eNOS and BH4; endothelial function or dysfunction. Importance of tetrahydrobiopterin (BH4)

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ABSTRACT

Tetrahydrobiopterin (BH4) is a multifunctional cofactor required for vital enzyme activity in the synthesis reactions of the neurotransmitters Dopamine and Serotonin as well as in the synthesis of the gaseous signaling agent Nitric Oxide (NO) involved in vascular health. BH4 must be maintained at continuously high levels including intracellular synthesis and recycling to prevent endothelial dysfunction with ongoing generation of free radicals and concomitant oxidative damage which leads to deleterious effects on the vascular wall including loss of vasodilation and protection against atherosclerotic pathogenesis.

Imbalances entail a drop in BH4 levels and occur in states of high blood sugar, high blood pressure, high blood lipids; their subsequent vascular disease states including diabetes, atherosclerosis, hypertension, hyperlipidemia, and hyperhomocysteinemia induce endothelial dysfunction with negative impacts on the NOS/BH4 enzyme system with resultant increased free radical formation, decreased NO production, and concomitant reduced NO vasodilatory and signaling bioactivity. In addition to vascular effects, impaired NO production also has a role in neurodegenerative diseases including impaired cerebral blood flow and decreased BDNF secretion (important for cognition, learning, and memory).

Endothelial dysfunction is oxidative stress driven by low levels of BH4 at the Nitric Oxide Synthase enzyme (NOS), called NOS-uncoupling; where NOS-Uncoupling is a perpetuating cycle of Superoxide (OO-) and Peroxynitrite (ONOO-) free radical formation. Oxidative stress in endothelial cells depletes BH4, switches NOS generation from NO to OO-, promotes formation of ONOO- from NO and OO-; dropping the intracellular ratio of BH4:BH2 inducing a feed forward cycle of more and more BH4 depletion and NOS-Uncoupling. Low levels of ONOO- exposure cause BH4 levels to drop by 60% in 500 seconds.

Vascular and cognitive health entails maintaining balanced redox ratios of BH4:BH2, sufficient arginine and citrulline levels for enzyme efficiency, as well as folate and antioxidants for cofactor rescue and anticipated free radical formation. Dietary essential vitamins and antioxidants acts as free radical scavengers and have successfully been shown to restore BH4/NO levels, endothelial NOS function, and protect against vascular and cognitive decline.

Key Words: *BH4 tetrahydrobiopterin; OO- superoxide anion; ONOO- peroxynitrite; Endothelial dysfunction; Oxidative stress; Free radicals; Antioxidants.*

Abbreviations: NOS: Nitric Oxide Synthase; BDNF: Brain derived Neurotrophic Factor.

INTRODUCTION

In the discussion of BH4 there are a few assumptions that need to be made. 1st assumption is the understanding of oxidative stress. Oxidative stress is a system which generates free radicals. The 2nd assumption is the understanding of what a free radical is. A free radical is a molecule which has lost its electron and wants it back so therefore steals an electron from any susceptible molecule near it. The 3rd assumption is required to talk about any system with oxidative stress and the generation of free radicals, and that is the basic understanding of redox reactions. A redox reaction occurs when a molecule gains or loses electrons. When a molecule has all of its electrons it's said to be reduced, and when it loses its electrons it's said to be oxidized [1-5].

The body does not want a system of free radicals except for short bursts for physiologic functions [6,7], then under homeostatic conditions the body utilizes built-in protective mechanisms of antioxidants to neutralize the free radicals. Under conditions of chronic oxidative stress with persistent generation of free radicals the intrinsic antioxidant enzyme systems get overwhelmed, thus creating a perpetual cycle of more and more free radical formation, insufficient antioxidant levels to neutralize and clear them, and more damage to local tissues from the chronic exposure of oxidative stress. Oxidative stress in blood vessels is called endothelial dysfunction. Endothelial dysfunction *is* oxidative stress [8-10].

Endothelial Function=Oxidative Stress-FREE environment=NOS-Coupling
Endothelial DysFunction=Oxidative Stress environment=NOS-UnCoupling

So then, what is endothelial function? Endothelial function is focal vasodilation during homeostasis [8,11].

NOS-enzyme and its cofactor BH4 during homeostasis:

BH4 is a cofactor for an enzyme called Nitric Oxide Synthase, NOS. Nitric Oxide Synthase generates the gaseous signaling molecule Nitric Oxide, NO. Nitric Oxide in blood vessel maintains and controls local vasodilation alongside deterring pathogenesis of atherosclerosis, and is an intracellular signaler [6,8].

BH4 is also a redox molecule. Redox reactions occur when molecules gain or loss electrons. In the case of BH4 it gains/losses (accepts/donates) electrons as Hydrogen atoms. In redox reaction nomenclature, a molecule with all its electrons is fully reduced, and BH4 is so called TetraHydroBiopterin, a fully reduced biopterin molecule; it possesses 4 electrons seen as having 4 Hydrogen atoms. This is the physiological and functional state of BH4 [1-3,12].

TetraHydroBiopterin = BH4 = fully reduced biopterin = NOS-enzyme cofactor

Only the fully reduced BH4 molecule can be a cofactor for NOS-enzyme to form the end-product NO for vasodilation and blood vessel homeostasis (8).

NOS-enzyme simplified and NOS-Coupling:

NOS simplified have 4 constituents: Arginine, BH4, Molecular Oxygen

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O2, NADPH. Arginine is the substrate. BH4 is the cofactor.

NOS-enzyme constituents = Arginine/BH4/O2/NADPH

Arginine is the precursor molecule to the formation of NO, meaning Arginine will enzymatically become Nitric Oxide. The Nitrogen adjacent to the amino end group of Arginine is cleaved off and replaced with an Oxygen as Arginine becomes the amino acid Citrulline and gaseous Nitric Oxide is formed.

This is called NOS-Coupling. Arginine and Molecular Oxygen enzymatically react to make Nitric Oxide.

BH4 stabilizes the NOS-enzyme in its fully reduced form and BH4 is NOT consumed.

Arginine is coupled to Molecular Oxygen, BH4 stabilizes the enzymatic reaction; then Citrulline, NO, and BH4 remain. This is an important feature of homeostasis and defines NOS-Coupling [8,13,14].

NOS-Coupling
N-Arginine-N + O_2 -> (NOS-BH4) -> NO+Citrulline-N+BH4

Once again; only BH4 in its fully reduced state can act in its physiological functional role as a cofactor for NOS-enzyme production of the vasodilator NO; this occurs during homeostasis in an oxidative stress-free environment.

BH4 under conditions of oxidative stress is highly susceptible to being oxidized by free radicals. Recall free radicals have lost their electrons. Molecules with Hydrogen atoms (as electrons) are easy targets and susceptible to oxidation (free radicals steal them). This renders BH4 a free radical scavenger instead of a cofactor, because to scavenge and neutralize free radicals it must give up its electrons which are the equivalent of losing its Hydrogen atoms to the free radical. This is oxidation of BH4. The resultant molecule is either BH2 as DiHydroBiopterin (partially reduced) with 2 Hydrogen atoms or Biopterin (fully oxidized) with no Hydrogen atoms. BH2 has the same binding affinity for the NOS-enzyme but cannot act as a cofactor to stabilize the coupling of Molecular Oxygen to Arginine [1,12,15-17].

BH4=fully reduced=NOS-enzyme cofactor=Function
BH2=partially reduced= NOS-enzyme competitor NOT cofactor=Dysfunction

In a system of homeostasis there are virtually undetectable levels of BH2 and all measurable biopterin exists as the fully reduced BH4. In a system of oxidative stress the ratio of BH4 is significantly decreased and BH2 is not only measurable but becomes the prominent biopterin [11,12].

Therefore, it becomes important to look at the BH4:BH2 ratio. Lower BH4:BH2 ratios are correlated with more enzyme derangement and more oxidative stress [9].

NOS-enzyme derangement and nos-uncoupling

The detrimental effects of NOS-enzyme derangement are vast and functionally insufficient. Recall the simplified version of NOS has 4 constituents: Arginine BH4 O_2 NADPH. Also recall its mentioned under homeostatic conditions. This is important because the NOS-enzyme structure has a reductase end to bind NADPH and an oxidase end to bind Molecular Oxygen. These 2 events occur with or without Arginine and/or BH4 and when the reaction occurs without them it's called NOS-Uncoupling where free radicals are formed and BH4 is oxidized [8,9,12,15].

NOS-UnCoupling generates free radicals as its end-products in the form of Reactive Species: Reactive Oxygen Species (ROS) Superoxide Anion and Reactive Nitrogen Species (RNS) Peroxynitrite.

NOS-enzyme Uncoupling Free Radical:
Reactive Oxygen Species => ROS-Superoxide Anion = OO-
Reactive Nitrogen Species => RNS-Peroxynitrite = ONOO-

Superoxide Anion OO- is an uncoupled Molecular Oxygen free radical. In a system where BH4 and OO- exist together, the OO- steals (oxidizes) the Hydrogen atoms (electrons) from BH4 readily oxidizing it to BH2. This creates a perpetuating cycle because BH2 *cannot* stabilize the reaction and more OO- gets created, oxidizing more BH4, further dropping the BH4:BH2 ratio (8,18).

Occurring simultaneously is the formation of an even more potent free radical, Peroxynitrite [24].

Peroxynitrite=Nitric Oxide+Superoxide Anion
NO+OO- => ONNO-

Peroxynitrite ONOO- is formed when NO and OO- exist in a system together. Even during decreased NOS-enzyme efficiency NO is still generated, but its bioavailability is decreased because instead of its physiological role in vasodilation NO is scavenged by OO- forming the free radical ONOO- further dropping the BH4:BH2 ratio *via* oxidation of BH4 [8,18].

The fate of BH4 under oxidative stress and endothelial dysfunction is oxidization by OO- and exceedingly faster oxidation by ONOO-; even under low levels of ONOO- exposure BH4 levels dropped by 60% in 500 seconds [18]. NOS-UnCoupling negatively effects bioavailability of both BH4 and NO from exposure to free radicals whose near proximity scavenges them; BH4 rendered BH2 and NO rendered ONOO- [3,9,18].

NOS-UnCoupling is the aberrant function of the enzyme due to low substrate (Arginine NO precursor) and/or low cofactor (BH4) with consequential production of free radical reactive species in a perpetuating cycle. Endothelial dysfunction results from NOS-UnCoupling; which IS oxidative stress [8].

Endothelial Dysfunction=NOS-UnCoupling
↓
Free Radical Formation (ROS/RNS)=OO-/ONNO-
↓
More NOS-UnCoupling/lower BH4:BH2/more BH4 oxidation
↓
more NOS-UnCoupling/more Free Radicals
↓
Endothelial Dysfunction
Vasoconstriction+Oxidative Stress

Taken altogether- Endothelial dysfunction from intracellular/extracellular vascular oxidative stress is correlated with oxidation of BH4, low ratio of BH4:BH2, simultaneous NOS-Uncoupling, persistent generation of OO- and ONOO- free radicals, decreased NO bioavailability for vasodilation, and resultant vasoconstriction [8-18].

Endothelial dysfunction and inflammatory conditions

Chronic exposure of endothelium to free radical reactive species is a driver of disease and underlying cause of cardiovascular and neurodegenerative disease states from endothelial dysfunction and its feed forward oxidative stress. The connection is simply mechanism of action [8,20-52].

Endothelial Function=Oxidative Stress-FREE environment
NOS-Coupling=Homeostasis
Endothelial DysFunction=Oxidative Stress environment
NOS-UnCoupling=Chronic Disease

A system of low substrate and/or low cofactor induces UnCoupling with resultant generation of free radicals which then continues to deplete the cofactor BH4 further inducing more UnCoupling and generating more free radicals; worsening and contributing to the UnCoupling and oxidative stress state.

An oxidative stress state causes UnCoupling by oxidizing BH4 which renders the molecule unable to fulfill its cofactor role to stabilize the enzymatic reaction therefore lowering the BH4:BH2 ratio ultimately looping the BH4-eNOS system into the perpetuating cycle of free radical formation, low levels of BH4, and persistent oxidative stress [8-10,12].

Chronic inflammatory systemic conditions such as hyperglycemia,

diabetes, hypertension, hyperhomocysteine, atherosclerosis/oxidized-LDL, viral/toxin load, and autoimmunity are sources of vascular free radicals and each individually induce NOS-UnCoupling [7,8,11,13,20].

Hyperglycemia as well as ONOO- decrease de novo synthesis of BH4 by inducing ubiquitination-induced GTPCH enzyme degradation; lowering levels of cofactor [16,21]. Diabetes patients present with one of the lowest levels of BH4, even oxidation to the level of undetectable BH2, where only biopterin exists, and their intrinsic enzyme defense mechanisms of Glutathione Reductase, Catalase, and Superoxide Dismutase become completely inhibited from high levels of free radicals [9,16,19]. AGEs from hyperglycemia [22], hypertension [11], hyperhomocysteine [20], and oxidized-LDL [23] each individually activate the endothelial NADPH-oxidase (NOX4) with significant production of OO- and concomitant ONOO [11,20-23]. NOX4 activation in endothelium is one of the largest contributors to vascular free radicals [24]. Viral/toxin load, autoimmunity, and atherosclerosis via oxidized-LDL activate the inflammation-induced NFkB cytokine pathway generating more free radicals which stimulates LOX/COX which in turn stimulates NOX4 [7,23,25,26]. In addition, viral/toxin load and autoimmunity activate the inducible iNOS of immune cells which depletes Arginine levels *via* uptake for macrophage-NO production; lowering levels of substrate [7,27].

Each one of these inflammatory states and their correlated mechanisms of free radical formation individually impact the BH4 levels indirectly as non-eNOS vascular free radicals (oxidation via exposure) and directly by NOS-UnCoupling BH4 oxidation. A person with several or all of these inflammatory states experience a compounding effect and degenerative barrage of NOS-UnCoupling, endothelial dysfunction, free radical formation and chronic oxidative stress [8-27].

Additionally, cognitive decline is largely correlated with NOS-UnCoupling. The cognition neurotrophin BDNF has been found to be synthesized from cerebral endothelium and dependent on eNOS function [28,29]. STZ-induced diabetes mellitus mice showed endothelial dysfunction (UnCoupling) as well as decreased expression of the enzyme, decreased BDNF, and vascular dementia [30]. Endothelial dysfunction is correlated with traumatic brain injury as well as secondary brain injury following trauma from NOS-UnCoupling and simultaneous activation of iNOS [31-33].

Endothelial dysfunction does not occur when there are sufficient levels of substrate/cofactor for enzyme coupling and sufficient levels of antioxidants to neutralize basal rate generation of and exposure to free radicals. This is homeostasis. To prevent and reverse endothelial dysfunction, homeostasis must be restored at these 2 levels [11,34]. Furthermore, chronic inflammatory disease states must be supported with dietary and supplemented antioxidants alongside revised lifestyles which decrease free radicals and restore antioxidant defense enzyme systems [35,36] to include improved sleep [37], exercise [38,39], and stress management [40].

Substrate levels of Arginine can be increased dietary by whey protein powder, nondairy pea protein, purified Arginine powder, purified Citrulline powder, and/or combination of Arginine/Citrulline powder; Arginine, Citrulline, and combo Arginine/Citrulline are also available as capsules [34,41]. Supplemented maternal Arginine in recipient cloned embryology improved levels of Arginine, its metabolites, NO, pregnancy rate, as well as embryo development [42]. Arginine in healthy population is considered semi-essential whereas in children and chronic disease states it is considered essential, hence the body cannot intrinsically produce it, and it must be consumed in diet or a deficiency presents [43]. BH4 supplementation is not available for purchase to the public, however in severe cases can be administered at hospitals as Sepiaterin [44]; however if the body's oxidative stress state is not addressed (antioxidants/lifestyle) the administered BH4 is rapidly oxidized [13].

For antioxidant support all of the following are beneficial and recommended to be included in dietary choices as well as vitamin supplemented: essential B-vitamins Pyridoxine B6 [45]/Folate B9 [46,47]/Cobalamin B12 [48], essential fat soluble vitamins Retinoic Vitamin A [49]/Tocopherol Vitamin E [23/]Cholecalciferol Vitamin D [50], essential water soluble Ascorbate Vitamin C [51], antioxidants Resveratrol [10]/Curcumin [52], and amino acid Cysteine as NAC. All of these are proven agents to combat free radicals, restore intrinsic antioxidant enzyme systems GPx/SOD/Catalase, replenish fully reduced BH4 levels, protect BH4 from future oxidation, and most important work synergistically to support ReCoupling of the NOS-enzyme [8,10,13,45-52].

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