

NEOPTERIN AND BIOPTERIN

Tetrahydrobiopterin (BH₄) is a cofactor that carries electrons for redox reactions. The pterins, neopterin and biopterin, are by-products of these reactions. BH₄ functions as a cofactor for the enzymes responsible for the production of monoamine neurotransmitters (epinephrine, norepinephrine, DOPA, serotonin), and as a cofactor in nitric oxide production.^{1,2}

The rate of BH₄ synthesis is important because it is so easily oxidized that its concentration may limit BH₄-dependent metabolic steps. Restricted BH₄ cofactor availability has been suggested as an etiologic factor in neurological diseases, including DOPA-responsive dystonia, Alzheimer's Disease, Parkinson's Disease, autism, schizophrenia, and depression; as well as in other conditions such as insulin resistance and cardiovascular disease.³⁻⁸ Endothelial BH₄ availability is essential for maintaining pulmonary vascular homeostasis, and it is a critical mediator in the pathogenesis of pulmonary hypertension. Because of its regulation of neuronal nitric oxide synthase (NOS), BH₄ may be a neuroprotective factor. Low BH₄ levels have been associated with impaired endothelial NOS activity.⁹ If BH₄ is limited, some cellular sources of NOS may generate superoxide while other BH₄ saturated NOS enzymes may be generating NO. Such a scenario could favor peroxynitrite generation. If peroxynitrite is not scavenged, e.g., by antioxidants such as reduced glutathione, irreversible damage to critical cellular enzymes could ensue. Such targets include components of the mitochondrial electron transport chain, alpha ketoglutarate dehydrogenase and possibly pyruvate dehydrogenase. Such a cascade of events has been hypothesized to occur in neurodegenerative conditions such as Parkinson's and Alzheimer's disease.⁷

Along with an assessment of neopterin and biopterin, functional markers for BH₄ adequacy include amino acids, such as phenylalanine, tyrosine, tryptophan, arginine and citrulline, and organic acids such as homovanillate, 5-hydroxyindolacetate and vanilmandelate. Each of these markers can be helpful in assessing BH₄ pathway activity. BH₄ is the cofactor for conversion of phenylalanine to tyrosine: Tyrosine is then converted into DOPA, dopamine, norepinephrine and epinephrine. Homovanillate is the breakdown product of dopamine, and vanilmandelate is the joint breakdown product of norepinephrine and epinephrine. Tryptophan's conversion to serotonin requires BH₄, 5-hydroxyindoleacetic acid is the breakdown product of serotonin. Arginine is the precursor for nitric oxide and citrulline.

NEOPTERIN

Neopterin is released by macrophages and is an immunologic marker for the activation of the cell-mediated immune system. Interferon gamma (secreted by T-lymphocytes) and tumor necrosis factor alpha are the key cytokines which lead to this immunologically triggered increase in neopterin levels.⁹ BH₄ synthesis is induced by proinflammatory cytokines. Thus, interferon-gamma is probably the most important activator of pteridine synthesis and release, and neopterin is useful for the monitoring of cell-mediated (Th1-type) immune activation. It has been known for some time that increased production and release of neopterin and 7,8-dihydroneopterin accompanies immune activation of macrophages both in vitro and in vivo.⁹ Neopterin is often simultaneously elevated along with quinolinic acid, since both increase in response to interferon gamma.^{10,11} Waist-to-hip ratio, which has been found to correlate positively with increased cardiovascular disease risk and mortality, was positively correlated with serum CRP and neopterin levels, and has been suggested in the assessment of cardiovascular risk in overweight and obese subjects.¹²

Neopterin has been utilized as a marker in inflammatory conditions and a measure of immune system activation. Neopterin is elevated in infections; cardiovascular disease; autoimmune diseases such as rheumatoid arthritis, systemic lupus, and atopic asthma; malignant diseases; immunomodulatory treatment monitoring; psychiatric disorders; and sleep-disordered breathing.^{2, 13-18} Autistic children were found to have significantly higher urinary neopterin levels than controls.¹⁹ Neopterin concentrations were found to correlate with cognitive decline in Alzheimer's disease patients.²⁰ Increased heavy metals due to occupational and environmental exposure have also been found to increase urinary neopterin. Glucocorticoids may cause suppression of cell-mediated immunity and consequently result in decreased neopterin levels.

BIOPTERIN

Biopterin and dihydrobiopterin (BH₂) are the oxidative products of BH₄. Defects in biopterin synthesis or regeneration can cause impairments in the biosynthesis of monoamine neurotransmitters. Autistic children were found to have significantly higher urinary biopterin (P<0.001) compared to control children; biopterin levels of siblings of autistic children were also higher than controls.¹⁹ Severely depressed or dystimic patients were found to have significantly lower plasma biopterin concentrations at baseline in comparison with healthy controls.²¹ Low biopterin levels have been associated with low BH₄ levels.²²

FIGURE I: TETRAHYDROBIOPTERIN (BH₄) SYNTHESIS

In the first step of BH₄ synthesis, GTP is converted to dihydroneopterin triphosphate which is then converted to 6-pyruvoyl-tetrahydropterin. 3,4-Neopterin is formed by a non-enzymatic reaction from dihydroneopterin triphosphate.

Through several further steps BH₄ is produced.

BH₄ is a co-factor in four enzymatic reactions:

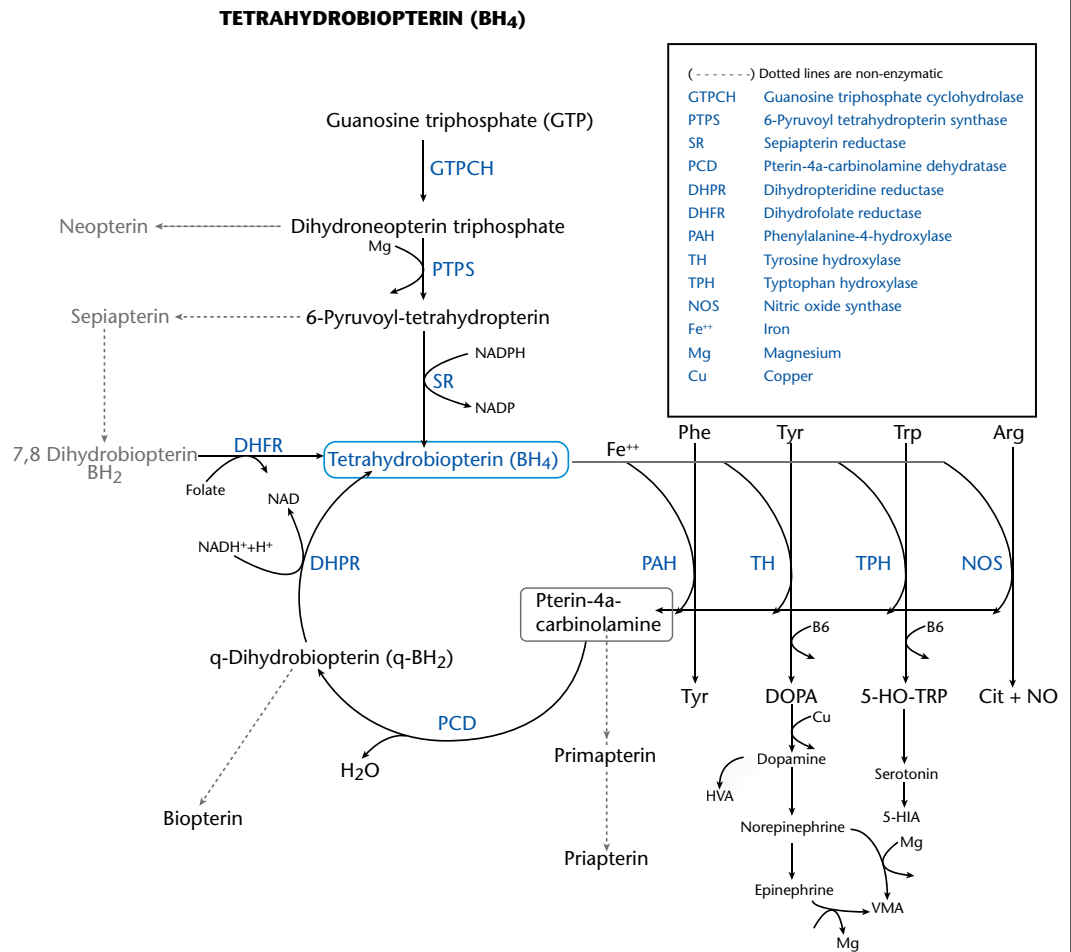
Phenylalanine to tyrosine via PAH;

Tyrosine to DOPA via TH;

Tryptophan to 5-OH-TRP via TPH;

Arginine to citrulline via NOS.

Following these enzymatic reactions BH₄ is converted to pterin-4a-carbinolamine tetrahydrobiopterin, which is then dehydrated and reduced to reform BH₄.^{2,4}



ENZYMATIC DEFECTS

The BH₄ pathway is rich with enzymes and is susceptible to several enzyme impairments or deficiencies, symptoms can range from mild to severe. In some instances increased amounts of BH₄ may increase enzyme function. The levels of neopterin and biopterin, along with urinary 5-hydroxyindoleacetic acid, formiminoglutamate, homovanillate, vanilmandelate, and blood levels of arginine, citrulline, phenylalanine, tyrosine, the Phe/Tyr ratio, and tryptophan, can help to indirectly measure BH₄ status and identify the location of a specific enzymatic defect (Table 1).³ A slowing of the metabolic conversion of phenylalanine to tyrosine can result from a BH₄ deficiency due to an enzyme defect. The accumulation of phenylalanine oxidation products can cause clinically significant symptoms and is recognized as a cause of hyperphenylalaninemia or PKU.²³ Patients with the classical PKU excrete generally more neopterin and biopterin in urine than normal controls.¹

TABLE 1 BH ₄ ENZYME DEFICIENCY ^{2,9}					
Enzyme Deficiency	Urinary Pterins		Low Phe diet	Therapy	Forms
GTPCH	Neopterin Very Low	Biopterin Very Low	No	BH ₄ , L-DoPA, 5-HTP	Severe
PTPS	Very High	Traces	No	BH ₄ , L-DoPA, 5-HTP	Severe, Mild, Transient
DHPR	Normal or slightly increased	Very High	Yes	L-DoPA, 5-HTP, Folinic Acid	Severe, Mild, Transient In a DHPR deficiency, biopterin synthesis is increased. ^{2,9}
PCD	Initially High	Low	No		Transient
DHFR17	Very High	Slightly elevated	No	Folic acid (folate) supplementation has been shown to increase intracellular BH ₄ levels. ¹¹⁻¹²	BH ₄ is produced via the reaction of dihydrofolate reductase (DHFR), it has been linked to megaloblastic anemia.

SUPPLEMENTATION

Administration of sepiapterin, BH₂, and BH₄ were comparably effective in raising BH₄ levels. Elevation in BH₄ by supplementation is primarily through the “salvage pathway” that includes BH₂ as a key intermediate in the production of BH₄ through the action of dihydrofolate reductase.^{24, 25} BH₄ accumulation in various tissues after supplementation with BH₄, BH₂ or sepiapterin can be inhibited by methotrexate.²⁶ Treatment of BH₄ deficiencies consists of BH₄ supplementation (2-20 mg/kg per day) or diet to control blood phenylalanine concentration and replacement therapy with neurotransmitters precursors (L-dopa and 5-hydroxytryptophan), and supplements of folinic acid in DHPR deficiency.^{24, 26, 27}

BH₄ is primarily available in the U.S. by prescription for patients with PKU and hyperphenylalanemia. Low dose BH₄ is available in supplements that focus on cardiac health. Supplements that may help recycle, and thereby increase BH₄ are folate, magnesium, copper, vitamin B6 and vitamin C. The primary cofactors in BH₄ reactions are iron and niacin. Supplementation of products that are low due to impaired enzyme function such as tyrosine, 5-hydroxytryptophan and citrulline are also recommended if testing identifies them as low.

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