Nitric oxide (NO) is a simple chemical compound—1 nitrogen and 1 oxygen atom coupled together—with complex biological actions (1,2). A singularly prominent feature of NO is its ability to cause vasodilation, a quality that is used pharmacologically when treating ischemic heart disease with NO precursors such as nitroglycerin.

In 1980, Furchgott and Zawadzki (3) showed that vascular relaxation induced by acetylcholine was dependent on the presence of endothelium and provided evidence for the release of a volatile humoral factor. This substance, later called endothelium-derived relaxing factor and now recognized as NO (4), is a significant component of the insulin-signaling cascade (Fig. 1), executing microvascular vasodilation stimulated by local NO production from the vascular endothelium (5). Vasodilation has the potential to decrease systemic blood pressure and increase local tissue blood flow in tissues such as muscle. The combination of decreased blood pressure and increased tissue blood flow, together with specific beneficial endothelial effects, may serve to prevent hypertension, cardiovascular disease, and insulin resistance (5,6). However, these effects remain excessively sensitive to impaired insulin signaling within the endogenous cardiovascular NO system and appear to be compromised in the presence of insulin resistance (7). So there is evidence that impaired NO-dependent vasodilation causes hypertension and insulin resistance and vice versa: that insulin resistance, such as that observed in diabetes, the metabolic syndrome, and hypertension, impairs endothelial function, it is of major importance to define and describe the underlying mechanisms leading to vascular dysfunction and insulin resistance. From a clinical point of view, there is a specific need for experimental human studies assessing NO synthesis and clearance rates and whether abnormalities of NO function relate to altered kinetics and impaired insulin stimulation.

In this issue of Diabetes, Tessari et al. (14) report basal and insulin-stimulated synthesis rates for mononitrogen oxides (NO and NO₂ – NOx) in 26 subjects covering a wide range of age, BMI, cholesterol, glucose tolerance, and renal function. In these experiments, the investigators use a precursor product, isotope dilution technique. Because arginine is the precursor for NO (Fig. 1), arginine labeled with nitrogen-15, a stable isotope, is infused, and the percentage of labeled arginine (precursor) and its product (NO) is measured by mass spectrometry. Once the whole system is at steady state, synthesis/appearance rates for arginine and NO can be calculated depending on the “intensity” of labeling. Compared with normal subjects, the new data showed that NO synthesis was lower in the elderly and in people with type 2 diabetes and generally increased after insulin stimulation. Regression analysis using data for all subjects showed that NOx synthesis was inversely correlated with arginine metabolites (ADMA, SDMA) and age, but not with insulin sensitivity. The authors conclude that whole-body NOx production is decreased in aging and type 2 diabetes and that arginine metabolites, not insulin resistance, appear to be negative regulators of in vivo NOx production.

These are timely and pertinent data from a well-conducted human study, and the findings not only expand our understanding of the field but also suggest that insulin resistance and NO dysfunction may not be as intimately linked as hitherto believed. A particular strength of the study is that it combines a complicated clinical setup in a relative large number of subjects with a state-of-the-art kinetic tracer technique. A recent study, using a saliva oral nitrate test and an intravenous glucose tolerance test, reported a correlation between insulin sensitivity and NOx synthesis (15), whereas the current study, using steady state isotope dilution and clamp techniques, fails to make this connection. Insulin sensitivity is expressed by a glucose clamp–derived M (or glucose infusion rate) value, which predominantly reflects glucose uptake in muscle (16). NO synthesis is measured systemically, leaving the contributing tissues unidentified.
Hence, the new results do not necessarily reflect conditions in muscle. Furthermore, the design of the study is quite complex, with many different subgroups and mixed pathologic features. It is therefore possible that the heterogeneity of the study sample may have underpowered the study. Whether clearance/disappearance is altered in insulin-resistant states is not known, and it is possible that NO-dependent alterations in blood flow might contribute to a feedback loop in which NO removal from its target tissues increases with increasing vasodilation and blood flow.

The clinical implication of the current study relates to the effect of insulin resistance on endothelial dysfunction. Although Tessari et al. (14) did not measure endothelial function or any balance between insulin-regulated NO-dependent vasodilator actions versus endothelin-1–dependent vasoconstrictor actions, the data support the idea that insulin resistance may only partly explain the impairment of endothelial function with increasing age, obesity, and type 2 diabetes. Several studies using multivariate analyses that adjust for other potential modulators of endothelial function have shown that insulin resistance may not be an independent predictor of endothelial function (17–19). On the other hand, the UK Prospective Diabetes Study clearly showed that metformin intervention, aimed at improving insulin sensitivity and endothelium-dependent vasodilation, led to a significant reduction in cardiovascular events in patients with insulin resistance (20). The extent to which other interventions that improve insulin sensitivity, such as caloric restriction, physical activity, and pharmacological agents, act via NO to improve cardiovascular outcomes are issues to be addressed by future studies.

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