# 7 Insulin Action

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**Keypoints**

- Insulin signaling is accurately viewed as involving a matrix of interacting pathways, allowing for extensive modulation and divergence in signal transduction, rather than a linear cascade of sequential reactions.
- Diverse cell types commonly share the proximal steps in insulin signal transduction, including the insulin receptor, insulin receptor substrate molecules (IRS), phosphatidylinositide 3 kinase and Akt/protein kinase B.
- Insulin action is negatively modulated by multiple cellular mechanisms that impair tyrosine phosphorylation of insulin receptors and IRS; foremost among these are serine–threonine kinases and protein tyrosine phosphatases.
- The tissue-specific biologic effects of insulin are explained by effector systems which are uniquely expressed in differentiated target tissues such as the insulin-responsive glucose transport system in skeletal muscle, enzymatic systems mediating antilipolysis in adipose tissue and regulated gene expression leading to suppression of gluconeogenesis in liver.
- Insulin action is highly regulated by three pathways for nutrient sensing: the hexosamine biosynthetic signaling pathway; the mammalian target of rapamycin signaling pathway; and the AMP-activated protein kinase signaling pathway.
- Oxidative stress, inflammation and endoplasmic reticulum stress are associated with insulin resistance, obesity and metabolic syndrome, and impair insulin action via activation of serine–threonine protein kinases.

## General aspects of insulin action

The peptide hormone insulin is exclusively synthesized in and secreted from pancreatic β-cells. Insulin exerts a broad spectrum of anabolic effects in multiple tissues. The regulation of whole body fuel homeostasis primarily involves insulin action in skeletal muscle, adipose tissue, and liver where insulin promotes uptake and storage of carbohydrate, fat, and amino acids, while at the same time antagonizing the catabolism of these fuel reserves. In skeletal muscle, insulin stimulates glucose transport and glucose storage as glycogen, as well as glycolysis and tricarboxylic acid cycle activity. Insulin lowers hepatic glucose output by inhibiting glycogenolysis and gluconeogenesis, and augments glycogen formation. In adipocytes, insulin promotes glucose uptake, glycerol synthesis, and triglyceride formation, while at the same time exerting an antilipolytic effect. During periods of fasting, a fall in circulating insulin combined with increased secretion of counter-regulatory hormones leads to breakdown of stored fuels and increased availability of metabolic substrates for cellular energy. In this way, alterations in insulin levels in the fed and fasting states have a key role in fuel metabolism and maintain blood glucose levels within a narrowly defined range. Insulin diminishes protein catabolism and increases translation, and also enhances cell growth, differentiation, and survival as a consequence of mitogenic and anti-apoptotic processes. Thus, the action of insulin at the level of cells and tissues affects substrate flux and coordinates the function of multiple organs as whole organisms adapt to the nutritional environment.

In mediating its pleiotropic actions, insulin binds to cell surface receptors, activates multiple signal transduction networks, and engages effector systems responsible for specific biologic functions. Over the last decade it has become clear that the classic perspective of insulin signaling as a linear cascade of sequentially interacting signal transduction molecules is short-sighted. Rather, the promulgation of insulin action is more accurately viewed as alterations in a network of interactions involving a matrix of signal cascades that engage in cross-talk. The final biologic action represents the net synergism of the combined facilitative, inhibitory and complementary signaling pathways that interact with more terminal functional systems in cell biology. While there is relative commonality in signal transduction networks, differentiated insulin target cells express a variety of unique effector systems, which are primarily responsible for mediating the cell-

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specific and organ-specific biologic functions of insulin. Effector systems include rate-limiting enzymes, enzymatic pathways, membrane transport systems, gene expression, processes regulating the cellular trafficking of proteins and vesicles, and systems governing the translation, post-translational modification and degradation of proteins. Certain aspects of linear insulin signal transduction are evolutionarily conserved; however, complex patterns of interactions between signal and evolved effector systems are more pronounced in mammals and allow for greater plasticity in adaptive responses [1].

This chapter first discusses insulin signaling pathways and networks that are common to multiple target cell types. Recent advances in our understanding of cell processes and pathways that inhibit insulin signaling are delineated. Subsequently, unique aspects of insulin action are described, in particular key effector systems, which are properties of skeletal muscle, adipocytes, and liver, and explain the distinct effects on biologic functions in these tissues. Finally, nutrient sensing pathways and cell stress responses are discussed in terms of their interaction with insulin signaling and role in the pathogenesis of insulin resistance.

## Insulin action: proximal signaling pathways

Proximal steps in insulin signaling, including the insulin receptor, insulin receptor substrate proteins (IRS), phosphatidylinositol 3 ($PI_3$) kinase, Akt/protein kinase B (Akt/PKB), and mitogen activated protein kinase (MAPK) are globally operative in multiple cell types. These proteins also serve as points of divergence or nodes in an expanding matrix of signal transduction pathways, and are highly regulated, both positively and negatively, via cross-talk with other signaling systems and modulatory pathways (Figure 7.1).

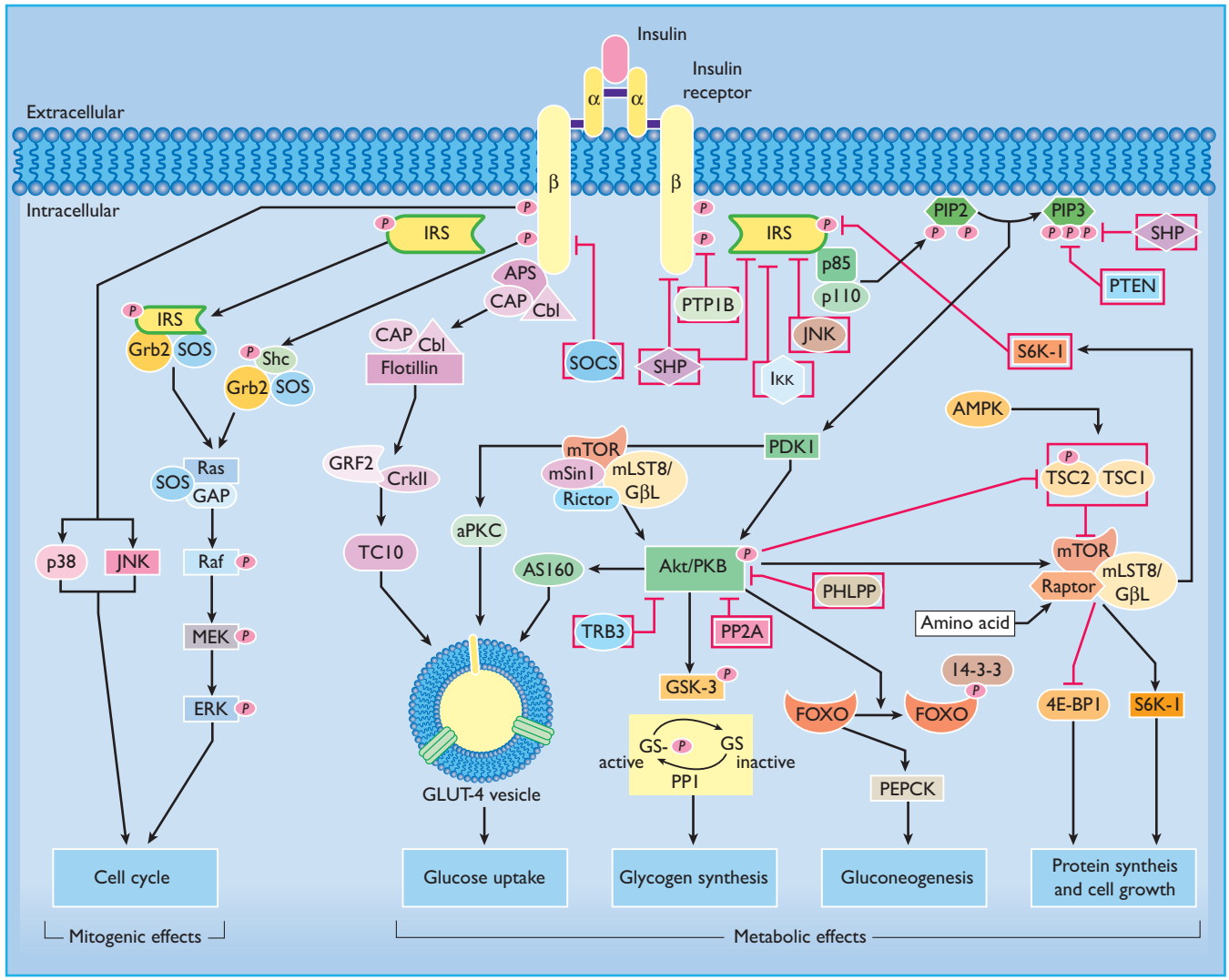


**Figure 7.1** A schematic illustration of insulin signaling pathways involved in both metabolic and mitogenic effects. Arrows represent an activation process; blocked arrows represent an inhibition process.

## Insulin receptor molecules

Insulin action is initiated by specific binding to high-affinity receptors on the plasma membrane of its target cells. The insulin receptor is a large transmembrane glycoprotein consisting of two α- and two β-subunits which form a heterotetramer. The insulin receptor is synthesized from a single gene that consists of 22 exons and 21 introns. Following translation of its mRNA, it is processed into two separate subunits (α and β) that assemble as a disulfide-linked holoenzyme of $(\alpha\beta)_2$ stoichiometry [2]. The 135 kDa α-subunits, derived from the amino-terminal portion of the proreceptor, reside entirely on the outside of the cell, tethered to the membrane via the 95 kDa β-subunits that span the membrane. Insulin binds to the extracellular α-subunits. This binding results in conformational changes that bring the α-subunits closer together, and enables ATP to bind to the intracellular domain of the β-subunit leading to autophosphorylation of distinct tyrosine residues on the β-subunit. Autophosphorylation augments the intrinsic activity of the β-subunit as a tyrosine kinase, directed against other tyrosines within the receptor as well as tyrosine phosphorylation of exogenous substrates. Ligand-dependent stimulation of the β-subunit tyrosine kinase activity is critical for promulgation of the insulin signal. At least six tyrosine residues in the β-subunit undergo phosphorylation and have been shown to serve different roles in insulin signaling. They lie within three functional groups. Phosphorylation of $Tyr^{972}$ establishes a recognition motif and docking site that provides sufficient stability of the receptor–substrate complex for intracellular substrate phosphorylation. Tyrosine phosphorylation sites at positions $Tyr^{1158}$, $Tyr^{1162}$ and $Tyr^{1163}$ are essential for mediating an increase in subunit tyrosine kinase activity and signal transduction. Phosphorylation sites $Tyr^{1328}$ and $Tyr^{1334}$ affect the sensitivity of Ras/MAPK pathway activation, and are thus involved in the receptor's mitogenic responses [3].

The number of cell-surface insulin receptors is downregulated by chronic exposure to high insulin concentrations *in vitro*, and receptor loss is observed in target cells from hyperinsulinemic insulin-resistant humans. Receptor loss per se can impair maximal insulin responsiveness. This is illustrated in the extreme by genetic ablation of the insulin receptor in mice which results in lethality at 4–5 days after birth as a result of severe diabetic ketoacidosis [4]. Insulin-stimulated glucose uptake and activation of glycogen synthase in muscle are severely impaired in muscle-specific insulin receptor knockout mice [5]. These latter animals also have features of the metabolic syndrome including increases in fat mass, serum triglycerides and serum free fatty acids, but retain normal basal and contraction-stimulated glucose transport [6]. Transgenic mice expressing dominant-negative insulin receptors also develop obesity, hyperinsulinemia, glucose intolerance, and hypertriglyceridemia. These phenotypes are analogous to those seen in insulin-resistant humans. Patients with genetic mutations in the insulin receptor gene (type B insulin resistance) or circulating antibodies directed against the insulin receptor that block ligand binding (type A insulin resistance) develop severe insulin resistance, acanthosis nigricans and glucose intolerance. In addition to receptor downregulation, the intrinsic activity of the insulin receptor tyrosine kinase is impaired in insulin-resistant humans and patients with type 2 diabetes mellitus (T2DM) via a number of regulatory and pathophysiologic processes which are described in this chapter. Clearly, the number and functional activity of insulin receptors is critical for effective insulin action.

The insulin receptor is similar in structure to the insulin-like growth factor 1 receptor (IGF1R) and several other growth factor and cytokine receptors, which have in common an extracellular ligand-binding domain that activates an intracellular tyrosine kinase domain. The mammalian insulin-like signaling system includes three well-defined ligands: insulin, insulin-like growth factor 1 (IGF-1), and insulin-like growth factor 2 (IGF-2), and all three ligands can bind and activate cell-surface insulin receptors [7]. Alternative splicing involving exon 11 of the insulin receptor gene determines the insertion or deletion of 12 amino acids near the COOH-terminus of the α-subunit. Isoform A lacking the 12 amino acids has high affinity for IGF-2, predominates during fetal development, and promotes growth as a consequence of IGF-2 binding. Isoform B containing the 12 amino acids predominates postnatally and is activated mainly by insulin. Some evidence supports the contention that dysregulated expression towards the fetal pattern could occur in adult tissues and result in insulin resistance [8].

## Insulin receptor substrate molecules

Following insulin binding and receptor autophosphorylation, the next committed step in signal transduction is tyrosine phosphorylation of intracellular proteins. At least 11 intracellular substrates have been identified that are rapidly phosphorylated on tyrosine residues by ligand-bound insulin receptors, including six insulin receptor substrate (IRS) proteins, Grb2-associated binder 1 (Gab1), Cas-Br-M (murine) ecotropic retroviral transforming sequence homolog (Cbl), and the various isoforms of Src-homology-2-containing protein (Shc) [9].

The IRSs are immediate substrates for the insulin receptor tyrosine kinase, and are of predominant importance for insulin action. IRS proteins have an N-terminal pleckstrin-homology (PH) domain, a phosphotyrosine-binding (PTB) domain and a COOH-terminal region of variable length that contains multiple tyrosine and serine phosphorylation sites [10]. The PH domain helps position IRS for coupling with the insulin receptor, possibly by binding to charged headgroups of certain phosphatidylinositides in adjacent membrane structures. PTB domains recognize the phosphotyrosine in the amino acid sequence asparagine-proline-any amino acid-phosphotyrosine (NPXpY), which encompasses $Tyr^{972}$ in the juxtamembranous domain of the insulin receptor β-subunit, and facilitates the formation of the IRS–insulin receptor complex. The center and C-terminus of IRS proteins contain up to 20 potential tyrosine phosphorylation sites that, after phosphorylation by the insulin receptor, bind to other intracellular molecules that contain Src-homology-2 domains (SH2 domains). The SH2-containing proteins that bind to phos-

phorylated IRS proteins could be adaptor molecules, such as the regulatory subunit of $PI_3$ kinase and the adaptor molecule Grb2, or enzymes with kinase or phosphatase activities such as SH2-domain-containing tyrosine phosphatase 2 (SHP2) and the cytoplasmic tyrosine kinase Fyn. Non-covalent attachment of multiple SH2-containing signaling molecules allows IRS to function as a docking protein that physically apposes proteins involved in downstream transduction cascades. Thus, IRS propagates insulin signal transduction via the docking, apposition, interaction, and activation of downstream signal molecules, rather than as a consequence of any intrinsic enzymatic activity per se. Thus, IRSs provide a major point of divergence of insulin signal transduction pathways, for example, leading to activation of mitogenic (Ras/MAP kinase) and metabolic ($PI_3$ kinase) pathways [11].

Among the family of IRS proteins, IRS1 and IRS2 exhibit a wide range of tissue expression, including muscle, fat, liver, and pancreatic islets. IRS3 is expressed in adipose tissue, fibroblasts, and liver, while IRS4 is detected in brain, thymus, and embryonic kidney. IRS5 is ubiquitously expressed but most abundant in kidney and liver. IRS6 expression is highest in skeletal muscle. Deletion of IRS1 produces small insulin-resistant mice with nearly normal glucose homeostasis because of β-cell expansion and compensatory hyperinsulinemia. Mice lacking IRS2 display nearly normal growth but develop life-threatening diabetes at 8–15 weeks of age because of reduced β-cell mass and insufficient compensatory insulin secretion. IRS3 knockout mice do not have an obvious phenotype, and IRS4 null mice also appear normal with the exception of reduced fertility. Therefore, IRS1 and IRS2 are considered to be the IRS isoforms critically important in glucose homeostasis. IRS1 functions as the principal IRS in skeletal muscle, and IRS2 predominates in liver and β-cell, where insulin action is required for normal β-cell growth and development.

IRS proteins are a key locus for regulation of insulin action. One mechanism occurs at the level of IRS protein expression; for example, IRS1 and IRS2 proteins are decreased by hyperinsulinemia [12]. Cell loss of IRS could occur through accelerated protein degradation by induction of ubiquitin-mediated degradation of IRS1 and IRS2 by suppressor of cytokine signaling (SOCS) proteins [13], or by inhibition of IRS gene transcription. Regardless of the mechanism, decreased levels of IRS proteins in hyperinsulinemic states, coupled with downregulation of the insulin receptor itself, certainly contribute to the insulin resistance in diabetes [14]. The function of IRS proteins can also be negatively regulated by serine–threonine kinases and protein tyrosine phosphatases such as such as PTP1B and SHP2, as described below [15]. In addition, IRS1 can be post-translationally modified by either O-linked N-acetylglucosamine adducts (O-GlcNAc) on serine–threonine residues under hyperglycemic conditions [16], or S-nitrosylation as a consequence of nitric oxide generation [17]. These modifications induce the proteasomal downregulation of IRS1 and insulin resistance.

## $PI_3$ kinase

Among several proteins that to bind to IRS1/2, $PI_3$ kinase is critical for signal transduction mediating the metabolic effects of insulin, including stimulation of glucose uptake into skeletal muscle and adipose tissues. $PI_3$ kinase activity represents a family of related enzymes that are capable of phosphorylating the hydroxyl groups in the inositol ring of membrane-associated phosphatidylinositol (PtdIns) [18]. The generation of $PI(3,4,5)P_3$ propagates the insulin signal, and therefore the class I subset of PI3K is responsible for the downstream action of receptor tyrosine kinases (including the insulin receptor) and Ras/MAPK [19]. Class I $PI_3$ kinases are heterodimeric molecules composed of one regulatory and one catalytic subunit. There are currently five known regulatory subunits, designated p85α, p55α, p50α, p85β or p55γ (known collectively as the p85 subunit), and one of these regulatory subunits is conjoined with one of four known p110 catalytic subunits, p110α, p110β, p110δ or p110γ [20]. Under normal conditions, p85 regulatory subunits are present in excess compared with the amount of the p85–p110 complex, and can serve as negative regulators of insulin action. One explanation pertains to the stoichiometry of free p85 and p85–p110 complexes because free p85 may be able to compete with the p85–p110 complex for recruitment to IRS phosphotyrosine docking sites (i.e. the "free p85" model) [21]. Accordingly, increased expression of p85 can worsen insulin sensitivity as demonstrated in patients with gestational diabetes or obesity who have increased levels of p85 in skeletal muscle. The p85 subunit can also exert negative modulatory effects via cross-talk with stress-kinase pathways. Recent studies have shown that p85 is required for the insulin-stimulated activation of c-Jun $NH_2$-terminal kinase (JNK) that occurs in states of insulin resistance, including high-fat diet-induced obesity and JNK overexpression [22]. Because the JNK pathway suppresses insulin action, the involvement of p85 in JNK activation provides a mechanism for cross-talk between the $PI_3$ kinase signaling pathway and JNK-mediated stress or inflammatory responses. Also, p85 is able to suppress insulin action via positive regulation of the phosphatase and tensin homolog (PTEN), a phosphoinositide phosphatase that degrades $PI(3,4,5)P_3$ and inhibits downstream insulin signaling [23].

### 3-Phosphoinositide-dependent protein kinase 1

Insulin-mediated activation of $PI_3$ kinase results in phosphorylation of the inositol ring at the 3′ position of phosphatidylinositol in membrane glycolipids, generating $PI(3,4,5)P_3$. This leads to recruitment of certain signaling proteins with PH domains to the plasma membrane. Binding to membrane-associated phosphoinositides both activates these proteins and positions them for downstream signal transduction. 3-Phosphoinositide-dependent protein kinase 1 (PDK1) can interact with $PI(3,4,5)P_3$, and is responsible for downstream activation of Akt/PKB and aPKCs. PDK1 phosphorylates the activation loops of Akt/PKB on $Thr^{308}$, and PKCζ on $Thr^{410}$, enhancing the activity of these kinases.

## Akt/protein kinase B

Phosphorylation and activation of Akt/PKB mediates various insulin- and growth factor-induced cellular responses, such as the stimulation of GLUT-4 translocation to the plasma membrane, the inhibition of glycogen synthase kinase 3 (GSK-3), induction of triglyceride synthesis via increasing the expression of sterol regulatory element-binding proteins 1c (SREBP-1c), and the promotion of cell survival by inhibiting apoptosis. Akt/PKB is a serine–threonine kinase with multiple substrates including kinases, signaling proteins, and transcription factors, such as cyclin-dependent kinase inhibitor p21kip, GSK3β, Bcl-2 antagonist of cell death, AS160, endothelial NO synthase, forkhead box class O1 (Foxo 1) and others. There are three isoforms of Akt/PKB in mammals, each encoded by a different gene (Akt1, Akt2 and Akt3). Akt1 and Ak2 are widely distributed; however, Akt2 is predominant in insulin-sensitive tissues such as liver and fat. Akt3 is largely expressed in the nervous system and testis.

Several lines of evidence implicate a role for Akt/PKB activation in the stimulation of glucose transport and other insulin-induced biologic processes. Expression of constitutively active membrane-bound forms of Akt/PKB results in persistent translocation of GLUT-4 to the plasma membrane in muscle and fat cells [24]. Deletion of Akt1 results in growth retardation and reduced lifespan without metabolic abnormalities [25]. In contrast, Akt2-deficient mice display insulin resistance and develop diabetes as a result of the inability of insulin to stimulate glucose utilization and decrease hepatic glucose output [26]. Mechanistic studies have suggested that Akt2 is the key isoform transducing effects on GLUT-4 glucose uptake; however, Akt/PKB activity alone cannot fully explain the ability of insulin to promote GLUT-4 translocation and stimulate glucose transport [27].

Akt/PKB activation is involved in multiple other insulin responses. One of the first substrates indentified for Akt/PKB was GSK3β. Phosphorylation of GSK3β decreases its activity towards glycogen synthase, which leads to increased glycogen synthesis [28]. Akt/PKB seems to stimulate glucose transport concomitant with the phosphorylation of Akt substrate of 160 kDa (AS160), a Rab-GTPase-activating protein (GAP) [29]. Under basal conditions, a functional GAP domain within AS160 is necessary to maintain the intracellular localization of GLUT-4 [30]. Following insulin stimulation, phosphorylation of AS160 shuts off its GAP activity, shifting the equilibrium of its target Rab(s) to an active GTP-bound form, and this enables GLUT-4 translocation by releasing GLUT-4 from intracellular retention mechanisms [30]. As expected, this effect is blocked by pre-exposure to the $PI_3$ kinase inhibitor wortmannin, indicative of signaling through $PI_3$ kinase. This response involving AS160 is impaired in skeletal muscle collected from insulin-resistant patients.

## Atypical protein kinase C

Protein kinase C isoforms are categorized as conventional (α,β,γ) (cPKC), novel (δ,θ,ε,η,μ) (nPKC), and atypical (ζ,λ) (aPKC) depending on their ability to be activated by calcium and diacylglycerol (DAG). cPKCs and nPKCs serve primarily as negative feedback inhibitors of the insulin receptor and IRS, and are discussed below. The aPKCs participate in signal transduction, and like Akt/PKB, are activated by the $PI_3$ kinase pathway and help mediate insulin's metabolic effects. The two aPKC isoenzymes ζ and λ vary in their tissue-specific expression and among various mammals; for example, PKCλ is the main aPKC in the skeletal muscle and adipose tissue of the mouse, whereas PKCζ is more prominent in rats, monkeys, and humans. Activation of PKCζ and PKCλ occurs proximal to Akt/PKB as a result of direct interaction with 3′ phosphoinositides and/or through phosphorylation and activation by PDK1. aPKCs have been shown to have a role in insulin-stimulated glucose uptake and GLUT-4 translocation in adipocytes and muscle [31]. Stimulation of glucose transport and translocation of GLUT-4 vesicles by aPKC may be brought about by effects on the actin cytoskeleton, because PKCλ/ζ can impinge on Rac and actin dynamics [32]. Overexpression of constitutively active forms of PKCζ and PKCλ increases, and expression of dominant negative forms of these aPKCs reduces, both glucose transport activity and GLUT-4 translocation in response to insulin (31). Decreased activation of aPKCs has been reported in the muscle of humans with T2DM and rodents with insulin resistance [33]. Compellingly, a mouse model with a muscle-selective PKCλ gene deletion displays whole-body insulin resistance, impaired insulin-stimulated glucose uptake into muscle and reduced GLUT-4 translocation. The mechanism by which aPKC participates in metabolic signaling remains to be fully elucidated.

## CAP/Cbl/TC10 pathway

In parallel to the $PI_3$ kinase pathway, substantial evidence has confirmed the participation of the CAP/Cbl/TC10 pathway in glucose transport stimulation [34]. This pathway diverges at the level of the insulin receptor kinase, which mediates tyrosine phosphorylation of the Cbl proto-oncogene through a process that does not involve IRSs. This phosphorylation step requires recruitment of the adapter protein APS, which contains SH2 and PH domains, to the insulin receptor β-subunit and the subsequent binding of Cbl to APS [35]. APS interacts via its SH2 domain with phosphotyrosines in the activation loop of the insulin receptor. Upon binding to the receptor, APS is phosphorylated on a C-terminal tyrosine, which permits the recruitment of Cbl to APS via the SH2 domain of Cbl, and subsequent tyrosine phosphorylation of Cbl. The Cbl associated protein (CAP) is also recruited with Cbl to the insulin receptor–APS complex via tandem SH3 domains in the COOH-terminus of CAP that bind to a proline-rich domain in Cbl. CAP also contains a sorbin homology (SoHo) domain in the NH2-terminal region. After disengagement of CAP/Cbl from the insulin receptor, the CAP SoHo domain binds to flotillin in caveolin-containing lipid rafts in the plasma membrane. Lipid rafts are plasma membrane domains, enriched in cholesterol, glycolipids, and sphingolipids, that coordinate signaling events by accumulating specific protein constituents.

Once the CAP/Cbl complex is bound to flotillin, tyrosine phosphorylated Cbl presents a recognition site for recruitment of the CrkII–C3G complex to the lipid raft. CrkII binds to specific phosphorylation sites on Cbl via its SH2 domain and is constitutively associated with the nucleotide exchange factor C3G via its SH3 domain. C3G is a guanyl nucleotide exchange factor for TC10 and other small molecular weight GTP-binding proteins. TC10 is a member of the Rho family of GTPases, and can target to lipid raft domains as a result of its capacity to undergo post-translational modification by farnesylation and palmitoylation. The Rab proteins cycle between GTP- and GDP-bound states to affect vesicle budding from donor membranes and fusion with acceptor membranes. TC10 is known to regulate the actin cytoskeleton [36], and downstream effectors of TC10 have been identified which have been proposed to have a role in the translocation of GLUT-4 [37].

### Ras-p38 MAPK pathway

Another component of signal divergence emanating from IRS docking proteins is the engagement of the Ras/MAPK signaling pathway, which has a critical role in cell growth and mitogenesis. Following insulin-mediated tyrosine phosphorylation of IRS, one of the SH2 domain-containing proteins that docks with IRS is Grb-2, a small cytosolic adapter protein. Grb-2 also contains an SH3 binding domain that binds proteins containing proline-rich sequences, and one of these proteins is SOS (mammalian homolog of the *Drosophila* son-of-sevenless protein), a GDP/GTP exchange factor. This interaction positions SOS for activation of the Ras/MAPK pathway. SOS facilitates GTP activation of membrane-bound Ras, the 21 kDa small molecular weight GTPase, and the GTP-bound form of Ras complexes with and activates Raf-1 kinase. Raf-1 kinase then initiates a cascade leading to sequential phosphorylation and activation of the dual-specificity kinase MEK (MAPK/ERK kinase), which in turn phosphorylates extracellular regulated kinases (ERK1 and ERK2) on threonine and tyrosine residues in their activation loops. Activated ERKs phosphorylate multiple targets that mediate the mitogenic actions of the Ras/MAPK pathway and the growth promoting effects of insulin [38]. Insulin receptors can also mediate activation of the Ras/MAPK pathway through another substrate docking molecule, SHC. Independent of IRS, SHC can also activate the Ras/Raf-1/MEK phosphorylation cascade by forming a complex with Grb-2/SOS in response to insulin. Whether activated through IRS or SHC, ERK1/2 translocates into the nucleus and phosphorylates transcription factors, such as ELK-1, thus modulating DNA binding properties and regulation of gene transcription. ERK also phosphorylates p90 ribosomal protein S6 kinase (p90 S6 kinase), which can phosphorylate and regulate the activity of transcription factors such as c-fos. In addition, the MAPK cascade is one of the pathways with the potential to stimulate glycogen synthase because p90 S6 kinase is able to activate the glycogen-associated protein phosphatase-1, which in turn dephosphorylates and activates glycogen synthase [39]. In this way, the MAPK pathway has the potential to interact with metabolic signaling pathways. However, the MAPK pathway is not necessary for stimulation of glucose transport, and is not viewed as being critically related to the metabolic effects of insulin [40].

## Inhibition of insulin signal transduction

The promulgation of insulin signaling pathways does not proceed unabated; rather, there is an extensive array of mechanisms that dampen or inhibit signal transduction. These inhibitory mechanisms can represent normal dynamic functioning of insulin action as organisms adapt to changing physiologic conditions, or, when unbalanced, can lead to pathophysiologic consequences and the development of insulin resistant states. The elucidation of inhibitory processes has provided insight into the extensive network of regulated insulin action pathways, and has also identified potential therapeutic targets because blocking these inhibitory mechanisms could enhance insulin sensitivity.

### Inhibition of the insulin receptor

As the first critical component in the insulin signaling network, there are also multiple mechanisms that can desensitize insulin action at the level of the insulin receptor. For example, it has long been known that persistent insulin stimulation can lead to loss of cell surface insulin receptors and this downregulation event impairs insulin sensitivity. In addition, multiple cellular proteins and processes can negatively regulate the intrinsic tyrosine kinase activity of the insulin receptor. Two mechanisms (serine–threonine phosphorylation and protein tyrosine phosphatases) act to impair both insulin receptors and IRS docking molecules.

#### Downregulation of insulin receptor number

Chronic exposure to high insulin concentrations leads to loss of insulin receptors, resulting in a rightward shift in insulin dose–response curves (i.e. impaired insulin sensitivity). This is caused by increased ligand-mediated internalization of insulin receptors followed by lysosomal degradation, as well as diminished gene expression. Insulin stimulation results in phosphorylation of Foxo1, which disrupts its interaction with the insulin receptor promoter, decreasing insulin receptor gene transcription, thus contributing to receptor loss and insulin resistance [41].

#### Plasma differentiation factor 1

Plasma differentiation factor 1 (PC-1), also referred to as ectonucleotide pyrophosphatase phosphodiesterase 1, is a membrane glycoprotein with pyrophosphatase activity that appears to act as an intrinsic inhibitor of the insulin receptor tyrosine kinase [42]. PC-1 binds to amino acids 485–599 of the insulin receptor connecting domain, a region required for the conformational change in receptor β-subunits that permit autophosphorylation upon insulin binding. This interaction with PC-1 interferes with the close opposition of the two β-subunits required for transphosphorylation. Muscle expression of PC-1 is elevated in patients with diabetes and in obesity, and it correlates with diminished

insulin receptor tyrosine phosphorylation and muscle glucose uptake. Analysis of PC-1 gene polymorphisms in a variety of individuals and family cohorts suggests that alterations in this gene are associated with the risk for development of childhood and adult obesity as well as T2DM [43].

### Grb proteins

The growth factor receptor-bound proteins (Grb proteins) constitute a family of structurally related multi-domain adapters with diverse cellular functions but lacking intrinsic enzymatic activity. Grb10 and Grb14 can bind to phosphotyrosine residues on the insulin receptor and alter receptor tyrosine kinase activity [44]. Overexpression of Grb10 and Grb14 in cells inhibits insulin-stimulated phosphorylation of IRS1, IRS2 and Shc [45]. However, the physiologic role of Grb proteins is not fully clear, and their actions may be tissue specific with capabilities as an inhibitory factor or a positive mediator in the insulin signaling pathway.

## Inhibition of insulin receptor substrate proteins

### Protein phosphotyrosine phosphatases

Endogenous protein phosphotyrosine phosphatases (PTPases) are able to dephosphorylate tyrosine residues on the insulin receptor β-subunit and insulin receptor substrate docking molecules, resulting in a dampening of insulin signal transduction. Two PTPases in particular, PTP-1B and leukocyte common antigen-related phosphatase (LAR), contribute to insulin receptor dephosphorylation in insulin target cells [46]. Membrane-associated PTPase activity is increased in skeletal muscle from patients with T2DM [47], principally because of increments in cytosolic PTPase-1B and membrane-associated LAR [48]. The ability of PTPases to modulate insulin signaling has been demonstrated in mice with genetic ablation of PTPase-1B, which exhibit enhanced insulin sensitivity, increased insulin-mediated tyrosine phosphorylation of the receptor and of IRS1, and a failure to develop insulin resistance when fed a high fat diet [46]. Muscle-specific overexpression of PTP-1B in mice induces tissue insulin resistance with decreased capacity for insulin receptor autophosphorylation [49]. Thus, available data consistently demonstrate that PTPase-1B is able to modulate insulin signaling negatively through dephosphorylation of tyrosine residues in the insulin receptor and IRSs. The role of the LAR in insulin receptor function remains less well defined.

### Serine–threonine phosphorylation

Serine–threonine phosphorylation of insulin receptors and IRS docking proteins is a major mechanism for negative modulation of insulin signal transduction. Serine phosphorylation diminishes insulin receptor tyrosine kinase activity and decreases receptor–IRS coupling by inhibiting insulin-mediated tyrosine phosphorylation of IRS-1, binding and activation of $PI_3$ kinase, and stimulation of glucose transport. There are consensus sequences in IRS-1 that make it susceptible to a wide variety of serine–threonine kinases including PKC, PKA, Akt/PKB, MAPK, S6 kinase, GSK3, casein kinase II, Cdc2 kinase, JNK and IκB kinase (IKKβ). Several of these kinases have been shown to function as physiologic modulators causing desensitization of insulin signaling pathways under conditions of nutrient excess, inflammation and cell stress responses. For example, JNK and IKKβ are activated by inflammatory stimuli (e.g. TNFα) contributing to insulin resistance, and PKC is activated by DAG which accumulates with increased availability of free fatty acids.

#### *Protein kinase C*

PKCs are serine–threonine kinases with multiple substrates, including IRS docking proteins and the insulin receptor [50]. Serine–threonine phosphorylation of IRS impairs its ability to associate with the insulin receptor and with $PI_3$ kinase, resulting in desensitization of the $PI_3$ kinase pathway. Hyperinsulinemia, hyperglycemia and elevated circulating free fatty acids (e.g. nutrient excess) leads to increased intracellular DAG, which in turn activates conventional and novel PKC isoforms principally via recruitment to the plasma membrane. These conditions are associated with increased serine–threonine phosphorylation and diminished function of insulin receptors and IRS proteins. In addition, insulin activates atypical PKCs, such as PKCζ, via the $PI_3$ kinase pathway, which is also capable of phosphorylating and desensitizing IRS [51].

#### *Tumor necrosis factor α*

Tumor necrosis factor α (TNF-α) is a cytokine produced by immune cells and also by adipocytes and muscle tissue. While having little impact on systemic circulating concentrations, TNF-α expression is increased in adipose and muscle tissues as a function of insulin resistance [52]. In adipose tissue, TNF-α and other proinflammatory cytokines are produced by adipocytes and also by macrophages that infiltrate adipose tissue under conditions of obesity and insulin resistance. This raises the possibility that the cytokine could be inducing cellular insulin resistance via autocrine and/or paracrine effects. TNF-α induces serine phosphorylation of IRS1, thereby decreasing its ability to be phosphorylated by the insulin receptor tyrosine kinase and impairing downstream insulin signal transduction [53]. Several pathways activated by TNF-α are implicated in increased serine phosphorylation of IRS-1, including the stress-induced kinases, JNK and IKKβ.

#### *JNK*

Three JNK-encoding genes have been described in mammals; JNK1 and JNK2 are expressed ubiquitously, whereas JNK3 expression is restricted to neuronal tissues. The JNK isoforms belong to the extended family of MAPKs, and control many cellular functions through regulation of activator protein 1 (AP-1). In addition to TNF-α signaling, insulin also activates JNK1 and JNK2 [54], which then display increased serine kinase activity against multiple intracellular substrates including IRS1, IRS2 and Shc. The ability of insulin to activate JNK represents a negative feedback mechanism by which insulin inhibits its own signaling.

JNK activity is also augmented during cellular stress responses, such as endoplasmic reticulum stress, and is increased in insulin-resistant states. Illustrative data include the observation that high-fat diets increase $Ser^{307}$ phosphorylation of IRS1 in wild-type mice but not in JNK1–/– mice, while the JNK1–/– mice are characterized by decreased adiposity, increased insulin receptor signaling and improved insulin sensitivity [55]. Thus, JNK has been implicated in the pathogenesis of insulin resistance in the metabolic syndrome and T2DM.

#### *NF-κB*

Activation of NF-κB-mediated pathways has been shown to inhibit insulin signaling through enhanced serine phosphorylation of IRS1 [56]. NF-κB is a transcription factor that functions as a proinflammatory "master switch" during inflammation, upregulating the transcription of a wide range of inflammatory mediators. NF-κB is normally retained in the cytoplasm by binding to members of the inhibitor of κB (IκB) protein family. During inflammatory or metabolic stress, NF-κB is activated by the IκB kinase (IKK) complex, which consists of two catalytic subunits, IKKα and IKKβ, and a regulatory subunit, IKKγ. The IKKβ subunit phosphorylates IκB, resulting in its ubiquitination and subsequent proteasomal degradation and in the release of NF-κB. The free NF-κB is then able to translocate into the nucleus and activate the transcription of at least 125 genes, most of which are proinflammatory [57]. There exists a substantial body of data implicating NF-κB in the pathogenesis of insulin resistance. Activation of NF-κB accompanies insulin resistance following high fat feeding and obesity, and in the metabolic syndrome. Proinflammatory cytokines such as TNF-α and interleukin 6 (IL-6) can also activate NF-κB. Circulating mononuclear cells in the obese display increased transcription of proinflammatory genes regulated by NF-κB with a decrease in IκB. Weight loss, caloric restriction and exercise training can lead to a reduction in transcription of the proinflammatory genes regulated by NF-κB [58]. Drugs with anti-inflammatory properties, such as thiazolidinediones, statins and salicylates, can inhibit proinflammatory cytokine secretion by interferring with the NF-κB pathway.

#### *IκB kinase-β*

IKKβ is a serine kinase that has the ability to desensitize insulin signaling through serine phosphorylation of IRS or the insulin receptor. The desensitizing effect of TNF-α, as a consequence of IRS serine phosphorylation, may in part be mediated through IKKβ. Consistent with this hypothesis, heterozygous ablation of the IKKβ gene in mice fed a high-fat diet, or in obese leptin-deficient *ob/ob* mice, prevents insulin resistance [59,60]. Mice that selectively express constitutively active IKKβ in hepatocytes (LIKK mice) exhibit a T2DM phenotype, characterized by hyperglycemia, profound hepatic insulin resistance, and moderate systemic insulin resistance including effects in muscle [56]. The hepatic production of proinflammatory cytokines, including IL-6, IL-1β and TNF-α, is increased in LIKK mice to a similar extent as induced by high-fat feeding in wild-type mice. However, no difference in obesity-induced insulin resistance is detectable in muscle-specific IKKβ knockout mice compared with wild type [61], which suggests that IKKβ may be more directly involved in the development of hepatic insulin resistance rather than in skeletal muscle.

An important biologic effect of salicylates is the inhibition of the IKKβ [62]. Predictably, salicylates would then enhance insulin sensitivity by causing a subsequent decrease in IKKβ-mediated serine phosphorylation of IRS. In fact, treatment with high doses of salicylates improves glucose tolerance and enhances insulin sensitivity in humans and rodents [59].

### Accelerated catabolism of $PI(3,4,5)P_3$ by phosphoinositide phosphatases

Increased production of $PI(3,4,5)P_3$ as a result of activated $PI_3$ kinase is key to metabolic insulin signaling, and the downstream activation of PDK1. PTEN can dephosphorylate $PI(3,4,5)P_3$ on the 3 position, and SH2-containing inositol phosphatases (SHIPs) can dephosphorylate $PI(3,4,5)P_3$ on the 5 position of the inositol ring; in either instance, the ability of $PI(3,4,5)P_3$ to stimulate PDK1 is lost. Therefore, PTEN and SHIP can exert negative regulatory influences on insulin signaling, particularly with respect to its metabolic actions [63]. While multiple studies support the view that PTEN can participate as a negative regulator of insulin action in pathophysiologic states, its role in human insulin resistance is yet to be defined.

### Inhibition of insulin signaling by protein–protein interactions

#### *Suppressor of cytokine signaling 3*

The SOCS family of proteins (CIS and SOCS 1–7) was originally described as a negative feedback loop for cytokine receptors involving Janus kinase (JaK). Following ligand activation of cytokine and growth factor receptors, JaK phosphorylates and activates signal transducer and activator of transcription (STAT), and these phosphorylated STAT family members then form homodimers or heterodimers that translocate to the cell nucleus where they act as transcription activators. In the nucleus, STAT augments SOCS gene expression. SOCS proteins then feedback to inhibit tyrosine-phosphorylated cytokine receptors, via either competitive binding through their SH2 domain preventing phosphorylation of cytokine receptor substrates, or by binding and inhibiting the action of JaK tyrosine kinases. Evidence suggests that a similar mechanism may be operative for the insulin receptor [46]. Studies have indicated that SOCS-1, SOCS-3 and SOCS-6 can bind to the COOH-terminus of the insulin receptor β-subunit, and block interaction between the insulin receptor and IRS [64]. Interestingly, several factors that induce cellular insulin resistance also induce SOCS-3 expression, including TNF-α, growth hormone, and leptin.

#### *Tribbles*

The Tribbles (TRB) gene family in mammals is comprised of three proteins that have a truncated kinase domain lacking an

ATP binding site. Accordingly, TRBs are "pseudokinases" that lack detectable kinase activity, but can bind to kinase substrates in phosphorylation cascades and inhibit their phosphorylation. TRB proteins, for example, can bind to Akt/PKB and inhibit its phosphorylation and activation in response to insulin [65], while downregulation of TRB3 improves insulin sensitivity [66].

## Tissue-specific insulin action: the role of insulin effector systems

Insulin regulates whole-body fuel homeostasis via specific effects in multiple target tissues. The nature of these biologic actions varies dramatically from tissue to tissue, and these variations, for the most part, are not brought about by differences in insulin signal transmission (described above). Rather, tissue-specific insulin effects are principally explained by effector systems that are uniquely expressed in a variety of differentiated target cells. The biochemical basis of these effects is described in skeletal muscle, adipose tissue, and liver, three organs primarily responsible for fuel storage and oxidation as well as counter-regulatory metabolism.

### Skeletal muscle

#### Insulin stimulation of glucose transport

Skeletal muscle accounts for the bulk of insulin-stimulated glucose uptake *in vivo*, and the hallmark of insulin action in this tissue is the ability to stimulate the glucose transport effector system (Figure 7.2). Glucose transport across the plasma membrane is facilitated by glucose transport proteins (GLUT); to date, 13 members of the GLUT/SLC2 family have been identified. All GLUT proteins are intimately embedded in membranes, and the most highly conserved regions are the putative membrane-spanning domains that serve a common function, the creation of a pore for facilitative diffusion of monosaccharides. Each glucose transporter isoform has a specific role in glucose metabolism determined by its pattern of tissue expression, substrate specificity and affinity, transport kinetics, and regulated expression in different physiologic conditions.

The major transporter isoforms that mediate glucose transport in cells with an insulin-responsive glucose transport system are GLUT-1 and GLUT-4. In unstimulated cells, GLUT-1 predominates at the cell surface and facilitates glucose diffusion across the plasma membrane into the cytosol where glucose is rapidly phosphorylated by hexokinase and metabolized. GLUT-4 contributes minimally to glucose transport in unstimulated target cells, because >90% of the cell content of GLUT-4 resides in intracellular membranes in the basal state. The mechanism by which insulin augments glucose transport activity is by recruiting intracellular GLUT-4 to the plasma membrane, a rate-limiting step for insulin-stimulated glucose uptake and metabolism in peripheral target tissues. Upon dissipation of the insulin signal, deactivation of glucose transport activity is the result of a net reverse translocation of GLUT-4 transporters back into the cell interior. Thus, GLUT-4 is the major transporter mediating insulin-stimulated glucose transport activity in tissues such as skeletal and cardiac muscle and adipose tissue.

In unstimulated muscle or adipose cells, a component of GLUT-4 resides in an inducible tubulo-vesicular storage compartment that includes the trans-Golgi network and endosomal vesicles located near the endofacial surface of the plasma membrane. However, another component of cellular GLUT-4 exists in an active endocytosis–endosomal recycling pathway that cycles GLUT-4 between endosomes and the plasma membrane. The recycling pathway results in the localization of approximately 4–10% of GLUT-4 in the basal plasma membrane, and this steady-state distribution is the balance of rapid endocytosis and slow recycling. Insulin shifts the distribution of GLUT-4 from intracellular pools towards the plasma membrane, both by elevating the exocytotic rate of GLUT-4 in the recycling pathway and by recruiting GLUT-4 from the inducible storage compartment to the cell surface. Deactivation of transport is accomplished via a slowing of the exocytotic rate and an acceleration of the endocytotic rate, as GLUT-4 is retrieved from the plasma membrane through clathrin-dependent and -independent mechanisms [67,68].

GLUT-4 vesicle trafficking involves the actin and microtubule cytoskeletons. Regarding actin, insulin stimulates cytoskeletal rearrangement with the appearance of cortical β-actin fiber projections that subtend the plasma membrane, and this actin remodeling is under the control of small G-proteins in the Rho, Rab and Rac families. Microtubules surround the inducible intracellular depot of GLUT-4 and microtubule proteins such as dynein and kinesin have been co-purified with GLUT-4. Inhibition of actin remodeling or disruption of microtubules using depolymerizing agents inhibits GLUT-4 translocation and glucose transport stimulation. Data suggest that exocytotic movement of GLUT-4 begins with its transfer to actin scaffolds that connect the microtubule cytoskeleton with the plasma membrane, which positions GLUT-4 vesicles for docking and membrane fusion.

The complete pathway linking insulin signal transduction to stimulation of the glucose transport system has not been fully elucidated. Activation of transport is critically dependent on insulin-mediated autophosphorylation of insulin receptors, tyrosine phosphorylation of IRS1 and activation of $PI_3$ kinase. The production of $PI(3,4,5)P_3$ by $PI_3$ kinase activates PDK1. At this point, PDK1 activates two separate kinase pathways that contribute to GLUT-4 translocation and stimulation of glucose transport activity, Akt2 and atypical PKCs (PKCζ and PKCλ). Akt2 then phosphorylates and inhibits AS160 (TBC1D4) and TBC1D1 [69], which are Rab-GTPase-activating proteins. The modulation of AS160 activates Rab small GTPases that in turn regulate aspects of GLUT-4 vesicle docking and cytoskeletal organization [70]. At the same time, the ligand-bound insulin receptor activates the CAP/Cbl/TC10 pathway upstream of IRS1 phosphorylation, and activates TC10, a Rho family member that regulates the actin cytoskeleton. Importantly, the factors

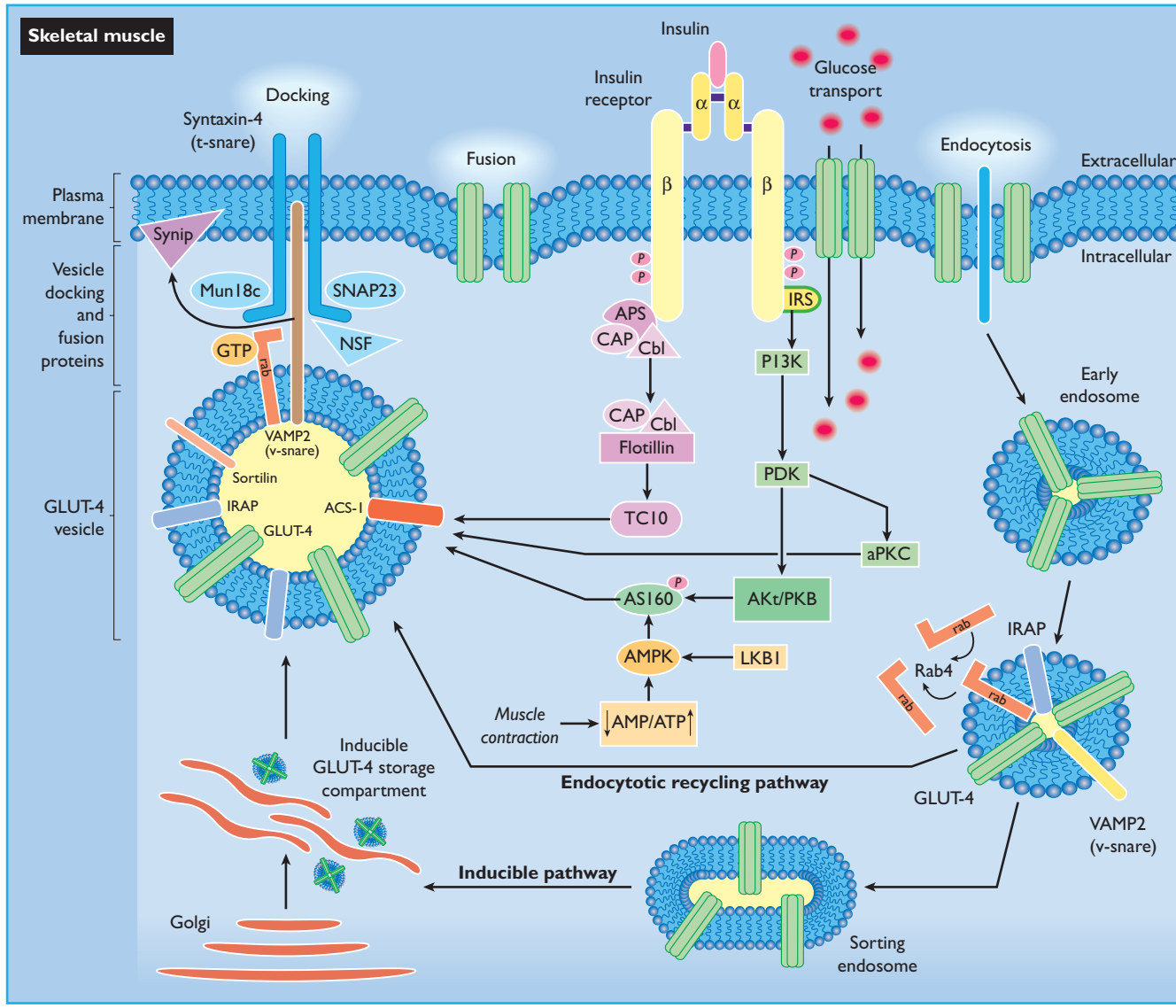


**Figure 7.2** An overview of insulin signaling pathways in skeletal muscle. Arrows represent an activation process.

that link TC10, AS160, atypical PKCs, and other relevant factors with the glucose transport effector system, and which impel the complex changes in GLUT-4 vesicle trafficking associated with transporter translocation, are unknown.

### Muscle contraction: stimulation of glucose transport independent of insulin signaling

Muscle contraction results in GLUT-4 translocation and stimulates muscle glucose transport activity [71]. This effect occurs without any change in serum insulin concentration and does not involve activation of insulin receptors, $PI_3$ kinase or Akt/PKB. The mobilization of GLUT-4 to the cell surface by acute exercise involves a different intracellular pool of GLUT-4 than that recruited by insulin, and the effects of acute exercise and insulin are partially additive. These observations indicate that signaling systems mediating glucose transport stimulation are different in response to acute exercise versus insulin [72]. This is underscored by the finding that, in muscle from insulin resistant humans and rodents, GLUT-4 translocation is impaired in response to insulin but normal in response to acute exercise.

While signal transduction mechanisms are not fully understood, this response appears to be at least partially dependent on increments in intracellular 5′AMP, and subsequent activation of adenosine 5′monophosphate-activated protein kinase (AMPK) [73]. AMPK is a serine–threonine kinase that responds to fluctuations in cellular energy levels and functions to maintain energy homeostasis. When ATP levels are low and 5′AMP is elevated, AMPK activates pathways for ATP regeneration and limits further ATP utilization by modifying activity of multiple metabolic enzymes, including acetyl-CoA carboxylase (ACC), hydroxymeth-

ylglutaryl-CoA reductase, creatine kinase, and hormone sensitive lipase. Exercise, ischemia, and hypoxia will activate AMPK via increments in AMP:ATP and creatine:phosphocreatine ratios. AMPK can also be activated, however, though allosteric modification and α-subunit phosphorylation by one or more upstream kinases, including LKB1.

## Adipose tissue

Adipose tissue is the predominant site for fuel storage as triglyceride, and effector systems responsible for the anabolic effects of insulin on lipogenesis and antilipolysis are key aspects of adipocyte biology (Figure 7.3).

### Lipogenesis

Fat accumulation in adipocytes is determined by the balance between triglyceride synthesis (fatty acid uptake and lipogenesis) and breakdown (lipolysis/fatty acid oxidation). Insulin is a critical stimulator of lipogenesis. Insulin augments availability of both glycerol and fatty acids for triglyceride synthesis by increasing the uptake of glucose in the adipose cell as well as by activating lipogenic and glycolytic enzymes. These enzymes constitute the effector system for the biologic effects of insulin on lipogenesis, and are modulated by insulin both through post-translational modifications and alteration of gene expression. Regarding post-translational effects, insulin activates Akt/PKB via phosphorylation, and its role in lipogenesis as illustrated by the observation that constitutively active Akt results in high levels of lipogenesis in 3T3-L1 adipocytes. Substrates for activated Akt/PKB include the phosphorylation and inhibition of GSK3, and this in turn abrogates GSK3 inhibition of ATP citrate lyase; the resulting increase in ATP citrase lyase activity enhances conversion of citrate to acetyl-CoA in the cytosol. Acetyl-CoA is then available as the "building block" for fatty acid synthesis.

Insulin also induces gene expression of two key lipogenic proteins: fatty acid synthase (FAS) and SREBP-1. FAS is the central enzyme participating in *de novo* lipogenesis and catalyzes the

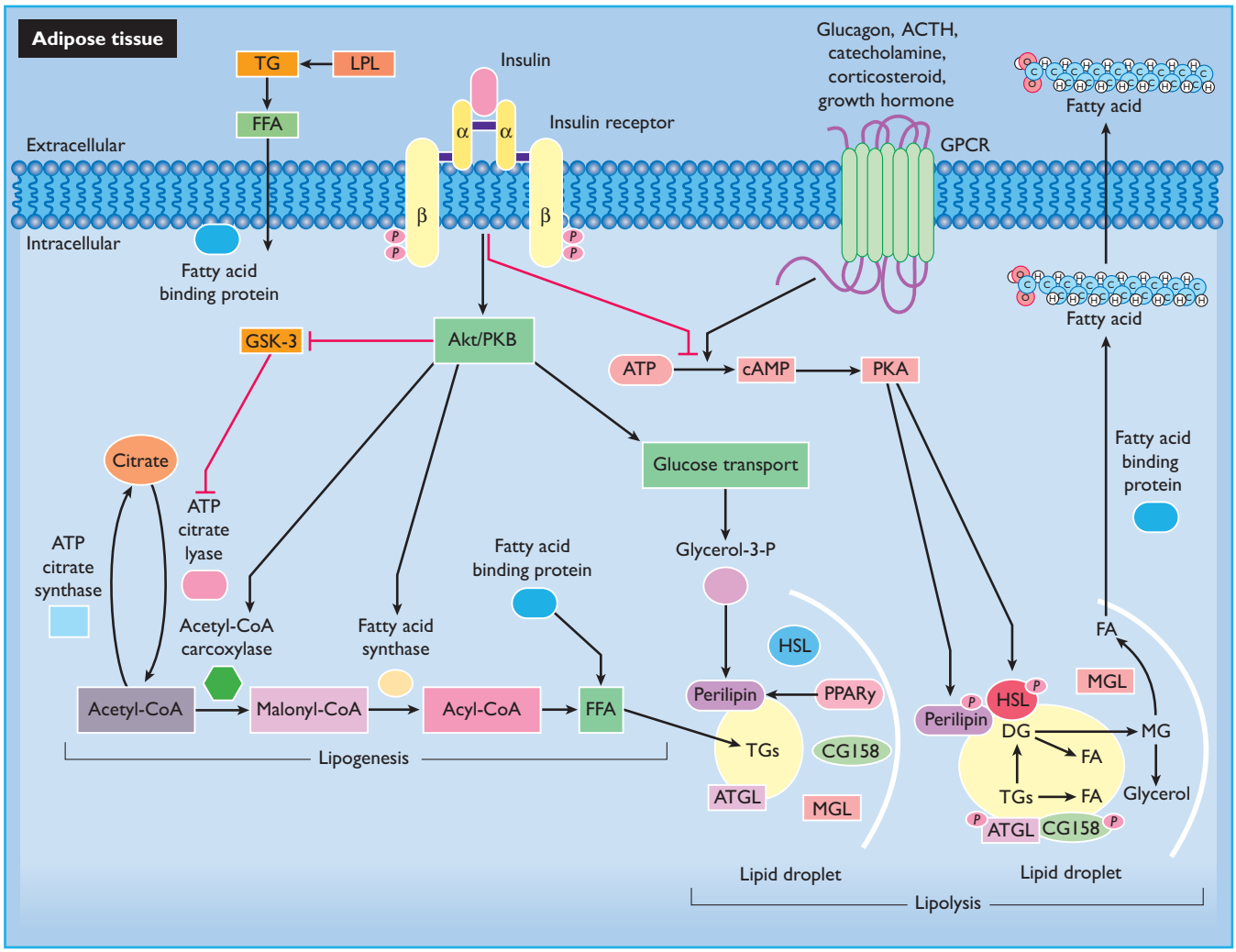


**Figure 7.3** Summary of insulin function involved in lipogeneis and lipolysis in adipose tissue. Arrows represent an activation process; blocked arrows represent an inhibition process.

conversion of malonyl-CoA and acetyl-CoA to long-chain fatty acids. Regulation of FAS activity by insulin occurs mainly at the level of gene transcription. SREBP-1 belongs to the class C bHLH (helix-loop-helix) transcription factor family based on its ability to bind to the sterol regulatory element (SRE) of the low-density lipoprotein receptor gene promoter. It activates a battery of genes involved in the uptake and synthesis of fatty acids and triacylglycerides. SREBP-1c is under transcriptional control by insulin [74], and overexpression of SREBP-1c mimics the effect of insulin on the expression of FAS. SREBP-1 also regulates FAS expression as evidenced by the failure of FAS and other lipogenic enzyme genes to be induced by fasting/refeeding in SREBP-1c–/– mice [75].

### Lipolysis and antilipolysis

Lipolysis in adipose tissue is tightly regulated to assure that partitioning of metabolic fuels, glucose and free fatty acids (FFA), is adapted to energy needs. During fasting, lipolysis is enhanced to make available FFAs that are the main oxidative fuel for the liver, the heart and skeletal muscle, and are metabolized by the liver to ketones that replace glucose as the principal fuel for nervous tissue. Upon feeding, lipolysis is abated and adipocytes convert to triglyceride storage. The rise (after a meal) and fall (with fasting) of insulin has a central role in this regulatory process as a result of its antilipolytic action in adipocytes. Lipolysis in normal subjects is exquisitely sensitive to inhibition by insulin, such that half-maximal suppression of lipolysis occurs at insulin concentrations well below those needed for significant stimulation of glucose uptake by skeletal muscle. Higher concentrations of insulin can reduce adipocyte release of FFA to nearly zero, although at high insulin concentrations there will still be some appearance of glycerol and FFA from the stimulatory effect of insulin on lipoprotein lipase which acts on triglycerides in circulating lipoproteins.

Hormones regulate lipolysis in adipocytes via coordinated action involving two major effector systems: hormone sensitive lipase (HSL) and perilipins localized to the surface of lipid droplets. Lipolytic and antilipolytic (i.e. insulin) hormones exert opposite effects on HSL and perilipins by determining cAMP availability and protein kinase A (PKA) activity. By way of illustration, catecholamines induce lipolysis and release FFAs from adipocytes by binding to β-adrenergic receptors coupled by heterotrimeric G-proteins to adenylate cyclase, which increases production of cAMP and activates PKA. The two main targets for PKA phosphorylation are HSL and the perilipins [76]. The ability of insulin to antagonize hormone-induced lipolysis is to a large extent accounted for by its ability to lower cAMP levels and thereby reduce PKA activity. The decrease in cAMP is mainly the result of an insulin-mediated phosphorylation and activation of phosphodiesterase 3B (PDE3B) via Akt/PKB. HSL is a key enzyme for the mobilization of triglycerides deposited in adipose tissue following its activation by cAMP/PKA-dependant phosphorylation [77]. HSL is an enzyme with three isoforms ranging from 84 to 130 kDa, yet all isoforms have three domains, a catalytic domain, a regulatory domain with several serine phosphorylation sites required for activation, and an N-terminal variable domain involved in protein–protein and protein–lipid interactions [78]. HSL in muscle can also be stimulated by adrenaline via β-adrenergic activation of PKA, or by muscle contraction via phosphorylation by PKC at least partly activated through the ERK pathway.

Perilipins are localized at the surface of the lipid droplet in adipocytes [79], and are essential in the regulation of triglyceride deposition and mobilization. In the absence of lipolytic stimulation, perilipin inhibits lipolysis by acting as a barrier against hydrolysis of the triacylglycerol by lipases. When PKA is activated, perilipin becomes phosphorylated and translocates away from the lipid droplet, which allows HSL to hydrolyze the lipid droplet triglyceride core [80]. Insulin blocks lipolysis by inhibiting PKA-mediated phosphorylation of HSL and perilipin, thus reducing both HSL activity and its access to triglycerides in the lipid droplet. In adipocytes there are two forms of perilipin, perilipin A and perilipin B, with perilipin A present at a higher concentration. Adipocyte perilipin content has an inverse correlation with lipolytic rates and a positive correlation with plasma glycerol in humans, and is reduced in obese women. Perilipin knockout mice are lean with increased basal lipolysis and are resistant to diet-induced obesity; however, these mice develop glucose intolerance and insulin resistance more readily, probably because of elevated levels of serum FFAs [81]. In addition, perilipin has a peroxisome proliferator-activated receptor γ (PPARγ) responsive element in its promoter region and is induced by thiazolidinedione agonists of PPARγ [82].

## Liver

Insulin regulates hepatic metabolism through acute post-translational modifications of enzymes, such as phosphorylation, and through changes in gene expression. The stimulation of glycogen formation and regulation of gluconeogenesis by insulin are the critical determinants of hepatic glucose output. In addition, because regulation of gene transcription is critical for the biologic effects of insulin on hepatic metabolism, mechanisms pertinent to transcriptional regulation are discussed (Figure 7.4).

### Glycogenesis/glycogenolysis

Insulin exerts dramatic effects on pathways of intracellular glucose metabolism. Under conditions of insulin stimulation, the major portion of glucose uptake is stored as glycogen in humans. Insulin promotes glycogen synthesis in muscle, adipocytes, and liver by activating glycogen synthase, which adds activated glucosyl groups to growing polysaccharide chains and thus catalyzes the final step in glycogen synthesis. The regulation of glycogen synthase is complex. It involves allosteric activators, translocation of glycogen synthase to the plasma membrane in the presence of glucose metabolites and insulin [83], inhibition by phosphorylation on serine residues by different kinases, and activation by dephosphorylation by serine–threonine phosphatases such as

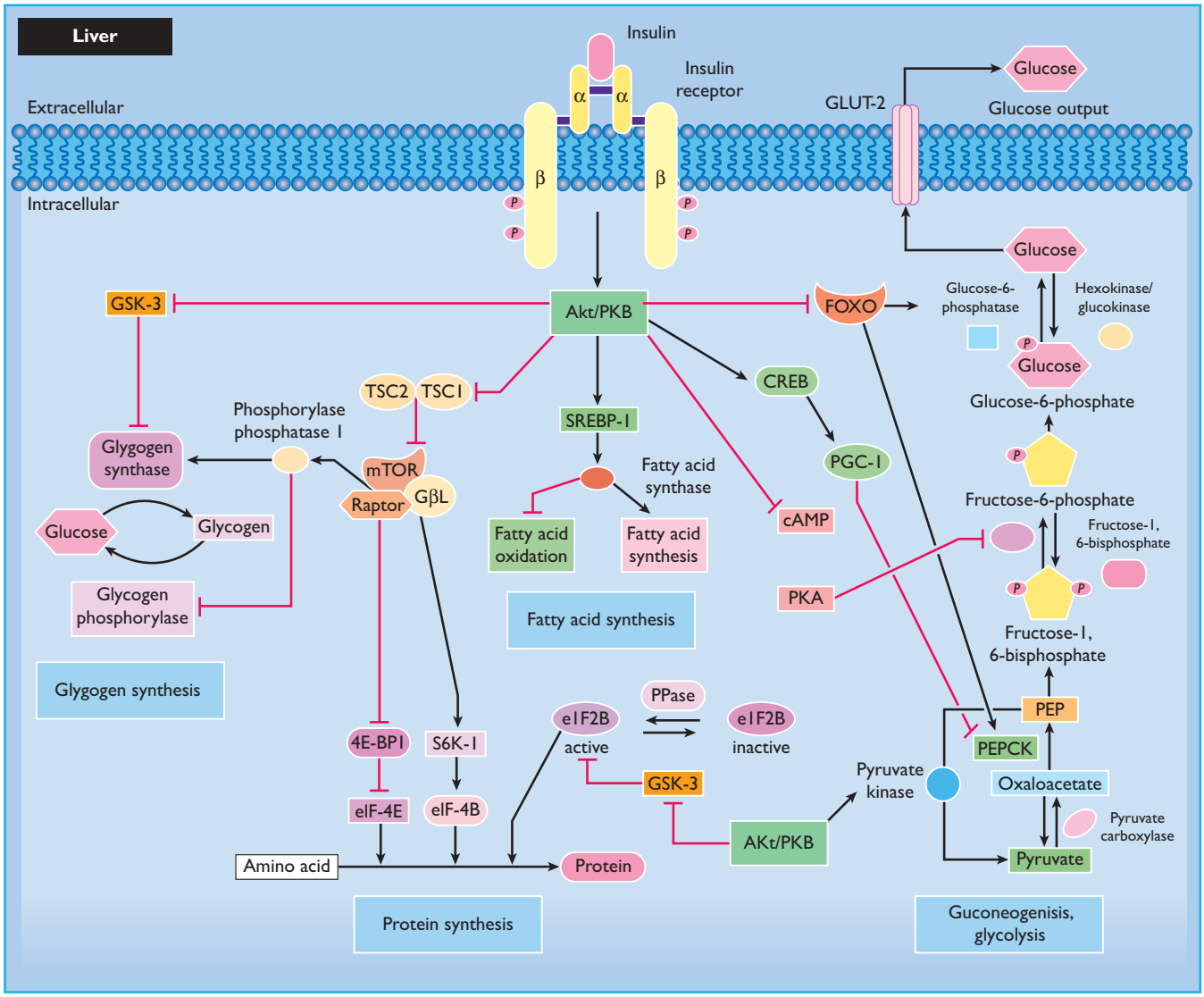


**Figure 7.4** Summary of insulin signaling pathways in the liver that are involed in glycogen synthesis, gluconeogenesis and glycolysis, and protein synthesis, respectively. Arrows represent an activation process; blocked arrows represent an inhibition process.

protein phosphatase 1 (PP1). The ability of insulin to stimulate glycogen synthase requires proximal signaling through activation of $PI_3$ kinase and Akt/PKB. One downstream pathway that activates glycogen synthase involves Akt/PKB-mediated phosphorylation and inactivation of GSK3, which results in a reduction in net phosphorylation of glycogen synthase. The reduction in glycogen synthase phosphorylation augments its activity. GSK3β inhibition is not insulin's only mechanism for stimulating glycogen accumulation. In 3T3-L1 adipocytes, insulin is capable of stimulating glycogen synthase even under experimental conditions when GSK3β is either not detectable or present in very low amounts. Moreover, lithium, a GSK3β inhibitor, is additive with insulin in activating glycogen synthesis and glycogen synthase activity [84]. These studies indicate the existence of additional pathways for glycogen synthase activation.

**Inhibition of gluconeogenesis and hepatic glucose output**

Hepatic glucose production is stimulated under fasting conditions by the counter-regulatory hormones glucagon, catecholamines, and glucocorticoids, which augment glucose output by promoting glycogenolysis and gluconeogenesis. During feeding and in response to exogenous insulin injections, hepatic glucose output is potently suppressed by insulin as a result of inhibition of glycogenolysis and gluconeogenesis. Gluconeogenesis is predominantly regulated through changes in gene expression for two key enzymes: phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G-6-Pase). PEPCK catalyzes one of the rate-limiting steps of gluconeogenesis, whereas G-6-Pase catalyzes the final step producing free glucose for transport out of liver via GLUT-2 glucose transporters. Gene transcription of PEPCK is tightly regulated by cAMP; counter-regulatory hor-

mones increase cAMP and induce PEPCK, whereas both are suppressed by insulin. In addition to direct hormonal effects on hepatocytes, hepatic glucose output is modulated by the delivery of gluconeogenic substrates to the liver such as lactate, amino acids, and FFA. For example, a reduction in FFA availability contributes to suppression of hepatic glucose output by insulin through its antilipolytic action in adipocytes, and insulin minimizes counter-regulatory effects of glucagon by inhibiting its secretion from the pancreatic α cell. Increased hepatic glucose production is an important determinant of fasting hyperglycemia in diabetes, and has been given greater focus because of the potential importance of regulatory pathways controlling hepatic glucose output as targets of drug therapy.

### *General considerations pertaining to gene regulation by insulin*

Suppression of hepatic glucose output by insulin relies heavily on induced changes in gene transcription. The ability of insulin to regulate gluconeogenesis via effects on gene transcription was first reported in 1963, with the observation that PEPCK expression is under negative regulation by insulin [85]. Over the ensuing five decades, it has become clear that insulin influences the expression of more than 800 genes in both positive and negative manners. Regulation of gene transcription can be brought about by effects on transcription factors that are directly modified via insulin signaling mechanisms, or that are indirect as the result of effects of substrate metabolism. The promoter elements (*cis*-acting sequences) that mediate changes in gene transcription are referred to as insulin response elements (IRE), of which multiple distinct consensus sequences have been defined. These include the serum response element, the activator protein 1 (AP-1) motif, the Ets motif, the E-box motif, and the thyroid transcription factor 2 (TTF-2) motif. All of these IREs mediate stimulatory effects of insulin on gene transcription. In contrast, an element with the consensus sequence T(G/A)TTT(T/G)(G/T), which can be referred to as the PEPCK-like motif, mediates the inhibitory effect of insulin on transcription of genes such as PEPCK, insulin-like growth factor binding protein 1 (IGFBP-1), tyrosine aminotransferase, and the G-6-Pase catalytic subunit. In addition, multiple insulin-responsive genes harbor an IRE containing GC-rich region, which binds the ubiquitous transcription factor Sp1, and this IRE is similarly considered to be an important mediator of insulin action [86].

Together with the *cis*-acting promoter sequences, it is also important to consider the identity of the transcription factors (*trans*-acting factors) activated by insulin that bind to the IREs and alter gene transcription rates. Inhibition of gluconeogenesis by insulin follows receptor-mediated tyrosine phosphorylation of IRS2, the IRS isoform that predominates in transducing the insulin signal in liver. Subsequent activation of $PI_3$ kinase and Akt/PKB are also required, and activation of Akt2 is critical because Akt2 knockout mice [26], but not Akt1 null mice [25], display hepatic insulin resistance, increased hepatic glucose output, and hyperglycemia. Activated Akt2 then phosphorylates and inhibits GSK-3, resulting in hepatic glycogen synthesis, and also suppresses PEPCK and G-6-Pase gene expression through a complex process involving multiple transcription factors including Foxo, SREBP-1c, liver X-activated receptor (LXR), Sp1, and others. The interplay of *cis*- and *trans*-acting factors in the regulation of hepatic metabolism is complex and remains to be fully elucidated.

### *Foxo transcription factors*

The Foxo family in mammals is composed of three expressed genes: Foxo1, Foxo3 and Foxo4, although Foxo1 is the most highly expressed isoform in insulin-responsive tissues such as liver, adipose tissue, and pancreatic β-cells. Foxo proteins, contain a winged-helix motif, comprising the DNA binding domain. Under basal conditions, Foxo proteins reside within the nucleus and actively regulate gene transcription. Upon stimulation by insulin, Akt/PKB phosphorylates Foxo family members at three different conserved serine and threonine residues ($Thr^{24}$, $Ser^{253}$ and $Ser^{319}$ in Foxo1), which is followed by the dissociation of Foxo proteins from their DNA binding sites and exclusion from the nucleus [87]. Nuclear exclusion appears to involve the binding of protein 14-3-3 to the phosphorylated Akt/PKB sites of Foxo, thus masking the nuclear localization signal [88]. The relocation of Foxo out of the nucleus represents an effective mechanism by which insulin can suppress transcriptional activity. The dependence on Akt/PKB phosphorylation for this effect is highlighted by the observation that replacement of $Thr^{24}$ or $Ser^{253}$ by alanine results in loss of phosphorylation, lack of Foxo1 nuclear export, and failure of insulin-mediated promoter suppression [89].

The G-6-Pase and PEPCK promoters contain IREs which mediate transcriptional activation by Foxo1 and Foxo3 in the liver. Phosphorylation of Foxo by Akt/PKB impairs Foxo binding to this *cis* element, results in nuclear exclusion of Foxo, and suppression of G-6-Pase and PEPCK gene expression. Foxo is not involved in the regulation of all genes suppressed by insulin, however, and cannot account fully for suppression of G-6-Pase and PEPCK. Under physiologic conditions, there is strong evidence that SREBP-1, LXR, and peroxisome proliferator-activated receptor gamma coactivator 1 (PGC-1) all contribute to regulation of gluconeogenic genes by insulin [90].

The importance of Foxo transcription factors in cell differentiation related to whole body metabolic homeostasis is being increasingly recognized. Foxo1 is involved in pancreatic β-cell proliferation and regulation of pancreatic-duodenal homeobox 1 (Pdx1) expression by insulin [91]. Foxo1 appears to contribute to the complex coordination of transcriptional events involved in adipocyte differentiation. Constitutively active Foxo1 prevents the differentiation of pre-adipocytes, while dominant-negative Foxo1 restores adipocyte differentiation of fibroblasts from insulin receptor deficient mice [92]. The binding of Foxo to its co-activator, PGC-1α, is disrupted by the phosphorylation of Foxo, and the disruption of this complex can in itself suppress effects on gene transcription [93].

***Sterol response element binding protein 1c***

The SREBP family of transcription factors is classically viewed as being involved in the regulation of genes in response to cholesterol availability; however, SREBP-1c is regulated primarily by insulin [92]. This protein is also involved in adipocyte differentiation which explains its alternative designation as adipocyte determination and differentiation factor 1 (ADD1). SREBP-1c/ADD1 is most highly expressed in liver, white adipose tissue, muscle, adrenal gland, and brain.

Insulin stimulates the transcription, post-translational processing, and nuclear translocation of SREBP-1c in cell lines and in adipose, liver, and muscle tissues. In the nucleus, SREBP-1c contributes to a positive autofeedback loop where SREBP-1 augments its own transcription in response to insulin involving a coordinated interaction of multiple transcription factors including LXRα, Sp1, and SREBP-1c itself [94]. This insulin effect is mediated through $PI_3$ kinase and Akt/PKB, although downstream targets and transcription factors mediating induction of SREBP-1c gene transcription have not been fully clarified. In addition, insulin signal transduction via the Ras/MAPK pathway plays a contributory part by increasing SREBP-1c expression independent of changes in its mRNA levels [94].

While multiple mechanisms may contribute to stimulation of SREBP-1 activity, once in the nucleus, SREBP-1c has an important role in the regulation of specific genes in response to insulin. Insulin suppresses gluconeogenesis and PEPCK expression in part through SREBP-1c. In experiments involving overexpression of wild-type, constitutively active and dominant interfering mutants, SREBP-1c has also been shown to be involved in the induction of FAS and leptin in adipose cells, and glucokinase, pyruvate kinase, FAS, and acetyl CoA carboxylase in the liver [95]. While these insulin-regulated genes contain SREs in their promoter regions, multiple additional hormones, metabolic substrates and transcription factors participate in their transcriptional regulation. Thus, SREBP-1c mediates a positive transcriptional effect on genes promoting glycolysis and lipogenesis, while suppressing genes involved in gluconeogenesis, and seems to prepare the liver for carbohydrate availability following a meal. In fact, excessive and persistent SREBP-1c action has been implicated in intrahepatocellular lipid accumulation and hepatic steatosis.

***Liver X receptors***

Liver X receptors (LXRs) are ligand-activated nuclear receptors that regulate genes involved in lipid and carbohydrate metabolism. The endogenous agonists are oxidized cholesterol derivatives referred to as oxysterols, and, when activated, LXR receptors stimulate reverse cholesterol transport and excretion of cholesterol as bile acids to protect hepatocytes from cholesterol excess. LXR activators are also known to stimulate lipogenesis through the concerted action of activated LXR and SREBP-1c resulting in intrahepatocellular triglyceride synthesis and storage. Despite this lipogenic effect, LXR agonism or hyperexpression also increases hepatic insulin sensitivity and lowers glycemia in diabetic animals [96], largely through suppression of genes encoding the key gluconeogenic enzymes PEPCK and G-6-Pase [96]. Insulin augments expression of LXRα primarily through stabilization of LXRα transcripts, consistent with the conclusion that LXRα contributes in part to the reductions in gluconeogenesis and hepatic glucose output in response to insulin [96]. In addition to its modulatory effects in liver metabolism, activation of pancreatic β-cell LXRβ with a synthetic agonist increased glucose-induced insulin secretion and insulin content, whereas deletion of the nuclear receptor in LXRβ knockout mice severely blunted insulin secretion [97]. Downregulation of SREBP-1 expression with specific small interfering RNA blocked LXRβ-induced expression of pancreatic duodenal homeobox 1, insulin, and GLUT-2 genes. Thus, activation of LXRβ in pancreatic β-cells increases insulin secretion and insulin mRNA expression via a SREBP-1-regulated pathway. These data support the role of LXR and SREBP-1 pathways in both the regulation of gluconeogenesis in liver and insulin secretion in pancreatic β-cells.

***PGC-1***

PEPCK and G-6-Pase gene expression is regulated not only by transcription factors, but also by co-activator proteins, such as PGC-1 [98]. PGC-1 was first identified as a factor involved in brown fat adipogenesis [99]; however, it also has an important role in the expression of gluconeogenic enzymes at physiologic concentrations within hepatocytes [98]. PGC-1 is induced in the liver by glucagon and glucocorticoids in the context of fasting, insulin deficiency, and diabetes, and then participates in the induction of the gluconeogenic program. Glucagon increases expression of PGC-1 via a cAMP-dependent process, and PGC-1 participates as a co-activator with Foxo1 to increase the Foxo1-dependent transcriptional activity of G-6-Pase and PEPCK expression. In this role, both Foxo1 and PGC-1 cooperate to promote fully the induction of gluconeogenic genes in liver.

***Sp1 transcription factors***

Sp1 also participates in a complex interplay with other nuclear receptors and co-factors to mediate the effects of insulin on hepatic expression of several genes [86]. There are three different known mechanisms by which Sp1 mediates insulin action: Sp1 may act alone in mediating the effects of insulin; Sp1 binding sites may be closely juxtaposed to those of other insulin-responsive transcription factors, effecting a cooperative interaction required for insulin induction; and Sp1 binding to an insulin-responsive promoter may lead to basal activity, but dissociation of Sp1 from this site may permit other factors to modulate gene activity in response to insulin.

## Nutrient sensing and insulin action

Fuel metabolism can interact with hormone signaling pathways to regulate a broad range of cell functions. With respect to insulin action, states of chronic nutrient excess, including glucose, amino acids, and fatty acids, impair signal transduction via transcrip-

tional and post-transcriptional mechanisms. These processes link nutrient availability with the ability of insulin to regulate metabolism together with cell growth and differentiation. There are three predominant pathways for nutrient sensing that are highly relevant to the regulation of insulin action: the hexosamine biosynthetic signaling pathway; the mammalian target of rapamycin (mTOR) signaling pathway; and the AMPK signaling pathway.

## Hexosamine biosynthetic signaling pathway

Defects in insulin signaling and impaired insulin secretion in T2DM have long been known to be at least partially reversible following a period (approximately 2–3 weeks) of therapeutic normalization of glycemia [100]. These observations, coupled with findings that elevated glucose concentrations can recapitulate these defects in cultured cell systems and rodent models, has given rise to the concept of "glucose toxicity" [101]. Marshall [102] and others have demonstrated that one important mechanism underlying glucose-induced insulin resistance involves glucose metabolism through a minor intracellular pathway, the hexosamine biosynthetic pathway. Glutamine: fructose-6-phosphate amidotransferase (GFA), the rate-limiting enzyme of this pathway transfers the amide group of glutamine to fructose 6-phosphate resulting in the production of glucosamine-6-phosphate. Glucosamine-6-phosphate is then metabolized to UDP-*N*-acetylglucosamine (UDP-GlcNAc). UDP-GlcNAc serves as the substrate for the enzyme *O*-linked *N*-acetylglucosamine transferase (OGT), which catalyzes the attachment of *O*-GlcNAc to proteins. The counterpart of OGT, *O*-GlcNAcase, catalyzes removal of the *O*-linked glycosyl adduct. Thus, increased glucose flux through the hexosamine biosynthetic pathway leads to increased post-translational modification of cytoplasmic and nuclear proteins by *O*-GlcNAc [103], which can modulate their enzyme activities, proteasomal degradation, and interaction with other proteins or DNA. UDP-GlcNAc levels can fluctuate with the availability of non-esterified fatty acids, uridine, glutamine and glucose, and in this way the hexosamine biosynthetic pathway acts as a nutrient sensor and regulator.

Increased glucose flux through the hexosamine biosynthetic pathway results in impaired ability of insulin to stimulate the glucose transport effector system in adipocytes and muscle cells, and represents a biochemical process by which hyperglycemia can induce insulin resistance [104]. In rodents, hyperglycemia induced by streptozotocin or by glucose infusions, and elevations in circulating FFAs induced by lipid infusion or high-fat feeding, lead to an increase in muscle UDP-GlcNAc levels and insulin resistance as demonstrated in hyperinsulinemic glucose clamp studies. In addition, overexpression of GFA in skeletal muscle and adipose tissue of transgenic mice enhanced glucose metabolism by the hexosamine biosynthetic pathway and induced insulin resistance. The mechanisms by which products of the hexosamine biosynthetic pathway induce insulin resistance, however, have not been fully elucidated. Increased metabolic flux through this pathway results in increased O-glycosylation of the insulin receptor, IRS1, Akt/PKB, GLUT-4 and GSK3β, and this can impair the functional capabilities of these proteins or accelerate their proteasomal degradation. For example, increased *O*-glycosylation of the insulin receptor is accompanied by a decrease in insulin-stimulated receptor autophosphorylation [105]. Glycosylation can also affect activities of nuclear transcription factors. O-GlcNAc adducts can decrease transcriptional activity of Sp1 but increase DNA binding and transcriptional regulation by Foxo1. *O*-glycosylation can impair phosphorylation and DNA binding of C/EBPβ, and decrease NK-κB binding with IκB freeing NF-κB for relocation to the nucleus thereby including transcription of proinflammatory genes. Finally, increased intracellular accumulation of glucosamine-6-P inhibits hexokinase and glycogen synthase activity rapidly via an allosteric interaction with these enzymes. Nevertheless, the extent to which these processes contribute to insulin resistance in patients with diabetes requires further study.

## Mammalian target of rapamycin signaling pathway

The mTOR signaling pathway, also known as FK506 binding protein 12-rapamycin associated protein 1 (FRAP1), is an evolutionarily conserved serine–threonine protein kinase and a member of the $PI_3$ kinase-related kinase (PIKK) family [106]. It is a central signal integrator that receives signals arising from growth factors, nutrients and cellular energy metabolism, and then activates pathways that control cell growth, proliferation and survival. mTOR contains a COOH-terminal region with strong homology to the catalytic domain of $PI_3$ kinase, functions as a protein kinase [107], and exists in two functionally distinct complexes dubbed mTOR complex 1 (mTORC1) and mTORC2. mTORC1 is a heterotrimeric protein kinase that consists of the mTOR catalytic subunit and two associated proteins, regulatory associated protein of mTOR (raptor) and mammalian LST8/G-protein β-subunit like protein (mLST8/GβL). Nutrients modulate the activity of the mTORC1 complex by affecting the interaction and association of the mTOR catalytic subunit with raptor. mTORC1 determines the activity of eukaryotic initiation factor 4E binding protein (4E-BP1) and ribosomal S6 kinase (S6K). The mTORC2 complex is comprised of mTOR, mLST8/GβL, rapamycin-independent companion of mTOR (rictor) and MAPK-associated protein (mSin1). The rictor-mTOR complex directly phosphorylates Akt/PkB on $Ser^{473}$ and facilitates $Thr^{308}$ phosphorylation by PDK1 [108]. This phosphorylation of Akt occurs at $Ser^{473}$ which is distinct from activation by PDK1 which phosphorylates $Thr^{308}$.

mTORC1 can impair insulin signaling via its ability to activate S6K, which then can phosphorylate serine residues of IRS1 resulting in desensitization of the $PI_3$ kinase/Akt pathway [109]. Indeed, IRS1 $Ser^{302}$, which is proximal to the IRS1 PTB domain, contains an S6K recognition motif and is able to be phosphorylated by S6K. In this way, an increase in mTOR activity can contribute to insulin resistance. A surfeit of glucose and amino acids, especially leucine, leads to an increase in mTOR activity, while depletion of

these metabolic substrates or mitochondrial (e.g. oligomycin) and glycolytic (e.g. 2-deoxyglucose) inhibitors attenuate mTOR activity [110]. Thus, activation of S6K by mTOR activity serves as a mechanism by which nutrient excess can desensitize insulin action through serine phosphorylation of IRS1. Data in rodent models are consistent with this formulation. S6K1 activity is enhanced in insulin resistant mice fed a high-fat diet and in genetically obese mouse models, while S6K1−/− mice maintained on a high-fat diet remained insulin sensitive [111].

Insulin and amino acids can activate the mTOR pathway in a wortmannin-sensitive manner indicative of signaling through $PI_3$ kinase; however, the wortmannin target is not class I $PI_3$ kinases. Rather, the wortmannin target is the class III $PI_3$ kinase, hVps34. This is substantiated by the observation that knockdown of hVps34 blocks amino acid- and insulin-induced S6K1 activation but has no effect on Akt/PKB activation [112]. Rapamycin can inhibit mTOR through association with its intracellular receptor FKBP12. The FKBP12–rapamycin complex binds directly to a FKBP12–rapamycin binding (FRB) domain in mTOR, and this interaction inhibits mTOR activity. A recent study demonstrated that rapamycin enhances insulin-mediated glucose uptake by inhibiting mTOR-mediated S6K phosphorylation and reducing IRS serine phosphorylation [113]. The proximal signaling component immediately responsible for activating the mTOR pathway is the tuberous sclerosis complex (TSC). TSC can integrate information from the insulin signaling cascade and the AMPK signaling pathway. These multiple steps involved in nutrient sensing by the mTOR signaling pathway are attractive targets for treating insulin resistance in metabolic diseases cause by nutrient excess, such as T2DM and obesity.

## AMPK signaling pathway

The AMPK signaling pathway is activated by elevation of the AMP:ATP ratio. The pathway is switched on by nutrient deprivation leading to accumulation of AMP, or by perturbations that interfere with ATP synthesis, such as glucose deprivation, hypoxia, metabolic inhibitors (2-deoxyglucose, arsenite), and disruption of oxidative phosphorylation. Hardie has described AMPK as a "fuel gauge" that regulates responses to extremes of nutrient availability at the levels of cells, tissues and the whole body [114]. Once activated, it switches on fuel metabolism pathways that generate ATP, while inhibiting anabolic processes that consume ATP. AMPK is a heterotrimeric enzyme complex comprised of α, β and γ-subunits, and each subunit has two or more isoforms that exhibit tissue-specific expression. The α-subunit is the catalytic component, and contains the $Thr^{172}$ residue that is phosphorylated by activating upstream kinases. The β-subunit has glycogen-binding C-terminal domains that help cement the trimolecular complex, and can also interact with high glycogen stores to exert an inhibitory effect on AMPK activity. The γ-subunit of AMPK allosterically binds one molecule of AMP or ATP in a mutually exclusive manner allowing for regulation of AMPK activity by changing ratios of AMP to ATP.

The upstream cascade leading to phosphorylation of $Thr^{172}$ in the AMPK α-subunit is initiated by the LKB1 complex. LKB1 is apparently not regulated by AMP and is constitutively active. The binding of AMP to the AMPK γ-subunit results in a net augmentation of phospho-$Thr^{172}$ by making this site less susceptible to phosphatases, as well as by allosterically activating the phosphorylated form of the kinase. These two mechanisms create a "multiplier effect" for AMPK activity, allowing a small increase in AMP to produce a large effect on kinase activity. Both effects are antagonized by high ATP concentrations, so that the AMP:ATP ratio becomes a sensitive regulator of AMPK. AMPK is also activated in response to cytosolic $Ca^{2+}$ levels via phosphorylation of $Thr^{172}$ by calmodulin-dependent kinase kinase-β (CaMKKβ); however, this action of CaMKKβ is independent of the AMP level.

AMPK switches on catabolic processes that generate ATP such as glucose uptake, glycolysis, fatty acid oxidation, and mitochondrial biogenesis, while switching off anabolic processes that consume ATP such as fatty acid and cholesterol biosynthesis, gluconeogenesis and glycogenesis. To conserve ATP, AMPK activation suppresses protein synthesis by inhibiting the mTOR pathway and initiation of translation, and by inhibiting translational elongation of proteins via activation of elongation factor 2 kinase. To enhance the capacity for ATP generation, AMPK appears to promote mitochondrial biogenesis through concerted action with another metabolic sensor, the $NAD^+$-dependent deacetylase SIRT1. Evidence indicates that AMPK can enhance SIRT1 activity by increasing cellular $NAD^+$ levels. SIRT1 then deacetylates various AMPK substrates and modulates their activity including PGC-1α and Foxo1. In this way, AMPK, SIRT1 and PGC-1α can act in an orchestrated way to regulate energy metabolism. In skeletal muscle, AMPK stimulates lipid oxidation, increases insulin sensitivity and mediates contraction-induced increments in glucose uptake during exercise [115]. In liver, AMPK activation suppresses gluconeogenic enzyme expression and leads to decreased blood glucose values, while fatty acid oxidation is stimulated and cholesterol and triglyceride biosynthesis is blocked. These biologic actions are the result of phosphorylation and inactivation of both ACC and 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase) by AMPK. The phosphorylation of ACC is central to the ability of AMPK to promote fatty acid oxidation, because this reduces its activity and results in decreased production of malonyl-CoA, an inhibitor of carnitine palmitoyltransferase I (CPT1). The reduction in malonyl-CoA relieves the inhibition of CPT1 and promotes CPT1-mediated transport of fatty acids into the mitochondria for oxidation. At the transcriptional level, AMPK blocks lipogenesis by limiting endogenous LXR ligand production, which inhibits LXR-dependent transcription of SREBP-1 [116]. AICAR is used experimentally to study the effects of AMPK activation on glucose and lipid metabolism and insulin signaling pathways. AICAR is an adenosine analog that is taken up by adenosine transporters and subsequently phosphorylated to ZMP (5-aminoimidazole-4-carboxamide-1-β-D-furanosyl

5′-monophosphate) within the cell, which mimics AMP and activates AMPK signaling through direct binding. In adipocytes, AICAR stimulates glucose uptake, increases adiponectin secretion, and inhibits production of cytokines such as TNF-α and IL-6. Two existing classes of antidiabetic drugs, biguanides (e.g. metformin) and the thiazolidinediones (e.g. rosiglitazone), both act (at least in part) by activation of AMPK. Furthermore, AMPK is activated by the insulin sensitizing hormones leptin and adiponectin. These actions are proof-of-principle that pharmacologic activators of AMPK could be used to treat patients with the metabolic syndrome and T2DM.

## Cell stress and insulin action

### Oxidative stress

Oxidative stress caused by increased reactive oxygen species (ROS) generation and/or compromised antioxidant systems represents an important factor in the development of insulin resistance and the related diseases of obesity, metabolic syndrome, and T2DM [117]. Nutrient excess, physical inactivity and hyperglycemia can lead to oxidative stress and low-grade inflammation. A considerable portion of total body oxidant stress and ROS production are generated by dysfunctional mitochondria in skeletal muscle. It is not clear, however, whether mitochondrial dysfunction is the consequence or the cause of increased ROS production. Production of excess ROS can occur in response to diverse stimuli including inflammation and proinflammatory cytokines (e.g. TNF-α). Oxidative stress can also be self-reinforcing, because ROS activates NF-κB and upregulates expression of proinflammatory genes such as TNF-α, IL-6, and C-reactive protein (CRP) [118]. Oxidants are believed to impair insulin signal transduction by inducing serine phosphorylation of IRS which impairs tyrosine phosphorylation and increases IRS protein degradation.

### Inflammation

Human insulin resistance is frequently accompanied by low-grade systemic inflammation, evidenced by elevated circulating markers of inflammation in these disorders such as white blood cell count, sedimentation rate, CRP, adhesion molecules (e.g. e-selectin), cytokines such as IL-6 and IL-8, and serum amyloid A. In adipose tissue, obesity and insulin resistance are associated with an infiltration of activated macrophages and paracrine cross-talk between macrophages and adipocytes involving multiple secreted factors (e.g. cytokines, adipokines). This process can influence secretion patterns of circulating adipokines that can affect other organs and generate aspects of the metabolic syndrome trait cluster. This includes secretion of IL-1 and IL-6 which helps mediate inflammation in metabolic disorders. For example, IL-1α and IL-1β exert strong proinflammatory functions [119] and the latter is able to reduce IRS-1 expression. Interestingly, in humans with T2DM, treatment with recombinant human IL-1 receptor antagonist improves glycemic control [120]. Circulating IL-6 levels are highly correlated with insulin resistance, and related studies demonstrate how proinflammatory cytokines can compromise insulin action, by a mechanism involving recruitment and activation of Jak, STAT proteins, and SHP-2 which helps to activate the ERK/MAP kinase pathway. Subsequent steps include increased transcription of SOCS genes, whose protein products induce insulin resistance at a proximal step at the insulin receptor or IRS proteins.

### Endoplasmic reticulum stress

Although endoplasmic reticulum (ER) stress has been recognized as an adaptive cellular response to an accumulation of unfolded or misfolded proteins in its lumen, the ER stress response can also be triggered by other factors, including chronic nutrient excess, high fatty acid concentrations, and oxidative stress, which are found in insulin resistant states. ER stress activates the JNK pathway with subsequent inhibitory serine phosphorylation of IRS-1, and the development of insulin resistance. The mechanism for JNK activation involves transmembrane ER proteins with luminal domains responsive to ER stress signals and cytoplasmic effector domains that influence intracellular pathways. One of these stress transducers, known as IRE1, links ER stress to JNK activation. Obesity and extended periods of high fat feeding can induce the ER stress response in adipose tissue and liver, as indicated by ER stress markers such as PERK, eIF2α phosphorylation, and JNK activation. Interestingly, small molecular weight "chemical chaperones" such as phenyl butyric acid which attenuate ER stress, can normalize fasting blood glucose, enhance insulin sensitivity and reduce ER stress in *ob/ob* mice [121]. In diabetes, ER stress has also been implicated in pancreatic β-cell failure [122]. Finally, oxidative stress, caused by excess availability of saturated fatty acids for mitochondrial oxidation, is linked to exacerbation of ER stress.

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