Insulin signalling and the regulation of glucose and lipid metabolism

Alan R. Saltiel* & C. Ronald Kahn†

**Life Sciences Institute, Department of Medicine, University of Michigan School of Medicine, Ann Arbor, Michigan 48109, USA (e-mail: saltiel@umich.edu)*
†Joslin Diabetes Centre, Harvard Medical School, Boston, Massachusetts 02215, USA (e-mail: c.ronald.kahn@joslin.harvard.edu)

The epidemic of type 2 diabetes and impaired glucose tolerance is one of the main causes of morbidity and mortality worldwide. In both disorders, tissues such as muscle, fat and liver become less responsive or resistant to insulin. This state is also linked to other common health problems, such as obesity, polycystic ovarian disease, hyperlipidaemia, hypertension and atherosclerosis. The pathophysiology of insulin resistance involves a complex network of signalling pathways, activated by the insulin receptor, which regulates intermediary metabolism and its organization in cells. But recent studies have shown that numerous other hormones and signalling events attenuate insulin action, and are important in type 2 diabetes.

Despite periods of feeding and fasting, plasma glucose remains in a narrow range between 4 and 7 mM in normal individuals. This tight control is governed by the balance between glucose absorption from the intestine, production by the liver and uptake and metabolism by peripheral tissues. Insulin increases glucose uptake in muscle and fat (see Box 1), and inhibits hepatic glucose production, thus serving as the primary regulator of blood glucose concentration. Insulin also stimulates cell growth and differentiation, and promotes the storage of substrates in fat, liver and muscle by stimulating lipogenesis, glycogen and protein synthesis, and inhibiting lipolysis, glycogenolysis and protein breakdown (Fig. 1). Insulin resistance or deficiency results in profound dysregulation of these processes, and produces elevations in fasting and postprandial glucose and lipid levels.

Insulin increases glucose uptake in cells by stimulating the translocation of the glucose transporter GLUT4 from intracellular sites to the cell surface (see Box 1). Up to 75% of insulin-dependent glucose disposal occurs in skeletal muscle, whereas adipose tissue accounts for only a small fraction[1]. Despite this, mice with a knockout of the insulin receptor in muscle have normal glucose tolerance[2], whereas those with a knockout of the insulin-sensitive glucose transporter in fat have impaired glucose tolerance, apparently owing to insulin resistance being induced in muscle and liver[3]. Both obesity and lipoatrophy also cause insulin resistance and predisposition to type 2 diabetes, demonstrating that adipose tissue is crucial in regulating metabolism beyond its ability to take up glucose[4]. Although insulin does not stimulate glucose uptake in liver, it blocks glycogenolysis and gluconeogenesis, and stimulates glycogen synthesis, thus regulating fasting glucose levels. Insulin action in tissues not normally considered insulin sensitive, including brain and pancreatic β-cell, may also be important in glucose homeostasis[2,5] (see below).

Proximal insulin-signalling pathways

The insulin receptor

The insulin receptor belongs to a subfamily of receptor tyrosine kinases that includes the insulin-like growth factor (IGF)-I receptor and the insulin receptor-related receptor (IRR)[6]. These receptors are tetrameric proteins consisting of two α- and two β-subunits that function as allosteric enzymes in which the α-subunit inhibits the tyrosine kinase activity of the β-subunit. Insulin binding to the α-subunit leads to derepression of the kinase activity in the β-subunit followed by transphosphorylation of the β-subunits and a conformational change that further increases kinase activity[6]. Insulin, IGF-I and the IRR receptors can form functional hybrids; thus, an inhibitory mutation in one receptor can inhibit the activity of the others[7].

Homologues of the insulin/IGF-I receptor have been identified in *Drosophila*, *Caenorhabditis elegans* and metazoan marine sponges[8]. These lower organisms use some of the same downstream signals critical to the regulation of mammalian cells, including phosphatidylinositol-3-OH kinase (PI(3)K), Akt and forkhead transcription factors. Inhibitory mutants of the insulin/IGF system in *C. elegans* live longer than normal animals, raising a number of interesting questions about the association of hyperinsulinaemia/insulin resistance with conditions that shorten life span, such as obesity, diabetes and accelerated atherosclerosis.

Insulin-receptor substrates

At least nine intracellular substrates of the insulin/IGF-I receptor kinases have been identified (Fig. 2). Four of these belong to the family of insulin-receptor substrate (IRS) proteins[9]. Other substrates include Gab-1, $p60^{dok}$, Cbl, APS and isoforms of Shc[10]. The phosphorylated tyrosines in these substrates act as 'docking sites' for proteins that contain SH2 (Src-homology-2) domains. Many of these SH2 proteins are adaptor molecules, such as the p85 regulatory subunit of PI(3)K and Grb2, or CrkII, which activate small G proteins by binding to nucleotide exchange factors. Others are themselves enzymes, including the phosphotyrosine phosphatase SHP2 and the cytoplasmic tyrosine kinase Fyn. Substrate binding to these SH2 proteins can regulate their activities, or in some cases their subcellular location.

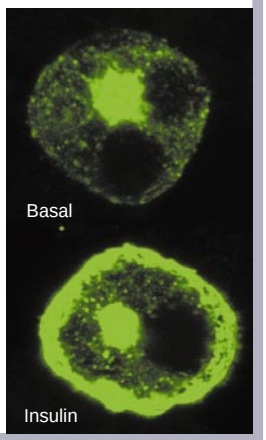
Although the IRS proteins are highly homologous, recent studies in knockout mice and cell lines suggest that they serve complementary, rather than redundant, roles in insulin/IGF-I signalling. IRS-1-knockout mice exhibit generalized pre- and post-natal growth retardation, as well as insulin resistance in peripheral tissues and impaired glucose tolerance[11,12]. IRS-2-knockout mice also exhibit insulin

Box 1
The regulation of glucose transport

Insulin increases glucose transport in fat and muscle cells by stimulating the translocation of the transporter GLUT4 from intracellular sites to the plasma membrane (see figure below). GLUT4 is found in vesicles that continuously cycle from intracellular stores to the plasma membrane. Insulin increases glucose transport by increasing the rate of GLUT4-vesicle exocytosis, and by slightly decreasing the rate of internalization[99]. Although the exact mechanisms are unknown, it is likely that the insulin-responsive GLUT4 vesicle is tethered to intracellular sites, perhaps defined by a microtubule network[100]. Phosphorylation events probably catalysed by PtdIns(3,4,5)P_3-dependent kinases release vesicles from these sites, allowing trafficking of GLUT4 to the cell surface. Recent evidence suggests that these vesicles move along microtubule tracks en route to the cell surface, perhaps via kinesin motors. The vesicles then dock and fuse with the plasma membrane, allowing the extracellular exposure of the GLUT4 protein.

It is likely that the actin cytoskeleton is also crucial in insulin-stimulated GLUT4 translocation. Insulin causes remodelling of cortical actin filaments just below the plasma membrane, and induces membrane ruffling. This effect probably reflects actin polymerization and depolymerization involving lamelipodia and/or filopodia formation. The actin depolymerizing agent cytochalasin D, as well as the actin monomer-binding toxins latrunculin A and B, partially inhibit the effects of insulin on actin and GLUT4 translocation.

The docking and fusion of the GLUT4 vesicle at the plasma membrane may also be subject to regulation by insulin. The v-SNARE protein VAMP2 physically interacts with its t-SNARE counterpart syntaxin 4 on GLUT4 vesicles during vesicle docking and fusion with the plasma membrane[99]. Although these SNARE interactions are essential, neither SNARE protein seems to be a direct target of insulin. Instead, the SNARE accessory proteins Synip and Munc18c may be involved in the control of GLUT4 docking and fusion in an insulin-dependent, PI(3)K-independent manner.



Box 1 Figure 3T3L1 adipocytes were transfected with a fusion construct of GLUT4 and enhanced green fluorescent protein. Cells were treated with or without insulin. The image is a confocal micrograph of single cells, showing the translocation of the GLUT4 protein from intracellular sites to the cell surface. For a three-dimensional image of these cells, visit http://www.medgen.med.umich.edu/labs/saltiel/.

resistance in both peripheral tissues and liver, but have defective growth in only some tissues, including certain regions of the brain, islets and retina[13,14]. In the IRS-2$^{-/-}$ mouse, this multifactorial insulin resistance combined with decreased β-cell mass leads to development of type 2 diabetes[14]. By contrast, IRS-3- and IRS-4-knockout mice have normal or near normal growth and metabolism[15].

The different IRS proteins seem to serve different functions at the cellular level, probably owing to differences in tissue distribution, subcellular localization and intrinsic activity of the proteins. IRS-1-knockout cells exhibit reduced IGF-I-stimulated DNA synthesis[11,12] and fail to differentiate into adipocytes in culture[16]. Likewise, the mitogenic response mediated by IRS-2 is weaker than that by produced by IRS-1 (ref. 17). IRS-2-knockout cells show a major defect in insulin-stimulated glucose transport[14]. The roles of IRS-3 and 4 are less clear in cultured cells, but some data suggest that these substrates may act as negative regulators of IRS-1 and -2 (ref. 18).

Inhibition of insulin-receptor signalling

In addition to tyrosine phosphorylation, both the insulin receptor and IRS proteins undergo serine phosphorylation, which may attenuate signalling by decreasing insulin-stimulated tyrosine phosphorylation[19] and promote interaction with 14-3-3 proteins[20]. These inhibitory phosphorylations provide negative feedback to insulin signalling and serve as a mechanism for cross-talk from other pathways that produce insulin resistance. Several kinases have been implicated in this process, including PI(3)K, Akt, glycogen synthase kinase (GSK)-3 and mammalian target of rapamycin (mTOR). Recent data indicate that obesity-induced attenuation of insulin signalling might arise from the sequential activation of protein kinase C (PKC) and inhibitor of nuclear factor-κB (IκB) kinase, although the details of this pathway have not been elucidated[21,22].

Insulin action is also attenuated by protein tyrosine phosphatases (PTPases), which catalyse the rapid dephosphorylation of the receptor and its substrates. A number of PTPases have been identified that catalyse dephosphorylation of the insulin receptor *in vitro*, some of which are expressed in insulin-responsive cells, or upregulated in states of insulin resistance. Most attention has focused on the cytoplasmic phosphatase PTP1B. Knockout of PTP1B leads to increased tyrosine phosphorylation of the insulin receptor and IRS proteins in muscle and improved insulin sensitivity[23]. PTP1B$^{-/-}$ mice are also resistant to diet-induced obesity, suggesting the brain as an important site of action. This combination of effects implicates PTP1B as a potential therapeutic target in diabetes and obesity.

PI(3)K and insulin action

PI(3)K has a pivotal role in the metabolic and mitogenic actions of insulin and IGF-I (ref. 24). Inhibitors of class Ia PI(3)K, or transfections with dominant negative constructs of the enzyme, block most metabolic actions of insulin, including stimulation of glucose transport, glycogen and lipid synthesis. PI(3)K consists of a p110 catalytic subunit and a p85 regulatory subunit that possesses two SH2 domains that interact with tyrosine-phosphorylated pYMXM and pYXXM motifs in IRS proteins[25]. At least eight isoforms of the regulatory subunits have been identified. These are derived from three genes (*p85α*, *p85β* and *P55^{PIK}*)[26] and alternative splicing of p85α to produce *AS53/p55α*[27] and *p50α*[28]. Of these, *p85α* is predominant and thought to be the main response pathway for most stimuli.

The exact roles of the different regulatory subunits of PI(3)K in insulin action are unclear. Splice variants possess differences in potencies for enzyme activation[27], tissue distribution[27,28] and sensitivity to insulin[29]. Knockout mice with a disruption of all three isoforms derived from the *p85α* gene die shortly after birth[30], whereas heterozygous knockout mice or mice lacking only full-length *p85α* are viable and exhibit improved insulin sensitivity (ref. 31, and F. Mauvais-Jarvis *et al.*, personal communication). Cell lines derived from heterozygous knockouts also exhibit increased insulin/IGF-I signalling, which seem to be due to improved stoichiometry of the interaction (see below).

The activation of PI(3)K may transmit multiple signals. PI(3)K catalyses the phosphorylation of phosphoinositides on the 3-position to produce phosphatidylinositol-3-phosphates, especially PtdIns(3,4,5)P_3, which bind to the pleckstrin homology (PH) domains of a variety of signalling molecules thereby altering their activity or subcellular localization[32]. Moreover, PI(3)K also possesses serine kinase activity, and both the regulatory and catalytic subunits of the enzyme can interact with other signalling proteins. Indeed, recent studies suggest that these proteins may be important in insulin action independent of PtdIns(3,4,5)P_3 generation[33].

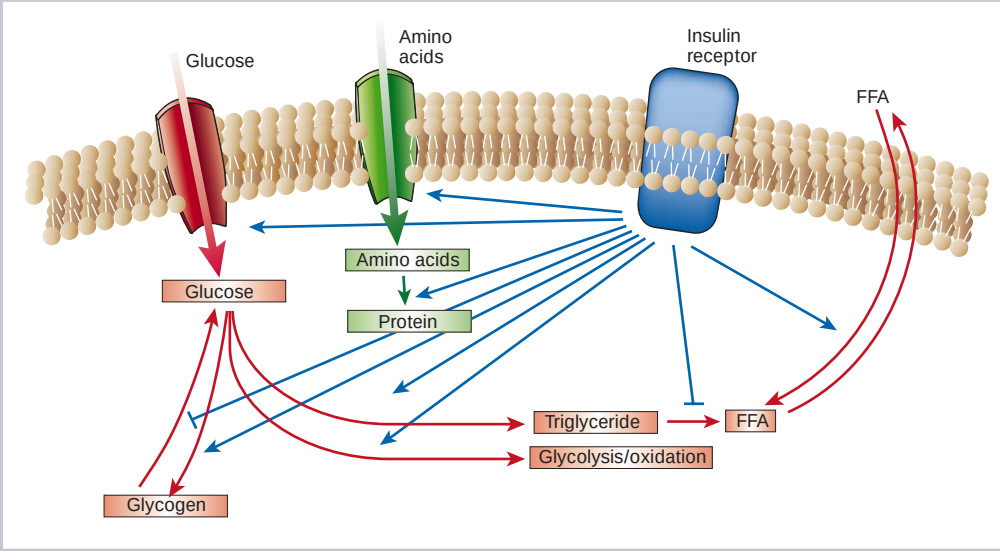


Figure 1 The regulation of metabolism by insulin. Insulin is the most potent anabolic hormone known, and promotes the synthesis and storage of carbohydrates, lipids and proteins, while inhibiting their degradation and release into the circulation. Insulin stimulates the uptake of glucose, amino acids and fatty acids into cells, and increases the expression or activity of enzymes that catalyse glycogen, lipid and protein synthesis, while inhibiting the activity or expression of those that catalyse degradation.

Phosphotidylinositol-3-phosphates regulate three main classes of signalling molecules: the AGC family of serine/threonine protein kinases[34], guanine nucleotide-exchange proteins of the Rho family of GTPases[35], and the TEC family of tyrosine kinases[36]. PI(3)K also activates the mTOR/FRAP pathway, and might be involved in regulation of phospholipase D, leading to hydrolysis of phosphatidylcholine and increases in phosphatidic acid and diacylglycerol. The best characterized of the AGC kinases is phosphoinositide-dependent kinase 1 (PDK1), one of the serine kinases that phosphorylates and activates the serine/threonine kinase Akt/PKB[37]. Akt possesses a PH domain that also interacts directly with PtdIns(3,4,5)P_3, promoting membrane targeting of the protein and catalytic activation. Akt has been suggested to be important in transmission of the insulin signal, by phosphorylation of the enzyme GSK-3 (see below), the forkhead transcription factors and cAMP response element-binding protein[38,39]. Although studies using inhibitory or activated forms of Akt have not uniformly inhibited or mimicked insulin actions[40], deletion of Akt2 produces hepatic insulin resistance in mice[41]. Other AGC kinases that are downstream of PI(3)K include serum- and glucocorticoid-regulated kinase and the atypical PKCs, PKC-ζ and -λ[42]. Akt and/or the atypical PKCs seem to be required for insulin-stimulated glucose transport.

Activity of this pathway is also determined by phosphatidylinositol-3-phosphates such as phosphatase and tensin homologue[43] and the SH2 domain-containing inositol-5-phosphatase SHIP2 (ref. 44). Overexpression of these enzymes leads to decreased levels of PtdIns(3,4,5)P_3. This might terminate signal transduction and/or change the nature of the phosphoinositides, altering the binding specificity to PH or phox homology domains. Disruption of these genes or reducing expression of these messenger RNAs yields mice with increased insulin sensitivity[45].

The CAP/Cbl pathway and lipid rafts

In addition to PI(3)K activity, other signals seem to be required for insulin-stimulated glucose uptake[10]. This second pathway appears to involve tyrosine phosphorylation of the Cbl protooncogene[46]. In

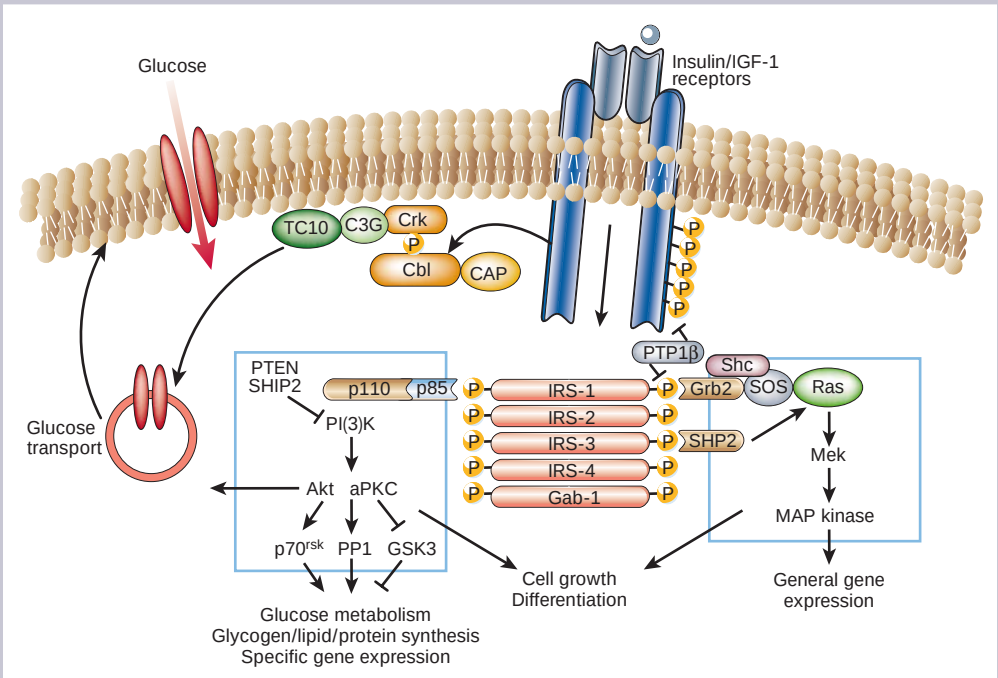


Figure 2 Signal transduction in insulin action. The insulin receptor is a tyrosine kinase that undergoes autophosphorylation, and catalyses the phosphorylation of cellular proteins such as members of the IRS family, Shc and Cbl. Upon tyrosine phosphorylation, these proteins interact with signalling molecules through their SH2 domains, resulting in a diverse series of signalling pathways, including activation of PI(3)K and downstream PtdIns(3,4,5)P_3-dependent protein kinases, ras and the MAP kinase cascade, and Cbl/CAP and the activation of TC10. These pathways act in a concerted fashion to coordinate the regulation of vesicle trafficking, protein synthesis, enzyme activation and inactivation, and gene expression, which results in the regulation of glucose, lipid and protein metabolism.

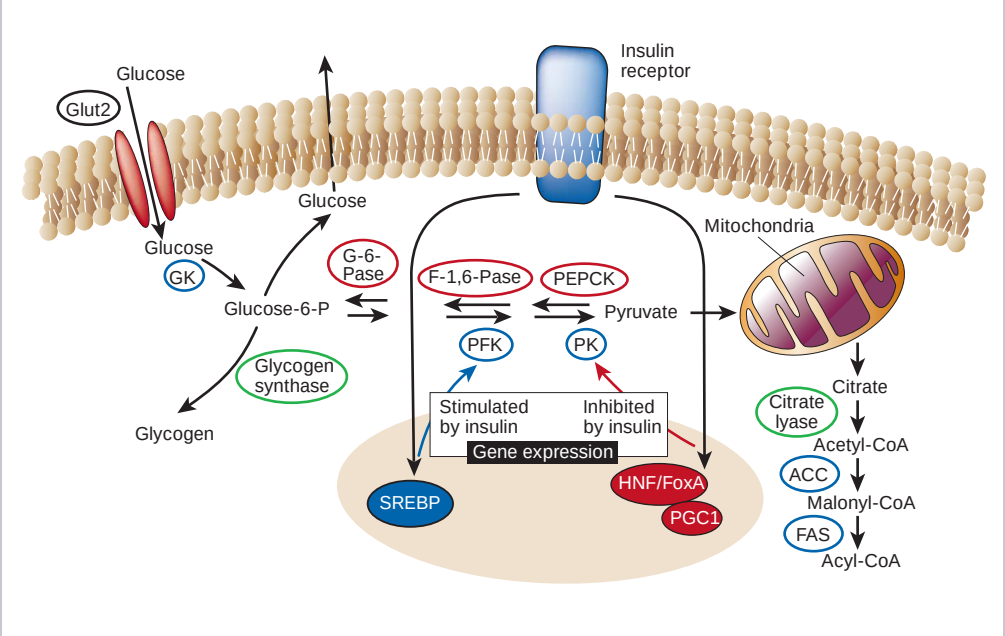


Figure 3 The regulation of glucose metabolism in the liver. In the hepatocyte, insulin stimulates the utilization and storage of glucose as lipid and glycogen, while repressing glucose synthesis and release. This is accomplished through a coordinated regulation of enzyme synthesis and activity. Insulin stimulates the expression of genes encoding glycolytic and fatty-acid synthetic enzymes (in blue), while inhibiting the expression of those encoding gluconeogenic enzymes (in red). These effects are mediated by a series of transcription factors and co-factors, including sterol regulatory element-binding protein (SREBP)-1, hepatic nuclear factor (HNF)-4, the forkhead protein family (Fox) and PPARγ co-activator 1 (PGC1). The hormone also regulates the activities of some enzymes, such as glycogen synthase and citrate lyase (in green), through changes in phosphorylation state. GK, glucokinase; Glucose-6-P, glucose-6-phosphate; G-6-Pase, glucose-6-phosphatase; F-1,6-Pase, fructose-1,6-bisphosphatase; PEPCK, phosphoenolpyruvate carboxykinase; PFK, phosphofructokinase; PK, pyruvate kinase; ACC, acetyl-CoA carboxylase; FAS, fatty-acid synthase.

most insulin-responsive cells, Cbl is associated with the adapter protein CAP, which binds to proline-rich sequences in Cbl through its carboxyl-terminal SH3 domain[47]. CAP is expressed in insulin-sensitive tissues, is markedly induced during adipocyte differentiation and its expression is increased by insulin-sensitizing peroxisome proliferator-activated receptor-γ (PPARγ) agonists[48].

CAP belongs to a family of adapter proteins with a common organization containing three SH3 domains and a region of similarity to the peptide sorbin, referred to as a sorbin homology (SoHo) domain[49]. Upon phosphorylation, the Cbl–CAP complex translocates to lipid raft domains in the plasma membrane, mediated by the interaction of the SoHo domain of CAP with the protein flotillin[50]. Expression of dominant-interfering CAP mutants that cannot bind to Cbl or flotillin inhibit Cbl translocation and insulin-stimulated glucose uptake. Translocation of phosphorylated Cbl recruits the adapter protein CrkII to the lipid raft via interaction of the SH2 domain of CrkII with phospho-Cbl[51]. CrkII also forms a constitutive complex with the guanyl nucleotide-exchange protein C3G. Once translocated into lipid rafts, C3G comes into proximity with the G protein TC10, and catalyses the exchange of GTP for GDP, resulting in the activation of the protein[51]. The localization of TC10 in rafts is required for its activation by insulin[52]. Once activated, TC10 seems to provide a second signal to the GLUT4 protein that functions in parallel with the activation of the PI(3)K pathway[51]. This may involve the stabilization of cortical actin, which seems to be important in GLUT4 vesicle translocation to the plasma membrane (see Box 1).

Insulin-stimulated phosphorylation cascades

As is the case for other growth factors, insulin stimulates the mitogen-activated protein (MAP) kinase extracellular signal-regulated kinase (ERK) (Fig. 2). This pathway involves the tyrosine phosphorylation of IRS proteins and/or Shc, which in turn interact with the adapter protein Grb2, recruiting the Son-of-sevenless (SOS) exchange protein to the plasma membrane for activation of Ras. The activation of Ras also requires stimulation of the tyrosine phosphatase SHP2, through its interaction with receptor substrates such as Gab-1 or IRS1/2. Once activated, Ras operates as a molecular switch, stimulating a serine kinase cascade through the stepwise activation of Raf, MEK and ERK. Activated ERK can translocate into the nucleus, where it catalyses the phosphorylation of transcription factors such as $p62^{TCF}$, initiating a transcriptional programme that leads to cellular proliferation or differentiation[53]. Blockade of the pathway with dominant negative mutants or pharmacological inhibitors prevents the stimulation of cell growth by insulin, but has no effect on the metabolic actions of the hormone[54].

Insulin increases synthesis and blocks the degradation of proteins through activation of mTOR. mTOR is a member of the PI(3)K family of proteins, but seems to act primarily as a serine, rather than lipid kinase[55]. The stimulation of this protein kinase involves PI(3)K activation, although another signal may also be required[56]. mTOR can control the mammalian translation machinery by direct phosphorylation and activation of p70 ribosomal S6 kinase ($p70^{rsk}$)[57], as well as phosphorylation of the initiation factor 4E for eukaryotic translation (eIF-4E) inhibitor, PHAS1 or 4E-binding protein 1 (ref. 58). $P70^{rsk}$ activates ribosome biosynthesis by phosphorylating the ribosomal S6 protein, producing increased translation of mRNAs with a 5′-terminal oligopyrimidine tract. $P70^{rsk}$ also requires a second PtdIns(3,4,5)P_3-dependent phosphorylation, presumably catalysed by PDK1. Phosphorylation of PHAS-1 by mTOR results in its dissociation from eIF-2, allowing cap-dependent translation of mRNAs with a highly structured 5′-untranslated region. Although the mechanism of activation of mTOR remains unclear, it seems to require the presence of amino acids in the media for full activation by growth factors, and thus may also represent a nutrient sensor[55].

Glucose and lipid regulation

Regulation of glycogen synthesis

Insulin stimulates glycogen accumulation through a coordinated increase in glucose transport and glycogen synthesis. The hormone activates glycogen synthase by promoting its dephosphorylation, through the inhibition of kinases such as PKA or GSK-3 (ref. 59), and activation of protein phosphatase 1 (PP1)[60]. Upon its activation downstream of PI(3)K, Akt phosphorylates and inactivates GSK-3, decreasing the rate of phosphorylation of glycogen synthase, thus increasing its activity state[59]. Insulin does not activate

PP1 globally, but rather specifically targets discrete pools of the phosphatase, primarily increasing PP1 activity localized at the glycogen particle. The compartmentalized activation of PP1 by insulin is due to glycogen-targeting subunits, which serve as 'molecular scaffolds', bringing together the enzyme directly with its substrates glycogen synthase and phosphorylase in a macromolecular complex, and in the process exerting profound effects on PP1 substrate-specific activity[61].

Four different proteins have been reported to target PP1 to the glycogen particle. Despite a proposed common function, no two targeting subunits share more than 50% sequence homology, and this is largely confined to the PP1- and glycogen-binding regions. Overexpression of these scaffolding proteins in cells or *in vivo* results in a marked increase in cellular glycogen levels[61]. Although the mechanism by which insulin activates glycogen-associated PP1 remains unknown, inhibitors of PI(3)K block this effect, suggesting that PtdIns(3,4,5)P_3-dependent protein kinases are involved. These scaffolding proteins have a critical permissive role in the hormonal activation of the enzyme, perhaps interacting with additional proteins that regulate the interaction of PP1 with glycogen synthase and phosphorylase.

Regulation of gluconeogenesis

Insulin inhibits the production and release of glucose by the liver by blocking gluconeogenesis and glycogenolysis (Fig. 3). This occurs through a direct effect of insulin on the liver[62], as well as by indirect effects of insulin on substrate availability[63]. Insulin can also influence glucose metabolism indirectly by changes in free fatty acids generated from visceral fat, the so called 'single gateway' hypothesis[64]. Because visceral fat is less sensitive to insulin than subcutaneous fat, even after a meal there is little suppression of lipolysis by the hormone in this fat depot. The resulting direct flux of fatty acids derived from these fat cells through the portal vein to the liver can stimulate glucose production, thus providing a signal for both insulin action and insulin resistance in the liver.

Insulin directly controls the activities of a set of metabolic enzymes by phosphorylation or dephosphorylation and also regulates the expression of genes encoding hepatic enzymes of gluconeogenesis and glycolysis[65]. It inhibits the transcription of the gene encoding phosphoenolpyruvate carboxylase, the rate-limiting step in gluconeogenesis[66]. The hormone also decreases transcription of the genes encoding fructose-1,6-bisphosphatase and glucose-6-phosphatase, and increases transcription of glycolytic enzymes such as glucokinase and pyruvate kinase, and lipogenic enzymes such as fatty acid synthase and acetyl-CoA carboxylase. Although the transcription factors that control the expression of these genes have remained elusive, new data suggest a potential role for the forkhead family of transcription factors through phosphorylation by Akt-related protein kinases[39], and the PPARγ co-activator PGC-1 (ref. 67).

Table 1 Mice phenotypes with single-gene knockouts in signalling pathways

Gene	Phenotype	Reference
Insulin receptor	Normal intrauterine growth; die of diabetic ketoacidosis at 3–7 days	76
IGF1 receptor	Intrauterine and postnatal growth retardation; normal glucose homeostasis	97
IRS-1	Insulin resistance/impaired glucose tolerance; IGF resistance/growth retardation	11,12
IRS-2	Insulin resistance/decreased β-cell development; type 2 diabetes	13,14
IRS-3	Normal growth/normal glucose tolerance	15
IRS-4	Normal growth/normal glucose tolerance	15
Akt2	Insulin resistance in liver and muscle	41
GLUT4	Cardiac hypertrophy/failure; normal glucose tolerance	81
P85α (hetero)	Increased insulin sensitivity; hypoglycaemia	30,31
PTP1B	Increased insulin sensitivity; resistance to diet-induced obesity	23
SHIP2	Increased insulin sensitivity	45

Regulation of lipid synthesis and degradation

As is the case with carbohydrate metabolism, insulin also promotes the synthesis of lipids, and inhibits their degradation. Recent studies suggest that many of these changes require an increase in the transcription factor steroid regulatory element-binding protein (SREBP)-1c[68]. Dominant negative forms of SREBP-1 can block expression of these gluconeogenic and lipogenic genes[69], whereas overexpression can increase their transcription[68]. Hepatic SREBP levels are increased in some rodent models of lipodystrophy, and this is coordinated with increases in fatty acid synthesis and gluconeogenesis, the exact phenotype observed in genetic models of obesity-induced diabetes[70]. Thus, increased expression of SREBP-1c might contribute to the insulin resistance observed in liver of diabetic rodents, with increased rates of both gluconeogenesis and lipogenesis. The pathways that account for the changes in SREBP-1c expression in response to insulin or other metabolic changes are not known, but probably lie downstream of the IRS/PI(3)K pathway.

In adipocytes, glucose is stored primarily as lipid, owing to increased uptake of glucose and activation of lipid synthetic enzymes, including pyruvate dehydrogenase, fatty acid synthase and acetyl-CoA carboxylase. Insulin also profoundly inhibits lipolysis in adipocytes, primarily through inhibition of the enzyme hormone-sensitive lipase[71]. This enzyme is acutely regulated by control of its phosphorylation state, which is activated by PKA-dependent phosphorylation, and inhibited as a result of a combination of kinase inhibition and phosphatase activation. Insulin inhibits the activity of the lipase primarily through reductions in cAMP levels, owing to the activation of a cAMP-specific phosphodiesterase in fat cells[72].

What causes insulin resistance?

The insulin resistance of obesity and type 2 diabetes is characterized by defects at many levels, with decreases in receptor concentration and kinase activity, the concentration and phosphorylation of IRS-1 and -2, PI(3)K activity, glucose transporter translocation, and the activity of intracellular enzymes[10]. Activation of the MAP kinase pathway by insulin is not reduced in type 2 diabetes, perhaps allowing for some of the detrimental effects of chronic hyperinsulinaemia on cellular growth in the vasculature[73].

Genetic and acquired factors can profoundly influence insulin sensitivity. Genetic defects in the insulin receptor are relatively rare, but represent the most severe forms of insulin resistance, and are exemplified by leprechaunism, the Rabson Mendenhall Syndrome, and the type A syndrome of insulin resistance[74]. Differences in clinical presentation may be due to the severity of the genetic defect, the ability of the mutant receptors to form hybrids with IGF-I or other receptors, and other background genetic or acquired factors that modify the insulin-resistant state. Type 2 diabetes is polygenic and may involve polymorphisms in multiple genes encoding the proteins involved in insulin signalling, insulin secretion and intermediary metabolism[75].

Targeted deletions of the components of insulin signalling *in vivo* using homologous recombination have yielded some insight into the complexity of these mechanisms. Although some single defects in the insulin-signalling pathway, such as knockout of the insulin receptor IRS-2 or Akt2, can produce diabetes, knockout of the p85 subunit of PI(3)K, IRS-1 or GLUT4 does not (summarized in Table 1). Conversely, knockout of single genes that are involved in turning off the insulin signal, such as PTP1B and SHIP2, ameliorate diabetes in obese rodents[23,45].

Combinatorial knockouts have been produced that mimic polygenic type 2 diabetes with heterozygous deletion of the insulin receptor and IRS-1 (ref. 76), of the insulin receptor, IRS-1 and IRS-2 (ref. 13), and of IRS-1 and glucokinase[77]. In some of these combina-

Table 2 Defects in tissue-specific knockout mice

Gene	Tissue	Phenotype	Reference
Insulin receptor	Skeletal muscle	Normal glucose tolerance; increased fat mass; increased triglycerides and FFAs	2,82
	Liver	Impaired glucose tolerance; hyperinsulinaemia and reduced insulin clearance; decreased hepatic function	62
	β-cell	Loss of glucose-stimulated insulin secretion; progressively impaired glucose tolerance; decreased β-cell growth in adults	5
	Brain	Increased appetite; increased fat and leptin; insulin resistance; hypothalamic hypogonadism	79
GLUT4 glucose transporter	Skeletal muscle	Reduced basal, insulin and contraction-stimulated glucose transport; severe insulin resistance; glucose intolerance	80
	Fat	Impaired glucose tolerance; hyperinsulinaemia; secondary insulin resistance in muscle and liver	3
Glucokinase	β-cell	Die within a few days of birth with severe diabetes	98
	Liver	Mild hyperglycaemia; pronounced defects in glycogen synthesis and glucose turnover; impaired glucose-stimulated insulin secretion	98

tions there has been clear evidence of genetic epistasis. For example, although heterozygous knockout of either the insulin receptor or IRS-1 alone does not produce diabetes, the double-heterozygous knockout produces diabetes in up to 50% of mice. This striking finding gives insight into human type 2 diabetes, where insulin-induced downregulation or genetic polymorphisms in the receptor or IRS-1 alone might produce only modest changes in signalling capacity, but when combined can lead to diabetes.

One genetic model that produced a surprising phenotype regarding glucose homeostasis emerged from the knockout of the p85α regulatory subunits of PI(3)K. Although, PI(3)K is central to the metabolic actions of insulin, p85α heterozygous knockout mice counter-intuitively exhibit improved insulin sensitivity[30,31]. Furthermore, superimposition of p85α heterozygosity on the insulin receptor/IRS-1 double-heterozygous knockout protects against diabetes (Mauvais *et al.*, personal communication). This surprising protection seems to be due to a unique feature of the insulin-signalling pathway in which the stoichiometric balance between p85α, the catalytic subunit p110 and IRS proteins is critical for optimal signal transduction.

The role of specific tissues in the pathogenesis of diabetes has been explored using the *Cre-lox* DNA-recombination technology to create tissue-specific knockouts of the insulin receptor[2,62,78,79] and GLUT4 (refs 3,80; see Table 2). Despite the absence of diabetes in mice with a global knockout of GLUT4 (ref. 81), tissue-specific knockouts of GLUT4 in muscle[80] and fat[3] have resulted in severely impaired glucose tolerance. Tissue-specific knockout of the insulin receptor has also produced often surprising results. As noted above, despite recognition that insulin stimulates glucose uptake primarily in muscle, mice with a knockout of the muscle insulin receptor have normal glucose tolerance[2]. This occurs, at least in part, as the result of a shift of glucose uptake into fat, with subsequent increases in adipose tissue mass, circulating free fatty acids (FFAs) and triglycerides[82]. Mice with a knockout of the fat-specific insulin receptor also have normal glucose tolerance, whereas the liver-specific insulin-receptor knockout shows both impaired glucose tolerance and decreased insulin clearance with marked hyperinsulinaemia[62]. Perhaps the most surprising results, however, have come from studies of mice with knockouts of the β-cell-specific insulin receptor and neural/brain-specific insulin receptor[79]. The former exhibit a marked defect in glucose-stimulated insulin secretion similar to that observed in type 2 diabetes, whereas the latter exhibit increased food intake, mild adiposity, insulin resistance and hypertriglyceridaemia, as well as reduced fertility due to hypothalamic hypogonadism. Taken together, these findings suggest a unifying hypothesis for type 2 diabetes in which insulin resistance in classic target tissues, such as liver, muscle and fat, coupled with insulin resistance in β-cell, brain and other tissues, combine to produce the pathophysiology of type 2 diabetes.

The fat cell regulates insulin sensitivity

Free fatty acids

Adipose tissue has a special role in insulin resistance (Fig. 4). Circulating FFAs derived from adipocytes are elevated in many insulin-resistant states and have been suggested to contribute to the

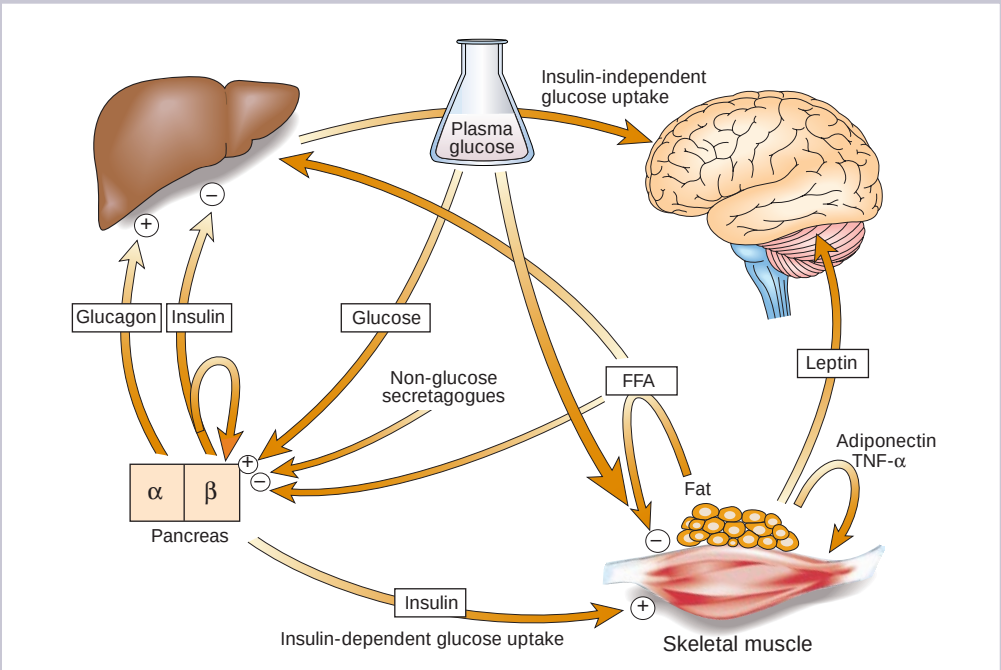


Figure 4 Cross-talk between tissues in the regulation of glucose metabolism. Insulin is secreted from the β-cells of the pancreas in response to elevations in plasma glucose. The hormone decreases glucose production from the liver, and increases glucose uptake, utilization and storage in fat and muscle. The fat cell is important in metabolic regulation, releasing FFAs that reduce glucose uptake in muscle, insulin secretion from the β-cell, and increase glucose production from the liver. The fat cell can also secrete 'adipokines' such as leptin, adiponectin and TNF, which regulate food intake, energy expenditure and insulin sensitivity.

insulin resistance of diabetes and obesity by inhibiting glucose uptake, glycogen synthesis and glucose oxidation, and by increasing hepatic glucose output[63]. Elevated FFAs are also associated with a reduction in insulin-stimulated IRS-1 phosphorylation and IRS-1-associated PI(3)K activity[83]. The link between increased circulating FFAs and insulin resistance might involve accumulation of triglycerides and fatty acid-derived metabolites (diacylglycerol, fatty acyl-CoA and ceramides) in muscle and liver. Nuclear magnetic resonance spectroscopy has shown a close correlation between intramyocellular triglyceride content and whole-body insulin resistance in patients with obesity and type 2 diabetes[84]. Transgenic mice with muscle- or liver-specific overexpression of lipoprotein lipase exhibit increases in tissue triglyceride content that correlate with decreases in insulin action and activation of IRS-associated PI(3)K[85]. Activation of PKC and/or IκB kinase and serine phosphorylation of the insulin receptor and its substrates might be important[83].

Adipokines

In addition to its role as a storage depot for lipid, the fat cell produces and secretes a number of hormones, collectively called adipokines, which may profoundly influence metabolism and energy expenditure. Expression of tumour-necrosis factor-α (TNF-α) is increased in fat of obese rodents and humans, and has been shown to produce serine phosphorylation of IRS-1, resulting in reduced insulin receptor kinase activity and insulin resistance[19]. In rodents, anti-TNF-α reagents significantly improved insulin resistance[86], although in humans the importance of this mechanism is much debated, as limited studies of anti-TNF reagents have shown little or no effect on the insulin-resistant state[87].

Leptin is a member of the cytokine family of hormones that is produced by adipose tissue and acts on receptors in the central nervous system and other sites to inhibit food intake and promote energy expenditure. Insulin resistance characterizes states of severe leptin deficiency or resistance, such as *ob/ob* or *db/db* mice, or genetic models of lipoatrophic diabetes. In some of these, administration of exogenous leptin improves glucose tolerance and insulin sensitivity independently of effects on food intake, probably by affecting neuroendocrine pathways that modulate insulin action in the liver[88,89], although this cytokine might also have direct effects on hepatic cells[90].

Adiponectin (also called Acrp30 or adipoQ) is a fat cell-derived peptide possessing a collagenous domain at the amino terminus and a globular domain that shares homology with complement factor C1q[91]. Recent studies have shown that expression of adiponectin mRNA is decreased in obese humans and mice and some models of lipoatrophic diabetes. Acute treatment of mice with this adipokine decreases insulin resistance, decreases plasma FFAs and the triglyceride content of muscle and liver, and increases expression of genes involved in fatty-acid oxidation and energy expenditure[92]. In lipoatrophic mice, insulin resistance is reversed by the combination of physiological doses of adiponectin and leptin, but only partially by either adiponectin or leptin alone. In isolated hepatocytes, adiponectin increased the ability of insulin to suppress glucose production[93]. A recent genome-wide scan in humans also mapped a susceptibility locus for type 2 diabetes and metabolic syndrome to chromosome 3q27 in a region near the adiponectin gene[94].

Resistin is the most recently discovered peptide hormone to be secreted by adipocytes. Resistin belongs to a family of related secreted proteins termed RELMs (resistin-like molecules) and FIZZ (found in inflammatory zone)[95]. Initial studies suggested that resistin might cause insulin resistance, as levels were increased in obese mice and reduced by antidiabetic drugs of the thiazolidinedione class. Furthermore, administration of antiresistin antibody seemed to improve blood sugar and insulin action in mice with diet-induced obesity. But subsequent studies have not confirmed these initial findings[96]. The potential role of resistin is further complicated by uncertainty about existence of a human homologue of the hormone.

Perspectives

There has been considerable progress over the past few years in unravelling the mechanisms of insulin action, and the molecular defects that give rise to insulin resistance. Recent advances dissecting the signalling pathways, cellular architecture and spatial compartmentalization of proteins, coupled with the sophisticated genetic analysis of the system, have yielded quantum leaps in our insight into how proteins and tissues interact to control glucose and lipid metabolism. But many gaps remain in our understanding of these processes, and in the pathophysiology underling insulin resistance. We need to define the missing steps in the insulin-signalling network, elucidate the mechanisms of cross-talk in the system, and determine the genetic susceptibility of insulin resistance and the interactions between genes and environment. Such studies will provide new insights into diabetes and insulin resistance, perhaps even allowing a more focused and individualized approach to therapy or prevention of these disorders. □

1. Klip, A. & Paquet, M. R. Glucose transport and glucose transporters in muscle and their metabolic regulation. *Diabetes Care* **13,** 228–243 (1990).
2. Bruning, J. C. *et al.* A muscle-specific insulin receptor knockout exhibits features of the metabolic syndrome of NIDDM without altering glucose tolerance. *Mol. Cell* **2,** 559–569 (1998).
3. Abel, E. D. *et al.* Adipose-selective targeting of the GLUT4 gene impairs insulin action in muscle and liver. *Nature* **409,** 729–733 (2001).
4. Gavrilova, O. *et al.* Surgical implantation of adipose tissue reverses diabetes in lipoatrophic mice. *J. Clin. Invest.* **105,** 271–278 (2000).
5. Kulkarni, R. N. *et al.* Tissue-specific knockout of the insulin receptor in pancreatic beta cells creates an insulin secretory defect similar to that in type 2 diabetes. *Cell* **96,** 329–339 (1999).
6. Patti, M. E. & Kahn, C. R. The insulin receptor—a critical link in glucose homeostasis and insulin action. *J. Basic Clin. Physiol. Pharmacol.* **9,** 89–109 (1998).
7. Butler, A. A. & LeRoith, D. Tissue-specific versus generalized gene targeting of the igf1 and igf1r genes and their roles in insulin-like growth factor physiology. *Endocrinology* **142,** 1685–1688 (2001).
8. Skorokhod, A. *et al.* Origin of insulin receptor-like tyrosine kinases in marine sponges. *Biol. Bull.* **197,** 198–206 (1999).
9. White, M. F. The IRS-signalling system: a network of docking proteins that mediate insulin action. *Mol. Cell. Biochem.* **182,** 3–11 (1998).
10. Pessin, J. E. & Saltiel, A. R. Signaling pathways in insulin action: molecular targets of insulin resistance. *J. Clin. Invest.* **106,** 165–169 (2000).
11. Tamemoto, H. *et al.* Insulin resistance and growth retardation in mice lacking insulin receptor substrate-1. *Nature* **372,** 182–186 (1994).
12. Araki, E. *et al.* Alternative pathway of insulin signaling in mice with targeted disruption of the IRS-1 gene. *Nature* **372,** 186–190 (1994).
13. Kido, Y. *et al.* Tissue-specific insulin resistance in mice with mutations in the insulin receptor, IRS-1, and IRS-2. *J. Clin. Invest.* **105,** 199–205 (2000).
14. Withers, D. J. *et al.* Disruption of IRS-2 causes type 2 diabetes in mice. *Nature* **391,** 900–904 (1998).
15. Fantin, V. R., Wang, Q., Lienhard, G. E. & Keller, S. R. Mice lacking insulin receptor substrate 4 exhibit mild defects in growth, reproduction, and glucose homeostasis. *Am. J. Physiol. Endocrinol. Metab.* **278,** E127–E133 (2000).
16. Fasshauer, M. *et al.* Essential role of insulin receptor substrate 1 in differentiation of brown adipocytes. *Mol. Cell. Biol.* **21,** 319–329 (2001).
17. Mendez, R., Myers, M. G. Jr, White, M. F. & Rhoads, R. E. Stimulation of protein synthesis, eukaryotic translation initiation factor 4E phosphorylation, and PHAS-I phosphorylation by insulin requires insulin receptor substrate 1 and phosphatidylinositol 3-kinase. *Mol. Cell. Biol.* **16,** 2857–2864 (1996).
18. Tsuruzoe, K., Emkey, R., Kriauciunas, K. M., Ueki, K. & Kahn, C. R. Insulin receptor substrate 3 (IRS-3) and IRS-4 impair IRS-1- and IRS-2-mediated signaling. *Mol. Cell. Biol.* **21,** 26–38 (2001).
19. Hotamisligil, G. S. *et al.* IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-alpha- and obesity-induced insulin resistance. *Science* **271,** 665–668 (1996).
20. Craparo, A., Freund, R. & Gustafson, T. A. 14-3-3 (ε) interacts with the insulin-like growth factor I receptor and insulin receptor substrate I in a phosphoserine-dependent manner. *J. Biol. Chem.* **272,** 11663–11669 (1997).
21. Yuan, M. *et al.* Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of *Ikkβ*. *Science* **293,** 1673–1677 (2001).
22. Kim, J. K. *et al.* Prevention of fat-induced insulin resistance by salicylate. *J. Clin. Invest.* **108,** 437–446 (2001).
23. Elchebly, M. *et al.* Increased insulin sensitivity and obesity resistance in mice lacking the protein tyrosine phosphatase-1B gene. *Science* **283,** 1544–1548 (1999).
24. Shepherd, P. R., Nave, B. T. & Siddle, K. Insulin stimulation of glycogen synthesis and glycogen synthase activity is blocked by wortmannin and rapamycin in 3T3-L1 adipocytes: evidence for the involvement of phosphoinositide 3-kinase and p70 ribosomal protein-S6 kinase. *Biochem. J.* **305,** 25–28 (1995).
25. Myers, M. G. Jr *et al.* IRS-1 activates phosphatidylinositol 3′-kinase by associating with src homology 2 domains of p85. *Proc. Natl Acad. Sci. USA* **89,** 10350–10354 (1992).
26. Pons, S. *et al.* The structure and function of p55PIK reveal a new regulatory subunit for phosphatidylinositol 3-kinase. *Mol. Cell. Biol.* **15,** 4453–4465 (1995).
27. Antonetti, D. A., Algenstaedt, P. & Kahn, C. R. Insulin receptor substrate 1 binds two novel splice variants of the regulatory subunit of phosphatidylinositol 3-kinase in muscle and brain. *Mol. Cell. Biol.* **16,** 2195–2203 (1996).

28. Fruman, D. A., Cantley, L. C. & Carpenter, C. L. Structural organization and alternative splicing of the murine phosphoinositide 3-kinase p85 alpha gene. *Genomics* **37,** 113–121 (1996).
29. Kerouz, N. J., Horsch, D., Pons, S. & Kahn, C. R. Differential regulation of insulin receptor substrates-1 and-2 (IRS-1 and IRS-2) and phosphatidylinositol 3-kinase isoforms in liver and muscle of the obese diabetic (*ob*/*ob*) mouse. *J. Clin. Invest.* **100,** 3164–3172 (1997).
30. Fruman, D. A. *et al.* Hypoglycaemia, liver necrosis and perinatal death in mice lacking all isoforms of phosphoinositide 3-kinase p85α. *Nature Genet.* **26,** 379–382 (2000).
31. Terauchi, Y. *et al.* Increased insulin sensitivity and hypoglycaemia in mice lacking the p85α subunit of phosphoinositide 3-kinase. *Nature Genet.* **21,** 230–235 (1999).
32. Lietzke, S. E. *et al.* Structural basis of 3-phosphoinositide recognition by pleckstrin homology domains. *Mol. Cell* **6,** 385–394 (2000).
33. Kessler, A., Uphues, I., Ouwens, D. M., Till, M. & Eckel, J. Diversification of cardiac insulin signaling involves the p85 alpha/beta subunits of phosphatidylinositol 3-kinase. *Am. J. Physiol. Endocrinol. Metab.* **280,** E65–E74 (2001).
34. Peterson, R. T. & Schreiber, S. L. Kinase phosphorylation: keeping it all in the family. *Curr. Biol.* **9,** R521–R524 (1999).
35. Mackay, D. J. & Hall, A. Rho GTPases. *J. Biol. Chem.* **273,** 20685–20688 (1998).
36. Ziegler, S. F., Bird, T. A., Schneringer, J. A., Schooley, K. A. & Baum, P. R. Molecular cloning and characterization of a novel receptor protein tyrosine kinase from human placenta. *Oncogene* **8,** 663–670 (1993).
37. Alessi, D. R. *et al.* Characterization of a 3-phosphoinositide-dependent protein kinase which phosphorylates and activates protein kinase Balpha. *Curr. Biol.* **7,** 261–269 (1997).
38. Cross, D. A. *et al.* The inhibition of glycogen synthase kinase-3 by insulin or insulin-like growth factor 1 in the rat skeletal muscle cell line L6 is blocked by wortmannin, but not by rapamycin: evidence that wortmannin blocks activation of the mitogen-activated protein kinase pathway in L6 cells between Ras and Raf. *Biochem. J.* **303,** 21–26 (1994).
39. Nakae, J., Park, B. C. & Accili, D. Insulin stimulates phosphorylation of the forkhead transcription factor FKHR on serine 253 through a Wortmannin-sensitive pathway. *J. Biol. Chem.* **274,** 15982–15985 (1999).
40. Brady, M. J., Bourbonais, F. J. & Saltiel, A. R. The activation of glycogen synthase by insulin switches from kinase inhibition to phosphatase activation during adipogenesis in 3T3-L1 cells. *J. Biol. Chem.* **273,** 14063–14066 (1998).
41. Cho, H. *et al.* Insulin resistance and a diabetes mellitus-like syndrome in mice lacking the protein kinase Akt2 (PKBβ). *Science* **292,** 1728–1731 (2001).
42. Standaert, M. L. *et al.* Protein kinase C-ζ as a downstream effector of phosphatidylinositol 3-kinase during insulin stimulation in rat adipocytes. Potential role in glucose transport. *J. Biol. Chem.* **272,** 30075–30082 (1997).
43. Ogg, S. & Ruvkun, G. The C. elegans PTEN homolog, DAF-18, acts in the insulin receptor-like metabolic signaling pathway. *Mol. Cell* **2,** 887–893 (1998).
44. Wada, T. *et al.* Role of the Src homology 2 (SH2) domain and C-terminus tyrosine phosphorylation sites of SH2-containing inositol phosphatase (SHIP) in the regulation of insulin-induced mitogenesis. *Endocrinology* **140,** 4585–4594 (1999).
45. Clement, S. *et al.* The lipid phosphatase SHIP2 controls insulin sensitivity. *Nature* **409,** 92–97 (2001).
46. Ribon, V. & Saltiel, A. R. Insulin stimulates tyrosine phosphorylation of the proto-oncogene product of c-Cbl in 3T3-L1 adipocytes. *Biochem. J.* **324,** 839–845 (1997).
47. Ribon, V., Herrera, R., Kay, B. K. & Saltiel, A. R. A role for CAP, a novel, multifunctional Src homology 3 domain-containing protein in formation of actin stress fibers and focal adhesions. *J. Biol. Chem.* **273,** 4073–4080 (1998).
48. Ribon, V., Johnson, J. H., Camp, H. S. & Saltiel, A. R. Thiazolidinediones and insulin resistance: peroxisome proliferator activated receptor gamma activation stimulates expression of the CAP gene. *Proc. Natl Acad. Sci. USA* **95,** 14751–14756 (1998).
49. Kimura, A., Baumann, C. A., Chiang, S. H. & Saltiel, A. R. The sorbin homology domain: a motif for the targeting of proteins to lipid rafts. *Proc. Natl Acad. Sci. USA* **98,** 9098–9103 (2001).
50. Baumann, C. A. *et al.* CAP defines a second signalling pathway required for insulin-stimulated glucose transport. *Nature* **407,** 202–207 (2000).
51. Chiang, S. H. *et al.* Insulin-stimulated GLUT4 translocation requires the CAP-dependent activation of TC10. *Nature* **410,** 944–948 (2001).
52. Watson, R. T. *et al.* Lipid raft microdomain compartmentalization of TC10 is required for insulin signaling and GLUT4 translocation. *J. Cell Biol.* **154,** 829–840 (2001).
53. Boulton, T. G. *et al.* ERKs: a family of protein-serine/threonine kinases that are activated and tyrosine phosphorylated in response to insulin and NGF. *Cell* **65,** 663–675 (1991).
54. Lazar, D. F. *et al.* Mitogen-activated protein kinase kinase inhibition does not block the stimulation of glucose utilization by insulin. *J. Biol. Chem.* **270,** 20801–20807 (1995).
55. Raught, B., Gingras, A. C. & Sonenberg, N. The target of rapamycin (TOR) proteins. *Proc. Natl Acad. Sci. USA* **98,** 7037–7044 (2001).
56. Nave, B. T., Ouwens, M., Withers, D. J., Alessi, D. R. & Shepherd, P. R. Mammalian target of rapamycin is a direct target for protein kinase B: identification of a convergence point for opposing effects of insulin and amino-acid deficiency on protein translation. *Biochem. J.* **344,** 427–431 (1999).
57. Thomas, G. & Hall, M. N. TOR signalling and control of cell growth. *Curr. Opin. Cell Biol.* **9,** 782–787 (1997).
58. Miron, M. *et al.* The translational inhibitor 4E-BP is an effector of PI(3)K/Akt signalling and cell growth in *Drosophila*. *Nature Cell Biol.* **3,** 596–601 (2001).
59. Cross, D. A., Alessi, D. R., Cohen, P., Andjelkovich, M. & Hemmings, B. A. Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. *Nature* **378,** 785–789 (1995).
60. Brady, M. J., Nairn, A. C. & Saltiel, A. R. The regulation of glycogen synthase by protein phosphatase 1 in 3T3-L1 adipocytes. Evidence for a potential role for DARPP-32 in insulin action. *J. Biol. Chem.* **272,** 29698–29703 (1997).
61. Newgard, C. B., Brady, M. J., O'Doherty, R. M. & Saltiel, A. R. Organizing glucose disposal: emerging roles of the glycogen targeting subunits of protein phosphatase-1. *Diabetes* **49,** 1967–1977 (2000).
62. Michael, M. D. *et al.* Loss of insulin signaling in hepatocytes leads to severe insulin resistance and progressive hepatic dysfunction. *Mol. Cell* **6,** 87–97 (2000).
63. Bergman, R. N. & Ader, M. Free fatty acids and pathogenesis of type 2 diabetes mellitus. *Trends Endocrinol. Metab.* **11,** 351–356 (2000).
64. Bergman, R. N. New concepts in extracellular signaling for insulin action: the single gateway hypothesis. *Recent Prog. Horm. Res.* **52,** 359–385 (1997).
65. Pilkis, S. J. & Granner, D. K. Molecular physiology of the regulation of hepatic gluconeogenesis and glycolysis. *Annu. Rev. Physiol.* **54,** 885–909 (1992).
66. Sutherland, C., O'Brien, R. M. & Granner, D. K. New connections in the regulation of PEPCK gene expression by insulin. *Phil. Trans. R. Soc. Lond. B* **351,** 191–199 (1996).
67. Yoon, J. C. *et al.* Control of hepatic gluconeogenesis through the transcriptional coactivator PGC-1. *Nature* **413,** 131–138 (2001).
68. Shimomura, I. *et al.* Insulin selectively increases SREBP-1c mRNA in the livers of rats with streptozotocin-induced diabetes. *Proc. Natl Acad. Sci. USA* **96,** 13656–13661 (1999).
69. Foretz, M. *et al.* ADD1/SREBP-1c is required in the activation of hepatic lipogenic gene expression by glucose. *Mol. Cell. Biol.* **19,** 3760–3768 (1999).
70. Shimomura, I. *et al.* Decreased IRS-2 and increased SREBP-1c lead to mixed insulin resistance and sensitivity in livers of lipodystrophic and ob/ob mice. *Mol. Cell* **6,** 77–86 (2000).
71. Anthonsen, M. W., Ronnstrand, L., Wernstedt, C., Degerman, E. & Holm, C. Identification of novel phosphorylation sites in hormone-sensitive lipase that are phosphorylated in response to isoproterenol and govern activation properties in vitro. *J. Biol. Chem.* **273,** 215–221 (1998).
72. Kitamura, T. *et al.* Insulin-induced phosphorylation and activation of cyclic nucleotide phosphodiesterase 3B by the serine-threonine kinase Akt. *Mol. Cell. Biol.* **19,** 6286–6296 (1999).
73. Cusi, K. *et al.* Insulin resistance differentially affects the PI 3-kinase- and MAP kinase-mediated signaling in human muscle. *J. Clin. Invest.* **105,** 311–320 (2000).
74. Taylor, S. I. & Arioglu, E. Syndromes associated with insulin resistance and acanthosis nigricans. *J. Basic Clin. Physiol. Pharmacol.* **9,** 419–439 (1998).
75. Stern, M. P. Strategies and prospects for finding insulin resistance genes. *J. Clin. Invest.* **106,** 323–327 (2000).
76. Bruning, J. C. *et al.* Development of a novel polygenic model of NIDDM in mice heterozygous for IR and IRS-1 null alleles. *Cell* **88,** 561–572 (1997).
77. Terauchi, Y. *et al.* Development of non-insulin-dependent diabetes mellitus in the double knockout mice with disruption of insulin receptor substrate-1 and β cell glucokinase genes. Genetic reconstitution of diabetes as a polygenic disease. *J. Clin. Invest.* **99,** 861–866 (1997).
78. Kulkarni, R. N. *et al.* Altered function of insulin receptor substrate-1-deficient mouse islets and cultured beta-cell lines. *J. Clin. Invest.* **104,** R69–R75 (1999).
79. Bruning, J. C. *et al.* Role of brain insulin receptor in control of body weight and reproduction. *Science* **289,** 2122–2125 (2000).
80. Zisman, A. *et al.* Targeted disruption of the glucose transporter 4 selectively in muscle causes insulin resistance and glucose intolerance. *Nature Med.* **6,** 924–928 (2000).
81. Katz, E. B., Stenbit, A. E., Hatton, K., DePinho, R. & Charron, M. J. Cardiac and adipose tissue abnormalities but not diabetes in mice deficient in GLUT4. *Nature* **377,** 151–155 (1995).
82. Kim, J. K. *et al.* Redistribution of substrates to adipose tissue promotes obesity in mice with selective insulin resistance in muscle. *J. Clin. Invest.* **105,** 1791–1797 (2000).
83. Shulman, G. I. Cellular mechanisms of insulin resistance. *J. Clin. Invest.* **106,** 171–176 (2000).
84. Hwang, J. H., Pan, J. W., Heydari, S., Hetherington, H. P. & Stein, D. T. Regional differences in intramyocellular lipids in humans observed by in vivo 1H-MR spectroscopic imaging. *J. Appl. Physiol.* **90,** 1267–1274 (2001).
85. Kim, J. K. *et al.* Tissue-specific overexpression of lipoprotein lipase causes tissue-specific insulin resistance. *Proc. Natl Acad. Sci. USA* **98,** 7522–7527 (2001).
86. Ventre, J. *et al.* Targeted disruption of the tumor necrosis factor-alpha gene: metabolic consequences in obese and nonobese mice. *Diabetes* **46,** 1526–1531 (1997).
87. Ofei, F., Hurel, S., Newkirk, J., Sopwith, M., Taylor, R. Effects of an engineered human anti-TNF-alpha antibody (SDP571) on insulin sensitivity and glycemic control in patients with NIDDM. *Diabetes* **45,** 881–885 (1996).
88. Halaas, J. L. *et al.* Weight-reducing effects of the plasma protein encoded by the obese gene. *Science* **269,** 543–546 (1995).
89. Shimomura, I., Hammer, R. E., Ikemoto, S., Brown, M. S. & Goldstein, J. L. Leptin reverses insulin resistance and diabetes mellitus in mice with congenital lipodystrophy. *Nature* **401,** 73–76 (1999).
90. Lee, Y. *et al.* Liporegulation in diet-induced obesity. The antisteatotic role of hyperleptinemia. *J. Biol. Chem.* **276,** 5629–5635 (2001).
91. Fruebis, J. *et al.* Proteolytic cleavage product of 30-kDa adipocyte complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice. *Proc. Natl Acad. Sci. USA* **98,** 2005–2010 (2001).
92. Yamauchi, T. *et al.* The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nature Med.* **7,** 941–946 (2001).
93. Berg, A. H., Combs, T. P., Du, X., Brownlee, M. & Scherer, P. E. The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. *Nature Med.* **7,** 947–953 (2001).
94. Vionnet, N. *et al.* Genomewide search for type 2 diabetes-susceptibility genes in French whites: evidence for a novel susceptibility locus for early-onset diabetes on chromosome 3q27-qter and independent replication of a type 2-diabetes locus on chromosome 1q21-q24. *Am. J. Hum. Genet.* **67,** 1470–1480 (2000).
95. Steppan, C. M. *et al.* A family of tissue-specific resistin-like molecules. *Proc. Natl Acad. Sci. USA* **98,** 502–506 (2001).
96. Nagaev, I. & Smith, U. Insulin resistance and type 2 diabetes are not related to resistin expression in human fat cells or skeletal muscle. *Biochem. Biophys. Res. Commun.* **285,** 561–564 (2001).
97. Accili, D., Nakae, J., Kim, J. J., Park, B. C. & Rother, K. I. Targeted gene mutations define the roles of insulin and IGF-I receptors in mouse embryonic development. *J. Pediatr. Endocrinol. Metab.* **12,** 475–485 (1999).
98. Postic, C. *et al.* Dual roles for glucokinase in glucose homeostasis as determined by liver and pancreatic β cell-specific gene knock-outs using Cre recombinase. *J. Biol. Chem.* **274,** 305–315 (1999).
99. Pessin, J. E., Thurmond, D. C., Elmendorf, J. S., Coker, K. J. & Okada, S. Molecular basis of insulin-stimulated GLUT4 vesicle trafficking. Location! Location! Location! *J. Biol. Chem.* **274,** 2593–2596 (1999).
100. Guilherme, A. *et al.* Perinuclear localization and insulin responsiveness of GLUT4 requires cytoskeletal integrity in 3T3-L1 adipocytes. *J. Biol. Chem.* **275,** 38151–38159 (2000).

Acknowledgements

We apologize to the many investigators whose work we could not cite owing to a limit on the number of references.