Perspectives in Diabetes

Hyperglycemic Pseudohypoxia and Diabetic Complications

JOSEPH R. WILLIAMSON, KATHERINE CHANG, MYRTO FRANGOS, KHALID S. HASAN, YASUOIDO, TAKAHIKO KAWAMURA, JENS R. NYENGAARD, MARIA VAN DEN ENDEN, CHARLES KILO, AND RONALD G. TILTON

Vasodilation and increased blood flow are characteristic early vascular responses to acute hyperglycemia and tissue hypoxia. In hypoxic tissues these vascular changes are linked to metabolic imbalances associated with impaired oxidation of NADH to NAD⁺ and the resulting increased ratio of NADH/NAD⁺. In hyperglycemic tissues these vascular changes also are linked to an increased ratio of NADH/NAD⁺, in this case because of an increased rate of reduction of NAD⁺ to NADH. Several lines of evidence support the likelihood that the increased cytosolic ratio of free NADH/NAD⁺ caused by hyperglycemia, referred to as pseudohypoxia because tissue partial pressure oxygen is normal, is a characteristic feature of poorly controlled diabetes that mimics the effects of true hypoxia on vascular and neural function and plays an important role in the pathogenesis of diabetic complications. These effects of hypoxia and hyperglycemia-induced pseudohypoxia on vascular and neural function are mediated by a branching cascade of imbalances in lipid metabolism, increased production of superoxide anion, and possibly increased nitric oxide formation. Diabetes 42:801–13, 1993

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addition, explanations will be suggested for observations discordant with the thesis of this perspective. Recent reviews of other hypotheses as well as supporting data relevant to this perspective have been reported previously (14, 26–42).

Several parallels between functional abnormalities associated with an increased NADH/NAD⁺ in diabetic tissues and in hypoxic or ischemic myocardium are depicted in Fig. 2 (albeit the metabolic imbalances that mediate these abnormalities may differ in hypoxic and hyperglycemic tissues). This redox imbalance in tissues of diabetic animals appears to result largely from an increased rate of oxidation of sorbitol to fructose by SDH. In hypoxic and ischemic myocardium, the same redox imbalance results from impaired mitochondrial oxidation of NADH to NAD⁺ because of decreased PO₂. Functional consequences associated with this redox imbalance in isolated perfused hearts include 1) electrophysiological dysfunction (arrhythmias), 2) impaired myocyte contractile function, 3) increased vascular permeability, and 4) increased blood flow after reflow after mild hypoxia or brief ischemia but decreased blood flow after prolonged hypoxia/ischemia. Corresponding functional changes in diabetic tissues include 1) electrophysiological dysfunction in peripheral nerve and retina; 2) impaired contractile function of heart, skeletal muscle, and vascular smooth muscle; 3) increased vascular permeability; and 4) increased blood flow early after the onset of diabetes but decreased blood flow later in the course of diabetes. All of these early changes in tissues of diabetic animals are prevented by inhibitors of AR (13, 26, 27, 43–48) (see APPENDIX, note 3).

An important feature of this glucose-induced redox imbalance is that it provides an explanation for the increased susceptibility of diabetic subjects to hypoxic and ischemic injury (32, 35, 49, 50) (see APPENDIX, note 4). Relatively mild hypoxic or ischemic episodes insufficient to cause dysfunction in nondiabetic subjects, when superimposed on preexisting pseudohypoxia induced by hyperglycemia, would result in a higher cytosolic NADH/NAD⁺ that would cause tissue dysfunction and injury in diabetic subjects.

IMBALANCES IN GLUCOSE METABOLISM CONTRIBUTING TO PSEUDOHYPOXIA

The ratio of free NADH to NAD⁺ modulates the activity of many metabolic pathways (54). Because the cytosolic pool of free NADH and NAD⁺ is in equilibrium with cytosolic lactate and pyruvate, and because oxidation of NADH to NAD⁺ by LDH is coupled to reduction of pyruvate to lactate (Fig. 1), an increased ratio of free NADH/NAD⁺ will be reflected by an increased lactate/pyruvate ratio. Indeed, the tissue lactate/pyruvate ratio is a more reliable parameter of the cytosolic ratio of free NADH/NAD⁺ than measurements of NADH and NAD⁺ in tissue extracts because it is not possible to distinguish between mitochondrial and cytosolic pools and between free and bound nucleotides in tissue extracts (23). Thus, for simplicity, in this perspective, changes in tissue lactate/pyruvate ratios are referred to as changes in cytosolic NADH/NAD⁺. In like manner, an increase in mitochondrial NADH/NAD⁺ is reflected by an increase in the ratio of β-hydroxybutyrate/acetocetate (23).

Increased sorbitol pathway metabolism. Increased metabolism of glucose via the sorbitol pathway is probably the most important mechanism by which hyperglycemia increases cytosolic NADH/NAD⁺ (Fig. 1). Because the Kₘ of AR for glucose is very high (70 mM), the rate of reduction of glucose to sorbitol increases with increasing glucose levels in tissues that do not require insulin for glucose uptake (33). Increased sorbitol levels, in turn, will increase the rate of oxidation of sorbitol to fructose coupled to a reduction of NAD⁺ to NADH. At elevated glucose levels, glucose metabolism via the sorbitol pathway accounts for 33% of glucose consump-

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**TABLE 1**

<table>
<thead>
<tr>
<th>Tissues manifesting hyperglycemic pseudohypoxia</th>
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<tbody>
<tr>
<td>Cornea*</td>
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<tr>
<td>Endoneurium*</td>
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<tr>
<td>Erythrocytes*</td>
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<tr>
<td>Glomeruli*</td>
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<td>Granulation tissue*</td>
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*Hyperglycemic pseudohypoxia is linked to increased flux of glucose via the sorbitol pathway.

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**FIG. 2.** Parallels between functional consequences of an increased cytosolic NADH/NAD⁺ linked to hyperglycemic pseudohypoxia in diabetic tissues and hypoxia or ischemia in myocardial tissue.

**Dysfunction**

1. Electrical
2. Mechanical
3. 1 Vascular Permeability
4. 1 → 1 Blood Flow
5. Vascular Sclerosis

**Dysfunction**

1. Electrical
2. Mechanical
3. 1 Vascular Permeability
4. 1 → 1 Blood Flow
5. Vascular Sclerosis
The likelihood that increased sorbitol pathway flux is the more important of these two mechanisms for increasing cytosolic NADH/NAD+* in diabetes is supported by evidence that inhibitors of AR prevent this glucose-induced redox imbalance in tissues in which glycolysis is increased as well as in those in which it is not (14,24,26,28), and decrease sorbitol levels and NADH/NAD+* in tissues exposed to normal glucose levels. The latter finding implies that flux of glucose via the sorbitol pathway modulates cytosolic NADH/NAD+* even at physiological glucose levels. Furthermore, exposure of human erythrocytes to elevated sorbitol levels (at normal glucose levels) even subclinical changes are very poorly reversible even by normalization of glucose levels; and the doses of ARIs...
used in diabetic humans and animals may not have completely inhibited metabolism of glucose via the sorbitol pathway.

NADH generated in the cytosol by glycolysis (coupled to oxidation of GAP to 1,3-DPG) or by oxidation of sorbitol to fructose must be reoxidized to NAD+, or glycolysis will be blocked at the level of GAP dehydrogenase as indicated above. In the cytosol, NADH is oxidized to NAD+ by LDH (coupled to reduction of pyruvate to lactate) and by sG3P dehydrogenase (coupled to reduction of DHAP to sG3P) (Fig. 3). Cytosolic reducing equivalents also are transported (by the sG3P and/or the malate-aspartate shuttle) into mitochondria for oxidation by the electron transport chain. The limited capacity of many cells and tissues to oxidize cytosolic NADH by the sum of these mechanisms is well documented as discussed above.

This discussion has emphasized hyperglycemia-induced changes in cytosolic NADH/NAD+ because of evidence linking this redox imbalance to increased production of reducing equivalents by cytosolic enzymes. An increase in cytosolic NADH/NAD+ may or may not be accompanied by corresponding increases in mitochondrial NADH/NAD+, because in the liver of diabetic rats, the ratio is increased in the cytosol but decreased in mitochondria (23). The same discordance between cytosolic and mitochondrial ratios of NADH/NAD+ is observed in isolated perfused hearts subjected to an increased work load at normal Po2 and constant glucose levels (58). In hypoxic tissues (and in cyanide-poisoned cells) the redox imbalance originates in mitochondria but also affects the cytosol because reducing equivalents generated in the cytosol can no longer be oxidized in the mitochondria.

If an increase in cytosolic NADH/NAD+ plays an important role in mediating vascular and neural dysfunction associated with diabetes and increased sorbitol pathway metabolism, the linkage between cytosolic ratios of lactate/pyruvate and NADH/NAD+ (depicted in Figs. 1 and 3) suggests that glucose-induced vascular dysfunction might be prevented simply by raising tissue pyruvate levels sufficiently to drive oxidation of NADH to NAD+ as rapidly as NAD+ is reduced by oxidation of sorbitol to fructose. Conversely, elevation of lactate levels should mimic the effects of hyperglycemia on vascular function. Both of these predictions have been confirmed experimentally. Elevation of tissue pyruvate levels prevents glucose-induced vascular dysfunction in the tissue chamber model as well as in nondiabetic rats with acute hyperglycemia of ~5-h duration induced by glucose infusion (61, 61a). Pyruvate also prevents sorbitol-induced vascular dysfunction in skin chamber granulation tissue (62). In contrast to inhibitors of AR and SDH, pyruvate has little impact on flux of glucose via the sorbitol pathway (63). Although pyruvate can function as a free-radical scavenger and can be oxidized in mitochondria via the citric acid cycle, these reactions would not decrease cytosolic NADH/NAD+ or normalize cytosolic redox-linked metabolites. In the skin chamber granulation tissue model, addition of lactate (at normal glucose levels) increases blood flow and vascular permeability. In nondiabetic and diabetic human subjects infusion of lactate increases renal blood flow and GFR independent of glucose levels (64). It is of interest that cyanide and ethanol (both of which increase tissue NADH/NAD+ at normal glucose levels) also are glumimetic in their effects on vascular function in the skin chamber model.

The implications of these observations are that 1) pseudohypoxia of any cause, i.e., elevated levels of glucose, sorbitol, lactate, cyanide, or ethanol, induces vascular responses, i.e., dysfunction (see APPENDIX, note 6), like those caused by true hypoxia; and 2) an important determinant of the susceptibility of cells/tissues to sorbitol pathway-mediated injury is whether they can reoxidize NADH to NAD+ rapidly enough to keep pace with the increased rate of reduction of NAD+ to NADH associated with increased flux of glucose via the sorbitol pathway. Although it remains unclear why some cells and tissues (i.e., retina, kidney, and nerve) appear to be more susceptible to injury by the diabetic milieu, their vulnerability may reflect unusual metabolic, structural, and/or functional characteristics in addition to a possible higher content of sorbitol pathway enzymes (resulting in more pronounced pseudohypoxia in response to elevated glucose levels). In tissues containing fructokinase (in addition to AR and SDH) fructose, produced from oxidation of sorbitol, may be phosphorylated and enter glycolysis directly (bypassing phosphofructokinase, which normally limits metabolism of glucose via glycolysis).

**METABOLIC CONSEQUENCES OF AN INCREASE IN CYTOSOLIC NADH/NAD+**

An increase in cytosolic NADH/NAD+ may impact on the activity of numerous cytoplasmic and mitochondrial enzymes that use NADH and NAD+ as cofactors and/or are inhibited or activated by NADH or NAD+. Thus, an important feature of this redox imbalance is that it has the potential to explain many metabolic imbalances associated with the diabetic milieu as well as the increased susceptibility of diabetic subjects to vascular and neural injury by factors independent of the diabetic milieu. Several imbalances in lipid metabolism linked to hyperglycemic pseudohypoxia have been implicated as mediators of diabetes-induced vascular and neural dysfunction. The roles of these imbalances in mediating vascular and neural dysfunction may vary in different cells and tissues depending on differences in their capacity for lipid uptake, synthesis, storage, and/or oxidation.

**Increased de novo synthesis of DAG.** Increased tissue levels of DAG and/or evidence of glucose- and diabetes-induced increased de novo synthesis of DAG (and associated activation of PKC) have been reported in retina, isolated glomeruli, heart, cultured retinal capillary endothelial cells, cultured aortic endothelial and smooth muscle cells, and granulation tissue (14,26,27,61,65–69). On the other hand, DAG levels were unchanged or decreased in peripheral nerve and aorta of diabetic rats (70,71), although PKC activity was increased in cultured aortic smooth muscle cells exposed to elevated glucose levels (72). An increased cytosolic NADH/NAD+ favors
increased de novo synthesis of DAG by two mechanisms. The increase in NADH favors reduction of DHAP to snG3P, which is the first step in one pathway for de novo synthesis of DAG (Fig. 3) (61). It also inhibits GAP dehydrogenase (Fig. 3), thereby impairing the oxidation of GAP to 1,3-DPG, which increases availability of DHAP (which is in equilibrium with fructose 1,6-bisphosphate and GAP) as substrate for DAG synthesis. Activation of PKC, presumably by DAG and/or by LCA-C (discussed below), has been linked to many metabolic and functional vascular and neural changes in diabetes, i.e., decreased Na⁺/K⁺-ATPase activity, increased PG synthetic activity, and vascular dysfunction in glomeruli, aorta, peripheral nerve, and granulation tissue (61,65,68,69).

Although the beneficial effects of myo-inositol supplementation on glucose- and diabetes-induced vascular and neural dysfunction have been attributed to normalization of PtdIns metabolism and Na⁺/K⁺-ATPase activity (34,40), they may also (or instead) accrue from concurrent decreases in DAG levels and PKC activity, because elevated myo-inositol levels favor incorporation of DAG (via CDP-DAG into PtdIns [Fig. 3]). This view is supported by evidence that 1) agonist-induced increases in DAG and CDP-DAG (resulting from hydrolysis of PIP₂) (Fig. 3) are prevented by myo-inositol (73–76), 2) glucose-induced vascular dysfunction in skin chamber granulation tissue (which is associated with an increase in DAG mass) is attenuated by an inhibitor of PKC (61), and 3) decreased neural Na⁺/K⁺-ATPase activity in diabetic mice is prevented by inhibitors of PKC (68). The prevention of glucose- and diabetes-induced decreased Na⁺/K⁺-ATPase activity and imbalances in PtdIns metabolism in aorta and nerve by ARLs also is consistent with the possibility that these changes are linked to sorbitol pathway-mediated pseudohypoxia (34,40).

Inhibition of fatty acid oxidation. The second step in β-oxidation of long-chain fatty acids is inhibited by an increase in mitochondrial NADH/NAD⁺ (31). As discussed earlier, mitochondrial NADH/NAD⁺ may be increased by impaired oxidation of NADH to NAD⁺ in the mitochondria (hypoxia) or by increased transport into the mitochondria of reducing equivalents generated in the cytosol (i.e., by oxidation of sorbitol to fructose); it also can result from increased oxidation of fatty acids (77,78). In hypoxic and ischemic myocardium, long-chain fatty acids accumulate as esters of CoA and of carnitine (Fig. 3) (14,26,31,32,49). These long-chain acyl esters, like DAG, are amphiphilic molecules and are potent modulators of many enzymes whose activities are altered by hypoxia and by diabetes, i.e., Na⁺/K⁺-ATPase (inhibition), PKC (activation), and Ca⁺⁺-ATPase (inhibition) (31,32). Accumulation of these esters in hypoxic and ischemic hearts and in hypoxic cultured myocytes is associated with impaired myocyte contractile function as well as electrophysiological dysfunction (31,49,79,80). These changes are more pronounced in hearts from diabetic animals and are made worse by elevating FFA levels in the perfusate (14,26,32,49). All of these changes are attenuated by addition of l-carnitine or short-chain esters of l-carnitine (acetyl-l-carnitine, propionyl-l-carnitine).

Exposure of skin chamber granulation tissue vessels to palmitoyl-l-carnitine (an LCA-C) in vivo mimics the effects of elevated glucose levels and of hypoxia on blood flow and vascular permeability (14,26,27,81); exposure of cultured myocytes to palmitoyl-l-carnitine also induces electrophysiological dysfunction like that caused by hypoxia (80). These effects of palmitoyl-l-carnitine on vascular and electrophysiological dysfunction are prevented by coadministration of l-carnitine, acetyl-l-carnitine, and/or propionyl-l-carnitine. Acetyl-l-carnitine also prevents vascular and neural (electrophysiological) dysfunction in diabetic rats (82,83). Note that plasma and myocardial levels of l-carnitine are decreased by diabetes (84). At this time, it is unclear how the beneficial effects of l-carnitine, acetyl-l-carnitine, and propionyl-l-carnitine are mediated. Several mechanisms have been proposed that include scavenging of free radicals, increasing the availability of free CoA for energy metabolism, and compensating for detergent effects of long-chain acylcarnitines on membranes. Recent observations in diabetic rats indicate that acetyl-l-carnitine prevents diabetes-induced decreases in endoneurial Na⁺/K⁺-ATPase and PKC activity (84a).

Increased PG synthetic activity. PG synthetic activity is increased in hypoxic and ischemic cells and tissues (85–87) as well as in glomeruli and aorta of diabetic and nondiabetic animals exposed to elevated glucose levels in vitro (48,88–92). The particular PGs synthesized (and the balance between vasodilator versus vasoconstrictor prostanoids) varies in different vascular cells and tissues and with the severity and duration of hypoxia and hyperglycemia (85,88). The increased GFR observed early after the onset of poorly controlled diabetes in experimental animals is associated with increased production of vasodilatory PGs (88–90); a decreased ratio of vaso-dilator/vasoconstrictor PGs is associated with decreased blood flow and GFR later in the course of diabetes (88). Impaired contractile function of aortic rings from diabetic and nondiabetic rabbits exposed to elevated glucose levels in vitro is associated with increased production of vasoconstrictor prostanoids (91,92).

Increased PG synthetic activity in glomeruli from diabetic rats is associated with increased de novo synthesis of DAG (67), activation of PKC (65), increased activity of some phospholipase A₂ isozymes (93), and increased GFR (13,14,88). All of these phenomena are attenuated to varying degrees by inhibitors of AR (13,14,26,89). A plausible linkage of these observations is depicted in Fig. 4.

EFFECTS OF AN INCREASED NADH/NAD⁺ ON FREE-RADICAL PRODUCTION

Increased free-radical production associated with hypoxia and ischemia. The importance of increased production of O₂⁻ and oxygen reactive species derived from it, i.e., hydrogen peroxide and hydroxyl radical, in mediating vascular injury during reperfusion after hypoxia and ischemia is well known (30,31,94,95). O₂⁻ production also is increased in cultured endothelial cells subjected to...
scavengers, ARIs, and inhibitors of cyclooxygenase (48,101) as noted earlier. At this time, it is unclear whether NO production is increased by diabetes or whether the effects of NO synthase inhibitors on vascular dysfunction associated with diabetes reflect correction of an imbalance in vasodilators versus vasoconstrictors (inhibition of NO production could compensate for increased vasodilator prostanoid production and/or decreased responsiveness to endothelin-1, a potent vasoconstrictor). Because plasma levels of endothelin-1 have been reported to be increased in diabetic humans, note that 1) endothelin-1 plasma levels are increased by insulin (105), 2) the biochemical effects of endothelin-1 on cultured pericytes are blunted by exposure to elevated glucose levels (106), and 3) low-affinity endothelin-1 receptors are downregulated in glomeruli from diabetic rats (107). This downregulation is prevented by an inhibitor of PKC given in vivo.

Mechanisms of increased \( \text{O}_2^- \) production by NADH.

Increased \( \text{O}_2^- \) production resulting from an increased rate of reduction of NADH to \( \text{NAD}^+ \) may be mediated by several different reactions. The likelihood that an increased NADH/\( \text{NAD}^+ \) will increase \( \text{O}_2^- \) production via increased PG synthetic activity is supported by evidence discussed earlier linking increased PG synthetic activity in diabetes to pseudohypoxia, together with evidence of \( \text{O}_2^- \) production coupled to PGH synthase activity (100,108,109). Vascular dysfunction induced by topical application of arachadonic acid is prevented by superoxide dismutase and cyclooxygenase inhibitors (100). Reduction of PGG2 to PGH2 by PG hydroperoxidase produces \( \text{O}_2^- \) via a sidechain reaction that uses NADH (and NADPH but not GSH) as a reducing cosubstrate (100). Subsequent autoinactivation of prostacyclin synthase by \( \text{O}_2^- \) (produced by PG hydroperoxidase) (108,109), would explain diabetes-induced decreased production of prostacyclin and other vasodilatory prostanoids reported by some investigators. This would result in a decreased ratio of vasodilator/vasoconstrictor prostanoids because thromboxane synthase is resistant to inactivation by \( \text{O}_2^- \) (108,109). Higher levels of oxygen free radicals also inactivate PG cyclooxygenase activity. Note that NO has been reported to inhibit lipid oxidation by lipoxygenase and cyclooxygenase (110). If increased PG synthetic activity associated with diabetes is indeed triggered by increased availability of arachadonic acid, then metabolism of arachadonic acid and associated formation of \( \text{O}_2^- \) by microsomal cytochrome \( P_450 \) also may be increased (30,111).

\( \text{O}_2^- \) is a normal byproduct of oxidation of NADH to NADH by the electron transport chain in mitochondria (30,31). Reduced electron transport chain components can undergo spontaneous auto-oxidation with \( \text{O}_2^- \) formation (97). If cytosolic-reducing equivalents produced by accelerated sorbitol pathway metabolism are transported into mitochondria rapidly enough, autooxidation of reduced electron transport components and \( \text{O}_2^- \) formation may be increased.

NADH also has been reported to increase \( \text{O}_2^- \) production by cultured human fibroblasts exposed to interleukin-1 (112). The nature of the reactions(s) that mediate
this effect of NADH are unclear but may be analogous to production of O2 by plasma membrane-associated NADPH oxidase in leukocytes. These observations raise the possibility that increased availability of NADH may favor increased O2 formation by other peroxidases, i.e., GSH peroxidase (100).

Note that increased oxidative stress and O2 formation by the above mechanisms may occur in the absence of an increased cytosolic ratio of NADH/NAD+ provided that the increased rate of reduction of NAD+ to NADH by SDH does not exceed the maximal rate at which the cell can reoxidize NADH to NAD+.

Mechanisms of increased NO production by NADH. Increased NO production associated with hypoxia and diabetes may be initiated by an increase in intracellular calcium (i.e., by increased O2 formation [113,114]), which would activate the constitutive isoform of NO synthase (98), and/or by activation of PKC (by increased levels of DAG and/or LCA-C and LCA-CoA), which has been reported to phosphorylate the constitutive isoform of NO synthase that results in a ~40% increase in activity (115,116). Note that a marked increase in NO production may increase NADH/NAD+ by inactivating aconitate and electron transport chain enzymes (98). It also is of interest that NO synthase, like AR, requires NADPH as a cofactor (98).

Many (but not all) investigators have reported that acute hyperglycemia and diabetes impair acetylcholine-induced (NO-mediated) relaxation of vessels in vitro (46,48,91,92,101,117-120). Most reports suggesting impaired NO production are based on experiments in which vessels were incubated for several hours in media lacking L-arginine as substrate for NO synthesis. The possibility that impaired NO production by tissues from diabetic animals (and from nondiabetic animals incubated at elevated glucose levels) in L-arginine-deficient media may be an artifact of the in vitro milieu is suggested by several lines of evidence: 1) when the concentration of L-arginine (or H2, bioterin, another cofactor required for NO synthesis) is suboptimal, activation of purified brain NO synthase results in production of hydrogen peroxide rather than NO (121); 2) glucose- and diabetes-induced decreases in Na+K+-ATPase activity in rabbit aortic rings are not observed in tissues incubated in media containing L-arginine (122); and 3) impaired acetylcholine-induced relaxation of arteries from hypercholesterolemic rabbits appears to be an in vitro artifact of increased free-radical production (catalyzed by trace metals in the buffer) (123); trace metal-catalyzed free-radical production would be even greater in hyperglycemic media because of autoxidation of glucose (29). Whereas glycated proteins and O2 react with (and inactivate) NO in vitro (98,124), the pathophysiological significance of such observations is unclear because the rate of NO quenching versus rates of NO production and advanced glycation product formation in vivo (in diabetic subjects) is unknown. Further studies are needed to resolve these discordant observations regarding the nature and time course of changes in NO production as well as changes in other vasodilators and vasoconstrictors induced by diabetes.

Second messenger effects and cell injury linked to increased O2 production. Increasing evidence attests to important roles for both O2 and NO in modulating normal cellular metabolism and mediating responses to vasoactive agents and injury (94,98,99,114). Therefore, if O2 production is increased in diabetic subjects, it could mediate many (otherwise apparently unrelated) diabetes-induced metabolic imbalances and biochemical changes, including extracellular matrix changes. For example, ascorbic acid–induced increases in collagen production and expression of procollagen α1(1) mRNA levels in human fibroblasts are mediated by reactive aldehyde products of lipid peroxidation (caused by O2) and are prevented by probucol (125) (which also prevents glucose-induced vascular dysfunction [104]). Thus, increased O2 production could potentially mediate glucose-induced increased gene expression (in cultured vascular and mesangial cells) for extracellular matrix constituents, i.e., type IV collagen, fibronectin, and β1 laminin (126,127). Increased O2 production linked to hyperglycemic pseudohypoxia also could mediate DNA damage and impaired cell replication in cultured endothelial cells and tissues exposed to elevated glucose levels (126,127). Oxygen free radicals have been reported to depress sarcosomeral Na+K+-ATPase and Ca2+-ATPase activity (52), increase intracellular Ca2+ (113,114), release arachidonic acid and stimulate cyclooxygenase activity (114,128), and inhibit NO synthase activity (129).

GALACTOSE-INDUCED PSEUDOHYPOTENSION Rodents fed galactose-enriched diets develop vascular and neural dysfunction and early vascular structural changes identical to those in diabetic animals and which, like those in diabetic animals, are prevented by inhibitors of AR (7,26,27,33). Unlike sorbitol (which is readily oxidized to fructose by SDH), galactitol does not appear to be further metabolized except in the liver. For this reason, many (if not most) investigators have attributed sorbitol pathway–linked diabetic complications to increased intracellular concentration of sorbitol (osmotic stress) and/or to redox changes and metabolic imbalances linked to reduction of glucose to sorbitol (33,36). Redox changes and metabolic imbalances associated with oxidation of sorbitol to fructose were considered unlikely candidate mechanisms for mediating sorbitol pathway–linked complications of diabetes.

In view of these considerations, it was of great interest to find that in human erythrocytes and in rat granulation tissue exposed to elevated galactose levels (in vitro and in vivo, respectively) cytosolic NADH/NAD+ and associated increases in triose phosphates and snG3P equal or exceed those induced by elevated glucose levels and are prevented by ARIs and by pyruvate (26,27,130; J.R.W., unpublished observations). Exposure of human erythrocytes to sorbitol also causes a marked increase in NADH/NAD+ that is attenuated by pyruvate (59); galactitol has no effect on NADH/NAD+. These observations suggest the existence of an as yet unidentified polyol pathway–linked mechanism for increasing cytosolic
NADH/NAD+. In any case, these findings are consistent with the likelihood that increased cytosolic NADH/NAD+ mediates polyol pathway–linked vascular and neural functional and early structural changes induced by galactosemia (33) as well as by hyperglycemia. It is of interest in this regard that elevated galactose levels (like elevated glucose levels) stimulate de novo synthesis of DAG in cultured vascular cells (G. King, unpublished observations).

Depletion of GSH levels and increased susceptibility to oxidative stress in selected tissues (i.e., lens and erythrocytes) of diabetic and galactose-fed animals have been widely attributed to competition between AR and GSH reductase for NADPH cofactor. Even though NADPH levels are modestly decreased by accelerated polyol pathway activity, recent studies indicate that the decrease is not rate limiting for reduction of oxidized GSH because of the much lower $K_m$ of GSH reductase versus AR for NADPH (131). The likelihood that sorbitol pathway–linked depletion of reduced GSH in these tissues reflects increased oxidative stress (i.e., an increased NADH/NAD+) that results from accelerated oxidation of sorbitol to fructose is consistent with depletion of reduced GSH levels linked to the increased NADH/NAD+ in hypoxic tissues of nondiabetic subjects (132).

In view of this new evidence of galactose-induced pseudohypoxia and earlier evidence that nonenzymatic glycation of lens crystallins by galactose is prevented by GSH (133), note that ARs prevent galactose-induced 1) depletion of lens GSH, 2) increased fluorescence (indicative of nonenzymatic glycation) and crosslinking of lens crystallins (134), and 3) pseudohypoxia. These observations are consistent with the putative importance of polyol pathway–induced oxidative stress mediated by pseudohypoxia (i.e., an increased NADH/NAD+) in the pathogenesis of diabetic cataracts and nonenzymatic glycation as well as in other complications of diabetes.

**IMPACT OF INCREASED CYTOSOLIC NADH/NAD+ ON THE SUSCEPTIBILITY OF VESSELS AND NERVES TO INJURY BY FACTORS INDEPENDENT OF DIABETES**

The nature of the metabolic imbalances and functional consequences linked to hyperglycemic pseudohypoxia suggests new insights for understanding the increased susceptibility of diabetic subjects to vascular and neural injury by risk factors independent of diabetes as well as by other systemic metabolic and biochemical imbalances associated with the diabetic milieu. Included among such factors are hypoxic and ischemic injury, elevated plasma levels of atherogenic lipoproteins and FFAs, hypertension, cigarette smoking, ethanol abuse, and increased oxygen-derived free radicals associated with nonenzymatic glycation and glycoxidation. Potential interactions between hyperglycemic pseudohypoxia and each of these factors are considered below.

First, the mechanism by which an increase in cytosolic NADH/NAD+ could increase the susceptibility of tissues to hypoxic and ischemic injury is depicted in Fig. 2. As discussed earlier, cells with an increased NADH/NAD+ attributable to hyperglycemic pseudohypoxia will require less severe hypoxia or ischemia to increase NADH/NAD+ to the same level caused by hypoxia or ischemia alone.

Second, because albumin permeation into the aorta is increased two- to threefold by diabetes, it is likely that vascular permeation by other macromolecules such as atherogenic lipoproteins also will be increased. Thus, even in diabetic subjects with normal cholesterol and lipoprotein profiles, permeation of atherogenic lipoproteins may be increased two- to threefold, which corresponds to the increased frequency of heart attacks in diabetic subjects (35). Any further increase in plasma levels of atherogenic lipoproteins would result in a corresponding increased flux into the vessel wall.

The likelihood that elevated plasma FFA levels in diabetic subjects may accentuate hyperglycemic pseudohypoxia and contribute to excessive accumulation of LCA-C and LCA-CoA, causing more vascular and neural injury, is supported by evidence that 1) elevation of FFAs leads to increased rates of fatty acid oxidation and reduction of NAD+ to NADH (resulting in an increase in mitochondrial NADH/NAD+) (77,78), which will accentuate oxidative stress and pseudohypoxia induced by hyperglycemia; 2) elevated FFAs increase hypoxic and ischemic injury to the isolated perfused heart; 3) levels of cytotoxic LCA-C and LCA-CoA are higher in hypoxic and ischemic hearts from diabetic than from nondiabetic subjects; and 4) microalbuminuria in diabetic humans and animals is significantly correlated with elevated plasma triglycerides (which also correlate with plasma nonesterified fatty acids) (14).

Third, the finding that blood flow in diabetic rats is preferentially increased in tissues prone to late complications, in the absence of an increase in systemic blood pressure, implies dilation of resistance arterioles and impaired smooth muscle contractile function in the affected tissues. Thus, systemic blood pressure will be transmitted further downstream causing microvascular hypertension (Fig. 5). Hypertension is a well-known stimulus for vascular collagen production (and is associated with increased O$_2$ production) (100), which will result in sclerosis and stiffening of the vessel wall (14,26,27). Impaired contractile function of resistance arterioles also will permit any increase in systemic blood pressure to be transmitted further downstream resulting in even more severe microvascular hypertension. These hemodynamic changes, coupled with increased vascular permeability, may account for the more severe and widespread hyalinization of arterioles in diabetic than nondiabetic subjects. The dilation of resistance arterioles induced by diabetes is in sharp contrast to the increased peripheral resistance (attributable to contraction of resistance arterioles) that contributes to hypertension in large arteries while tending to limit transmission of systemic blood pressure into the microvasculature distal to resistance arterioles. Thus, these observations predict that at any arterial blood pressure, microvascular blood flow will be higher in these (complication-prone) tissues of diabetic subjects than in nondiabetic subjects and much higher than in nondiabetic hypertensive subjects (until blood flow is reduced by vascular sclerosis).
Fourth, cigarette smoking has been implicated as a significant risk factor for vascular complications of diabetes. Increased CO levels in smokers could impact on diabetes-induced vascular injury in several ways. CO, like NO, reacts with iron-containing electron transport chain enzymes in the mitochondria, thereby impairing oxidation of NADH, binding of CO to Hb also would impair oxygen delivery to tissues. In addition, CO (like NO) activates guanylate cyclase (129,133), which could contribute to vasodilatation and increased blood flow.

Fifth, hyperglycemic pseudohypoxia also may provide an explanation for reports that neuropathy is more common and more severe in diabetic subjects who consume excessive amounts of ethanol (136) (assuming the presence of alcohol dehydrogenase in neural tissue). Oxidation of ethanol to acetaldehyde by alcohol dehydrogenase, like oxidation of sorbitol to fructose, is coupled to reduction of NAD⁺ to NADH. Thus, the impact of ethanol ingestion on cytosolic NADH/NAD⁺ is equivalent to higher glucose levels and an increased flux of glucose via the sorbitol pathway.

Sixth, numerous reports attest to increased O₂ production by glycated proteins and by autodissociative glycoxidation reactions (29,137–139). Glycated proteins induce vascular dysfunction (during euglycemia) like that caused by elevated glucose levels, which also is prevented by free-radical scavengers such as probucol and superoxide dismutase (104a; R.G.T., K.C., J.R.W., unpublished observations). These observations suggest that elevated glucose levels initiate production of free radicals by two independent mechanisms: 1) hyperglycemic pseudohypoxia, and 2) nonenzymatic glycation and autooxidative glycoxidation. Therefore, increased production of oxygen free radicals appears to be a strong candidate mechanism for effecting nonenzymatic as well as enzymatically mediated glucose effects on vascular and neural dysfunction and the late complications of diabetes (Figs. 4 and 5).

CONCLUSIONS
A considerable body of evidence 1) indicates that in many tissues hyperglycemia-induced metabolic imbalances increase the cytosolic ratio of free NADH/NAD⁺ (despite normal tissue pO₂) that results in pseudohypoxia; 2) attests to the similarity of vascular, neural, and redox changes induced by true hypoxia and hyperglycemia-induced pseudohypoxia; and 3) supports the importance of oxidative stress and a branching cascade of metabolic imbalances linked to pseudohypoxia in mediating vascular and neural dysfunction induced by diabetes. Imbalances in lipid metabolism, increased O₂ production, and perhaps increased NO formation appear to play important roles in mediating these functional disorders. Tentative scenarios for translation of these early metabolic imbalances and vascular dysfunction into irreversible progressive vascular sclerosis and organ failure are suggested in Figs. 2–5.

Increased production of O₂ and NO appears to play an important role in mediating early vascular and neural dysfunction linked to true hypoxia, hyperglycemic pseudohypoxia, and nonenzymatic glycation. Clearly, much more work is needed to delineate the mechanisms by which elevated glucose levels cause pseudohypoxia and the role of hyperglycemia-induced pseudohypoxia (and associated oxidative stress and metabolic imbalances) in mediating complications of diabetes.

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APPENDIX
First, because of the putative importance of early vascular dysfunction (and of vascular responses to hypoxia and pseudohypoxia) in the pathogenesis of diabetic vascular complications, it is appropriate at the outset to acknowledge and address discordant reports of decreased retinal and endoneurial blood flow in acutely diabetic/hyperglycemic rats (1–3). Such reports are derived largely from indirect assessments of blood flow (transit time of fluorescein-labeled albumin for retina [3], hydrogen clearance or laser Doppler methods for endoneuron [1,2]) and/or invasive procedures (hydrogen clearance and laser Doppler methods for endoneuron). The problem with indirect measures of blood flow is that they can be influenced by many variables independent of blood flow, i.e., changes in tissue blood volume and/or in vascular permeability. Hydrogen clearance values based on oxidation of hydrogen by a platinum electrode also
can be affected by tissue edema and by a number of metabolites, including ascorbic acid and oxygen-reactive species (4); thus, redox changes and increased production of oxygen-reactive species associated with the diabetic milieu may affect blood flow values based on hydrogen clearance. The problem with invasive procedures is that vasoreactivity in many tissues is altered by diabetes (5,6); thus, neural trauma and cooling inherent to surgical exposure required for hydrogen clearance and laser Doppler methods (and insertion of electrodes into endoneurium for the hydrogen clearance method) may cause a relative or absolute decrease in neural blood flow in diabetic versus control subjects. For these reasons, indirect assessments of blood flow obtained by invasive procedures lack credibility if they are discordant with more direct measures of blood flow (i.e., appropriately sized microspheres) obtained by noninvasive techniques.

Blood flow measurements based on radiolabeled microsphere injection methods are not subject to any of the above limitations. The major considerations in the use of microspheres are that the microspheres must be large enough to be trapped efficiently by the tissue vasculature examined, and enough microspheres must be captured to ensure reliable sampling and counting (this can be a problem in small tissues like retina and sciatic nerve of rats) (7). These potential confounding problems have been excluded in the experimental models we have used by the demonstration that molecular microspheres (i.e., $^{3}$H-desmethylimipramine, 266,000 M) yield blood flow values identical to those obtained with conventional 10- to 15-$\mu$m microspheres in retina and endoneurium of diabetic rats (8). These findings, coupled with elevated microvascular hematocrits (a rheological parameter of increased blood flow) in retina and sciatic nerve tissue of diabetic rats (9), strongly support the conclusion that blood flow in these tissues is increased by acute hyperglycemia and by diabetes of short duration.

Second, true hypoxia is defined as decreased tissue $pO_{2}$ levels (in the absence of restricted blood flow) that result from a decrease in $O_{2}$ content of blood (hypoxemia) delivered to tissue. Ischemia is defined as decreased tissue $pO_{2}$ levels that result from decreased volumetric flow of blood with a normal $pO_{2}$ content.

Third, despite these provocative similarities in neural, vascular, and myocyte contractile dysfunction induced by diabetes and hypoxia, it is clear that chronic hypoxic conditions (i.e., chronic lung disease, cyanotic heart disease, etc.) are not associated with diabetic complications and vice versa (although retinal capillary microaneurysms and diabetic vascular changes in retina, kidney, and muscle are not unique to diabetes). Clearly, the combined effects of chronically elevated glucose levels and pseudohypoxia are essential for development of the late complications of diabetes. In addition, other risk factors such as hypertension appear to play a critical role in the pathogenesis of late complications. Indeed, we have proposed that the major impact of the diabetic milieu on the vasculature and nerves is to induce metabolic imbalances and dysfunction, which make them more susceptible to injury by risk factors independent of the diabetic milieu (such as hypertension, hypercholesterolemia, cigarette smoking, ethanol abuse, etc.) as discussed later.

Fourth, in hearts from diabetic animals, basal myocardial contractile function is impaired (49,50), and global ischemia-induced contractile dysfunction develops more rapidly than in control hearts (49). Paradoxically, diabetes improves recovery of myocyte and vascular smooth muscle contractile function during reperfusion after ischemia (50,51). Whereas endothelial barrier functional integrity in diabetic hearts does not differ from control hearts before ischemia, albumin leakage in diabetic hearts during reflow exceeds that in control hearts by two- to threefold (50). These paradoxical effects of diabetes may be explained by differential responses of endothelium versus vascular smooth muscle and cardiac myocytes to the combination of preexisting pseudohypoxia and impaired $Ca^{++}$ transport function in the sarcolemma and endoplasmic reticulum (31,32,51,52). Impaired $Ca^{++}$ transport function could limit accumulation of $Ca^{++}$, which is believed to play an important role in mediating hypoxic injury (31,32,51). It is of interest that a similar paradoxical effect of ischemia is observed in peripheral nerve in which resistance to ischemic conduction failure is increased by diabetes (53).

Fifth, even the combined measurements of tissue fructose and sorbitol will tend to underestimate sorbitol pathway activity because fructose can be further metabolized and also readily diffuses out of cells to be carried away by the blood. About 66–75% of the fructose produced by incubated tissues is recovered in the medium.

Sixth, vasodilation and increased blood flow may be viewed as normal vascular responses to hypoxia and pseudohypoxia as well as to various vasoactive substances. The point at which these normal vascular responses should be considered vascular dysfunction is unclear. As long as the vessels are dilated and blood flow is increased in response to sustained pseudohypoxia, they may be considered to be reacting normally. On the other hand, vessels that are dilated in response to hyperglycemic pseudohypoxia may dilate and constrict less in response to vasodilating and vasoconstricting agents than vessels in a euglycemic milieu. Although the dampened responses of such vessels to vasoactive agents is generally viewed as evidence of vascular dysfunction, it may just as well be considered a normal response to the combined effects of pseudohypoxia and the particular vasoactive agent examined. In any case, as discussed later, chronic vasodilation and increased blood flow imply microvascular hypertension, which is an important risk factor for diabetic retinopathy and nephropathy.

Seventh, glycoxidation products are formed by reactions between sugar-derived autoxidation products (such as glucosone) and proteins, lipids, etc. Oxidative stress is defined as an increase in steady-state levels of oxygen-reactive species (including $O_{2}$, hydrogen peroxide, and hydroxyl radical) that result either from increased production of precursors of reactive oxygen species or decreased free-radical scavenger activity (29).


