Insulin controls systemic nutrient homeostasis by promoting anabolic processes in various tissues, including the stimulation of glucose influx (into muscle and adipose), protein and glycogen synthesis (in muscle and liver), lipid synthesis and storage (in liver and adipose), and the inhibition of fatty acid oxidation, glycogenolysis, gluconeogenesis, and apoptosis and autophagy (especially in a damaged heart). 1,2 Resistance to the action of insulin is associated with chronic physiological and inflammatory stress that develops during obesity and inactivity. In too many instances it progresses to type 2 diabetes mellitus when compensatory hyperinsulinemia fails to maintain glucose homeostasis. A combination of compensatory hyperinsulinemia and inconsistent levels of insulin resistance across body tissues can cause life-threatening sequelae, especially nonalcoholic fatty liver disease and atherosclerosis. 3,4 Thus, dissection of the insulin signaling pathways and the molecular mechanisms of tissue-specific insulin resistance might reveal novel strategies to arrest or reverse the progression of metabolic disease.

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Cell-based studies initiated decades ago and extended most recently with mouse genetics reveal a common insulin signaling cascade that begins by activation of the insulin receptor tyrosine kinase (IR) and propagates through the insulin receptor substrates (IRS1 and IRS2) to the phosphatidylinositol 3 kinase v-akt murine thymoma viral oncogene homolog (AKT) cascade 1. AKT plays a particularly broad role as it phosphorylates many protein substrates—including the direct phosphorylation and inactivation of FoxO (forkhead box protein O1 family of transcription factors, FoxO1 and FoxO3) and the indirect phosphorylation of CRT2 (cAMP response element binding protein–regulated transcription coactivator 2) that inactivates cAMP response element binding protein. Inactivation of these factors suppresses the expression of many hepatic genes, including phosphoenolpyruvate carboxykinase and glucose-6-phosphatase that promote gluconeogenesis (Figure). 5,6 Genetic disruption of hepatic insulin signaling by the ablation of the insulin receptor, 7 IRS1/2, 8 or AKT1/2 causes hyperglycemia, hyperinsulinemia, and systemic insulin resistance. Conversely, pharmacological or genetic suppression of cAMP response element binding protein or FoxO1 can largely normalize glucose homeostasis during insulin resistance. 9-11 Thus, the potential of targeting hepatic cAMP response element binding protein and FoxO1 activity deserves further investigation and therapeutic validation.

Insulin ordinarily inhibits hepatic fatty acid oxidation and promotes triglyceride and cholesterol synthesis, whereas reduced insulin signaling during periods of decreased calorie intake attenuates these processes. 12,13 Consistent with the effects of starvation, recent evidence suggests that AKT is required for normal lipid metabolism as Akt2 deficiency decreases de novo lipogenesis thus avoiding fatty liver disease that usually accompanies insulin resistance and hyperinsulinemia. 14 AKT promotes lipogenesis, at least in part, because it stimulates the mammalian target of rapamycin complex (mTORC)-1 sterol regulatory element-binding protein-1 cascade that promotes the expression of lipogenic genes including acetyl-CoA carboxylase and fatty acid synthase. 15-17 AKT also inactivates insulin-induced gene 2 (an endogenous inhibitor of sterol regulatory element-binding protein-1) and stimulates ATP citrate lyase (Figure). 18,19 However, this story is complicated by the finding that human subjects with defective AKT2 display not only insulin resistance and hyperglycemia but also elevated hepatic lipogenesis, circulating triglycerides, and hepatic steatosis. 20,21 The persistent lipogenesis during insulin resistance seems to teach against a canonical view of insulin action, which fuels the search for the cause of selective insulin resistance (Figure). 22,23

A network of protein and lipid phosphorylation regulates cellular metabolism, growth, and survival, which is modulated by kinases and phosphatases. 24 Dysregulation of discrete steps in the signaling cascade that mediate the effects of obesity and chronic physiological stress has been difficult to resolve. In this issue of Arteriosclerosis, Thrombosis, and Vascular Biology, Wu and Williams suggest that dysfunction of NOX4 (NAD(P)H oxidase 4) in McArdle 7777 rat hepatoma cells and primary hepatocytes recapitulates the partial insulin resistance observed in liver of obese db/db mice: diminished insulin-stimulated phosphorylation of the hydrophobic motif in AKT; reduced phosphorylation of FoxO1; but normal or augmented phosphorylation of other AKT substrates, including glycogen synthase kinase 3 beta, acetyl-CoA carboxylase, tuberous sclerosis protein 2, proline-rich AKT1 substrate 1, also known as PRAS40, and S6 kinase-1. 25 The atypical pattern of insulin signaling in the absence of NOX4 might promote lipogenesis through mTORC1 sterol regulatory element-binding protein-1c signaling (by stimulating lipogenic genes such as acetyl-CoA carboxylase, fatty acid synthase) and gluconeogenesis through constitutive FoxO1 activity (by sustained expression of phosphoenolpyruvate carboxykinase, glucose-6-phosphatase). Therefore, the authors propose that NOX4 dysfunction might
Figure. Possible mechanisms for selective insulin resistance in diabetes and NAD(P)H oxidase 4 (NOX4)-deficient hepatocytes. Insulin normally activates the IR (phosphorylated on serine 308, T308AKT)→IRS→PI3K signaling cascade that suppresses FoxO and the CREB (cAMP response element binding protein (CREB) complex and activates mammalian target of rapamycin complex 1 (mTORC1). A full activation of AKT requires phosphorylation of T308AKT and S473AKT by PDK1 and mTORC2, respectively. Impaired insulin action (or insulin resistance) dampens the IR→IRS→PI3K signaling, which promotes FoxO and CREB-mediated gluconeogenic gene expression (eg, phosphoenolpyruvate carboxykinase [PEPCK] and glucose-6-phosphatase [G6P])—the cause of hyperglycemia. However, lipid accumulates during insulin resistance, revealing a selective insulin responsiveness in diabetes mellitus characterized by hyperglycemia with hypertriglyceridemia. Wu and Williams suggest that the mechanism for such selective insulin resistance could include NOX4, the oxidase that elicits a reactive oxygen species (ROS) burst during insulin stimulation and inhibits protein tyrosine phosphatase 1B (PTP1B) and phosphatase and tensin homolog (PTEN)—inhibitors of canonical IR→IRS→PI3K signaling. Unexpectedly, NOX4 deficiency induces atypical AKT phosphorylation (strong T308AKT but weak pS473AKT), which is associated with FoxO1 and mTORC1 activation. That the NOX4 deficiency diminished pS473 could be a result of (1) inactivation of sirtuin (silent mating type information regulation 2 homolog) 1 (Sirt1) or mTORC2 or (2) activation of PH domain leucine-rich repeat protein phosphatase-1 (PHLPP) or other phosphatases that specifically dephosphorylate S473AKT. Genetic ablation of hepatic Sirt1 inactivates mTORC2, the kinase that funnels PI3K signaling cascade and promotes pS473AKT. Moreover, FoxO1 activity can cause mitochondrial dysfunction and dysregulation of NAD+/NADH, which impairs Sirt1 activity and lipid metabolism. The gray lines denote insulin signaling pathways that are inactive in db/db mice or cells lacking NOX4, and the black lines denote insulin signaling pathways that remain active in cells lacking NOX4 (molecules shown in gray circles are inactive). OHREBP indicates carbohydrate responsive element-binding protein.

underlie the paradoxical features (eg, hyperglycemia and hypertriglyceridemia) of metabolic disease.

How the inhibition of NOX4 might promote a distinct pattern of insulin resistance is unclear. Through an unknown mechanism, NOX4 generates a burst of reactive oxygen species such as H$_2$O$_2$, during insulin stimulation that can oxidize essential cysteine residues in the catalytic site of various protein and lipid phosphatases. Because NOX4 is prominently expressed in insulin-sensitive cells, the production of H$_2$O$_2$ inhibits protein tyrosine phosphatase 1B and phosphatase and tensin homolog—negative regulators of IR→IRS1/2 phosphorylation and the accumulation of phosphatidylinositol 3-phosphate by the PI3K, respectively—which enhances canonical insulin signaling (Figure). But the story is complicated in diabetic db/db mice, where phosphatase and tensin homolog is weakly inhibited during insulin stimulation while AKT is strongly phosphorylated in its catalytic domain (T308AKT) but weakly in the hydrophobic motif (S473AKT). Given that NOX4 deficiency is expected to suppress IR→IRS→PI3K signaling by promoting PTP1B and phosphatase and tensin homolog activity, the persistent and even augmented AKT phosphorylation in its catalytic domain (T308AKT) is difficult to understand (Figure).

Full activation of AKT requires phosphorylation of T308AKT and S473AKT by 3-phosphoinositide dependent protein kinase-1 and mTORC2, respectively. It is possible that a phosphatase targeted toward the hydrophobic motif in AKT is hyperactivated or retargeted when NOX4 is deleted, which reduces the S473AKT phosphorylation. Wu and Williams suggest serine/threonine-protein phosphatase 5 as one possibility, as it associates with NOX4. Another possibility is the activation or retargeting of AKT2-specific phosphatase PH domain leucine-rich repeat protein phosphatase-1 because it specifically dephosphorylates S473AKT, however, whether NOX4 deletion could accomplish these changes needs to be established (Figure).

Alternatively, NOX4 might be required for the activation of mTORC2—but what could this mechanism involve? Inactivation of mTORC2 attributable to hepatic sirtuin (silent mating type information regulation 2 homolog) 1 (Sirt1) deficiency also impairs pS473AKT and causes hyperglycemia and dyslipidemia as a result of FoxO1 and carboxydrate responsive element-binding protein activity (Figure). Whether NOX4 deficiency reduces the NAD$^+$/NADH ratio, which inhibits Sirt1 activity, is unknown, but it could contribute to reduced S473AKT phosphorylation. Moreover, such a mechanism could gain momentum from constitutive FoxO1 activity, which can dysregulate mitochondrial oxidative phosphorylation and attenuate the NAD$/\text{NADH}\rightarrow\text{Sirt1}$ cascade. Moreover, reduced Sirt1 activity can also impair mitochondrial function, which inhibits fatty acid oxidation and promotes lipid accumulation. Thus, redox imbalance owing to NOX4 deletion could modulate multiple pathways that lead to differential inhibition of the insulin signaling cascade and lipid accumulation.

Now that the principal elements of the insulin signaling cascade are in place, how they respond to systemic stress and compensatory changes needs to be vigorously explored. Understanding selective insulin responsiveness across body tissues might lead to new understanding of the paradoxical hypertriglyceridemia during insulin resistance. Exploration of these novel interactions has just begun and can benefit from the creative investigation of genetically modified mice. Sorting
out the selective insulin resistance that propagates across body tissues could open a new chapter in our search for the molecular mechanisms of life-threatening metabolic disease.

**Disclosures**

None.

**References**

kNOXing on the Door of Selective Insulin Resistance
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