Endothelial Insulin and IGF-1 Receptors: When Yes Means NO

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Cardiovascular (CV) disease is the leading cause of morbidity and mortality and a major driver of health care costs in patients with type 2 diabetes. Observational studies suggest that insulin resistance and hyperglycemia independently predict atherosclerosis (1,2). However, recent clinical trials have been disappointing in that intensive glycemic control does not reduce the risk of CV events in individuals with diabetes (3). Consequently, it has been suggested that therapies targeting hyperinsulinemia and/or insulin resistance (e.g., metformin) may lead to CV risk reduction (2). In addition to its metabolic actions, insulin has important vascular actions that stimulate endothelial production of nitric oxide (NO), an anti-inflammatory and antiatheroslcerotic molecule (4). In turn, endothelial insulin resistance leads to diminished glucose disposal, endothelial dysfunction, and atherosclerosis. Strategies that ameliorate endothelial insulin resistance may simultaneously augment metabolic and vascular actions of insulin, thereby reducing CV risk. However, molecular mechanisms regulating endothelial insulin action are still unclear.

Elegant studies from various laboratories have elucidated insulin signaling pathways that regulate NO production in the endothelium (4). Insulin binding to its receptor increases receptor tyrosine kinase activity and results in phosphorylation of insulin receptor (IR) substrate (IRS)-1 and sequential activation of phosphatidylinositol 3-kinase (PI3K) and 3-phosphoinositide-dependent protein kinase (PDK)-1. PDK-1, in turn, activates Akt, which then directly phosphorylates endothelial NO synthase (eNOS) at Ser1177, resulting in increased eNOS activity and NO production (Fig. 1). Although less potent, IGF-1, like insulin, activates the PI3K-Akt-eNOS pathway and stimulates NO production in endothelial cells (5,6).

Human endothelial cells express IR, IGF-1 receptors (IGF-1R), and hybrid receptors (IR/IGF-1R) composed of heterodimers containing a αβ-chain of the IR associated with a αβ-chain of the IGF-1R (7). IGF-1R are more abundant (10-fold higher) than IR (5,8). IR/IGF-1R have a low affinity for insulin, but they bind IGF-1 with the same affinity as IGF-1R. However, because of the low binding affinity of insulin to IGF-1R, physiological concentrations of insulin (100–500 pmol/L) selectively activate IR to release NO and increase microvascular perfusion in vivo (6). At supraphysiological concentrations, insulin and IGF-1 cross-react with each other's receptors, albeit at a significantly lower affinity than with their own receptors (7). In nonendothelial cells, IGF-1R expression modulates insulin signaling by altering the levels of hybrid receptors (7). Whether or not a similar dynamic affects insulin signaling in the endothelium was unknown.

In this issue of *Diabetes*, Imrie et al. (9) demonstrate a novel role for IGF-1R in modulating insulin signaling in the endothelium. They evaluated endothelial insulin sensitivity in mice overexpressing human IGF-1R in the endothelium (hIGFREO). Aorta from hIGFREO displayed reduced basal NO release and enhanced responsiveness to vasoconstrictors. Although basal total and active eNOS levels were similar, neuronal NO synthase (nNOS) expression in endothelial cells from hIGFREO was lower when compared with wild-type mice. In hIGFREO, endothelial levels of IR/IGF-1R were increased and associated with reduced insulin, but not IGF-1–stimulated NO production and eNOS activation. Data from the current study extend and confirm previous reports from this group that have demonstrated that reducing IGF-1R and IR/IGF-1R results in improved endothelial insulin sensitivity in insulin-resistant mice (10). Taken together, these novel findings suggest that IGF-1R negatively affects insulin-stimulated NO production in the endothelium by modulating the amount of IR/IGF-1R.

How does endothelial IGF-1R expression influence insulin signaling? Current models assume that IR, IGF-1R, and IR/IGF-1R are formed by random dimerization of receptor monomers (7). Consequently, relative distribution of the receptor species is determined by the monomeric ratio of IGF-1R and IR. Higher IGF-1R expression is associated with increased formation of IR/IGF-1R and a lower proportion of IR holoreceptors. Conversely, decreasing IGF-1R levels lowers the amount of IR/IGF-1R and thus a higher proportion of IR is available for ligand binding. This phenomenon does not appear to be cell-specific, since similar findings are observed in vascular smooth muscle cells, adipocytes, and osteoblasts (11–13). Likewise, fibroblasts from individuals with heterozygous IGF-1R mutation, Arg59Ter, manifest reduced IGF-1R as well as hybrid receptor expression and augmented insulin signaling (14). Thus, isolated changes in IR number may be sufficient to alter the strength of PI3K-Akt-eNOS signaling (Fig. 1). It is also possible that higher numbers of IR/IGF-1R may diminish coupling efficiency of IR to postreceptor signaling intermediates and reduce insulin responsivity.

In the study by Imrie et al., insulin-stimulated eNOS phosphorylation/activation was reduced in endothelial cells from hIGFREO mice. However, insulin-stimulated Akt activation was unaffected. Thus, the molecular mechanisms

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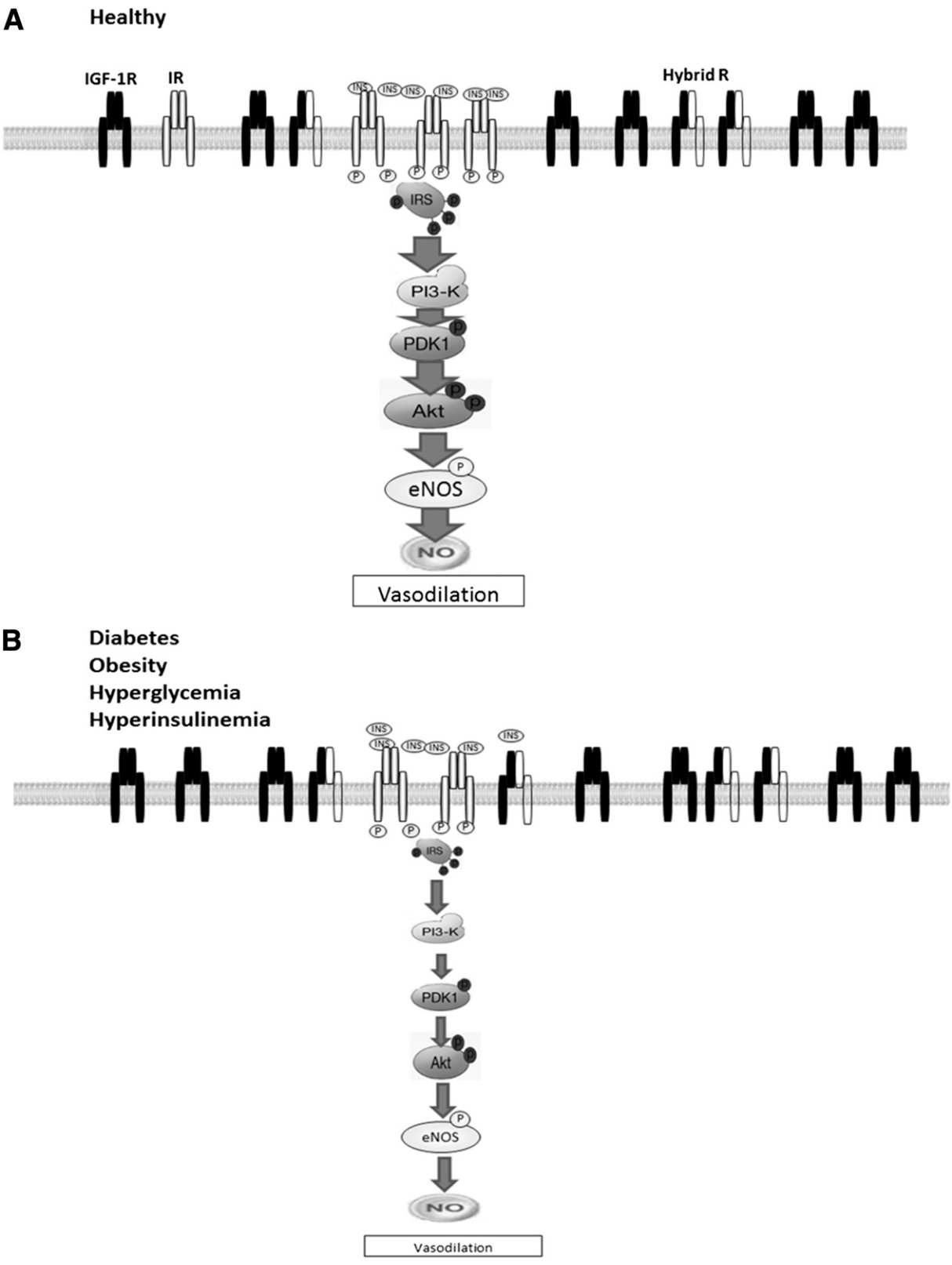

FIG. 1. Relative distribution of insulin (INS) and IGF-1R modulates insulin-stimulated NO production in the endothelium. *A*: In a healthy endothelium, IGF-1R are more abundant than IR. Physiological insulin concentrations selectively activate IR to stimulate the PI3K branch of insulin signaling to stimulate NO production and vasodilation. *B*: Increased IGF-1R expression is associated with increased IR/IGF-1R and reduced numbers of IR holoreceptors. The magnitude of insulin-stimulated NO production is reduced, leading to diminished vasodilation.

mediating reduced eNOS activation are unclear. These studies were performed in pulmonary endothelial cells and not in aortic endothelial cells where, surprisingly, insulin-mediated aortic vasodilation was not impaired. This heterogeneous response may be secondary to cellular differences in the relative abundance of receptor species (IR, IGF-1R, and IR/IGF-1R) in the two vascular beds. Moreover, in these in vitro studies, insulin was used at concentrations (150 nmol/L) known to activate endothelial IGF-1R and hybrid receptors. In adipocytes, IR is more efficient in activating IRS-1 and PI3K than IGF-1R (15). Thus, it is conceivable that physiological concentrations (<1 nmol/L) known to selectively activate IR may indeed show reduced Akt activation in endothelium of hIGFREO mice. Additional studies, particularly in aortic endothelial cells, are needed to delineate specific mechanisms that lead to reduced insulin activation of eNOS. The authors suggest that lower basal endothelial NO release in hIGFREO is due to reduced nNOS expression. However, the contribution of nNOS activity to basal NO release and the cause for diminished nNOS-protein expression need to be assessed in future studies. Finally, IGF-1 and high concentrations of insulin in a NO-dependent manner accentuates reendothelialization partly through enhanced mobilization of progenitor cells in injured arteries (16). Considering that endothelial IGF-1R/eNOS/NO pathway is functional and sensitive in hIGFREO mice, the observed increase in endothelial regeneration is confirmatory.

Despite these limitations, the findings by Imrie et al. are both novel and relevant. The current work suggests that the interaction of IR and IGF-1R to form IR/IGF-1R shapes the amplitude of insulin signaling in the endothelium. Vascular IGF-1R expression is increased in obese and diabetic rodent models (17). Interestingly, in these models insulin, but not IGF-1–mediated vasorelaxation, is impaired (18). Dysglycemia and activation of vascular renin-angiotensin-aldosterone system are characteristic of insulin-resistant states (19). Angiotensin II, aldosterone, and hyperglycemia are known to upregulate vascular IGF-1R expression (11,17,19). Similarly, type 2 diabetes and obesity are associated with increases in IR/IGF-1R expression in insulin-sensitive tissues (20). In these pathological states, interventions aimed at downregulating IGF-1R expression may augment endothelial insulin sensitivity. To that end, relevance of these findings to humans needs to be explored further. Such studies may provide important insight into strategies directed at improving insulin signaling in endothelial cells in a manner that results in reduced CV disease.

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