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.

Figure 1, illustrates the metabolic pathways relevant BH4.

**RESULTS**

- 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. Exclusion criteria were ages <18 or >40, body mass index (BMI) <18.5 or >29.3, 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 (a = .94).

**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.

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