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REVIEW ARTICLE

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Tetrahydrobiopterin treatment in phenylketonuria: A repurposing approach

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Abstract

In phenylketonuria (PKU) patients, early diagnosis by neonatal screening and immediate institution of a phenylalanine-restricted diet can prevent severe intellectual impairment. Nevertheless, outcome remains suboptimal in some patients asking for additional treatment strategies. Tetrahydrobiopterin (BH_4) could be one of those treatment options, as it may not only increase residual phenylalanine hydroxylase activity in BH_4-responsive PKU patients, but possibly also directly improves neurocognitive functioning in both BH_4-responsive and BH_4-unresponsive PKU patients. In the present review, we aim to further define the theoretical working mechanisms by which BH_4 might directly influence neurocognitive functioning in PKU having passed the blood-brain barrier. Further research should investigate which of these mechanisms are actually involved, and should contribute to the development of an optimal BH_4 treatment regimen to directly improve neurocognitive functioning in PKU. Such possible repurposing approach of BH_4 treatment in PKU may improve neuropsychological outcome and mental health in both BH_4-responsive and BH_4-unresponsive PKU patients.

KEYWORDS
phenylketonuria, tetrahydrobiopterin, treatment, brain, neurocognitive functioning, neurotransmitters

1 | INTRODUCTION

Given its success, treatment of phenylketonuria (PKU; OMIM 261600) has classically focused on controlling blood phenylalanine (Phe) concentrations to prevent irreversible intellectual disability. This is mainly done by institution of a Phe-restricted diet following a positive neonatal screening test for PKU.[1] Additionally, 20%-50% of PKU patients benefit from chaperone activity of tetrahydrobiopterin (BH_4) for the Phe hydroxylase enzyme (PAH; EC 1.14.16.1).[2] In these so-called BH_4-responsive patients, pharmacological treatment with BH_4 results in increased PAH activity, leading to a decrease in blood Phe concentrations and/or an increase in natural protein tolerance. Notwithstanding the effects of the Phe-restricted diet and/or BH_4 treatment to prevent severe intellectual disability, some early-treated PKU patients still show mild impairments in executive and social-cognitive functioning and social skills, and are prone to develop anxiety and depressive symptoms.[3-5] These findings have often been attributed to Phe neurotoxicity and cerebral neurotransmitter impairments.[6,7]

Interestingly, anecdotally, some BH_4-unresponsive PKU patients experience a better ability to concentrate and less behavioral problems on BH_4 supplementation during the short periods of time when being tested for BH_4-responsiveness, despite not showing a decrease in blood Phe concentrations.[8] Although these reports may simply indicate placebo effects, it raises the question whether BH_4 could directly improve neurocognitive and psychosocial functioning beyond its effect of reducing blood Phe concentrations in BH_4-responsive PKU patients. This hypothesis is further substantiated by a pilot study in late-diagnosed PKU patients

with maladaptive behavior, of whom most were BH_4-unresponsive, showing improved behavior after 1 year of BH_4 treatment (20 mg/kg/day).[9] Thereby, if BH_4 will indeed be shown to directly improve neurocognitive functioning beyond its effect on the PAH enzyme, such a repurposing approach may extend the target population of BH_4 treatment.

Different working mechanisms, substantiated by different levels of evidence, may underlie such a possible direct beneficial effect of BH_4 on neurocognitive functioning in PKU patients as summarized in Figure 1 and Table 1. In the present review, we aim to further define these theoretical working mechanisms in order to stimulate further research on the possible neurocognitive effects of BH_4 to ultimately be able to use BH_4 in PKU patients to its full potential.

2 | BH$_4$ AND THE BRAIN

Before discussing several mechanisms through which BH_4 could directly improve neurocognitive functioning, this review will focus on the question to what degree BH_4 supplementation can increase cerebral BH_4 concentrations. It is known that orally administered BH_4 is largely rapidly excreted through feces and urine, the latter being facilitated by high-capacity organic anion transporters in the kidney.[10,11] BH_4 furthermore shows slow transport across cell membranes, compared to related pterins such as sapropterin and dihydrobiopterin (BH_2).[10] Next to this, BH_4 is an instable molecule that is easily oxidized to BH_2, although it appears BH_2 is then intracellularly reconverted into BH_4.[12] Overall, BH_4 supplementation does increase plasma BH_4 concentrations,[13] albeit very inefficiently, but it is unclear to what extent BH_4 then crosses the blood-brain barrier (BBB). At least in part, this question is prompted by the experience of BH_4 treatment in patients with BH_4 deficiency, resulting in a disturbed function of PAH, as well as of tyrosine hydroxylase (TH) and tryptophan hydroxylase (TPH). Patients with BH_4 deficiency due to for example a defect in dihydropteridine reductase only benefit from BH_4 treatment with regard to liver Phe metabolism, and not with regard to cerebral TH and TPH activity,[14] suggesting BH_4 does not reach the brain. This similarly applies to patients with BH_4 deficiency as a result of 6-pyruvoyl tetrahydropterin synthase deficiency.[15] While this seems to contrast with the previously mentioned reports of direct neurocognitive effects of BH_4 in PKU patients, these discrepancies could be explained by a difference in BH_4 dosage. Namely, the dose of BH_4 in responsiveness testing and treatment in PKU can be much higher (up to 20 mg/kg body weight) compared to the dose of BH_4 in the treatment of BH_4 defects (usually below 10 mg/kg body weight). It has indeed been shown that BH_4 could pass the BBB and increase cerebral BH_4 in a dose-dependent manner in mice.[16] Moreover, some studies
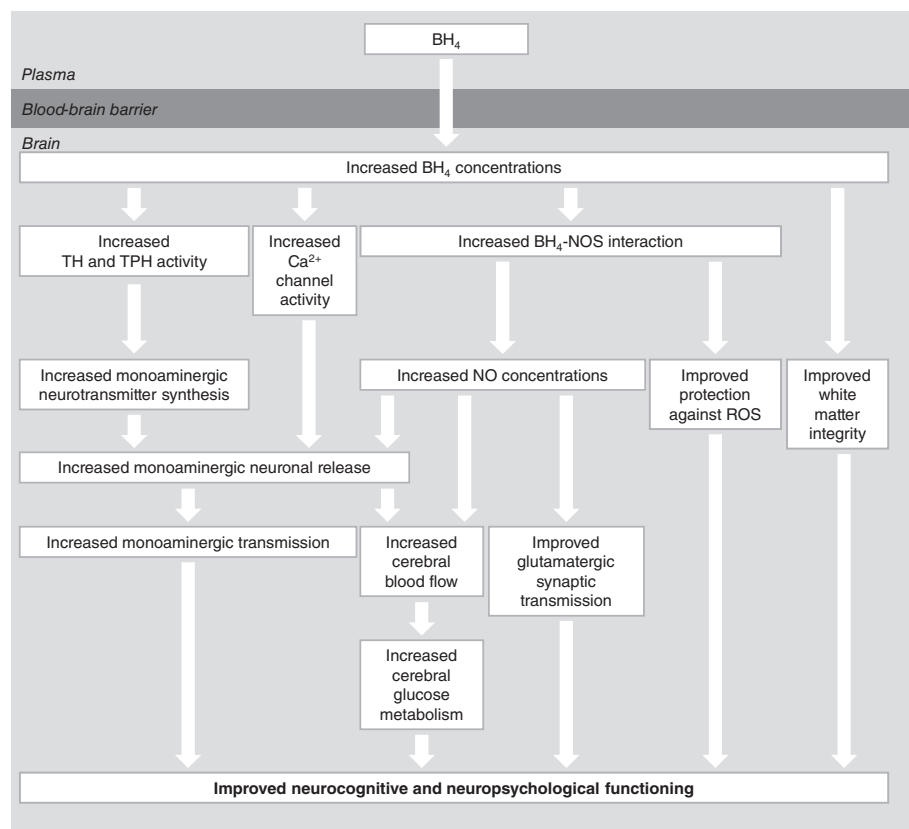

FIGURE 1 A simplified schematic overview of the different hypothesized working mechanisms that may underlie a possible direct beneficial effect of BH_4 on neurocognitive functioning in PKU patients

TABLE 1 An overview of human and non-human studies presenting results that are related to the possible neurocognitive effects of tetrahydrobiopterin treatment

	Topic	Monoaminergic neurotransmitter synthesis	Monoaminergic neurotransmitter release	Glutamatergic synaptic transmission	Oxidative stress	Glucose metabolism	Improved myelination
Hypothesized effect		BH$_4$ may increase monoaminergic neurotransmitter synthesis by stimulating TH and TPH activity.	BH$_4$ could increase monoaminergic neurotransmitter release through stimulating NO production and activation of Ca^{2+} channels.	BH$_4$ may improve glutamatergic synaptic transmission, without a clear underlying mechanism.	BH$_4$ may protect against oxidative stress.	BH$_4$ treatment may increase the BH$_2$/BH$_4$ ratio, thereby possibly increasing oxidative stress. BH$_4$ could increase glucose metabolism certain brain regions.	BH$_4$ may result in improved myelination, without a clear underlying mechanism.
Evidence Non-human studies	PKU	BH$_4$ resulted in partial recovery of brain serotonin concentrations.	BH$_4$ increased dopamine and serotonin metabolism in mice despite unaltered concentrations.				
	BH$_4$ deficiencies	Studies in mice show increased dopamine and concentrations after BH$_4$ treatment.			Increased BH$_4$ levels in mice brain were associated with decreased oxidative stress.		
	Other	Studies in healthy mice and rats show increased TH expression, and increased dopamine and serotonin concentrations after BH$_4$ treatment, although no increase in dopamine and serotonin concentrations was seen in another study.	Dopamine and serotonin release increased upon BH$_4$ infusion in multiple studies in rats.	BH$_4$ increased neuronal glutamate release in healthy rats.		In vitro studies and studies in healthy rats show that increasing the BH$_2$/BH$_4$ ratio increases oxidative stress.	

(Continues)

TABLE 1 (Continued)

	Topic	Monoaminergic neurotransmitter synthesis	Monoaminergic neurotransmitter release	Glutamatergic synaptic transmission	Oxidative stress	Glucose metabolism	Improved myelination
Human studies	PKU	Peripheral parameters of brain serotonin and dopamine were not altered by BH_4 treatment in one study, whereas results from another study did suggest increased brain dopamine concentrations.				BH_4 treatment resulted in increased glucose metabolism in one study.	BH_4 treatment improved white matter abnormalities in one study, and improved neuronal activation in another study.
	BH_4 efficiencies						BH_4 treatment was associated with fewer white matter abnormalities.
	Other				Cardiovascular studies on BH_4 treatment show inconclusive results.		

Abbreviations: BH_4, tetrahydrobiopterin; BH_2, dihydrobiopterin; NO, nitric oxide; PKU, phenylketonuria; TH, tyrosine hydroxylase; TPH, tryptophan hydroxylase.

suggest that BH_4 could also reach the brain in humans. BH_4 administration (20 mg/kg/day) in children with autism spectrum disorder has also been shown to improve social awareness, autism mannerisms, hyperactivity, and inappropriate speech.[17] High doses of BH_4 are probably necessary to cross the BBB, as shown by increased biopterin concentrations in cerebrospinal fluid (CSF) in humans and rhesus monkeys following BH_4 administration at 20 mg/kg or higher,[18-21] but not at lower doses.[18] Overall, while some studies indicate that BH_4 supplementation may increase cerebral BH_4 concentrations, it is important to emphasize that the extent to which this happens is not yet clear and this essential question requires further research. Higher dosages of BH_4 than currently used may be necessary to establish an increase in cerebral BH_4 that could result in relevant therapeutic effects. Alternatively, the use of sepiapterin, which is a precursor of BH_4, may be considered for this purpose. Although the exact mechanism has not yet been elucidated, it has been shown that administration of sepiapterin is more effective in increasing intracellular BH_4 concentrations compared to administration of BH_4 directly, with the latter resulting in a larger increase in intracellular BH_2 concentrations.[22] Moreover, a very recent clinical trial showed that administration of a therapeutic formula of sepiapterin was well-tolerated in healthy subjects, and increased plasma BH_4 concentrations more efficiently compared to BH_4 administration.[23]

3 | BH_4 TOXICITY

Some reports have warranted against the indiscriminate use of high-dose BH_4 treatment because of possible toxicity. In one study in BH_4-deficient mice, acute subcutaneous BH_4 administration at 300 mg/kg resulted in the death of two of 12 animals,[24] which has been suggested to be related to non-specific stimulation of NO synthesis.[24,25] Such apparent toxicity, however, has not been observed in mice at subcutaneous administration of lower doses (30 or 90 mg/kg)[24] or at subchronic oral administration up to 100 mg/kg[16,24] or acute oral administration up to 1300 mg/kg.[26] For subchronic intraperitoneal BH_4 administration in mice, the median lethal dose has been found to be 260 mg/kg.[26] In PKU patients, BH_4 treatment (up to 20 mg/kg/day) is considered safe, with a low rate of adverse effects.[27]

4 | BH_4 AND MONOAMINERGIC NEUROTRANSMITTER SYNTHESIS

As a first hypothetical working mechanism by which BH_4 may directly improve neurocognitive functioning in PKU patients, BH_4 is suggested to increase cerebral monoaminergic neurotransmitter synthesis. Cerebral monoaminergic neurotransmitter deficiencies are considered an important pathophysiological factor underlying brain dysfunction in PKU,[28] resulting from insufficient brain uptake of their amino acid precursors (tyrosine [Tyr] and tryptophan [Trp]) and/or inhibition of TH and TPH—the enzymes being responsible for the rate limiting steps in dopaminergic and serotonergic neurotransmitter synthesis—by elevated brain Phe concentrations.[29] In both living and deceased PKU patients, decreased Tyr and Trp levels in brain as well as reduced dopaminergic and serotonergic metabolites in CSF have been reported.[30,31] Moreover, a reduction in TH protein expression of 40% in medial prefrontal cortex of the BTBR *Pah-enu2* PKU mouse model has been observed.[32] No such reductions have been found for cerebral protein expression of TPH in this PKU mouse model, although enzyme activity levels were shown to be significantly reduced.[33]

BH_4 may directly stimulate cerebral monoaminergic synthesis in PKU patients, although the catecholaminergic and serotonergic system might respond differently to BH_4 administration. This hypothesis is substantiated by various findings. Firstly, with regard to the catecholaminergic system, TH activity and protein expression significantly increased in wild-type mice following oral BH_4 administration (20 and 100 mg/kg).[16] Also, in BH_4 knock-out mice, chronic intraperitoneal BH_4 administration (50 mg/kg) improved TH activity, thereby increasing brain dopamine concentrations,[34] and the reduction of TH protein expression in striatum was partly reversed by BH_4 administration (50 mg/kg).[34,35] Additionally, as observed by in vivo microdialysis, dopamine concentrations increased on BH_4 infusion in striatum of healthy rats, and this effect was further enhanced by continuous infusion of Tyr at a relatively low dose.[36] Secondly, with regard to the serotonergic system, acute BH_4 administration in BH_4 knock-out mice strongly increased brain serotonin concentrations without any effect on TPH activity.[34] In line with that study, BH_4 treatment (50 mg/kg/day) in *Pah-enu1/2* mice led to a partial recovery of brain serotonin levels, but again without increased TPH activity.[37]

Nevertheless, the effects of BH_4 administration in mice and rats are somewhat inconclusive, with BH_4 administration (20, 40, and 100 mg/kg) in one study not resulting in changed dopamine and serotonin concentrations in wild-type mice,[16] while other studies, in wild-type rats and BH_4 knock-out mice, respectively, showed increased biosynthesis of both monoaminergic neurotransmitters,[24,38] although this effect was only achieved at toxic dose (300 mg/kg) in one of these studies.[24]

More recently, blood and urine melatonin and urine dopamine concentrations, that are thought to reflect brain serotonin and dopamine availability in the CNS, were found

to be not changed by BH_4 administration in BH_4-responsive and BH_4-unresponsive patients.[39] On the other hand, observations in BH_4-responsive PKU patients suggested a direct positive effect of BH_4 on cerebral dopamine bioavailability beyond its effect through lowering blood Phe concentrations.[40] In this study, in male BH_4-responsive PKU patients, blood prolactin concentrations—as a peripheral parameter to reflect cerebral dopamine concentrations—were found to be significantly lower on BH_4 treatment if compared to treatment without BH_4, even when correcting for blood Phe concentrations, and tended to be lower at increasing BH_4 dose. It is important to emphasize, however, that both studies assessed indirect measures of brain monoaminergic neurotransmitters.

Overall, while reports are still inconclusive, studies in PKU mice and patients suggest a benefit of BH_4 treatment to directly improve brain monoaminergic neurotransmitter synthesis in PKU (Table 1), which consequently might lead to improved neurotransmitter release and neurotransmission. The different responses of the catecholaminergic and serotonergic system, as well as the possible beneficial effects on neurocognitive functioning in PKU patients, certainly warrant further investigations.

5 | BH_4 AND NEURONAL MONOAMINERGIC NEUROTRANSMITTER RELEASE

Besides stimulating monoaminergic neurotransmitter synthesis, BH_4 in PKU patients may also improve neurocognitive functioning by increasing neuronal dopamine and serotonin release either directly or through stimulation of NO production. Neuronal release of monoaminergic neurotransmitters has not been investigated in PKU patients, but research in the PKU mouse model has shown clear impairments in neuronal monoamine release.[32,33] Although impaired neuronal monoaminergic neurotransmitter release in PKU could partly be explained by insufficient neurotransmitter synthesis, this may not be the only underlying mechanism. Firstly, disturbed NO metabolism in PKU might contribute to the observed impairments in neuronal monoaminergic neurotransmitter release. Disturbed NO metabolism was reported in both PKU patients[41-43] and in PKU mouse brain,[44] and is hypothesized to result from increased oxidative stress. Cerebral NO is involved in both synaptic and nonsynaptic neurotransmission. As such, impaired cerebral NO metabolism in PKU can be suggested to contribute to dysfunctional monoaminergic neurotransmission. Secondly, alterations in neuronal Ca^{2+} channels have been reported in cell studies, which may be related to impaired neuronal monoamine release in PKU.[45,46] These alterations in Ca^{2+} channels are hypothesized to result from the disturbed amino acid balance in the PKU brain.[45,47,48]

Several studies, although mostly not related to PKU, suggest that BH_4 has an effect on monoaminergic neurotransmitter synthesis. in vivo microdialysis with intracerebral BH_4 infusion was found to increase neuronal dopamine and serotonin release in a dose-dependent manner in striatum from healthy rats.[49-53] For dopamine, a similar effect of BH_4 has been observed in rat frontal cortex.[52] Furthermore, a more recent study showed increased metabolism of serotonin and dopamine in PKU mice despite unaltered concentrations of these neurotransmitters, which might be explained by an increase in synaptic monoaminergic neurotransmitter release.[54]

This effect of BH_4 may be exerted by two different mechanisms that are theoretically related to the causes of impaired neuronal monoaminergic neurotransmitter release in PKU. Firstly, BH_4 is (together with flavin adenine dinucleotide and flavin mononucleotide) one of the essential cofactors for NOS, catalyzing the conversion of arginine into NO and citrulline, also in the brain. Research in both rats and mice indeed suggest an increase in cerebral NO production by NOS following BH_4 administration.[55,56] Extensive research, as reviewed by Kiss,[57] has shown that NO influences neuronal release of monoaminergic neurotransmitters.[57] Although controversy exists concerning the exact role of NO in monoaminergic neurotransmission, the majority of data has indicated that NO stimulates dopaminergic, noradrenergic, and serotonergic neuronal release.[57] Secondly, besides acting through NO, BH_4 has also been shown to directly enhance neuronal dopamine and serotonin release[49,50,53,58,59] independent of its cofactor activity. Such effect would be mediated by activation of Ca^{2+} channels[53,58,60] via the cAMP protein kinase A pathway.[58]

To conclude, the possible effect of BH_4 treatment on neuronal monoaminergic neurotransmitter release and thereby neurotransmission has only been investigated in animal studies, mostly not related to PKU (Table 1). However, given the similarities in the possible mechanisms underlying impaired monoaminergic neurotransmission in PKU and the modes of action of BH_4, this might well offer a potentially useful therapeutic objective for BH_4 that deserves further research.

6 | BH_4 AND GLUTAMATERGIC SYNAPTIC TRANSMISSION

Although most research has focused on the possible effect of BH_4 on synthesis and neuronal release of monoaminergic neurotransmitters, BH_4 could also improve glutamatergic neurotransmission. Glutamate is the primary excitatory neurotransmitter in the brain, and has been shown to regulate

neurogenesis, neurite outgrowth, synaptogenesis, and neuronal survival.[61] As such, glutamatergic synaptic transmission is crucial for normal brain functioning, and impaired glutamatergic synaptic transmission has been associated with a diverse group of neurological disorders.[61] Also in PKU, impaired glutamatergic synaptic transmission has been hypothesized to contribute to brain dysfunction.[62] Both in vitro and in vivo studies in rats and PKU mice have shown that high Phe concentrations impair glutamatergic synaptic transmission through both presynaptic and postsynaptic effects.[63-65] This mechanism has been suggested to be responsible for impaired learning and memory in PKU mice,[65] and has been associated with susceptibility of BTBR *Pah-enu2* mice to audiogenic seizures.[66]

As BH$_4$ has been found to induce neuronal glutamate release in striatum and frontal cortex from healthy rats,[52] BH$_4$ might improve glutamatergic synaptic transmission in PKU and thereby improve neuropsychological functioning. The exact mechanism by which BH$_4$ would stimulate neuronal glutamate release has not been fully elucidated yet. The finding that increased glutamatergic neuronal release on BH$_4$ treatment was abolished by concomitant administration of 6-hydroxydopamine, destroying the dopaminergic nerve terminals, may suggest that the possible effect of BH$_4$ on striatal glutamate release is mediated by proper functioning dopaminergic neurons.[52] Alternatively, it can be hypothesized that the effect of BH$_4$ is mediated by increased NO production, as NO has also been found to stimulate neuronal glutamate release.[67] Overall, this hypothesis is mostly based on a single animal study not related to PKU (Table 1). Therefore, whether BH$_4$ treatment could stimulate glutamatergic synaptic transmission in PKU and thereby improve neurocognitive functioning still remains to be established.

7 | BH$_4$ AND OXIDATIVE STRESS

Theoretically, BH$_4$ may improve neurocognitive functioning in PKU patients by protecting against oxidative stress. Oxidative stress is defined as an imbalance between free radicals and antioxidant defense systems and is usually followed by oxidative cell injury and death. Research on this subject in PKU patients and mice, as reviewed by Ribas et al[68] suggests that oxidative stress could be an import mechanism leading to brain damage in PKU as a result of both increased reactive species production and decreased antioxidant defenses.[68]

In oxidative stress, BH$_4$ plays a vital anti-oxidative role as a result of its interplay with nitric oxide synthase (NOS).[69,70] During oxidative stress, NOS generates superoxide, which is a reactive oxygen species, thereby further aggravating the oxidative situation. BH$_4$ is able to prevent the formation of superoxide by interacting with NOS, which

is called "NOS coupling." However, especially during oxidative stress, BH$_4$ is oxidized to BH$_2$ leading to "NOS uncoupling," thereby no longer protecting against free radicals. In case of oxidative stress, as observed in PKU, a high intracellular BH$_4$ level might therefore be critical to maintain homeostasis, so that increased BH$_4$ availability in the brain might lead to a better protection against oxidative damage. However, it should be noted that the extent to which peripherally administered BH$_4$ could protect against oxidative stress largely depends on the increase of BH$_4$ concentrations relative to that of BH$_2$, for example, the BH$_2$/BH$_4$ ratio, as studies in endothelial cells in vitro and in healthy rats indicate that an increase in this ratio would actually result in more oxidative stress.[71,72] Thus, while BH$_4$ itself may decrease oxidative stress, BH$_4$ treatment may ultimately lead to too high BH$_2$ levels and by this may increase oxidative stress. Therefore, this possible negative effect of BH$_4$ treatment should be kept in mind and may be very relevant when considering the hypothesized benefits of BH$_4$ treatment in general.

The effect of BH$_4$ on oxidative stress has been investigated in research areas other than PKU. Some studies focusing on the vascular system, performed in both humans an mice, indeed suggest that BH$_4$ decreases oxidative stress,[73,74] while other human studies show no effects of BH$_4$ on the cardiovascular system, possibly due to increased BH$_2$ levels.[75,76] Overall, BH$_4$ may in theory have positive effects on neurocognitive functioning of PKU patients by decreasing oxidative stress, but the effect of BH$_4$ on oxidative stress in the PKU brain has not yet been examined. Meanwhile, the possible negative effects of BH$_4$ treatment on oxidative stress deserve further attention as well.

8 | BH$_4$ AND CEREBRAL ENERGY METABOLISM

BH$_4$ might also have a positive effect on the brain by improving cerebral glucose metabolism through increased cerebral blood flow. Impaired cerebral energy status has been observed in both PKU patients[77-80] and PKU mice.[81] The role of such impaired cerebral energy metabolism on brain functioning in PKU is however largely unknown. Reduced cerebral energy metabolism has been suggested to relate to white matter abnormalities in PKU,[78] while increased cerebral glucose metabolism, which has been observed in particular brain areas in PKU patients,[77,80] has been suggested to reflect some compensatory mechanism.[77]

The effect of BH$_4$ (at 20 mg/kg/day) on brain glucose metabolism as measured by FDG-PET has been investigated in one study in BH$_4$-unresponsive PKU patients.[77] This study showed that, after 4 months of BH$_4$ treatment, glucose metabolism in left Broca's and right lateral temporal cortices

was increased, which was accompanied by enhanced performance in a phonemic fluency test. Ficicioglu et al. hypothesized that this could have been the result of BH_4-induced vasodilation. As previously mentioned, BH_4 might lead to increased synthesis of NO, dopamine and serotonin. Whilst serotonin has a vasoconstrictive effect, dopamine and especially NO have strong vasodilatory qualities. Possibly, the increased blood flow enables certain brain regions to compensate for imbalances in glucose metabolism. Overall, this hypothesis is only supported by a single study in PKU patients (Table 1). Therefore, further research is necessary to establish the effects of BH_4 on cerebral energy metabolism, and to investigate whether these effects could indeed be beneficial for neurocognitive functioning.

9 | BH$_4$ AND WHITE MATTER

Finally, some evidence points towards a possible role for BH_4 in ameliorating neurocognitive functioning in PKU by influencing white matter. White matter abnormalities are one of the neuroradiological features characterizing PKU and have been observed in both untreated and early-treated (especially if not treated optimally) PKU patients.[82] While white matter pathology in untreated PKU is generally accepted to reflect hypomyelination, the observed white matter pathology in early-treated PKU patients is suggested to reflect intramyelinic edema rather than demyelination.[82] The clinical significance of the observed white matter abnormalities is still highly debated. However, PKU-related white matter abnormalities have been correlated to slowed information processing,[83,84] which has been found to partly account for the executive function impairments seen in PKU.[85]

A possible relationship between BH_4 supplementation and improved myelination has been described in BH_4 deficiency patients.[86] When comparing their own results with a previous study on myelination in BH_4 deficiency patients,[87] Wang et al[86] showed more white matter abnormalities. In contrast to these differences in neuroradiological findings, age at which dietary treatment was initiated was comparable for both patient groups. BH_4 as well as neurotransmitter precursor treatment (levodopa and 5-HTP), however, was started at later age in the patient group presenting with more white matter abnormalities, suggesting a role for BH_4 and neurotransmitter precursor treatment in the reversal of white matter pathology.[86] More recently, institution of BH_4 treatment in early-diagnosed and early-treated PKU patients has been shown to improve (and in some cases even fully correct certain aspects of) white matter abnormalities, which were significantly associated with reductions in blood Phe concentrations.[88] Whether these clear improvements were completely due to the blood Phe lowering effect of BH_4 remains to be established, as the study was only performed in BH_4-responsive PKU patients.

In addition, neuroimaging findings by functional MRI (fMRI) in early-treated PKU patients have shown improved neural activation after 4 weeks of BH_4 treatment (20 mg/kg) even when blood Phe concentrations had not decreased.[89] Although additional research should further elucidate the possible relationship between these deficiencies of functional connectivity and white matter abnormalities in PKU, the results obtained by fMRI studies are in good agreement with the white matter abnormalities observed in PKU.[90,91] Taken together, these results from studies in BH_4-treated PKU patients hold some promise for BH_4 treatment to improve neurocognitive functioning in PKU by influencing white matter. The underlying mechanism for such possible effect might be multifactorial and is not fully understood.

10 | CONCLUSION

Besides lowering blood Phe concentrations in BH_4-responsive PKU patients, findings suggest that BH_4 treatment in PKU may also directly improve neurocognitive functioning. While the important question to what extent which peripherally administered doses of BH_4 can increase cerebral BH_4 concentrations in humans necessitates additional investigation, the present review describes the working mechanisms that, theoretically, may underlie this possible direct neurocognitive effects of BH_4 in PKU. It should be emphasized that these hypothesized mechanisms are in large part based on studies in animal models and non-PKU-related research, but, taken together, they definitely justify further research on this topic. This research should at least focus on (a) further elucidating the possible beneficial effects of BH_4 treatment on neurocognitive functioning in PKU; (b) examining possible negative or toxic effects of BH_4 or its metabolites on brain function; and (c) the most effective way to increase BH_4 concentrations in the brain. Regarding the first aim, we suggest that the *Pah-enu2* mouse model for BH_4-unresponsive PKU could be used to identify if, and by which of the described mechanisms, BH_4 might improve neurocognitive functioning in PKU, beyond its effect through lowering blood Phe concentrations. Regarding the second aim, both this mouse model as well as in vitro studies could be used to investigate possible toxicity of BH_4 at different concentrations, especially focusing on the effect of BH_4, and the balance between BH_4 and BH_2, on oxidative stress. Thirdly, animal studies should also be used to investigate the optimal treatment regimen to increase cerebral BH_4 concentrations. This will possibly require higher BH_4 dosages than currently studied, or, alternatively, treatment with sepiapterin as this may be more effective in increasing intracellular BH_4 concentrations. Ultimately, the effect of BH_4 treatment on objective neurocognitive tasks in BH_4-unresponsive patients should be assessed in a placebo-

controlled setting. If BH$_4$ indeed has beneficial neurocognitive effects, this may extend the target population of BH$_4$ treatment.

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AUTHOR CONTRIBUTIONS

All authors were involved in the design and drafting of this review.

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