

# **NADPH Oxidase May be the Primary Mediator of Organic Erectile Dysfunction**

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## **Abstract**

Increased production of nitric oxide by cavernosal endothelial cells, provoked by shear stress and cholinergic neurons, is necessary for sustained penile tumescence during the erectile process. Non-neurogenic organic erectile dysfunction (ED) is often associated with vascular risk factors that induce endothelial dysfunction, and amelioration of these risk factors often has a favorable impact on ED. These considerations suggest that cavernosal endothelial dysfunction may be at the root of many cases of ED – a view supported by rodent models of this syndrome. These models also reveal that increased oxidant stress is a key mediator of this dysfunction, and the likely primary source of this stress is endothelial NADPH oxidase, typically activated in the vascular disorders that have been linked to ED. Oxidants derived from this complex act in a variety of ways to impair the production and bioactivity of endothelial NO. Thus, drugs or phytonutrients which inhibit the activation or activity of NADPH oxidase may have great potential for preventing or treating ED.

## **Erectile Dysfunction as an Endotheliopathy**

There is increasing reason to suspect that, in the majority of instances, organic erectile dysfunction (ED) is an endotheliopathy characterized by a failure of shear-mediated endothelium-dependent vasodilation; the dysfunctional endothelial cells responsible for erectile failure are those which line the small resistance helicine arteries that feed the corpus cavernosum, as well as those those within the corpus cavernosum that regulate cavernosal smooth muscle contraction.<sup>1</sup> These endothelial cells are exposed to shear stress when upstream arteries are dilated by stimulation of post-ganglionic cavernous neurons that are “non-adrenergic, non-cholinergic” (NANC); these neurons employ nNOS-derived nitric oxide (NO), as well as HO-2-derived carbon monoxide,<sup>2</sup> as neurotransmitters. This shear stress provokes endothelial release of NO, via a signaling pathway entailing sequential activation of PI3K, Akt, and eNOS;<sup>3-5</sup> inhibitors of this signaling pathway have an adverse impact on the erectile capacity of mice.<sup>5</sup> Acetylcholine released by cavernous cholinergic neurons during the erectile process can also induce NO release by cavernosal endothelium.<sup>6</sup> This NO is believed to be the chief mediator of the cavernosal smooth muscle relaxation required for sustained penile tumescence – albeit prostacyclin and endothelium-derived hyperpolarizing factor (EDHF) are other shear-induced endothelial mediators that can contribute to this dilation.<sup>1;7-9</sup> The NO/CO released by NANC neurons is now thought to act briefly as a trigger for erection – whereas the sustained shear stress-mediated activation of eNOS in cavernosal endothelium is required for durable tumescence.<sup>5;10</sup> The key role of NO in erectile

physiology is underlined by the fact that the phosphodiesterase 5-inhibitory drugs currently used to treat ED function to potentiate the impact of NO on cGMP levels.<sup>11</sup> Adenoviral transfer of the eNOS gene to the cavernosum of aged or diabetic rats markedly improves their erectile function.<sup>12;13</sup>

Evidently, traumatic or metabolic damage to the NANC neurons – or upstream neural pathways which regulate their activity – is the key mechanism underlying some cases of ED. For example, diabetic neuropathy contributes to the ED experienced by many diabetics.<sup>14</sup> In aging mice, a thinning of the NANC innervation of the penis has been observed.<sup>15</sup> However, the majority of middle-aged or elderly subjects who experience ED have not experienced significant neural trauma, nor do they suffer from metabolic neuropathy. Such patients are however highly prone to risk factors typically associated with endothelial dysfunction and increased coronary risk – such as hypercholesterolemia, hypertension, metabolic syndrome, obesity, diabetes, hyperhomocysteinemia, smoking, elevated C-reactive protein, and aging.<sup>16-29</sup> It is notable that, in rodents rendered hyperlipidemic, hypertensive, hyperhomocysteinemic, obese, or diabetic, erectile function is characteristically impaired.<sup>30-42</sup> Moreover, in many human subjects with organic ED, effective treatment of associated cardiovascular risk factors is often accompanied by an improvement in erectile function.<sup>21;25;26;43</sup> In these cases, it is reasonable to suspect that cavernosal endotheliopathy is the primary cause of ED<sup>1;6</sup> – albeit stenotic lesions of upstream arteries (a longer term consequence of arterial endotheliopathy) also plays a role in many cases of ED associated with vascular risk factors.<sup>44;45</sup> In several recent studies, endothelium-dependent flow-mediated vasodilation of the brachial artery was only about half as great in subjects with ED as in control subjects who did not differ greatly in risk factor profile.<sup>46-48</sup> This suggests that ED can be the first symptomatic manifestation of a systemic endotheliopathy that may or may not be associated with increases in established vascular risk factors. In patients who are otherwise asymptomatic, ED is now viewed as an ominous “early warning” sign that significant atherogenic vascular disease may be in the offing.<sup>23;24;28</sup>

## **Role of Oxidative Stress and NADPH Oxidase in Erectile Dysfunction**

All of the vascular risk factors cited above are characterized by increased endothelial oxidant stress, the chief source of which appears to be NADPH oxidase.<sup>49-67</sup> (In diabetes, increased mitochondrial superoxide generation contributes in this regard.<sup>68;69</sup>) The effects of diabetes on erectile function in rats are mediated in part by advanced glycation end-products,<sup>70-72</sup> which likewise stimulate endothelial NADPH oxidase activity.<sup>73;74</sup> Oxidant stress impairs endothelium-dependent vasodilation through a diverse array of complementary mechanisms. Superoxide directly quenches NO, generating the potent oxidant peroxynitrite in the process. One the targets of peroxynitrite is tetrahydrobiopterin, a crucial cofactor for eNOS;<sup>75</sup> oxidative loss of tetrahydrobiopterin not only impairs the ability of eNOS to generate NO, but also converts this enzyme into a secondary source of superoxide.<sup>76</sup> Oxidant stress also inhibits dimethylarginine dimethylaminohydrolase (DDAH), while boosting the expression of protein arginine methyltransferase I<sup>77;78</sup> – thereby increasing levels of asymmetric dimethylarginine (ADMA), a competitive inhibitor of eNOS.<sup>79</sup> (Not surprisingly, elevated plasma levels of

ADMA are common in patients with ED.<sup>80-82</sup>) And peroxynitrite inhibits prostacyclin synthetase,<sup>83-85</sup> thereby suppressing prostacyclin-mediated smooth muscle dilation. Clearly, excessive endothelial oxidative stress, stemming from activation of NADPH oxidase, can have a very negative impact on the endothelial production of NO and prostacyclin required for effective erectile function. Whether it can influence production of EDHF is less clear.<sup>86:87</sup>

The possibility that endothelial oxidative stress is a key mediator of ED finds support in rodent models of ED. Thus, intracavernosal injection of an adenoviral vector carrying the gene for extracellular superoxide dismutase, was found to normalize erectile function in streptozotocin-diabetic rats; in untreated diabetic rats, confocal microscopy demonstrated increased superoxide in cavernosal endothelial and smooth muscle cells.<sup>42</sup> Analogous studies demonstrated that superoxide excess was at the root of senile ED in rats.<sup>88</sup> Similarly, in rats rendered hyperhomocysteinemic with a high-methionine diet, the impairment of endothelium-dependent dilation of cavernosal smooth muscle, assessed *ex vivo*, was associated with a marked increase in superoxide production, and was corrected by adding superoxide dismutase to the incubation.<sup>37</sup> Cavernosal smooth muscle was normally responsive to exogenous NO in this study, demonstrating that reduced NO production, rather than an impairment of smooth muscle capacity to respond to NO, was responsible for the dysfunction. In hypercholesterolemic rabbits, cavernosal tissue studied *ex vivo* produced increased superoxide and was resistant to carbachol-stimulated relaxation; these abnormalities were reversed if the tissue was pre-incubated with two inhibitors of NADPH oxidase, diphenylene iodonium and apocynin.<sup>32</sup> Thus, the ED associated with hypercholesterolemia in rabbits appears to be mediated by increased NADPH oxidase activity.

In specific circumstances, other mechanisms may contribute to impaired NO production or bioactivity in the corpus cavernosum. As has been noted, mitochondria may contribute to endothelial superoxide production in diabetics. Arginase activity is reported to be elevated in the cavernosum of human diabetics; conceivably, this can reduce the availability of arginine as a substrate of eNOS.<sup>89</sup> Down-regulation of the PI3K-Akt signaling pathway in the cavernosal endothelium of aging mice may blunt the efficiency of eNOS activation.<sup>90</sup> In spontaneously hypertensive rats, cavernosal smooth muscle is less responsive to exogenous NO, and decreased expression of superoxide dismutase may contribute to the observed increased in oxidant stress.<sup>2</sup> Thus, it is hardly likely that increased NADPH oxidase activity is the sole cause of organic ED linked to vascular risk factors or aging. Nonetheless, the central role of NADPH oxidase overactivity in vascular endotheliopathies strongly suggests that this makes a substantial pathogenic contribution in most cases of non-neurogenic organic ED.

### **Therapeutic Implications**

Further studies are needed to confirm a central role for NADPH oxidase overactivity in rodent models of ED associated with various metabolic conditions that induce endotheliopathy. If these studies continue to indict NADPH oxidase, it will be reasonable to propose NADPH as a target for the clinical management of organic ED;

drugs or phytonutrients which inhibit this enzyme complex may have considerable potential in this regard.<sup>32;91-95</sup> Remarkably, sildenafil may accomplish this to a degree, as there is evidence that cGMP down-regulates expression of certain subunits of the NADPH oxidase complex in endothelial and smooth muscle cells;<sup>92;96</sup> thus, NADPH oxidase and NO/cGMP may be mutual antagonists.

Measures which reconstitute eNOS activity by replacing tetrahydrobiopterin – or “pinch hitting” for it with 5-methyltetrahydrofolate<sup>97-99</sup> – may also be useful for controlling cavernosal oxidant stress while boosting NO production. A single oral dose of tetrahydrobiopterin (200-500 mg) has been reported to increase duration of penile rigidity in ED patients receiving visual erotic stimulation.<sup>100</sup> Of related interest is a case history noting that a patient with ED, initially unresponsive to sildenafil, became highly responsive to this drug after receiving folic acid (5 mg daily) for one month.<sup>101</sup> Although high-dose supplemental arginine has the potential to improve eNOS function in patients with elevated ADMA levels, and has been evaluated as a treatment for ED, with varying results,<sup>102-107</sup> the wisdom of such an approach must be re-evaluated in light of a recent randomized trial that observed increased mortality in post-MI patients receiving arginine<sup>108</sup> (possibly an effect of increased iNOS activity?) Finally, as has been noted, measures which control the risk factors that activate endothelial NADPH oxidase are useful in the management of ED.<sup>21;25;26</sup>

It should be borne in mind that, inasmuch as ED is often an “early warning” of incipient cardiovascular disease, measures for controlling it which get to the root of the underlying endotheliopathy are likely to have a very favorable impact on subsequent vascular health.

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