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.

Journal of Endocrinology (2014) **220**, T1–T23

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 2005*a*). 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

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This paper is one of four papers that form part of a thematic review section on Energy, Insulin Resistance and Metabolic Syndrome. The Guest Editor for this section was Shaodong Guo, Texas A&M University, USA. He was not involved in the handling of this paper, on which he is listed as an author.
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Table 1 Clinical criteria for the diagnosis of metabolic syndrome

Metabolic parameters	ATP III	WHO	IDF	Diabetes
Abdominal obesity (cm)				
Men: waist circumference	>102	>102	>94	
Women: waist circumference	>88	>88	>80	
Fasting glucose (mg/dl)	>110, <126	>110	>100	>130
Blood pressure (mmHg)	>130/85	140/90	>130/85	
Triglycerides (mg/dl)	150	150	150	
HDL cholesterol (mg/dl)				
Men	<40	<35	<40	
Women	<50	<39	<50	
References	National Cholesterol Education Program (NCEP) 2002	2004, *Lancet*	Alberti & Zimmet (1998)	

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

(Table 1; Alberti & Zimmet 1998, National Cholesterol Education Program (NCEP) 2002, Alberti *et al*. 2005, Grundy *et al*. 2005, Simmons *et al*. 2010). Metabolic syndrome is a major risk factor for type 2 diabetes mellitus, which afflicts 8% of Americans and 11% of Chinese and threatens public health worldwide (Alberti *et al*. 2005, Eckel *et al*. 2005, Cornier *et al*. 2008, Roger *et al*. 2011). An estimated 366 million people had diabetes worldwide in 2011, and this number is predicted to rise to 522 million by 2030, with a high economic cost for disease management (Whiting *et al*. 2011).

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 2005*b*, 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 $\alpha_2\beta_2$ complex in the cell membrane and autophosphorylation of the β-subunit at Tyr^{1158},

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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 NH_2-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).

mTORC1 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). Additionally, 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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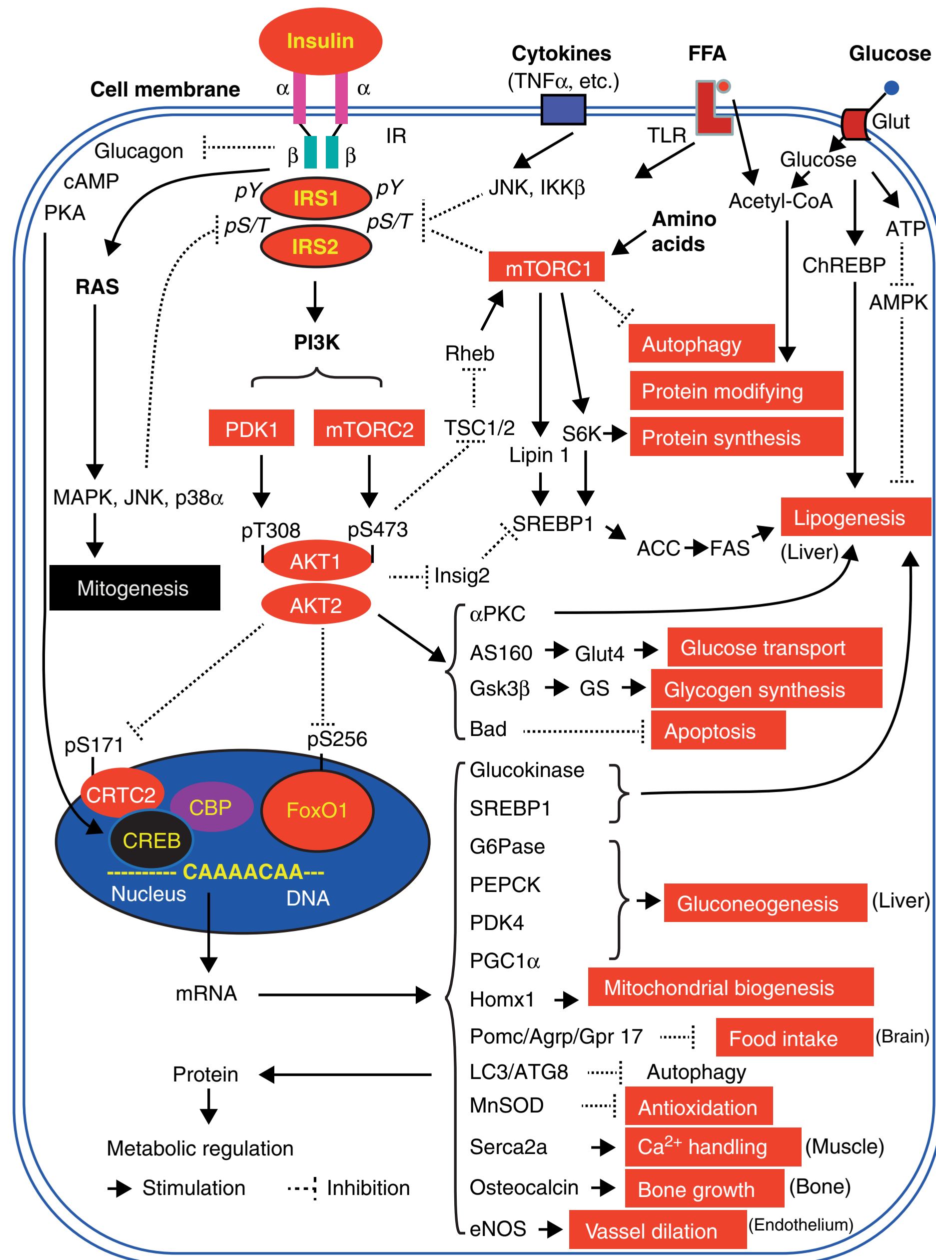

Figure 1

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 cofactor 2; Foxo1, fork-head/winged helix transcription factor O class member 1; SREBP1, sterol response element-binding protein 1; *Insig2*, insulin induced gene 2; S6K, ribosome protein p70 S6 kinase; Gsk3, glycogen synthase kinase 3; GS, glycogen synthase; mTORC, mammalian target of rapamycin complex; TSC1/2, tuberous sclerosis complex 1/2; Rheb, Ras homolog enriched in brain; aPKC, 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 α; pS/T, phosphorylated serine or threonine; *Pomc*, pro-opiomelanocortin; *Agrp*, Agouti-related peptide; Gpr 17, G-protein-coupled receptor 17; *Serca2a* (*Atp2a2*), sarco/endoplasmic reticulum Ca^{2+}-ATPase; PGC1α, peroxisome proliferator-activated receptor gamma coactivator 1α; *Homx1*, heme oxygenase 1; ATG8, autophagy-regulated gene 8; LC3 (*MAP1L3A*), microtubule-associated protein 1A/1B-light chain 3; eNOS, endothelial nitric oxide synthase; Glut, glucose transporter; JNK, c-Jun N-terminal kinase; IKKβ, inhibitor of NFκB kinase.

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maintaining cellular energy levels (Liu *et al*. 2009*b*). 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 RhebGTPase 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 Rab10GTPase 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 Srepb1c, 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 S^{256} 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 SKP2, 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, Matsuzaki *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

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muscle and liver, and lipid synthesis and storage in the liver and adipose tissue, while it inhibits fatty acid oxidation, glycogenolysis, and gluconeogenesis, as well apoptosis and autophagy in insulin-responsive tissues. During the fasting state, insulin secretion decreases, and tissues coordinate with counter-regulatory hormones, such as glucagon in the liver and adipose tissue, in favor of using fatty acids largely derived from adipocyte lipolysis for the generation of ATP and maintenance of glucose homeostasis. The substrate preferences for metabolic adaptation, during the transit from the fasting to the postprandial state, are tightly controlled by insulin under physiological conditions (Randle *et al.* 1963). This adaptive transition reflects the action of insulin in insulin-responsive organs, while it is largely blunted in organs with insulin resistance preceding the development of type 2 diabetes (Johnson & Olefsky 2013).

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.

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^{473}) 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* 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,

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

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

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; αMhc, myosin heavy chain α; Ins2, insulin 2; Tie2, angiopoietin 2 receptor.

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

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inactivation of Foxo1 in either the liver of mice with type 2 diabetes reversing hyperglycemia (Lu *et al.* 2012) or the heart of animals with type 2 diabetes preventing heart failure (Battiprolu *et al.* 2012) indicates that the activation of Foxo1 is both sufficient and necessary for the induction of hyperglycemia and organ failure following insulin resistance or type 2 diabetes.

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.* 2009*a*). 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 p38α 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 S^{307} and desensitizes insulin action in the liver and other tissues, acting as a mechanism for insulin resistance (Lee *et al.* 2003). The deletion of *Jnk1* (*Mapk8*), in mice, reduced blood glucose levels and improved insulin sensitivity following HFD treatment (Tuncman *et al.* 2006). Although ERK1/2 was thought to have a minor effect on metabolic regulation (Gabbay *et al.* 1996), recent data indicate that ERK1/2 mediated upstream MEK activation, reduced hepatic Akt phosphorylation, and contributed to insulin resistance (Jager *et al.* 2011, Jiao *et al.* 2013). It is likely that the activation of MAPK phosphatase 3 (MKP3) or phosphatase 2A (PP2A) following ERK1/2 activation may result in Foxo1 dephosphorylation at S^{253}, promoting gluconeogenesis. Indeed, either MKP3 or PP2A interacts with Foxo1 and contributes to Foxo1 dephosphorylation at S^{253} and activation (Yan *et al.* 2008, Wu *et al.* 2010).

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Additionally, some PKC isoforms, such as PKCδ and PCKθ, 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 (T^{24}, S^{256}, and S^{319}) enhances Foxo1 stability and transcriptional activity, stimulating gluconeogenesis and resulting in hyperglycemia. An increase in nuclear dephosphorylated Foxo1-S^{253} 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 number of target genes (Fig. 1). Foxo1 promotes hepatic glucose production via the expression of *Pepck* and *G6pase* and inhibits lipogenesis, resulting from the suppression of *Srebp1c*, and glucokinase 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 S^{253} 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 S^{212}, 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 R^{251} and R^{253} by protein arginine methyltransferase 1 (PRMT1) at the Akt consensus motif RXRXXS/T blocks Akt-mediated phosphorylation of Foxo1 at S^{253}, 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 T^{317} via *O*-GlcNac modification in response to glucose increased Foxo1 transcriptional activity for the expression of gluconeogenic genes (*Pepck* 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*-GlcNAc 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-T^{317} 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-S^{253} phosphorylation following insulin resistance.

The acetylation of Foxo1 at several lysine residues has been identified, including at K^{242}, K^{245}, and K^{262}, and the reversible acetylation is regulated by histone acetyl-transferase CBP/p300 and NAD^+-dependent histone

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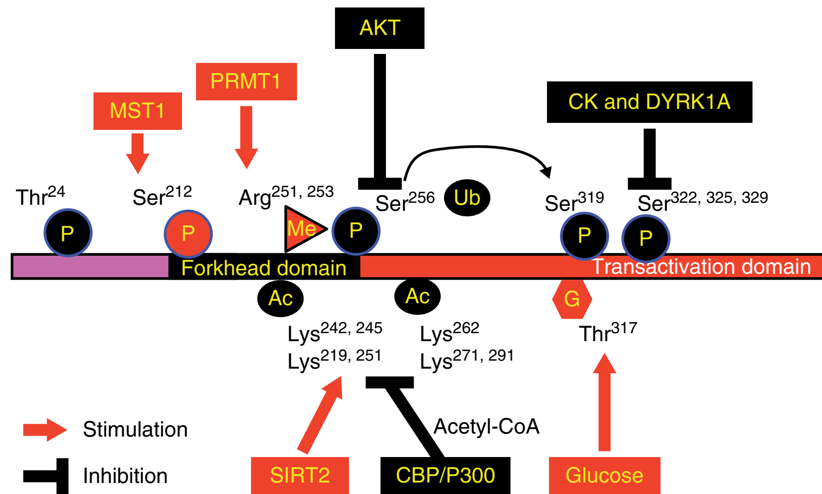

Figure 2
Human Foxo1 phosphorylation, ubiquitination, methylation, acetylation, and glycosylation at amino acid residues via different pathways and enzymes. PRMT1, protein arginine methyltransferase 1; MST1, mammalian sterile 20-like kinase 1; CK, casein kinase; DYRK1A, dual-specific tyrosine-phosphorylated 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.

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 histone deacetylases (HDACs), promoting hepatic glucose production (Mihaylova *et al.* 2011). Nuclear HDAC4, HDAC5, and HDAC7 are phosphorylated and excluded from the nucleus by AMP-dependent protein kinase (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 Foxo transcription factors. The loss of class IIa HDACs in murine liver results in the inhibition of Foxo 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

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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 anorexigenic 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

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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 IKKb 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 2005*a*). 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 PKCξ, 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 *Irs1* or *Irs2* in the liver maintained glucose homeostasis, but the deletion of both *Irs1* and *Irs2* (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 *Irs1* 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.

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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 (Biddinger *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

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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 exhibit 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-S^{473} 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

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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, Muoio & Neufer 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

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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.

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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.

Funding

This research was supported by grants from the American Diabetes Association (JF-7-07-27), American Heart Association (BGIA-7880040), Faculty Start-up from Texas A&M University Health Science Center College of Medicine, and National Institutes of Health (RO1 DK095118). This research was also supported by resources and the use of facilities at the Central Texas Veterans Health Care System, Temple, Texas, USA.

Acknowledgements

The author thanks Drs Kenneth M Baker and Yajuan Qi for reading/editing the manuscript.

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DOI: 10.1530/JOE-13-0327
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Received in final form 30 October 2013
Accepted 22 November 2013
Accepted Preprint published online 26 November 2013

http://joe.endocrinology-journals.org
DOI: 10.1530/JOE-13-0327
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