that exacerbate the phenotypes caused by the gene specific knockout and unravel the complex genetic interactions underlying insulin resistance.[129]

5.2. Laboratory Models for Experimental Use

Besides the genetic mouse models for understanding the functional importance of key genes in insulin signaling and glucose metabolism, there are several commonly used diabetic or insulin-resistant models. These models were developed on the basis of the naturally occurring phenotypes due to genetic mutations when the specific mutations had not yet been identified. Because these animals exhibit the typical phenotypes of obesity coupled with insulin resistance and/or overt diabetes, they are historically favored as standard laboratory testing animals for addressing scientific questions of the diabetic nature. These models have an advantage in that obesity, insulin

resistance, and diabetes develop in the first few months of life, in contrast to the situation in humans and nonhuman primates, in whom type 2 diabetes takes many years to develop.[130]

5.2.1. Ob/ob and db/db Mice

The obese (*ob*) gene is an autosomal recessive mutation that occurred in a non-inbred stock in the early 1950s and was later established and maintained in the C57BL/6J (BL/6) strain.[131,132] Mice homozygous for the *ob* mutation on chromosome 6, known as *ob/ob*, develop mild diabetes with marked obesity, hyperphagia, and transient hyperglycemia.[132,133] The wild-type *ob* gene, also known as leptin, is a 167 amino acid protein with a putative signal peptide sequence and highly expressed in adipose tissue.[134] The expression of the *ob* gene is markedly reduced in *ob/ob* mice,[134] which inhibits the leptin-mediated signaling pathway that stimulates energy expenditure.[135] The diabetes (*db*) autosomal recessive mutation occurred in the C57BL/KsJ inbred strain.[132] The mutation is in the leptin receptor on chromosome 4,[132] which is required for the leptin-mediated metabolic pathway for energy expenditure.[136,137]

Given the importance of the leptin-mediated pathway for energy consumption, both the *ob* and the *db* mutations result in reduced energy expenditure and lead to diabetes and obesity. When maintained on the same genetic background, both mutations exhibit identical syndromes from 3 weeks of age onward.[132] However, the C57BL/KsJ background appears to enhance the severity of diabetes, possibly due to genetic interactions with the leptin pathway.[132] The commonly used *ob*/*ob* strain is on the C57BL/6J background, whereas the *db*/*db* mouse is on the C57BL/KsJ background. Therefore, the *db*/*db* mouse, although displaying a degree of obesity at younger ages similar to that of the *ob/ob*, exhibits a more severe diabetic phenotype with marked hyperglycemia and hyperphagia.[132] Although both strains exhibit significant hyperinsulinemia in the first few months of life (much more than seen in environmental models of insulin resistance), the high plasma insulin level is sustained in the *ob/ob* mouse but is transient in the *db*/*db* strain with β-cell failure exemplified by hyperplasia and hypertrophy of the β cells. The *db*/*db* mice are characterized by marked glycosuria after plasma insulin levels decline and have a markedly decreased lifespan. Both animal models are used for studies of diabetes and obesity but, depending upon the need for the severity of diabetes, a choice of the two can be made. Homozygous mutants of both sexes for either *ob*/*ob* or *db*/*db* are infertile, and obese homozygous mutants are obtained by mating known heterozygotes.[132]

5.2.2. Obese Zucker Fatty (Fa/fa) Rat

Similar to the *db*/*db* mouse, the obese Zucker fatty rat harbors the *fa* autosomal recessive mutation in the leptin receptor[138−140] and is a rat obesity model. The Zucker fatty rat, homozygous for the *fa* mutation and known as *fa/fa*, develops massive obesity after weaning associated with hyperphagia, hyperinsulinemia, and hypertriglyceridemia.[138] Additional metabolic abnormalities in the Zucker fatty rat include increased fatty acid synthesis in liver and adipose tissue and high fat-storage capacity.[138] Unlike the *db*/*db* mouse, the obese Zucker fatty rat is not diabetic but has impaired glucose tolerance (IGT), mild hyperglycemia, pronounced hyperinsulinemia, and marked reduction in insulin sensitivity.[141] It is therefore widely used as a model for tests of glucose tolerance.

5.2.3. Zucker Diabetic Fatty (ZDF) Rat

The Zucker diabetic fatty (ZDF) rat harbors the same mutation in the leptin receptor as the Zucker fatty rat but, in addition, it has a defect in the pancreatic β cells that affects insulin production, which later progresses to a state of insulin deficiency.[142] The ZDF rat develops overt diabetes with severe hyperglycemia, polyuria, and polydipsia, similar to human NIDDM.[142−144] Therefore, the ZDF rat is a good type 2 diabetic model, and it has been used extensively for testing small molecule antidiabetic compounds.[143,145]

5.2.4. KK and KA^y Mouse

The inbred mouse strain KK was established in the 1960s in Japan.[146] The KK mice have inherent glucose intolerance and insulin resistance mainly in the peripheral tissues.[146] They become modestly obese with aging and further develop overt diabetes with frank hyperglycemia.[146,147] The symptoms of glucose intolerance and insulin resistance are exacerbated in KKA^y mice, a congenic strain harboring the A^y allele at the agouti locus.[148] The A^y allele facilitates the expression of the agouti peptide, which acts as an antagonist of the melanocortin-4 receptor (MC4-R), leading to maturity onset obesity.[149−151] Plasma triglyceride, total cholesterol, and free fatty acids are elevated in KK mice.[147,148] No specific gene responsible for the diabetic phenotype has been identified, but quantitative trait loci that regulate plasma lipid concentrations and suggestive loci for glucose intolerance have been revealed.[148,152] The KK mouse has been used as a model for studies of progressive obesity and complications associated with diabetes.[153]

5.2.5. Obese Rhesus Monkey

Rhesus monkeys develop obesity and insulin resistance by aging when allowed free access to chow. As is the case with humans, a subset of monkeys progresses to frank diabetes with advancing age. The insulin resistance is partly due to defective glucose uptake caused by dysfunctional insulin activation of protein kinase C in skeletal muscle.[154] The spontaneous development of obesity and insulin resistance in rhesus monkeys make them an attractive model for examining the sequence of metabolic changes associated with the development and onset of diabetes.[155,156] In addition, these animals have increased plasma triglyceride, increased very low-density lipoprotein (VLDL), deceased high-density lipoprotein (HDL), hypertension, and hyperinsulinemia, but they are normoglycemic in the prediabetic state.[155,156] These

symptoms resemble the human metabolic syndrome X that eventually progresses in some individuals to overt type 2 diabetes.[157] More importantly, the rhesus monkey is a good model to study the natural history of the development of type 2 diabetes from which a better understanding of the human disease progression can be gained. Eight phases in the progression from normal lean young adult to overt type 2 diabetes have been defined in these animals, and the biological changes concomitant with the disease progression have been revealed.[158] The contribution of obesity and β-cell dysfunction to the onset of the disease has been delineated using this model.[159,160] The rhesus monkey is a good primate model for syndrome X and suitable for studies of insulin resistance and dyslipidemia, especially for evaluation of leading small molecule drug candidates refined through screening of compounds in rodent models.[161,162]

5.3. Environment-Induced Insulin Resistance Models

Accumulated evidence has linked the development of insulin resistance to environmental, nongenetic factors. These factors include dietary treatments that affect insulin sensitivity or pathways involved in insulin sensitivity. On the basis of these understandings or hypotheses, several rodent models have been developed to address specific questions in insulin resistance and the development of diabetes. However, it is important to note that these models, although with insulin resistance, have their own limitations because they resemble only a fraction of the symptoms in prediabetic or diabetic state.

5.3.1. Glucosamine-Induced Insulin Resistance Model

Hyperglycemia is known to induce insulin resistance in vivo. Numerous in vitro studies have demonstrated that high concentrations of glucose impair insulin-stimulated glucose transport in rat adipocytes.[163] Additional studies suggested that an increased flux through the hexosamine biosynthetic pathway might be the mechanism by which hyperglycemia causes insulin resistance.[164,165] Excess hexosamine flux has been shown to cause insulin resistance in cultured cells.[165] Overexpression of glutamine:fructose-6-phosphate amidotransferase (GFAT), the first and rate-limiting enzyme in the hexosamine biosynthetic pathway, led to insulin resistance.[166,167] The increased flux through the hexosamine pathway produces increased UDP-*N*-acetylglucosamine, which serves as a substrate in the formation of glycoproteins and proteoglycans. On the basis of these findings, it was hypothesized that increasing the flux through the hexosamine biosynthetic pathway can generate an insulin resistant animal model.[168] Because glucosamine enters the pathway downstream of the rate-limiting step and mediates insulin desensitization,[164] it was used to test the hypothesis in vivo and confirmed that it can induce insulin resistance in skeletal muscle of normoglycemic rats.[168,169] Exposure of rats to hyperglycemia or glucosamine in vivo results in accumulation of hexosamine pathway end products in insulin sensitive tissues with a time course that precedes the onset of insulin resistance.[168,170,171] This model can be used to test small molecule insulin sensitizers.[172]

5.3.2. Fructose-Induced Insulin Resistance Model

The effects of fructose feeding are exerted on the liver. Golden Syrian hamsters fed diets containing 60% fructose or sucrose develop obesity and glucose intolerance.[173] Fructose feeding also increases fasting plasma nonesterified fatty acids (NEFA), plasma, and liver triglycerides.[173] Because it takes only two weeks to feed the animals with a high-fructose diet to induce insulin resistance, fructose feeding is a convenient way to produce insulin resistance in vivo. The insulin resistance induced by fructose feeding is reportedly due to the diminished ability of insulin to suppress hepatic glucose output but not a decreased insulin-stimulated glucose uptake by the muscle,[174] suggesting the phenotype is characterized primarily by hepatic insulin resistance. Thus, this model has limited value for testing drug candidates that act primarily on adipose tissue or skeletal muscle. Rats fed >60% fructose for the same period also develop insulin resistance with hyperinsulinemia, hypertriglyceridemia, and, interestingly, hypertension,[175] but the fructose-induced hypertension is not associated with the hyperinsulinemia and hypertriglyceridemia.[175] Both the hamster and the rat models have been utilized to test antidiabetic small molecules improving hepatic insulin sensitivity.[176,177]

5.3.3. High-Fat-Fed (HFF) Insulin Resistance Model

Rats fed a high fat diet (60% of calories as fat) develop insulin resistance with reduced basal glucose metabolism.[178] The insulin resistance is exemplified by >50% reduction in net whole-body glucose utilization at physiological insulin levels and the failure to suppress liver glucose output.[179] The major suppressive effects on glucose transport are in oxidative skeletal muscle and brown adipose tissue (BAT), suggesting these tissues contribute mainly to the overall insulin resistance.[179] The HFF model is suitable for studies of mild insulin resistance because it is closer to normal animals than diabetic animals. If caloric intake is carefully controlled to avoid obesity, this model does not exhibit hyperglycemia even after several weeks on the diet.[178,179] Insulin resistance develops within a few weeks, with associated hyperinsulinemia and impaired glucose tolerance, but the development of frank hyperglycemia takes much more time. High-fat-fed mice also develop insulin resistance with glucose intolerance.[180,181] The susceptibility to develop obesity and diabetes varies among different rodent strains.[182,183] High-fat diets have been widely used to study processes involved in the development of insulin resistance, in screening candidate antiobesity and antidiabetic drugs, and as an environmental stressor to probe the roles of specific genes in knockout and transgenic mice. For example, the HFF rat model is widely used to test the antidiabetic thiazolidinediones.[184,185]

The mechanism of dietary fat-induced insulin resistance is not clear. One hypothesis is that the fat-induced insulin resistance is acquired by increasing the flux of the hexosamine biosynthetic pathwah, but

this was demonstrated at only maximally effective insulin levels.[170] At physiological insulin levels, the flux through the hexosamine biosynthetic pathway is not affected by high-fat diet or increased plasma free fatty acids.[186] Recent findings suggest that high-fat feeding impairs glucose uptake in the peripheral tissues.[187] A number of additional putative mechanisms have been identified, such as operation of the glucose−fatty acid cycle,[188] increased glucocorticoid action,[189] activation of the protein kinase C[190] and/or NF-kappaB pathway,[191] shunting of glucose into the hexosamine biosynthetic pathway,[170] modulation of the expression of adipocytokines such as TNF,[192] resistin,[193] and adiponectin,[194−197] or other mechanisms secondary to oversupply of free fatty acids to insulin's target tissues.

5.3.4. TNFα-Induced Insulin Resistance in Rats

The mechanisms by which obesity causes insulin resistance have not been conclusively demonstrated. One hypothesis is that the increased flux of substrates into adipose tissue results in elevated levels of secreted factors that may induce insulin resistance.[192] Consistent with this hypothesis, increased TNFα mRNA and protein levels have been observed in rodent models of obesity and diabetes.[192] Evidence from the molecular level indicates that TNFα inhibits the signaling events mediated by the insulin receptor.[198,199] In vivo studies demonstrated that TNFα caused insulin resistance,[200,201] and neutralization of TNFα in obese *fa/fa* rats significantly improved insulin sensitivity in peripheral tissues.[192]

On the basis of these findings, TNFα infusion has been used to create insulin resistance in rats. In this model, rats are infused with a high level of TNFα for 4−5 days.[202] The infused animals have higher basal plasma insulin and free fatty acids and develop peripheral insulin resistance.[202] This model has been used to test small molecule compounds with insulin-sensitizing activities.[202] Although it is not entirely clear how TNFα induces insulin resistance, accumulating evidence suggests that the TNFα effect is mediated by serine phosphorylation of IRS-1, which inhibits its tyrosine phosphorylation and activation and insulin signaling.[203] In addition, TNFα also inhibits the phosphorylation of Akt, a downstream kinase in the insulin signaling pathway.

6. Current Treatments for Diabetes

6.1. Introduction

Dietary modifications and exercise are the first line of treatment for type 2 diabetics. Hypocaloric diets to induce weight loss result in lowering of plasma glucose, in some cases normalizing blood sugar levels.[204,205] The mechanism for improved glucose homeostasis following weight loss includes amelioration of hepatic and peripheral insulin resistance.[206] A review of the weight loss literature is beyond the scope of this review; readers are referred to previous review articles for more information.[207,208]

Weight loss and exercise enhance insulin sensitivity and glucose utilization and improve lipid and lipoprotein profiles, but in many patients compliance is a significant problem.[209] If these interventions fail to adequately control blood sugar levels, oral medication is prescribed. There are mainly five categories of orally active antidiabetic drugs on the market: metformin, a biguanide for type 2 diabetes; thiazolidinediones, including pioglitazone and rosiglitazone, peroxisome proliferator-activated receptor gamma (PPARγ) activators; the α-glucosidase inhibitors that delay intestinal carbohydrate absorption and blunt postprandial glucose excursions; and sulfonylureas (SU) and non-sulfonylurea (non-SU) insulin secretagogues that stimulate insulin secretion by pancreatic β cells. With increasing severity of diabetes, insulin administration is prescribed; many patients progress to insulin therapy with time. Combination therapy for the treatment of type 2 diabetes has become quite prevalent. Clinically, the effectiveness of therapy is monitored by measurement of fasting blood glucose concentrations and levels of glycosylated hemoglobin; the former measure can be influenced by acute changes in glucose homeostasis, whereas the latter measure provides a surrogate of longer term glucose control.

6.2. Biguanides

Biguanides, including phenformin and metformin, were introduced in 1957 as oral antidiabetic drugs.[210] Phenformin was later withdrawn in many countries due to its side effect of lactic acidosis. Metformin is now a widely used biguanide for the therapy of type 2 diabetes. Metformin lowers blood glucose level without causing overt hypoglycemia or stimulating insulin secretion. In addition, metformin is weight neutral or causes weight loss rather weight gain[210] and has beneficial effects on plasma lipid profiles.

The beneficial effects of metformin are supported by a large number of clinical studies. In a study with 10 obese NIDDM patients who received 2660 mg of metformin daily for 12 weeks, significant reductions of hemoglobin A_{1c} (HbA_{1c}) and fasting plasma glucose concentrations were achieved.[211] The rate of conversion of lactate to glucose decreased by 37%, suggesting that metformin likely lowers glucose levels by inhibiting gluconeogenesis. In addition, the treated subjects experienced weight loss. In the landmark United Kingdom Prospective Diabetes Study (UKPDS) trial in type 2 diabetics, metformin was found to have a greater effect on any diabetes-related endpoint than insulin intensive therapy or sulfonylureas.[212] The mechanism by which metformin lowers hepatic glucose production was further investigated in poorly controlled type 2 diabetic patients with ^{13}C nuclear magnetic resonance (NMR) combined with ingested $^{2}H_2O$ to measure gluconeogenesis and glycogenolysis.[213] The study demonstrated that the elevated rates of endogenous glucose production in diabetics were reduced following metformin therapy. Metformin lowered the rate of glucose production by suppressing the gluconeogenic pathway[213] and by reducing the overall rate of glycogenolysis. These data are consistent with the previous report on the inhibitory effect of metformin on gluconeogenesis.[211] The direct effect of metformin on peripheral glucose transport was examined with skeletal muscle isolated

from NIDDM patients and normal subjects.[214] Metformin potentiated insulin-stimulated glucose transport in insulin-resistant human skeletal muscle.[211] The concentration of metformin needed for this effect is higher than the therapeutic concentration administered to patients. Therefore, the issue of whether metformin directly activates skeletal muscle glucose uptake at clinically relevant doses has not been resolved. In addition to the hypoglycemic effects that require the presence of insulin, metformin has insulin-independent effects on fatty acid oxidation, reducing the energy supply for gluconeogenesis and correcting hypertriglyceridemia.[215] Some studies have also reported that metformin therapy also enhances glucose uptake into skeletal muscle in vivo; it is not clear whether this is a direct effect of metformin or whether this effect is secondary to reduction of hyperglycemia, which is known to improve insulin action.

The main side effect of metformin monotherapy is gastrointestinal (GI) symptoms. At 2550 mg daily dosage, patients experienced abdominal discomfort, bloating, and metallic taste.[211] The GI side effects are the primary reason for discontinuation but can be lessened by gradual dose titration and administration with food. Although there is a risk of lactic acidosis with metformin therapy, the incidence rate is extremely low. The drug is contraindicated in patients with increased risks of lactic acidosis (such as those with renal impairment), congestive heart failure, and hepatic dysfunction. In light of efficacy and safety, a multiple dose-ranging study was conducted in a 14-week double blind and placebo-controlled trial.[216] A total of 451 patients participated in the study, receiving metformin at 500, 1000, 1500, 2000, and 2500 mg daily. At week 7, between-group differences were significant for fasting glucose and HbA_{1c}.[216] These data suggest that metformin can lower plasma glucose and HbA_{1c} in a dose-dependent manner and benefits can be achieved with as low as 500 mg daily. This leaves a relatively large dosing range to minimize side effects while maintaining efficacy. For patients whose diabetes is not adequately treated by metformin alone, the drug can be combined with sulfonylureas or insulin.

A number of hypotheses have been proposed to explain the beneficial effects on metformin. Perhaps the most compelling explanation is provided in a recent study by Zhou et al., which examined the molecular mechanism through which metformin exerts its antidiabetic effects.[217] These investigators found that metformin activates AMP-activated protein kinase (AMPK) in primary hepatocytes but has no direct effect on the partially purified enzyme in an in vitro kinase assay.[217] This finding suggests that metformin indirectly activates AMPK by acting on a target other than AMPK itself. After AMPK activation, a variety of downstream biochemical changes occur, including the down-regulation of SREBP1 expression and consequently decreased hepatic fatty acid synthesis. In addition, VLDL synthesis decreases as a result of reduced acetyl-CoA carboxylase (ACC) activity. In the meantime, AMPK activation results in the suppression of hepatic glucose production and increased glucose uptake in skeletal muscle.[217] These effects are similar to those following treatment of tissues with AICAR, a nonspecific AMPK activator.[218] The identification of AMPK as the mechanism for metformin raised the possibility of selecting the AMPK pathway as a target for type 2 diabetes, but it should be noted that increased AMPK activity can lead to the development aberrancy of cardiac conduction.[218] Furthermore, AMPK is believed to regulate activity of the CFTR.[218]

6.3. Thiazolidinediones (TZDs)

TZDs are a class of antidiabetic compounds that are derived from the antilipidemic and weakly anti-hyperglycemic clofibrate.[219] TZDs have increased anti-hyperglycemic activity while maintaining some antilipidemic effects. TZDs reduce insulin resistance, increase insulin-stimulated glucose disposal, and improve glycemic control by increasing peripheral glucose uptake and suppressing hepatic glucose production. TZDs are ligands of PPARγ, a master gene controlling the expression of genes involved in adipocyte differentiation and lipoprotein metabolism. Through activation of PPARγ, TZDs induce adipocyte differentiation and improve glucose utilization and sensitivity in adipose tissue. Because PPARγ is expressed at low levels in skeletal muscle and not expressed in hepatocytes, TZDs are believed to improve insulin sensitivity in skeletal muscle indirectly through actions on adipocytes. Among the factors secreted from differentiated adipocytes, adiponectin has been shown to suppress hepatic glucose production and stimulate glucose uptake in skeletal muscle.[220] Other adipocyte-secreted proteins regulated by TZDs include resistin and leptin.[193,221] Therefore, the key effect of TZDs appears to be mediated by adipose tissue, which is increasingly believed to be an endocrine organ. Like metformin, TZDs do not induce insulin secretion or cause hypoglycemia.

The clinical efficacy of the TZD troglitazone was evaluated in a 6-month, randomized, double-blind, placebo-controlled study in 24 hospitals and outpatient clinics in the United States and Canada.[222] A total of 402 patients with type 2 diabetes participated in this study in which troglitazone at doses of 100, 200, 400, and 600 mg or placebo were given to the patients daily.[222] In the treatment groups of 400 and 600 mg, troglitazone significantly decreased fasting serum glucose and HbA_{1c}. In addition, significant reductions in plasma triglycerides and free fatty acids were observed in these groups. In the 600 mg group, increased HDL was significant. This study suggests that troglitazone is an efficacious therapy for type 2 diabetes. However, after its approval in 1997, troglitazone was withdrawn due to a rare but clinically serious incidence of hepatic toxicity.[223] The mechanism of troglitazone-induced hepatoxicity is not clear but it is likely related to inflammation via an immunological mechanism.[223] The toxicity does not appear to be associated with the other TZDs approved later, pioglitazone and rosiglitazone, both of which proved to be efficacious in clinical trials in lowering fasting plasma glucose, HbA_{1c}, and triglycerides and increasing HDL.[224−228]

The main side effects of TZDs are weight gain and edema. Weight gain is associated with their ability to stimulate adipocyte differentiation that results in the increased synthesis of fat in patients treated with the drug. The mechanism of edema is not clear. TZDs are indicated as monotherapy or in combination with metformin and SUs. Pioglitazone is also used in combination with insulin.

6.4. α-Glucosidase Inhibitors

The α-glucosidase inhibitors (AGIs) are a class of nonsystemic drugs that do not target specific pathophysiological defects in type 2 diabetes. The enzyme is located in the brush border of the small intestine and is required for the final step in the breakdown of carbohydrates such as starches, dextrins, and maltose to absorbable monosaccharides.[229] As inhibitors of this enzyme, the α-glucosidase inhibitors delay but do not prevent the absorption of ingested carbohydrates and reduce the postprandial insulin and glucose peaks.[229] Patients with type 2 diabetes demonstrate sluggish or unmatched insulin response following a meal (glucose load). Delaying glucose absorption helps to match the pancreatic insulin response and reduce postprandial hyperglycemia, which has been associated with cardiovascular mortality.[230] However, the long-term effects of these compounds on chronic complications have not been examined.

The long-term efficacy of acarbose, a potent competitive α-glucosidase inhibitor, was investigated in a 1-year randomized, double-blind, and placebo-controlled study involving 354 type 2 diabetic patients.[231] The treatment of acarbose or placebo was on four different regimens: diet alone, diet and metformin, diet and sulfonylurea, and diet and insulin. Acarbose significantly improved glycemic control regardless of the treatment regimen used. Acarbose clearly blunted the rapid increase in plasma glucose following a meal with carbohydrates. Although the lowering of HbA_{1c} is significant, acarbose is less effective than metformin and TZDs.[216,226,231] This is consistent with another clinical study with multiple doses of acarbose.[232] A second α-glucosidase inhibitor, miglitol, was examined in a trial with African-American patients with type 2 diabetes.[233] Significantly lower HbA_{1c}, postprandial insulin and glucose levels were achieved.

The adverse effects of α-glucosidase inhibitors are mainly GI symptoms such as abdominal pain, flatulence, and diarrhea.[231,232] This is due to the passing of the unabsorbed carbohydrates into the large intestine where an osmotic fluid retraction occurs and where GI flora metabolize the sugars to produce gaseous waste products. Starting with a low dose and slowly increasing the dosage can significantly reduce the GI side effects. An advantage of the α-glucosidase inhibitors is that they are not associated with hypoglycemia and weight gain.

6.5. Effect of Exercise on Disease

Physical inactivity is an important risk factor for the development of obesity, insulin resistance, and type 2 diabetes.[234] Indeed, some researchers have argued that the explosion of obesity, cardiovascular disease, and diabetes in modern society reflects a disease of exercise deficiency.[235] Exercise, particularly endurance exercise, enhances glucose metabolism in normal, insulin resistant, and diabetic individuals. The beneficial effects of endurance exercise include weight loss, increased capacity to generate energy aerobically, improved insulin action and glucose homeostasis, improved plasma lipid profiles (e.g., lowered triglycerides, increased HDLc), and improved cardiac function. Thus, exercise positively impacts a wide range of factors that are dysregulated in type 2 diabetes and, along with dietary modification, is the first treatment for newly diagnosed diabetics. In the United States and many industrialized countries, support systems for initiation and maintenance of exercise programs are minimal, compliance is inadequate, and many patients fail to achieve adequate control of their diabetes through lifestyle changes and progress to oral medications. Yet numerous studies have shown that exercise programs can impart substantial benefits when compliance is adequate.

Exercise enhances skeletal muscle glucose metabolism in at least three different ways.[236] Muscle contractions activate an insulin-independent signaling pathway that enhances glucose uptake during and for some time after a bout of exercise. In addition, a single bout of exercise enhances the ability of insulin and other stimuli to activate glucose transport and glycogen storage, an effect that can persist for a prolonged period after cessation of exercise. When exercise is performed on a regular basis (i.e., exercise training), skeletal muscle undergoes an adaptive response, characterized by increased gene expression, that further enhances the activation of glucose transport by insulin and other stimuli. These latter two effects of exercise result in a more sustained enhancement of glucose metabolism and are critical components of the effect of exercise to improve glucose homeostasis in diabetes.

Potential signaling mechanisms by which contractile activity acutely stimulates the insulin-independent glucose transport process include the rise in intracellular calcium, AMPK, protein kinase C, and nitric oxide.[237] AMPK is activated by exercise and in situ or in vitro muscle contractions.[238,239] AMPK activation is believed to mediate the insulin-independent effect of exercise to stimulate Glut4 translocation and glucose uptake.[240,241] Incubation of skeletal muscle with the AMPK activator AICAR results in the stimulation of glucose transport in a process that is not blocked by PI3K inhibitors.[242] Activation of AMPK independent of insulin stimulation in rats resulted in the increased skeletal muscle glucose uptake that was associated with an increase in the translocation of Glut4.[243] The downstream mechanism by which AMPK acutely stimulates glucose transport has not been conclusively demonstrated, although some data point to a potential role of phosphorylation of the endothelial isotype of nitric oxide synthase,[244,245] and others suggest the potential activation of p38 kinase[246] or ERK/Pyk2/atypical PKC.[247]

Skeletal muscle insulin resistance in obesity and type 2 diabetes does not result from a decrease in the total tissue pool of glucose transporters, but rather from a failure of insulin to recruit an adequate number of Glut4 transporters to the cell surface[248−251] (see also section 3.6). In both liver and muscles of Zucker fatty rats, the expression of both IRS-1 and IRS-2 is down-regulated, resulting in the decreased tyrosine phosphorylation and drastically decreased PI3K activities.[252] Studies in rat skeletal muscle from animal models of diabetes[248] as well as from type 2 diabetics[253] demonstrate that the ability of contractile activity to stimulate Glut4 translocation and the glucose transport process is not substantially impaired in insulin-resistant muscle. Contractile activity activates the α2 isoform of AMPK in muscle from diabetic animals[254] and humans.[241] Furthermore, insulin-resistant animal models and subjects respond to exercise training in a similar fashion as insulin-sensitive individuals, that is, with an enhancement of insulin signaling, an expansion of the Glut4 cellular pool, and increased insulin-mediated appearance of Glut4 on the cell surface.[255]

The effect of a single bout of exercise to enhance insulin action occurs rapidly and is independent of the synthesis of Glut4 or insulin signaling proteins.[255] This effect is not specific for insulin-mediated glucose transport; after exercise, the ability of other stimuli such as IGF-1, contractile activity, and hypoxia is enhanced.[256,257] Presumably this reflects an effect of exercise to augment downstream events where the signaling pathways for these different stimuli converge, for example, Glut4 translocation. The ability of exercise to enhance this process appears to require a permissive humoral factor. Contraction of isolated muscles in vitro activates the insulin-independent transport pathway but does not enhance insulin sensitivity, whereas exercise and in situ muscle contractions activate the insulin-independent pathway and also enhance insulin action;[256] when in vitro contractions are performed in the presence of serum from sedentary animals, insulin sensitivity is enhanced.[258] A single bout of exercise increases insulin action in normal and diabetic subjects.[255]

When exercise is performed on a repeated basis (training), skeletal muscle adapts to the chronic increase in energy demand by increasing the expression of key enzymes needed for glucose (and fat) uptake and glucose (and fat) metabolism. Levels of hexokinase II and Glut4 are increased in muscles actively involved in exercise, but not in nonexercising muscles.[236,255,259] Both the transcription and translation of Glut4 are increased in response to training.[260] Thus, more Glut4 is available for translocation in response to various stimuli, and the capacity of muscle to utilize incoming glucose is enhanced. Insulin signaling is also enhanced in the trained state. For example, after training, insulin-induced phosphorylation of IRS-1 and IRS-2, the amount of activated PI3K, and Akt-1 all increased in skeletal muscle of male rats.[261] These effects of exercise have been demonstrated in nondiabetic and diabetic individuals as well as nondiabetic and diabetic animal models. Training also enhances Glut4 expression and glucose uptake into adipose tissue[262] and enhances the ability of insulin to inhibit hepatic glucose production.[263]

It was previously thought that prolonged periods of training were required to stimulate the adaptive increases in skeletal muscle Glut 4 levels. Studies over the past decade have revealed that Glut4 expression and glucose transport capacity can be rapidly up-regulated given a sufficiently intense training stimulus. In rodents one 6-h intense bout of exercise up-regulated Glut4 to the same extent as a 3-month progressive training program.[264] These findings have been extended to human subjects, although the time for maximal adaptation takes somewhat longer than in rodents, who have higher metabolic rates and can undergo more strenuous exercise bouts. The increases in skeletal muscle Glut4 levels following 7−10 days of exercise were similar to those reported in many studies in which subjects had trained for several months.[265,266] The converse of this finding is that reversal of training-induced increases in Glut 4 can occur fairly rapidly, so exercise needs to be performed regularly in order to maximize its antidiabetic effects (but note that training induces additional effects on glucose metabolism when accompanied by weight loss).

In addition to glucose transport, exercise has a beneficial effect on fatty acid uptake. Fatty acid uptake into skeletal muscle is mediated by the translocation of the fatty acid translocase, CD36, from intracellular compartments to the plasma membrane, a process similar to that of glucose uptake by Glut4. The translocation of CD36 is triggered by muscle contraction and insulin. Although whether CD36 is subject to recycling like Glut4 is unknown, the increased muscle fatty acid uptake during exercise could be associated with its increased translocation.[267] The expression of CD36, as well as other proteins involved in lipid metabolism, was increased by exercise training.[268] These data help to explain the long-known finding that exercise increases the transport and β oxidation of fatty acids in skeletal muscle.[269]

The potential of exercise for disease prevention has been assessed in two large clinical trials.[270,271] The Da Qing Impaired Glucose Tolerance (IGT) and Diabetes Study demonstrated that exercise significantly reduced the incidence of diabetes among subjects with IGT.[270] The Finnish Diabetes Prevention Study further demonstrated that exercise lowered cardiovascular risks.[271] More U.S. trials are ongoing to examine whether intensive lifestyle intervention can prevent or delay the development of type 2 diabetes among individuals with IGT.[272]

7. Diabetes Clinical Trials

7.1. Clinical Trials for Diabetes Treatment

Given the large number of clinical trials that have been conducted, only the proof-of-concept trials for the effects of intensive glycemic control or specific drug therapy on the progression or the prevention of diabetic macrovascular and microvascular complications are reviewed here.

7.2. Glycemic Control with Intensive Insulin Therapy

Both type 1 and type 2 diabetes are characterized by long-term microvascular complications such as retinopathy, neuropathy, and nephropathy as well as macrovascular complications such as coronary heart disease and cerebrovascular disease. A number of landmark diabetic clinical trials were designed to dissect the relationship of chronic levels of glycemia on the incidence and progression of diabetic complications. A key question is whether reduction of hyperglycemia per se can prevent or delay the onset and progression of these microvascular complications.

A small-scale clinical trial in IDDM patients was designed to evaluate whether improved glycemic control by means of intensive insulin treatment lessens the incidence of diabetic complications, as compared to standard therapy. A total of 102 IDDM patients were randomly assigned to intensified insulin treatment (three injections a day with physician education) or standard insulin treatment (two injections per day with standard hospital visits).[273] The patients were evaluated for microvascular complications after periods of 1.5, 3, 5, and 7.5 years.[273] In the intensified insulin therapy group, the patients had significantly lower rates of serious retinopathy and nephropathy than those in the placebo group. This study suggested that long-term intensified insulin treatment of IDDM patients retards the development of microvascular complications. This conclusion is further supported by data from the larger scale landmark Diabetes Control and Complications Trial (DCCT), in which 1441 IDDM patients were included for intensive insulin therapy and the microvascular complications were monitored for 6.5 years.[274] Compared to standard therapy, the intensive treatment reduced the risk for the development of and slowed the progression of retinopathy. In addition, the intensive therapy reduced the occurrence of microalbuminuria. Overall, the DCCT trial demonstrated that intensive insulin therapy delays the onset and slows the progression of diabetic retinopathy, neuropathy, and nephropathy in IDDM patients. The reduction of the risks for microvascular complications persisted in the 4-year follow-up study after the end of the 6.5-year DCCT trial.[275]

The Kumamoto trial evaluated the importance of glycemic control in type 2 diabetes patients; 110 Japanese NIDDM patients were assigned to either intensive insulin treatment or conventional (standard) insulin treatment.[276] The onset and progression of diabetic complications were significantly slowed in the intensive treatment group, suggesting that the reduction and control of hyperglycemia in type 2 diabetic patients has an important impact on diabetic microvascular complications.[276] These data are supported by the findings from the UKPDS trial, in which 3867 NIDDM patients were enrolled in a study of intensive blood glucose control by means of sulfonylurea or insulin treatment compared to standard glucose control achieved with conventional treatment.[277] The patients were followed up for over 10 years. Compared with the conventional treatment group, the intensive treatment group had an 11% additional reduction of HbA_{1c}), 10% reduction in diabetes-related death, and 25% risk reduction in microvascular endpoints. No difference in the microvascular endpoints between the insulin and the sulfonylurea groups was observed. Interestingly, the risk of macrovascular disease was not changed in the intensively treated group, suggesting the macrovascular risks are independent of hyperglycemia and other forms of therapies such as controlling blood pressure and lowering plasma lipoproteins are needed. The UKPDS trial reinforces the notion that blood glucose control with either insulin or sulfonylureas substantially reduces the risk of microvascular complications. However, as found in other studies, the intensive insulin treatment was associated with a higher risk of hypoglycemia.[274,277] This risk was not significant in the Kumamoto trial, possibly due to the lower dosage used in this trial and subsequent dosage adjustment based on the degrees of glycemic control.[276] This suggests that the risk of hypoglycemia can be minimized by flexible dosing strategy in the intensive treatment. The UKPDS trial also compared the effect of metformin with those of insulin and sulfonylureas.[278] The data demonstrate that metformin is more effective than sulfonylurea or insulin for any diabetes-related endpoints. Because metformin is associated with less weight gain and a lower risk of hypoglycemia than insulin or sulfonylurea, it is considered a more preferred therapy for type 2 diabetic patients.[278] Overall, the UKPDS trial strongly suggests that intensive therapy of glycemic control in type 2 diabetes is beneficial.[279] This is consistent with findings from the Skaraborg Hypertension and Diabetes Project that poor glucose control is one of the major factors that predict mortality in type 2 diabetes.[280] Although in some patients the control of hyperglycemia in type 2 diabetes can be achieved with insulin or other oral agents alone, combination of several agents is recommended if treatment goals are not achieved within several months.[281] In practice, the use of combination therapy has expanded significantly in recent years as multiple antidiabetic therapies with complementary mechanisms of action have become available.

7.3. Prevention of Macrovascular Complications

Correction of hyperglycemia helps to prevent diabetic microvascular complications but has only modest effect on macrovascular complications, including coronary heart disease, peripheral arterial disease, and cerebrovascular disease. Because atherosclerosis is largely responsible for mortality in patients with type 2 diabetes, medical treatments to reduce cardiovascular risks are important in disease management. Medical treatments of dyslipidemia and hypertension, common morbidities occurring in type 2 diabetics, are two main approaches to lower macrovascular complications.[282] Patients with type 2 diabetes are characterized by abnormalities in their lipoprotein profiles, such as hypertriglyceridemia, low levels of HDL, and increased levels of small dense low-density lipoproteins (LDL). VLDL production in the liver tends to be elevated as a result of increased plasma free fatty acid concentrations.[282] The Diabetes Ath-

erosclerosis Intervention Study (DAIS) was designed to examine the effect of correction of lipoprotein abnormalities on coronary artery disease.[283] In this study, 418 patients were randomly assigned to fenofibrate or placebo treatment for at least 3 years. At the end of the study, the fenofibrate group had a significantly smaller increase in percentage diameter stenosis than the placebo group, suggesting that fenofibrate (an agent that reduces triglyceride levels and results in a modest increase in HDL) treatment reduces the angiographic progression of coronary artery disease in patients with type 2 diabetes.[283] Atorvastatin is an inhibitor of HMG-CoA reductase that substantially lowers LDL cholesterol and triglyceride levels and modestly increases HDL cholesterol. Aggressive lipid lowering was also tested in the Diabetes Atorvastatin Lipid Intervention (DALI) trial. Although significant lipid control was achieved, the endothelial dysfunction characteristic of diabetes was not reversed.[284] Additional lipid control trials in type 2 diabetes are in progress. The Lipids in Diabetes Study (LDS) was designed to examine the effects of combination therapy using a statin and a fibrate in ~5500 men and women without clinical evidence of cardiovascular disease.[285]

Control of hypertension is another important intervention that limits morbidity and mortality more effectively than tight glycemic control.[286] Several classes of orally active anti-hypertensive agents have been evaluated in patients with type 2 diabetes. Both an angiotensin-converting enzyme (ACE) inhibitor (captopril) and a calcium channel β blocker (atenolol) were examined in a multicenter, randomized, and placebo-controlled trial embedded within the UKPDS study.[287,288] In this study, 1148 hypertensive patients with type 2 diabetes were assigned to either tight or less tight control of blood pressure with a median follow up of 8.4 years. The group with tight control of blood pressure showed a 24% reduction in diabetes-related endpoints. In addition, there was a 32% reduction in the risk of deaths related to diabetes, 44% risk reduction in strokes, and 37% reduction in microvascular complications.[287] These data demonstrate that tight control of blood pressure in type 2 diabetes significantly reduces the risks of both macrovascular and microvascular complications.[287] Furthermore, the study indicates that lowering blood pressure with captopril and atenolol had similar effects in reducing the diabetic complications and, therefore, the type of treatment used is not as important as blood pressure reduction itself.[288]

7.4. Other Interventions To Reduce Microvascular Complications

7.4.1. Retinopathy

The Diabetes Retinopathy Study (DRS) investigated the effect of laser therapy (photocoagulation), which is a major means to delay the progression of retinopathy, in both type 1 and type 2 diabetic patients with proliferative or severe proliferative retinopathy.[289] The study randomly assigned patients to receive pan-retinal argon or xenon arc laser therapy to the eyes or no therapy. Over the 2-year follow-up period, the laser treatment resulted in a 50% reduction in severe vision loss, although there were side effects such as decreased peripheral fields and night vision. This study implicates the importance of photocoagulation treatment in delaying the development of diabetic retinopathy.[289] The Early Treatment Diabetic Retinopathy Study (ETDRS) used a similar study design but also investigated the benefit of aspirin therapy. The study assigned one eye to either focal or scatter photocoagulation, depending upon the presence of early diabetic complication (i.e., macular edema) and the other eye as control. The study found that focal photocoagulation is more beneficial in the treatment of macular edema.[290] The study also concluded that aspirin did not have any beneficial effect.[290]

7.4.2. Nephropathy

Diabetic nephropathy remains another leading microvascular complication in both type 1 and type 2 diabetic patients. Diabetes is responsible for ~40% of all patients beginning renal replacement therapy.[291] Diabetic nephropathy is associated with increased risks of cardiovascular morbidity and mortality. Given that anti-hypertensive therapies significantly reduce cardiovascular events,[291] ACE inhibitors were tested in clinical trials for the treatment of diabetic renal disease. A clinical trial to test the renal-protective properties of the ACE inhibitor captopril, independent of its effect on blood pressure, was conducted with 472 type 1 patients with diabetic nephropathy.[292] The captopril treatment was associated with a 50% reduction in the risk of combined endpoints of death, dialysis, and kidney transplantation. It was more effective than blood pressure control alone.[292] This trial suggested that ACE inhibitors slow the progression of advanced renal disease but provided no data on early stage intervention. The effect of ACE inhibitors on the early stage albuminuria was investigated in the EURODIAB Controlled trial of Lisinopril in Insulin Dependent diabetes (EUCLID) study.[293] The randomized, double-blind, placebo-controlled trial included 530 men and women with type 1 diabetes aged 20−59 years with normoalbuminuria or microalbuminuria. The lisinopril treatment group showed a 19% reduction in albumin excretion rate (AER) compared with the control group. The EUCLID study demonstrated that lisinopril slows the progression of renal disease in type 1 diabetics with little or no albuminuria without increasing the risk of hypoglycemia.[293] A similar trial was conducted in type 2 patients with the ACE inhibitor enalapril. Treatment with enalapril slowed the decline of renal function and reduced the extent of albuminuria.[294] Collectively, these studies suggest that ACE inhibitors are effective in slowing the progression of nephropathy in either type 1 or type 2 diabetes, and the results have been incorporated into diabetes treatment guidelines.[295]

7.4.3. Neuropathy

Clinical trials involving the treatment of diabetic neuropathy include the Diabetes Control and Complications Trial (DCCT), in which intensive insulin

treatment resulted in significant nerve conduction improvement in the intensive treatment group.[296] These data indicate that intensive insulin therapy in IDDM patients prevent and delay the progression of diabetic neuropathy.[296] Trials aimed at treatment via different mechanisms were also conducted, but the changes in nerve function were similar in most trials, suggesting that the proposed mechanisms contribute equally to the development of neuropathy or there are overlaps among the mechanisms.[297] In addition, management of pain associated with diabetic neuropathy has been another focus of clinical trials. Gabapentin was tested in an 8-week randomized trial for its effect on diabetic peripheral neuropathy[298] with a primary endpoint of daily pain severity as measured on an 11-point Likert scale.[298] Patients in the treatment group had significantly lower mean daily pain scores as compared with the control group.[298] Furthermore, gabapentin significantly improved quality of life measurement.[298] Several other agents were also tested in clinical studies for selective therapy and more effective outcomes.[299]

7.5. Diabetes Prevention Trials

Besides intervention, prevention plays an important role in slowing the diabetic complications. In the Da Qing IGT and Diabetes Study, 577 prediabetic subjects with IGT were randomly assigned to dietary intervention only, exercise only, or diet plus exercise.[270] Follow-up examinations were conducted every 2 years over a 6-year period. The cumulative incidence of diabetes at the end of the study was 68% in the control group, but 44% in the diet group, 41% in the exercise group, and 46% in the diet plus exercise group. The data from this study indicate that diet and/or exercise significantly reduce the incidence of diabetes among subjects with IGT. The similar Finnish Diabetes Prevention Study confirmed these results and further demonstrates that diet and exercise also lower cardiovascular risks such as blood pressure and serum triglyceride.[271] In an ongoing U.S. trial, the Diabetes Prevention Program is examining whether intensive lifestyle intervention or metformin prevents or delays the development of type 2 diabetes among those with impaired glucose tolerance.[272]

8. Emerging Therapeutic Targets

A number of potential antidiabetic targets are under study, particularly at the preclinical stage. This section is not intended to be comprehensive; instead, selected targets are discussed in order to illustrate the diversity of approaches.

8.1. Insulin Receptor Mimetic

Once the insulin receptor was cloned in 1985,[300,301] it was immediately obvious that screening for an orally available small molecule (i.e., <1000 Da) insulin mimetic would be a major medical advance that could supplant the need for daily insulin injections with a more palatable option. On the surface, the design of such a compound seems to be a daunting task. How could a small molecule perform the same role as a 110 amino acid protein? Numerous pharmaceutical companies have screened for such an agent. However, it was not until 1999, 14 years after the cloning of the insulin receptor, that such a compound appeared in print from a group at Merck Research Laboratories.[302] This important work described a nonpeptidyl, small molecule fungal metabolite (designated demethylasterriquinone B-1, DMAQ-B1, or L-783,281) from *Pseudomassaria* sp., a tropical endophytic fungus. L-783,281 was shown to be a selective insulin receptor activator (and did not activate other receptor tyrosine kinases) that mimicked insulin effects including phosphorylation of IRS-1, activation of PI3K and Akt, and increased glucose uptake in skeletal muscle and adipocytes. Furthermore, oral administration to *db*/*db* and *ob*/*ob* mice resulted in significant lowering of blood glucose levels.[302]

An emerging field of cerebral insulin action has unraveled a previously underappreciated role for insulin in the control of the appetite. Indeed, over 20 years ago, it was demonstrated that brain administration of insulin reduced food intake and body weight in baboons.[303] Consistent with these results, ablation of neuronal insulin receptors either by selective tissue knockout in mice[304] or introduction of specific antisense oligonucleotides in rats[305] results in dysregulation of energy balance with animals that are hyperphagic, obese, and insulin resistant. Interestingly, Merck's insulin mimetics also reduce food intake and body weight when administered intracerebroventricularly into rats.[306]

Other compounds, which are apparently insulin sensitizers rather than true mimetics, have been described. These compounds appear to increase the number of insulin receptors that are tyrosine-phosphorylated in response to insulin and have glucose-lowering effects in several animal models of diabetes.[307,308] Such proof of concept for small molecule insulin mimetics holds significant promise as a treatment modality for diabetic patients.

8.2. Protein Tyrosine Phosphatase 1B (PTP1B)

The insulin receptor is activated via autophosphorylation on tyrosine residues, so it follows then that a tyrosine phosphatase would be required to shut off the receptor. A number of candidate enzymes have been studied both in vitro and in vivo, but none have had the stunning phenotype exhibited by PTP1B knockout mice.[309] The homozygous PTP1B$^{-/-}$ mice were generally healthy with normal weight gain and food intake and had plasma glucose levels after feeding that were 13% lower and insulin levels that were ~50% lower than those of controls. They exhibited increased insulin sensitivity as verified by glucose and insulin tolerance tests. The knockout mice also demonstrated a decreased tendency toward obesity when placed on a high-fat diet. Enhanced IR and IRS-1 phosphorylation was observed in the liver and skeletal muscle of PTP1B$^{-/-}$ mice, suggesting that this target has the potential to address the defects in hepatic glucose output as well as the defect in muscle glucose uptake. Indeed, the phenotype exhibited by the PTP1B knockout mouse is a hopeful one when such a set of studies is aimed at identifying

targets for diabetes medications. Therefore, therapeutic intervention via small molecule inhibitors of PTP1B would appear to be a promising treatment option. This approach is complicated, however, in that all protein tyrosine phosphatases share an active site cysteine residue that is critical for catalysis. This fact creates a major challenge for the design of specific small molecule inhibitors. Although numerous reports have appeared describing small molecule inhibitors of PTP1B, none have yet to emerge from phase III clinical testing. Another treatment possibility is to utilize an antisense oligonucleotide approach to specifically lower the expression of PTP1B. Indeed, after 6 weeks of antisense treatment, PTP1B expression was knocked down ~50% in liver and adipose tissue in vivo in both *ob*/*ob* and *db*/*db* mice, resulting in a normalization of plasma glucose, glucose excursion, and HbA_{1c}. In addition, knockdown of PTP1B resulted in improved insulin sensitivity as assessed by increased Akt Ser^{308} phosphorylation in response to insulin and increased down-regulation of the gluconeogenic enzymes phosphoenolpyruvate carboxykinase and fructose-1,6-bisphosphatase.[310] Furthermore, PTP1B antisense treatment of *ob*/*ob* mice resulted in reduced adiposity concomitant with the down-regulation of genes involved in lipogenesis.[311]

8.3. Adiponectin

Adipose tissue, once viewed simply as a storage depot for excess energy in the form of triacylglycerols, is now thought to act as an endocrine organ in that numerous hormones and "adipokines" are secreted in response to a variety of stimuli. One such secretory protein known as adiponectin (also known as Acrp30, AdipoQ, OBG3, or APM1) was first identified in the mid 1990s independently by two laboratories as an adipose-specific gene encoding a 247 amino acid 30 kDa secretory protein that exists in plasma in trimeric and multimeric forms.[194,312] The adiponectin gene is localized on mouse chromosome 16, which is syntenic to human 3q27, a known susceptibility locus for type 2 diabetes and metabolic syndrome X.[313] Pharmacological studies on the physiological role of this protein did not appear until 2001, 6 years after the identification of adiponectin, presumably due to the technical difficulties in purifying sufficient quantities of the protein, especially challenging because circulating adiponectin levels can rise in mice to ~20 μg/mL, roughly 1000 times higher than circulating insulin or other adipokines such as leptin. In humans, circulating adiponectin varies from about 1 to 15 μg/mL and is inversely proportional to adiposity and fasting insulin concentrations and positively correlated with insulin sensitivity.[195−197] Adiponectin gene expression is reduced in obese, insulin-resistant rodents, monkeys, and humans. When obese patients undertake weight reduction therapy, adiponectin expression is restored toward normal levels. Thiazolindinedione treatment of rodents and diabetic patients also increases adiponectin expression, raising the possibility adiponectin may mediate (some of) the beneficial effects of TZDs. Knockout of the adiponectin gene in rodents enhances the susceptibility to develop diet-induced insulin resistance and impairs clearance of free fatty acids.[314] Although these findings were not universal,[315] treatment of knockout mice with adenovirus expressing adiponectin ameliorated diet-induced insulin resistance.[314]

Treatment of rodents with recombinantly generated adiponectin results in beneficial effects on glucose and lipid metabolism. The molecular mechanisms of adiponectin's actions are under intense investigation and appear to be diverse and complex. A single injection of purified recombinant adiponectin yielding levels ~2−3-fold above normal into wild type, *ob*/*ob*, NOD, or streptozotocin-treated mice elicited an acute and transient decrease in basal serum glucose levels, thereby abolishing hyperglycemia in the three diabetic animal models.[220] The effect was not due to a compensatory increase in insulin levels, indicating that adiponectin does not act by stimulating insulin secretion. In isolated mouse hepatocytes, adiponectin sensitized the cells to insulin in that it increased the ability of insulin to suppress gluconeogenesis.[220] A second study published back to back with the previous one observed similar hyperglycemia-ameliorating effects in *db*/*db* and KKA^y mice treated for a few weeks with adiponectin.[316] Furthermore, chronic adiponectin treatment for several weeks elicited significant decreases in skeletal muscle and liver triglyceride content and decreased serum free fatty acids concomitant with modest increases in the transcription of select fatty acid metabolism genes in liver (e.g., fatty acid translocase/CD36, acyl-Co A oxidase, and uncoupling protein-2). Infusion of the globular domain of adiponectin sensitized skeletal muscle to insulin as assessed by increased phosphorylation of insulin receptor, insulin receptor substrate 1, and Akt relative to untreated controls. Yamauchi et al. demonstrated that a carboxy-terminal globular portion of adiponectin was also efficacious in improving glucose and lipid metabolism.[316] A third study compared both full-length adiponectin with a 143 amino acid globular head domain in C57BL/6 mice fed a high-fat/sucrose diet. Whereas globular adiponectin decreased glucose, free fatty acids, and triglycerides, full-length adiponectin was without effect.[317] Thus, the issue as to which precise form or forms of adiponectin mediate the different beneficial effects remains to be clarified.

The identification of an adiponectin receptor has recently been reported,[318] which should greatly facilitate understanding of the adiponectin signaling pathway(s). Adiponectin was first recognized to share sequence homology with complement-related protein C1q, a protein known to be involved in the recognition of invading microbial surfaces and complexes of antigen with antibody, but upon its crystallization at 2.1 Å resolution, it was revealed to have an unexpected homology to tumor necrosis factor α.[319] Such a homology could not have been predicted on the basis of primary amino acid sequence comparisons.

The results from the aforementioned studies suggest that adiponectin is a potent insulin sensitizer that links adipose tissue with whole body glucose metabolism. Several groups are racing to produce enough adiponectin for human safety and proof of

concept clinical trials. Given the impressive and quickly amassing preclinical data, adiponectin injection appears to present an exciting new treatment modality for type 2 diabetes. However, despite many rapid and impressive advances in adiponectin biology, clearly much work remains to determine whether the full-length, globular head domain or fragments thereof of adiponectin are the predominant player in mediating the beneficial therapeutic effects of this protein. It is not yet clear whether recombinant variants of adiponectin will possess pharmacokinetic properties compatible with an injectable antidiabetic agent.

8.4. 11β-Hydroxysteroid Dehydrogenase Type 1 (11βHSD1)

The liver is the major "glucostatic" organ in that it provides glucose from glycogenolysis and gluconeogenesis to maintain circulating concentrations of ~6 mM (110 mg/dL). Insulin normally attenuates hepatic glucose output by specifically inhibiting the transcription of key gluconeogenic genes such as phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase) and via inhibition of glycogen phosphorylase. In type 2 diabetic patients who present with elevated circulating glucose levels, the insulin resistance exists on several major levels: diminished glucose uptake into skeletal muscle and adipose, which is exacerbated by continued hepatic glucose output via glycogenolysis and gluconeogenesis. These two pathways are normally inhibited by insulin. In normal individuals the distribution of hepatic glucose output is typically 25% gluconeogenic and 75% glycogenolytic, whereas in type 2 diabetics as much as 90% of hepatic glucose output has been attributed to increased gluconeogenic activity.[3] Glucocorticoids, on the other hand, increase hepatic glucose production via induction of PEPCK and G6Pase, and excessive concentrations of glucocorticoids can cause glucose intolerance and insulin resistance, presumably through dysregulated hepatic gluconeogenesis. In addition, elevated circulating levels of glucocorticoids can induce insulin resistance in skeletal muscle and adipose tissue. The enzymes 11βHSD types 1 and 2 interconvert glucocorticoids between inactive and active forms, thereby regulating the agonist concentration and activation of corticosteroid receptors.[320] A working hypothesis is that local production of active glucocorticoid from the inactive form can impair glucose metabolism and that selective inhibition of 11βHSD1 could ameliorate excessive hepatic glucose output and improve peripheral glucose uptake via preventing the conversion of cortisone to cortisol, the active glucocorticoid. Indeed, clinical studies in healthy individuals with the nonselective 11βHSD1 inhibitor carboxenelone resulted in enhanced whole-body insulin sensitivity.[321] Mice with a homozygous disruption of 11βHSD1 displayed attenuated activation of PEPCK and G6Pase and were resistant to obesity- or stress-provoked hyperglycemia, presumably because of a relative hepatic glucocorticoid insufficiency.[322] Furthermore, a recent report presented data in which KKA^y mice treated for 7 days with a selective 11βHSD1 inhibitor showed decreased hepatic PEPCK and G6Pase mRNA content concomitant with lowered blood glucose and serum insulin concentrations.[323] In contrast, transgenic overexpression of 11βHSD1 selectively in adipose tissue elicited visceral obesity, hyperglycemia, glucose intolerance, and insulin resistancd, thereby recapitulating many of the symptoms of metabolic syndrome X.[324] Taken together, inhibition of 11βHSD1 appears to be an intriguing target.

8.5. I Kappa Kinase β (IKKβ or IKK2) and c-Jun Amino-Terminal Kinase 1 (JNK1)

Kinases that can serine phosphorylate IRS-1 at sites that inhibit subsequent IRS tyrosine phosphorylation represent potential therapeutic targets. As discussed under section 3.3, JNK1 could be a potential drug target for type 2 diabetes. Another kinase that has gained recent attention is IKKβ. The diabetes literature is speckled with reports of high-dose salicylate treatment, including acetylsalicylic acid (aspirin) and sodium salicylate, normalizing high glucose levels in type 2 diabetics.[325,326] Initially, it was thought that salicylates somehow triggered insulin secretion, but recent evidence suggests that this compound improves insulin action. Although the obvious targets for aspirin are the cyclooxygenases 1 and 2 (COX1 and COX2), other data suggest that both aspirin and salicylate can also inhibit the enzyme IKKβ.[327] Following up on this work a recent study showed that high doses of salicylates reversed hyperglycemia, hyperinsulinemia, and dyslipidemia in both Zucker fatty rats and *ob/ob* mice while concomitantly sensitizing the animals to insulin signaling.[191] Furthermore, *ob/ob* mice heterozygous for IKKβ were resistant to diet-induced obesity, supporting the hypothesis that the molecular target of salicylates is IKKβ.[191] IKKβ is activated in response to a number of stimuli such as TNFα and elevations in plasma free fatty acids, which are also elevated in type 2 diabetes. Thus, an attractive and potentially unifying pathophysiological mechanism for several conditions associated with insulin resistance is the aberrant activation of IKKβ.

One molecular mechanism mediating insulin resistance may be the serine phosphorylation of IRS-1. The structural basis for this effect is largely unknown, but evidence is emerging that serine phosphorylation of IRS-1 seems to impair insulin's ability to tyrosine phosphorylate (and activate) IRS-1 by preventing its interaction with the juxtamembrane region of the insulin receptor, thereby attenuating downstream signaling events.[328] Several cell culture studies have further implicated IKKβ in this proposed scenario of events. For example, TNFα treatment of HepG2 cells elicited the serine phosphorylation of IRS-1 concomitant with activation of IKKβ, and it was inhibited by 15-deoxyprostaglandin J2, an IKKβ inhibitor.[329] In 3T3-L1 adipocytes and Fao hepatoma cells, indirect activation of IKKβ with either TNFα or calyculin, a serine phosphatase inhibitor, inhibited the tyrosine phosphorylation of both insulin receptor β subunit, IRS-1, and IRS-2, whereas IKKβ inhibition with aspirin or salicylate reversed insulin resistance. In the Fao hepatoma cells

neither ibuprofen, naproxen, nor a COX2 inhibitor, all treatments for general inflammation, was able to reverse the TNFα-induced decrease in IRS-2 phosphorylation, further implicating IKKβ as the molecular target of aspirin and salicylates.[191] Interestingly, high concentrations of aspirin have also been shown to inhibit the activation of JNKs.[330] Because IKKβ is apparently not involved in the activation of JNKs, the implication is that high-dose aspirin treatment likely inactivates more than one potentially beneficial therapeutic target. Whether IKKβ, JNK, or some other kinase proves to be pharmaceutically tractable as a diabetes treatment remains to be determined.

8.6. Glucagon-like Peptide 1 (GLP-1) and Dipeptidylpeptidase IV (DPPIV)

It has long been known that oral administration of glucose results in increased insulin secretion relative to intravenous glucose administration. The increased insulin secretion in response to oral nutrients is known as the "incretin" effect and results from gastrointestinal secretion of humoral factors that enhance insulin secretion. The primary incretin hormones are GLP-1 and gastric inhibitory polypeptide (GIP; named for another biological activity of this molecule that was the first to be characterized). GIP is also known as glucose-dependent insulinotropic peptide, although in fact both GLP-1 and GIP display glucose-dependent insulinotropic properties. GIP and GLP-1 share a common effect of enhancing insulin secretion,[331] but their other biological activities differ and are mediated via distinct receptors. The feasibility of GIP as an antidiabetic target is not fully clear in that its insulinotropic properties are impaired in experimental[332] and clinical[333,334] diabetes, and the extrapancreatic effects of GIP to promote fatty acid synthesis and lipid accumulation are undesirable in the diabetic state.[335,336]

GLP-1 is a 30 amino acid hormone produced by L-cells of the intestinal mucosa that results from tissue-specific processing of the proglucagon gene.[337] This is the same gene that is present in A cells of the endocrine pancreas. Posttranslational processing of the proglucagon gene transcript in A cells produces the 29 amino acid hormone glucagon as the primary bioactive product, along with other proglucagon-derived fragments. The primary products of the L-cell, GLP-1 and GLP-2 are ~50% homologous to glucagon. GLP-2 regulates gastric acid secretion and intestinal motility, possesses intestinal trophic activity, and will not be discussed further. Secretion of GLPs is regulated by nutrients, neural factors, and somatostatin. GLP-1 levels are only transiently elevated following a meal due to rapid cleavage and inactivation by the enzyme DPP-IV, renal clearance, and other mechanisms, such that the plasma half-life is on the order of ~4−6 min.

The actions of GLP-1 on pancreatic β cells are mediated through a specific seven-transmembrane G-protein-coupled receptor (GLP-1 receptor[338]). Activation of this receptor by GLP-1 stimulates cAMP production[339] followed by an increase in free intracellular Ca^{2+} concentration, membrane depolarization, and insulin secretion.[340] GLP-1 possesses multiple biological effects, however, that act in concert to regulate blood glucose levels to a greater extent than the insulinotropic effect alone.[341] In addition to its effect on β cells, GLP-1 also inhibits glucagon secretion from pancreatic α cells[342,343] (reducing hepatic glucose output) and slows gastric emptying[343,344] (slowing the delivery of glucose to the intestine, allowing more time for glucose clearance to keep pace with glucose appearance in the bloodstream). The importance of these latter biological activities is evident in the antidiabetic effects of GLP-1 treatment in type 1 diabetics alone.[341] Like its effect on β cells, the effects of GLP-1 on α cells and on gastric emptying are glucose-dependent, which is a very attractive property for an antidiabetic agent. When glucose levels diminish toward the hypoglycemic range, the effects of GLP-1 on insulin secretion, glucagon secretion, and gastric emptying are diminished, lessening the tendency for hypoglycemia;[345] this contrasts sharply with effects of sulfonylureas, for example. GLP-1 also possesses additional actions in animal models of diabetes, which have been less extensively validated in humans. Preclinical data suggest that chronic GLP-1 therapy may result in an increase in pancreatic β cell mass.[337] If this effect occurs in humans, it would be of substantial clinical importance given the gradual deterioration of β cell function in type 2 diabetes and would further distinguish this mechanism from the other insulin secretagogues (sulfonylureas and glitinides). GLP-1 treatment also reduces food intake in preclinical models[337] (independent of the effect to lower food intake after reduce urinary spillage of glucose), but data supporting such an effect in humans are currently sparse.

The increase in plasma levels of biologically active GLP-1 following meal challenge is diminished in type 2 diabetics,[346] but diabetics retain responsiveness to exogenously administered GLP-1. Short-term administration of GLP-1 via subcutaneous or intravenous routes rapidly lowers blood glucose levels in animals, nondiabetic humans, and type 2 diabetics. When administered in a manner that achieves sustained plasma levels, GLP-1 treatment produces striking effects on blood glucose levels. When GLP-1 was continuously infused in type 2 diabetic patients, it reduced their diurnal glucose concentrations to levels similar to nondiabetic control subjects, and these levels were maintained throughout the 8 h study period.[347] No other antidiabetic agents can normalize circulating glucose concentrations with such precision timing. Furthermore, chronic subcutaneous infusion of GLP-1 for 6 weeks in type 2 diabetics lowered fasting glucose, meal-regulated glucose, free fatty acids, and HbA_{1c} and improved β cell function.[348] Potential complications of the GLP-1 approach include GI side effects such as nausea and vomiting,[349,350] which could result in a narrow therapeutic window.

Several approaches to therapeutic intervention are possible. The most expeditious route to the clinic, being pursued by multiple companies, involves production of recombinant GLP-1 for injection. Given its rapid clearance, therapy utilizing unmodified

native GLP-1 is not practical. A number of GLP-1 analogues engineered to reduce plasma clearance are under development. One strategy to protect GLP-1 from degradation and increase its circulating half-life is to increase its association with albumin, either non-covalently by derivatizing GLP-1 with fatty acids[351,352] or by covalent attachment of GLP-1 to albumin.[353] Another strategy is to identify related synthetic[354] or natural peptides[355] that retain the ability to activate the GLP-1 receptor but are resistant to DDPIV degradation. The most advanced of these is the natural GLP-1 analogue exendin-4. Exendin-4 is a 39 amino acid peptide originally isolated from the salivary venom of the lizard *Heloderma suspectum*.[355] Exendin-4 exhibits 52% structural identity to GLP-1. It acts as an agonist for the GLP-1 receptor and displays potency similar to that of GLP-1 in stimulating insulin secretion from isolated pancreatic islets in vitro.[214,356] Exendin-4 retains all of the biological activities of GLP-1 in animal models but has a markedly longer half-life in vivo[357] and extended duration of biological efficacy.[358,359] Clinical studies have demonstrated that acute treatment of nondiabetics and type 2 diabetics with exendin-4 enhances insulin secretion, lowers blood glucose,[360,361] and substantially reduces HbA_{1c} in just 28 days, thereby strengthening the case for the efficacy of the GLP-1 mechanism.[362] Issues with the recombinant approach include patient willingness for, and compliance with, daily injections as needed for the lead molecules currently in development.

A second general approach to this target could be development of a small molecule GLP-1 receptor agonist. Development of small molecules to supplant the function of a polypeptide is very difficult, and successful efforts to identify small molecule GLP-1 agonists have not been reported. A third approach would involve stimulation of the gut to produce the hormone; the feasibility of identifying an agent with the selectivity to stimulate the exclusive transcription of just a single gene seems daunting. Another approach is to inhibit GLP-1 degradation in the plasma, thereby prolonging its activity.[363] As noted, GLP-1 is rapidly cleaved by the serine protease DPPIV and cleared from the circulation through the kidneys such that steady state intact, active GLP-1 levels may be only $\sim$10−20%.[364] Thus, cleavage by DPPIV rapidly eliminates GLP-1 action, and intense efforts to develop orally available DPPIV inhibitors and thereby raise the level of endogenously produced GLP-1 are underway. The preliminary feasibility of this approach has been demonstrated. Long-term (2−3 months) treatment of rodents with diet-induced[365] or genetic[366−368] obesity/diabetes with DPPIV inhibitors improved glucose tolerance and insulin secretion concomitant with increased plasma GLP-1 levels. Preliminary proof of principle has recently been demonstrated in type 2 diabetics.[369] Several potential issues regarding the feasibility of DDPIV inhibition, however, need to be resolved:[363,370] (1) It is not clear whether this approach will result in efficacy comparable to that obtained with exogenous GLP-1 administration. Because the levels of endogenous GLP-1 are reduced in diabetes, the DPPIV approach may not be able to raise GLP-1 levels high enough to attain peak efficacy. (2) DPPIV cleaves numerous (upward of 50) substrates in vitro, raising the concern that inhibition of DPPIV may lead to mechanism-based side effects due to accumulation of peptides other than GLP-1. The specificity and safety of DDPIV inhibition for GLP-1 in vivo remain to be rigorously established. (3) There are a number of structurally related peptidases with overlapping enzyme activity that may complicate efforts to develop DPPIV-specific inhibitors. (4) DPPIV is expressed in numerous tissues and may have important roles in immune function and in metastasis, so significant side effects of DPPIV inhibition are possible. DPPIV activity is altered in a number of different disease states.

8.7. AMP Kinase

Treatment of diabetic animals with the AMPK activator AICAR lowers blood glucose levels,[371,372] and AMPK is a potential target for diabetes.[240] AMPK is a heterotrimeric protein; each of its subunits exists as multiple isoforms, with differential tissue expression.[373] AMPK itself is regulated in several ways: by phosphorylation via an upstream kinase (AMPKK) and by allosteric regulation via AMP/ATP and creatine/phosphocreatine. Target proteins for phosphorylation by AMPK include acetyl-CoA carboxylase, HMG-CoA reductase, and endothelial nitric oxide synthase. Recent data suggest that the effects of metformin,[217] adiponectin,[374] and other agents that regulate glucose and fatty acid metabolism[375] may be mediated at least in part via AMPK activation.

Chronic activation of AMPK mimics several effects of exercise in skeletal muscle, such as induction of mitochondrial oxidative enzymes, enhancement of glucose transport, and induction of Glut4 expression.[376] Given that some of the targets of AMPK phosphorylation are involved in the control of lipid and lipoprotein metabolism, AMPK activation in diabetics might favorably modulate lipid metabolism as well as glucose metabolism, although AICAR treatment of some preclinical models resulted in some adverse effects on lipid metabolism.[371,372]

9. Conclusions

Type 2 diabetes is a complex disease characterized by uncontrolled hepatic glucose output and insulin resistance and impaired glucose tolerance in peripheral tissues. The insulin resistance in the liver and the peripheral tissues, mainly skeletal muscle and the adipose tissue, causes the pancreatic β cells to secrete more insulin to compensate for the resistance. As a result, patients often develop hyperglycemia, hyperinsulinemia, dyslipidemia, and hypertension. As these symptoms escalate, further complications such as atherosclerosis, retinopathy, and neuropathy develop and become main causes for morbidity and mortality. Most current therapies address these defects only individually. For an antidiabetic agent to be broadly applicable, it must address these multiple mechanisms. Currently marketed drugs do not have such broad therapeutic properties; hence, the trend in current therapy is increasingly one of polypharmacy.

The insulin signaling pathways have been the central focus in order to understand how insulin regulates hepatic glucose production and peripheral glucose utilization. Major advances have been made in the past decade. By binding to its receptor, insulin triggers a cascade of phosphorylation and activation events that lead to the activation of glucose transport and the suppression of hepatic glucose production. The defective regulation of these pathways by insulin in the disease state has been under intense scrutiny using animal models that mimic certain aspects of type 2 diabetes. These models, although each with their own limitations, have been important tools in understanding the individual aspects of the disease and defining the pathways mediating insulin resistance. Clinical trials have helped to understand the disease in the human context and validate treatment hypotheses and strategies. The new knowledge gained further advances our understanding of the pathophysiology of the disease so that better prevention and treatment approaches can be developed. With the information garnered from animal studies and in subsequent human clinical trials, new treatments such as TZDs have been developed to better manage insulin resistance and hyperglycemia. Several emerging therapeutic targets for disease treatment promise to lead to more efficacious and safer clinical therapies.

Future research lies in the further understanding of insulin resistance and its potential causal relationship to the disease. The prevention or reversal of insulin resistance and glucose intolerance before the onset of overt type 2 diabetes will likely become the next target for intervention. This requires better understanding of the mechanisms underlying insulin resistance, especially association with obesity. Improved prognostic and diagnostic tools are needed to identify those at risk so that treatments may be started prior to the development of type 2 diabetes.

10. References

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