Insulin signaling, resistance, and metabolic syndrome: insights from mouse models into disease mechanisms

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Abstract

Insulin resistance is a major underlying mechanism responsible for the ‘metabolic syndrome’, which is also known as insulin resistance syndrome. The incidence of metabolic syndrome is increasing at an alarming rate, becoming a major public and clinical problem worldwide. Metabolic syndrome is represented by a group of interrelated disorders, including obesity, hyperglycemia, hyperlipidemia, and hypertension. It is also a significant risk factor for cardiovascular disease and increased morbidity and mortality. Animal studies have demonstrated that insulin and its signaling cascade normally control cell growth, metabolism, and survival through the activation of MAPKs and activation of phosphatidylinositide-3-kinase (PI3K), in which the activation of PI3K associated with insulin receptor substrate 1 (IRS1) and IRS2 and subsequent Akt → Foxo1 phosphorylation cascade has a central role in the control of nutrient homeostasis and organ survival. The inactivation of Akt and activation of Foxo1, through the suppression IRS1 and IRS2 in different organs following hyperinsulinemia, metabolic inflammation, and overnutrition, may act as the underlying mechanisms for metabolic syndrome in humans. Targeting the IRS → Akt → Foxo1 signaling cascade will probably provide a strategy for therapeutic intervention in the treatment of type 2 diabetes and its complications. This review discusses the basis of insulin signaling, insulin resistance in different mouse models, and how a deficiency of insulin signaling components in different organs contributes to the features of metabolic syndrome. Emphasis is placed on the role of IRS1, IRS2, and associated signaling pathways that are coupled to Akt and the forkhead/winged helix transcription factor Foxo1.

Introduction

Obesity, hyperglycemia, hyperlipidemia, and hypertension clustered together have been described as ‘insulin resistance syndrome’ or ‘syndrome X’ by Reaven et al. (Reaven 1988, Moller & Kaufman 2005). The constellation of metabolic abnormalities tightly correlates with cardiovascular dysfunction, resulting in high morbidity and mortality rates (Reaven 2005a). The term ‘metabolic syndrome’ has been adopted (Reaven 1988, DeFronzo & Ferrannini 1991, Kahn et al. 2005) and the clinical features of the syndrome have been established...
Table 1  Clinical criteria for the diagnosis of metabolic syndrome

<table>
<thead>
<tr>
<th>Metabolic parameters</th>
<th>ATP III</th>
<th>WHO</th>
<th>IDF</th>
<th>IDF</th>
<th>Diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdominal obesity (cm)</td>
<td>&gt; 102</td>
<td>&gt; 102</td>
<td>&gt; 94</td>
<td>&gt; 88</td>
<td>&gt; 80</td>
</tr>
<tr>
<td>Men: waist circumference</td>
<td>&gt; 88</td>
<td>&gt; 110, &lt; 126</td>
<td>&gt; 110</td>
<td>&gt; 100</td>
<td>&gt; 130</td>
</tr>
<tr>
<td>Women: waist circumference</td>
<td>&gt; 130/85</td>
<td>140/90</td>
<td>130/85</td>
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<tr>
<td>Fasting glucose (mg/dl)</td>
<td>150</td>
<td>150</td>
<td>150</td>
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<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>&lt; 40</td>
<td>&lt; 35</td>
<td>&lt; 40</td>
<td></td>
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</tr>
<tr>
<td>Men</td>
<td>&lt; 50</td>
<td>&lt; 39</td>
<td>&lt; 50</td>
<td></td>
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</tbody>
</table>

ATP III, Adult Treatment Panel III based on the National Cholesterol Education Program (NCEP); WHO, World Health Organization; IDF, International Diabetes Foundation.


Patients with type 1 diabetes suffer from insulin deficiency, owing to pancreatic β-cell failure, and insulin is a primary and effective therapy to decrease hyperglycemia and reduce the risk of cardiovascular dysfunction, as demonstrated by the Diabetes Control and Complications Trial (DCCT) (Nathan et al. 2005, Wilson 2011). However, patients with type 2 diabetes are non-insulin-dependent, in these patients intensive insulin therapy lowers blood glucose levels, but increases body weight and cardiovascular risk, as demonstrated in the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial (Wilson 2011). Intensive insulin therapy does not provide much cardioprotective benefit in adults, and two-thirds of patients with type 2 diabetes die of heart failure. Understanding the action of insulin and finding an effective management strategy for metabolic syndrome, type 2 diabetes mellitus, and associated cardiovascular dysfunction have important clinical implications.

Hyperinsulinemia, a major characteristic of metabolic syndrome, results from the oversecretion of insulin from pancreatic β-cells and is recognized as a primary contributor to the development of type 2 diabetes and cardiovascular dysfunction (Reaven 2005b, Battiprolu et al. 2010, Cao et al. 2010, Qi et al. 2013). Understanding the mechanisms responsible for insulin action and resistance will be critical for the management of metabolic syndrome and development of therapeutic interventions to prevent or treat type 2 diabetes. In this review, we provide mechanistic insights from animal studies as to how insulin resistance in different organs contributes to metabolic syndrome at the molecular, biochemical, and physiological levels.

Part 1: molecular basis of insulin signaling

Insulin and signal transduction studies have resulted in breakthroughs in the area of diabetes and biomedical research. Innovative attempts at insulin purification from the pancreas of animals, DNA and protein sequencing, crystallography, and RIA have been made by Banting, Sanger, Hodgkin, and Yalow, who all received Nobel prizes in 1923, 1958, 1969, and 1977 respectively (Yalow & Berson 1960). With the advent of molecular cloning technology in 1980, the genes encoding insulin receptor (IR (INSR)) and IR substrate (IRS) proteins were identified and sequenced (Kasuga et al. 1983, White et al. 1985, Sun et al. 1991, White & Kahn 1994).

IRS1 and IRS2

IR, a glycoprotein consisting of an extracellular α-subunit (135 kDa) and a transmembrane β-subunit (95 kDa), is an allosteric enzyme in which the α-subunit inhibits tyrosine kinase activity of the β-subunits. Insulin binding to the α-subunit results in the dimerization of the receptor to form the α2β2 complex in the cell membrane and autophosphorylation of the β-subunit at Tyr1158,
Try^{1162} and Tyr^{1163}, the first step in the activation of IR. The activation of IR tyrosine kinase recruits and phosphorylates several substrates, including IRS1–4, SHC, Grb-2-associated protein (GAB1), DOCK1, CBL, and APS adaptor proteins, all of which provide specific docking sites for the recruitment of other downstream signaling proteins, leading to the activation of both the Ras→MAPKs and phosphatidylinositide-3-kinase (PI3K)→Akt signaling cascade (White 2003).

IR and its homologous insulin-like growth factor 1 receptor (IGF1R) can also form heterodimers (IR/IGF1R) that modulate the selectivity and affinity for insulin and IGF1 in the activation of downstream signaling molecules (White 2003). Moreover, a recent report has indicated that IR forms a hybrid complex with Met, a transmembrane tyrosine kinase cell-surface receptor for hepatocyte growth factor (HGF) and structurally related to IR (Fafalios et al. 2011). The IR/Met hybrid complex results in robust signal output, by activating IR downstream signaling cascades, and mediates the metabolic effects of insulin (Fafalios et al. 2011).

IRS proteins and the docking proteins for IR provide interfaces by which insulin, IGF1, or HGF signaling propagates and engages with similar intracellular signaling components. IRS proteins are characterized by the presence of a NH2-terminal pleckstrin homology (PH) domain adjacent to a phosphotyrosine-binding (PTB) domain, followed by a COOH-terminal tail that contains numerous tyrosine and serine/threonine phosphorylation sites (Copps & White 2012). The PH domain mediates cell membrane interactions and the PTB domain binds to the phosphorylated NPXpY motif (Asn-Pro-Xaa-Tyr (pi); X, any amino acid and pi, inorganic phosphate) of the activated IR. The COOH terminal of each IRS protein has about 20 potential tyrosine phosphorylation sites that act as on/off switches to transduce insulin action, recruiting downstream signaling proteins, including PI3K subunit, phosphotyrosine phosphatase SHP2, and adaptor molecules such as GRB2, SOCS3, NCK, CRK, SH2B, and other molecules (White 2003, Sun & Liu 2009).

The activation of Ras→MAPKs mediates the effect of insulin on mitogenesis and cell growth; however, the activation of PI3K generates phosphatidylinositol (3,4,5)-triphosphate (PIP3), a second messenger activating 3-phosphoinositide-dependent protein kinase 1 (PDK1) and PDK2, which mediate the effect of insulin on metabolism and pro-survival. PDK1 and PDK2, in turn, activate the protein kinase Akt (PKB), by inducing phosphorylation at T^{308} and S^{473} respectively, and both PDK1 and PDK2 are crucial for the activation of Akt (Fig. 1).

PDK1 and TORC2 → Akt → TORC1 signaling cascades

Although PDK1 phosphorylates T^{308} of Akt resulting in the activation of Akt and has a profound effect on cell survival and metabolism (Alessi et al. 1997, Williams et al. 2000, Kikani et al. 2005), the action of PDK2 remains more of an enigma (Dong & Liu 2005). Mammalian target of rapamycin complex 2 (mTORC2), which interacts with rictor adaptor protein, is a rapamycin-insensitive companion of mTOR and has been identified to be PDK2 that phosphorylates the S^{473} of Akt (Alessi et al. 1997, Sarbassov et al. 2005, 2006). mTOR is a highly conserved protein kinase that controls cell growth and metabolism in response to nutrients, growth factors, and energy status and exists as two distinct complexes called complex 1 (mTORC1) and mTORC2 (Sengupta et al. 2010).

mTORC2 phosphorylates and activates Akt and other protein kinases, such as protein kinase C (PKC), controlling cell survival and energy homeostasis (Sarbassov et al. 2006, Hagiwara et al. 2012). mTORC2, through Akt, promotes the expression and activation of the sterol regulatory element-binding protein 1 (SREBP1) transcription factor, a family member of the SREBPs that promote lipid and cholesterol synthesis (Yecies et al. 2011). Moreover, mTORC2 and PDK1 suppress the Foxo1 forkhead transcription factor that promotes gluconeogenesis, mediating the effect of insulin on the suppression of hepatic glucose production (Hagiwara et al. 2012; Fig. 1).

mTORC2 is the mTOR interacting with the raptor adaptor protein, which is rapamycin-sensitive and is activated by Ras homolog enriched in brain GTPase (RhebGTPase), via the suppression of tuberous sclerosis protein 2 (TSC2) following the activation of Akt (Sengupta et al. 2010). mTORC1, which is not required for hepatic gluconeogenesis (Li et al. 2010), has as its substrates ribosomal protein S6 kinase (S6K) and eukaryotic initiation factor 4E-binding protein (4E-BP), both of which control protein synthesis. Recent data indicate that mTORC1 promotes lipogenesis via the phosphorylation of a phosphatidic acid phosphatase Lipin 1 and nuclear translocation of Lipin 1, stimulating SREBP1c and lipogenesis (Li et al. 2010, Peterson et al. 2011). S6K is required for the stimulation of SREBP1c in rat hepatocytes (Owen et al. 2012).

mTORC1 is also activated by nutrients, such as amino acids, suppressing cellular autophagy. Autophagy is a basic catabolic mechanism that involves the degradation of unnecessary or dysfunctional cellular components through lysosomal machinery and expression of a number of autophagy genes (Klionsky 2007). The breakdown of cellular components ensures cell survival during starvation by

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Figure 1
Insulin signaling cascade and interaction with intracellular signaling components from nutrients and cytokines involved in the control of cell metabolism, including the synthesis of glucose, glycogen, lipids and proteins, as well as other biological responses, such as autophagy, apoptosis, mitochondrial biogenesis, food intake, antioxidation, calcium handling, bone growth, and vascular dilation. PKA, protein kinase A; IR, insulin receptor; IRS, IR substrate; PI3K, phosphatidylinositol (PI)-3-kinase; PDK1, phosphoinositide-dependent protein kinase 1; CREB, cAMP response element-binding protein; CBP, CREB-binding protein; CRTC2, CREB-regulated co-factor 2; Foxo1, forkhead/winged helix family member O class member 1; SREBP1, sterol response element-binding protein 1; AS160, Akt substrate 160 kDa protein; Bad, BCL2-associated agonist of cell death; PDK4, pyruvate dehydrogenase kinase 4; PEPCK, phosphoenolpyruvate carboxykinase; G6Pase, glucose-6-phosphatase; FAS, fatty acid synthase; MnSOD, manganese superoxide dismutase; TLR, Toll-like receptor; FFA, free fatty acids; ChREBP, carbohydrate-responsive element-binding protein; AMPK, AMP-dependent protein kinase; pY, phosphorylated tyrosine; TNFα, tumor necrosis factor alpha; pS/T, phosphorylated serine or threonine; Pomc, pro-opiomelanocortin; Agrp, Agouti-related peptide; Gpr17, G-protein-coupled receptor 17; eNOS, endothelial nitric oxide synthase; Glut, glucose transporter; JNK, c-Jun N-terminal kinase; IKKβ, inhibitor of NFκB kinase; TSC1/2, tuberous sclerosis complex 1/2; Rheb, Ras homolog enriched in brain; pPKC, atypical protein kinase C; AS160, Akt substrate 160 kDa protein; Bad, BCL2-associated agonist of cell death; PDK4, pyruvate dehydrogenase kinase 4; ACC, acetyl-CoA carboxylase; PEPCK, phosphoenolpyruvate carboxykinase; G6Pase, glucose-6-phosphatase; FAS, fatty acid synthase; MnSOD, manganese superoxide dismutase; TLR, Toll-like receptor; FFA, free fatty acids; ChREBP, carbohydrate-responsive element-binding protein; AMPK, AMP-dependent protein kinase; pY, phosphorylated tyrosine; TNFα, tumor necrosis factor alpha; pS/T, phosphorylated serine or threonine; Pomc, pro-opiomelanocortin; Agrp, Agouti-related peptide; Gpr17, G-protein-coupled receptor 17; eNOS, endothelial nitric oxide synthase; Glut, glucose transporter; JNK, c-Jun N-terminal kinase; IKKβ, inhibitor of NFκB kinase.
maintaining cellular energy levels (Liu et al. 2009b). Thus, TORC1 and TORC2 serve as sensors and mediators for the action of both nutrients and hormones in cells.

**Targets of Akt in metabolic control**

Akt phosphorylates a number of downstream targets, including the inhibitors of macromolecular synthesis as follows: i) it phosphorylates and inhibits glycogen synthase kinase 3β (Gsk3b), which, in turn, dephosphorylates and activates glycogen synthase (GS) and ii) it inhibits TSC2, thereby activating Rheb GTPase for the activation of mTORC1 and S6K, which promote protein synthesis (Inoki et al. 2002). Akt also phosphorylates many other mediators involved in the control of numerous biological responses, including AS160 for Rab10 GTPase activation and Glut4 translocation; Bad for apoptosis inhibition; and PDE3B for cAMP degradation. Akt phosphorylates and inhibits CAMP response element-binding protein (CREB)-regulated transcription coactivator 2 (CRTC2), a CREB coactivator that increases hepatic gluconeogenesis (Wang et al. 2010). Most importantly, Akt regulates metabolism and survival by controlling the expression of a number of genes through transcription factors, such as SREBP1c and Foxo1.

Akt phosphorylates and stimulates Srebplc, promoting liver lipogenesis through the suppression of INSIG2, a protein of the endoplasmic reticulum that blocks the activation of SREBP1c by binding to SREBP cleavage-activating protein (SCAP) and preventing it from escorting SREBPs to the Golgi (Yabe et al. 2002). In contrast, Akt phosphorylates Foxo1 at S256 and inhibits Foxo1 transcriptional activity, suppressing glucose production in the liver and promoting cell survival in the heart (Guo et al. 1999, Hannenhalli et al. 2006, Matsumoto et al. 2007, Evans-Anderson et al. 2008, Battiprolu et al. 2012, Zhang et al. 2012). Many of these phosphorylation events are indicators of insulin signaling, and Akt→Foxo1 phosphorylation serves as a powerful indicator of insulin sensitivity in metabolic regulation in a variety of cells and tissues (Guo et al. 2006, 2009, Gonzalez et al. 2011, Qi et al. 2013; Fig. 1).

**Forkhead transcription factor Foxo1 signaling**

Foxo1, a member of the O class of forkhead/winged helix transcription factors (Foxo), was first identified as an Akt substrate in insulin signaling (Guo et al. 1999, Rena et al. 1999). Insulin suppresses the gene expression of IGF-binding protein 1 (IGFBP1) through a conserved insulin response element (IRE: CAAAACAA), located on the IGFBP1 promoter region (Cichy et al. 1998, Guo et al. 1999). A similar sequence is present in the promoter regions of a number of genes, including phosphoenolpyruvate carboxykinase (Pepck, Pck1) and glucose-6-phosphatase (G6pase, G6pc), two rate-limiting enzymes for gluconeogenesis (Schmoll et al. 2000, Yeagley et al. 2001). We demonstrated that Foxo1 serves as the endogenous transcription factor interacting with the IRE for the activation of target gene expression (Guo et al. 1999, Zhang et al. 2012). Foxo1 has three Akt phosphorylation sites at T24, S256, and S319 (Rena et al. 1999), and the phosphorylation of these residues, by insulin, promotes Foxo1 cytoplasmic translocation from the nucleus and interaction with SKIP2, a subunit of the SKIP1 (TRIB1)/CUL1/-f-box protein for Foxo1 ubiquitination and inhibits Foxo1-mediated gene transcription, by removing Foxo1 from gene transcriptional machinery (Biggs et al. 1999, Nakae et al. 1999, Rena et al. 2001, Woods et al. 2001, Rena et al. 2002, Matsuizaki et al. 2003, Huang et al. 2005). This provides a molecular link by which Foxo1 integrates cell-surface receptor signaling with gene transcriptional activity (Guo et al. 1999). Other members of the O class of forkhead family include Foxo3, Foxo4, and Foxo6, sharing the conserved Akt phosphorylation motif – RXRXXS/T (R, arginine; X, any amino acid; and S/T, Akt phosphorylation site of serine or threonine). Mice lacking Foxo1 displayed embryonic lethality and failed to complete embryonic angiogenesis, while mice lacking Foxo3 or Foxo4 survived beyond parturition (Hosaka et al. 2004). Mice lacking hepatic Foxo1, rather than Foxo3 or Foxo4, exhibited lower hepatic glucose production and blood glucose levels, and mice lacking both Foxo1 and Foxo3 or Foxo1, Foxo3, and Foxo4 exhibited a further reduction in blood glucose levels (Haeusler et al. 2010, Estall 2012, Zhang et al. 2012). Similarly, mice lacking Foxo6 also exhibited impaired hepatic glucose production (Kim et al. 2011, 2013). Thus, each of the members of the Foxo family has redundant as well as distinct roles in the regulation of physiological functions, the mechanisms of which are incompletely understood, but the inhibition of Foxo transcription factors mediates many of the metabolic effects of insulin (Fig. 1).

**Part 2: mechanisms for insulin resistance**

During the postprandial state, insulin secretion from the pancreatic β-cells controls systemic nutrient homeostasis by promoting anabolic processes in a variety of tissues. Insulin stimulates glucose influx into the muscle and adipose tissue, protein, and glycogen synthesis in the
mice lacking IR and prevented premature postnatal death (Okamoto et al. 2004, Lin & Accili 2011), suggesting that the liver, pancreatic β-cells, and brain are crucial for the maintenance of glucose homeostasis.

Recently, we have demonstrated that the deletion of both Irs1 and Irs2 genes in the liver of mice, designated as L-DKO mice (liver double Irs1 and Irs2 gene knockout mice), prevented the activation of hepatic Akt—Foxo1 phosphorylation and resulted in the development of hyperglycemia, hyperinsulinemia, insulin resistance, and hypolipidemia (Dong et al. 2008, Guo et al. 2009). The deletion of both Irs1 and Irs2 in the cardiac muscle diminished the phosphorylation of Akt (T^308 and S^173) and Foxo1 (S^253) and caused sudden death of male animals at the age of 6–8 weeks (Qi et al. 2013; Table 2). These results indicate that the loss of Irs1 and Irs2 may serve as a key component for insulin resistance and cardiac failure.

Loss of Irs1 and Irs2 results in insulin resistance

Gene knockout experiments in mice have helped to elucidate the role of IR, IRS1, and IRS2 in the control of growth and nutrient homeostasis (Guo 2013). Mice lacking the Ir gene were born with slight growth retardation, but rapidly developed hyperglycemia and hyperinsulinemia, followed by diabetic ketoacidosis and early postnatal death (Accili et al. 1996, Joshi et al. 1996). Although both Irs1 and Irs2 null mice displayed embryonic lethality (Withers et al. 1999), systemic Irs1 null mice displayed growth retardation and peripheral resistance to insulin and IGF1, mainly in the skeletal muscle, but did not develop diabetes because of Irs2-dependent pancreatic β-cell growth and compensatory insulin secretion (Araki et al. 1994). Systemic Irs2 null mice displayed metabolic defects in the liver, muscle, and adipose tissue, but developed diabetes secondary to pancreatic β-cell failure (Withers et al. 1998).

Tissue-specific gene knockout studies in mice provided new insights into the action of IR and control of glucose homeostasis and body weight (Nandi et al. 2004, Biddinger & Kahn 2006, Rask-Madsen & Kahn 2012). Mice lacking Ir in the liver, pancreatic β-cells, adipose tissue, or brain developed hyperglycemia, hyperlipidemia, hyperinsulinemia, and obesity (Kulkarni et al. 1999, Bruning et al. 2000, Michael et al. 2000, Boucher & Kahn 2013). The deficiency of Ir in the skeletal muscle also impaired glucose tolerance, even though circulating blood glucose levels were normal (Bruning et al. 1998, Kulkarni et al. 1999, Katic et al. 2007). Moreover, reconstitution of IR in the liver, β-cells, and brain prevented diabetes in mice lacking Ir and prevented premature postnatal death (Okamoto et al. 2004, Lin & Accili 2011), suggesting that the liver, pancreatic β-cells, and brain are crucial for the maintenance of glucose homeostasis.

Loss of Irs1 and Irs2 is linked to the inactivation of PI3K and Akt

IRS1 and IRS2 are associated tightly with PI3K and Akt activation and minimally with MAPK activity. The deficiency of Irs1 and Irs2 causes biased PI3K inactivation and sustained MAPK activation in the liver and heart of mice (Dong et al. 2008, Guo et al. 2009, Qi et al. 2013). Differential PI3K inactivation and MAPK activation by the loss of Irs1 and Irs2 in vivo may act as a fundamental mechanism to elucidate the prevalence of insulin resistance and association with type 2 diabetes, obesity, and cardiovascular dysfunction. The inhibition of IRS1 and IRS2 inactivates PI3K, disrupting nutrient homeostasis, and prolongs the activation of MAPKs (ERK1/2, p38, and JNK), promoting mitogenesis and overgrowth, resulting in obesity. Supporting this concept, mice lacking either the PI3K catalytic subunit or Akt2 exhibited insulin resistance and type 2 diabetes (Cho et al. 2001, Brachmann et al. 2005), while in mice lacking Erk1 (Mapk3), the growth of adipocytes was prevented and insulin resistance was improved following high-fat diet (HFD) treatment (Bost et al. 2005). Furthermore, in mice lacking Gab1, which is an ERK activator, insulin sensitivity was enhanced with elevated hepatic Akt activity (Bard-Chapeau et al. 2005).

Inactivation of PI3K → Akt → Foxo1 signaling causes diabetes and heart failure

The activation of PI3K and Akt plays a central role in metabolic regulation, which is supported by studies in animals and humans. Hepatic inactivation of PI3K,
PDK1, mTORC2, or both Akt1 and Akt2 is sufficient for the induction of hyperglycemia, hyperinsulinemia, and hypolipidemia (Miyake et al. 2002, Mora et al. 2005, Hagiwara et al. 2012, Lu et al. 2012). Mice lacking Akt2 developed type 2 diabetes mellitus (Cho et al. 2001), and AKT2 mutation has also been described in patients with type 2 diabetes mellitus (George et al. 2004). The expression of constitutively active Foxo1, when three Akt sites were mutated to alanine, blocked phosphorylation in either the liver, causing insulin resistance (Zhang et al. 2002), or the heart, resulting in embryonic lethality in mice (Evans-Anderson et al. 2008). Conversely, the

### Table 2 Phenotypes of conditional Irs knockout and Foxo knockout mice using the Cre-LoxP genetic approaches

<table>
<thead>
<tr>
<th>Tissue-specific Irs or Foxo null mouse genotype</th>
<th>Phenotype</th>
<th>Cre-mice</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypothalamic and β-cell Irs2−/−</td>
<td>Obesity; hyperglycemia; insulin resistance</td>
<td>RIP-cre</td>
<td>Lin et al. (2004)</td>
</tr>
<tr>
<td>Hypothalamic (AGRP neuron) Foxo1−/−</td>
<td>Leanness; reduced food intake; increased insulin and leptin sensitivity</td>
<td>Agrp-cre</td>
<td>Ren et al. (2012)</td>
</tr>
<tr>
<td>Hypothalamic (POMC neuron) Foxo1−/−</td>
<td>Leanness; reduced food intake; increased insulin and leptin sensitivity</td>
<td>Pomc-cre</td>
<td>Plum et al. (2009)</td>
</tr>
<tr>
<td>Leptin receptor neuron Irs2−/−</td>
<td>Obesity; hyperglycemia; insulin resistance</td>
<td>Lep-R-cre</td>
<td>Sadagurski et al. (2010, 2012)</td>
</tr>
<tr>
<td>Leptin receptor neuron Foxo1−/−::Irs2−/−</td>
<td>Leanness; prevented obesity and hyperglycemia from Irs2 deficiency</td>
<td>Lep-R-cre</td>
<td>Sadagurski et al. (2010, 2012)</td>
</tr>
<tr>
<td>Liver Irs1−/−</td>
<td>Normal glucose levels; severe insulin resistance on a high-fat diet</td>
<td>Alb-cre</td>
<td>Guo (2013)</td>
</tr>
<tr>
<td>Liver Irs1−/−::Irs2−/−</td>
<td>Normal glucose levels</td>
<td>Alb-cre</td>
<td>Guo et al. (1999, 2006, 2009)</td>
</tr>
<tr>
<td>Liver Foxo1−/−</td>
<td>Reduced blood glucose levels</td>
<td>Alb-cre</td>
<td>Guo et al. (1999, 2006, 2009) and Kubota et al. (2008, 2011)</td>
</tr>
<tr>
<td>Liver Foxo3−/−</td>
<td>Normal glucose levels</td>
<td>Alb-cre</td>
<td>Zhang et al. (2012)</td>
</tr>
<tr>
<td>Liver Foxo4−/−</td>
<td>Normal glucose levels</td>
<td>Alb-cre</td>
<td>Zhang et al. (2012)</td>
</tr>
<tr>
<td>Liver Foxo1−/−::Foxo3−/−::Foxo4−/−</td>
<td>Reduced blood glucose levels; increased triglyceride levels; hepatic steatosis</td>
<td>Alb-cre</td>
<td>Haeusler et al. (2010) and Zhang et al. (2012)</td>
</tr>
<tr>
<td>Liver Foxo1−/−::Irs1−/−::Irs2−/−</td>
<td>Prevented hyperglycemia from hepatic Irs1 and Irs2 deficiency</td>
<td>Alb-cre</td>
<td>Dong et al. (2008)</td>
</tr>
<tr>
<td>Skeletal and cardiac muscle Irs1−/−::Irs2−/−</td>
<td>Normal glucose levels; normal insulin levels; die 2 weeks after birth</td>
<td>MCK-cre</td>
<td>Long et al. (2011)</td>
</tr>
<tr>
<td>Cardiac Irs1−/−::Irs2−/−</td>
<td>Males die of heart failure at the age of 7 weeks; hyperlipidemia</td>
<td>Mhc-cre</td>
<td>Qi et al. (2013)</td>
</tr>
<tr>
<td>Cardiac Foxo1−/−</td>
<td>Prevented heart failure from a high-fat diet</td>
<td>Mhc-cre</td>
<td>Battiprolu et al. (2010, 2012)</td>
</tr>
<tr>
<td>Cardiac Foxo3−/−</td>
<td>Did not prevent heart failure from a high-fat diet</td>
<td>Mhc-cre</td>
<td>Battiprolu et al. (2010, 2012)</td>
</tr>
<tr>
<td>Pancreatic β-cell Foxo1−/−</td>
<td>Reduced β-cell regeneration; β-cells dedifferentiate into progenitor-like cells or α-cells; hyperglycagominemia; hyperglycemia</td>
<td>Ins2-cre</td>
<td>Talchai et al. (2012)</td>
</tr>
<tr>
<td>Endothelium Irs1−/−::Irs2−/−</td>
<td>Reduced Akt and eNOS phosphorylation; impaired skeletal muscle glucose uptake; insulin resistance</td>
<td>Tie2-cre</td>
<td>Kubota et al. (2011)</td>
</tr>
<tr>
<td>Endothelium Foxo1−/−::Foxo3−/−::Foxo4−/−</td>
<td>Increased ENOS phosphorylation; reduced inflammation and oxidative stress of endothelium; prevented atherosclerosis</td>
<td>Tie2-cre</td>
<td>Tsuchiya et al. (2012)</td>
</tr>
<tr>
<td>Bone osteoblast Foxo1−/−</td>
<td>Increased osteocalcin and insulin production; reduced blood glucose concentration</td>
<td>Collagen</td>
<td>Rached et al. (2010)</td>
</tr>
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</table>

Abbreviation of promoters driving Cre expression: RIP, rat insulin promoter; Agrp, Agouti-regulated peptide; Pomc, pro-opiomelanocortin; Lep-R, leptin receptor; Alb, albumin; MCK, muscle creatine kinase; Mhc, myosin heavy chain α; Ins2, insulin 2; Tie2, angiopoietin 2 receptor.
Mechanism of insulin resistance by hyperinsulinemia

Insulin resistance occurs at multiple levels in cells, from the cell surface to the nucleus, including desensitization of IR, suppression of IRS proteins and functionality, inhibition of PI3K cascades, and failure to restrain Foxo1-activated gene transcriptional profiling, all of which can result from the inhibition of IRS1 and IRS2.

IRS1 and IRS2 each contain 40 potential serine/threonine sites, which are phosphorylated by p38α MAPK, JNK, mTOR, and PKC, stimulating IRS protein degradation or inhibiting IRS-associated PI3K activation under pathological conditions (Sun & Liu 2009, Copps & White 2012, Guo 2013, Qi et al. 2013). Even under physiological conditions, there is a 50% reduction in hepatic IRS2 protein levels under feeding conditions, compared with fasting conditions (Ide et al. 2004). This observation suggests that the liver is probably more insulin resistant during a feeding state than during a fasting state, in which serine/threonine phosphorylation of IRS2 may decrease the expression and function of IRS2 protein. It is of note that PI3K→Akt signaling serves as a common platform for multiple hormone and growth factor signaling events (Hirsch et al. 2007, Sussman et al. 2011). Our recent studies have demonstrated that IRS1 and IRS2 are the major endogenous mediators activating the PI3K→Akt signaling cascade in the liver and heart of animals (Guo et al. 2009, Qi et al. 2013). Normal expression and functionality of IRS activating the PI3K→Akt signaling pathway are essential for animals to maintain nutrient homeostasis and cardiac function, while many factors can result in insulin resistance.

Hyperinsulinemia has profound effects on the induction of insulin resistance, which is supported by several lines of recent evidence: i) prolonged insulin treatment is sufficient for preventing the acute action of insulin on Foxo1 phosphorylation or Glut4 cellular membrane trafficking in myocardium and adipocytes (Gonzalez et al. 2011, Qi et al. 2013). ii) Insulin inhibits Irs2 gene transcription in the liver (Zhang et al. 2001) and promotes IRS2 ubiquitination or degradation in murine embryonic fibroblasts (Rui et al. 2001, Guo et al. 2006). The activation of mTORC1 following insulin stimulation is a major pathway that results in IRS2 ubiquitination and the mTORC1 inhibitor rapamycin completely prevents insulin- or IGF1-induced IRS2 degradation (Rui et al. 2001, Guo et al. 2006). Moreover, the deletion of hepatic S6k (Rps6k), a downstream target of mTORC1, improved insulin resistance, enhancing Irs1 and Irs2 gene expression and preventing diabetes in mice (Um et al. 2004, Bae et al. 2012). In contrast, the deletion of Torc2 in the liver of mice resulted in a diabetic phenotype, similar to that of L-DKO mice lacking both Irs1 and Irs2 in the liver (Guo et al. 2009, Hagiwara et al. 2012). It is of note that long-term treatment with rapamycin blocks mTORC2-mediated Akt phosphorylation/activation and the use of rapamycin for the treatment type 2 diabetes is a clinical challenge (Sarbassov et al. 2005). iii) Hyperinsulinemic treatment induces insulin resistance and is associated with oxidative stress and mitochondrial dysfunction in the skeletal muscle and liver of mice with type 1 diabetes (Liu et al. 2009a). iv) Decreased IRS1 and IRS2 expression levels are observed in the tissues of animals and patients with hyperinsulinemia or type 2 diabetes (Kerouz et al. 1997, Rondinone et al. 1997, Qi et al. 2013). v) The activation of p38 MAPK following prolonged insulin treatment in cardiomyocytes mediates insulin resistance by increasing IRS1 and IRS2 serine/threonine phosphorylation and degradation, as demonstrated in our recent studies (Qi et al. 2013). vi) p38 MAPK also mediates the induction of inflammatory cytokines that promote insulin resistance (Li et al. 2005, Shoelson et al. 2006). vii) Many, if not all, MAPKs can induce IRS serine/threonine phosphorylation and degradation, particularly when animals are fed a HFD. The activation of JNK induces IRS1 phosphorylation at S253 and activation (Yan et al. 2008, Wu et al. 2010).
Additionally, some PKC isoforms, such as PKCδ and PCK9, also have important roles in the induction of IRS serine/threonine phosphorylation, resulting in insulin resistance in tissues following HFD treatment (Gao et al. 2007, Bezy et al. 2011). Currently, there are about 1100 protein kinases found in mouse or human genome sequences. It is important to identify these kinases and activation mechanisms under different cellular and environmental conditions for the induction of IRS serine/threonine phosphorylation and inactivation of insulin signaling.

Foxo1 activation following insulin resistance

During the development of insulin resistance and diabetes mellitus, following the loss of Irs and inactivation of the PI3K→Akt signaling pathway, the inhibitory mechanism of Foxo1 by the activation of Akt upon feeding or insulin stimulation is uncontrolled. Thus, the dephosphorylation of Foxo1 at the conserved Akt phosphorylation sites (T24, S256, and S319) enhances Foxo1 stability and transcriptional activity, stimulating gluconeogenesis and resulting in hyperglycemia. An increase in nuclear dephosphorylated Foxo1-S253 levels was detected in the liver and heart of animals with type 2 diabetes (Altomonte et al. 2003, Battiprolu et al. 2012). The deletion of Foxo1 in the liver of L-DKO mice and db/db mice reduced hepatic glucose production and ameliorated diabetes (Dong et al. 2008, Zhang et al. 2012), and the deletion of Foxo1 in the heart of HFD mice prevented heart failure (Battiprolu et al. 2012). These results indicate that IRS→Akt→Foxo1 signaling cascades are critical to nutrient homeostasis and organ survival.

The aberrant activation of Foxo1 disrupts metabolic homeostasis and promotes organ failure, by regulating the expression of a target genes (Fig. 1). Foxo1 promotes hepatic glucose production via the expression of Pegck and G6pase and inhibits lipogenesis, resulting from the suppression of Srebp1c, and glucokinin and fatty acid synthase (Zhang et al. 2006, Zhang et al. 2012, Deng et al. 2013). Recently, we have identified a novel Foxo1 target gene – hemeoxygenase 1 (Hmox1), an enzyme catalyzing the degradation of heme to produce biliverdin, iron, and carbon monoxide. Heme is a component of the mitochondrial electron transport chain complexes III and IV, and constitutive Foxo1 activation, following the loss of Irs1 and Irs2, is a key component for heme degradation and impairment of mitochondrial biosynthesis and function (Cheng et al. 2009, Qi et al. 2013). This impairment results in reduced fatty acid oxidation and ATP generation, significantly contributing to triglyceride accumulation, resulting in organ steatosis or energy deficiency, as often observed in type 2 diabetes mellitus.

Activation of Foxo1 by multiple signaling mechanisms

The phosphorylation of Foxo1 at S253 by Akt promotes Foxo1 cytoplasmic retention and ubiquitination, which serve as a central mechanism controlling Foxo1 stability and activity (Guo 2013). However, Foxo1 can also be phosphorylated at different serine or threonine residues by other protein kinases, enhancing transcriptional activity. For example, mammalian sterile 20-like kinase 1 (MST1) promotes Foxo1 phosphorylation at S212, which promotes neuronal cell apoptosis (Yuan et al. 2009) or anti-oxidative stress responses, extending lifespan in Caenorhabditis elegans (Lehtinen et al. 2006). In addition to the phosphorylation-based pathway, the activity of Foxo1 can also be regulated by other post-translational modifications, including methylation, glycosylation, and acetylation (Fig. 2).

The methylation of Foxo1 at arginine R251 and R253 by protein arginine methyltransferase 1 (PRMT1) at the Akt consensus motif RXRXXS/T blocks Akt-mediated phosphorylation of Foxo1 at S253, resulting in long-lasting Foxo1 retention in the nucleus and activation of Foxo1 transcriptional activity (Yamagata et al. 2008, Takahashi et al. 2011). However, whether PRMT1 expression and Foxo1 methylation are altered in diabetics is unclear.

The glycosylation of Foxo1 at threonine T317 via O-GlcNac modification in response to glucose increased Foxo1 transcriptional activity for the expression of glucoenicogenes (Pegck and G6pase) and anti-oxidative stress genes (Mnsod, Sod2 and catalase) (Housley et al. 2008). The flux of glucose through the hexosamine biosynthetic pathway provides a substrate for the glucosamine-6-phosphate forming UDP-GlcNac (UDP-N-acetylglucosamine). O-GlcNacA modification of proteins results in an enzymatic addition of the N-acetyl glucosamine (GlcNAc) moiety of UDP-GlcNac on the hydroxyl oxygen of serines and threonines (Kuo et al. 2008). Foxo1-T317 is GlcNAcylated in the liver and it is a modification that is increased in diabetic animals (Housley et al. 2008), indicating that hyperglycemia further enhances Foxo1 activity in the absence of Foxo1-S253 phosphorylation following insulin resistance.

The acetylation of Foxo1 at several lysine residues has been identified, including at K242, K245, and K262, and the reversible acetylation is regulated by histone acetyltransferase CBP/p300 and NAD+-dependent histone
deacetylase SIRT2 (Matsuzaki et al. 2005). Early studies indicate that p300 acetylates Foxo1 and enhances Foxo1-induced transcription (Perrot & Rechler 2005), which may also involve histone acetylation by p300 for the activation of basal transcriptional machinery, while the deacetylation of Foxo1 by SIRT1 represses Foxo1 (Motta et al. 2004, Yang et al. 2005). In contrast, recent studies indicate that the acetylation of Foxo1 suppresses Foxo1 activity, while deacetylation by SIRT1 increases it (Matsuzaki et al. 2005, Jing et al. 2007), which is supported by a report that mutations of the lysines to glutamines (Q) in Foxo1, mimicking acetylation, resulted in the loss of Foxo1 function and embryonic lethality, while mutations of the lysines to arginines (R) prevented acetylation and potentiated Foxo1 activity (Banks et al. 2011).

Moreover, Foxo1 is deacetylated and activated by class IIa HDACs (Mihaylova et al. 2011). Nuclear HDAC4, HDAC5, and HDAC7 are phosphorylated and excluded from the nucleus by AMPK, but fasting hormone glucagon rapidly dephosphorylates and translocates the HDACs to the nucleus, where they associate with the promoters of gluconeogenic enzymes, such as Pepck and G6pase. In turn, HDAC4 and HDAC5 recruit HDAC3, which results in acute transcriptional induction of these genes via the deacetylation and activation of Foxo1 transcription factors. The loss of class IIa HDACs in murine liver results in the inhibition of Foxo1 target genes and lowers blood glucose levels (Mihaylova et al. 2011). Thus, the suppression of class IIa HDACs in mouse models of type 2 diabetes ameliorates hyperglycemia, indicating that the inhibitors of class I/II HDACs may serve as a potential therapeutic modality for metabolic syndrome. Moreover, with food intake, cells accumulate acetyl-CoA from glucose oxidation, providing substrate for the acetylation of Foxo1 and suppression of Foxo1 activity, in addition to insulin-induced inhibitory phosphorylation. Thus, Foxo1 merges the nutritional and hormonal signaling into a well-controlled metabolic regulation (Fig. 2).

It is of note that Foxo1 stimulates the expression of manganese superoxide dismutase (MnSOD) and catalase and enhances antioxidant responses. In rodents, the activation of Foxo1 following Irs2 deficiency in the brain enhanced longevity, but promoted obesity and diabetes (Taguchi et al. 2007). Also, the activation of Foxo1 enhanced myocardial survival upon the induction of oxidative stress (Sengupta et al. 2009, 2011, 2012) and autophagy for the control of cell size following serum starvation (Sengupta et al. 2009). Mice lacking systemic Foxo1 display embryonic lethality, since Foxo1 is required for endothelial cell lineage during cardiovascular development (Hosaka et al. 2004, Sengupta et al. 2012). In C. elegans, the Foxo1 ortholog Daf-16 enhances longevity when IR/IGF1R signaling is inactivated and potentially increases the expression of antioxidative genes (MnSOD) and also stimulates lipid droplet accumulation (Ogg et al. 1997). Together, these data indicate that the activation of Foxo1 is required for the maintenance of the life cycle under stressful conditions.

Figure 2
Human Foxo1 phosphorylation, ubiquitination, methylation, acetylation, and glycosylation at amino acid residues via different pathways and enzymes. PRMT1, protein arginine methyltransferase 1; MST1, mammalian sterile 20-like kinase 1; CK, casein kinase; DYRK1A, dual-specific tyrosine-phosphorylated and -regulated kinase 1A; Ub, ubiquitin; SIRT2, NAD⁺-dependent histone deacetylase silent information regulator 2; CBP, CREB-binding protein; p300, global transcription factor cofactor; P, phosphorylation; Me, methylation; G, glycosylation; Ac, acetylation.
conditions, such as prolonged fasting, in the liver for hepatic glucose production and activation of anti-oxidative mechanisms promoting survival in *C. elegans*. However, Foxo1 is activated through multiple layers of regulatory mechanisms, contributing to the development of type 2 diabetes mellitus and organ failure, following insulin resistance.

**Part 3: insulin resistance differentially contributes to metabolic syndrome phenotype**

**CNS insulin resistance causes obesity**

Human appetite is tightly controlled by the action of insulin in the CNS. The hypothalamus at the base of the forebrain comprises numerous small nuclei, each with distinct connections and neurochemistry, which regulate food intake, hormone release, sleep and wake cycles, and other biological functions. When an action potential, traveling along an axon, arrives at a neuronal synapse, it causes neurotransmitter release triggering biological responses in target cells (Myers & Olson 2012). A low dose of insulin delivery by i.c.v. infusion decreased both food intake and hepatic glucose production, effects which were blocked by PI3K inhibitors (Woods et al. 1979, Obici et al. 2002). Combined with evidence that mice with neuron-specific *Ir* deletion are overweight and insulin resistant (Bruning et al. 2000), current data indicate that neuronal insulin signaling is required for both body weight control and glucose homeostasis.

The functional significance of brain insulin signaling is further evidenced by the deletion of *Irs2* in the hypothalamus resulting in hyperglycemia and obesity in mice (Lin et al. 2004, Taguchi et al. 2007). The deletion of *Irs1* in the hypothalamus did not disrupt glucose homeostasis and obesity did not develop in young mice (Table 2; Guo & White, unpublished data 2009). Similar to the action of leptin, an adipocyte-derived hormone that inhibits food intake through CNS leptin receptor neurons activating the Jak2→Stat3 signaling cascade (Bates et al. 2003, Myers & Olson 2012), brain insulin signaling reduced food intake by the activation of PI3K via IRS2 and inactivation of Foxo1, which can be independent of the Jak2→Stat3 pathway (Taguchi et al. 2007). However, both leptin and insulin promoted IRS2 tyrosine phosphorylation and PI3K activation in the brain (Warne et al. 2011), and the deletion of *Irs2* in leptin receptor-expressing neurons caused diabetes and obesity, in which the inactivation of Foxo1 completely reversed the metabolic dysfunction (Sadagurski et al. 2012).

Hypothalamic neurons expressing Agouti-regulated peptide (*Agrp*) stimulate food intake (orexigenic: appetite stimulant) during the fasting state. Foxo1 stimulates orexigenic *Agrp* expression, an effect reversed by leptin delivery, in which the activation of Stat3 abrogates Foxo1 occupancy on the *Agrp* promoter region (Kitamura et al. 2006). The deletion of *Foxo1* in AGRP neurons of mice resulted in reduced food intake, leanness, and decreased hepatic glucose production, involving the suppression of a G-protein-coupled receptor *Gpr17*, a Foxo1 target gene in AGRP neurons (Ren et al. 2012). By antagonizing the effect of Agrp, hypothalamic neurons expressing pro-opiomelanocortin (*Pomc*) inhibit food intake during the feeding state (anorexic: lack of appetite). The deletion of *Foxo1* in POMC neurons resulted in reduced food intake and body weight, by increasing the expression of obesity susceptibility gene, carboxypeptidase E (*Cpe*), and subsequent production of β-endorphin, which mediates anorexic effects in mice (Plum et al. 2009).

**Insulin resistance in adipose tissue, hyperlipidemia, and the role of inflammation**

A key feature of metabolic syndrome is hyperlipidemia, which probably results from insulin resistance in adipose tissue. Insulin promotes fat cell differentiation, enhances adipocyte glucose uptake, and inhibits adipocyte lipolysis. Mice lacking adipocyte *Torc2* exhibited hyperglycemia, hyperinsulinemia, failure to suppress lipolysis in response to insulin, elevated circulating fatty acid and glycerol levels, and insulin resistance in the skeletal muscle and liver (Kumar et al. 2010). Recent studies have shown that mice lacking *Ir* in adipose tissue, created by the adiponectin promoter-driven Cre/LoxP system, developed severe lipoatrophic diabetes, a 95% reduction of white adipose tissue, hyperglycemia, hyperinsulinemia, hyperlipidemia, and liver steatosis (Boucher & Kahn 2013). These data indicate that when insulin action fails in the adipose tissue, adipocyte development is retarded and lipids are unable to convert from carbohydrates for storage. Thus, both glucose and lipids will redistribute into the circulation and organs, resulting in hyperlipidemia and fatty organs. These studies significantly underscore the contribution of insulin resistance in adipose tissue, via the inactivation of Akt signaling, to the control of systemic nutrient homeostasis.

Adipose tissue is also an endocrine organ secreting cytokines and hormones, including TNFα (TNF), IL6, leptin, adiponectin, and many other factors, influencing food intake, systemic insulin sensitivity, and nutrient
homeostasis. However, obesity from fat expansion disrupts a proper balance of cytokine and hormone generation, promoting insulin resistance. For example, TNFα, IL6, and leptin are pro-inflammatory factors and their levels are markedly increased in obesity, where the levels of adiponectin, which has anti-inflammatory effects on the enhancement of insulin sensitivity, are markedly reduced (Hotamisligil et al. 1993, Shoelson et al. 2006, Hotamisligil & Erbay 2008, Romeo et al. 2012). The overexpression of IκkB for the activation of NFκB (a key player in the control of pro-inflammatory responses) in the liver of mice is sufficient for inducing insulin resistance and type 2 diabetes (Cai et al. 2005). TNFα reduces IRS1 protein levels by the activation of JNK or S6K, resulting in insulin resistance (Gao et al. 2002, Zhang et al. 2008). Thus, the suppression of inflammation increases insulin sensitivity and reduces metabolic dysfunction in type 2 diabetes mellitus (Hotamisligil et al. 1996). However, the outcome of anti-inflammatory therapy in treating insulin resistance deserves a cautionary note for several reasons, which are as follows: i) inflammation is involved in the deployment and mobilization of immune cell leukocytes to defend against infections or toxins. Many inflammatory actors, such as TNFα, reduce body weight and increase energy expenditure (Ye & McGuinness 2013). The overexpression of IL6, in the liver, increased energy expenditure and insulin sensitivity in mice (Sadagurski et al. 2010). ii) During physical exercise, inflammatory factors, such as TNFα and IL6, are secreted resulting in the inhibition of anabolic metabolism (insulin action) and promoting catabolic metabolism (fat lipolysis) to meet the fuel requirements of the muscle. iii) NFκB is essential for hepatocyte proliferation and survival, and mice lacking the p65 subunit of NFκB die of liver failure (Geisler et al. 2007, Malato et al. 2012). iv) Inflammation not only triggers pro-inflammatory responses, but also activates anti-inflammatory processes. Together, these data indicate that a balance between inflammation and anti-inflammation is required for proper insulin actions and nutrient homeostasis. Thus, correcting the imbalance of hormones, nutrients, and inflammation may provide opportunities and challenges for the prevention and treatment of metabolic syndrome and type 2 diabetes.

In general, excess energy storage in tissues, particularly lipids, is now believed to be a primary factor contributing to metabolic syndrome (Reaven 2005a). Free fatty acids derived from nutritional intake or conversion from carbohydrates not only act as an important energy source, but also act as signaling molecules in the modulation of intracellular protein kinases (PKC, JNK, etc.) for the inactivation of insulin signaling (Oh et al. 2010, Holzer et al. 2011). Excess lipid accumulation in several organs, including adipose tissue, liver, muscle, heart, and blood vessels, results in insulin resistance and triggers metabolic inflammation, a low-grade and chronic inflammatory response (Samuel et al. 2010, Samuel & Shulman 2012). An acute lipid or fatty acid infusion or chronic HFD directly induces insulin resistance in mice via the activation of PKCθ (Griffin et al. 1999, Boden 2011). Saturated fatty acids also interact with a liver-secreted glycoprotein fetuin A that binds and activates Toll-like receptor 4, resulting in NFκB activation (Pal et al. 2012) and c-SRC recruitment for the activation of JNK and inhibition of insulin action (Holzer et al. 2011). Moreover, saturated fatty acids induce apoptosis in hepatocytes and pancreatic β-cells, by activating PKCζ, JNK, and oxidative stress, inhibiting IRS1/2 tyrosine phosphorylation, and blocking insulin signaling (Fig. 1; Wrede et al. 2002, Malhi et al. 2006, Wong et al. 2009, Galbo et al. 2013). In contrast, unsaturated fatty acids interact with the G-protein-coupled receptor GRP120, inhibiting inflammation and obesity and increasing insulin sensitivity (Ichimura et al. 2012). In the liver, lipid accumulation (hepatic steatosis) is a risk factor for non-alcoholic steatohepatitis, fibrosis, cirrhosis, and liver cancer (Kumashiro et al. 2011, Samuel & Shulman 2012).

Hepatic insulin resistance results in hyperglycemia

Hyperglycemia is caused by insulin resistance not only in the brain and adipose tissue, but also in the liver, which is a central organ controlling blood glucose and lipid homeostasis. Insulin promotes the synthesis of the macromolecules glycogen, lipids and protein in the liver and suppresses hepatic glucose production by inhibiting gluconeogenesis. The deletion of either Ir1s1 or Ir2s2 in the liver maintained glucose homeostasis, but the deletion of both Ir1s1 and Ir2s2 (L-DKO mice) blocked the induction of Akt and Foxo1 phosphorylation by insulin or feeding and resulted in unrestrained gluconeogenesis for hepatic glucose production, resulting in hyperglycemia, with a reduction in hepatic lipogenesis and blood lipid levels (Kubota et al. 2008, Guo et al. 2009). Moreover, a HFD severely impaired IRS2 expression and tyrosine phosphorylation in the hepatocytes of liver-specific Ir1s1 null mice and the mice developed severe diabetes (Guo et al. 2009). Overnutrition or a HFD can modify intracellular signaling, affecting IRS2 expression and functionality, altering metabolic gene expression, and impairing glucose homeostasis.
Hepatic insulin resistance also results in insulin resistance in other tissues, which is demonstrated in L-DKO mice. The L-DKO mice exhibited not only inhibition of the hepatic Akt signaling cascade, but also blunted brain i.c.v. insulin action on the reduction of hepatic glucose production in i.c.v. clamp experiments (Guo et al. 2009). Moreover, L-DKO mice exhibited features of heart failure, probably secondary to hyperinsulinemia, resulting in cardiac IRS1 and IRS2 suppression (Qi et al. 2013). Similarly, mice lacking hepatic Ir displayed pro-atherogenic lipoprotein profiles with reduced HDL cholesterol and VLDL particles, and within 12 weeks of being placed on an atherogenic diet, they developed severe hypercholesterolemia (Biddingger et al. 2008). These data indicate that hepatic insulin resistance is sufficient to produce dyslipidemia and increased risk of atherosclerosis and cardiac dysfunction.

The role of Foxo1 activation in the control of the development of diabetes is supported by findings in L-TKO mice, which lack Irs1, Irs2, and Foxo1 genes in the liver. L-TKO mice demonstrated a significant reversal of elevated blood glucose levels, glucose intolerance, and the fasting-feeding effect on hepatic gene expression, which were observed in L-DKO mice (Dong et al. 2008). Similarly, mice lacking both Akt1 and Akt2 in the liver (Akt-DLKO) or lacking Pdk1 or Mtorc2 (which blocks Akt activation) developed a similar diabetic phenotype to that seen in L-DKO mice (Mora et al. 2005, Guo et al. 2009, Hagiwara et al. 2012, Lu et al. 2012). Moreover, mice lacking Akt1, Akt2, and Foxo1 (TLKO) rescued diabetes in the Akt-DLKO mice (Lu et al. 2012). It is of interest that, L-TKO and TLKO mice had normal glucose tolerance and responses to the fasting-feeding challenge and suppressed Pepck and G6Pase gene expression to a degree similar to that of control mice (Chai et al. 2008, Lu et al. 2012), indicating that there is an Akt and Foxo1-independent pathway regulating blood glucose homeostasis, the mechanism of which is unclear. It is likely that hepatic Foxo1 deletion may sensitize brain insulin signaling to reduce hepatic glucose production, even though Akt activity is not controlled.

**Cardiac insulin resistance promotes heart failure**

The loss of Irs1 and Irs2 in the liver and brain resulted in hyperglycemia, while loss in other tissues, such as the heart and pancreas, resulted in organ failure. Thus, it is likely that diabetes may serve as a link to the development of heart failure via the loss of IRS proteins. The heart is an insulin-responsive and energy-consuming organ that requires a constant fuel supply to maintain intracellular ATP levels for myocardial contraction. The deletion of both cardiac Irs1 and Irs2 (H-DKO mice: heart-specific double Irs1 and Irs2 gene knockout) diminished cardiac Akt and Foxo1 phosphorylation and resulted in heart failure and death of male animals at 7–8 weeks of age (Qi et al. 2013). The deletion of both Irs1 and Irs2 in the skeletal and cardiac muscle caused heart failure and diminished Akt and Foxo1 phosphorylation in the skeletal muscle, but the mice had normal blood glucose levels and insulin sensitivity (Long et al. 2011), indicating that insulin resistance in the skeletal muscle is not necessary for the disruption of glucose homeostasis in mice. In contrast, cardiac muscle requires either Irs1 or Irs2 for the maintenance of endogenous Akt activity and Foxo1 inactivation to promote cardiac function and survival. The overexpression of cardiac Foxo1, which caused heart failure in mice (Evans-Anderson et al. 2008), was also observed in failing human hearts (Hannenhalli et al. 2006).

The loss of Irs1 and Irs2 following chronic insulin stimulation and p38 MAK activation contributes to insulin resistance in the heart (Qi et al. 2013). Based on our recent studies, we proposed that the regulation of IRS1 and IRS2 has a major role in the control of cardiac homeostasis, metabolism, and function. This concept was based on the following observations: i) metabolic adaptation during physiological conditions (phase I); ii) metabolic remodeling following the development of insulin resistance and mild cardiac dysfunction (phase II); and iii) maladaptive metabolic and cardiac remodeling, leading to cardiac failure and sudden death (phase III).

During phase I in the postprandial setting, insulin stimulates glucose transport and oxidation, resulting in effective cardiac utilization of glucose as a substrate for the supply of ATP. A 20–40% reduction in IRS2 protein levels was found in mouse liver and heart, compared with those in the fasting state (Guo et al. 2009). In phase II when insulin resistance occurs, the heart undergoes adaptive responses to limit glucose utilization (insulin-dependent) and responds to lipid oxidation (less insulin-dependent). The heart is capable of generating ATP for myocardial contraction and changes in gene expression patterns, with unaltered cardiac morphology. During this period, the metabolic adaptation or remodeling compensates for cardiac energy demand, even without overt indications of heart failure. With continued insulin resistance resulting from hyperinsulinemia and/or other metabolic and mechanical stresses, cardiac dysfunction develops, as exhibited by L-DKO mice, which have a 60–70% reduction in cardiac IRS1 and IRS2 levels in the heart in association with cardiac dysfunction (Qi et al. 2013). During phase III
in H-DKO mice, when maladaptive metabolic remodeling occurs, there is a lack of compensation for cardiac energy demand, secondary to the loss of Irs1 and Irs2, with Akt inactivation, utilization of both glucose and fatty acids being restrained, resulting in hyperlipidemia and cardiac ATP deficiency and sudden death (Qi et al. 2013). In this phase, the failing heart may exhibit a loss of mitochondrial biogenesis, a process required for fatty acid and glucose utilization via mitochondrial oxidative phosphorylation. In addition, unknown myocardial factors, which are derived from the loss of Irs1 and Irs2 and released to cardiofibroblasts, may also contribute to the onset of interstitial fibrosis. Thus, sensitizing myocardial Akt→Foxo1 signaling, by integrating insulin therapy and blocking the p38→IRS1/2 signaling cascade, may serve as a new treatment modality for heart failure, during insulin resistance, type 2 diabetes mellitus, and other chronic physiological stresses (Guo 2013, Qi et al. 2013).

**Insulin resistance in pancreas impairs β-cell regeneration**

Pancreatic β-cell failure is essential for the development of hyperglycemia in type 1 diabetes, but β-cell failure is also observed in patients with type 2 diabetes (Rhodes 2005, Rhodes et al. 2013). The β-cells secret insulin, reducing blood glucose levels, and the α-cells secret glucagon, increasing blood glucose levels to meet bodily metabolic requirements. Recent studies have shown that insulin enhances glucose-stimulated insulin secretion in healthy humans (Bouche et al. 2010) and mice lacking Ir in β-cells exhibited impaired insulin secretion (Kulkarni et al. 1999). However, whether insulin has a direct autocrine action on β-cells in promoting insulin secretion is unclear (Rhodes et al. 2013).

The deletion of whole-body Irs2 in mice resulted in diabetes owing to pancreatic β-cell failure (Withers et al. 1998), while the inactivation of Foxo1 in Irs2 null mice prevented β-cell apoptosis and diabetes (Nakae et al. 2002), indicating that IRS2→Foxo1 signaling or Foxo1 inactivation is required for β-cell survival. On the other hand, the deletion of Irs2 in β-cells triggered β-cell repopulation or regeneration, leading to a restoration of insulin secretion and resolution of diabetes in aged mice (Lin et al. 2004), indicating that Foxo1 activation following IRS2 inactivation in β-cells promotes β-cell regeneration or differentiation. Conversely, the inactivation of Foxo1 in β-cells resulted in reduced β-cell mass, hyperglycemia, and hyperglucagonemia, owing to the dedifferentiation of β-cells into progenitor-like cells or pancreatic α-cells (Talchai et al. 2012, Kitamura 2013).

Insulin resistance and/or hyperinsulinemia is the main cause of type 2 diabetes, but more recently, there has been evidence for a failure of functional β-cell mass to meet metabolic demand, the mechanism of which is unclear (Rhodes 2005, Kahn et al. 2006). On the other hand, antagonizing glucagon receptor action in type 1 diabetes induced by streptozotocin and type 2 diabetes mellitus in mice markedly reduced blood glucose levels and completely prevented diabetes (Liang et al. 2004, Sorensen et al. 2006, Ali & Drucker 2009, Lee et al. 2011). Thus, an abnormality at the level of the pancreas is critical for the development of diabetes, and the correction of the imbalance of hormones between insulin (β-cells) and glucagon (α-cells) may provide a potential strategy to prevent diabetes.

**Insulin resistance in skeletal muscle shortens lifespan**

Skeletal muscle is an important fuel storage tissue for glucose uptake, converting it to glycogen and triglycerides, a process stimulated by insulin. Skeletal muscle demonstrates remarkable metabolic flexibility to consume and store glucose and lipids. Mice lacking muscular Ir display elevated fat mass, serum triglyceride levels, and free fatty acid levels, but blood glucose levels, serum insulin levels, and glucose tolerance are normal. Thus, insulin resistance in muscle contributes to the altered fat metabolism associated with type 2 diabetes, but tissues other than muscle appear to be more involved in insulin-regulated glucose disposal than previously recognized (Bruning et al. 1998). Mice lacking Mtorc2 exhibited decreased insulin-stimulated phosphorylation of Akt-S473 and glucose uptake and mild glucose intolerance (Kumar et al. 2008), while mice lacking Mtorc1 displayed dystrophic muscle, mild glucose intolerance, and shortened lifespan (Bentzinger et al. 2008). Mice lacking both Irs1 and Irs2 in the skeletal and cardiac muscle died at 3 weeks of age, and had a much shorter lifespan than mice lacking both Irs1 and Irs2 in only the cardiac muscle (H-DKO mice), which died at 7 weeks of age (Qi et al. 2013), indicating that insulin action in skeletal muscle has a key and unrecognized role in the control of lifespan and mTORC1 may also contribute to this observed effect.

Mice lacking both Irs1 and Irs2 in the skeletal and cardiac muscle did not develop hyperglycemia or hyperinsulinemia, though insulin-induced glucose uptake was diminished. However, AMP levels were elevated in the skeletal muscle, resulting in the activation of AMPK (Long et al. 2011). AMPK stimulates glucose uptake in an insulin-independent manner, by phosphorylating and...
activating the Rab GAP family member AS160, which promotes Glut4 translocation (Taylor et al. 2008, Pehmoller et al. 2009). AMPK also induces acetyl-CoA carboxylase (ACC) phosphorylation and inhibits ACC activity, preventing the conversion of acetyl-CoA to malonyl-CoA, disrupting lipid synthesis, and enhancing fatty acid oxidation (Hoehn et al. 2010). Together, these studies underscore the flexibility of skeletal muscle in the control of glucose homeostasis and longevity. Since skeletal muscle actively secretes hormones (myokines), such as irisin, a hormone that systemically regulates glucose homeostasis and obesity (Bostrom et al. 2012, Muolo & Neuf 2012), it would be of interest to determine whether a skeletal muscle-derived hormone affects longevity in animals.

**Insulin resistance in vascular endothelium promotes hypertension and disrupts glucose homeostasis**

Vasodilator actions of insulin are mediated by PI3K-dependent signaling pathways that stimulate the production of nitric oxide from vascular endothelium (Muniyappa et al. 2008, Xu & Zou 2009). Insulin resistance in vascular endothelium stimulates vasoconstriction, promotes hypertension and atherosclerosis, and impairs systemic insulin sensitivity and glucose homeostasis. The inactivation of IR in vascular endothelium diminished insulin-induced eNOS phosphorylation and blunted aortic vasorelaxant responses to acetylcholine and calcium ionophore in normal mice (Duncan et al. 2008) and accelerated atherosclerosis in apolipoprotein E null mice (Rask-Madsen et al. 2010). Vascular endothelium deficient in Irs2 or both Irs1 and Irs2 reduced endothelial Akt and eNOS phosphorylation and impaired skeletal muscle glucose uptake, resulting in systemic insulin resistance (Kubota et al. 2011). The activation of Foxo following the deficiency of Irs2 or both Irs1 and Irs2 may play a key role in the stimulation of endothelial cell dysfunction. In fact, the deletion of Foxo1, Foxo3, and Foxo4 in the endothelium enhanced eNOS phosphorylation, reduced inflammation and oxidative stress of endothelial cells, and prevented atherosclerosis in HFD or LDL receptor null mice (Tsuchiya et al. 2012). Endothelium-targeted deletion of Ir or Foxo genes in mice barely disrupted glucose homeostasis (Duncan et al. 2008, Rask-Madsen et al. 2010, Tsuchiya et al. 2012); however, we have recently shown that endothelium-targeted deletion of the transcription factor-related transcriptional enhancer factor 1 (RTEF1, known as Tead4) increased blood glucose levels and insulin resistance. RTEF1 has the potential to interact with the IRE and Foxo1 in cells (Messmer-Blust et al. 2012). Thus, vascular endothelium serves as an organ that potentially regulates glucose homeostasis.

**Insulin resistance in bone impairs glucose homeostasis**

Insulin promotes the formation of bone and differentiation of osteoblasts that synthesize osteocalcin, a bone-derived insulin secretagogue that regulates pancreatic insulin secretion and systemically controls glucose homeostasis. Mice lacking Ir in osteoblasts exhibited reduced bone formation, increased peripheral adiposity, and insulin resistance, primarily by reduced gene expression and activity of osteocalcin (Ferron et al. 2010, Fulzele et al. 2010). The results of these studies indicate that in osteoblasts insulin may stimulate osteocalcin by suppressing Foxo1, which affects bone remodeling and glucose homeostasis control. Foxo1 inhibits osteocalcin expression and activity by increasing the expression of ESP, a protein tyrosine phosphatase that inhibits the bioactivity of osteocalcin by favoring its carboxylation. Moreover, osteoblast-specific Foxo1 null mice exhibit increased osteocalcin expression and insulin production and reduced blood glucose levels (Rached et al. 2010). Collectively, these data indicate that bone serves as an endocrine organ involved in the control of glucose homeostasis, through bone–pancreas crosstalk, in which Foxo1 plays a key role in insulin action regulating osteocalcin expression and activity in osteoblasts.

**Part 4: other considerations**

**Mouse models**

A large body of evidence related to the mechanisms of diabetes, obesity, and cardiovascular diseases has been derived from mouse studies. However, mice have a high heart rate: 600 vs 70 beats/min in humans; brain glucose intake in mice is much less than that in humans, 15 vs 65% respectively; and mice are nocturnal animals and inactive during daytime when many data are often collected for analyses. Also, experimental mice have immune gene transcriptional programs that are divergent from those of humans (Shay et al. 2013). Humans live in a mobile environment. Recent studies have indicated that gastrointestinal microbiota may trigger inflammation and insulin resistance (Kau et al. 2011, Nicholson et al. 2012, Johnson & Olefsky 2013) and increased levels of circulating bacteria or bacterial products derived from microbiota, such as
lipopolysaccharides, can initiate infection and metabolic inflammation that induce insulin resistance and promote metabolic syndrome (Burcelin 2012).

Genetic approaches often rely on the Cre/LoxP system. Since tissue-specific deletion of a gene of interest is dependent on the tissue specificity and intensity of Cre-recombinase expression, a tissue-specific promoter that drives Cre-recombinase is critical to achieve a partial or complete deletion of the target gene to affect the phenotype observed in animals. For example, myosin heavy chain-Cre-driven Irs1 and Irs2 deletion is almost complete and the heart failure phenotype striking, while myocyte enhancer factor-Cre-driven Irs1 and Irs2 deletion is partial and there is no observed phenotype. Similarly, adiponectin-Cre-driven Ir gene deletion is much stronger than aP2-Cre-driven Ir gene deletion and a diabetic phenotype is evident. The interpretation of the role of insulin in adipose tissue and contribution to nutrient homeostasis may be affected. For example, RIP-Cre is a rat insulin promoter-driven Cre transgenic mouse model, but Cre exhibits leaky expression in the hypothalamus of the brain (Lin et al. 2004). Thus, the deletion of Irs2 by the RIP-Cre system resulted in a phenotype that is derived not only from pancreatic β-cells, but also from the brain hypothalamus (Rhodes et al. 2013). Thus, tissue specificity and intensity of Cre-recombinase expression, though advancing our understanding of mouse genetic engineering, also have a significant role in the analysis of gene function.

Integrative physiology of insulin resistance and hyperlipidemia

Insulin inhibits hepatic glucose production and stimulates lipid synthesis, and the deletion of Ir or both Irs1 and Irs2 in the liver of mice results in hyperglycemia, hyperinsulinemia, and hypolipidemia (Michael et al. 2000, Guo et al. 2009). A valid question is whether the mouse disease models created by genetic engineering accurately reflect the clinical features of metabolic syndrome and type 2 diabetes. Many patients with metabolic syndrome and type 2 diabetes have hyperglycemia, hyperinsulinemia, and hyperlipidemia (Brown & Goldstein 2008). Given that the IRS→PI3K→PDK1/2→Akt→Foxo1 branch of the insulin signaling pathway has a central role in the control of glucose homeostasis and organ survival, suppression will result in unchecked hepatic glucose production and hyperglycemia. Although the inhibition of this signaling branch also limits hepatic TOCR2 or Akt-stimulated lipogenesis, suppression in adipose tissue may block the insulin inhibitory effect on fat lipolysis, contributing to hyperlipidemia in patients with type 2 diabetes mellitus, in whom other alternative pathways promoting lipogenesis remain active. For example, insulin-independent mTORC1 activation and carbohydrate-activated lipogenic gene expression profiles via Chrebp and AMPK facilitate the progression of lipogenesis in patients with metabolic syndrome and type 2 diabetes mellitus (Fig. 1). The identification of these and other novel mediators in the control of lipid homeostasis is important for understanding disease mechanisms and developing interventions for the control of metabolic syndrome, type 2 diabetes mellitus, and their complications.

Bariatric and metabolic surgery

More than 60% of patients with type 2 diabetes are obese; thus, body weight loss is an attractive but challenging therapeutic option (Zimmet et al. 2011, Dixon et al. 2012). Bariatric surgery, designed to achieve and sustain substantial weight loss and reduce food intake, effectively prevents and remediates type 2 diabetes (Sjostrom et al. 2012). Moreover, gastric bypass surgery reduces adverse cardiovascular events, not only in obese adults (Sjostrom et al. 2012), but also in patients suffering from type 2 diabetes without severe obesity (Cohen et al. 2012). The actions of metabolic surgery on metabolic control are unclear (Rubino et al. 2010), but it is likely that the surgery resets metabolic parameters in a balanced way, such that energy intake and expenditure are controlled.

Part 5: conclusion

Mouse studies have demonstrated that Akt inactivation and Foxo1 activation following the suppression of IRS1 and IRS2 act as a fundamental mechanism for insulin resistance, which occurs in insulin-responsive tissues, impairing systemic glucose and lipid homeostasis and body weight control and serving as an important mechanism for the development of metabolic syndrome. Metabolic syndrome includes insulin resistance in different organs of the body, such as the brain, liver, pancreas, adipose tissue, muscle, and the cardiovascular system. The IRS→Akt→Foxo1 signaling cascade and its regulatory network require further exploration under different cellular and environmental contexts. Hyperinsulinemia, pro-inflammation, and overnutrition are important environmental factors that affect this system, contributing to type 2 diabetes and cardiovascular dysfunction.
Although genome-wide association analyses have identified a number of genes that control the development of diabetes and obesity (Doria et al. 2008, Wagner et al. 2013), metabolic syndrome is a result of complex interactions between genetic and environmental factors, among which are protein modifications by environmental stimuli, such as overnutrition through phosphorylation (hormones), ubiquitination, acetylation (excess acetyl-CoA), and glycosylation (hyperglycemia), all of which modify the IRS→Akt→Foxo1 branch. Current anti-diabetic therapeutics, such as glucagon-like peptide, pioglitazone, and metformin, as well as metabolic surgery, may affect this pathway directly or indirectly, helping to correct the imbalance of hormones, nutrients, and inflammation. Targeting IRS1 and IRS2 by activating the Akt→Foxo1 signaling cascade, associated protein kinases, and gene expression profiles may provide important therapeutic modalities in the pursuit of a balanced action at the level of hormones, nutrients, and inflammation for the treatment or prevention of metabolic syndrome, type 2 diabetes mellitus, and cardiovascular dysfunction.

Declaration of interest
The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review reported.

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