Opportunity "Nox": A Novel Approach to Preventing Endothelial Dysfunction in the Context of Insulin Resistance

J. David Symons

Cardiovascular complications are more prevalent in individuals with conditions associated with insulin resistance. Knowledge is evolving concerning the mechanistic link between insulin resistance and vascular disease, but complete clarity does not exist. This is a high-priority area of investigation because individual and societal costs associated with diabetic vascular complications are increasing, and the need to elucidate effective therapeutic targets and intervention strategies with minimal off-target side effects is urgent.

Insulin-resistant conditions such as type 2 diabetes and diet-induced obesity are associated with an altered endothelial cell phenotype, i.e., endothelial dysfunction. A crucial component of endothelial cell dysfunction is reduced nitric oxide (NO) bioavailability. Reduced NO bioavailability contributes importantly to pathologies specific to large (atherosclerosis, cardiomyopathy) and small (retinopathy, nephropathy, neuropathy) blood vessels (1). Oxidative stress, hyperglycemia, lipotoxicity, activation of the renin-angiotensin system, and increased proinflammatory cytokines are systemic disturbances in patients with conditions associated with insulin resistance. Each of these factors contributes independently and synergistically to decreasing NO bioavailability by impairing its synthesis and/or by enhancing its degradation (2). In this issue of *Diabetes*, Sukumar et al. (3) demonstrate that endothelial cell–specific insulin resistance increases NADPH oxidase (Nox) isoform 2 (Nox2) expression to an extent that elevates superoxide anion ($O_2^{\cdot -}$) production and precipitates endothelial dysfunction.

Reactive oxygen species (ROS) are highly reactive metabolites of oxygen that participate in oxidation-reduction reactions (4). A traditional definition of oxidative stress is when ROS accumulation overwhelms the cellular antioxidant defense mechanisms and the redox state of the biological compartment is shifted toward one that is more oxidizing (5,6). Cellular sources of ROS include NADPH-dependent oxidases, xanthine oxidase, lipoxygenases, mitochondrial oxidases, and NO synthases (7). Nox is a major source of $O_2^{\cdot -}$ in insulin-resistant humans (8,9) and mice (10,11). Seven isoforms of Nox have been described in mammals (Fig. 1). Each isoform comprises a core catalytic subunit, i.e., Nox1–Nox5 and dual oxidase (DUOX) 1 and DUOX 2 (4). Each Nox catalytic isoform contains up to five regulatory subunits that determine *1*) maturation and expression of Nox and DUOX subunits in biological membranes (e.g., p22phox), *2*) enzyme activation (e.g., p67phox, RAC1/RAC2), and *3*) spatial organization (e.g., p47phox). Endothelial cells express Nox1, Nox2 ($gp91^{phox}$), Nox4, and Nox5; vascular smooth muscle cells express Nox1, Nox4, and Nox5; and adventitial fibroblasts express Nox2 and Nox4 (5).

Earlier work from this laboratory group indicates that mice with germ-line haploinsufficiency of the insulin receptor ($IR^{+/-}$ mice) display hypertension and an age-dependent attenuation of arterial vasorelaxation that is associated with elevated Nox-mediated $O_2^{\cdot -}$ production (12,13). Importantly, impaired endothelial function in $IR^{+/-}$ mice has physiological relevance in models of endothelial injury (14) and atherosclerotic plaque development (15). A valid concern when interpreting these results is that metabolic disruptions known to exist in $IR^{+/-}$ and apoE mice might contribute to impaired endothelial function. To address this, mice with endothelium-targeted overexpression of a dominant-negative mutant human insulin receptor (endothelium-specific mutant insulin receptor overexpressing mice [ESMIRO]) were studied (10). Compared with wild-type littermates, ESMIRO mice have preserved glucose homeostasis and are normotensive. However, endothelium-dependent vasorelaxation and endothelial NO synthase (eNOS) phosphorylation are blunted in aortae from ESMIRO versus wild-type mice, despite similar eNOS protein expression. Because mRNA expression of Nox2 and Nox4 was increased in endothelial cells and aortae of ESMIRO mice, and endothelium-dependent dysfunction was normalized when aortae from ESMIRO mice were incubated with a superoxide dismutase mimetic, the authors concluded that Nox-mediated $O_2^{\cdot -}$ generation contributed importantly to dysfunction observed in arteries from mutant mice (10).

Sukumar et al. investigated a relatively new approach to the challenge of preventing arterial dysfunction in the context of insulin resistance, i.e., to suppress $O_2^{\cdot -}$ generation locally by disrupting an NADPH enzyme complex located in the endothelial cell. First, the authors confirmed the preserved metabolic phenotype and disrupted vascular phenotype that exists in $IR^{+/-}$ and ESMIRO mice, and demonstrated that exaggerated $O_2^{\cdot -}$ production and endothelial dysfunction exhibited in aortae from both groups could be attenuated by acute or chronic Nox inhibition using gp91ds-tat (16). Because Nox2 mRNA expression was elevated in endothelial cells from $IR^{+/-}$ and ESMIRO mice and Nox2 siRNA suppressed $O_2^{\cdot -}$ generation in endothelial cells from ESMIRO mice, the authors sought to determine

From the College of Health, University of Utah, Salt Lake City, Utah, and the Division of Endocrinology, Metabolism, and Diabetes, School of Medicine, University of Utah, Salt Lake City, Utah.
Corresponding author: J. David Symons, j.david.symons@hsc.utah.edu.
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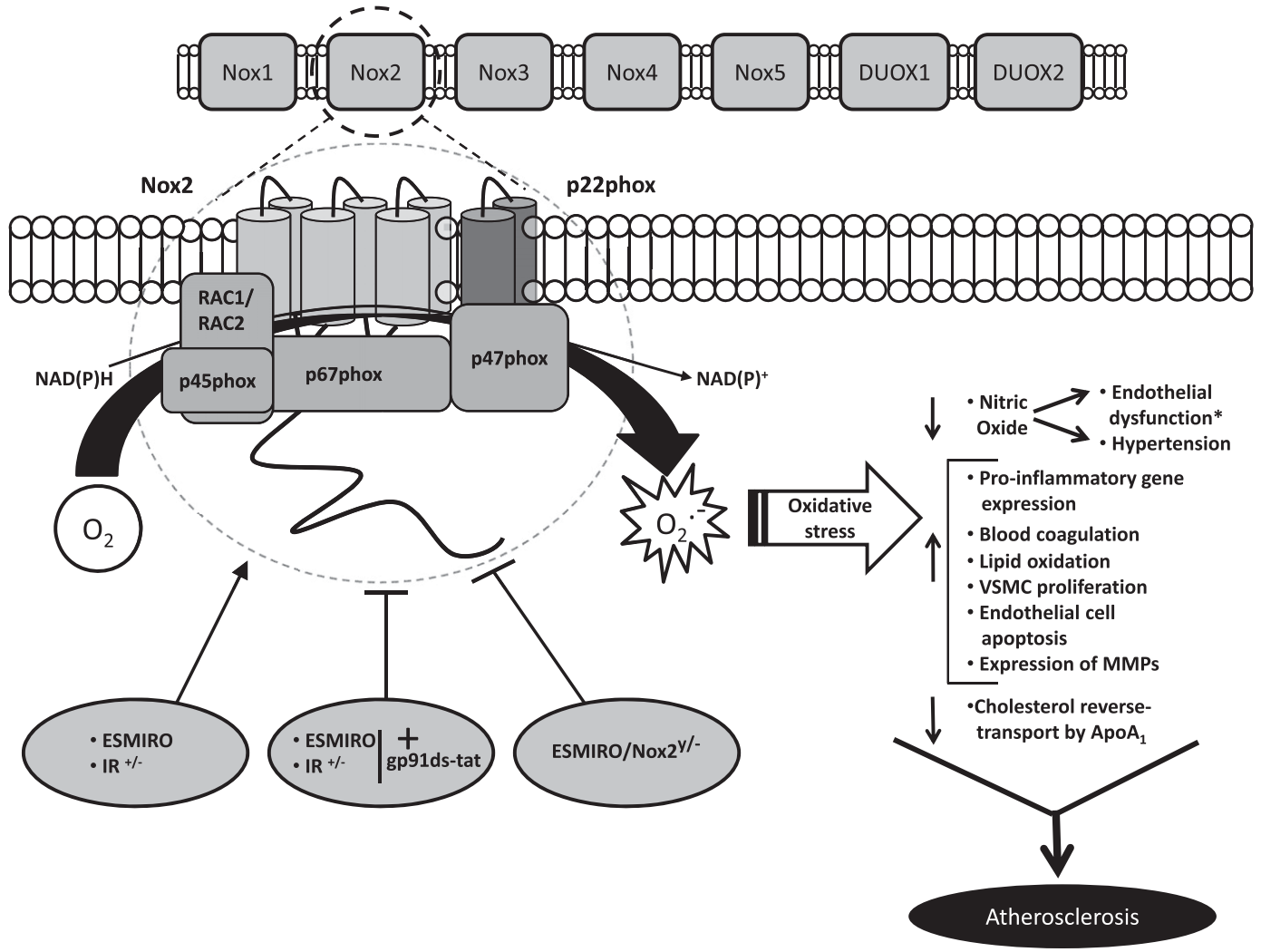

FIG. 1. Hypothesized contributions from Nox2-generated oxidative stress to the process of atherosclerosis. When NADPH donates electrons to molecular oxygen via Nox2, $O_2^{\cdot -}$ generation occurs. Oxidative stress occurs when $O_2^{\cdot -}$ production exceeds the antioxidant capacity of the immediate environment. Oxidative stress can contribute to atherosclerosis progression by various mechanisms (7). ↑, increase; ↓, decrease. Mice with endothelial cell–specific insulin resistance (ESMIRO and $IR^{+/-}$ mice) exhibit increased Nox2-mediated $O_2^{\cdot -}$ generation to an extent that evokes endothelial dysfunction. Treatment of ESMIRO and $IR^{+/-}$ mice with the Nox inhibitor gp91ds-tat (+gp91ds-tat) or endothelial cell–specific knockout of Nox2 in ESMIRO mice (ESMIRO / $Nox2^{y/-}$) reduces $O_2^{\cdot -}$ production and restores endothelial function. *Endothelial function was assessed in the current study (3), but the influence of endothelial cell–specific Nox2 deletion on other potential contributors to atherosclerosis depicted in the figure has not been investigated. VSMC, vascular smooth muscle cell; MMPs, matrix metalloproteinases.

whether Nox2–mediated $O_2^{\cdot -}$ production is sufficient to contribute to arterial dysfunction in the context of insulin resistance. To explore this, mice with endothelial cell–specific insulin resistance and deletion of Nox2 were generated i.e., ESMIRO / $Nox2^{y/-}$ mice. As hypothesized, increased $O_2^{\cdot -}$ generation and vascular dysfunction were prevented in endothelial cells and/or aortae from ESMIRO/$Nox2^{y/-}$ versus ESMIRO mice. Importantly, systemic glucose homeostasis and blood pressure were similar between groups. These findings provide strong evidence to consider the Nox2 isoform as a therapeutic target for the treatment or prevention of vascular disease in the context of insulin resistance.

Strengths of this particular study include *1*) the use of double transgenic mice wherein insulin resistance and Nox2 deletion is created specifically in the endothelial cell; *2*) demonstrating that systemic glucose homeostasis and arterial pressure was not altered in double transgenic versus ESMIRO mice, *3*) using redundant methodologies to confirm $O_2^{\cdot -}$ generation; and *4*) verifying that Nox2 expression and $O_2^{\cdot -}$ production in the context of insulin resistance was negated in denuded arteries. Further exploration of *1*) the systemic metabolic environment (with particular focus on factors known to alter Nox2 expression, e.g., angiotensin II), *2*) arterial function (e.g., acetylcholine-evoked vasorelaxation and receptor/nonreceptor-mediated contraction ± NOS inhibition ± endothelial denudation), *3*) the impact of Nox2 inhibition/deletion on the function of other tissues known to express high levels of Nox2 (e.g., pancreatic β-cells) (17), and *4*) indices of vascular signal transduction (e.g., in vivo and/or in vitro insulin-mediated arterial eNOS phosphorylation and NO production) in ESMIRO/$Nox2^{y/-}$ and ESMIRO mice could yield interesting findings to provide even greater insight. Future research questions arising from this important contribution to the field include *1*) Does Nox2 deletion in endothelial cells influence the expression/function of other Nox isoforms throughout the arterial wall and/or unmask beneficial

effects of vasoprotective (?) isoforms such as Nox4 (5,18,19)? *2*) Will endothelial cell–specific deletion of Nox2 mitigate the endothelial dysfunction and hypertension that exist in the context of obesity in high-fat diet–fed mice (11,20) and/or atherosclerosis in ApoE mice (15)? *3*) Because females have lower Nox expression and activity than males (21), do sex-specific differences concerning Nox2-mediated $O_2^{\cdot -}$ generation exist?

The prevalence of diagnosed and undiagnosed cases of type 2 diabetes—a condition characterized by insulin resistance and endothelial dysfunction—is estimated to increase to 33% by 2050 (22). Sukumar et al. have provided novel mechanistic insight into defining a molecular target that should be pursued further in an effort to delay the onset and/or lessen the severity of vascular disease that exists in patients with conditions associated with insulin resistance.

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