

ESM Table 1. Cramér-Rao bound for ¹H- and ³¹P-MRS analysis.

	Right	Left
NAA	3.9 ± 3.0	4.1 ± 4.1
Cr	10.8 ± 7.8	11.9 ± 14.0
Cho	9.5 ± 3.7	8.3 ± 5.7
B-ATP	38.4 ± 16.2	36.8 ± 25.1
α-ATP	18.5 ± 3.6	19.1 ± 4.2
γ-ATP	22.4 ± 5.0	21.6 ± 3.9
PCr	11.2 ± 2.4	10.9 ± 2.1
Pi	45.4 ± 32.5	41.2 ± 15.2
PME	70.0 ± 43.9	72.9 ± 49.3
PDE	41.4 ± 18.0	44.8 ± 26.0

ATP, adenosine triphosphate; Cho, choline; Cr, creatine; NAA, N-acetylaspartate; PCr, phosphocreatine; PDE, phosphodiester; PME, phosphomonoesters; Pi, inorganic phosphate.

ESM Table 2. German pain research network (DFNS) results for each study cohort.

	HV (n=11)	No-DPN (n=9)	Painless-DPN (n=11)	Painful-DPN (n=18)	ANOVA
CDT (z-score)	-0.61 ± 1.48	-0.47 ± 0.93	-2.32 ± 1.25	-2.70 ± 0.65	<0.001 ^{*†§}
WDT (z-score)	-0.59 ± 0.93	-0.32 ± 0.94	-1.86 ± 0.98	-1.98 ± 0.52	<0.001 ^{*†§}
TSL (z-score)	-1.04 ± 1.38	-0.60 ± 0.88	-2.32 ± 1.31	-2.41 ± 0.63	<0.001 ^{*†§}
CPT (z-score)	-0.86 ± 0.45	-0.96 ± 0.25	-0.88 ± 0.44	-0.89 ± 0.27	0.932
HPT (z-score)	0.49 ± 1.52	-0.09 ± 1.24	-1.10 ± 1.32	-1.38 ± 0.58	0.005 ^{*†§}
PPT (z-score)	0.88 ± 0.84	1.22 ± 0.98	1.01 ± 2.04	0.58 ± 2.01	0.797
MPT (z-score)	1.55 ± 2.08	1.43 ± 1.64	-2.62 ± 1.00	-1.70 ± 1.76	<0.001 ^{*†§}
MPS (z-score)	-0.38 ± 1.38	-0.60 ± 1.47	-1.79 ± 0.55	-1.62 ± 0.54	0.002 ^{*†§}
MDT (z-score)	1.06 ± 1.39	0.94 ± 1.22	-2.51 ± 1.76	-2.37 ± 1.41	<0.001 ^{*†§}
VDT (z-score)	-0.00 ± 1.30	-0.22 ± 1.13	-2.47 ± 2.42	-2.78 ± 1.73	<0.001 ^{*†§}
DMA present (%)	0	0	0	0	n/a

Groups were compared using ANOVA for continuous data. Boldface denotes p<0.05. CDT, cold detection threshold; CPT, cold pain threshold; DMA, dynamic mechanical allodynia; HDT, heat detection threshold; HPT, heat pain threshold; MDT, mechanical detection threshold; MPS, mechanical pain sensitivity; MPT, mechanical pain threshold; PPT, pressure pain threshold; VDT, vibration detection threshold. *p < 0.05 HV vs Painless-DPN; † p < 0.05 No-DPN vs Painless-DPN; ‡ p < 0.05 Painless-DPN vs Painful-DPN; § p < 0.05 HV vs Painful-DPN; || p < 0.05 No-DPN vs Painful-DPN.

ESM Table 3. MR analysis results for left, right and mean (total) thalamic structural, 1H- and 31P-MRS results.

	HV (n=11)	No-DPN (n=9)	Painless-DPN (n=11)	Painful-DPN (n=18)	ANOVA
Left Volume, mL	6.51 ± 0.93	6.81 ± 0.90	6.63 ± 0.67	6.84 ± 1.12	0.808
Left NAA:Cho [§]	2.23 ± 0.32	1.88 ± 0.32	1.72 ± 0.34	2.04 ± 0.35	0.022^{§*‡}
Left NAA:Cr [¶]	2.37 ± 0.69	2.38 ± 0.84	2.08 ± 0.62	2.40 ± 0.64	0.721
Left Cho:Cr [¶]	1.15 ± 0.28	1.37 ± 0.56	1.65 ± 1.05	1.33 ± 0.38	0.411
Left Pi:ATP	0.91 ± 0.63	1.31 ± 0.28	1.41 ± 0.41	0.90 ± 0.40	0.010^{*‡}
Left PCr:ATP	2.38 ± 0.47	2.19 ± 0.45	2.33 ± 0.41	2.13 ± 0.38	0.406
Left PME:PDE	0.96 ± 0.47	1.17 ± 0.39	1.00 ± 0.46	1.05 ± 0.49	0.768
Left pH	7.08 ± 0.08	7.05 ± 0.06	7.06 ± 0.08	7.04 ± 0.03	0.351
Right Volume, mL	5.89 ± 0.74	5.97 ± 0.54	5.73 ± 0.58	5.85 ± 0.68	0.873
Right NAA:Cho [§]	2.00 ± 0.24	1.93 ± 0.31	1.80 ± 2.74	2.00 ± 0.32	0.515
Right NAA:Cr [¶]	2.57 ± 0.71	2.45 ± 0.59	1.99 ± 0.35	2.72 ± 1.01	0.219
Right Cho:Cr [¶]	1.18 ± 0.33	1.30 ± 0.61	1.19 ± 0.37	1.23 ± 0.29	0.941
Right Pi:ATP	0.85 ± 0.39	1.10 ± 0.59	1.25 ± 0.65	0.89 ± 0.52	0.241
Right PCr:ATP	2.28 ± 0.24	2.37 ± 0.35	2.13 ± 0.34	2.04 ± 0.27	0.036[¶]
Right PME:PDE	1.15 ± 0.98	0.87 ± 0.31	0.96 ± 0.25	1.04 ± 0.59	0.759
Right pH	7.12 ± 0.09	7.05 ± 0.05	7.02 ± 0.03	7.05 ± 0.10	0.051
Mean Volume, mL	6.20 ± 0.82	6.39 ± 0.69	6.18 ± 0.47	6.34 ± 0.86	0.890
Mean NAA:Cho [§]	2.11 ± 0.26	1.90 ± .031	1.76 ± 0.24	2.00 ± 0.30	0.081
Mean NAA:Cr [¶]	2.47 ± 0.65	2.41 ± 0.71	2.07 ± 0.31	2.56 ± 0.73	0.390
Mean Cho:Cr [¶]	1.17 ± 0.27	1.34 ± 0.58	1.42 ± 0.61	1.42 ± 0.61	0.678
Mean Pi:ATP	0.88 ± 0.41	1.21 ± 0.30	1.33 ± 0.46	0.90 ± 0.36	0.012^{*‡}
Mean PCr:ATP	2.33 ± 0.30	2.28 ± 0.37	2.23 ± 0.34	2.09 ± 0.26	0.197
Mean PME:PDE	1.06 ± 0.59	1.02 ± 0.23	0.98 ± 0.30	1.04 ± 0.45	0.972
Mean pH	7.10 ± 0.07	7.05 ± 0.05	7.04 ± 0.04	7.04 ± 0.06	0.050[*]

Groups were compared using ANOVA for continuous data. Boldface denotes p<0.05. ATP, adenosine triphosphate; Cho, choline; Cr, creatine; NAA, N-acetyl aspartate; PCr, phosphocreatine; Pi, inorganic phosphate. * p<0.05 HV vs Painless-DPN; † p<0.05 No-DPN vs Painful-DPN; ‡ p<0.05 Painless-DPN vs Painful-DPN; § p<0.05 HV vs No-DPN; || p<0.05 HV vs Painful-DPN. ¶ 11 people excluded from analysis due to inadequate ¹H-spectra (HV n=2, No-DPN n=2, Painless-DPN n=3, Painful-DPN n=4).

ESM Table 4. Correlation analysis of NAA:Cho and clinical/neurological variables.

	Right (r, p-value)	Left (r, p-value)	Mean (r, p-value)
Age, years	-2.06, 0.214	-0.26, 0.877	-0.122, 0.542
Duration of Diabetes, years	0.085, 0.662	-0.276, 0.147	0.122, 0.528
Body Mass Index, kg/m ²	-0.294, 0.073	-0.444, 0.005	-0.416, 0.009
Systolic blood pressure, mmHg	0.076, 0.649	0.076, 0.651	0.084, 0.618
HbA1c, mmol/mol	0.254, 0.124	-0.131, 0.432	0.042, 0.803
Total Cholesterol, mmol/l	0.018, 0.915	0.009, 0.957	0.014, 0.933
ACR, mg/mmol	0.130, 0.546	-0.148, 0.490	-0.021, 0.924
Creatinine, μ mol/l	0.021, 0.901	-0.094, 0.575	-0.048, 0.776
TCNS	0.076, 0.650	-0.105, 0.531	-0.028, 0.868
NIS-LL	0.060, 0.721	-0.227, 0.170	-0.111, 0.506
Peroneal CMAP (mV)	0.095, 0.598	0.253, 0.155	0.0200, 0.265
Peroneal MNCV (m/s)	0.169, 0.364	0.438, 0.014	0.348, 0.055
Peroneal MNDL (msec)	-0.212, 0.236	-0.331, 0.060	-0.300, 0.089
Tibial MNDL (msec)	0.104, 0.546	-0.103, 0.548	-0.013, 0.940
Sural SNAP (mV)	0.006, 0.977	-0.025, 0.898	-0.012, 0.950
NIS(LL)+7	0.147, 0.385	-0.223, 0.184	-0.067, 0.693
ESC (μ S)	0.027, 0.875	0.224, 0.189	0.151, 0.378
CDT, z-score	-0.217, 0.191	-0.050, 0.764	-0.136, 0.417
WDT, z-score	-0.097, 0.564	0.123, 0.462	0.029, 0.862
TSL, z-score	-0.073, 0.662	0.102, 0.541	0.028, 0.869
CPT, z-score	-0.224, -0.159	-0.87, 0.602	-0.162, 0.331
HPT, z-score	-0.159, 0.341	0.023, 0.893	-0.063, 0.709
PPT, z-score	-0.361, 0.026	-0.336, 0.039	-0.381, 0.018
MPT, z-score	-0.274, 0.096	0.082, 0.623	-0.081, 0.627
MPS, z-score	-0.036, 0.828	0.110, 0.509	-0.050, 0.763
WUR, z-score	-0.008, 0.976	-0.127, 0.638	-0.32, 0.903
MDT, z-score	0.055, 0.742	0.258, 0.117	0.186, 0.264
VDI, z-score	0.144, 0.390	0.313, 0.056	0.262, 0.112

CDT, cold detection threshold; CMAP, compound muscle action potential; CPT, cold pain threshold; DMA, dynamic mechanical allodynia; ESC, electrochemical skin conductance; HDT, heat detection threshold; HPT, heat pain threshold; MDT, mechanical detection threshold; MNCV, motor nerve conduction velocity; MNDL, motor nerve distal latency; MPS, mechanical pain sensitivity; MPT, mechanical pain threshold; NIS-LL, Neuropathy Impairment score of the lower limb; NIS(LL)+7, Neuropathy Impairment score of the lower limb plus 7 neurophysiological tests; PPT, pressure pain threshold; SNAP, sensory nerve action potential; TCNS, Toronto clinical neuropathy score; VDT, vibration detection threshold.

ESM Table 5. Correlation analysis of PCr:ATP and clinical/neurological variables.

	Right (r, p-value)	Left (r, p-value)	Mean (r, p-value)
Age, years	-0.032, 0.826	0.190, 0.191	0.111, 0.448
Duration of Diabetes, years	0.005, 0.975	0.035, 0.834	0.025, 0.880
Body Mass Index, kg/m ²	-0.105, 0.475	-0.047, 0.750	-0.83, 0.571
Systolic blood pressure, mmHg	-0.247, 0.087	-0.041, 0.779	-0.150, 0.304
HbA1c, mmol/mol	-0.233, 0.107	-0.310, 0.030	-0.322, 0.024
Total Cholesterol, mmol/l	-0.002, 0.988	-0.051, 0.729	-0.035, 0.812
ACR, mg/mmol	-0.220, 0.218	0.046, 0.798	-0.82, 0.649
Creatinine, µmol/l	-0.105, 0.472	0.019, 0.896	-0.039, 0.788
TCNS	-0.424, 0.002	-0.115, 0.430	-0.287, 0.046
NIS-LL	-0.380, 0.007	-0.048, 0.746	-0.220, 0.129
Peroneal CMAP (mV)	-0.506, <0.001	0.147, 0.340	-0.340, 0.024
Peroneal MNCV (m/s)	0.112, 0.481	-0.066, 0.676	0.009, 0.954
Peroneal MNDL (msec)	0.017, 0.910	0.246, 0.107	0.175, 0.257
Tibial MNDL (msec)	-0.535, <0.001	-0.157, 0.291	-0.368, 0.011
Sural SNAP (mV)	-0.353, 0.030	0.066, 0.694	0.211, 0.205
NIS(LL)+7	-0.470, <0.001	-0.059, 0.692	-0.270, 0.063
ESC (µS)	0.264, 0.073	0.137, 0.358	0.221, 0.136
CDT, z-score	0.374, 0.009	0.042, 0.776	0.215, 0.143
WDT, z-score	0.316, 0.029	0.024, 0.873	0.173, 0.239
TSL, z-score	0.172, 0.242	-0.083, 0.575	0.031, 0.835
CPT, z-score	0.084, 0.571	-0.073, 0.621	-0.007, 0.963
HPT, z-score	0.257, 0.078	0.042, 0.779	0.156, 0.290
PPT, z-score	0.143, 0.331	0.269, 0.065	0.250, 0.086
MPT, z-score	0.452, 0.001	0.115, 0.435	0.302, 0.037
MPS, z-score	0.235, 0.108	0.115, 0.437	0.194, 0.187
MDT, z-score	0.404, 0.004	0.088, 0.553	0.260, 0.074
VDT, z-score	0.181, 0.217	-0.031, 0.836	0.070, 0.636

CDT, cold detection threshold; CMAP, compound muscle action potential; CPT, cold pain threshold; DMA, dynamic mechanical allodynia; ESC, electrochemical skin conductance; HDT, heat detection threshold; HPT, heat pain threshold; MDT, mechanical detection threshold; MNCV, motor nerve conduction velocity; MNDL, motor nerve distal latency; MPS, mechanical pain sensitivity; MPT, mechanical pain threshold; NIS-LL, Neuropathy Impairment score of the lower limb; NIS(LL)+7, Neuropathy Impairment score of the lower limb plus 7 neurophysiological tests; PPT, pressure pain threshold; SNAP, sensory nerve action potential; TCNS, Toronto clinical neuropathy score; VDT, vibration detection threshold

ESM Table 6. Correlation analysis of Pi:ATP and clinical/neurological variables.

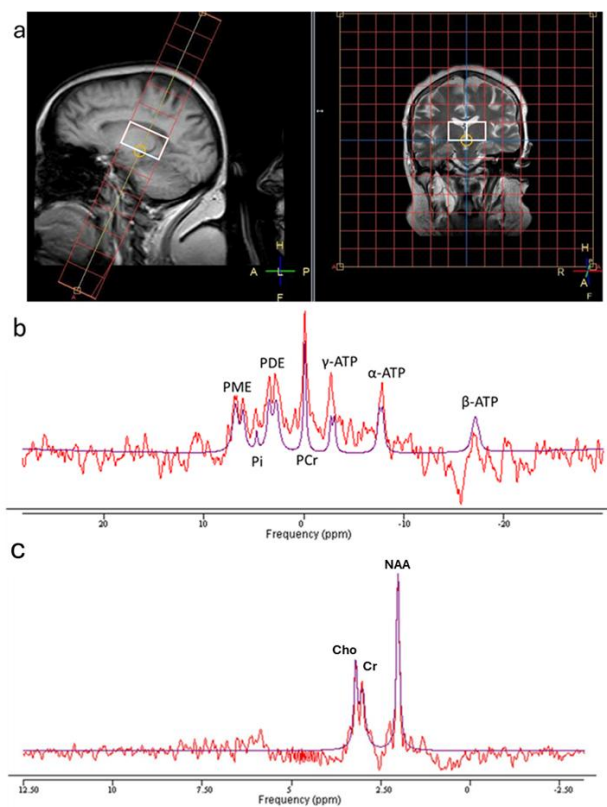
	Right (r, p-value)	Left (r, p-value)	Mean (r, p-value)
Age, years	-0.176, 0.225	-0.008, 0.958	-0.119, 0.415
Duration of Diabetes, years	0.268, 0.104	0.295, 0.073	0.341, 0.036
Body Mass Index, kg/m ²	-0.079, 0.589	0.346, 0.015	0.149, 0.306
Systolic blood pressure, mmHg	0.066, 0.651	0.261, 0.070	0.194, 0.181
HbA1c, mmol/mol	0.356, 0.012	0.134, 0.359	0.309, 0.013
Total Cholesterol, mmol/l	-0.127, 0.386	-0.092, 0.530	-0.135, 0.353
ACR, mg/mmol	0.054, 0.766	-0.121, 0.502	-0.024, 0.892
Creatinine, μ mol/l	0.075, 0.610	0.220, 0.128	0.176, 0.226
TCNS	0.017, 0.910	-0.090, 0.539	0.041, 0.778
NIS-LL	-0.003, 0.986	-0.041, 0.781	-0.025, 0.863
Peroneal CMAP (mV)	-0.024, 0.876	0.010, 0.947	-0.010, 0.947
Peroneal MNCV (m/s)	-0.015, 0.925	-0.215, 0.173	-0.139, 0.380
Peroneal MNDL (msec)	0.024, 0.877	0.323, 0.033	0.204, 0.184
Tibial MNDL (msec)	0.014, 0.924	0.091, 0.542	0.062, 0.677
Sural SNAP (mV)	-0.167, 0.316	-0.113, 0.498	-0.176, 0.289
NIS(LL)+7	0.032, 0.831	-0.053, 0.722	-0.010, 0.946
ESC (μ S)	0.181, 0.224	0.140, 0.347	0.198, 0.183
CDT, z-score	0.057, 0.702	0.034, 0.821	0.055, 0.708
WDT, z-score	-0.029, 0.844	0.003, 0.984	-0.017, 0.908
TSL, z-score	0.085, 0.566	-0.033, 0.826	0.037, 0.805
CPT, z-score	0.196, 0.181	-0.070, 0.638	0.088, 0.554
HPT, z-score	-0.069, 0.642	0.068, 0.648	-0.006, 0.965
PPT, z-score	-0.080, 0.590	0.027, 0.854	-0.036, 0.807
MPT, z-score	-0.256, 0.079	0.024, 0.869	-0.179, 0.223
MPS, z-score	-0.164, 0.266	-0.096, 0.516	-0.160, 0.277
MDT, z-score	0.014, 0.927	0.093, 0.528	0.061, 0.679
VDT, z-score	0.057, 0.702	-0.016, 0.916	0.028, 0.851

CDT, cold detection threshold; CMAP, compound muscle action potential; CPT, cold pain threshold; DMA, dynamic mechanical allodynia; ESC, electrochemical skin conductance; HDT, heat detection threshold; HPT, heat pain threshold; MDT, mechanical detection threshold; MNCV, motor nerve conduction velocity; MNDL, motor nerve distal latency; MPS, mechanical pain sensitivity; MPT, mechanical pain threshold; NIS-LL, Neuropathy Impairment score of the lower limb; NIS(LL)+7, Neuropathy Impairment score of the lower limb plus 7 neurophysiological tests; PPT, pressure pain threshold; SNAP, sensory nerve action potential; TCNS, Toronto clinical neuropathy score; VDT, vibration detection threshold

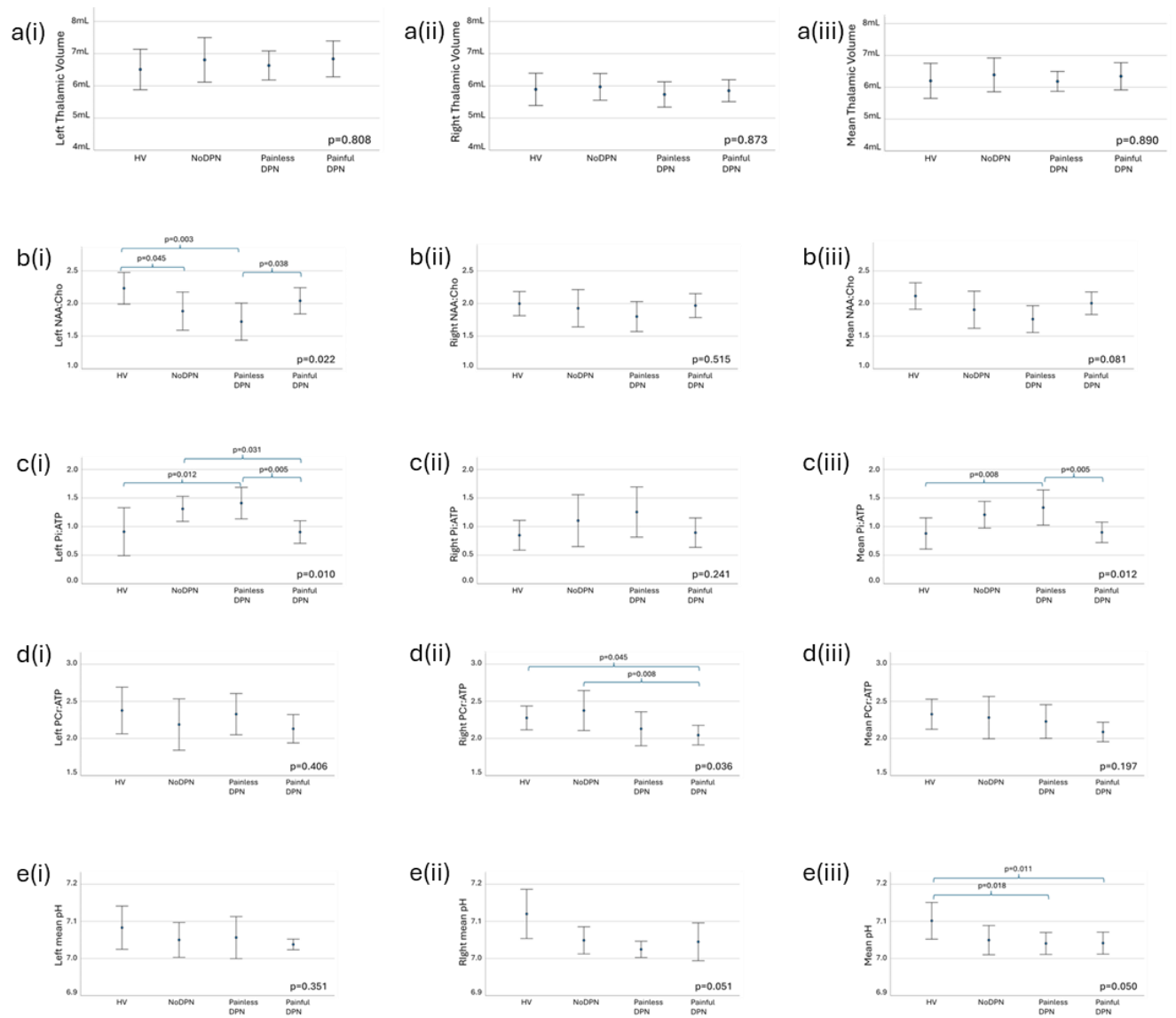
ESM Table 7. Correlation analysis between 1H- and 31P-MRS measures at right thalamus (r, p-value).

	PCr:ATP	Pi:ATP
NAA:Cho	-0.368, 0.023	0.218, 0.189
Cho:Cr	0.334, 0.040	0.0053, 0.754

Cho, Choline; Cr, Creatine; PCr, Phosphocreatine; Pi, Inorganic Phosphate



ESM Fig. 1.a) Voxel Positioning. b) Example spectra of ^{31}P -MRS (red line) and fitting by AMARES (blue line). c) Example spectra of ^1H -MRS (red line) and fitting by AMARES (blue line).



ESM Fig. 2. MR analysis for left (i), right (ii) and mean (iii) thalamic structural (a), ^1H -MRS (b) and ^{31}P -MRS (c-f) results. ATP, adenosine triphosphate; Cho, choline; Cr, creatine; NAA, N-acetyl aspartate; PCr, phosphocreatine; Pi, inorganic phosphate.