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Hippocampal neurometabolic and structural changes from pre-to post-COVID-19: A case-series study

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ABSTRACT

Background: Neurological complications of the COVID-19 infection may be caused in part by local neurochemical and structural abnormalities that could not be detected during routine medical examinations. We examined within subject neurometabolic and structural brain alterations from pre-to post-COVID-19 in the hippocampal region of three elderly individuals (aged 63–68 years) who had a COVID-19 infection with mild symptoms. Patients were participating in an interventional study in which they were closely monitored at the time they were diagnosed with COVID-19. Patients 1 and 2 just completed 18–20 resistance training sessions prior to their diagnosis. Patient 3 was assigned to a non-training condition in the same study.

Methods: Whole brain magnetic resonance imaging (MRI) images and proton magnetic resonance spectroscopy (^{1}H-MRS) of the left hippocampus were collected before and after infection. Structural and spectroscopic imaging measures post-COVID-19 were contrasted to the pre-COVID-19 measures and were compared with values for Minimal Detectable Change at 95% (MDC_{95}) and 90% (MDC_{90}) confidence from a group of six elderly (aged 60–79 years) without COVID-19 that participated in the same study.

Results: After SARS-COV-2 infection, we observed a reduction of glutamate-glutamine (Glx) in Patients 1 and 2 ($\geq$ 42.0%) and elevation of myo-inositol (mIns) and *N*-acetyl-aspartate (NAA) in Patient 3 ($\geq$ 36.4%); all > MDC_{90}. MRI findings showed increased (Patients 1 and 2) or unchanged (Patient 3) hippocampal volume.

Conclusions: Overall, findings from this exploratory study suggest that mild COVID-19 infection could be associated with development of local neuroinflammation and reduced glutamate levels in the hippocampus. Our ^{1}H-MRS findings may have clinical value for explaining chronic neurological and psychological complaints in COVID-19 long-haulers.

1. Introduction

Coronavirus 2019 disease (COVID-19) is characterized by multiple neurological symptoms including anosmia and ageusia, and in more severe cases, encephalitis, cerebral infarctions and syncope [1–3]. Recent neuroimaging studies and brain autopsies have shown structural and neurochemical brain abnormalities associated with COVID-19 that may lead to these complications [4–9]. The overall observations from these studies suggest that hemorrhagic infractions and moderate to severe microglial activation were the most frequent pathological findings in COVID-19 non-survivors [4,7]. Neuroimaging studies in living COVID-19 patients revealed, in addition to inflammatory and

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cerebrovascular brain lesions [5,6], also neurometabolic abnormalities in white matter [8] and prefrontal cortex [9,10].

Post-acute COVID-19 neuronal abnormalities such as hypoxic injury, hemorrhage, and non-specific inflammatory effects are likely to be more prevalent in patients with severe illness than in mild cases of COVID-19 [8,11]. Long-term neurological and psychiatric conditions were also found in non-hospitalized COVID-19 patients which included besides mood and anxiety disorders [12], reduction in attention and working memory functions [11–13]. Cognitive and functional declines found in those COVID-19 long-haulers could be associated, at least in part, by local neurochemical abnormalities such as increased levels of myo-inositol [8] and decreased levels of glutamate and glutamate/glutamine ratio [9] that may not be detected during routine medical examinations. However, most neuroimaging studies on COVID-19 patients often overlook the effects of SARS-CoV-2 infection on brain metabolites even though a large body of evidence exists to support the links between neurometabolic abnormalities and cognitive dysfunctions as consequence of viral infections [14]. Here, we present preliminary observations from proton magnetic resonance spectroscopy (^{1}H-MRS) of the left hippocampus and MRI volumetric measurements of bilateral hippocampal regions in the same individuals before and after SARS-CoV-2 infection.

2. Methods

2.1. Patients and control subjects

Patients were three elderly adults (aged 63, 66 and 68 years; 2 females; Table 1) who, at the time of infection, were participating in a randomized controlled trial (RCT) that was specifically designed to examine the effects of resistance training on brain and blood biomarkers and cognitive/motor functions in elderly adults. All three patients were Lithuanian citizens from the region of Kaunas. All three patients were considered cognitively intact (all scored 28 or higher on the Montreal Cognitive Assessment - MoCA) and physically abled-bodied with no severe morbidity conditions at the time of inclusion and had no medical history of COVID-19. The experimental protocol was approved by the local Medical Ethics Committee for Biomedical Research (No. BE-10-7), and a written informed consent was obtained from all participants prior to their inclusion in the original study. Before COVID-19 data collection was performed 60–79 days before participants were reported to be diagnosed with the SARS-CoV-2 virus infection. Exposure to high-risk contacts was 3–5 days before onset of symptoms in Patient 1 and 3 days in Patient 2 (no information exists for Patient 3). All three patients presented mild COVID-19 symptoms that did not require hospitalization. Reported illness durations were 12 to 19 days. The second (post COVID-19) ^{1}H-MRS and MRI scanning sessions, blood collection, and behavioral tests were performed 19–31 days after positive COVID-19 Polymerase Chain Reaction (PCR) test was taken, and 7–16 days after patients had been cleared by their general practitioners. Concerning the originally designed study, Patient 1 completed 20 resistance-training sessions and Patient 2 completed 18 resistance-training sessions prior to their diagnosis with COVID-19. Patient 3 was assigned as a passive control in the same study and did not undergo any training program prior to his infection. Clinical assessments for depression and anxiety diagnoses were performed by a qualified, mental health care specialist (co-author SK) using the Hospital anxiety and depression scale (HADS). None of the three patients presented neuro long-COVID symptoms after being declared healthy by their general practitioners. Lastly, we included six elderly adults (age range 60–79 years; 3 females; MoCA $\geq$26) that were randomly selected for the purpose of this study. They were all control subjects in the original RCT. These participants underwent the same data collection protocol as that of the experimental group but did not undergo any training. All six controls had no severe morbidity conditions at the time of their inclusion and, to our knowledge, were not diagnosed with or developed COVID-19 symptoms prior or during their participation in the RCT. The mean (SD) time between first and follow-up scanning sessions was 110 $\pm$ 13 days.

Table 1
Clinical information and testing chronology of the three patients with COVID-19.

Patient 1 (63 yr./F)	*Background:* The patient enrolled into the study in August 2020. She was in good health, has no known chronic illness, and was not taking any medication. First MRI session was performed on the 22nd of August 2020. Laboratory tests were performed on the 27th of August 2020. The patient completed 20 resistance-training sessions prior to her infection with COVID-19. Post COVID-19 MRI and laboratory tests were performed on the 5th and the 9th of December 2020, respectively. *COVID-19 diagnosis & symptoms:* Possible moment of a high risk contact was 3 to 5 days before symptoms. Polymerase chain reaction (PCR) test was performed, upon onset of symptoms, on the 9th of November 2020 and the patient was diagnosed with COVID-19 on the same day. Symptoms included anosmia, ageusia, arthralgia, myalgia, nausea and somnolence. No fever, no blood pressure changes. The patient recovered within 2 weeks and returned to work on the 23rd of November 2020. *Treatment & medication:* The patient was prescribed naproxen 550 mg once per day for pain relief for 4 days.
Patient 2 (68 yr./F)	*Background:* The patient enrolled into the study in October 2020. She was diagnosed with glaucoma (medication: travaprost 40 μg/ml, daily drops into eyes) but was otherwise in good health and was not taking any peroral medications. First MRI session was performed on the 17th of October 2020. Laboratory tests were performed on the 23rd of October 2020. The patient completed 18 resistance-training sessions prior to her infection with COVID-19. Post COVID-19 MRI and laboratory tests were performed on the 16th and the 21st of January 2021, respectively. *COVID-19 diagnosis & symptoms:* Possible moment of a high risk contact was 3 days before symptoms. PCR test was performed (upon onset of symptoms) on the 28th of December 2020 and the patient was diagnosed with COVID-19 on the same day. The symptoms included anosmia, ageusia, somnolence, subfebrile, and hypotension. The patient was declared healthy by her general practitioner (GP) on the 9th of January 2021. *Treatment & medication:* paracetamol 500 mg per day for two days and ibuprofen 400 mg every 4 h for two days. The patient took vitamin C, vitamin D and Zinc supplements.
Patient 3 (66 yr./M)	*Background:* The patient enrolled into the study in October 2020. He has hypertension but was otherwise in good health and was taking ramipril 10 mg daily (non-centrally active ACE inhibitor). First MRI session was performed on October 24th, 2020. Laboratory tests were performed on the 29th of October 2020. The patient was enrolled as a control subject and did not undergo resistance training. Post COVID-19 MRI and laboratory tests were performed on the 23rd and the 27th of January 2021, respectively. *COVID-19 diagnosis & symptoms:* No reported high risk contacts. First symptoms (myalgia, arthralgia) appeared 2–3 days before PCR test. Patient reported subfebrile on the 22nd of December 2020. PCR test was performed on the 23rd of December 2020 and the patient was diagnosed with COVID-19 on the same day. The symptoms included anosmia, ageusia, somnolence, headache, subfebrile, and hypotension. The patient was declared healthy by his GP on the 11th of January 2021. *Treatment & medication*: Single dose of paracetamol 500 mg. Vitamin C, vitamin D and Zinc supplements.

2.2. Neuroimage acquisition and processing

MR images and ^{1}H-MRS spectra were acquired on a Siemens 3 T MAGNETOM Skyra scanner (Siemens Healthineers, Erlangen, Germany) with a 32-channel receiver head coil. The scanning protocol included: high resolution T1-weighted (T1W) whole brain scan (TR/TE = 2200/2.48 ms, 0.9 × 0.9 × 1.0 mm^3 voxels, field of view: 230 × 256 mm, 176 sagittal slices), T2-weighted (T2W) turbo-spin echo scan, fluid-attenuated inversion recovery (FLAIR), and susceptibility weighted imaging (SWI). Images were reviewed by an experienced radiologist with >10 years of experience (co-author KV). ^{1}H-MRS spectra were

acquired using a Point Resolved Spectroscopy (PRESS) sequence with the following parameters: TR/TE = 2000/30 ms, 128 averages with and without water suppression, acquired from a 26 × 12 × 12 mm^3 voxel positioned in the left hippocampus. ^{1}H-MRS spectra from the three COVID-19 patients pre- and post-COVID-19 are illustrated in Fig. 1.

Spectra were processed using the LCModel software package, Version 6.3 (http://www.lcmodel.com/) and data were analyzed to quantify absolute (water-referenced) concentrations of *N*-acetyl-aspartate (NAA), choline (Cho), myo-inositol (mIns), glutamate-glutamine complex (Glx), and total creatine (Cr). Spectra with FWHM (full width at half maximum) > 0.1 ppm or SNR (signal-to-noise ratio) < 5 were excluded. All neurometabolites were quantified with a Cramér-Rao lower bound (CRLB) < 20%. We were interested specifically in examining changes in levels of NAA, mIns, and Glx from pre- to post-COVID-19 scanning sessions. NAA has been demonstrated to be a predictor of neuronal density and NAA levels decline can be taken as a marker for neuronal damage or neuronal loss [8,9,14]. Myo-inositol can be taken as an indicator for astrogliosis and inflammation [8,14]. Glx represents the sum of glutamate which is main neurotransmitter involved in cognitive/motor control [15,16] and glutamine. The aforementioned neurometabolites were found to be valuable biomarkers of cognitive and motor dysfunctions in normal aging [15–17] and neurodegenerative disorders [18,19]. One MRS scan from a healthy control was excluded due to low-quality (before COVID-19 scan: FWHM = 0.12 ppm, SNR = 3). Therefore, longitudinal ^{1}H-MRS data were available from only five healthy controls (see Table S1 in the Supplementary Appendix). Test-retest reliability for Glx were estimated from a sample of four subjects (one data point was considered as an outlier; see supplemental Table S1).

Hippocampal volume changes (both left and right) were analyzed using FreeSurfer v7.1.1 (Harvard, MA, USA, http://surfer.nmr.mgh.harvard.edu/). Isotropic 3D T1W images (0.9 mm slice thickness) from 18 scans of nine subjects (i.e., first and follow-up scans from the three COVID-19 patients and the six healthy controls) were first processed separately with the cross-sectional fully automated script. This step was then followed by joint analysis of each subjects' pairs of initial and follow-up scans in the automatic longitudinal analysis pipeline with an additional command to automatically segment the hippocampal subfields. Hippocampal volumes were divided by intracranial volume for each subject to adjust for differences in head size.

2.3. Interleukin-6 (IL-6)

Blood samples (10 ml) were collected via venipuncture at the antecubital vein, and were centrifuged immediately (10 min, at 3500 rpm, 4 °C). Serum was stored at −80 °C until the analysis. IL-6 concentrations were measured using a commercially available ELISA kit (DIAsource ImmunoAssays S.A., Belgium, KAP1216). Lower limit of detection was 2

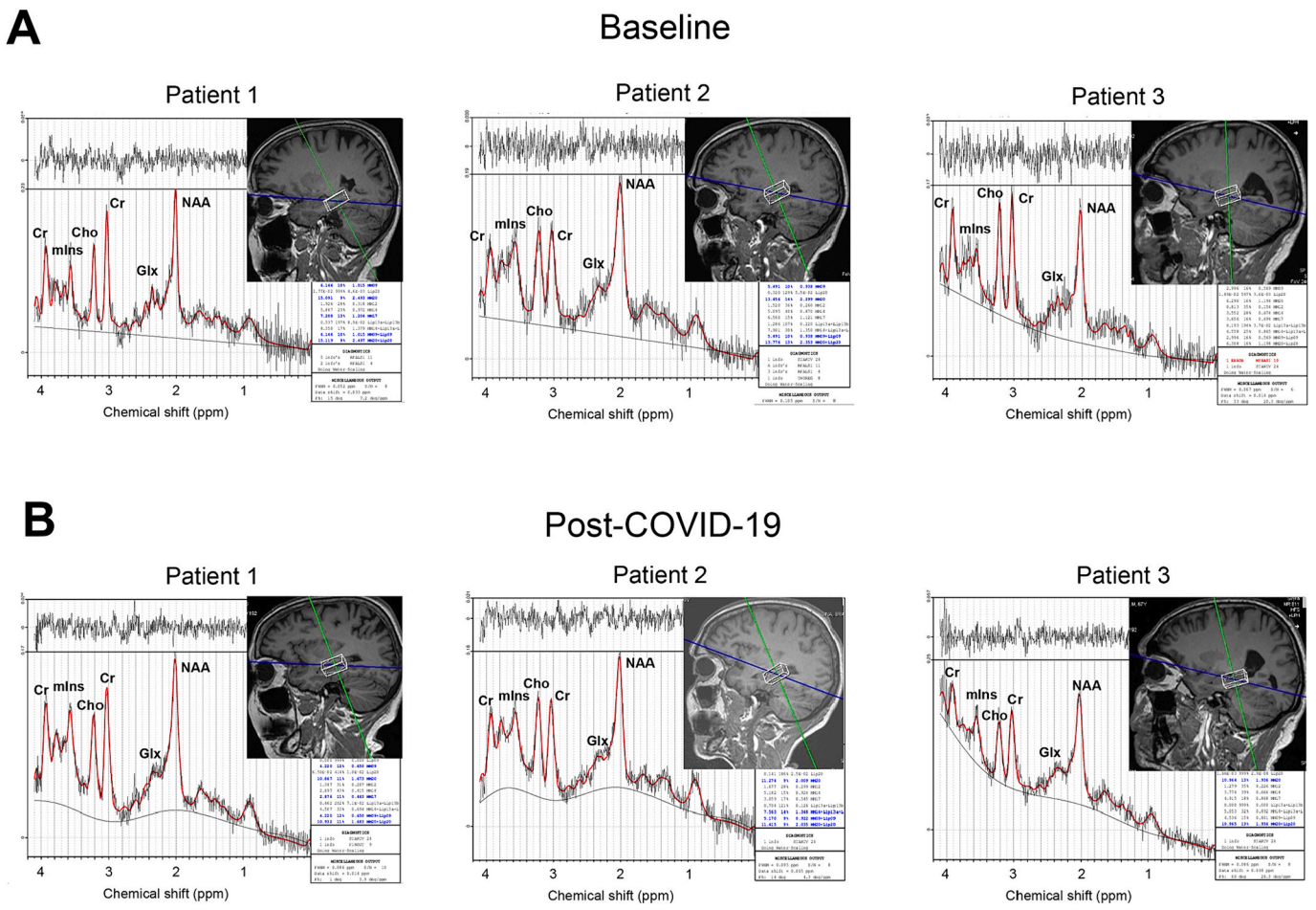

Fig. 1. ^{1}H-MRS spectra from the left hippocampus of the three COVID-19 patients before COVID-19 (A) and after recovery from COVID-19 (B). Spectral quantification was performed with LCModel (version 6.3). Pre MRS acquisitions were taken 79 days (Patient 1), 72 days (Patent 2), and 60 days (Patient 3) before infections. Post-COVID-19 MRS sessions were performed 12 days (Patient 1 and Patient 3) and 7 days (Patent 2) after the reported recovery (see Table 1 for details). Abbreviations: NAA = *N*-acetyl aspartate; Glx = glutamate-glutamine complex; Cho = total choline; mIns = myo-inositol; Cr = creatine + phosphocreatine. Corresponding spectrum (black) and LCModel fit (red) from each patient are illustrated. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

pg/ml. Absorbance was measured using spectrophotometer at 450 nm absorbance.

2.4. Statistical analysis

A single measure two-way random effects model for absolute agreement between first and follow-up measurements from the healthy controls was used to estimate the interclass correlation coefficient (ICC) for test/retest reliability of hippocampal metabolites and volumetric data. The standard error of measurement (SEM) and the minimal detectable change (MDC) values were calculated for each of the neurometabolites and left/right hippocampal volumes, serum IL-6 levels, and HADS scores (Table 2). The MDC values were calculated from SEM at 90% confidence (MDC_{90}) to indicate a trend and at 95% confidence (MDC_{95}) to indicate a real change for individual patients.

3. Results

Pre- and post-COVID-19 (patients) or follow-up (controls) measures of the absolute water-referenced concentrations of NAA, Glx, Cr, Cho, and mIns are shown in Table 2A. Our findings indicated alterations in ^{1}H-MRS spectral profiles of the left hippocampus after recovery from COVID-19. Specifically, we observed reductions in Glx concentration in Patients 1 and 2 ($\geq$ 42.0% reduction from before COVID-19; both $>$ MDC_{95}), a trend towards an increased mIns in Patient 1 (32.4% elevation; $<$ MDC_{90}) and a visible increase of mIns and NAA levels in Patient 3 ($\geq$ 36.4% elevation; both $>$ MDC_{90}). No pre-to post-COVID-19 changes or trends were observed for Cho and Cr (all three patients), Glx (Patient 3), mIns (Patient 2), or NAA (Patients 1 and 2). Similar trends were observed when examining pre- and post-COVID measures of metabolite ratios to Cr (see supplemental Table S2) with the exception of reduction of Cho/Cr ratio in Patient 3 (16.6% reduction; $>$ MDC_{95}).

Both Patients 1 and 2 showed an overall increase in the volume of left hippocampus body ($\geq$ 4.94% increase from before COVID-19; both $>$ MDC_{95}). A detailed inspection of the hippocampus subfields (see supplemental Table S3) revealed that these increases can be attributed primarily to enlargements of the left CA3-body, left CA4-body, left GC-ML-DG-body, and left parasubiculum regions ($\geq$ 6.85%; all $>$ MDC_{95}). An increase was also noted in the total volumes of left hippocampal head of Patient 1 (4.29%) and right hippocampal body of Patient 2 (4.07%); both $>$ MDC_{95}. There were no overall pre to post differences for the change in hippocampal volume of Patient 3. However, inspection of the hippocampus subfields revealed enlargements of right and left parasubiculum ($\geq$ 9.16%) and reduction of the left subiculum body (7.46%); all $>$ MDC_{95}.

Since an increased mIns concentration may be casually linked with elevated expressions of pro-inflammatory cytokines we further tested serum samples obtained from the three patients before and after recovery from COVID-19 to examine if there was an increase in serum levels of interleukin 6 (IL-6) relative to their pre-infection levels. The levels of IL-6 were observed to drop in all three patients (Table 2C). However, elevated levels of IL-6 were found in Patient 3 (76.7 pg/ml) before SARS-COV-2 infection. This suggests that Patient 3 may have developed an inflammatory condition prior to his inclusion in the RCT that was not worsened by the COVID-19 infection.

Finally, all three subjects showed higher scores on the HADS for depression and/or anxiety after recovery from COVID-19 as compared to their before COVID-19 levels (Table 2D). However, changes in HADS-anxiety and HADS-depression categories were not consistent across the three patients. Noticeably, the greatest increase in the combined anxiety and depression scores were observed in Patients 1 (total of +7 point) and 2 (total of +6 points) for whom Glx levels were found to decline following their recovery from COVID-19.

4. Discussion

We described findings from three recovered COVID-19 patients who underwent ^{1}H-MRS and anatomical MRI scanning of the brain to examine neurometabolic and structural changes that may be caused by the disease. The results of the post-COVID-19 scanning sessions were compared with pre-COVID-19 data, which were obtained from the same individuals prior to their infection by the SARS-CoV-2 virus. Two of the three patients showed a general reduction of Glx concentration in agreement with the findings of a case report by Yesilkaya et al. [9], who reported decreased prefrontal NAA, glutamate, and glutamate/glutamine ratio in a patient after acute COVID-19. In addition, Patient 1 and Patient 3 demonstrated elevated mIns levels, in line with observations from a ^{1}H-MRS study by Rapalino et al. [8] who reported elevated mIns levels in severe COVID-19 patients with leukoencephalopathy during the acute phase of the disease.

Increased levels of mIns is expected to be associated with an increased expression of pro-inflammatory cytokines in astrocytes and microglial cells similar to that seen in HIV infection [14], Alzheimer's disease [18] or neurodegenerative disorders such as amyotrophic lateral sclerosis [19]. Elevated IL-6 levels are commonly reported in COVID-19 patients as an expression of cytokine-triggered inflammatory reaction [20–22]. We examined whether increased levels of mIns in Patient 1 and Patient 3 would be accompanied by elevation of serum levels of IL-6. However, we found no association between elevation of mIns and increased serum levels of IL-6 in these two patients. The elevated levels of mIns (but not serum levels of IL-6) in two of three COVID-19 patients in our study could be interpreted as a marker of local neuroinflammation without a (remaining) systemic inflammatory response; implying that blood samples may be insufficient to detect those neuroinflammatory responses in mild or asymptomatic COVID-19 cases. Therefore, we propose that ^{1}H-MRS should be considered as a diagnostic tool to detect inflammatory foci in the brain of COVID-19 patients with neurological symptoms. Specifically, mIns levels should be considered as potential biomarkers for neurodegenerative abnormalities [8] and persisting cognitive consequences of COVID-19.

Infection of the central nervous system (CNS) can be explained in part as a response to binding of the SARS-CoV-2 virus to angiotensin-converting enzyme 2 (ACE2) receptors in the CNS [3,23]. After binding, the virus is recognized by immune cells on the neural surface that then start an inflammatory response possibly leading to increased glial cell activity and glial inflammation [24–26]. Glial inflammation in the hippocampus could partly be associated with the observed elevation of mIns in Patient 3 and more generally explain the observed declines in memory and mental functions or hippocampal volume loss seen in COVID-19 patients during and/or after the acute phase [2,3,10–12,14,23–27]. In contrast to our expectation we found no hippocampal volume loss. Rather we observed a consistent increase of hippocampal volume (specifically but not exclusively in the left hippocampal body subfields) in two of our patients (Patients 1 and 2) who were included in a resistance-training program prior to their infection. The question remains open whether exercise can partly protect against pro-inflammatory processes and volume loss [28] that may be caused by COVID-19 infection.

The expression of ACE2 receptors in glutamatergic and GABAergic neurons [23] could suggest that inflammatory reactions triggered by the SARS-CoV-2 virus infection may also interrupt signaling pathways in the pre- and postsynaptic excitatory and inhibitory neurons which could possibly lead to glutamate excitotoxicity as seen in other viral infections [14,29]. Evidence for glutamate excitotoxicity as manifested by elevated levels of glutamate/glutamine in the brain tissue were previously reported in intubated COVID-19 patients [8]. However, we found no evidence for glutamate excitotoxicity in our patients as Glx levels measured in the post-COVID-19 scanning session decreased (Patients 1 and 2) or remained stable (Patient 3) relative to their pre-COVID-19 levels. The decrease in glutamatergic regulation is in line with

Table 2
Longitudinal comparison of left hippocampus metabolite concentrations **(A)**, hippocampal volumes **(B)**, serum levels of interleukin 6 (IL-6) **(C)**, and Hospital Anxiety and Depression Scale (HADS) scoring **(D)** before and after infection with SARS-COV-2. Percentage changes relative to before (% Δ) or absolute changes (Δ) from before SARS-COV-2 infection are presented for the three COVID-19 patients (left-hand panel). Test-retest reliability assessments for first and follow-up data from six older controls with no history of COVID-19 (right-hand panel).

	Patient 1			Patient 2			Patient 3			Controls					
	Pre-COVID-19	Post-COVID-19		Pre-COVID-19	Post-COVID-19		Pre-COVID-19	Post-COVID-19		Mean (SD) before	Mean (SD) follow-up	$ICC_{2,1}$ (n)	SEM	MDC_{95}	MDC_{95} (% before)
(A) **^{1}H-MRS**†			Δ (%)			Δ (%)			Δ (%)						
NAA	5.33	5.94	+11.3	7.03	5.76	−18.1	4.81	6.57	**+36.4**	6.16 (0.66)	6.33 (0.50)	0.149 (n = 5)[1]	0.61	1.69	27.5
Glx	10.9	6.34	**−42.0**	9.18	4.64	**−49.5**	7.90	7.75	−1.89	9.55 (1.09)	9.88 (0.51)	0.527 (n = 4)[2]	0.75	2.08	21.7
Cho	1.34	1.58	+14.7	1.96	1.80	−8.36	1.55	1.39	−10.4	1.80 (0.22)	1.87 (0.26)	0 (n = 5)	0.22	0.61	33.8
mIns	5.44	7.21	**+32.4**	7.60	6.35	−16.4	3.75	5.85	**+56.1**	7.14 (0.90)	7.94 (1.54)	0 (n = 5)	0.90	2.49	34.9
Cr	6.06	6.50	+7.30	5.85	5.61	−4.17	5.27	5.66	+7.50	6.19 (0.92)	6.03 (0.64)	0 (n = 5)	0.92	2.55	41.2
(B) **MRI-based hippocampal volumetrics (mm^3)**‡			Δ (%)			Δ (%)			Δ (%)						
Left body	1402	1477	**+5.34**	1118	1173	**+4.94**	1245	1215	−2.42	1094 (68)	1111 (69)	0.944 (n = 6)	16.1	44.8	4.09
Left head	2005	2092	**+4.29**	1549	1579	+1.97	1865	1866	+0.04	1656 (163)	1642 (194)	0.979 (n = 6)	23.7	65.6	3.96
Left total	4119	4310	**+4.63**	3189	3273	2.63	3641	3605	−0.98	3279 (236)	3292 (272)	0.972 (n = 6)	39.5	109.4	3.34
Right body	1455	1470	+0.98	1144	1190	**+4.07**	1270	1279	+0.71	1152 (100)	1149 (113)	0.980 (n = 6)	14.2	39.3	3.41
Right head	1816	1816	−0.01	1686	1745	+3.48	1976	2003	+1.41	1701 (171)	1712 (198)	0.960 (n = 6)	34.3	95.0	5.58
Right total	4032	4062	+0.74	3441	3568	**+3.67**	3836	3892	+1.46	3456 (354)	3460 (390)	0.984 (n = 6)	44.7	124	3.59
(C) **Inflammatory biomarkers**#			Δ			Δ			Δ						
IL-6 (pg/ml)	10.8	8.80	−2.10	11.7	6.30	−5.4	76.7	39.6	**−37.1**	17.2 (11.7)	17.6 (13.9)	0.350 (n = 5)[3]	9.41	26.1	
(D) HADS §			Δ			Δ			Δ						
Depression	2	3	+1	1	5	**+4**	0	3	+3	1.83 (3.06)	3.00 (3.41)	0.840 (n = 6)	1.22	3.39	
Anxiety	2	8	**+6**	2	4	+2	4	4	0	4.00 (3.52)	4.83 (4.12)	0.601 (n = 6)	2.22	6.17	

Abbreviations: NAA = *N*-acetyl aspartate; Glx = glutamate-glutamine complex; Cho = total choline; mIns = myo-inositol; Cr = creatine + phosphocreatine.
$ICC_{2,1}$ was calculated using a two-way random model for absolute agreement. Negative ICC values are set to zero. n = number of samples. [1,2]Missing ^{1}H-MRS data from one (NAA, Cho, mIns, and Cr) or two (Glx) healthy controls; for details, see supplemental Table S1. [3] A blood-sample from one healthy control was not collected at follow-up.
Standard error of measurement (SEM) was calculated as follows: SEM = SD(before COVID-19) × $\sqrt{(1 - ICC_{2,1})}$.
Minimum detectable change (MDC) at 90% (MDC_{90}; not shown) and 95% (MDC_{95}) levels of confidence were calculated as follows: $MDC_{95} = 1.96 \times SEM \times \sqrt{2}$; $MDC_{90} = 1.645 \times SEM \times \sqrt{2}$.
Pre-to-post changes of > MDC_{95} were interpreted as true changes (**bold ± underlined text**). Pre-to-post percentage difference of >30% and/or > MDC_{90} were considered as trends (**bold text**).
† Values presented are the absolute (water-referenced) concentrations (in institutional units). Metabolic ratios to Cr are reported in Supplemental Table S2.
‡ Segmented subfield volumes are reported in Supplemental Table S3.
§ Hospital Anxiety and Depression Scale (HADS) scores considered normal if <8.
# Interleukin 6 (IL-6) normal range: 5–15 pg/ml.

findings from TMS studies in COVID-19 long-haulers with neurological symptoms, where cortical hypoexcitability has been described [30,31]. However, to our knowledge, none of our participants developed neurological post-COVID-19 symptoms. Downregulation of glutamatergic and GABAergic neurotransmission (as well as lower levels of glutamate and GABA) has been implicated in a variety of age-related cognitive/motor declines [32], psychiatric disorders [33,34], and neuroplasticity [35] which may partly explain manifestation of persistent neuronal symptoms such as depression, attentional disorders and fatigue after the acute phase of the illness [5,27,36]. Based on the observations from this case study (specifically, increase of HADS-Anxiety and HADS-depression scores in Patients 1 and 2), we suggest that in addition to pro-oxidative and pro-inflammatory effects [37,38], cognitive changes observed in COVID-19 patients may be attributed partly to reduced hippocampal glutamate.

In addition to the possible role of inflammation, recent evidence from neuroimaging studies also suggested that alterations in brain structural and functional properties observed in COVID-19 patients could be caused by conditions with hypoperfusion (e.g., [39]; for a review see Cull et al., 2023 [40]). Thus, it is tempting to speculate that some of the metabolic changes observed in the current study could be explained at least in part by decreased cerebral blood flow (CBF) and hypoperfusion (e.g., [41]). For example, decreased NAA levels in Patient 2 or increased level of Cho in Patient 1. This presumption is supported by evidence from animal models [42,43] and observations in patients with circulatory impairments [44–46]). For example, findings from animal studies have shown that decreased cerebral blood flow (CBF) was accompanied by a significant reduction in hippocampal NAA concentration [42,43]. Interventional studies in patients with circulation impairments or at high risk of developing recurrent infraction reported that post-intervention recovery of blood reperfusion in the brain was associated with recovery of NAA signal [44,45] or reduction in choline Cho/Cr and increase of NAA/Cho ratios [46]. We need to emphasize, nevertheless, that the MRI protocol used in our study did not include monitoring of CBF. Therefore, our data cannot provide supporting evidence for a direct link between tissue metabolism and blood perfusion.

It should be noted that this study has several limitations: This study was not planned or preregistered before the results were obtained. Only three COVID-19 patients were included, all three of them suffered only mild symptoms, and we expect potential confounding effects of the exercise program on the results of two of the three patients, as was discussed. Finally, only ^{1}H-MRS changes in the left hippocampus were investigated. Therefore, our results should be regarded as an exploratory basis, which allows readers to draw hypotheses. However, readers should not draw final conclusions.

To summarize we have shown that SARS-CoV-2 infection may cause changes to the neurometabolic state of the hippocampus, indicating neuroinflammation and metabolic abnormalities that may persist beyond the acute phase of the disease. To the best of our knowledge, our study is the first one to offer observations from longitudinal ^{1}H-MRS measures that were acquired from the same subjects before their infection and immediately after the acute phase of COVID-19. The study has some limitations, specifically: small sample size, inclusion of only mild COVID-19 patients, and the possible confounding effects of exercise. Therefore, generalization of the present findings should be made with caution. However, they may serve others to formulate new hypotheses and guide future studies. In addition, we argue that more studies should examine the role of ^{1}H-MRS as a diagnostic, prognostic and predictive tool for assessment of long-lasting neurological and neuropsychiatric impacts of COVID-19.

CRediT authorship contribution statement

Wouter A.J. Vints: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Kristina Valatkevičienė:** Methodology, Investigation, Formal analysis, Data curation. **Oron Levin:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Akila Weerasekera:** Supervision, Methodology, Conceptualization. **Simonas Jesmanas:** Investigation, Formal analysis, Data curation. **Simona Kušleikienė:** Project administration, Investigation. **Vida J. Česnaitienė:** Supervision, Project administration, Methodology, Investigation. **Uwe Himmelreich:** Methodology, Formal analysis, Data curation, Conceptualization. **Jeanine A. Verbunt:** Writing – original draft, Supervision. **Eva-Maria Ratai:** Supervision, Methodology, Conceptualization. **Rymantė Gleiznienė:** Supervision, Resources, Project administration, Methodology. **Nerijus Masiulis:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Investigation, Funding acquisition, Conceptualization.

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Disclosure statement

The authors disclose no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mri.2024.03.032.

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