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Acute Supplementation of Beta-Hydroxybutyrate Increases Visual Cortical Excitability in Humans: A Combined Electro-EncephaloGraphy and Magnetic Resonance Spectroscopy Study

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ABSTRACT

Increasing plasma levels of ketone bodies via supplementation has been recently found to modulate the neurometabolic profile in the healthy human brain. Here, we aimed to explore the physiological consequences of these neurometabolic changes by assessing visual cortical function. Ten young adult human volunteers (mean age 27 years, range 23–34) were orally administered a single dose of a β-hydroxybutyrate (βHB) ester (one of the main ketone bodies), and we measured neurometabolic change after supplementation. We used Electroencephalography (EEG) to assess cortical responsivity to visual stimuli and endogenous rhythms, and magnetic resonance spectroscopy (MRS) to quantify glutamate and GABA+ concentrations in the occipital cortex. βHB supplementation increased the amplitude of steady-state visual evoked potentials and increased resting-state EEG alpha power (8–13 Hz). These electrophysiological changes were paralleled by an increase in glutamate (but not GABA+) concentration in the occipital cortex. The glutamate increase was correlated with the increased steady-state visual evoked potentials amplitude. This suggests that acute βHB supplementation increases the excitability of the brain cortex, as assessed neurometabolically and electrophysiologically. We discuss how these effects of acute supplementation may differ from the long-term effects of chronic interventions in healthy or pathological brains.

Abbreviations: βHB, β-Hydroxybutyrate; BMI, Body mass index; CRLB, Cramér–Rao lower bound; CSF, Cerebrospinal fluid; EEG, Electro-encephalography; FFT, Fast Fourier transform; GABA, Gamma amino-butyric acid; GLP-1, Glucagon-like peptide-1; GLX, Combined concentration of glutamate and glutamine in the brain; MRI, Magnetic resonance imaging; MRS, Magnetic resonance spectroscopy; NAA, N-acetylaspartate.

Giuseppe Daniele and Paola Binda shared senior authorship.

1 | Introduction

Brain metabolism primarily relies on glucose; however, during fasting and strenuous physical exertion, ketone bodies serve as the primary alternative source of energy for brain cells (Owen et al. 1967). Ketogenesis predominantly occurs in hepatocytes, where free fatty acids undergo a process of β-oxidation regulated by insulin and glucagon (Rui 2014). The three final products, acetone, acetoacetate, and β-hydroxybutyrate (βHB), are released into the bloodstream, and they cross the blood–brain barrier via monocarboxylic acid transporters (Morris 2005).

The level of ketone bodies in the bloodstream can also be steadily elevated through exogenous supplementation of ketone esters or salts and through ketogenic diets (Clarke et al. 2012; Stubbs et al. 2017; Soto-Mota et al. 2020). However, even a single dose of acute βHB supplementation produces a substantial raise of βHB levels in the bloodstream and increases βHB concentrations in the brain, as estimated with magnetic resonance spectroscopy (^{1}H-MRS) (Mujica-Parodi et al. 2020; Hone-Blanchet et al. 2023). Evidence that peripherally infused βHB is metabolized in brain cells, particularly neurons, also came from MRS with ^{13}C labeling (Pan et al. 2002).

This raises the question of whether and how elevated ketone bodies, particularly through acute βHB supplementation, affect brain function, as a recent human study with 7 T magnetic resonance spectroscopy (^{1}H-MRS) (Hone-Blanchet et al. 2023) showed that such supplementation alters brain neurometabolism. Specifically, Hone-Blanchet et al. (2023) showed that βHB supplementation reduces GABA levels within the cingulate cortices, paralleling the increase of βHB concentration in the same voxel. However, the average GABA change was about 50%, very large compared to physiological variations (Maddock 2023). The GABA decrease was accompanied by a glutamate decrease, leaving their ratio unaffected. Crucially, the decrease of GABA and glutamate was strongly dependent on age, with the largest decrease observed for older participants and negligible variations for the youngest participants in the sample. These features make it hard to predict whether and how these large but coordinated glutamate and GABA changes could influence neuronal activity and function.

Estimates of neurotransmitter concentration obtained with MRS cannot be taken as direct indices of neural signaling because the very same molecule often serves many different functions across multiple compartments (Rae 2014). For example, MRS estimates include both the neurotransmitter glutamate and the glutamate used to produce energy; they include both the cytoplasmatic glutamate and the glutamate stored in vesicles or released in the synaptic cleft, etc. For these reasons, measuring a change in neurotransmitter concentration with MRS does not allow for concluding that a shift of brain function has occurred; drawing such conclusions requires measures of brain activity and response to stimulation, possibly acquired in the same conditions and participants (Stagg et al. 2011).

One established methodology to noninvasively assess the activity and function of the brain cortex is through electro-encephalography (EEG), either performed in combination with visual stimulation or in resting-state conditions. A visual stimulus that oscillates in time elicits a reliable neuronal rhythmic response readily measured with EEG and termed steady-state visual evoked potential. Its amplitude provides a sensitive index of visual cortex excitability (Hudnell and Boyes 1991), indicative of the overall balance between glutamate and GABA (Boker and Heinze 1984; Bartel et al. 1988; Bale et al. 2005; Geller et al. 2005). By superimposing visual stimuli of different orientations (cross-orientation stimuli), steady-state visual evoked potentials reveal a strong reciprocal inhibition, previously shown to depend on GABAergic signaling (Morrone et al. 1982; Morrone and Burr 1986; Morrone et al. 1987; Smith et al. 2006). The resting-state EEG power spectrum does not directly index neuronal responsiveness, but it relates to endogenous rhythms—which characterize brain activity in both resting conditions and during task engagement and attentional allocation. The largest of these is the alpha rhythm, primarily recorded from posterior (occipital and parietal) electrodes and prominently increased in periods of reduced alertness and in the absence of sensory stimulation (Womelsdorf et al. 2014).

Here, we used steady-state visual evoked potentials and resting-state EEG to evaluate the acute effects of a single-dose supplementation of a βHB ester in healthy young adult humans. In the same session and participants, we used MEGA-PRESS MRS to measure GABA+ and glutamate levels in the occipital (visual) cortex. Our aim was to acquire evidence of concurrent neurometabolic and neurophysiological changes induced by acute βHB supplementation in healthy young human brains. Given the recent evidence discussed above (Hone-Blanchet et al. 2023), we expected a change in glutamate and GABA+ concentrations. However, the age dependency of the Hone-Blanchet et al.'s results prevented us from making definite predictions on the direction of such changes—as GABA+ decreases were mainly characteristic of participants aged 40 years or more, providing less clear information about young adult participants in the 20–40 years of age, on which we focused. A correlation between increased concentration of glutamate (or decreased GABA+) and increased neuronal responsiveness would suggest an overall increase in neuronal excitability.

2 | Methods

2.1 | Participants

Experimental procedures were approved by the regional ethics committee (*Comitato Etico Regionale per la sperimentazione clinica della Regione Toscana—Sezione Area Vasta Nord Ovest*; protocol "Studio BetaidrossiBrain: effetto del betaidrossibutirrato sulla attività della corteccia cerebrale") and are in line with the declaration of Helsinki. Participants gave written informed consent prior to inclusion and completed a confidential medical screening questionnaire to determine eligibility.

Sample size was defined by a power analysis based on a recent study measuring the impact of βHB supplementation upon neurometabolites concentrations through MRS

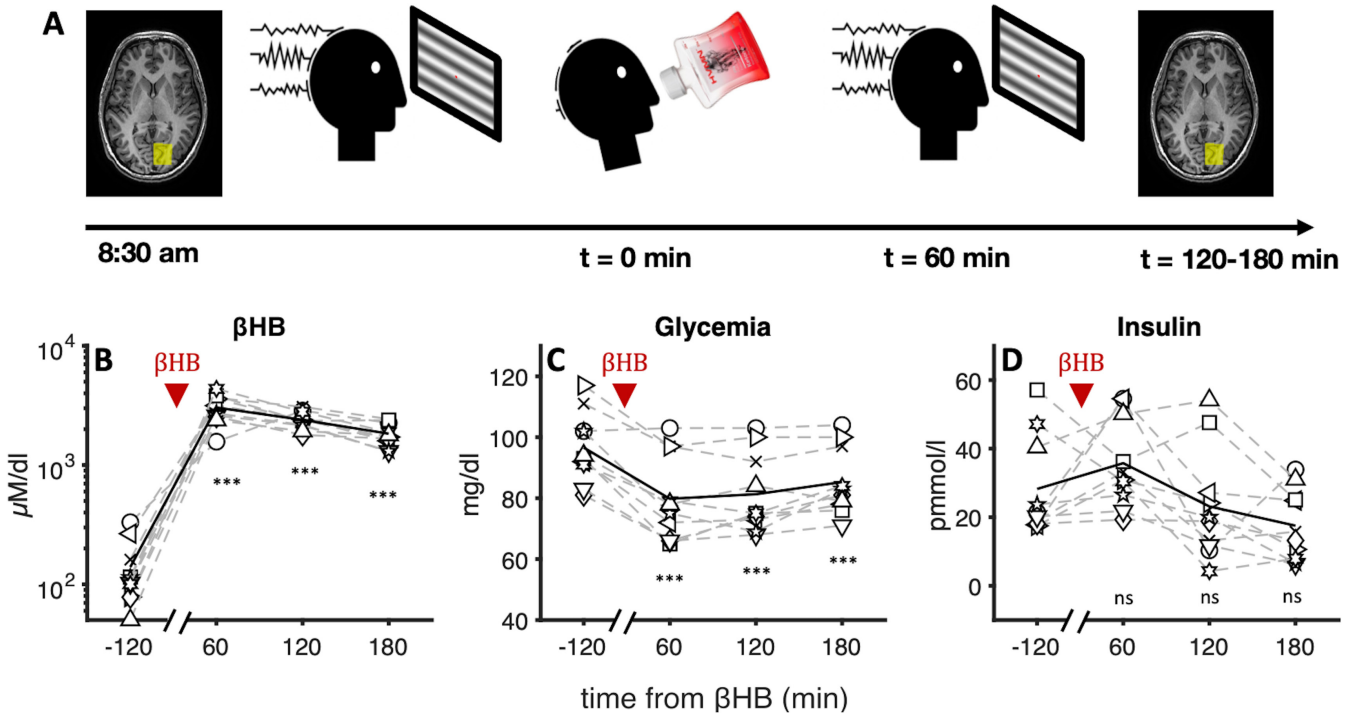

FIGURE 1 | Experimental design and time-courses of metabolic parameters. A) Experimental procedure. The experiment lasted about 6 h per participant, with metabolic, EEG and MRS measurements taken at baseline and after βHB supplementation. B) Plasma concentrations of βHB. C) Glucose. D) Insulin, at baseline and 60, 120, and 180 min after βHB supplementation (marked by red triangle). In each panel, the solid black line shows the average time-course across participants, with individual participants shown as open symbols (symbol-participant pairing is consistent across all figures). Text insets give the significance of post hoc tests comparing post supplementation measures with baseline (ns, nonsignificant, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, Bonferroni corrected).

(Hone-Blanchet et al. 2023), and reporting a large modulation of glutamate concentrations (Cohen's $d = 1.13$, computed from their Figure 3B). Given an a priori chosen alpha level of 0.05 (two-tailed) and power of 0.80, this sets the minimum required sample size to 9.

We recruited 12 healthy volunteers (2 females, 10 males). The selection criteria included normal or corrected-to-normal vision and no known neurological, psychiatric, or metabolic condition and age between 18 and 40 years. One male participant was excluded due to abnormal fasting glycemia (> 140 mg/dL). The recruitment was initially limited to male volunteers, given the possible dependence of neurometabolic profiles on the menstrual cycle (as shown in: Harada et al. 2011; De Bondt et al. 2015; but note: Song et al. 2025); however, given the difficulty of reaching the planned sample size, we eventually included two female volunteers, one of whom had to be excluded because of an anatomical abnormality encountered (incidental MRI finding of partial agenesis of the corpus callosum).

Participants came to the CNR-Fondazione Regione Toscana G. Monasterio (Pisa, Italy) at 8:00 a.m., after an overnight fast of 12 h. Blood samples were collected upon arrival, followed by the MRI (magnetic resonance imaging) acquisitions and the EEG recordings. These measurements were all repeated after the βHB supplementation. This was achieved by orally administering a single dose of a βHB ester (HVMN), containing 25 g of D-βHB. One hour later, EEG recordings were repeated and, at about 2 h after βHB supplementation, the second MRI acquisition was performed. The whole experimental procedure could last up to 8 h per participant, which limited the sample size we could achieve—however, the final sample size ($N = 10$) was still larger than the minimum sample size defined by the a priori power analysis.

2.2 | Material and Apparatus

2.2.1 | Collection and Analysis of Blood Samples

Venous blood samples for pharmacokinetic analysis were collected before starting the experimental procedure and at 60, 120, and 180 min after βHB supplementation (Figure 1A). Blood samples were stored on ice, centrifuged (3000 rpm for 15 min at 4°C), and duplicate plasma aliquots were stored at −80°C. Plasma concentrations of βHB, glucose, insulin, C-peptide, glucagon, GLP-1 total, and active were assessed for each time point using the following procedures. βHB was stored in a K2E (EDTA) 7.2 mg BD Vacutainer test tube and assayed using the commercially available ß-hydroxybutyrate LiquiColor (EKF Diagnostics). Plasma glucose was stored in an FX 10 mg 8 mg BD Vacutainer and was assessed using a commercial glucometer (YSI 2300 STAT Plus). Insulin and C-peptide were stored in Litio/Heparina 102 IU BD Vacutainer and measured using a commercially available ELISA assay (Mercodia, DBA Italia S.r.l.). Glucagon, GLP-1 total, and active were stored using a BD P800 Blood Collection System test tube. Glucagon was assayed using a commercially available ELISA assay (Mercodia, DBA Italia S.r.l.), whilst GLP-1 levels were assessed with a dedicated commercially available ELISA kit (Merck Life Science S.r.l.).

2.2.2 | EEG Recordings

EEG was recorded using a wireless g. Nautilus system, with a sampling rate of 250 Hz. The scalp electrodes were positioned according to the 10–20 international system, and the reference electrode was positioned on the right earlobe. The impedance was checked before each recording and kept below 50 kΩ. For the present study, we recorded from 8 electrodes, including central electrodes *FZ*, *CZ*, and the posterior and occipital electrodes *PZ*, *OZ*, *PO3*, *PO7*, *PO4*, and *PO8*. Electrode *PO7* failed over the course of the study after recording seven participants; the *PO7* EEG data were eliminated for all participants.

Visual stimuli were generated with Psychtoolbox for Matlab (Matlab r2017b, The Mathworks Inc.) and displayed on a gamma-calibrated Barco monitor (Barco CDCT 6551, 800×600 pixels, 100 Hz) through a ViSaGe (CRS, Cambridge Research Systems, Rochester, UK). A custom-made trigger connected the ViSaGe with the g. Nautilus system. Participants were positioned at 114 cm from the screen and maintained their gaze on a black fixation spot (0.5 deg. diameter, <1 cd/m^2) visible at the screen center.

We measured steady-state visual evoked potentials with a whole-screen (20 × 15°) horizontal sinusoidal grating (spatial frequency of 1 c/°, Michaelson contrast of 35%, mean luminance of 51.8 cd/m^2), modulated over time according to a sinusoidal function with a frequency of 8.33 Hz. In a separate recording, we measured steady-state visual evoked potentials masked by superimposing the same test stimulus with a vertical grating mask (spatial frequency of 0.8 c/°, Michaelson contrast of 45%) modulated sinusoidally at a frequency of 7.1 Hz (Morrone and Burr 1986). In this condition, the response to the test is strongly attenuated, and the final response is proportional to the product of excitation by inhibition (Atallah et al. 2012).

In addition, EEG recordings in resting state conditions were acquired while participants kept their eyes open, gazing at the fixation spot shown against an otherwise homogeneous grey screen (size 20 × 15°, luminance 51.8 cd/m^2, matched to the mean luminance of the visual stimuli used for steady-state visual evoked potentials).

2.2.3 | MRI and MRS Acquisitions

We acquired MRI data on a 3-T scanner (GE HDx TWINSPEE, GE Medical Systems, Wisconsin, Milwaukee, USA) at CNR-Regione Toscana G. Monasterio (Pisa, Italy) using an 8-channel head coil equipped with TwinSpeed gradients.

We collected a three-dimensional (3D) fast spoiled gradient recall T1-weighted images (*3D SPGR*, TR/TE = 10.7/4.9 ms, FOV = 25.6 cm, acquisition matrix = 256 × 256, voxel size = 1 mm isotropic, slice gap = 0 mm, BW = 15.6 kHz, NEX = 1) covering the entire brain. We collected MRS data with a MEGA-PRESS (Mescher et al. 1998) sequence (TE = 68 ms; TR = 1500 ms; 320 transients of 4096 data points acquired in 8-min experiment time; 16-ms Gaussian editing pulse applied at 1.9 (ON) and 7.46 (OFF) ppm); for further information, please see Supplemental Table S1, which was set up following consensus guidelines (Bell et al. 2021).

We acquired spectra from two MRS voxels (25×25×25 mm): before and after βHB supplementation. We manually positioned the voxel based on each participant's anatomical T1 scan, ensuring to maintain the same voxel placement before and after βHB supplementation. To cover visual areas, we centered the MRS voxel on the right the calcarine sulcus (Figure 3A) in all but two participants, where the voxel was centered on the left calcarine sulcus to minimize inclusion of the ventricular space. During the MRS acquisitions, participants kept their eyes closed.

2.3 | Quantification and Statistical Analysis

2.3.1 | EEG Analysis

Offline analyses were performed using EEGlab (14.1.2b) and custom-made MATLAB scripts (MATLAB 2019a; MathWorks). The raw EEG signal was visually inspected, and epochs with obvious artefacts were discarded from further analyses; these include epochs contaminated by muscle activity (detectable as a high-frequency signal spreading across multiple channels) or by large eye movements and blinks (large and characteristic voltage variations in frontal-central electrodes).

For steady-state visual evoked potentials, the EEG signal was divided into epochs of 240 ms (2 cycles of stimulation), synchronized with the alternation of the grating stimulus. Based on the artefact detection criteria above, 5.6% of epochs were excluded before supplementation and 4.9% after supplementation. Valid epochs were averaged (separately for each participant and condition, pre vs. post βHB supplementation), and a fast Fourier transform (FFT) was applied to the average trace. The amplitude of the second harmonic (twice the stimulus frequency, i.e., 16.6 Hz) was used to index the visually evoked response. The resting state EEG signal was divided into 10-s-long epochs, of which 2.5% were excluded pre supplementation and 3.9% in post supplementation.

An FFT was then applied to each epoch, and the amplitude spectrum was averaged across epochs (separately for each participant and condition). The power in three frequency ranges of interest (theta: 4–8 Hz, alpha: 8–13 Hz, and beta: 13–30 Hz; frequency ranges include the lower limit and exclude the upper one) was integrated in each range and then square-rooted to obtain amplitude estimates.

2.3.2 | MRS Analysis

MRS data preprocessing was performed using MRSpa v1.5 (https://www.cmrr.umn.edu/downloads/mrspa/). Eddy current, frequency, and phase correction were applied before subtracting the average ON and OFF spectra, resulting in one edited spectrum per participant and condition. We used LCModel (Provencher 2001), with a dedicated MEGA-PRESS basis set ("mega-press-3") to fit the spectra within the chemical shift range from 1.9 to 4 ppm (Figure 3B) with the aim of avoiding artefacts contamination. We estimated the concentration of the following neurometabolites: glutamate (Glu), glutamine (Gln), γ-aminobutyric acid (GABA+), glutathione (GSH), and *N*-acetylaspartate (NAA). We refer to GABA concentration

TABLE 1 | Anthropometrics and metabolic variables reported as mean (SD) [range].

Variables	Baseline	60 min	120 min	180 min
BMI in kg/m^2	22 (2.15) [19.23–25.83]	—	—	—
βHB in μmol/L	140 (90.14) [50.23–331.80]	3036 (826.09) [1571.43–4351.60]	2393 (414.99) [1790.24–3078.34]	1834 (407.76) [1302.44–2433.18]
Glucose in mg/dl	97 (11.46) [81.00–117.00]	80 (14.21) [65.00–103.00]	81 (12.79) [68.00–103.00]	85 (11.12) [71.00–104.00]
Insulin in μU/mL	28 (14.47) [17.50–57.12]	36 (13.01) [19.29–54.57]	23 (16.18) [4.24–54.14]	18 (10.37) [6.31–33.91]
C-peptide in ng/mL	1.2 (0.48) [0.23–1.63]	1.3 (0.45) [0.54–2.06]	1.1 (0.39) [0.40–1.59]	0.8 (0.36) [0.18–1.25]
Glucagon in ng/mL	7 (3.97) [0.50–15.00]	9.7 (10.13) [1.10–37.30]	10.8 (9.78) [3.30–37.30]	—
GLP1-total in ng/mL	37.9 (18.94) [18.00–84.00]	38.6 (24.40) [17.10–100.10]	40.8 (23.39) [18.50–100.20]	—
GLP1-active in (ng/mL)	4.8 (1.65) [0.50–6.30]	2.5 (2.44) [0.40–6.30]	3.7 (2.46) [0.50–7.40]	—

Anthropometrics and metabolic variables. For each variable, the first numerical value is the arithmetical mean of the concentration at the time point specified in the header, the values in round brackets are standard deviations, and those in the square brackets are the lower and upper values across the participant population.

as "GABA+," as MRS measurements of GABA with MEGA-PRESS are known to include coedited macromolecules (Mullins et al. 2014).

We focused on glutamate rather than GLX because βHB has been shown to directly affect glutamate's levels (Hone-Blanchet et al. 2023). Previous work using MEGA-PRESS at 3 T has shown that glutamate and glutamine signals can be separable and reliably quantifiable using LCModel (Sanaei Nezhad et al. 2018). Our glutamate measurements are in line with the spectral quality criteria outlined in previous work (Sanaei Nezhad et al. 2018; Jia et al. 2022). Both glutamate and GABA+ had Cramér-Rao lower bound (CRLB) values smaller than 10%. As we were not sure that βHB supplementation would not have affected other metabolites, like NAA, we elected as reference for our GABA+ and glutamate concentrations the water peak. To avoid variability in voxel tissue composition, metabolite concentration values were further corrected using the alpha-method described by Harris et al. (Harris et al. 2015):

$$\text{Metab\_corr} = \frac{\text{Metab\_measured}}{(\text{fGM} + \alpha\,\text{fWM})} \times \frac{\mu\text{GM} + \alpha\mu\text{WM}}{\mu\text{GM} + \mu\text{WM}}$$

where fGM and fWM are the fractions of individual grey and white matter content in each participant's voxel, μGM and μWM are the GM and WM fractions of the group average voxel fractions, and α value is set to 0.5 (Harris et al. 2015).

CSF, grey (fGM), and white matter (fWM) fractions were extracted through co-registering high resolution three-dimensional T1 images (segmented with FreeSurfer: Fischl et al. 2002) with a binary MRS voxel mask. There was no significant difference between CSF ($fCSF_{pre} = 5.2\%$, $fCSF_{post} = 5.5\%$, $t(9) = 0.4$), grey ($fGM_{pre} = 47.9\%$, $fGM_{post} = 48.4\%$, $t(9) = 0.8$), and white ($fWM_{pre} = 47.2\%$, $fWM_{post} = 45.9\%$, $t(9) = 0.6$), matter segmentation values between baseline and post βHB supplementation.

3 | Results

3.1 | Acute βHB Supplementation Increases βHB Levels in the Bloodstream

Before and after supplementation of a βHB ester, we monitored the main metabolic parameters through hourly blood sampling for 3 h (Table 1).

Plasma βHB concentrations (Figure 1B) rose from a mean baseline level of 140 μmol/L to a maximum of 3036 μmol/L 1 h after βHB supplementation, in line with previous reports (Clarke et al. 2012; Mikkelsen et al. 2015; Stubbs et al. 2017). The variation was significant (one-way ANOVA for repeated measures, $F(3,27) = 74.3$, $p < 0.001$). After the peak response, plasma βHB decreased nonlinearly (Stubbs et al. 2017) and was still significantly elevated 180 min following ingestion (post hoc $t = 8.31$, Bonferroni corrected $p < 0.001$).

Plasma glucose levels (Figure 1C) decreased with time ($F(3,27) = 24.8$, $p < 0.001$) and remained below baseline throughout the experiment (all post hoc $t > 5.2$, all Bonferroni corrected $p < 0.001$).

Plasma insulin concentrations (Figure 1D) showed a subtler and more variable change ($F(3,27) = 5.3$, $p = 0.005$), with a nonsignificant rise at 60 min after βHB supplementation (post hoc $t = 1.59$, Bonferroni corrected $p = 0.755$), followed by a progressive nonsignificant decreasing trend (all post hoc $t < 2.2$, all Bonferroni

corrected $p > 0.197$). The plasma C-peptide levels showed a similar trend as insulin levels.

The active fraction of GLP1 was significantly modulated ($F(2,18) = 4.1$, $p = 0.034$), with a decrease at 60 min (post hoc $t = 2.87$, Bonferroni corrected $p = 0.030$). The other parameters (GLP1-total and glucagon) did not show reliable changes (both $F(2,18) < 1.45$, both $p > 0.261$ after correction with Greenhouse–Geisser).

3.2 | Acute βHB Supplementation Increases Steady-State Visual Evoked Potentials Amplitude and Resting-State Alpha Amplitude

To investigate whether acute βHB supplementation modulates brain activity, we used EEG to measure steady-state visual evoked potentials and endogenous rhythms in resting state with eyes open. We acquired these measures immediately before βHB supplementation (blue lines in Figure 2) and 60 min afterward (red lines), corresponding to the peak βHB plasma level.

Figure 2A–D show the steady-state visual evoked potentials for the occipital electrode *OZ*. Figure 2A displays the voltage modulation elicited by an alternating sinusoidal grating; as expected, this is mainly at the second harmonic (16.6 Hz) of the stimulus temporal frequency (8.3 Hz). Figure 2C shows the voltage modulation elicited by the same grating, coupled with an orthogonal mask alternating at a different frequency (7.1 Hz) that strongly attenuates the response—in line with previous cross-orientation inhibition studies (Morrone et al. 1982; Morrone and Burr 1986; Morrone et al. 1987). Figure 2B, D report the amplitude of the second harmonic for both stimulus types, pre and post supplementation, again tightly correlated and supporting the reliability of the measurements (grating alone: Pearson's $r = 0.96$, $p < 0.001$; grating plus mask: Pearson's $r = 0.94$, $p < 0.001$). We evaluated the impact of βHB supplementation on the amplitude of responses to both stimulus types, with a two-way ANOVA for repeated measures with factors: time (pre and post supplementation) and stimulus (grating alone and grating plus orthogonal mask). This revealed a significant main effect of time ($F(1,9) = 9.28$, $p = 0.014$) and stimulus ($F(1,9) = 24.38$, $p < 0.001$), and a significant interaction between factors ($F(1,9) = 8.36$, $p = 0.018$). Post hoc t-tests showed that βHB supplementation selectively affected steady-state visual evoked potentials elicited by the individual grating ($t = 4.2$, Bonferroni corrected $p = 0.003$), while leaving responses to the grating plus orthogonal mask unaffected ($t = 0.25$, Bonferroni corrected $p = 1$); importantly, responses to both stimuli were tightly correlated before vs. after the βHB supplementation (Pearson's $r = 0.96$, $p < 0.001$ for the individual grating; $r = 0.94$, $p < 0.001$ for the grating plus orthogonal mask). The observed effect of βHB supplementation on steady-state visual evoked potentials elicited by the individual grating is large (Cohen's $d = 1.04$), as the increment is an average of 17% of the amplitude before βHB supplementation (regression line: post supplementation = 0.4 + 1 × pre supplementation).

Figure 2E shows the amplitude spectrum of the resting state EEG for the occipital electrode *OZ*. There is a marked amplitude increase within the alpha band (8–13 Hz, indicated by vertical dashed lines) after βHB supplementation, while slower and faster rhythms are less clearly modulated (see also Figure S2A,B for resting-state spectra across all recording electrodes). Figure 2F shows that the alpha-amplitude (quantified as integral of amplitude values over the alpha band) was reliably higher after βHB supplementation than before ($t(9) = 4.62$, $p < 0.001$; regression line: post supplementation = 0.22 + 1.14 * pre supplementation), while theta and beta rhythms were nonsignificantly affected (theta: $t(9) = 2.5$, $p = 0.033$; beta: $t(9) = 1.7$, $p = 0.12$; both p-values are above the $0.05/3 = 0.016$ Bonferroni corrected threshold). A similar effect was seen across all recording electrodes, both occipital and fronto-parietal (Figure S2C–H). Across all these electrodes, pre and post supplementation alpha-amplitudes were correlated: all Pearson's $r > 0.93$ and $p < 0.001$, supporting the reliability of the measurements. The alpha change in OZ (Figure 2E–F) was not correlated with the change of steady-state visual evoked potentials amplitude (Figure 2A–B): Pearson's $r(10) = 0.12$ and $p = 0.74$.

3.3 | Acute βHB Supplementation Increases Glutamate in Occipital Cortex

We used a dedicated MRS MEGA-PRESS sequence to evaluate GABA+ and glutamate concentrations within a 25 × 25 × 25 mm voxel placed in the occipital cortex (Figure 3A). Figure 3B shows the MR spectra (mean across participants) before βHB supplementation and an average of 120 min after it (blue and red lines, respectively).

After βHB supplementation, there was an increase in glutamate concentration (Figure 3C, Glu/water, $t(9) = 2.52$, $p = 0.033$) with no change in GABA+ (Figure 3D, GABA+/water, $t(9) = 0.11$, $p = 0.91$) or glutamine levels (Gln/water, $t(9) = -1.16$, $p = 0.274$). A two-way ANOVA for repeated measures with factors time (pre and post supplementation) and neurometabolite (glutamate and GABA+) revealed a significant time by neurometabolite interaction ($F(1,9) = 5.88$, $p = 0.038$), with no significant main effects (time: $F(1,9) = 4.03$, $p = 0.075$; neurometabolite: $F(1,9) = 3.04$, $p = 0.115$). The increase in glutamate levels in the face of unaltered GABA+ levels is also seen as a significant increase in the glutamate to GABA+ ratio ($t(9) = 2.32$, $p = 0.046$); this ratio has been interpreted as an index of excitation/inhibition balance in the cortex (Steel et al. 2020; Jia et al. 2022), although this issue is controversial (Jia et al. 2022). Note that the glutamate concentration change is unlikely to result from changes in spectral fitting quality, as glutamate concentration levels estimated before and after βHB supplementation were correlated ($r(10) = 0.70$, $p = 0.025$). The glutamate increase is 13% of the glutamate levels before βHB supplementation (Cohen's $d = 0.8$).

3.4 | The Increases of Glutamate Concentration and Steady-State Visual Evoked Potentials Amplitude Following Acute βHB Supplementation Are Correlated

We found a significant correlation between the increase of glutamate concentration measured with MRS (referenced on

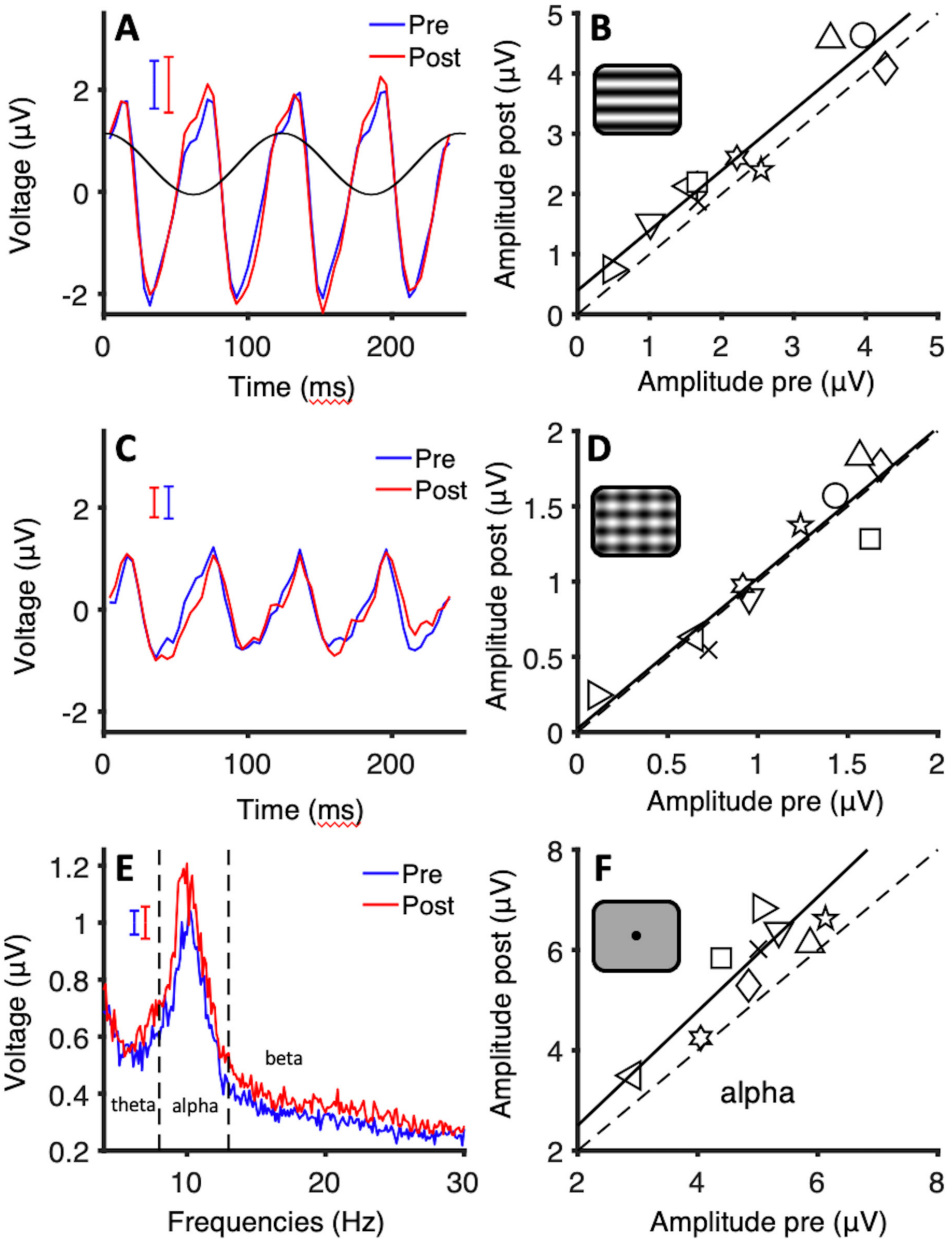

FIGURE 2 | Effects of βHB supplementation on EEG recordings in the OZ electrode. A) Waveforms of steady-state visual evoked potentials, elicited by horizontal grating oscillating in time at 8.33 Hz. B) Amplitude of steady-state visual evoked potentials in each participant (second harmonic of the stimulus frequency), post vs. pre supplementation, again showing a consistent enhancement in most participants. C) Waveforms of steady-state visual evoked potentials, elicited by combining the same horizontal grating oscillating in time at 8.33 Hz with an orthogonal grating oscillating at 7.1 Hz, yielding markedly smaller responses (compare with panel A), as expected from cross-orientation inhibition. D) Amplitude of steady-state visual evoked potentials elicited by the combined grating and orthogonal mask stimulus, showing no change after βHB supplementation. E) Amplitude spectra of EEG recordings measured in resting-state with eyes open, immediately before (blue) and 60 min after (red) βHB supplementation; the vertical dashed lines mark the frequency range used to extract the amplitude of the alpha rhythm (8–13 Hz). F) Alpha amplitude in each participant, values acquired after βHB supplementation plotted against the corresponding values acquired before it. All data points are above the bisection of the axes (dashed line), implying that alpha amplitude was consistently increased. In panels A, C, and E, curves are averages across participants and the floating error bars show the S.E.M. across participants. In panels B, D, and F, the dashed line marks the y = x function and the continuous black line gives the best fitting linear function across the data points; icons in the top-left corners represent the visual stimulation conditions: a single horizontal grating in B and the same horizontal grating overlaid with a vertical grating in D, no stimulus in F. All results are from the OZ electrode.

water and alpha corrected) and the increase of the steady-state visual evoked potential measured with EEG (individual grating), as shown in Figure 4A (Pearson's $r = 0.67$, $p = 0.035$) across participants. Interestingly, the levels of glutamate measured after βHB supplementation (Glu/water, alpha-corrected value) correlated with the rate of change in βHB plasma levels between 120 and 180 min (Figure 4B, Pearson's $r = 0.69$, $p = 0.026$), which is the time window where spectra were acquired. We also explored correlations between MRS parameters and the other significant change measured with EEG, the

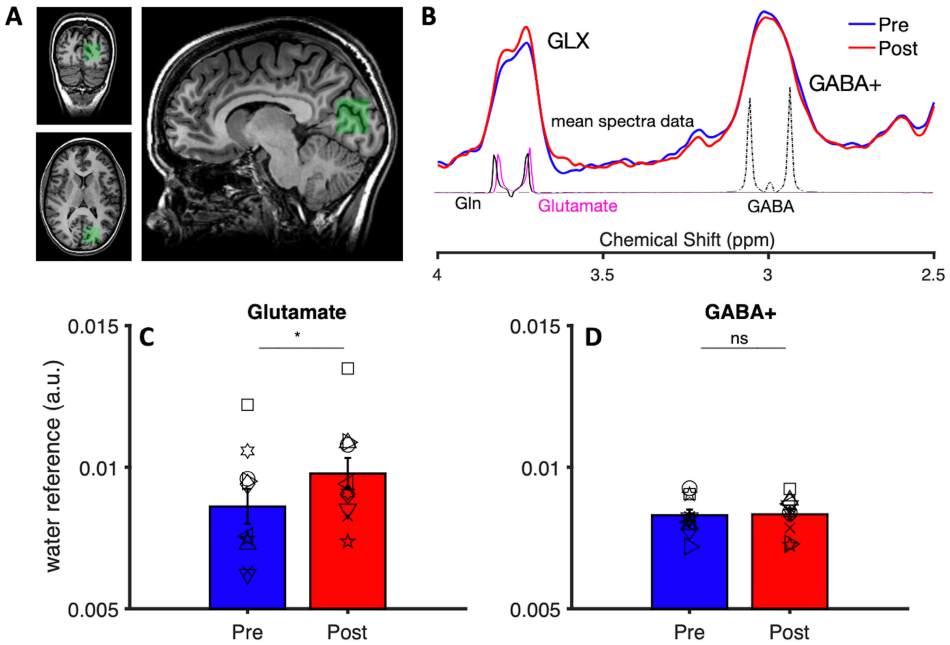

FIGURE 3 | Effects of βHB supplementation on MEGA-PRESS MRS estimates of neurometabolite concentrations in the occipital cortex. A) Example of MRS voxel positioning for one example participant. B) MEGA-PRESS MRS spectra averaged across participants, acquired before and after βHB supplementation (blue and red, respectively), with the GLX and GABA+ peaks marked. The lower traces illustrate the basic functions used by LCModel to model the contribution of GABA+ (dotted black line), glutamate (Glu, pink solid line), and glutamine (Gln, solid black line) to the measured spectra. C–D) Estimated concentrations of glutamate and GABA+ (referenced to water). In all plots, open symbols show individual participants and error bars report S.E.M. Text insets give the significance of post hoc t-tests comparing post-supplementation measures with baseline (ns, nonsignificant, $*p < 0.05$).

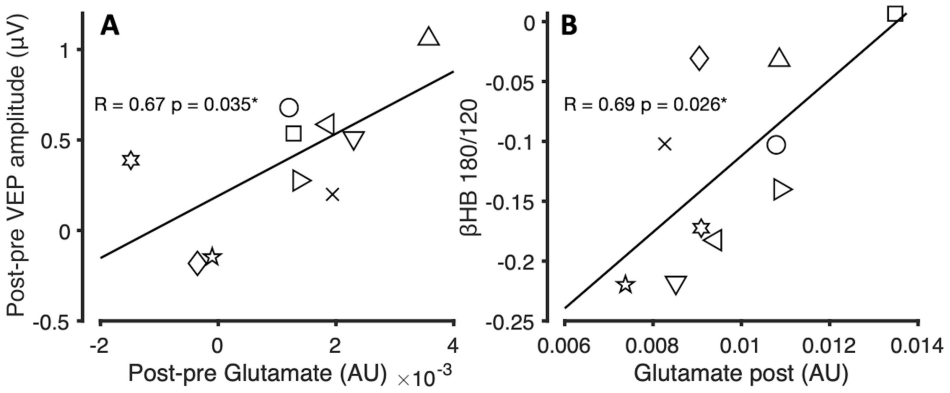

FIGURE 4 | Correlations between MRS, EEG and βHB plasma levels. A) The amplitude change of steady-state visual evoked potentials is directly correlated with the change of glutamate concentration (referenced to water) post-pre βHB supplementation. B) Glutamate concentrations after βHB supplementation (referenced to water) are directly with the rate of βHB clearance, measured as the ratio between βHB plasma levels at 2 and 3 h after the supplementation. In both panels, the continuous black line gives the best fitting linear function across the individual participants data (open symbols); text insets give the Pearson's correlation coefficients with associated p-value ($*p < 0.05$).

increased alpha-amplitude in resting state. However, no significant correlation emerged with either glutamate or GABA+ concentration changes (all $|r| < 0.4$, all $p > 0.25$). In addition, we found that our participants' age was not significantly correlated with either the neurometabolic or the EEG changes (all $|r| < 0.17$, all $p > 0.63$); note that this may be predominantly due to the much smaller age range explored here compared to previous studies (Hone-Blanchet et al. 2023).

4 | Discussion

Although there have been investigations of how acute ketone supplementation affects brain metabolism (Hone-Blanchet et al. 2023), the present study is the first that combines neurometabolic measures with established physiological measures of brain activity in healthy young adult humans. We found that acute βHB supplementation increased glutamate concentrations

in the visual cortex, leaving GABA+ levels unchanged, enhancing cortical responses to visual stimulation and endogenous alpha rhythms. This provides strong evidence that this metabolic intervention promotes a functional shift of cortical circuits, compatible with the neurometabolic shift.

We evaluated visual cortical function through an established measure, the amplitude of steady-state visual evoked potentials. This is tightly linked with the balance between excitatory and inhibitory cortical signaling: Steady-state visual evoked potentials were enhanced when the glutamate to GABA ratio was increased (Hudnell and Boyes 1991; Bale et al. 2005; Geller et al. 2005). Consistent with this, we observed enhanced steady-state visual evoked potentials paralleling the increased concentration of occipital glutamate levels, with the two increases positively correlated across our participants. Although MRS estimates of glutamate concentrations cannot be taken as a direct index of glutamatergic signaling (Rae 2014), there is evidence that glutamate concentrations estimated with MRS are systematically and positively correlated with cortical excitability, both in humans (where excitability was indexed by TMS: Stagg et al. 2011) and in animal models (where it was monitored by calcium imaging: Takado et al. 2022). Our results provide further support for this concept, showing a positive relationship between glutamate levels and an established electrophysiological index of cortical responsivity, steady-state visual evoked potentials.

In contrast with the enhanced steady-state visual evoked potentials to individual grating stimuli, we found that the (attenuated) responses observed with the superposition of an orthogonal mask (yielding cross-orientation inhibition: Morrone et al. 1982) were unaffected by βHB supplementation. This dissociation could be expected if the balance between excitation and inhibition elicited by the cross-orientation stimulus was unaltered. If βHB supplementation enhanced both the grating and the mask response in a similar way (i.e., enhancing the excitatory drive to the cortex) and given the divisive nature of the cross-orientation inhibition phenomenon (Morrone and Burr 1986; Atallah et al. 2012), this would explain the observed unaltered responses to the composite stimulus. Thus, the results from our cross-orientation inhibition stimuli may be interpreted as indirect evidence that βHB supplementation did not alter GABAergic signaling, in line with our MRS GABA concentration estimates.

We also measured the amplitude of the endogenous rhythms in resting-state with eyes open. Across the frequency range of interest (from 4 to 30 Hz, including theta, alpha, and beta), we found that only the alpha rhythm was reliably enhanced by βHB supplementation. The alpha modulation was not correlated with the change of steady-state visual evoked potentials, and it was not correlated with the concentration of neurotransmitters in the visual cortex measured with MRS (in contrast with the positive association between the increase of steady-state visual evoked potentials and the glutamate increase). The alpha rhythm is the most prominent resting EEG pattern recorded from the occipital and parietal cortex; it is known as the "idling" rhythm because it is most prominent in resting-state conditions, in the absence of sensory stimulation (Womelsdorf et al. 2014). Alpha oscillations are also measurable during task performance, when alpha amplitude is often inversely related to the amplitude of sensory responses—e.g., alpha is enhanced in cortical regions representing distractor stimuli, away from the focus of spatial attention (Kelly et al. 2006). While alpha generation is often associated with GABAergic signaling, the underlying circuitry is very complex and still incompletely understood (Womelsdorf et al. 2014). It is likely that distinct circuits support the generation of alpha rhythms in resting-state versus task performance (Bastiaens et al. 2025). Moreover, the circuitry is not only localized within the cortex but also involves thalamo-cortical projections (Womelsdorf et al. 2014; Bastiaens et al. 2025). This opens the possibility that resting-state alpha amplitudes are better predicted by neurotransmitter levels in the thalamus. Because our MRS measurements were restricted to the cortex, we cannot address this question, which remains open for future studies.

Very few previous studies measured the effects of acute (βHB supplementation) or chronic (ketogenic diets) interventions on brain activity or function in healthy humans. A large study with 30 healthy participants used resting-state fMRI to test the effects of an acute single-dose βHB supplementation; their functional connectivity analysis revealed enhanced network stability, which they interpreted as a functional correlate of cognitive acuity (Mujica-Parodi et al. 2020). Given the fundamental differences between their fMRI technique and our EEG measurements, it is difficult to draw parallels between the two sets of findings. Another smaller study used EEG and Transcranial Magnetic Stimulation over the motor cortex to test the effects of a 2-week ketogenic-diet. Sensitivity to single TMS pulses was unaffected, but paired-pulse integration was reduced; in addition, the amplitude of the beta rhythm was enhanced in frontal electrodes (Cantello et al. 2007). This beta enhancement is qualitatively consistent with the enhancement of endogenous rhythms that we report here; however, the unaltered responsivity to TMS pulses does not support the excitability increase suggested by the enhanced steady-state visual evoked potentials we observed. This discrepancy could be related to the protocol used for increasing ketone levels: acute supplementation here versus ketogenic dieting in Cantello et al. (2007) (see also below on the importance of this difference).

The number of studies measuring the impact of ketone supplementation or dieting on clinical populations and animal models is much larger, and their results are exceedingly heterogeneous (Machowiec et al. 2022). Many of these studies focused on epilepsy, given the long-established effect of ketogenic dieting or fasting as anticonvulsants (Helmholz and Keith 1933). The beneficial outcome of dieting is commonly interpreted as an enhancement of inhibition by increasing the GABA/Glutamate ratio. However, the direct physiological evidence in support of such an increase is sparse. For example, elevated βHB levels have been associated with GABA increases (in humans: Dahlin et al. 2005, or rodents: Roy et al. 2015; Calderon et al. 2017; Qiao et al. 2024), or no GABA changes (in humans: Wang et al. 2003, or rodents: Yudkoff et al. 2001; Melo et al. 2006; Zhang et al. 2015). Similarly, elevated βHB levels have been associated with glutamate increases (in humans: Wiers et al. 2021, or rodents: Bough et al. 2006), glutamate decreases (in rodents: Melo et al. 2006), or no glutamate change (in rodents: Yudkoff et al. 2001; Calderon et al. 2017). Findings in patients, particularly with epilepsy, are difficult to relate to findings in healthy

controls, given that the neurometabolism is likely to be globally and profoundly shifted in pathological conditions like epilepsy (Bartolomei et al. 2017).

Recognizing the challenge of using a pathological brain condition, one recent study measured healthy rodents undergoing ketogenic dieting and reported no GABA changes but increased glutamate (Gzielo et al. 2020). The first and only study to measure the effects of a single dose of βHB supplementation in healthy humans (before ours) was performed by Hone-Blanchet et al. (2023). Using ^{1}H-MRS at 7 T, they found a decrease in both GABA and glutamate levels in the cingulate cortices; however, the largest decreases were observed for their oldest participants (40-year-old and above). Here, we used a similar βHB supplementation approach combined with MEGA-PRESS MRS at 3 T and selectively tested young adults (aged between 23 and 34 years old), observing a significant increase in glutamate, with no change in GABA+ concentrations. This suggests that the effects of βHB supplementation are critically dependent on age (Hone-Blanchet et al. 2023) and might even take opposite directions in young vs. older individuals. It would be important to directly test this hypothesis, collecting the same EEG and MRS measures we acquired here in a sample of elderly participants, to directly compare the results with our young participants. Without this information, we speculate that an age-dependent change in glucose metabolism might be responsible for this reversal. In vitro work shows that the effect of delivering βHB to cultured glutamatergic neurons is radically different depending on the co-administration of glucose. When βHB and glucose are delivered at equimolar concentrations, glutamate increases (through a shift in the equilibrium of the aspartate–glutamate aminotransferase reaction toward glutamate); however, when βHB is delivered alone, implying lower glucose availability, the effect is reversed (Lund et al. 2009). This might be related to glutamate being exploited to produce energy when glucose levels are critically low (Yudkoff et al. 2008). Based on the established knowledge that physiological aging is accompanied by glucose hypometabolism (Mosconi 2013), we suggest that age differences in glucose metabolism may account for the opposite effects of βHB supplementation in older individuals (included in the sample of Hone-Blanchet et al. (2023)) versus younger individuals (selectively tested here).

One final biochemical consideration concerns the link between glutamate and GABA, the former being a precursor of the latter (Yudkoff et al. 2008). Increasing glutamate levels, for example, by shifting the equilibrium of the aspartate–glutamate aminotransferase reaction, implies that more glutamate becomes accessible to the glutamate decarboxylase reaction to yield GABA. Thus, an acute increase in glutamate concentration (as we observe) could potentially lead to a secondary increase in GABA concentration over a longer time-frame (Yudkoff et al. 2008). Moreover, a persistent increase in βHB levels (e.g., achieved through repeated supplementation or dieting) enhances the expression of monocarboxylic acid transporters (Jensen et al. 2020), responsible for the passage of βHB across the blood–brain barrier. This could enhance the βHB uptake and metabolism in neurons and potentially promote further changes in the neurotransmitter profile. These considerations could help reconcile our observations of increased glutamate concentrations and enhanced cortical excitability following acute βHB supplementation with the long-term beneficial effects of ketogenic diets in some forms of epilepsy, which are interpreted as resulting from a decreased cortical excitability. However, the current results offer no direct evidence on the long-term effects of βHB supplementation.

Our acute protocol produced dramatic changes in plasma βHB levels that are compatible with previous studies (Clarke et al. 2012; Mikkelsen et al. 2015; Stubbs et al. 2017). However, we also observed a minor decrement in plasma glucose. We believe that the latter is not a likely explanation for the observed changes in glutamate concentrations for the following three main reasons: 1) Our glucose levels decreased very mildly and never reached hypoglycemia; 2) reduced glucose availability predicts a decrease in glutamate levels (Yudkoff et al. 2008), opposite to what we found; 3) Hone-Blanchet et al. (2023) showed that a very marked increase in glucose levels, achieved by supplementation, does not systematically alter glutamate or GABA levels as assessed with ^{1}H-MRS. We did not directly measure βHB levels in the brain; however, there is clear evidence that acute βHB supplementation increases βHB levels in the brain since about 30 min following the oral administration (Mujica-Parodi et al. 2020), closely following plasma βHB concentrations. In our results, plasma βHB levels varied little across participants, and the small differences are likely due to normal variations in gastrointestinal function, which may affect βHB hydrolysis and absorption (Clarke et al. 2012). As expected based on Clarke et al. (2012), plasma βHB decayed slowly and showed only a minor change between 2 and 3 h after supplementation. This rate of plasma βHB clearance was correlated with the glutamate levels measured in the visual cortex, that is, individuals with slower clearance showed higher glutamate concentrations following βHB supplementation. This correlation strengthens the link between plasma βHB concentration and glutamate concentration in the brain cortex, which in turn correlates with the amplitude of steady-state visual evoked potentials, closing the loop between our metabolic intervention and the observed changes in brain metabolism and function.

It is important to acknowledge that the effects of acute βHB supplementation can be radically different from the effects of chronic interventions like dieting. Chronically elevated plasma levels of ketone bodies could increase the blood–brain barrier permeability to βHB (by enhancing the expression of its transporter: Leino et al. 2001) and produce epigenetic changes in neural cells (Jang et al. 2023); there is also evidence for changes in the gut microbiota composition with ketogenic dieting (Santangelo et al. 2023), which could impact the brain neurochemistry and function through the gut-brain axis (Olson et al. 2018). As discussed above, the increased availability of glutamate initially triggered by increased levels of ketone bodies (as seen here) may eventually lead to increased GABA synthesis, given that glutamate is the GABA precursor. Thus, studying the effects of acute βHB supplementation does not allow for predicting the consequences of repeated βHB supplementation and of long-term dietary interventions. We acknowledge that this limits the scope of our study, which is not directly relevant for understanding the neural underpinning of clinical applications of ketogenic dieting and supplementation. However, understanding the transient effects of a single dose is a necessary first step toward unraveling the complex consequences of these metabolic interventions.

Another important limitation of our study lies in the small sample size. Although adequate given an a priori power analysis, our $N = 10$ prevented us from investigating fundamental dimensions of inter-individual variability, including sex. Our sample was strongly biased toward the male population, like many previous MRS studies (due to the reported variability of neurometabolite concentrations with the menstrual cycle: Harada et al. 2011; De Bondt et al. 2015; but note: Song et al. 2025). For this reason, our results cannot be directly generalized to the general population. Future studies should address these crucial dimensions.

In conclusion, our combined EEG and MEGA-PRESS MRS results show that βHB supplementation modulates both visual cortical responses and the neurochemical profile of the occipital cortex. The correlated increase of glutamate concentration and of steady-state visual evoked potentials across our young adult participants suggests that a single dose of acute βHB increased their cortical excitability. These findings provide novel evidence that acute βHB supplementation can influence human cortical function; this is important both from a translational perspective (highlighting its potential as a neuromodulation strategy) and for fundamental research, for furthering our understanding of the links between neurometabolism (indexed through MRS) and cortical function (indexed through EEG).

Author Contributions

P.B., M.C.M., D.M., F.F., D.M., G.D.: methodology and resources. C.S., P.B., F.F.: investigation. P.B., C.S.: formal analysis. P.B., C.S., M.C.M.: writing—original draft. P.B., M.C.M., E.F., G.D.: conceptualization. P.B.: Funding. All authors: writing—review and editing.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data and Availability Statement

The experimental data that support the findings of this study are available on Zenodo at the following link: https://doi.org/10.5281/zenodo.16871670.

The dataset includes individual participants' age and other anthropometric variables, as well as individual EEG and MRS results.

Peer Review

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/ejn.70270.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** Individual participants' steady-state visual evoked potentials. **Figure S2:** resting-state EEG rhythms across recording electrodes. **Table S1:** Minimum Reporting Standards in MRS checklist. **Table S2:** MRS metabolite concentrations and quality measures: Glutamate and GABA+ values before alpha-correction and referenced on water, Cramer–Rao lower bound (CRLB), water peak linewidth and signal-to-noise ratio (SNR) are shown for the two MRS voxels (pre and post βHB supplementation).