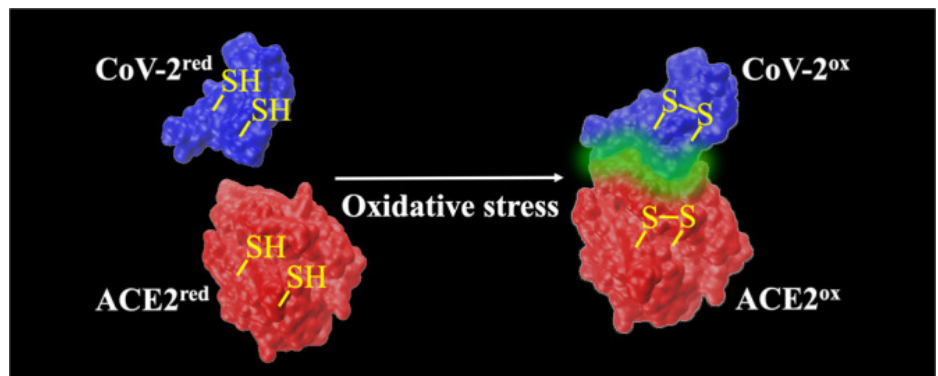


N-acetylcysteine and SARS-Cov-2 spike

Structurally, SARS-CoV-2 contains surface spike proteins, membrane proteins and envelope proteins, as well as internal nucleoproteins that package RNA. The spike protein is a homotrimeric glycoprotein complex with diverse roles realized through dynamic conformational modifications, based in part on disulfide bonds¹. It enables infection of target cells by binding to receptors of human angiotensin-converting enzyme (ACE2), among others, which triggers proteolysis by the transmembrane protease serine 2 (TMPRSS2), furin and possibly other proteases, leading to fusion of the virion to the host cell membrane.²



Virus entry into mammalian cells, or "virus internalization," is a key mechanism of enveloped virus infection and is based on dynamic conformational changes in their surface glycoproteins mediated by disulfide bond reduction and regulated by cell surface oxidoreductases and proteases³.

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7346263/>

It has been shown that entry of SARS-CoV-2 into host cells begins with destabilization of the spike protein through allosteric mechanical transition, which induces a conformational change from the closed "down" to the open "up" state of the receptor binding domain (RBD) of the spike protein⁴. The conformational changes in the RBD and virus binding are induced by TMPRSS2 or cathepsin L, which trigger the transition from the pre-fusion to the post-fusion state.

¹ Akhter J, Quéromès G, Pillai K, et al.

The Combination of Bromelain and Acetylcysteine (BromAc) Synergistically Inactivates SARS-CoV-2.

Viruses. 2021;13(3):425. Published 2021 Mar 6. doi:10.3390/v13030425

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7999995/>

Cai Y, Zhang J, Xiao T, et al.

Distinct conformational states of SARS-CoV-2 spike protein.

Science. 2020;369(6511):1586-1592. doi:10.1126/science.abd4251

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7464562/>

² Coutard B, Valle C, de Lamballerie X, Canard B, Seidah NG, Decroly E.

The spike glycoprotein of the new 2019-nCoV coronavirus contains a furin-like cleavage site absent in CoV of the same clade.

Antiviral Res. 2020 Apr;176:104742. doi: 10.1016/j.antiviral.2020.104742. Epub 2020 Feb 10. PMID: 32057769; PMCID: PMC7114094.

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7114094/>

Vankadari N, Wilce JA.

Emerging WuHan (COVID-19) coronavirus: glycan shield and structure prediction of spike glycoprotein and its interaction with human CD26.

Emerg Microbes Infect. 2020 Mar 17;9(1):601-604. doi: 10.1080/22221751.2020.1739565. PMID: 32178593; PMCID: PMC7103712.

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7103712/>

³ Hati S, Bhattacharyya S.

Impact of Thiol-Disulfide Balance on the Binding of Covid-19 Spike Protein with Angiotensin-Converting Enzyme 2 Receptor.

ACS Omega. 2020;5(26):16292-16298. Published 2020 Jun 23. doi:10.1021/acsomega.0c02125

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7346263/>

Mathys L, Balzarini J.

The role of cellular oxidoreductases in viral entry and virus infection-associated oxidative stress: potential therapeutic applications.

Expert Opin Ther Targets. 2016;20(1):123-43. doi: 10.1517/14728222.2015.1068760. Epub 2015 Jul 15. PMID: 26178644.

<https://pubmed.ncbi.nlm.nih.gov/26178644/>

Wrapp D, Wang N, Corbett KS, et al.

Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation.

Science. 2020;367(6483):1260-1263. doi:10.1126/science.abb2507

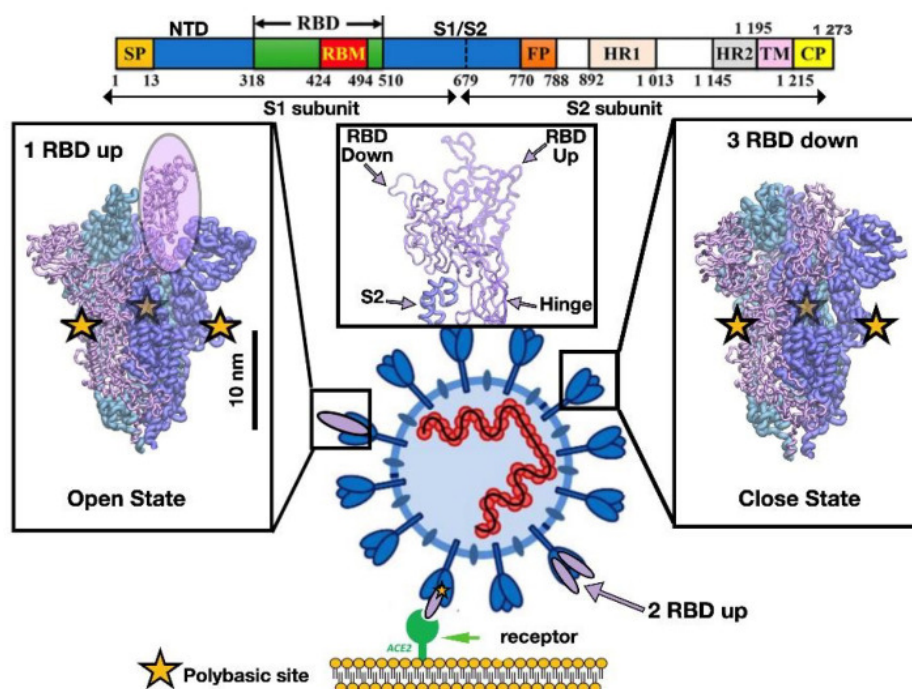
<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7164637/>

⁴ Moreira RA, Chwastyk M, Baker JL, Guzman HV, Poma AB.

Quantitative determination of mechanical stability in the novel coronavirus spike protein.

Nanoscale. 2020 Aug 21;12(31):16409-16413. doi: 10.1039/d0nr03969a. Epub 2020 Jul 29. PMID: 32725017.

<https://pubmed.ncbi.nlm.nih.gov/32725017/>



<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7730245/>

Representation of the different conformations of the receptor-binding domain (RBD) in the acute respiratory syndrome-new spike protein of Coronavirus 2 (SARS-CoV-2). Cellular recognition is initiated by the transition of RBD from the downward (down) to the upward (up) conformation involving RBD detachment from S2 mediated by the hinge region as illustrated in the middle panel. Then, due to the high affinity between RBD in the up conformation and the angiotensin-converting enzyme 2 (ACE2) receptor, binding occurs. The sequence of a spike protein chain is shown above as well as the residue numbers for different protein domains. The bar shows the typical length scale for the entire system. Fusion of the viral and cell membrane occurs via surface proteases that cleave each chain at the polybasic sites (yellow stars) located at the interface of the S1/S2 subunits

The energy released by the reduction of the disulfide bond increases protein flexibility, which is maximal when the reduced state is complete, thus allowing fusion of host virus membranes, which is otherwise impossible due to the repulsive hydration forces present prior to reduction. ⁵

The use of acetylcysteine as an antiviral therapy ⁶

Moreira RA, Guzman HV, Boopathi S, Baker JL, Poma AB.

Characterization of Structural and Energetic Differences between Conformations of the SARS-CoV-2 Spike Protein.

Materials (Basel). 2020;13(23):5362. Published 2020 Nov 26. doi:10.3390/ma13235362

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7730245/>

⁵ Cai Y, Zhang J, Xiao T, Peng H, Sterling SM, Walsh RM Jr, Rawson S, Rits-Volloch S, Chen B

Distinct conformational states of SARS-CoV-2 spike protein.

Science. 2020 Sep 25; 369(6511):1586-1592.

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7464562/>

Hati S, Bhattacharyya S

Impact of Thiol-Disulfide Balance on the Binding of Covid-19 Spike Protein with Angiotensin-Converting Enzyme 2 Receptor.

ACS Omega. 2020 Jul 7; 5(26):16292-16298.

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7346263/>

⁶ Elhidsi, M., Fachrucha, F., & Yudha Irawan, R..

N-Acetylcysteine for COVID-19: A Potential Adjuvant Therapy.

Journal of Health Sciences, (2021) 11(1), 1-6. <https://doi.org/10.17532/jhsci.2020.1156>

<https://www.jhsci.ba/ojs/index.php/jhsci/article/view/1156>

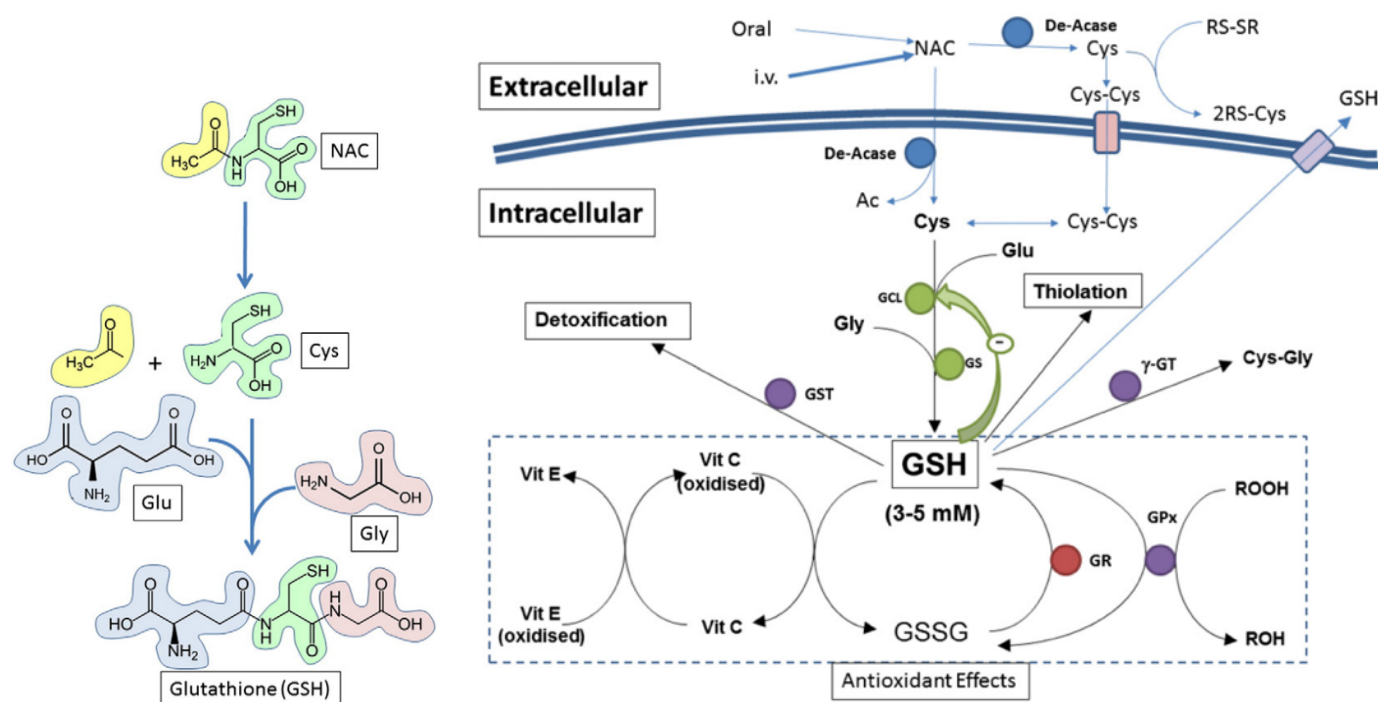
Rushworth GF, Megson IL.

Existing and potential therapeutic uses for N-acetylcysteine: the need for conversion to intracellular glutathione for antioxidant benefits.

Pharmacol Ther. 2014 Feb;141(2):150-9. doi: 10.1016/j.pharmthera.2013.09.006. epub 2013 Sep 28. PMID: 24080471.

<https://www.sciencedirect.com/science/article/abs/pii/S0163725813001952>

GSH, a tripeptide compound γ -L-glutamyl-L-cysteinyl-glycine or GSH, is the most important antioxidant produced by living cells. Severe cases of COVID-19 are associated with lower GSH levels, higher ROS levels and higher redox status (ROS/GSH ratio) than mild-to-moderate cases⁷. Cysteine in GSH has a sulfhydryl/thiol group (-SH), which has the ability to reduce and conjugate in the removal of other peroxides and xenobiotics⁸. Cysteine is also a substrate that determines the rate of GSH synthesis, i.e. when there is oxidative stress in COVID-19, the synthesis of GSH will increase through the Nrf2 activator and requires the availability of an adequate amount of cysteine⁹. NAC, the GSH precursor, functions as an oxygen-deprived radical scavenger and also replenishes depleted GSH stores, enhancing endogenous antioxidant defense. In experimental animals infected with influenza, NAC can promote the production of GSH¹⁰. N-acetylcysteine acts as an antioxidant directly or indirectly by releasing its cysteine or thiol groups or by breaking sulfide bonds. NAC easily penetrates into cells where it is deacetylated to L-cysteine so that it can be a precursor to GSH in the cell.¹¹



<https://www.sciencedirect.com/science/article/abs/pii/S0163725813001952>

⁷ Polonikov A.

Endogenous Deficiency of Glutathione as the Most Likely Cause of Serious Manifestations and Death in COVID-19 Patients.

ACS Infect Dis. 2020 Jul 10;6(7):1558-1562. doi: 10.1021/acinfecdis.0c00288. Epub 2020 May 28. PMID: 32463221; PMCID: PMC7263077.

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7263077/>

⁸ Forman HJ, Zhang H, Rinna A.

Glutathione: overview of its protective roles, measurement, and biosynthesis.

Mol Aspects Med. 2009 Feb-Apr;30(1-2):1-12. doi: 10.1016/j.mam.2008.08.006. epub 2008 Aug 30. PMID: 18796312; PMCID: PMC2696075.

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2696075/>

⁹ Lu SC.

Regulation of glutathione synthesis.

Mol Aspects Med. 2009 Feb-Apr;30(1-2):42-59. doi: 10.1016/j.mam.2008.05.005. Epub 2008 Jun 14. PMID: 18601945; PMCID: PMC2704241.

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2704241/>

¹⁰ Garozzo A, Tempera G, Ungheri D, Timpanaro R, Castro A.

N-acetylcysteine synergizes with oseltamivir in protecting mice from lethal influenza infection.

Int J Immunopathol Pharmacol. 2007 Apr-Jun;20(2):349-54. doi: 10.1177/039463200702000215. PMID: 17624247.

<https://journals.sagepub.com/doi/pdf/10.1177/039463200702000215>

¹¹ Aldini G, Altomare A, Baron G, Vistoli G, Carini M, Borsani L, Sergio F.

N-Acetylcysteine as an antioxidant and disulphide breaking agent: the reasons why.

Free Radic Res. 2018 Jul;52(7):751-762. doi: 10.1080/10715762.2018.1468564. Epub 2018 May 9. PMID: 29742938.

<https://www.tandfonline.com/doi/full/10.1080/10715762.2018.1468564>

Impact of NAC on GSH synthesis and utilization pathways. De-Acase - deacetylase; GCL - glutathione cysteine ligase; GS - glutathione synthase; GPx - glutathione peroxidase; GR - glutathione reductase; GST - glutathione-S-transferase; -GT - glutamyl transpeptidase.

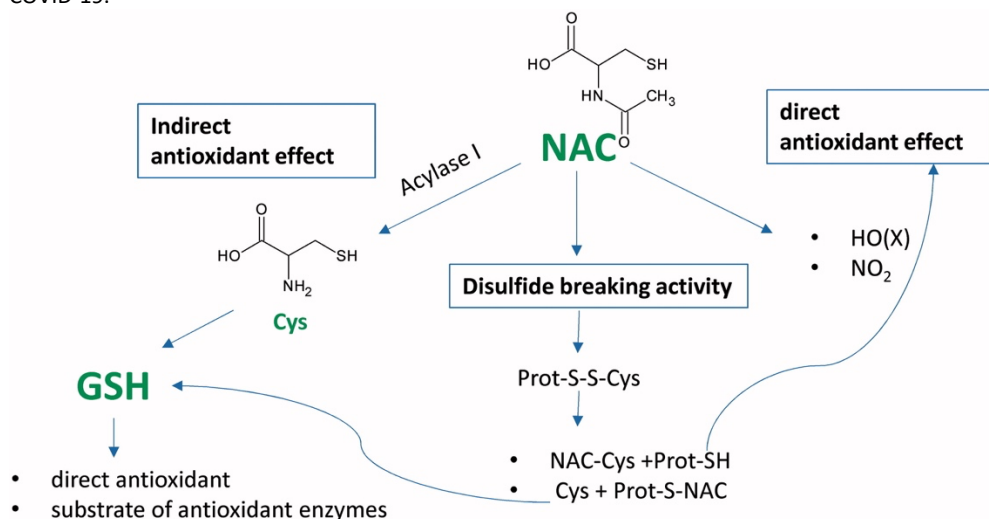
Human clinical trials have shown that NAC (N-acetylcysteine) improves cellular redox status. NAC has been reported to inhibit gene expression of TNF- α and IL-6, and ¹²has also been used to prevent and treat microvascular thrombosis events in COVID-19. ¹³

The [vonWillebrandfactor](#) multimers are disintegrated by NAC, acting in "medical revascularization" in COVID-19 patients with intravascular thrombosis.

Based on its antioxidant and anti-inflammatory mechanism, Poe et. al, hypothesized that NAC could be a potential therapeutic molecule for the treatment of COVID-19.¹²

Recently, Debnath et al ¹⁴proposed a different mechanism of action of NAC against SARS-CoV-2. Reduction of solvent-accessible disulfide bonding followed by NAC conjugation leads to disruption of the functionally active structure of the spike protein of SARS-CoV-2 and thus reduces the infectivity of the virus.

This effect could work in synergy with the other activities reported by NAC such as antioxidant and anti-inflammatory activity in combating COVID-19.



<https://www.tandfonline.com/doi/full/10.1080/10715762.2018.1468564>

Overview of the antioxidant action of NAC. The antioxidant effect is due to indirect (GSH synthesis) and direct antioxidant activity, as well as disulfide breaking activity. The indirect activity refers to NAC's ability to act as a precursor to GSH, which in turn is a known direct antioxidant and a substrate of numerous antioxidant enzymes. When a state of oxidative stress depletes SH pools, NAC can act as a direct scavenger of certain oxidants such as NO(X) and NO₂. NAC breaks down thiolate proteins thus releasing free thiols, which have better antioxidant activity than NAC and enhance the synthesis of GSH and reduced proteins, which in some cases, as with mercaptoalbumin, have important direct antioxidant activity.

¹² Cuadrado A, Pajares M, Benito C, Jiménez-Villegas J, Escoll M, Fernández-Ginés R, García Yagüe AJ, Lastra D, Manda G, Rojo AI, Dinkova-Kostova AT. Can Activation of NRF2 Be a Strategy against COVID-19? Trends Pharmacol Sci. 2020 Sep;41(9):598-610. doi: 10.1016/j.tips.2020.07.003. Epub 2020 Jul 14. PMID: 32711925; PMCID: PMC7359808. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7359808/>

¹³ Jhang JJ, Yen GC.

The role of Nrf2 in NLRP3 inflammasome activation.

Cell Mol Immunol. 2017 Dec;14(12):1011-1012. doi: 10.1038/cmi.2017.114. Epub 2017 Nov 13. PMID: 29129911; PMCID: PMC5719138.

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5719138/>

¹⁴ Debnath U, Dewaker V, Prabhakar YS, Bhattacharyya P, Mandal A.

Conformational Perturbation of SARS-CoV-2 Spike Protein Using N-Acetyl Cysteine, a Molecular Scissor: A Likely Strategy to Combat COVID-19.

ChemRxiv. Cambridge: Cambridge Open Engage; 2020;

<https://chemrxiv.org/engage/api-gateway/chemrxiv/assets/orp/resource/item/60c753a54c8919810fad4380/original/conformational-perturbation-of-sars-co-v-2-spike-protein-using-n-acetyl-cysteine-a-molecular-scissor-a-probable-strategy-to-combat-covid-19.pdf>

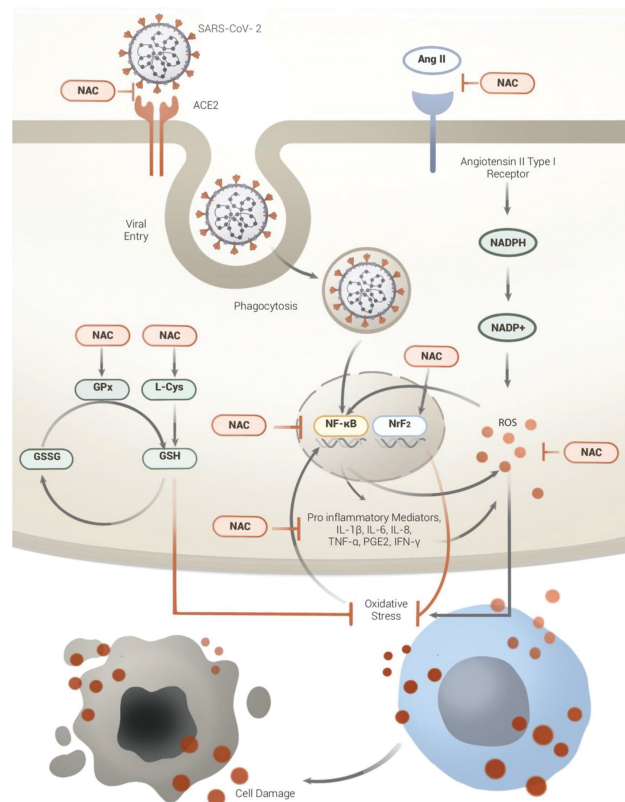
Debnath U, Mitra A, Dewaker V, Prabhakar YS, Tadala R, Krishnan K, et al.

N-acetyl cysteine: A tool to perturb SARS-CoV-2 spike protein conformation.

ChemRxiv. Cambridge: Cambridge Open Engage; 2021;

<https://chemrxiv.org/engage/api-gateway/chemrxiv/assets/orp/resource/item/60c753ec4c89190f3bad43ca/original/n-acetyl-cysteine-a-tool-to-perturb-sars-co-v-2-spike-protein-conformation.pdf>

Interestingly, almost all SARS-CoV-2 variants discovered to date retain cysteine residues in their spike protein, so it can be expected that all SARS-CoV-2 variants have an identical pattern of disulfide bonds. In addition, the observed inhibition in viral replication indicates that it might be worth investigating the role of NAC in the pharmacoprevention and treatment of COVID-19 in a clinical setting.



<https://www.jhsci.ba/ojs/index.php/jhsci/article/view/1156>

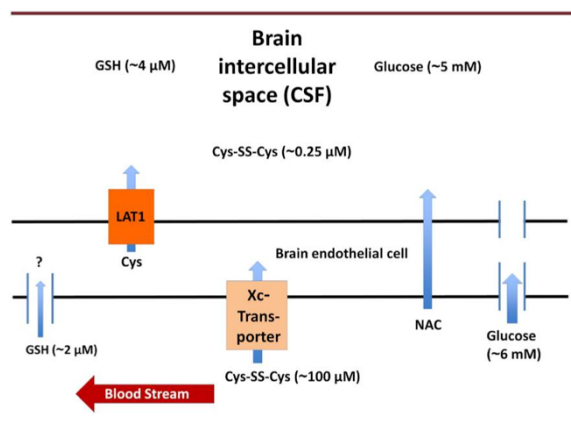
Schematic representation of the potential mechanisms of NAC as an antioxidant and anti-inflammatory in SARS-CoV2 infection. NAC: N-acetylcysteine, SARS-CoV2: severe acute respiratory syndrome coronavirus 2, ACE2: angiotensin-converting enzyme 2, Ang II: angiotensin II, GPx: glutathione peroxidase, GSSG: glutathione disulfide, GSH: glutathione, L-Cys: L -Cysteine, NF-κB: Nuclear factor-κB, Nrf2: Nuclear factor 2 related to erythroid, NADPH: Reduced nicotinamide-adenine dinucleotide phosphate, ROS: Reactive oxygen species, TNF-α: Tumor necrosis factor-alpha, IL: Interleukin, PGE2: Prostaglandin E2, IFN-γ: Interferon-gamma

TABLE 1. Clinical trials using systemic NAC as a therapeutic agent for COVID-19

Study	Clinical trial ID	Intervention	Primary outcome
A study of NAC in patients with COVID-19 infection (57)	NCT04374461 Phase 2	NAC IV 6 g/day	Number of patients who are successfully extubated and/or transferred out of critical care due to clinical improvement and discharged from the hospital due to clinical improvement
Efficacy of NAC in preventing COVID-19 from progressing to severe disease (58)	NCT04419025 Phase 4	Inpatients: • NAC 25 mg/kg oral q 4 h until discharge • NAC 1200 mg oral Outpatients: NAC 2400 mg oral then 1200 mg oral twice a day × 2 weeks	<ul style="list-style-type: none"> • Decrease in dyspnea measured by respiratory rate • Hospital length of stay • Need for mechanical ventilation • Length of time intubated • Need for hospitalization • Outpatients on NAC needing admission to the hospital • Recovery disposition
Inflammatory regulation effect of NAC on COVID-19 treatment (INFECT-19) (59)	NCT04455243 Phase 3	NAC 150 mg/kg every 12 h for 14 days (oral/intravenous)	Time to recovery
A study to evaluate OP-101 (Dendrimer N-acetyl-cysteine) in severe coronavirus disease 2019 (COVID-19) patients (PRANA) (60)	NCT04458298	OP-101 (Dendrimer N-acetyl-cysteine) 2-8 mg/kg	Number of participants with treatment-emergent adverse events

NAC: N-acetylcysteine

The effect of NAC on the SARS-Cov-2 spike can also be exploited to treat possible central and peripheral toxic reactions due to receptor binding of the spike to cells in various tissues. Of particular interest in the case of COVID and SARS-Cov-2 vaccine damage is its "antidotal" action towards spike neurotoxicity, both due to its disulfide bridge breaking effect and antioxidant action.



Indeed, it is known that GSH depletion is a feature of a wide range of neurological and ¹⁵neuropsychiatric disorders. ¹⁶

Although there are subtle differences in GSH metabolism within the CNS compared to other tissues, the basic concepts are the same: GSH is synthesized predominantly in the cytoplasm of cells and depends on cysteine influx to drive the rate-limiting step in GSH synthesis.

The major player in cysteine transport in neurons is the excitatory amino acid transporter C1 (EACAAC1); astrocytes also employ the antiporter cystine/glutamate (Xc-) to supplement the intracellular thiol pool.

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3497002/>

The schematic describes potential modes of delivery of GSH (and the rate-limiting amino acid cyst(e)ine) through the BBB. These are considered in the studies of [37], but an explicit and quantitative estimate of the net transport of GSH from plasma to CSF is not known. From the limited data provided for GSH influx and efflux with endothelial cell and astrocyte models, it seems unlikely that direct GSH transport would contribute

substantially to brain GSH content, especially since most of the GSH uptake studies by Kannan et al. were conducted with an extracellular GSH concentration of 1 mM, almost 3 orders of magnitude higher than the typical GSH concentration in plasma or cerebrospinal fluid [38,39]. The molecules cross the BBB (here represented only by the endothelial cell) at various concentrations and through different mechanisms. The drug N-acetylcysteine (NAC) can cross the BBB by passive non-ionic diffusion without a transporter.

The estimated concentrations of cystine [40,41], GSH [42] and glucose [43] in plasma and cerebrospinal fluid (CSF) are shown in the diagram. Due to its hydrophilic nature, glucose must cross the BBB using GLUT transporters even though the chemical gradient favors diffusion through the BBB. Importantly, cystine is transported into endothelial cells via the Xc- transporter and the corresponding cysteine is transported into the cerebrospinal fluid via LAT1. Some studies report that intact GSH can pass through the BBB through the use of a transport mechanism, however, characterization of the actual transporter is limited and the relative contribution of this putative mechanism to the delivery of GSH to brain cells is likely to be very small

Because cysteine is critical for neuronal synthesis of GSH, NAC has been tested in a number of neurological and neuropsychiatric disorders in which redox imbalance has been implicated in the etiology. ¹⁷

¹⁵ Bavarsad Shahripour R, Harrigan MR, Alexandrov AV.

N-acetylcysteine (NAC) in neurological disorders: mechanisms of action and therapeutic opportunities.

Brain Behav. 2014;4(2):108-122. doi:10.1002/brb3.208

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3967529/>

¹⁶ Johnson WM, Wilson-Delfosse AL, Mieyal JJ.

Dysregulation of glutathione homeostasis in neurodegenerative diseases.

Nutrients. 2012 Oct 9;4(10):1399-440. doi: 10.3390/nu4101399. PMID: 23201762; PMCID: PMC3497002.

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3497002/>

¹⁷ Unnithan AS, Choi HJ, Titler AM, Posimo JM, Leak RK.

Rescue from a two-hit, high-throughput model of neurodegeneration with N-acetyl cysteine.

Neurochem Int. 2012 Aug;61(3):356-68. doi: 10.1016/j.neuint.2012.06.001. Epub 2012 Jun 9. PMID: 22691629.

<https://pubmed.ncbi.nlm.nih.gov/22691629/>

Disease	Mechanism
Neurodegenerative disorders: SCD, tardive dyskinesia, myoclonus epilepsy, Unverricht–Lundborg type	Antioxidant effect by free-radical scavenging and increased levels of glutathione (Arakawa and Ito 2007)
Down syndrome	Increase and modulation of the level of super oxidase dismutase (Busciglio and Yankner 1995 ; Behar and Colton 2003)
Multiple sclerosis	Free-radical scavenging and inhibition of TNF toxicity (Lehmann et al. 1994 ; Stanislaus et al. 2005)
Amyotrophic lateral sclerosis	Increasing the level of glutathione peroxidase and free-radical scavenging (Rosen et al. 1993 ; Louwerse et al. 1995)
Parkinson's disease	Increasing the level of glutathione and free-radical scavenging (Schapira et al. 1990 ; Martínez et al. 1999)
Huntington's disease	Free-radical trapping and preventing mitochondrial dysfunction (Fontaine et al. 2000 ; Stanislaus et al. 2005)
Alzheimer's disease	Increasing the level of glutathione (Adair et al. 2001 ; Tchanchou et al. 2005 ; Tucker et al. 2005)
Focal cerebral ischemia	NOS inhibition, regeneration of endothelium-derived relaxing factor, increasing glutathione levels, improving microcirculatory blood flow, and tissue oxygenation (Dawson and Dawson 1997 ; Cuzzocrea et al. 2000b)
Subarachnoid hemorrhage	Free-radicals scavenger, endothelial apoptosis inhibition, lipid peroxidation reduction, increasing glutathione levels, and SOD enzymatic activities, endothelial integrity protection (Findlay et al. 1989 ; Sen et al. 2006)
Traumatic brain injury	Repair of TBI-induced mitochondrial dysfunction, increasing the reduced antioxidant enzyme and glutathione levels, inhibition of the activation of NF- κ B and TNF- α (Hoffer et al. 2002 ; Akca et al. 2005 ; Hsu et al. 2006 ; Chen et al. 2008)

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3967529/>

Summary of the mechanisms of action of NAC in different neurological disorders.

In some cases, it has been suggested that NAC might be useful in this context, not only because of GSH repletion, but also because NAC-derived cystine has the potential to lead to increased glutamate release from astrocytes via Xc⁻ antiporters, resulting in activation of neuronal glutamate receptors and dopamine release.¹⁸

A further positive effect has been found against proteinopathies. It is known that postmortem tissues from patients with neurodegenerative diseases demonstrate protein misfolding by oxidative stress (transition from alpha helix to beta leaflet conformation) and reduced proteasome activity that degrades misfolded proteins. This wide-ranging effect of proteotoxic stress has led to the term "proteinopathies" for neurodegenerative diseases. Unnithan and his team believe that toxicity-related proteinopathies with GSH loss have a good response to NAC as it can restore GSH loss and prevent toxicity associated with proteotoxic stress.¹⁹

¹⁸ Dean O, Giorlando F, Berk M.

N-acetylcysteine in psychiatry: current therapeutic evidence and potential mechanisms of action.

J Psychiatry Neurosci. 2011;36(2):78-86. doi:10.1503/jpn.100057

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3044191/>

Berk M, Malhi GS, Gray LJ, Dean OM.

The promise of N-acetylcysteine in neuropsychiatry.

Trends Pharmacol Sci. 2013 Mar;34(3):167-77. doi: 10.1016/j.tips.2013.01.001. Epub 2013 Jan 29. PMID: 23369637.

<https://pubmed.ncbi.nlm.nih.gov/23369637/>

¹⁹ Unnithan AS, Choi HJ, Titler AM, Posimo JM, Leak RK.

Rescue from a two-hit, high-throughput model of neurodegeneration with N-acetyl cysteine.

Neurochem Int. 2012 Aug;61(3):356-68. doi: 10.1016/j.neuint.2012.06.001. Epub 2012 Jun 9. PMID: 22691629.

<https://pubmed.ncbi.nlm.nih.gov/22691629/>

Katz M, Won SJ, Park Y, Orr A, Jones DP, Swanson RA, Glass GA. Cerebrospinal fluid concentrations of N-acetylcysteine after oral administration in Parkinson's disease.

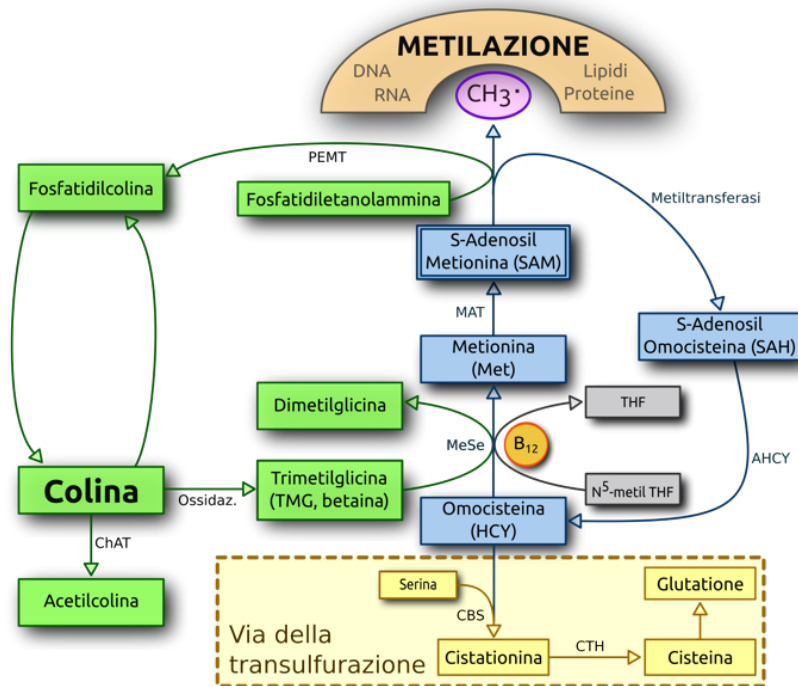
Antioxidant therapy proposal

The team working on the proposed therapy has been providing support to scientific research for years through its studies and research, carried out with national and international bodies of excellence, disseminated through peer reviewed scientific journals.²⁰

The scientific hypothesis of the research group is that the virus is activated due to the depletion of reduced glutathione and consequent inhibition of catalase at the intracellular level, in an organism in which the cellular redox-homeostatic relationship is in imbalance, i.e. in subjects under permanent oxidative stress.

The imbalance of the oxidoreductase system in the reactions of oxidation and blocking of methylation activates the virus and its replication, causing inflammation and an aggravation of the patient's state of health, until death.

For the sake of completeness, the following figure shows the biochemical cycles that lead to the production of both glutathione and methionine.



https://it.wikipedia.org/wiki/Ciclo_del_metile

Parkinsonism Relat Disord. 2015 May;21(5):500-3. doi: 10.1016/j.parkreldis.2015.02.020. Epub 2015 Feb 28. PMID: 25765302.
<https://escholarship.org/uc/item/5nf226nc>

²⁰ Cristoni S, Bernardi LR, Malvandi AM, Larini M, Longhi E, Sortino F, Conti M, Pantano N, Puccio G.
 A case of personalized and precision medicine: pharmacometabolomic applications to rare cancer, microbiological investigation, and therapy.
 Rapid Commun Mass Spectrom. 2020 Oct 14:e8976. doi: 10.1002/rcm.8976. Epub ahead of print. PMID: 33053249.

Ballabio C, Cristoni S, Puccio G, Kohler M, Sala MR, Brambilla P, Martinez-Lozano Sinues P.
 Rapid identification of bacteria in blood cultures by mass-spectrometric analysis of volatiles.
 J Clin Pathol. 2014 Aug;67(8):743-6. doi: 10.1136/jclinpath-2014-202301. Epub 2014 May 9. PMID: 24817704.

Cristoni S, Rossi Bernardi L, Larini M, Natale G, Didomenico N, Varelli M, Conti M, Dorna I, Puccio G.
 Predicting and preventing intestinal dysbiosis on the basis of pharmacological gut microbiota metabolism.
 Rapid Commun Mass Spectrom. 2019 Jul 30;33(14):1221-1225. doi: 10.1002/rcm.8461. PMID: 31013543.

Puccio G, Brambilla P, Conti M, Bartolini D, Noonan D, Albini A.
 Surface-activated chemical ionization-electrospray mass spectrometry in the analysis of urinary thiodiglycolic acid.
 Rapid Commun Mass Spectrom. 2013 Feb 15;27(3):476-80. doi: 10.1002/rcm.6471. PMID: 23280980.

²¹ <https://www.holisticheal.com/media/downloads/autism-pathways-to-recovery-book.pdf>
https://mednat.news/cure_natur/metilazione-acidi-nucleici_Yasko.pdf

The imbalance in the oxidant phase (oxidants are greater than the reducing elements) in subjects who show high parameters of homocysteine (hyperhomocysteinemia) this causes the block (inhibition) of catalase and the depletion of reduced glutathione (intracellular).

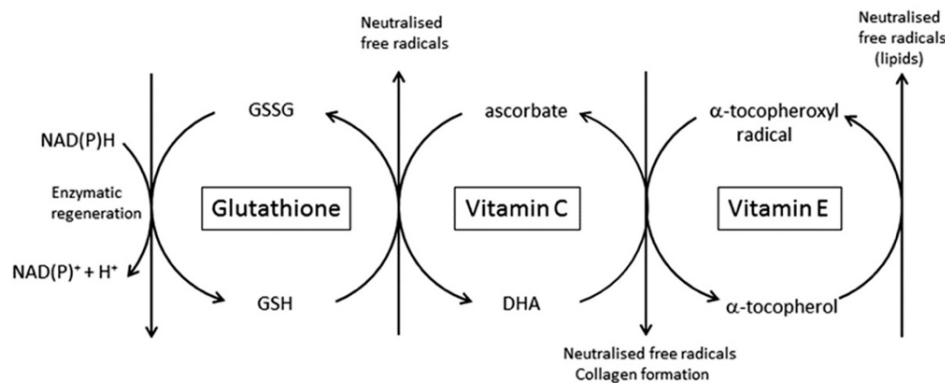
This imbalance in the oxidoreductase group system, secondary oxidation reactions similar to those catalysed by peroxidase, causes a malfunction of methylation and the consequent replication of the virus, activating a state of permanent inflammation due to the peroxides released by our immune system, which fails to fight the virus and releases harmful elements that lead to the collapse of the organism. The release of peroxides can cause multi-organ syndrome and death by cardiac arrest.

It should be noted that while GSH is produced by the human body, ascorbate must be taken in through the diet, and it is important that you take it in because it is an essential nutrient, as it can eliminate highly damaging free radicals such as OH^- , RO^- , ROO^- or GS^- . This makes ascorbate defined as an antioxidant molecule that can also reduce several redox active metals such as iron and copper.

In addition to being the most important and active non-enzymatic antioxidant molecule in the cell, eliminating hydroxyl and superoxide radicals, GSH also has several other functions: it is a cofactor for several detoxifying enzymatic reactions and is involved in the regeneration of other important antioxidants such as vitamins C and E, with two GSH molecules oxidizing to GSSG.

The reducing system resulting from the cooperation of GSH with the oxidoreductive activity of ascorbate, is in normal conditions continuously regenerated through a sequence of reactions that involves the intervention of two enzymes: glutathione peroxidase and glutathione reductase.

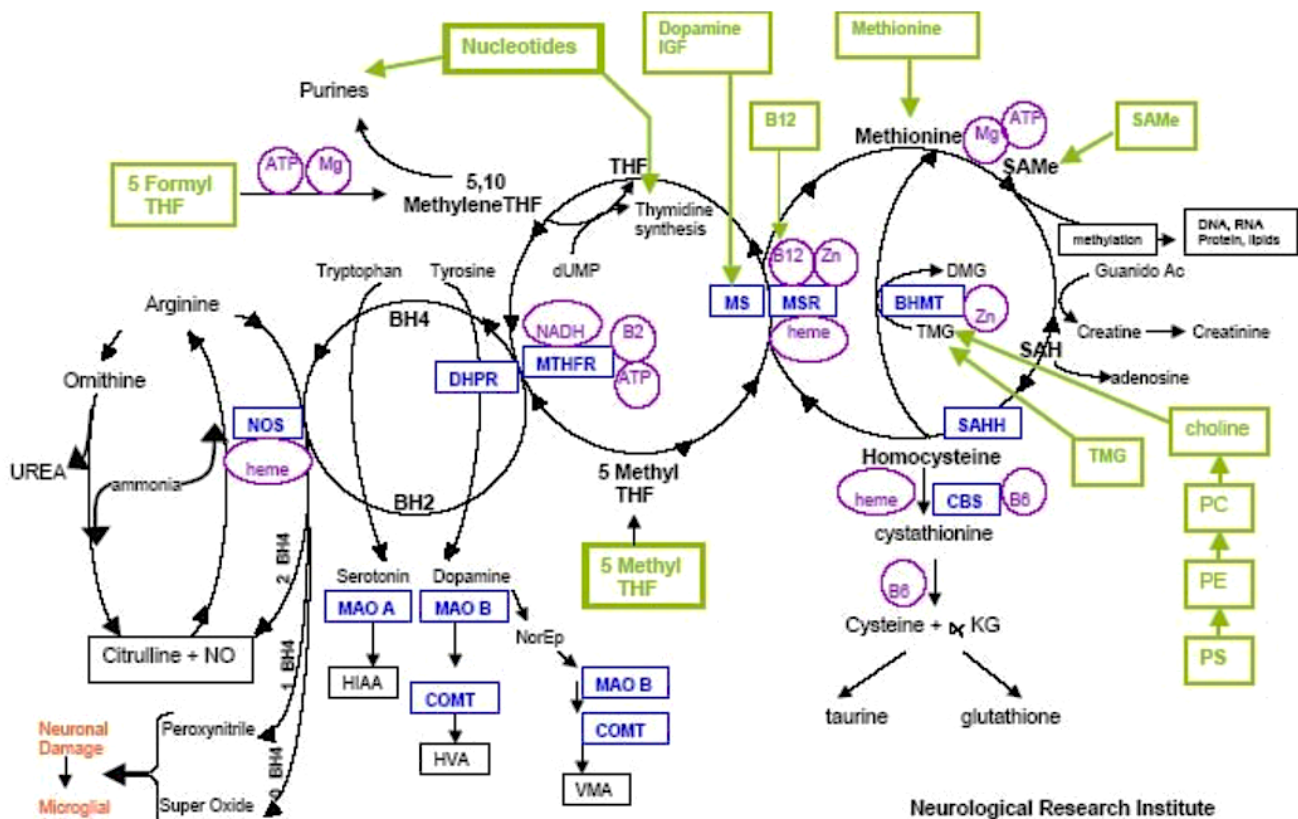
Antioxidant cycle:



<https://www.mdpi.com/2072-6643/9/8/866/htm>
GSSG: oxidised glutathione; DHA: dehydroascorbic acid

The following figure shows:

in the **BLUE** boxes: the main enzymes; **VIOLET** circles: enzyme cofactors; **GREEN** boxes: supplements



https://mednat.news/cure_natur/metilazione-acidi-nucleici_Yasko.pdf

The proposed therapy for critically ill patients involves the following protocol,²² for which please refer to the research group for more information.

medications	administration	Biological effect
Glutathione (GSH)	intravenous	Supports the liver producing GSH to reduce oxidative stress
N-acetylcysteine (NAC)	intravenous	It is partially involved in the biosynthesis of cysteine which is in turn involved in the biosynthesis of GSH and the reduction of oxidative stress
Vitamin C	intravenous	It is involved in the biochemical synthesis of GSH in the liver, reduces oxidative stress, and improves the efficiency of the immune system
Vitamin B complex	oral	Catalyzes the transformation of homocysteine to methionine thus reducing bacterial and viral resistance through DNA/RNA (de)methylation
Antivirals/antibiotics	intravenous	Used to fight viral and bacterial infections

Phlebotomy

Compose an IV with the following elements:

²² Cristoni S, Bernardi LR, Malvandi AM, Larini M, Longhi E, Sortino F, Conti M, Pantano N, Puccio G.

A case of personalized and precision medicine: pharmacometabolomic applications to rare cancer, microbiological investigation, and therapy. Rapid Commun Mass Spectrom. 2020 Oct 14:e8976. doi: 10.1002/rcm.8976. Epub ahead of print. PMID: 33053249.

John Puccio. (2021).

SARS-COV-2 and COVID-19: from RESEARCH to PREVENTION. <http://doi.org/10.5281/zenodo.4555178>

https://zenodo.org/record/4555178#.YN5T_RMzbXQ

- n. 1 glucosate solution 5 of 500ml (in case of hyperglycaemia use physiological solution. Once the blood values are restored, return to the glucose solution)
- n. 3 vials with 600 mg of GSH;
- n. 2 vials with 300 mg of N-acetylcysteine;
- n. 1 vial of 1 gram ascorbic acid, should be increased up to 4 grams depending on neutrophils or high LDH value, to block peroxides and avoid damage to the heart muscle.

It is necessary to modify the treatment on the basis of some of the patient's parameters, which will be monitored before the beginning of the therapy and in the intermediate phase of administration in order to intervene on the dosages and on the integration of other elements.

C.R.A.Pu Therapy for Prophylaxis

Before proceeding with prophylactic treatment, it is essential to perform:

- plasma homocysteine analysis;
- vitamin D analysis;

If high homocysteine values (concentration > 13 ng/mL) and low vitamin D values (concentration < 20 mcg/mL) are found, the following therapy is carried out:

Oral treatment

- Vit. D at least 10,000 units per day (15,000 to over 70) and at a distance of 5 hours Vit. K2 in a ratio of 10:1;
- Betaine anhydrous (Trimethylglycine) - has been approved by the NDA (New Drug Application), the recommended dose is one gram per day, in Italy the Ministry of Health has deemed it appropriate to establish the maximum daily dose of 250 mg;
- Magnesium, 500 mg a day;
- B6 - 1.5 mg per day;
- B9 - 400 mcg per day;
- B12 - 33 mcg per day.

If homocysteine is not lowered, methylfolate (calcium mefolinate 15 mg per day) is used.
