Defective nitric oxide synthesis: a link between metabolic insulin resistance, sympathetic overactivity and cardiovascular morbidity

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Abstract
Epidemiological studies demonstrate an association between insulin resistance, hypertension and cardiovascular morbidity. In addition to its metabolic effects, insulin also has important cardiovascular actions. The sympathetic nervous system and the nitric oxide–L-arginine pathway have emerged as central players in the mediation of these actions. Over the past decade, the underlying mechanisms and the factors that may govern the interaction between insulin and these two major cardiovascular regulatory systems have been studied extensively in healthy people and insulin-resistant individuals. Here we summarize the current understanding and gaps in knowledge on these interactions. We propose that a genetic and/or acquired defect of nitric oxide synthesis could represent a central defect triggering many of the metabolic, vascular and sympathetic abnormalities characteristic of insulin-resistant states, all of which may predispose to cardiovascular disease.

Introduction
Epidemiological observations demonstrate an association between obesity, insulin resistance and hypertension (1, 2) and indicate that insulin is an independent predictor of coronary artery disease (3). The underlying mechanism relating these disorders is not known. Over the past decade evidence has accumulated indicating that insulin, in addition to its metabolic action, exerts cardiovascular effects that are mediated by the sympathetic nervous system and the nitric oxide–L-arginine pathway. Insulin-resistant states are characterized by alterations in both of these functions. Recent evidence indicates that these two regulatory systems interact closely and a defect in nitric oxide synthesis may have important consequences with regard to sympathetic function. In this paper we will review studies examining vascular and sympathetic function in insulin-resistant states. We will then try to demonstrate how a genetic and/or acquired defect in nitric oxide synthesis could represent a central defect triggering many of the metabolic, vascular and sympathetic abnormalities that characterize insulin-resistant states.

Impaired nitric oxide synthesis: a defect underlying sympathetic overactivity and impaired vasodilatation in insulin-resistant states

Physiology: interplay between nitric oxide and the sympathetic nervous system in the mediation of the cardiovascular actions of insulin in normal individuals

Short-term infusion of insulin (4–14) and carbohydrate ingestion (15–17) stimulate sympathetic nerve activity in normal individuals. Insulin stimulation of sympathetic outflow is targeted specifically at the skeletal muscle tissue (17, 8, 18) — the main target of the metabolic action of insulin. This sympathoexcitatory effect appears to be mediated by a central neural action of insulin (4, 6, 11, 19–22) possibly involving the release of specific neuropeptides such as corticotrophin releasing hormone (10).

In healthy individuals, insulin also stimulates blood flow and decreases vascular resistance in skeletal muscle (9, 10, 14, 22–35) but not in skin (29, 35). Insulin
stimulation of blood flow is characterized by inter-individual variability (29) that appears to be determined, at least in part, by the relative limb muscle content and related phenomena such as physical fitness and capillary fibre ratio (36, 37). Insulin vasodilatation is related primarily to insulin itself rather than to stimulation of carbohydrate metabolism (14).

Nitric oxide release accounts for the vasodilator action of insulin (22). In vitro, insulin activates L-arginine transport and stimulates nitric oxide release in cultured vascular endothelial cells (38, 39). In vivo, insulin-induced vasodilatation is abolished by the stereospecific inhibitor of nitric oxide synthase, Nω-monomethyl-L-arginine (L-NMMA) (31, 40), and by inhibition of the synthesis of tetrahydrobiopterin, a cofactor necessary for nitric oxide synthesis (41). Insulin potentiates endothelium-dependent but not endothelium-independent vasodilatation, indicating that its vasodilator effect is related specifically to stimulation of nitric oxide release, and not to facilitation of vascular responsiveness to nitric oxide (42, 43).

Insulin may stimulate nitric oxide release either by a direct, local effect at the vascular endothelium or by stimulating sympathetic nitrergic fibres. Comparison of vasodilatation during local, intra-arterial and systemic, intravenous insulin infusion has provided conflicting results (22). In patients who have undergone regional sympathectomy, insulin stimulates nitric oxide release and blood flow in the denervated limb, indicating that it stimulates blood flow by a direct action at the vasculature (44). Consistent with this hypothesis, insulin causes hypotension in patients with autonomic failure (45). In innervated limbs, however, stimulation of sympathetic vasodilator outflow by insulin appears to be necessary to induce vasodilatation, because the prevention of insulin-induced sympathetic activation by means of dexamethasone abolished the insulin-induced vasodilatation (10).

The sympathetic nervous system modulates insulin-induced vasodilatation, as indicated by the much more rapid vasodilatation in the denervated than in the innervated limb in patients with regional sympathectomy (46, 47). Moreover, it is possible that there exists a balance between the central neural sympathoexcitatory (via stimulation of neural peptide release) and sympathoinhibitory (by stimulating nitric oxide release) actions of insulin, as nitric oxide inhibits central neural sympathetic vasoconstrictor outflow (48, 49).

In summary, insulin causes vasodilatation by stimulating release of nitric oxide through a direct local effect at the vasculature and by stimulating neural vasodilator outflow. The sympathetic vasoconstrictor tone restricts the insulin-induced vasodilatation.

**Pathophysiology: insulin resistance is associated with a defect in nitric oxide synthesis and sympathetic overactivity**

Many studies have examined insulin cardiovascular effects in insulin-resistant states (9, 23, 25, 32, 43, 50–81) and most, but not all, of these studies show that insulin-induced vasodilatation is impaired in obesity, essential hypertension and non-insulin-dependent diabetes mellitus (NIDDM) (Table 1). The reason for the divergent findings between studies is not clear, but could be related to confounding factors that were not controlled for, such as glycaemic control (82), dyslipidaemia (52), dysautonomia (78, 79), long-term complications of NIDDM and differences in the techniques and pharmacological agents used to assess endothelial function.

There is increasing evidence that in insulin-resistant individuals, in addition to insulin vasodilatation, endothelium-dependent vasodilatation is also impaired (Table 1). Insulin stimulation of nitric oxide flux in skeletal muscle tissue is defective in obese persons (83). Impaired endothelial nitric oxide synthesis has been shown to be directly related to metabolic insulin resistance (84). In vitro, insulin stimulation of nitric oxide release and glucose metabolism appear to share common signalling pathways in vascular endothelial cells, suggesting that the vascular and metabolic actions of insulin may be coupled (39). Alternatively, insulin resistance is associated with metabolic abnormalities such as dyslipidaemia that, in turn, may alter endothelial function. The mechanism underlying the endothelial dysfunction is not known.

Insulin resistance is associated with sympathetic overactivation in both animals and humans (13, 15, 16, 85–88). The mechanism causing this sympathetic overactivity is not known. Hyperinsulinaemia is a candidate mechanism, but is not invariably associated with sympathetic overactivity in humans, as demonstrated by the normal sympathetic nerve activity in patients with insulinoma (89, 90) and alternative mechanisms need to be considered. Nitric oxide inhibits central neural vasoconstrictor outflow in animals (91–93) and humans (49, 48). It is therefore possible that the defect in nitric oxide synthesis found in many insulin-resistant states (83, 94) may contribute to sympathetic overactivity. This defect in nitric oxide synthesis could be acquired and/or inherited. With regard to an inherited defect, recent studies indicate that polymorphisms in the endothelial nitric oxide synthase gene are risk factors for cardiovascular diseases associated with insulin resistance such as hypertension (95) and coronary artery disease (96, 97).

In summary, there is increasing evidence that nitric oxide synthesis is impaired in insulin-resistant states. This defect may be inherited and/or acquired and could contribute to both impaired insulin-induced vasodilatation and sympathetic overactivity characteristic of insulin-resistant states.
Pathophysiological consequences of defective nitric oxide synthesis (and sympathetic overactivity)

Metabolic consequences of impaired nitric oxide synthesis: flow-dependent and -independent effects

As insulin increases muscle blood flow, the obvious question arises whether this effect has a role in its main action – namely the promotion of glucose disposal in skeletal muscle tissue. Stimulated by the pioneering study by Laakso and colleagues, who provided evidence consistent with this hypothesis (23), many subsequent investigations have examined the effects of an acute augmentation or inhibition of muscle blood flow on insulin-stimulated muscle glucose uptake in both healthy and insulin resistant individuals. Since our last review (22) this issue has remained highly controversial and is far from being definitively settled (82, 98, 99). These conflicting results may be related, at least in part, to differences in study design, dose and pharmacology of the vasoactive agents used, the absence of reliable tools for the direct assessment of muscle microcirculation (100), confounding effects of these drugs on other systems known to modulate glucose metabolism, and confounding flow-independent effects of these agents on glucose uptake (22, 94).

Specifically, the role of insulin stimulation of nitric oxide release and vasodilatation in the regulation of
muscle glucose uptake is not clear. Stimulation of blood flow, and presumably the release of nitric oxide, by infusion of methacholine into the femoral artery has been reported to potentiate insulin-induced stimulation of regional glucose uptake during steady-state physiological and pharmacological hyperinsulinaemia (101), whereas acute reduction of femoral blood flow, and presumably nitric oxide release, by intra-arterial infusion of L-NMMA had the opposite effects (102). Conversely, induction of hypertension and inhibition of nitric oxide synthesis by infusion of L-NMMA did not alter stimulation of whole-body glucose uptake and glucose oxidation during insulin/glucose infusion in healthy individuals (31, 44, 103) and stimulation of muscle blood flow by intra-arterial infusion of bradykinin did not augment regional muscle glucose uptake in normal individuals (34).

Nitric oxide may have effects on glucose metabolism that are independent of its vascular actions (104, 105). It is synthesized in skeletal muscle where it regulates metabolic and contractile processes (106). In incubated rat skeletal muscle, inhibition of nitric oxide synthase activity impaired contraction-stimulated (but not insulin-stimulated) glucose transport (104, 105, 107, 108), whereas nitric oxide donors had the opposite effect (105). In humans, improved glucose tolerance and insulin sensitivity characteristic of the trained state may be related to an augmented expression of nitric oxide synthase protein in skeletal muscle tissue (106). In keeping with this hypothesis, infusion of l-arginine augments insulin sensitivity by a mechanism independent of insulin-mediated vasodilatation (109). In addition to these peripheral actions, central neural nitric oxide-dependent pathways may also modulate insulin sensitivity, as indicated by the induction of insulin resistance in rats by intracerebroventricular administration of L-NMMA (110). Finally, and most importantly, mice with disruption of the gene encoding endothelial nitric oxide synthase are insulin resistant, as demonstrated by a roughly 40% smaller insulin-stimulated whole-body glucose uptake than occurs in their wild-type littermates (H Duplain, C Sartori & U Scherrer, unpublished observations). The underlying mechanism relating nitric oxide deficiency and insulin resistance is still not known. Insulin resistance, however, is believed to play an important part because it often persists during antihypertensive treatment (118) and, although not found in secondary hypertension (119, 120), is present in offspring of hypertensive parents (who are prone to develop hypertension later in their life) at a time when they are still normotensive (121).

In humans, short-term infusion of insulin does not increase arterial pressure (8, 10, 12, 14, 60) because the sympathetic pressor effects are offset by vasodilatation, as demonstrated by the induction of hypertension by insulin/glucose infusion during nitric oxide synthase inhibition (31, 47). The observation in patients with autonomic failure that unopposed insulin-induced vasodilatation is associated with marked hypotension further demonstrates the importance of this balance between the pressor and depressor actions of insulin for blood pressure regulation (45). It is therefore possible that the endothelial dysfunction found in insulin-resistant states (43, 52, 61, 66) may tip the balance between the pressor and depressor actions in favour of the former and ultimately lead to hypertension. In obese insulin-resistant individuals, the conjunction of sympathetic overactivity and impaired nitric oxide synthesis not only may favour hypertension, but could potentiate overall cardiovascular risk. Sympathetically mediated trophic effects on the vasculature (122) and stimulation of platelet number and aggregability (123) together with the loss of nitric oxide inhibition of such actions (124–126) could trigger acute cardiovascular events. In the presence of preserved insulin-stimulated atherogenesis (82), impaired insulin-stimulated nitric oxide synthesis and, in turn, inhibition of atherogenesis, could provide a link between insulin resistance and macrovascular disease (3). Finally, an inherited defect (127) in nitric oxide synthase not only may alter sympathetic regulation and impair antithrombotic and antiatherogenic defence mechanisms, but may also underly the poor capillary development that characterizes young adults predisposed to arterial hypertension.

**Cardiovascular consequences of impaired nitric oxide synthesis**

There exists an association between obesity, insulin resistance and hypertension (1, 2) and insulin is an independent predictor of coronary artery disease (3). The underlying mechanism relating these disorders is not known. Insulin resistance, however, is believed to play an important part because it often persists during antihypertensive treatment (118) and, although not found in secondary hypertension (119, 120), is present in offspring of hypertensive parents (who are prone to develop hypertension later in their life) at a time when they are still normotensive (121).
(128), as vasodilatation and stimulation of blood flow are determinants of angiogenesis (129). Such an inherited impairment of nitric oxide synthesis may then be further aggravated by acquired factors such as obesity and physical inactivity.

In summary, animal species and human populations in whom insulin-resistance is associated with a high incidence of cardiovascular complications are characterized by possibly causally related sympathetic overactivity and a defect in nitric oxide synthesis. The mechanisms governing the interaction between these two systems are still incompletely understood. What is already clear, however, is that the conjunction of these two defects greatly potentiates the overall cardiovascular risk.

**Conclusion**

The data summarized in this review are consistent with the novel concept that a genetic and/or acquired impairment of nitric oxide synthesis may represent a central defect causing many of the metabolic, vascular, and sympathetic abnormalities associated with insulin resistance (Fig. 1). Impaired nitric oxide synthesis explains the defect in endothelium-dependent and insulin-induced vasodilatation that characterizes many insulin-resistant states and which in part may be related to poor development of capillary density and, in turn, vasodilator reserve. Nitric oxide inhibits sympathetic vasoconstrictor outflow. Thus defective nitric oxide synthesis may contribute to the sympathetic overactivity that characterizes these conditions. Finally, mice with disruption of the gene encoding for endothelial nitric oxide synthase are insulin resistant. The underlying mechanism by which a defect in nitric oxide synthesis causes metabolic insulin resistance is not yet known, but impaired insulin stimulation of muscle blood flow (and in turn substrate delivery), sympathetic overactivity and defective insulin signalling in the skeletal muscle cell may represent candidate mechanisms. In the clinical setting, impaired nitric oxide synthesis, sympathetic overactivity and hyperinsulinaemia concur in their proatherogenic actions. This unfavorable triad could promote hypertension by stimulation of vascular smooth muscle cell growth and vasoconstriction, and trigger acute coronary events by facilitating coronary vasoconstriction and platelet aggregation. We suggest that the pursuit of this important avenue of research will generate important new insight into the underlying mechanisms linking cardiovascular and metabolic disease.

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