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Cytosolic NADH/NAD$^+$, free radicals, and vascular dysfunction in early diabetes mellitus

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To the extent that early vascular dysfunction and end-stage diabetic vascular disease are mediated by common pathogenetic factors (although early increases in blood flow may not cause end-stage vascular disease), elucidation of metabolic imbalances that mediate the earliest manifestations of vascular dysfunction may provide insights into the pathogenesis of vascular complications of diabetes. The earliest detectable evidence of vascular dysfunction induced by diabetes is increased blood flow in retina, kidney, and peripheral nerve. In non-diabetic humans and animals increased blood flow in these tissues is demonstrable after only a few hours of acute hyperglycaemia induced by intravenous glucose infusion [1]; loss of endothelial barrier function develops later. The earliest metabolic imbalance linked to increased blood flow in these tissues in animal models of diabetes is cytosolic reductive stress, i.e. an increased ratio of cytosolic free NADH/NAD$^+$. This 'hypoxia-like' redox change is caused by increased oxidation of substrates coupled to reduction of the cofactor NAD$^+$ to NADH (Fig. 1). Candidate substrates include sorbitol, non-esterified fatty acids, and glucuronic acid pathway metabolites (UDP glucose, L-gulonate, and xylitol) [1].

In animal models of early diabetes the most important metabolic pathway contributing to cytosolic reductive stress appears to be increased flux of glucose via the sorbitol pathway [1–4]. In the first step of this pathway glucose is reduced to sorbitol by aldose reductase. In the second step of the pathway sorbitol is oxidized to fructose coupled to reduction of NAD$^+$ to NADH by sorbitol dehydrogenase. In humans, increased oxidation of non-esterified fatty acids (in peroxisomes in addition to mitochondria) and/or glucuronic acid pathway metabolites may be equally or more important than oxidation of sorbitol. In contrast to cytosolic reductive stress induced by increased oxidation of substrates coupled to reduction of NAD$^+$ to NADH, reductive stress induced by hypoxia is largely the consequence of impaired oxidation of NADH to NAD$^+$ by the mitochondrial electron transport chain. Thus, reductive stress arising in the mitochondria due to hypoxia and that in the cytosol due to increased substrate oxidation are mediated by independent mechanisms and are additive. Two to four weeks after the onset of diabetes, when sciatic nerve blood flow is increased, metabolite couples that reflect mitochondrial NADH/NAD$^+$ do not differ from normal rats. This finding indicates that cytosolic reductive stress in these nerves (manifested by

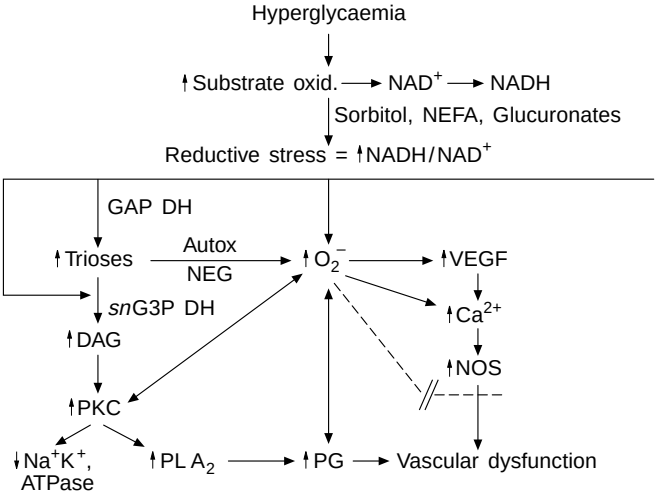

Fig. 1. See text.

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Abbreviations: GAPDH, Glyceraldehyde 3-phosphate dehydrogenase; G3PDH, glycerol 3-phosphate dehydrogenase; DHAP, dihydroxyacetone phosphate; VEGF, vascular endothelial growth factor.

increased lactate/pyruvate ratios which are in equilibrium with NADH/NAD$^+$ via lactate dehydrogenase) is not attributable to hypoxia; it is, instead, the consequence of metabolic imbalances arising in the cytosol.

The importance of the NADH/NAD$^+$ redox couple in mitochondrial and cytosolic energy metabolism, and evidence that cytosolic free NADH/NAD$^+$ is modulated by mitochondrial NADH/NAD$^+$ and by intra- and extracellular lactate and pyruvate (via lactate dehydrogenase and plasma membrane transporters of lactate and pyruvate), indicate that cytosolic free NADH/NAD$^+$ is a sensor of the (NADH/NAD$^+$) redox state of mitochondria, cytosol, and the extracellular milieu. It is unlikely to be a coincidence, therefore, that cytosolic NADH/NAD$^+$ also appears to play an important role in regulating tissue blood flow in response to changes in oxygen tension, energy metabolism, and the extracellular lactate/pyruvate ratio. Reductive stress, regardless of the cause, is associated with increased blood flow. In addition to increased oxidation of glucose-derived metabolites in diabetes, increased oxidation of ethanol, electrochemical or mechanical work, hyperlactataemia, experimental galactosaemia, cyanide and carbon monoxide poisoning, and fasting all cause reductive stress and are associated with increased blood flow in the affected tissue(s).

The importance of cytosolic reductive stress is that it impacts on the activity of many dehydrogenase enzymes, several of which have been implicated in the pathogenesis of diabetic complications, that require NAD$^+$ or NADH as cofactors and are regulated by NADH/NAD$^+$. Several lines of evidence suggest that intracellular production of oxygen reactive species, glycation reactions, and activation of protein kinase C are mediated in large part by a cascade of events initiated by the effects of cytosolic reductive stress on glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and glycerol 3-phosphate dehydrogenase (G3PDH) [1, 4].

Because of the equilibrium between NADH/NAD$^+$ and glyceraldehyde 3-phosphate/1,3-bisphosphoglycerate established by GAPDH, an increased ratio of NADH/NAD$^+$ favours increased levels of glyceraldehyde 3-phosphate which is in equilibrium with other trioses (dihydroxyacetone phosphate [DHAP], and fructose 1,6-bisphosphate, referred to collectively as 'triose phosphates'). Triose phosphates are highly reactive sugars which undergo autoxidation (with production of free radicals including superoxide) resulting in non-enzymatic glycation and oxidative damage to intracellular proteins, DNA, and membrane lipids (Fig. 1).

As a result of the corresponding equilibrium between NADH/NAD$^+$ and glycerol 3-phosphate/DHAP established by G3PDH, an increased ratio of NADH/NAD$^+$ favours reduction of DHAP to glycerol 3-phosphate, the first step in one pathway for de novo synthesis of diacylglycerol (which in turn activates protein kinase C). Activation of protein kinase C has been implicated in mediating vascular dysfunction in the retina, aorta, and kidney, since inhibitors of protein kinase C and/or of its sequelae prevent vascular dysfunction in all three tissues in diabetic rats [1, 4].

Reductive stress, whether induced by elevated glucose levels or by hypoxia, can promote free radical production by several mechanisms including: 1)inhibition of xanthine dehydrogenase which will shift oxidation of xanthine and hypoxanthine to xanthine oxidase which yields superoxide; 2) autoxidation of NADH; 3) glycation of CuZn-superoxide dismutase by triose phosphates which inactivates the enzyme [1].

Several lines of evidence support a physiological role for superoxide in modulating numerous metabolic pathways, gene transcription, and biological functions including increased blood flow in response to reductive stress [1]. We have hypothesized that the metabolic need for increased blood flow is sensed by reductive stress (regardless of the cause) which initiates a cascade of events that increase blood flow [1, 4] as depicted in Figure 1. Reductive stress increases superoxide levels which increase intracellular calcium and vascular endothelial growth factor (VEGF). Increased intracellular calcium activates constitutive nitric oxide synthase to produce small amounts of nitric oxide which increases blood flow. This scenario is supported by evidence that: 1) VEGF expression is increased in cultured cells exposed to elevated glucose levels; 2) increased blood flow in granulation tissue induced by elevated glucose levels is prevented by polyclonal and monoclonal antibodies to VEGF. The role of each of the participants in this cascade is supported by evidence that increased blood flow induced by elevated glucose levels is also prevented by inhibitors of aldose reductase, sorbitol dehydrogenase, nitric oxide synthase, prostaglandin synthase, and superoxide dismutase [1–4]. Increased blood flow in soleus muscle of rats induced by acute hyperglycaemia is also prevented by superoxide dismutase. With increasing duration of diabetes, increased production of superoxide by various mechanisms may scavenge nitric oxide to the point that blood flow returns to normal or may even be decreased.

In conclusion, cytosolic reductive stress produced by increased oxidation of glucose metabolites (via selected metabolic pathways) coupled to reduction of NAD$^+$ to NADH appears to play an important role in mediating early vascular dysfunction induced by diabetes. With longer duration of diabetes, sustained reductive stress and associated increased production of free radicals and growth factors will lead to vascular sclerosis (and angiogenesis in selected tissues such as the retina) resulting in obliterative end-stage vascular disease.

References

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