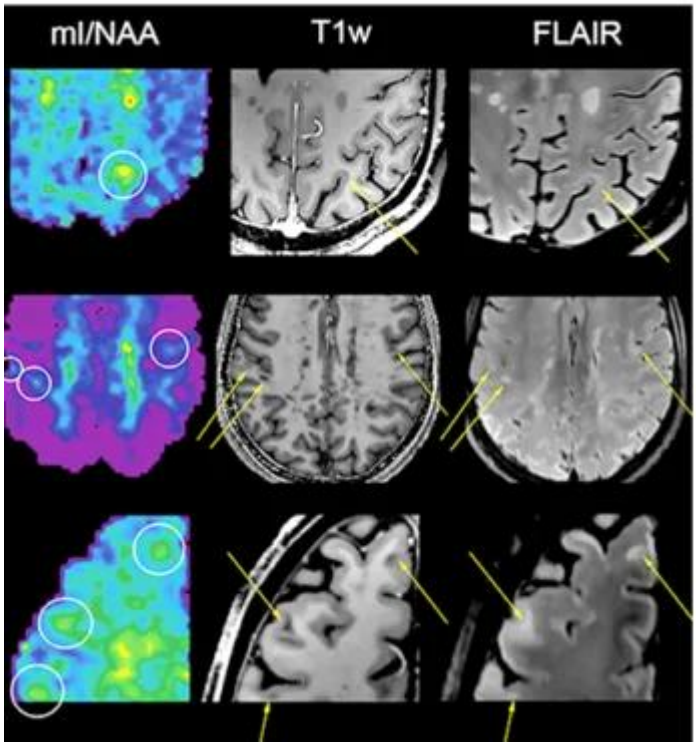
In this study, the authors from the United States used brain proton magnetic resonance spectroscopy (MR spectroscopy) to investigate neurometabolites that indicate neuroinflammation, neuronal damage, and glial dysfunction in patients with neuropsychiatric symptoms of long COVID syndrome. MR spectroscopy is a supplement to standard diagnostic MR imaging. Protons in different molecules have slightly different magnetic properties, and this difference enables MR spectroscopy to detect small molecules. Therefore, MR spectroscopy can reveal metabolic changes that precede pathological structural changes in the brain tissue.

Glutamate (Glu) is the most abundant excitatory neurotransmitter, and gamma-aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the mature central nervous system (CNS). The nervous system-specific metabolite *N-acetyl aspartate* (NAA) is synthesized from aspartate and acetyl-coenzyme A in neurons and is present at exceptionally high concentrations in the brain. It is a direct precursor for the enzymatic synthesis of the most concentrated neuropeptide in the human brain, neuron-specific dipeptide *N-acetyl aspartyl-glutamate*.

In addition to the water signal, the dominant peaks in the MR spectra of the brain are from choline (Cho), creatine (Cr), and NAA, which appear in all spectra. The changes in these signals can often be linked to pathology. Neuroinflammation and the proliferation of microglia are typically associated with increased glial marker myo-inositol (MI). Increased MI levels are associated with concomitantly increased levels of Cho and total Cr due to their higher concentration in glia than in neurons.

A multicellular metabolic pathway known as the Glu/GABA/glutamine (Gln) cycle maintains the balance between metabolites that play critical roles in numerous brain functions, such as learning, memory, pain, synaptogenesis, motor stimuli, and neuronal synaptic transmission. A decrease in total *N*-acetyl compounds (NAA + *N-acetyl aspartyl-glutamate*) and Glu/Gln levels shows irreversible neuronal dysfunction, injury, or loss in affected brain areas.

An altered Glu/GABA/Gln balance is associated with brain damage in several neurodegenerative diseases, such as Alzheimer's, Parkinson's, and neuroHIV. In neuro HIV, changes in Glu/GABA/Gln balance contribute to neuronal and glial dysfunction, and cognitive impairment identified in HIV-associated neurocognitive disorder (HAND). The increase in Cho and MI levels and a decrease in Cr, total *N*-acetyl compounds, and Glu/Gln levels are the most common MR spectroscopy findings in neuroHIV.



Brain Proton Magnetic Resonance Spectroscopy: Metabolic maps showing the ratio of MI and NAA

About the study

The study included participants with at least one neuropsychiatric symptom that emerged after COVID-19 and healthy controls matched by age, sex, and education. The participants with significant neurologic or psychiatric disorders, such as stroke, encephalitis from any cause except COVID-19, neurodegenerative disorder, schizophrenia, uncontrolled major depression or anxiety disorder requiring medication before COVID-19, traumatic brain injury with loss of consciousness for more than one hour that required hospitalization, severe substance use disorders except for tobacco or cannabis use, and any contraindication for MRI were excluded.

Participants diagnosed with long COVID syndrome had acute COVID-19 more than six weeks before enrollment (a mean of 242 ± 156 days). Healthy controls did not have COVID-19. They tested negative for SARS-CoV-2 on the polymerase chain reaction test within a week or had a negative rapid antigen test on the evaluation day. More healthy controls than patients with long COVID received COVID-19 vaccines.

All patients underwent brain proton MR spectroscopy of the anterior cingulate cortex gray matter and frontal white matter. According to the authors, the anterior cingulate cortex was

selected as the primary node of the attention network necessary for all cognitive tasks. The frontal white matter was chosen because neuroinflammation is often found in this brain region during viral neuroinfections (e.g., HIV, John Cunningham virus, and hepatitis C virus). Most neurons (~80%) in the frontal white matter are glutamatergic. All participants were evaluated with the National Institutes of Health Toolbox- Cognition and Motor Batteries.

Results

The study included 54 participants, 29 diagnosed with neuropsychiatric symptoms of long COVID syndrome, and 25 healthy controls. The mean age of patients with long COVID was 42.4 years. Nine of them were hospitalized and required supplemental oxygen and/or ventilation during acute COVID-19.

The most frequent neuropsychiatric symptoms were difficulties with concentration (93%) and memory (79%), fatigue (86%), and depression or anxiety (68%). Despite the high prevalence of complaints regarding concentration and memory, their performance in all domains assessed by the NIH Toolbox–Cognition Battery was similar to that observed in healthy controls. Nonetheless, on the PROMIS surveys, individuals diagnosed with long COVID syndrome had a greater number of symptoms, such as depression, fatigue, anxiety, and pain, and poorer global mental and physical health scores.

Importantly, participants with long COVID syndrome had poorer scores on the Motor Battery tests, especially on the 2-minute endurance walk test, the 4-meter walk gait speed test, and the 9-hole pegboard dexterity test (test of dominant hand). These findings are consistent with a recent functional MRI study that investigated brain activation during a working memory task and showed that participants with neuropsychiatric symptoms of long COVID performed worse than healthy controls in the domains of endurance, locomotion, and manual dexterity of dominant hand.
https://discovermednews.com/patients-with-post-covid-syndrome-and-neuropsychiatric-symptoms-have-different-brain-activation-during-the-working-memory-task/

Brain proton MR spectroscopy

Brain proton MR spectroscopy demonstrated changes in brain neurometabolites in patients with neuropsychiatric symptoms of long COVID. They had a decrease in total *N*-acetyl compounds, Glu/Gln, and MI levels in the frontal white matter compared to healthy controls. As most neurons (~80%) in the frontal white matter are glutamatergic, the marked decrease

in Glu/Gln level suggests damage or loss of glutamatergic neurons.

Since NAA, the major component of total *N*-acetyl compounds, is synthesized in mitochondria, the authors suggested that a reduced level of total *N*-acetyl compounds could potentially result from mitochondrial dysfunction.

A decrease in total *N*-acetyl-compound levels in the frontal white matter predicted poorer performance of all long COVID patients on several cognitive measures. Additionally, lower *N*-acetyl-compound level in the gray matter of the anterior cingulate cortex was associated with lower endurance on the 2-minute walk test.

Only participants with neuropsychiatric symptoms of long COVID who were hospitalized during acute COVID-19 had increased MI, Cho compounds, and total Cr levels. Since elevated MI level is typically associated with neuroinflammation, increased MI levels in participants with neuro-long COVID hospitalized during acute COVID-19 might indicate persistent neuroinflammation and glial activation. On the contrary, lower MI levels actually represent glial dystrophy or dysfunction rather than neuroinflammation and glial activation.

These findings are consistent with the results of brain MR spectroscopy found in patients positive for HIV-1. A decrease in Cr, NAA, and Glu levels and an increase in Cho and MI levels in neuroHIV indicate neuroinflammation, microglial proliferation, and neuronal injury or loss. A decrease in NAA and Glu/Gln levels in the cortical gray matter found in early HIV-1 infection indicates that the virus causes neuronal and astroglial dysfunction within a short time after infection. In HIV-1-infected individuals, a decreased Glu/Cr ratio was shown to correlate with worse performance on verbal recall, psychomotor speed, and reaction time.

Conclusion

In this study, brain proton MR spectroscopy demonstrated decreased tNAA and Glu/Gln levels, increased Cho and tCr levels, and altered MI levels (decreased or increased depending on the severity of acute COVID-19) in the frontal brain regions of patients with neuropsychiatric symptoms of long COVID. The observed changes in Glu/Gln balance, total *N*-acetyl-compound levels, and MI provided evidence of neuronal injury or loss, as well as of persistent neuroinflammation and glial dysfunction in the brains of long COVID patients. These findings are consistent with the results of proton MR spectroscopy in other neurodegenerative diseases, such as neuroHIV.

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