Supplementary materials (NPP-22-0253)

Supplementary Tables

Table 1: MRS measurements (mean ± SD) in arbitrary units (A.U.) with associated sample sizes (N), and voxel tissue fraction from the voxel positioned on the anterior cingulate region in **Experiment 1**. Metabolites included are GABA, Glu, glutamine (Gln), myo-inositol (mI), glutathione (GSH), total Choline (tCho), total n-acetylaspartate (tNAA) and total creatine (tCr). Results are shown in absolute concentrations and relative to tCr.

		D-βHB bolus	
		Pre-bolus	Post-bolus
MRS measurement	GABA	0.94±0.38 N=16	0.41±0.38 N=13
	GABA/tCr	1.51±0.74 N=16	0.64±0.63 N=13
	Glu	5.99±1.10 N=16	5.37±0.78 N=16
	Glu/tCr	9.27±2.11 N=16	8.09±1.10 N=16
	Gln	2.34±0.68 N=16	2.08±0.31 N=16
	Gln/tCr	3.61±1.01 N=16	3.14±0.46 N=16
	mI	5.23±1.14 N=16	5.00±0.65 N=16
	mI/tCr	7.86±0.62 N=16	7.50±0.59 N=16
	GSH	0.87±0.45 N=16	0.88±0.21 N=16
	GSH/tCr	1.26±0.59 N=16	1.32±0.29 N=16
	tCho	1.25±0.33 N=16	1.19±0.22 N=16
	tCho/tCr	1.87±0.31 N=16	1.78±0.27 N=16
	tNAA	5.69±1.20 N=16	5.39±0.90 N=16
	tNAA/tCr	8.91±2.55 N=16	8.15±1.45 N=16
	tCr	5.19±1.35 N=16	4.26±1.51 N=16
	bHB	N/A	1.40±0.97 N=14
MRS voxel tissue fraction	GM	0.81±0.06 N=16	0.81±0.06 N=16
	WM	0.12±0.06 N=16	0.13±0.17 N=16
	CSF	0.10±0.06 N=16	0.10±0.05 N=16

Table 2: blood levels of D-βHB (mM) and glucose (mg/dL) at all timepoints in **Experiment 1**.

Blood levels	**Baseline**	**Post-bolus**	**Final**	**ANOVA result**
D-βHB	0.15±0.08	2.00±1.44 (*P*<0.001 vs Baseline)	3.76±1.1 (*P*<0.001 vs Post-bolus)	**F(2,48)=92.45, *P*<0.001**
Glucose	93.4±10.4	92.8±12.4 (*P*=0.833 vs Baseline)	74.2±11.2 (*P*<0.001 vs Post-bolus)	**F(2,48)=70.78, *P*<0.001**

Table 3: MRS measurements (mean ± SD) in arbitrary units (A.U.) with associated sample sizes (N), and voxel tissue fraction from the voxel positioned on the posterior cingulate region in **Experiment 2**. Metabolites included are GABA, Glu, glutamine (Gln), myo-inositol (mI), glutathione (GSH), total Choline (tCho), total n-acetylaspartate (tNAA) and total creatine (tCr). Results are shown in absolute concentrations and relative to tCr.

		D-βHB bolus		Glucose bolus	
		Pre-bolus	Post-bolus	Pre-bolus	Post-bolus
MRS measurement	GABA	1.03±0.32 N=24	0.52±0.25 N=25	0.97±0.27 N=24	0.94±0.28 N=24
	GABA/tCr	0.16±0.05 N=24	0.08±0.04 N=25	0.15±0.04 N=24	0.15±0.05 N=24
	Glu	6.40±0.58 N=26	5.65±0.81 N=26	6.38±0.69 N=26	6.3±0.76 N=26
	Glu/tCr	1.01±0.1 N=26	0.88±0.14 N=26	0.99±0.14 N=26	1.00±0.11 N=26
	Gln	2.04±0.34 N=26	1.96±0.31 N=26	2.11±0.37 N=26	2.05±0.34 N=26
	Gln/tCr	0.32±0.06 N=26	0.30±0.05 N=26	0.33±0.06 N=26	0.33±0.05 N=26
	mI	5.38±0.70 N=26	5.41±0.61 N=26	5.50±0.93 N=26	5.28±0.93 N=26
	mI/tCr	0.85±0.07 N=26	0.84±0.07 N=26	0.84±0.06 N=26	0.84±0.05 N=26
	GSH	0.88±0.25 N=26	0.91±0.25 N=26	0.81±0.16 N=26	0.77±0.22 N=26
	GSH/tCr	0.14±0.03 N=26	0.14±0.03 N=26	0.13±0.02 N=26	0.12±0.03 N=26
	tCho	0.90±0.12 N=26	0.90±0.14 N=26	0.92±0.19 N=26	0.89±0.11 N=26
	tCho/tCr	0.14±0.02 N=26	0.14±0.02 N=26	0.14±0.02 N=26	0.14±0.02 N=26
	tNAA	8.90±0.95 N=26	8.83±0.89 N=26	8.93±1.40 N=26	8.81±0.81 N=26
	tNAA/tCr	1.40±0.11 N=26	1.36±0.09 N=26	1.39±0.13 N=26	1.40±0.11 N=26
	tCr	6.36±0.52 N=26	6.47±0.51 N=26	6.49±0.80 N=26	6.31±0.44 N=26
	βHB	N/A	2.91±1.22 N=24	N/A	N/A
MRS voxel tissue fraction	GM	0.74±0.09 N=26	0.73±0.09 N=26	0.74±0.10 N=26	0.74±0.09 N=26
	WM	0.17±0.05 N=26	0.17±0.06 N=26	0.17±0.05 N=26	0.17±0.09 N=26
	CSF	0.10±0.08 N=26	0.10±0.08 N=26	0.09±0.08 N=26	0.09±0.08 N=26

Table 4: blood levels of D-βHB (mM) and glucose (mg/dL) in **Experiment 2**.

	D-βHB bolus				Glucose bolus			
Blood levels	**Baseline**	**Post-bolus**	**Final**	**ANOVA result**	**Baseline**	**Post-bolus**	**Final**	**ANOVA result**
D-βHB	0.18±0.13	2.06±1.44 (*P*<0.001 vs Baseline)	4.19±0.99 (*P*<0.001 vs Post-bolus)	**F(2,60)=167.58, *P*<0.001**	0.19±0.23	0.22±0.27 (*P*=0.72 vs Baseline)	0.1±0.06 (*P*=0.002 vs Post-bolus)	**F(2,54)=6.091, *P*=0.004**
Glucose	100±12.3	96.1±12.5 (*P*=0.13 vs Baseline)	75.2±10.4 (*P*<0.001 vs Post-bolus)	**F(2,60)=135.48, *P*<0.001**	98.9±10.2	107±12.8 (P=0.004 vs Baseline)	129±41.2 (*P*<0.001 vs Post-bolus)	**F(2,29)=13.25, *P*<0.001**

Table 5: descriptive statistics for quality control metrics in MRS measurements in **Experiment 1** and **Experiment 2**. CRLB values are given as percentages. Also show are full width half maximum (FHWM) values of highest peak (NAA) given in ppm (and converted to Hz), and values for signal to noise ratio (S/N).

		Experiment 1		**Experiment 2**			
		D-βHB bolus		D-βHB bolus		Glucose bolus	
		Pre-bolus	Post-bolus	Pre-bolus	Post-bolus	Pre-bolus	Post-bolus
MRS measurement CRLBs	GABA	11±4.32	12.1±3.56	9.83±2.5	11.75±2.71	9.61±2.33	9.48±1.69
	Glu	2.5±0.62	3.11±1.18	2.03±0.21	2.0±0.02	2.04±0.21	2.1±0.3
	Gln	5.39±1.61	6.67±2.5	4.78±1.53	5.13±0.99	4.61±1.1	4.95±1.63
	mI	2.61±0.99	3.39±2.3	2.35±0.49	2.25±0.46	2.39±0.58	2.38±0.59
	GSH	8.07±2.77	9.88±3.37	7.22±1.95	7.75±1.16	7.22±1.73	7.71±2.83
	tCho	3.89±1.6	4.17±1.62	4.0±1.19	3.75±0.71	3.78±0.85	3.9±1.14
	tNAA	1.83±0.71	2.11±0.58	1.09±0.29	1.0±0.02	1.13±0.34	1.09±0.3
	tCr	2.11±0.96	2.61±0.6	1.26±0.45	1.13±0.35	1.26±0.45	1.29±0.46
	βHB	N/A	20.6±19.1	N/A	12.5±6.5	N/A	N/A
Additional outputs	FWHM (NAA peak)	0.05±0.01ppm 16.34±3.56Hz	0.06±0.02ppm 17.43±4.97Hz	0.03±0.007ppm 9.7±2.23Hz	0.03±0.007ppm 10.1±2.06Hz	0.03±0.006ppm 9.46±1.93Hz	0.03±0.007ppm 10±1.96Hz
	S/N	30.8±6	28.5±5	44.2±9.9	40±9.1	45.1±8.8	42.6±7

Supplementary Methods

Detailing of D-βHB pharmacodynamics

D-β-hydroxybutyrate (D-βHB) is one of three basic ketone bodies along with acetoacetate and acetone. It is utilized by the body for energy, as cells convert it to acetyl-coenzyme A (acetyl-CoA) which then enters the TCA cycle. For human ingestion, D-βHB is combined with (R)-1,3-butanediol; the resulting compound (R)-3-hydroxybutyl (R)-3-hydroxybutyrate is commonly referred to as ketone monoester [1]. Upon ingestion, the ester is broken down in D-βHB and (R)-1,3-butanediol, of which the latter gets metabolized in the liver by dehydrogenases and oxidized into D-βHB. The D-βHB produced this way is then secreted from the liver and enters the same pathway as the original D-βHB component of the ketone ester [1, 2].

Detailing of MRS data analysis performed in Experiment 1 and Experiment 2.

Metabolites obtained with LC Model (version 6.3, Copyright S.W. Provencher) include: alanine (Ala), aspartate (Asp), ascorbate (Asc), beta-hydroxybutyrate (βHB), creatine (Cr), gamma-aminobutyric acid (GABA), glucose (Glc), Glutamine (Gln), Glutamate (Glu), glycerolphosphocholine (GPC), glutathione (GSH), myo-inositol (Ins), scyllo-inositol (sIns), lactate (Lac), phosphocreatine (PCr), N-acetylaspartate (NAA), N-acteylaspartylglutamate (NAAG), taurine (Tau). Basis set was provided by Dr. Dinesh Deelchand and used in prior works with 7T MRS [3].

Regarding the estimation of levels of βHB with MRS, we disregarded the 15% CRLB cutoff used for *standard* metabolites. Because pre-bolus levels of MRS βHB in the D-βHB condition should be zero, relying on CRLBs for quality control with a stringent cutoff value would exclude most βHB measurements and prevent calculation of ΔD-βHB with MRS levels of βHB. As suggested in Kreis (2016), relying on cutoff CRLB values may induce error [4].

Supplementary References

1. Clarke, K., et al., *Kinetics, safety and tolerability of (R)-3-hydroxybutyl (R)-3-hydroxybutyrate in healthy adult subjects.* Regulatory Toxicology and Pharmacology, 2012. **63**(3): p. 401-408.
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