The Contrary Impact Of Diabetes And Exercise On Endothelial Nitric Oxide Synthase Function

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Article

Introduction
Cardiovascular disease is the leading cause of mortality in noninsulin dependent diabetes mellitus patients (NIDDM). Although originally thought to be a metabolic problem, widespread systemic complications are now recognized. The cardiovascular complications include intermittent claudication, atherosclerosis, hypertension, retinopathy, nephropathy, and congestive heart failure. The vascular problems of NIDDM individuals are largely traceable to alterations in endothelial function. Diabetes results in significant impairment of endothelium-dependent vasodilation in response to acetylcholine or increases in flow. This impairment is likely the result of decreased effectiveness of nitric oxide (NO) mediated functions within the vasculature. There appears to be an interrelationship between NO metabolism and diabetes. Transgenic mice in which the eNOS enzyme has been knocked out exhibit insulin resistance and metabolic syndrome [1].

Exercise has been very useful in the management of diabetes. Although most human studies have focused more on skeletal muscle metabolism, exercise has also been shown to improve vascular as well as cardiac function in NIDDM patients. Whereas some of the physiological consequences of the diabetes on the vasculature are understood, less is known about the molecular mechanisms responsible. And even less is understood about the effects of exercise on the diabetic microvasculature. A clearer understanding of the molecular mechanisms that are influenced by diabetes and exercise will ultimately serve to improve health care management of diabetic individuals.

NO and Endothelial Function
In addition to regulating vascular tone, NO inhibits components of the atherogenic process including platelet aggregation, monocyte adhesion, and vascular smooth muscle migration [2-4]. Additionally, NO influences myocardial energy consumption [5-7]. Different mechanisms of endothelial dysfunction related to altered NO release have been proposed and include changes in the rate of NO breakdown, decreased synthesis of nitric oxide synthase (NOS), altered NOS sensitivity, and down regulation of the signal transduction pathways that either activate NOS or maintain its synthesis [8-11]. All of these proposals involve significant changes in the endothelial phenotype and central to most of them are changes in NOS function as the rate-limiting step in NO production [12-14].

NO synthesis is synthesized by the nitric oxide synthase enzyme. Three isoforms of the NOS enzyme are known; that are the products of three separate genes and include endothelial NOS (eNOS), inducible NOS (iNOS), and neuronal NOS (nNOS). These isoforms are similar and have a sequence identity of about 50-60% at the protein level. All use L-arginine, O2, and NADPH to catalyze the synthesis of NAP, citrulline, and NO as well as superoxide. Biochemical structural studies have identified different regions that separate the oxygenase and reductase domains within the NOS molecule [15]. eNOS function is subject to several layers of regulation. The eNOS enzyme can function either as a dimer or as a monomer in the membrane. Dimerization is a requirement for NO synthesis but full catalytic activity requires the formation of a complex that incorporates calmodulin, FAD, tetrahydrobiopterin (BH4), and iron protoporphyrin IX (haem) [15]. Only eNOS undergoes palmitoylation and myristoylation and it is this latter modification which is required for insertion into the caveolae of endothelial cells [16, 17]. Localization of eNOS to the caveolae is important for rapid modulation of eNOS activity by different stimuli including shear stress. Regulation by phosphorylation is also significant in that eNOS has multiple phosphorylation sites. Phosphorylation of the serines 617, 635 and 1179 will activate eNOS, while phosphorylation of the Thr497 site appears to serve as an intrinsic switch to enhance coupling of eNOS in favor of NO production at the expense of superoxide [18]. All of these factors point towards a complex regulation of eNOS allowing it to respond to varying environments.

The composition of the eNOS complex is critical for the formation of NO as opposed to superoxide formation. Whereas the dimer complex produces NO, the monomer preferentially synthesizes superoxide [19]. BH4 preserves the eNOS dimer conformation and supports eNOS dimer conformation and supports eNOS activity [20]. The mechanisms responsible for uncoupling remain unclear, however, increases in glucose or peroxynitrite have been shown.
to reduce the dimer:monomer ratio [19]. More recently, Chen reported that peroxynitrite promotes the oxidation of tetrahydrobiopterin leading to the destabilization of the eNOS dimer [21]. Increased oxidative stress is evident within the diabetic vasculature. Diabetes is thought to have a significant impact on dimerization and a decrease in the dimer:monomer eNOS ratio have been reported in tissues from diabetic animals [19]. Supplementation of tetrahydrobiopterin (BH4) using sepiapterin can restore NO mediated flow-dependent dilation to type I diabetic rat arterioles [22]. Acute infusion of BH4 restored acetylcholine induced vasodilation in type II diabetic subjects [23]. These findings are important because the shift could contribute to increased cellular oxidative stress, which could exacerbate the decline in NO bioavailability.

eNOS protein levels are altered in response to different stimuli. Decreases were found in different forms of pathological overload including hypertension or heart failure, whereas chronic exercise increased vasculature eNOS protein levels [24, 25]. Aging is associated with decreased eNOS protein in the endothelium of coronary microvessels [26]. Control of eNOS expression lies at the transcriptional level. Both shear stress and VEGF will induce eNOS expression [27-34]. Gender differences in arteriole function have been linked to estrogen, and are also thought to act at the level of transcription [35-37]. The 5’ flanking region of the eNOS gene contains several transcription factor binding sites, including SP1, AP1, and cAMP response elements [28, 38]. Of these a VEGF response element that is highly similar to a consensus AP1 binding site appears responsible for the initial increases in eNOS expression [39]. This element is separate and distinct from a shear stress response element within the eNOS gene. Shear stress has also been reported to increase eNOS-mRNA stability a separate mechanism for the up regulation of eNOS expression [40, 41]. The impact of diabetes on eNOS expression is unclear, but several reports indicate that elevated glucose will increase eNOS-mRNA [42-44]. Insulin has also been shown to increase eNOS expression in the vascular stroma of lean Zucker rats but not insulin-resistant Zucker fatty rats [45]. This suggests that insulin resistance negatively impacts on the vasculature. Because NO inhibits smooth muscle growth, it is also possible that the loss of insulin sensitivity may result in a proatherosclerotic state. Both increases and decreases in eNOS expression have been reported from different models of diabetes including streptozotocin-diabetes [46, 47], alloxan-diabetes [42], in fatty Zucker rats [45] and in GK rats [48]. However, these findings were from analysis of tissues and the larger conduit arteries, and less is known about the microvasculature, which is responsible for regulation of blood pressure and the distribution of blood flow. A decrease in eNOS would link lower NO bioavailability to gene expression levels. An increase or no changes in eNOS would support the idea that diabetes may result in an inactivation of eNOS. Clarification of these issues will be important for understanding the molecular mechanisms responsible for vasculature dysfunction.

Hyperglycemia has been shown to act as a NO scavenger [49]. Although the mechanism is not entirely clear, an acute change in eNOS conformation may be involved. In normal individuals, a single oral glucose challenge can reduce NO-specific vasodilatation, an action that was blocked by supplementation with tetrahydrobiopterin (BH4) [50]. It is known that shifts in BH4 concentration will directly influence the rate of NO or superoxide anion O2⁻ production [51-55]. Using an inhibitor of tetrahydrobiopterin (2,4-diamino-6-hydroxypyrimidine), Yamashio demonstrated a reduction in endothelium-dependent vasodilatation [56]. Menninger et al. isolated endothelial cells from diabetes-prone and non-diabetes-prone rats, a model of IDDM [57]. They determined that tetrahydrobiopterin concentration, GTP-cyclohydrolase-1 enzyme activity, and GTP-cyclohydrolase-1 protein levels were all decreased in the endothelial cells of diabetic rats compared to age matched control rats. Similarly in the Goto-Kakizaki rat (a nonobese model of NIDDM), Biter et al demonstrated that tetrahydrobiopterin was significantly decreased while oxidized bioppterin was increased [12]. In conjunction with this oxidant stress in the form of superoxide was significantly increased in the Goto-Kakizaki vasculature [12, 58]. BH4 preserves the eNOS dimer conformation which supports NO-dependent eNOS activity, and a decline in BH4 results in an increase in superoxide formation [20]. Supplementation with sepiapterin, a precursor of tetrahydrobiopterin, restored NO-dependent endothelial function to skeletal muscle microvessels from type 1 diabetic rats [22]. These findings suggest that diabetic-induced changes in eNOS function rather than a direct change in eNOS protein content are more important in the decline in NO bioavailability in the diabetic vasculature.

Exercise and NIDDM

There is considerable evidence to demonstrate the benefits of exercise in the management of diabetes including improved glycemic control, an increase in quality of life and a reduction of cardiovascular risk factors. Exercise with and without dietary changes resulted in a significant reduction in
glycosylated hemoglobin (HbA1c), increased insulin sensitivity, improved blood lipid levels, and lowered blood pressure [59-63]. Even low intensity forms of exercise such as walking will benefit NIDDM patients [59]. This is good news since diabetic-related complications such as obesity, peripheral neuropathies, and ischemic heart disease represent real limitations on the exercise capacity of many NIDDM patients.

Vascular responsiveness in diabetics is compromised at the onset of exercise and during submaximal exercise compared to healthy individuals suggestive of microvascular pathology [64-67]. Not all exercise programs are equally efficacious in that adaptations have been observed in response to endurance training protocols but not after strength or speed training programs [68, 69]. The influence of chronic exercise extends across several levels including induction of angiogenesis and shifts in vasculature sensitivity [70-73]. The endothelial cells are an important target for these adaptations. Increased NO production is an early adaptation and has been observed as soon as one week after the start of the training program [71, 74]. There are several potential mechanisms that may be responsible for this increase in NO release. Exercise increases the sensitivity to endothelium-dependent relaxation by acetylcholine, but not the endothelium-independent response to sodium nitroprusside indicating that the impact lies more at the endothelium rather than smooth muscle [13, 75]. At the onset of exercise, blood flow to the working muscles increases dramatically and in proportion to the intensity of exercise. Blood flow to other organ systems such as the kidney, G-I tract, and liver is compromised by exercise and all decrease as exercise intensity and sympathetic outflow increases. Earlier studies observed that training increased eNOS protein [24, 25, 76]. Training effects appear limited to the vasculature of the working muscles since no effect was observed in the mesenteric arterioles (a nonworking vascular bed) [13, 77-79]. From this it has been suggested that flow-induced increases shear stress may be responsible for inducing increases in eNOS expression [27-30]. There is ample evidence from both in vivo and in vitro studies to support this concept [13, 77, 79, 80]. However, studies of diabetic animals have yielded somewhat different results. In OLETF rats (an obese model of NIDDM) exercise improved endothelium dependent vasodilatation was observed in the mesenteric arteries [81]. Similarly, in a human study, exercise and diet modification improved flow-mediated dilation (FMD) of the brachial artery during an exercise protocol recruiting the lower limbs [82]. These findings are very important as they suggest that mechanisms other than localized stimuli (such as increased shear stress or localized VEGF release) are important for diabetic individuals.

eNOS function is subject to complex regulation. Exercise-induced increases in vascular eNOS protein has been shown by several investigations [24, 25, 76, 83]. Chronic exercise increases the sensitivity to insulin-stimulated phosphorylation of eNOS. [84]. Zhang et al. [84] reported that the shift in eNOS phosphorylation (ser1179) status appears mediated through the Akt signaling pathway and significantly enhances myocardial contractility. However, shifts in phosphorylation status do not fully explain the findings improved myocardial contractility. To date no one has reported exercise-induced alterations in eNOS-Thr497 phosphorylation status. Further, Fulton et al demonstrated that phosphorylation of ser1179 was not required for correct intracellular localization [85]. Myristoylation is required for initial targeting of eNOS to the plasma membrane, while palmitoylation is thought to serve to stabilize eNOS in the membrane [86-88]. To date no one has examined the issue of exercise-induced changes in myristoylation or palmitoylation of eNOS.

Chronic exercise improves eNOS dimerization in the diabetic heart [83]. Increased dimerization of the eNOS protein increases coupling of the enzyme and shifts its enzymatic activity towards improved NO generation, at the expense of superoxide production. Any reduction in oxidant stress should serve to promote eNOS dimerization. Plasma levels of angiotensin II (Ang II) are elevated in diabetics with poor glucose control and in untreated diabetic animal models [89-93]. Chronically elevated Ang II levels are associated with increased expression of NADPH oxidase components and oxidative stress [94]. Conversely, normalization of Ang II levels can be achieved with improved glucose handling, ACE inhibition, as well as by exercise [89, 90, 94, 95]. Exercise training has long been known to improve glycemic control in diabetics and it may serve a multi-faceted role to lower angiotensin II impact and more locally to decrease hyperglycemic scavenging of NO [96, 97]. Exercise has also been shown to decrease NADPH oxidase activity [83]. Evidence that oxidant stress reduces tetrahydrobiopterin is significant in that preservation to tetrahydrobiopterin may restore its function. In aged skeletal muscle but not young muscle, chronic exercise elevates tetrahydrobiopterin levels [98]. In conjunction with this supplementation improved vasodilatation in aged sedentary individuals but not those that were exercise trained suggesting that training restores tetrahydrobiopterin levels [99]. These several factors;
improved glycemic control, reduction of angiotensin II activity, reduction of oxidant stress will all promote increased eNOS dimerization leading to better coupling of the eNOS enzyme [83]. Although increases in eNOS protein have been reported by several investigations, our own results observed a paradox in that exercise training of diabetic animals lead to an increase in eNOS protein, but a decrease in eNOS-mRNA. Others have reported that in comparison to controls within the diabetic heart eNOS protein was decreased while eNOS-mRNA was elevated in the vasculature or the heart[12, 42, 83]. And similar results have also been observed in cultured endothelial cells chronically exposed to elevated media glucose [44]. In cultured endothelial cells, exogenous H2O2 increased eNOS transcription and eNOS-mRNA stability [100]. While diabetic-induced oxidative stress depresses eNOS function it also serves to activate compensatory mechanisms. Exercise training reverses this. Both eNOS-driven and NADPH oxidase derived ROS are lowered by exercise training and this should serve to diminish eNOS transcription [83]. Exercise-induced improvements in glycemic control are well known and should have the effect to improve NO bioavailability [96, 97]. In conjunction with this, increased NO bioavailability is also a negative feedback mechanism to decrease eNOS transcription [101, 102]. As a result, the decreases in eNOS-mRNA following exercise training were possibly the result of the reduced drive for eNOS transcription.

Summary

Although diabetes was originally thought to be a metabolic problem, widespread systemic complications related to vascular dysfunction are now recognized. Exercise has been highly useful in the management of diabetes. Both human and animal studies have found significant evidence to indicate that exercise improves cardiovascular function in the diabetic. Central to these improvements is increased NO bioavailability. Nitric oxide synthase is the rate limiting step in NO bioavailability, and it is eNOS function that has the largest impact on cardiovascular function. Diabetes disrupts the complex regulation of eNOS function on several levels. Although it remains unclear if enzyme localization is influenced by chronic exercise; changes in protein content, phosphorylation status, and enzyme conformation are sensitive to exercise training. Several mechanisms are likely to contribute to exercise-induced improvements in eNOS function in diabetics and many orginate from exercise-induced improvements in glycemic control. This one change brings about a host of intracellular changes centered on decreasing oxidant stress that leads to improved eNOS function.

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