Aging and Insulin Resistance: Just Say iNOS

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The ubiquitous presence of insulin resistance cannot be understated. First brought to light by Himsworth and Kerr (1) in 1939, insulin resistance, defined as a subnormal response to a given dose of insulin, was ushered into prime time by Gerald Reaven (2), where it has remained at center stage. Insulin resistance is a major feature of type 2 diabetes (2). Insulin resistance is also associated with obesity, essential hypertension, dyslipidemia, nonalcoholic fatty liver disease, obstructive sleep apnea, and cancer (3). This cluster of maladies has been termed by Reaven as the "insulin resistance syndrome." Therefore, an individual with insulin resistance is strongly predisposed to an increased risk of life-threatening clinical conditions, including cardiovascular disease.

As Reaven points out, the clinical consequences of insulin resistance are not due to insulin resistance per se but come from the hyperinsulinemia that occurs as the individual with insulin resistance attempts to maintain normoglycemia. In reality, compensatory hyperinsulinemia is akin to cutting a deal with the devil. Chronic hyperinsulinemia may be beneficial to resistant tissues requiring it, for example to maintain insulin action in liver, muscle, and adipose tissues; however, it may wreak havoc with tissues that have normal sensitivity to insulin. Even within the same tissue, some of the insulin-regulated pathways, such as the glucose metabolic pathway, are more resistant to insulin than others, including the mitogenic pathway (4). Thus, it is likely that chronic overstimulation of the mitogenic pathway by insulin also plays a causative role in mediating the clinical consequences of insulin resistance. Therefore, intensive efforts are being directed toward identifying novel nutritional and pharmacological approaches that improve insulin sensitivity in target tissues.

Our knowledge of the molecular mechanism(s) of insulin action has increased dramatically over the past 30 years (5–10). Impaired insulin signaling can result from mutations or posttranslational modifications of the insulin receptor tyrosine kinase or its downstream effector proteins, although this does not appear to be the cause in the majority of insulin resistance in individuals (5). Additional candidate molecules that have been implicated in insulin resistance in humans include plasma cell membrane glycoprotein-1 (11) and protein tyrosine phosphatase-1B (12).

The strongest mechanistic evidence for the negative regulation of insulin action has emerged for stimuli that activate inflammatory serine/threonine kinases (e.g., c-Jun NH2-terminal kinase and inhibitor of κB kinase). These stimuli include nutrient oversupply (especially lipids), endoplasmic reticulum stress, and oxidative stress (6,8–10). Many of these stressors have previously been linked to mitochondrial dysfunction and/or reduced mitochondria content (Fig. 1) (10,13). However, in contrast, numerous studies have reported that insulin resistance may cause mitochondrial dysfunction. Holloszy and colleagues (14) have pointed out that reduced mitochondrial content is still sufficient to oxidize fatty acids in the resting state and is unlikely to cause accumulation of fat or insulin resistance.

The insulin receptor substrate (IRS) proteins mediate the metabolic effects of insulin (7). Serine/threonine phosphorylated forms of IRS molecules are less able to associate with the insulin receptor and downstream effector molecules, such as phosphatidylinositol 3-kinase, resulting in the attenuation of insulin action on glucose metabolism and other metabolic functions (7,15). In addition, the serine/threonine phosphorylated forms of IRS molecules are more susceptible to proteasome-mediated degradation (16).

Increased nitric oxide (NO) production, especially as a consequence of inducible nitric oxide synthase (iNOS), has also been implicated in insulin resistance, especially in the context of obesity (17). iNOS is markedly increased in macrophages and other inflammatory cells stimulated by proinflammatory cytokines. In the presence of O_2, NO covalently attaches to cysteine residues of target proteins forming *S*-nitrosothiol adducts in a reversible posttranslational modification termed protein *S*-nitrosation (or *S*-nitrosylation). Dietary (obesogenic diet) and pharmacological induction of NO production increases *S*-nitrosation of the insulin receptor IRS-1 and AKT/PKB (a key enzyme in the regulation of glucose metabolism) in rodent skeletal muscle along with inducing insulin resistance in the metabolic pathway (18). In adipocytes from insulin-resistant obese mice and humans, increased *S*-nitrosation of the insulin receptor and AKT/PKB have recently been reported (19). Thus, protein *S*-nitrosation resulting from NO overproduction or impaired denitrosation can be regarded as a causative molecular mechanism for insulin resistance.

Insulin resistance increases with aging, and in this issue of *Diabetes*, Ropelle et al. (20) report that aging in mice was associated with increased iNOS expression and *S*-nitrosation of the insulin receptor IRS-1 and AKT/PKB in skeletal muscle, along with insulin resistance. Elegantly using three independent approaches, including iNOS-null mice, pharmacological inhibition of iNOS, and acute exercise to reduce iNOS expression, they report that each method protected against iNOS-mediated protein *S*-nitrosation and insulin resistance. These results provide further evidence implicating protein *S*-nitrosation mechanistically with insulin resistance and extend the mechanism from insulin resistance in the context of obesity to aging.

This article has a number of notable strengths, including the use of three independent approaches to reduce iNOS

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DOI: 10.2337/db12-1239

See accompanying brief report, p. 466.

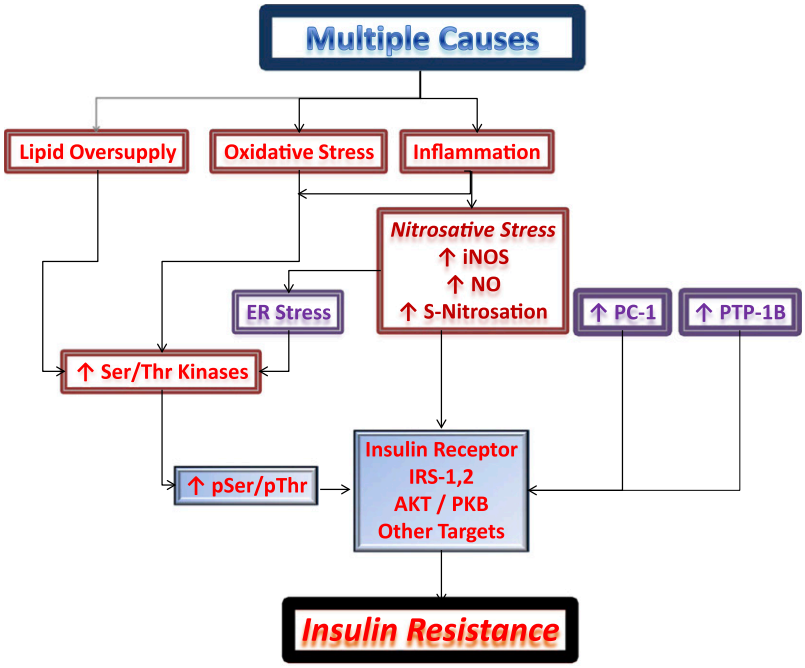

FIG. 1. Overview of the major stimuli resulting in insulin resistance. Multiple factors including reduced mitochondrial content and/or function may predispose certain individuals to intramyocellular lipid accumulation and insulin resistance. IMCL promotes the production of diacylglycerol, activation of protein kinase CΘ, and increased phosphorylation of IRS-1. Impaired mitochondrial function along with nutrient oversupply can also result in the overproduction of reactive oxygen species and the activation of stress-sensitive signaling pathways. Consequently, these activated serine/threonine kinases phosphorylate the insulin receptor and IRS-1 and -2, resulting in insulin resistance. Chronic inflammation, as is observed in obesity, type 2 diabetes, and other conditions, is associated with increased production of tumor necrosis factor-α (and other proinflammatory cytokines) and iNOS. Tumor necrosis factor-α stimulates the activation of the inflammatory serine/threonine kinases c-Jun NH2-terminal kinase and inhibitor of κB kinase, while iNOS overproduction results in nitrosative stress. Each of these can trigger endoplasmic reticulum (ER) stress and the unfolded protein response. Plasma cell membrane glycoprotein-1 (PC-1) binds to the connecting domain of the insulin receptor α-subunit that is located in residues 485–599. The connecting domain transmits insulin binding in the α-subunit to activation of tyrosine kinase activation in the β-subunit. When plasma cell membrane glycoprotein-1 is overexpressed, it inhibits insulin-stimulated insulin receptor β-subunit tyrosine kinase activity. Protein tyrosine phosphatase-1B (PTP-1B) is a negative regulator of insulin (and leptin) signaling, which causes reduced tyrosine phosphorylation of the insulin receptor and other substrates and the attenuation of insulin action. See text for the references upon which these associations have been based.

expression and activity along with the simultaneous determination of *1*) whole-body insulin sensitivity (euglycemic-hyperinsulinemic clamp method), *2*) glucose transport in isolated muscle, and *3*) characterization of the insulin receptor IRS-1 and AKT/PKB with respect to phosphorylation state and *S*-nitrosation. Limitations of the study include *1*) the use of only soleus muscle and not other insulin target tissues, *2*) the use of only male mice, and *3*) lack of information regarding the extent of protein nitration or serine/threonine phosphorylation, which has also been linked to insulin resistance in aging.

Future work needs to include an assessment of the role of protein *S*-nitrosation in other conditions and insulin target tissues and on other molecules in the insulin-signaling pathways. The molecular mechanisms of insulin resistance are diverse and likely to be context specific. For example, the decreased whole-body glucose infusion rates during a hyperinsulinemic clamp (a measure of whole-body insulin sensitivity) that are observed in type 2 diabetic patients are also observed in other conditions including obesity, aging, polycystic ovary syndrome, and hypertension. Are the molecular mechanisms of insulin resistance identical in all these conditions? And are those mechanisms the same for each insulin-regulated pathway? Ropelle et al. have convincingly linked protein *S*-nitrosation to insulin resistance in aging mice, whereas, previously, it had been linked principally to obesity-related insulin resistance. The emergence of protein *S*-nitrosation as a causative link to insulin resistance is a welcome advance in the field and suggests additional research and molecular targets for intervention.

ACKNOWLEDGMENTS

No potential conflicts of interest relevant to this article were reported.

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