Tetrahydrobiopterin (BH4) Plasma Level Is Reduced In A Clinically Stable Cohort Of Relapsing Remitting Multiple Sclerosis (RRMS)

Zaenab Dhari MD[1,2], Marco Pisa MD[3], Lindsay Neto MPH[1,2], Ashley Paro BS[1], Mary Bailey MD[1,2], Gabriele C. De Luca MD DPhil[3], Jennifer A. Ruiz DPT[1,2]

(1)Mandell Center for Multiple Sclerosis, Mount Sinai Rehabilitation Hospital, Trinity Health Of New England, Hartford, CT, USA(2)Frank H. Netter MD School of Medicine at Quinnipiac University, North Haven, CT, USA (3)Nuffield Department of Clinical Neuroscience, Oxford, UK

ABSTRACT

Background: Higher efficacy disease modifying therapies (DMTs) demonstrate diminished inflammatory burden in multiple sclerosis (MS). Such progress is observed through reduced clinical and radiological activity. However, evidence suggests disability progression later in life remains independent of currently available indicators of MS disease breakthrough. There is an unmet need for feasible and quantifiable molecular markers for the MS population. Tetrahydrobiopterin (BH4), an endogenous metabolite, is crucial for maintaining inflammatory homeostasis through nitric oxide synthase coupling (NOS). When inflammation occurs, BH4 is depleted causing NOS uncoupling which further induces cellular insults. Although BH4 is known to impact inflammatory levels, little is known about its influences on patient outcomes in MS. **Methods:** Forty participants including a control group (healthy, n=20) and MS group (stable relapsing remitting MS [RRMS], n=20) were enrolled. Stable MS participants were defined as currently on Natalizumab, a high efficacy DMT for at least 6 months before enrollment, with no evidence of MS activity within the last 6 months. Exclusion criteria were ages < 18 and > 40, body mass index (BMI) < 18.5 and > 29.9, any physical disability affecting mobility, comorbidities other than MS, excessive use of alcohol and cigarette smoking. An additional exclusion for the MS group was any history of fumaric acid esters usage. Blood was collected through venipunctures and processed immediately to preserve BH4 stability. Preanalytical methods validation was performed. Finally, Liquid Chromatography Mass Spectrometry (LC-MS) assays were performed to quantify plasma BH4 in two blinded experiments (α =0 .94). Independent samples t- test was performed to measure significance of variance. **Results:** MS group descriptive statistics were 65% female, with age (34 ± 5) and BMI (25 ± 2.3) and were similar to control group characteristics. Plasma BH4 peak area was significantly reduced in MS group (p<0.01, Cohen's d > 0.8). Following absolute quantification of BH4 metabolite in plasma using a reference compound, BH4 concentration was (7.7 ± 2) versus (6 ± 2) ng/mL in control and MS groups, respectively, suggesting reduced plasma BH4 in MS group (p<0.01, Cohen's d > 0.8). **Conclusions:** BH4 plasma level was reduced in this MS cohort, despite being clinically stable and on one of the higher efficacies DMTs. Our findings provide a novel insight of BH4 level alterations present in MS, expanding the potential to further explore its contribution to MS pathology.

CONTACT:

Zaenab Dhari, MD, Research Fellow
Mandell Center for Comprehensive Multiple Sclerosis Care & Neuroscience Research,
Mount Sinai Rehabilitation Hospital, Trinity Health Of New England
Zaenab.Dhari@TrinityHealthOfNE.org
W 860-714-2623
490 Blue Hills Avenue
Hartford, CT 06112

INTRODUCTION

An unmet need exists for feasible and quantifiable molecular markers for the MS population. Tetrahydrobiopterin (BH4), an endogenous metabolite, is crucial for maintaining inflammatory homeostasis through nitric oxide synthase coupling (NOS). When inflammation occurs, BH4 is depleted causing NOS uncoupling which further induces cellular insults. Although BH4 is known to impact inflammatory levels, little is known about its influences on patient outcomes in MS.

Figure 1, illustrates the metabolic pathways relevant BH4.

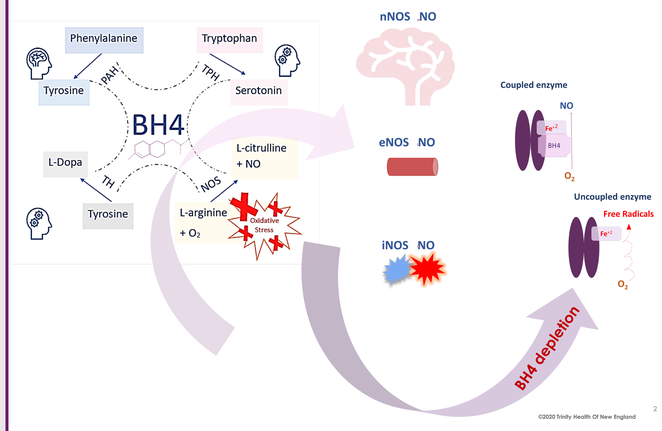


Figure 1 BH4: (6R) 5, 6,7, 8 Tetrahydrobiopterin. **PAH**: phenylalanine hydroxylase, **TPH**: Tryptophan hydroxylase, **TH**: Tyrosine hydroxylase**, iNOS**: inducible nitric oxide synthase, **eNOS**: endothelial nitric oxide synthase, **nNOS**: neuronal nitric oxide synthase, **NO**: nitric oxide.

BH4 is an important facilitator for many pathways. Aromatic amino acid hydroxylases such as PAH requires BH4 as catalyst for phenylaniline to be converted to tyrosine, a precursor for hormones and neurotransmitters such as dopamine through TH, which also requires BH4 to synthesize L-Dopa, which is converted to dopamine, a precursor for catecholamines. TPH, another aromatic hydroxylase, requires BH4 to facilitate the conversion of tryptophan to serotonin. BH4 plays an important role in maintaining redox homeostasis through the binding of NOS to oxygen, to facilitate the conversion of L-arginine to L- citrulline, yielding NO.

METHODS AND MATERIALS

- Forty individuals including a control group (healthy, n=20) and MS group (stable relapsing remitting MS [RRMS], n=20) participated. Stable RRMS was defined as currently on Natalizumab, for at least 6 months, with no evidence of MS activity within the last 6 months.
- Blood was collected through venipunctures and processed immediately to preserve BH4 stability. Preanalytical methods validation was performed.
- Finally, Liquid Chromatography Mass Spectrometry (LC-MS) assays were performed to quantify plasma BH4 in two blinded experiments (α =0 .94).

Figure2, represents the study design and methods.

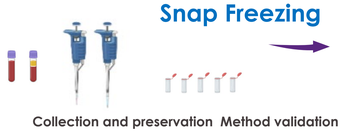
A. *Inclusion Criteria*

- Ages (18-40)
- BMI (18.5-29.9)
- EDSS <3
- Either diagnosis of RRMS, or healthy controls
- For RRMS: no evidence of clinical or radiological activity within the last 6 months
- For RRMS: being on a uniform high efficacy DMT

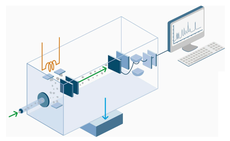
Exclusion Criteria

- Any comorbidity (CVD, renal, hematological, autoimmune, substance abuse, malignancy , psychiatric and other neurological diseases)
- For RRMS: History of FAES.
- Intake Alcohol, medications and supplements, along with smoking, within 24 hours from blood draw.
- Pregnancy and or lactation

B. Recruitment → **Sample Preparation**



Storage → **Quantification**



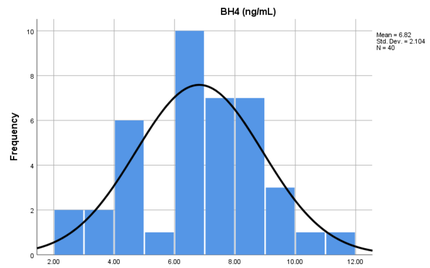
Mass Spectrometry

Figure 2, Pilot study design. **(A)** Inclusion Criteria (**B**) Blood collection steps. **BMI**: Body mass index, **RRMS**: relapsing remitting multiple sclerosis, **EDSS**: expanded disability status scale, **DMT**: disease modifying therapy, **CVD**: cardiovascular diseases, **FAEs**: fumaric acid esters.

RESULTS

Plasma BH4 level is decreased in a clinically stable young cohort of RRMS

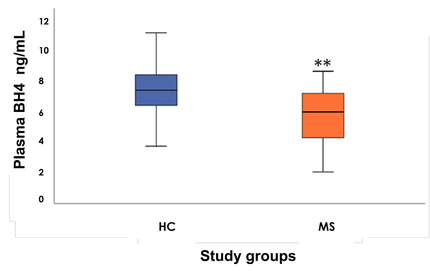
A.



Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
BH4 (ng/mL)	.110	40	.200*	.983	40	.799

B.



Group Statistics

	Sample Name	N	Mean	Std. Deviation	Std. Error Mean
BH4 (ng/mL)	HC	20	7.6765	1.91643	.42853
	MS	20	5.9585	1.96384	.43913

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means					
									95% Confidence Interval of the Difference
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
BH4 (ng/mL)	Equal variances assumed	.198	.659	2.800	38	.008	1.71800	.61357	.47589	2.96011
	Equal variances not assumed			2.800	37.977	.008	1.71800	.61357	.47587	2.96013

Figure 3. Results from our pilot study. **(A)** BH4 distribution across the study cohort and normality tests. Following absolute quantification of BH4 metabolite in plasma using a reference compound, BH4 concentration (**B**) was (7.7 ± 2) versus (6 ± 2) ng/mL in control and MS groups, respectively (p<0.01, Cohen's d), data expressed as mean ± SD, independent t-test, **p < 0.01).

CONCLUSIONS

- **BH4 plasma level was reduced in this MS cohort, despite being clinically stable and on one of the higher efficacy DMTs.**
- **Our findings provide a novel insight of BH4 level alterations present in MS, expanding the potential to further explore its contribution to MS pathology.**

ACKNOWLEDGMENTS:

The authors would like to thank the Mandell Family Foundation and Weldon Family for their generous contributions to fund this project.