Inflammation, SARS-CoV-2 infection and mental health disorders impact on systemic serotonin and dopamine metabolism

Manuscript

Katharina Hüfner1,✉, Sophia Vedova, Piotr Tymoszuk2, Philipp Nelles1, Markus Anliker3, Tobias Bruckner1, Eberhard Deisenhammer1,4, Jonas Egeter1, Alexander Egger3, Matyas Galffy1, Johannes Giesinger1, Jens Lehmann4, Maria Oberhammer4, Joachim Rockenschaub1, Magdalena Sacher5, Bernhard Holzner1, Johanna M Gostner6, and Barbara Sperner-Unterweger1

2023-05-23

1 Department of Psychiatry, Psychotherapy, Psychosomatics and Medical Psychology, University Hospital for Psychiatry II, Medical University of Innsbruck, Anichstrasse 35, Innsbruck, Austria  
2 Data Analytics As a Service Tirol, Innsbruck, Austria  
3 Central Institute of Medical and Chemical Laboratory Diagnostics, University Hospital Innsbruck, Anichstrasse 35, Innsbruck, Austria  
4 Department of Psychiatry, Psychotherapy, Psychosomatics and Medical Psychology, University Hospital for Psychiatry I, Medical University of Innsbruck, Anichstrasse 35, Innsbruck, Austria  
5 Department of Visceral, Transplant and Thoracic Surgery, Center of Operative Medicine, Medical University of Innsbruck, Innsbruck, Austria  
6 Institute of Medical Biochemistry, Biocenter, Medical University of Innsbruck, Innrain 80/82, Innsbruck, Austria

✉ Correspondence: [Katharina Hüfner <[katharina.huefner@tirol-kliniken.at](mailto:katharina.huefner@tirol-kliniken.at)>](mailto:katharina.huefner@tirol-kliniken.at)

# Keywords

SARS-CoV-2, inflammation, serotonin, dopamine, tryptophan, kynurenine, psychiatric disorders

# Abstract

**Background:** Serotonin and dopamine metabolism poses a junction of mental and physical health. We aimed to identify demographic, clinical, psychometric and SARS-CoV-2 infection-related factors affecting systemic serotonin and dopamine turnover.

**Methods:** The cross-sectional SIMMUN (n = 165, Austria) and longitudinal INCOV cohort (n = 167, Su et al. 2022) were investigated. Explanatory variables encompassed age, sex, clinical and inflammatory variables, SARS-CoV-2 infection, signs of anxiety and depression (HADS), and mental stress scoring (PSS-4). Their effects on serotonin biosynthesis markers (serotonin, tryptophan) and competitor kynurenine pathway products (kynurenine, quinolinate), and dopamine availability readouts (dopamine sulfate, phenylalanine, tyrosine) were assessed by linear modeling, correlation analysis and two-tailed T tests.

**Results:** In the SIMMUN cohort, the inflammatory marker neopterin, SARS-CoV-2 infection, age, depression symptoms and mental stress were positively associated with catabolism of the serotonin precursor tryptophan to kynurenine. Inflammation and age were also suppressed the first step of dopamine biosynthesis, phenylalanine - tyrosine conversion. In the INCOV cohort, inflammation was associated with lowered serotonin (IL6: = -0.24 [95% CI: -0.4 to -0.089]) and the dopamine metabolite, dopamine sulfate (interferon-gamma: = -0.13 [95% CI: -0.24 to -0.014]). Serotonin ( = 0.72 [95% CI: 0.3 to 1.1]) and dopamine sulfate ( = 0.64 [95% CI: 0.28 to 1]) were significantly increased during SARS-CoV-2 infection recovery.

**Conclusion:** Inflammation limits systemic serotonin and dopamine by activating the competitor kynurenine pathway and inhibiting the phenylalanine - tyrosine conversion, respectively, and may link SARS-CoV-2 infection with mental health. Advanced age and psychiatric disorders can additionally suppress neurotransmitter synthesis.

# Introduction

Physical conditions such as infections, trauma, frailty, autoimmune illness or malignancy are often accompanied by sings of depression, anxiety, metal stress and other psychiatric disorders (1–8). Imbalance of two depressiolytic and anxiolytic neurotransmitters, serotonin and dopamine, elicited by inflammation as proposed as a connex of physical pathology and mental health deficits (2,9,10). Serotonin is synthesized from the essential aminoacid tryptophan (TRP) via reactions catalyzed by tryptophan hydroxylase (TPH) and aromatic L-amino acid decarboxylase. Yet the great majority of TRP is by the so called kynurenine (KYN) pathway, whose first step is the breakdown of TRP to KYN catalyzed by indoleamine 2,3-dioxygenase (IDO) (11,12). Expression of an inducible and highly active form of IDO, IDO1, is strongly stimulated by a plethora of cytokines, pathogen products and other inflammatory stimuli (11,13–15). During systemic or central nervous system-localized inflammation, TRP is depleted by high IDO1 activity, which, in turn, suppresses serotonin biosynthesis (7,9,10,16). Consequently, elevated blood levels of KYN pathway metabolites, decreased TRP and serotonin were reported for numerous physical conditions and associated with symptoms of psychiatric deficits (3,8,9,17–22). Dopamine synthesis involves the conversion of another essential amino acid phenylalanine (PHE) to tyrosine (TYR) catalyzed by phenylalanine hydroxylase (PAH) followed by hydroxylation of TYR by tyrosine 5-hydroxylase (TH) (23). Both PAH and TH as well as the serotonin-synthesizing enzyme TPH rely on tetrahydrobiopterin (BH4) as an essential cofactor. During inflammation, BH4 is depleted by oxidation to its inactive derivative and gets consumed in a competitor reaction of nitric oxide formation. As a result of such functional BH4 deficiency, biosynthesis of dopamine and serotonin is further suppressed (6,16,24). Accordingly, imbalance of PHE and TYR levels suggestive of systemic dopamine deficiency was reported in cancer, infections and inflammatory conditions and associated with depression and anxiety (6,8,16,24,25).

SARS-CoV-2 virus is the causal pathogen of coronavirus disease 2019 (COVID-19). Apart from sustained physical disability, psychiatric disorders in millions of SARS-CoV-2 patient amount to the persistent burden of the COVID-19 pandemic (26–30). Of note, high activity of the KYN pathway and signs of inflammatory BH4 deficiency manifesting by distorted PHE and TYR levels were reported for COVID-19 patients and associated with inflammation and disease severity (31–37). Furthermore, reduced systemic availability of serotonin and dopamine was postulated to contribute to mental health deficits during SARS-CoV-2 infection recovery (38–40)

Still, the impact of inflammation on systemic metabolism of serotonin and dopamine availability in acute SARS-CoV-2 infection and recovery, along with its mutual interaction with psychiatric disorders is still incompletely characterized. To address that, we explored effects of demographic and clinical factors, inflammation, SARS-CoV-2 infection, anti-SARS-CoV-2 humoral response and psychiatric disorder symptoms on readouts of systemic serotonin and dopamine availability and kynurenine pathway activity in two cross-sectional cohorts of SARS-CoV-2 patients: the SIMMUN cohort and the published longitudinal INCOV study (41).

# Materials and Methods

## Ethics statement

The SIMMUN study was conducted in accordance with the Declaration of Helsinki and European Data Policy. All participants gave written informed consent prior to enrollment. Participants’ data were stored and analyzed in anonymized form. This study was approved by the ethics committee of the Medical University Innsbruck (Austria, approval number: 1132/2020). No approval by the ethics committee was required for analysis of the published INCOV dataset (41).

## Study cohorts

Details on study cohorts, procedures and analyses are provided in **Supplementary Methods**.

### SIMMUN study

Individuals tested for SARS-CoV-2 via PCR at the University Hospital of Innsbruck and patients of the University Clinic for Psychiatry I and II (Innsbruck, Austria) undergoing routine SARS-CoV-2 PCR were invited to participate. The study was conducted between June 2020 and May 2021. The inclusion criteria were age of 18 - 70 years, proficiency in German, residence in the study region (Tyrol, Austria), and a SARS-CoV-2 PCR test conducted at the study site. The exclusion criteria were active SARS-CoV-2 infection (< 14 days after diagnosis), pregnancy, active malignancy, organ transplantation, surgery in the past 3 months, inflammatory illness and oral corticosteroid treatment. The analysis inclusion criterion was the complete study variable dataset (**Figure 1**, **Supplementary Table S1**). Significant differences between the analyzed and excluded participants are listed in **Supplementary Table S2**.

### INCOV study

Proteome and metabolome data and clinical information for the INCOV cohort were extracted from supplementary tables accompanying the report by Su and colleagues (41). In the current analysis, samples obtained for individuals with the complete age information and complete study variable dataset (**Figure 1**, **Table 2**, **Supplementary Table S3**).

## Procedures

### SIMMUN study

The SIMMUN study data were gathered during a single on-site study visit including a general medical assessment, supervised completion of self-rating questionnaire and a blood sample collection (**Supplementary Table S1**). The median time interval between the study visit and SARS-CoV-2 PCR was 139 days (interquartile range: 119 - 157).

Demographic and clinical variables: age, sex, body mass index, professionally diagnosed psychiatric disorders, self-reported physical disorders, smoking and alcohol consumption, result and date of the SARS-CoV-2 PCR test were surveyed during the study visit or extracted from electronic patient records. Inflammatory markers: plasma neopterin concentrations (NEO) and neutrophil - lymphocyte ratio (NLR) were determined by the certified clinical routine laboratory at the University Hospital of Innsbruck. Plasma concentrations of tryptophan (TRP), kynurenine (KYN), phenylalanine (PHE) and tyrosine (TYR) were determined by high-performance liquid chromatography, and the KYN/TRP and PHE/TYR ratios calculated (22,25,42). Plasma titer of immunoglobulin gamma against receptor binding domain S1/S2 protein (anti-RBD IgG) were quantified by ELISA (43). Laboratory measurements at and beyond the detection limits were substituted with the lower or upper detection limit value, respectively. Mental stress was scored with the 4-item perceived stress scale (PSS-4) (44). Anxiety and depression signs were scored with the hospital anxiety and depression scale (HADS) including 7 items for anxiety and 7 items for depression (45). Clinically relevant signs of anxiety or depression were identified with the cutoff of 8 points (45,46).

In order to improve normality of some numeric study variables prior to linear modeling and statistical hypothesis testing with parametric tools, logarithm or square root transformations were applied (**Supplementary Table S1**)

### INCOV cohort

Plasma proteomes and metabolomes in the INCOV cohort were measured by proximity extension assay (Olink, Sweden) and ultra-high-performance liquid chromatography/tandem accurate mass spectrometry (Metabolon, USA) (41,47). Normalized, age- and sex-adjusted, log2-transformed plasma concentrations of metabolites and cytokines, and clinical information were extracted from [supplementary tables](https://data.mendeley.com/datasets/96v329bg7g/1) of the report by Su et al. (41). Metabolites implicated in systemic serotonin availability (serotonin, TRP), KYN pathway activity (KYN, quinolinate [QUIN]) and dopamine availability (PHE, TYR and dopamine 3-O-sulfate [DA sulfate]), cytokine markers of inflammation (interleukin-6 [IL6], interleukin-10 [IL10], tumor necrosis factor-alpha [TNF] and interferon-gamma [IFNG]) and age were analyzed (**Supplementary Table S3**).

Plasma features were sampled in uninfected controls and SARS-CoV-2 individuals during acute (median: 10) and sub-acute (median: 14) infection, and recovery (median: 64 days after diagnosis of SARS-CoV-2 infection via PCR) (**Supplementary Table S3**).

## Analysis endpoints

Our analysis pursued two endpoints. The first endpoint was to determine demographic, clinical, psychometric, inflammation- and SARS-CoV-2-related factors influencing plasma levels of serotonin and dopamine precursors and KYN pathway metabolites (TRP, KYN, KYN/TRP, PHE, TYR, PHE/TYR). This endpoint was addressed by multi-parameter modeling in the SIMMUN cohort. The second endpoint was to investigate how more direct readouts of systemic serotonin and dopamine availability, plasma serotonin (21,48–50) and DA sulfate (23,51,52), are influenced by age, inflammatory cytokines, timepoint of the SARS-CoV-2 infection, neurotransmitter precursors (TRP, PHE, TYR) and KYN pathway products (KYN, QUIN). This endpoint was addressed by multi-parameter robust linear modeling, time course modeling and correlation analysis in the INCOV cohort (**Figure 1**).

## Statistical analysis

R version 4.2.3 was used for statistical analysis.

Numeric variables were presented as medians with interquartile ranges and ranges. Categorical variables are presented as percentages and counts within the complete observation set. Distribution normality and variance homogeneity was assessed by Shapiro-Wilk and Levene test, respectively. In the SIMMUN cohort, non-normally distributed numeric variables were logarithm- or square root-transformed prior to modeling and parametric tests (**Supplementary Table S1**). Since most of the INCOV study variables were non-normally distributed, robust linear modeling and non-parametric testing were employed. Since the tau-equivalence assumption investigated by factor analysis for the HADS scales was violated, consistency of psychometric tools was investigated by global McDonald’s (53–55). Except for multi-parameter modeling, p values were corrected for multiple testing with the false discovery rate method (56) separately for each analysis task. Effects with p < 0.05 were considered significant.

Correlation was assessed by Spearman’s rank or Pearson test. Significance of comparisons of numeric variables between two groups was determined by Mann-Whitney test with r effect size statistic or by two-tailed T test with Cohen’s d effect size metric. Comparisons of categorical variable distributions were evaluated by test with Cramer V effect size statistic. Correlation was assessed by Pearson’s or Spearman’s rank test (57,58).

In the SIMMUN cohort, effects of age, sex, body mass class, physical and psychiatric disorders, body mass class, smoking and alcohol consumption, inflammation markers (NEO, NLR), SARS-CoV-2 infection, anti-RBD IgG titer, depression and anxiety signs (HADS), mental stress (PSS-4) on TRP, KYN, KYN/TRP, PHE, TYR and PHE/TYR were assessed by multi-parameter linear regression with backward elimination. Modeling responses and explanatory variables were subjected to normality-stabilizing transformations (**Supplementary Table S1**) and normalized. Normality and homogeneity of model residuals were evaluated by Shapiro-Wilk and Levene test, respectively, and visual inspection (residuals versus fitted and quantile-quantile plots). In the INCOV cohort, effects of age, cytokine markers of inflammation (IL6, IL10, TNF, IFNG), SARS-CoV-2 infection timepoint (acute, sub-acute, recovery versus uninfected control), neurotransmitter precursors (TRP, PHE, TYR) and KYN pathway products (QUIN, KYN) on plasma serotonin and DA sulfate were modeled by multi-parameter robust linear regression with the MM algorithm and Huber psi function (59,60). Reproducibility and proper parameterization of the multi-parameter linear and robust models was investigated by RMSE and statistics in 10-fold cross-validation (61). Significance of the model estimates was assessed by two-tailed T test.

Differences in cytokines and metabolites between SARS-CoV-2 infection timepoints in the INCOV collective were investigated by robust linear modeling (MM algorithm, Huber’s psi function) with uninfected subset or acute infection serving as baselines.

Pairwise Spearman’s correlation coefficients were calculated for cytokines and metabolites in the INCOV cohort. The correlation matrices were subsequently scaled into the [0, 1] range and converted to undirected force directed graphs (62). The graphs were visualized as two-dimensional network plots with the node proximity determined by the value and distance-dependent repulsion, and edge color and width coding for the value and sign (63).

# Results

## Characteristic of the study cohorts

Herein, two cohorts of uninfected and SARS-CoV-2-positive individuals were analyzed. Out of 215 individuals enrolled in the SIMMUN study, 165 participants with a complete study variable dataset were analyzed (**Figure 1**, **Supplementary Table S1**). The excluded individuals were characterized by more frequent psychiatric disorders, depression and anxiety signs, elevated mental stress scoring and less frequent SARS-CoV-2 infections as compared with the analyzed participants (**Supplementary Table S2**). SARS-CoV-2-positive individuals accounted for 61% of the analyzed SIMMUN cohort. None of the participants had received anti-SARS-CoV-2 vaccination prior to enrollment. Males represented 38% of the cohort and the median age was 50 years. The gender and age structure of the SARS-CoV-2-negative and -positive subsets was comparable. In the entire SIMMUN cohort, 47% of participants were overweight or obese, the physical disorder rate was 51%; these figures were comparable between SARS-CoV-2-negative and -positive individuals. Psychiatric disorders affected 41% of participants and were significantly more common in SARS-CoV-2-negative (50%) than in SARS-CoV-2-positive individuals (28%, p = 0.021, effect size: p = 0.021). Signs of depression and anxiety, and intensity of mental stress were gauged with the HADS depression, HADS anxiety and PSS-4 tools, respectively (44,45). The psychometric tools displayed good-to-excellent internal consistency ( = 0.74 - 0.96) (53) (**Supplementary Figure S1**). Clinically relevant anxiety signs (HADS 8) (45,46) were more frequent in the uninfected (43%) than in the SARS-CoV-2-positive subset (20%, p = 0.013, effect size: V = 0.23). Depression signs were more common in SARS-CoV-2-negative (31%) than in SARS-CoV-2-positive participants (16%), yet this effect was no significant. Scores of mental stress were comparable in both SARS-CoV-2 subsets (negative: median 6, positive: median 5 points). As expected, titer of antibodies against the S1/S2 SARS-CoV-2 protein (anti-RBD IgG) was significantly higher in the convalescent subset (median: 16 AU) as compared with uninfected individuals (median: 0.31 AU, p < 0.001, effect size: r = 0.84). In 73% of SARS-CoV-2-infected SIMMUN study participants, the infection was mild and ambulatory (**Table 1**).

Out of 645 individuals initially enrolled in the INCOV study (41,47), 167 participants with 354 samples with a complete study variable dataset were analyzed (**Figure 1**, **Supplementary Table S3 - S4**). SARS-CoV-2-positive individuals comprised 84% of the analyzed INCOV collective. Males constituted 56% of participants, the median age was 60 years. SARS-CoV-2-positive participants (median age: 62 years) were significantly older than the uninfected subset (median age: 56 years, p = 0.044, effect size: r = 0.17). Shares of overweight or obese individuals were higher among SARS-COV-2-positive (74%) than SARS-CoV-2-negative participants (55%), yet this effect missed statistical significance. Nearly all (97%) of SARS-CoV-2-positive INCOV individuals were hospitalized due to a moderately or critically severe infection (**Table 2**).

As compared with the SIMMUN collective, the INCOV cohort was characterized by a significantly higher percentage of males (p = 0.0014, effect size: V = 0.18), more advanced age (p < 0.001, effect size: p < 0.001), elevated rates of overweight or obesity (p < 0.001, effect size: V = 0.28), higher share of SARS-CoV-2 cases (p < 0.001, effect size: V = 0.46) and higher hospitalization rates (p < 0.001, effect size: V = 0.72) (**Supplementary Table S5**).

## Inflammation, SARS-CoV-2 infection, age, mental stress and depression influence systemic levels of neutrotransmitter precursors and kynurenine pathway activity

We employed the SIMMUN dataset in an initial search for predictors of systemic neurotransmitter availability. As markers of serotonin turnover, we modeled plasma levels of the serotonin precursor TRP and readouts of the competitor KYN pathway, KYN and KYN/TRP (11,12,22). Plasma concentrations of PHE, TYR and PHE/TYR were investigated as markers of dopamine availability (6,23,25). The candidate explanatory variables were age, sex, body mass, physical and psychiatric disorders, alcohol and tobacco consumption, SARS-CoV-2 infection, anti-SARS-CoV-2 antibody titer, NEO and NLR as inflammatory markers, mental stress, depression and anxiety signs (**Supplementary Table S1**). All those variables were recorded at a single timepoint, median 139 days after the SARS-CoV-2 test (interquartile range: 119 - 157).

Full multi-parameter linear models were optimized by backwards elimination of non-significant terms (60) (**Supplementary Table S6**). Meaningful models could be established for TRP, KYN, KYN/TRP, TYR and PHE/TYR. The PHE model had a negligible explanatory performance, proved non-significant as compared with the intercept-only null model and was hence not further analyzed. The remaining models were characterized by good reproducibility and proper parameterization as indicated by comparable fit errors in the genuine dataset and cross-validation. The KYN and KYN/TRP ratio models had the best explanatory performance measured by cross-validated of 0.21 and 0.3, respectively. The TRP, TYR and PHE/TYR models could explain between 10% and 15% of the cross-validated response variances (, **Supplementary Figure S2**).

The inflammatory marker NEO ( = -0.19 [95% CI: -0.34 - -0.04]) and depression signs (HADS 8, = -0.48 [95% CI: -0.83 - -0.14]) were independently associated with reduced plasma TRP. Increased KYN and KYN/TRP were significantly associated with SARS-CoV-2 infection (KYN: = 0.48 [95% CI: 0.2 - 0.76], KYN/TRP: = 0.42 [95% CI: 0.16 - 0.68]), elevated NEO (KYN: = 0.35 [95% CI: 0.2 - 0.49], KYN/TRP: = 0.47 [95% CI: 0.34 - 0.61]) and age (KYN: = 0.21 [95% CI: 0.067 - 0.35], KYN/TRP: = 0.26 [95% CI: 0.12 - 0.39]). Additionally, high mental stress measured by PSS-4 was linked to increased KYN/TRP ( = 0.18 [95% CI: 0.055 - 0.31]).  
Low NLR, in turn, was related to increased KYN (-0.19 [95% CI: -0.32 - -0.051]) (**Figure 2A**). Plasma TYR concentrations were negatively associated with the inflammatory marker NEO ( = -0.2 [95% CI: -0.35 - -0.048]) and rose significantly with age ( = 0.37 [95% CI: 0.21 - 0.52]). SARS-CoV infection ( = -0.38 [95% CI: -0.68 - -0.08]) and age ( = -0.27 [95% CI: -0.41 - -0.12]) were linked to lower PHE/TYR (**Figure 2B**).

By univariable analysis, we could corroborate significant, positive, moderate-to-strong correlations of age and NEO with increased KYN and higher KYN/TRP in the SIMMUN dataset. Participant’s age was paralleled by elevated circulating TYR and lowered PHE/TYR in univariable correlation analysis (**Supplementary Figure S3 - S4**, **Supplementary Tables S7 - S8**).

## Inflammatory cytokines, SARS-CoV-2 infection course, age and availability of biosynthesis precursors regulate systemic serotonin and dopamine turnover

Next, we sought to validate the effects of inflammation, SARS-CoV-2 infection and age on systemic serotonin and dopamine availability in the longitudinal INCOV cohort (41). The outcome variables in such analyses were plasma levels of serotonin (12,21,48–50,64), and DA sulfate, a the main circulating dopamine catabolite (23,51,52). The explanatory variables were age, infection timepoint, major cytokines induced by inflammatory signaling (IL6, IL10, TNF, IFNG), the serotonin precursor TRP, KYN pathway metabolites (KYN, QUIN) (11,12,22), and dopamine precursors (PHE, TYR) (23,25) (**Supplementary Table S3**). Metabolites and cytokines were sampled in SARS-CoV-2 INCOV patients at three timepoints of infection: acute (median 10), sub-acute (median 14) and recovery (median 64 days after infection diagnosis via PCR) (**Supplementary Table S4**).

Multi-parameter robust regression models (59,60) could explain 13% and 11% of cross-validated variance () of serotonin and DA sulfate, respectively. Comparable fit errors in the training dataset and cross-validation indicated good reproducibility and proper parameterization of the models (**Figure 3A**). In the INCOV collective, 4 predictors of plasma serotonin were identified. Age ( = -0.15 [95% CI: -0.25 - -0.041]) and the inflammatory cytokine IL6 ( = -0.24 [95% CI: -0.4 - -0.089]) were associated with significantly lower serotonin levels, whereas TRP ( = 0.17 [95% CI: 0.048 - 0.29]) and the SARS-CoV-2 infection recovery ( = 0.72 [95% CI: 0.3 - 1.1]) were predictors of increased plasma serotonin. Infection recovery ( = 0.64 [95% CI: 0.28 - 1]) and TNF ( = 0.14 [95% CI: 0.032 - 0.24]) predicted higher DA sulfate levels, and IFNG was associated with significantly decreased DA sulfate concentrations ( = -0.13 [95% CI: -0.24 - -0.014]) (**Figure 3**, **Supplementary Table S9**).

This association of serotonin and DA sulfate levels wuth inflammation was confirmed by time course modeling. The maximum plasma concentrations of inflammation markers IL6, IL10, TNF and IFNG were observed during acute infection. They were paralleled by a significant decrease of the serotonin precursor TRP ( = -1.9 [95% CI: -2.7 - -1]) and upregulated TRP catabolism products KYN ( = 1.5 [95% CI: 0.99 - 2]) and QUIN ( = 1.8 [95% CI: 1.3 - 2.4]) as compared with uninfected individuals. The inflammatory milieu of acute infection was associated with a significant rise in PHE ( = 1.4 [95% CI: 0.97 - 1.9] versus uninfected), suggestive of an inhibited PHE - TYR conversion (25). Resolution of systemic inflammation during sub-acute SARS-CoV-2 infection and recovery was reflected by decreasing plasma levels of IL6, IL10, TNF and INFG as compared with acute disease. The inflammation resolution was paralleled by decreased KYN (sub-acute: = -0.31 [95% CI: -0.6 - -0.022], recovery: = -1.3 [95% CI: -1.6 - -0.9]) and QUIN (sub-acute: = -0.29 [95% CI: -0.61 - 0.022], recovery: = -1.4 [95% CI: -1.8 - -0.97]), paralleled by elevated TRP (sub-acute: = 0.75 [95% CI: 0.25 - 1.3], recovery: = 1.9 [95% CI: 1.2 - 2.5]) and serotonin (sub-acute: 0.22 [95% CI: -0.1 - 0.54], recovery: = 1.2 [95% CI: 0.81 - 1.6]) as compared with acute SARS-CoV-2 infection. Similarly, infection recovery was associated with significantly decreased PHE (recovery: = -1.2 [95% CI: -1.5 - -0.83]) and elevated DA sulfate (recovery: = 1.5 [95% CI: 1.1 - 2]) as compared with acute infection.

In correlation analysis, the serotonin precursor TRP was negatively associated with all investigated cytokines during acute (: -0.38 - -0.24) and sub-acute infection (: -0.51 - -0.33). Plasma serotonin levels correlated negatively with IL6 concentrations during acute infection ( = -0.23) and was negatively associated with IL6, IL10, TNF and IFNG in sub-acute infection (: -0.44 - -0.26). By contrast, the TRP catabolites KYN (acute, : 0.42 - 0.58; sub-acute. : 0.56 - 0.65) and QUIN (acute, : 0.39 - 0.69; sub-acute, : 0.59 - 0.71) correlated positively with circulating IL6, IL10, TNF and IFNG. Serotonin levels correlated with TRP during acute ( = 0.2) and sub-acute infection ( = 0.3) with weak and moderate effect size, respectively. This may indicate that systemic availability of tryptophan may be particularly crucial for serotonin synthesis at the peak of the infection-related inflammation (**Figure 5A**, **Supplementary Figure S5**). Concerning the dopamine availability readouts, positive correlations of PHE with the cytokine markers of inflammation IL6, IL10 and TNF were observed in acute infection (: 0.24 - 0.39). PHE was positively associated with IL10 in sub-acute SARS-CoV-2 infection ( = 0.29). TYR plasma concentrations correlated in turn negatively with TNF and IFNG during acute ( = -0.23) and sub-acute infection (: -0.26 - -0.22). DA sulfate was negatively associated with IFNG and IL6 in acute (IFNG, = -0.22) and sub-acute infection (IL6, = -0.23), respectively (**Figure 5B**, **Supplementary Figure S5**).

# Discussion

Herein, we explored effects of demographic, clinical, psychometric, infection-, inflammation- and SARS-CoV-2-related factors on systemic availability of serotonin and dopamine in SARS-CoV-2 infection. Our results underline the pivotal role of SARS-CoV-2-dependent inflammation in systemic neurotransmitter metabolism. Furthermore, they put forward plasma TRP levels as a one of limiting factors for systemic serotonin biosynthesis during SARS-CoV-2 infection. Age, mental stress and depression were proposed as infection-independent factors contributing to activity of the KYN pathway, lowered TRP and, consequently, reduced serotonin formation. Our data suggest similar inhibitory effects of inflammation and age on PHE - TYR conversion, which is the initial step of dopamine formation.

Two discrepancies of our results need a more thorough discussion. First, in the SIMMUN cohort, the inflammatory marker NEO was associated with higher plasma levels of KYN (22). Unexpectedly, another inflammatory variable, NLR could be linked to lower KYN. This may be explained e.g. by sustained suppression of neutrophil counts reported for up to 1 year after infection (65). Second, effects of inflammatory markers on neurotransmitter-associated metabolites were stronger in the INCOV than in the SIMMUN cohort. This phenomenon can be traced back to the more severe infection in INCOV study participants.

Mechanistically, multiple inflammatory cytokines such as type I and type II interferon, TNF or IL6, identified here as a predictor of plasma serotonin in the INCOV cohort, signal via JAK/STAT or NK-B pathways, which in turn activate transcription of the *IDO1* gene (11,13–15). The IDO1 protein, together with inflammation-independent enzyme forms, TDO and IDO2, catalyze the first step of the KYN pathway, which catabolizes >90% TRP in the body (11,12,66). As such, conversion of the serotonin precursor TRP to KYN and subsequent breakdown to downstream KYN pathway products such as 3-hydroxykynurenine, 3-hydroxyanthanilic acid, QUIN or kynurenic acid is believed to limit systemic serotonin production (11,12). Additionally, such KYN pathway intermediates were ascribed standalone activity in neuronal signaling, e.g. by interfering with glutamatergic NMDA receptors (9,10,16). Elevated KYN and KYN pathway products were reported for multiple inflammatory conditions (11). In COVID-19, activity of the KYN pathway was found to correlate positively with inflammatory markers (33,36,37), disease severity (32,36,37) and was implicated in persistent symptom presence (31,35,40,67) Interestingly, our INCOV data demonstrating the positive association of serotonin and TRP levels suggest the KYN pathway activity may especially efficiently compete for TRP with systemic serotonin synthesis in the highly inflammatory milieu of acute and sub-acute COVID-19. Increasing TRP and serotonin in infection recovery in INCOV study participants suggest re-routing of the from IDO-mediated catabolism to the serotonin biosynthesis (11,12,22). The results of multi-parameter modeling in the SIMMUN cohort demonstrate an additional, inflammation-independent effect of SARS-CoV-2 infection on KYN and KYN/TRP. While its mechanism remains obscure, an analogical sustained upregulation of KYN in absence of the inflammatory marker C-reactive protein was described by Bizjak et al. in long-term COVID-19 recovery (35).

Tetrahydrobiopterin (BH4) is a co-factor of PAH, the enzyme catalyzing the PHE - TYR conversion, and of TH, catalyzing the TYR - L-DOPA (L-3,4-dihydroxyphenylalanine) conversion (6,24). Importantly, BH4 serves also as a co-factor of TPH, which converts TRP to 5-hydroxytryptophan, an intermediate in serotonin synthesis from TRP (23,24). BH4 poses, apart from IDO1, another junction between neurotransmitter metabolism and inflammation (6,23,24,68,69). Activity of GTP cyclohydrolase I, the enzyme catalyzing the rate limiting step of the BH4 synthesis is strongly stimulated by IFNG and other inflammatory stimuli (24,68,69). However, during inflammation, BH4 is oxidized to the inactive derivative dihydrobiopterin and consumed during nitric oxide formation by the BH4-dependent nitric oxide synthase, which restricts BH4 from neurotransmitter synthesis (3,6,16,24,25,70). Furthermore, upon high activity of GTP cyclohydrolase I, NEO, a side product of BH4 biosynthesis exploited as an inflammation marker in the SIMMUN cohort, is secreted by myeloid leukocytes (6,16,24,70). In line with a previous report (34), we could demonstrate an increase in circulating PHE in SARS-CoV-2 infection and associate lowered TYR with NEO. Additionally, we observed negative correlations of plasma levels of the dopamine derivative DA sulfate (23,52) and TYR with the canonical GTP cyclohydrolase I and neopterin inducer, IFNG. Collectively, these phenomena support the model of inflammatory BH4 deficiency culminating at inefficient PHE - TYR conversion, hyperphenylalaninemia and impaired catecholamine biosynthesis during acute SARS-CoV-2 infection. By contrast, the rise in DA sulfate during infection recovery in the INCOV cohort and the significantly lower PHE/TYR in SARS-CoV-2 infection convalescents in the SIMMUN collective may indicate a restored BH4 homeostasis leading to an efficient PHE - TYR conversion, dopamine synthesis and subsequent dopamine sulfonylation (6,23,25,51,52).

Rising activity of the KYN pathway with individual’s age was evident in both the SIMMUN and INCOV cohort data. This phenomenon was attributed to ‘inflammaging’, a chronic low-grade inflammation observed in elderly (1,22,71). Our multi-parameter modeling results suggest, that the lowered TRP and elevated KYN and KYN/TRP in older SIMMUN study participants, and plasma serotonin levels decreasing with age of the INCOV cohort are at least partly independent of inflammatory marker levels. This may implicate e.g. increased KYN formation by inflammation-independent IDO2 and TDO in elderly participants. This fits well to the data of Martilla and colleagues, who describe similar expression of *IDO1* in immune cells from young and elderly individuals (72). Contrary to the expected age-related suppression of catecholamine synthesis (1,73), we observed higher blood levels of TYR and lower PHE/TYR in older participants of the SIMMUN study. However, reproducibility and clinical significance of this phenomenon and is questionable. The effect of age on PHE and TYR could not be corroborated in the INCOV collective (not shown), and no significant effects of age on circulating levels of the dopamine derivative DA sulfate were discerned.

Depression (8,9,17–22), depression treatment resistance (48–50,74), schizophrenia (17), suicidal behavior (75),  
pathological mental stress (4) were associated with decreased circulating TRP and serotonin, high KYN pathway activity and increased blood concentrations of neuroactive KYN pathway metabolites. Systemic and central nervous system inflammation was put forward as a neuroimmune mechanism contributing to those mental health disorders (2,5,7,9,10,12,16,18,22,76). In infections, inflammation was proposed to suppress serotonin biosynthesis and trigger KYN pathway activity that mediate the ‘sickness behavior’ characterized by reduced locomotor activity, social avoidance, reduced appetite, lethargy and concentration problems (5). Inflammatory stimuli were also found to reduce dopamine availability measured by PHE/TYR ratio and hence contribute to depression in cancer (8) and trauma patients (3). In the SIMMUN cohort, depression signs (45,46) and mental stress (44) along with the inflammatory marker NEO could be linked to decreased TRP and higher KYN/TRP, respectively. In the INCOV collective, inflammation was also identified as the main diver of alterations in circulating serotonin, its precursor TRP and the competitor pathway products KYN and QUIN. Others also identified lowered TRP in COVID-19 convalescents with signs of depression and anxiety (39) and linked KYN pathway activity to depressive symptoms (40) and cognitive impairment (67) during long-term COVID-19 recovery. These observations may hence support the neuroimmune model assuming inflammation as a link between physical conditions, neurotransmitter disturbance and psychiatric disorders. Still, most evidence for this mechanism and relevance of the peripheral neurotransmitter availability is delivered by observational and in vitro studies, whereas in vivo experimental reports are scarce (9,10,16). In the periphery, liver, mesenteric organs and vasculature are the main sites of serotonin and dopamine synthesis and catabolism, and KYN pathway activity (11,12,23,51,52). Although the neurotransmitter precursors TRP and TYR, and neuroactive KYN pathway products were postulated to pass the blood-brain barrier (9,10,16), psychiatric disorders are not consistently paralleled by changes in dopamine, serotonin and neuroactive KYN metabolites or expression of KYN pathway enzymes in the central nervous system (9,77,78). Of note, quality of evidence of the serotonin theory of depression was also criticized in a recent systematic review (64). Hence, the hypothesis that inflammatory stimuli impact on systemic and central nervous system serotonin and dopamine availability and, hence, contribute to psychiatric disorders, like those frequently observed during COVID-19 recovery (26–29,38), needs validation in a robust experimental or prospective setting.

# Limitations

Our study has limitations. First, circulating serotonin, dopamine or any abundant dopamine metabolites (e.g. DA sulfate) (23,51,52), i.e. more direct readouts of neurotransmitter turnover than TRP, KYN, PHE and TYR, were not measured in the SIMMUN cohort. Additionally, the SIMMUN study variables were recorded at a single timepoint with a highly variable infection - sampling interval. For the INCOV collective, KYN/TRP and PHE/TYR could not be computed with normalized metabolome data, and measurements of stress, depression and anxiety were not available. This incompatibility of the datasets precluded development of comprehensive multi-parameter models in one of the cohorts and subsequent direct validation in the other. Second, relevance of circulating markers serotonin and dopamine availability, and KYN pathway metabolites for the central nervous system and psychiatric disorders is controversial, as discussed above. Third, the SIMMUN cohort suffered from a selection bias due to enrichment in psychiatric patients with a high rate of physical and psychiatric disorders. Analogically, hospitalized COVID-19 patients constituted the majority of the INCOV cohort. Hence, none of the collectives is representative for the entire pandemic population. Fourth, both the SIMMUN and INCOV cohorts were recruited during initial phases of the pandemic and do not include any (SIMMUN) or systematically vaccinated patients (INCOV). Similarly, the analyzed cohorts were exposed to wild-type-like SARS-CoV-2 variants and do not allow to assess effects of repeated, seasonal infections with highly transmissible but far less virulent omicron pathogens. For these reasons, studies with recent, real world post-pandemic collectives are urgently needed to validate our findings. Finally, the cross-sectional SIMMUN and INCOV cohorts encompassed uninfected controls and SARS-CoV-2 infections ranging from asymptomatic to critical disease. In particular, the shares of pathogen-positive individuals and the infection severity differed significantly between the analyzed collectives, which may have contributes to stronger effects of inflammation on neurotransmitter metabolism observed in the INCOV cohort.

# Conclusions

SARS-CoV-2-dependent inflammation can lower systemic availability of serotonin and dopamine by depletion of the tryptophan via the competitive kynurenine pathway and inhibition of the phenylalanine - tyrosine suppression, respectively. Those effects can be further amplified by advanced age, mental stress and depression.  
It remains to be investigated, if and how this mechanism may contribute to neurotransmitter metabolism in the central nervous system and, consequently, to psychiatric disorders following SARS-CoV-2 infection.

# Acknowledgements

We thank all participants and patients for the participation in the study.

# Funding

The study was supported by the Science Fund of the Land Tirol (grant number GZ71134 to Katharina Hüfner).

# Conflict of interest

Katharina Hüfner has received research grants from Austria Wirtchaftsservice (AWS) and the State of Tyrol as well as lecturer’s honoraria from Forum Medizinische Fortbildung (FOMF), the Anton Proksch Institute and the Hospital of Schwaz. Piotr Tymoszuk owns a data science company, Data Analytics as a Service Tirol, and receives payments from statistical data analysis, bioinformatic and scientific writing services. Other authors declare that no conflict of interest exists.

# Data and code availability

Anonymized patient data will be made available upon request to the corresponding author. The INCOV cohort data are publicly available at <https://data.mendeley.com/datasets/96v329bg7g/1>. The analysis pipeline code is available at <https://github.com/PiotrTymoszuk/simmun>.

# Tables

Table 1: Characteristic of the local SIMMUN cohort. Numeric variables are presented as medians with interquartile ranges. Categorical variables are presented as percentages and counts within complete observation sets.

| **Variablea** | **Uninfected** | **SARS-CoV-2** | **Test** | **Significanceb** | **Effect size** |
| --- | --- | --- | --- | --- | --- |
| Participants, n | 101 | 64 |  |  |  |
| Sex | female: 64% (65) male: 36% (36) | female: 58% (37) male: 42% (27) | χ² | ns (p = 0.54) | V = 0.066 |
| Age, years | 50 [IQR: 35 - 58] range: 18 - 69 | 50 [IQR: 32 - 56] range: 20 - 68 | Mann-Whitney | ns (p = 0.45) | r = 0.069 |
| Body mass classc | normal: 53% (54) overweight: 32% (32) obesity: 15% (15) | normal: 53% (34) overweight: 27% (17) obesity: 20% (13) | χ² | ns (p = 0.6) | V = 0.079 |
| Physical disorder | 54% (55) | 45% (29) | χ² | ns (p = 0.43) | V = 0.089 |
| Psychiatric disorder | 50% (50) | 28% (18) | χ² | p = 0.021 | V = 0.21 |
| HADS anxiety score | < 8: 57% (58) ≥ 8: 43% (43) | < 8: 80% (51) ≥ 8: 20% (13) | χ² | p = 0.013 | V = 0.23 |
| HADS depression score | < 8: 69% (70) ≥ 8: 31% (31) | < 8: 84% (54) ≥ 8: 16% (10) | χ² | ns (p = 0.078) | V = 0.17 |
| Depression or anxiety signs, HADS ≥ 8 | 45% (45) | 20% (13) | χ² | p = 0.0078 | V = 0.25 |
| PSS-4 stress score | 6 [IQR: 3 - 9] range: 0 - 14 | 5 [IQR: 3 - 8] range: 1 - 13 | Mann-Whitney | ns (p = 0.33) | r = 0.095 |
| anti-RBD SARS-CoV-2, IgG, AU | 0.31 [IQR: 0.28 - 0.34] range: 0.21 - 0.99 | 16 [IQR: 14 - 17] range: 0.42 - 20 | Mann-Whitney | p < 0.001 | r = 0.84 |
| COVID-19 severity |  | ambulatory: 73% (47) hospitalized: 27% (17) |  |  |  |
| aHADS: hospital anxiety and depression scale; PSS-4: perceived stress scale, 4 item; RBD: receptor binding domain; IgG: immunoglobulin gamma; AU: arbitrary urnits. | | | | | |
| bcorrected for multiple testing with the false discovery rate method. | | | | | |
| coverweight: body mass index (BMI) 25 - 30 kg/m²; obesity: BMI > 30 kg/m² | | | | | |

Table 2: Characteristic of the INCOV cohort. Numeric variables are presented as medians with interquartile ranges. Categorical variables are presented as percentages and counts within complete observation sets.

| **Variable** | **Uninfected** | **SARS-CoV-2** | **Test** | **Significance** | **Effect size** |
| --- | --- | --- | --- | --- | --- |
| Participants, n | 27 | 140 |  |  |  |
| Sex | female: 56% (15) male: 44% (12) | female: 41% (58) male: 59% (82) | χ² | ns (p = 0.25) | V = 0.1 |
| Age, yearsa | 56 [IQR: 49 - 60] range: 32 - 70 | 62 [IQR: 48 - 73] range: 26 - 89 | Mann-Whitney | p = 0.044 | r = 0.17 |
| Body mass class | normal: 44% (12) overweight: 22% (6) obesity: 33% (9) | normal: 26% (36) overweight: 36% (51) obesity: 38% (53) | χ² | ns (p = 0.17) | V = 0.16 |
| Ethnicsb | Asian: 0% (0) Black or African-American: 12% (3) White: 88% (21) Other: 0% (0) | Asian: 16% (22) Black or African-American: 9.3% (13) White: 46% (65) Other: 29% (40) | χ² | p = 0.0013 | V = 0.33 |
| COVID-19 severity |  | mild: 2.9% (4) moderate: 59% (83) severe: 25% (35) critical: 13% (18) |  |  |  |
| aoverweight: body mass index (BMI) 25 - 30 kg/mm², obesity: BMI > 30 kg/mm² | | | | | |
| bWHO ordinal scale for clinical improvement; mild: 1 - 2, moderate: 3 - 4, severe: 5 - 6, critical: 7 | | | | | |

# Figures

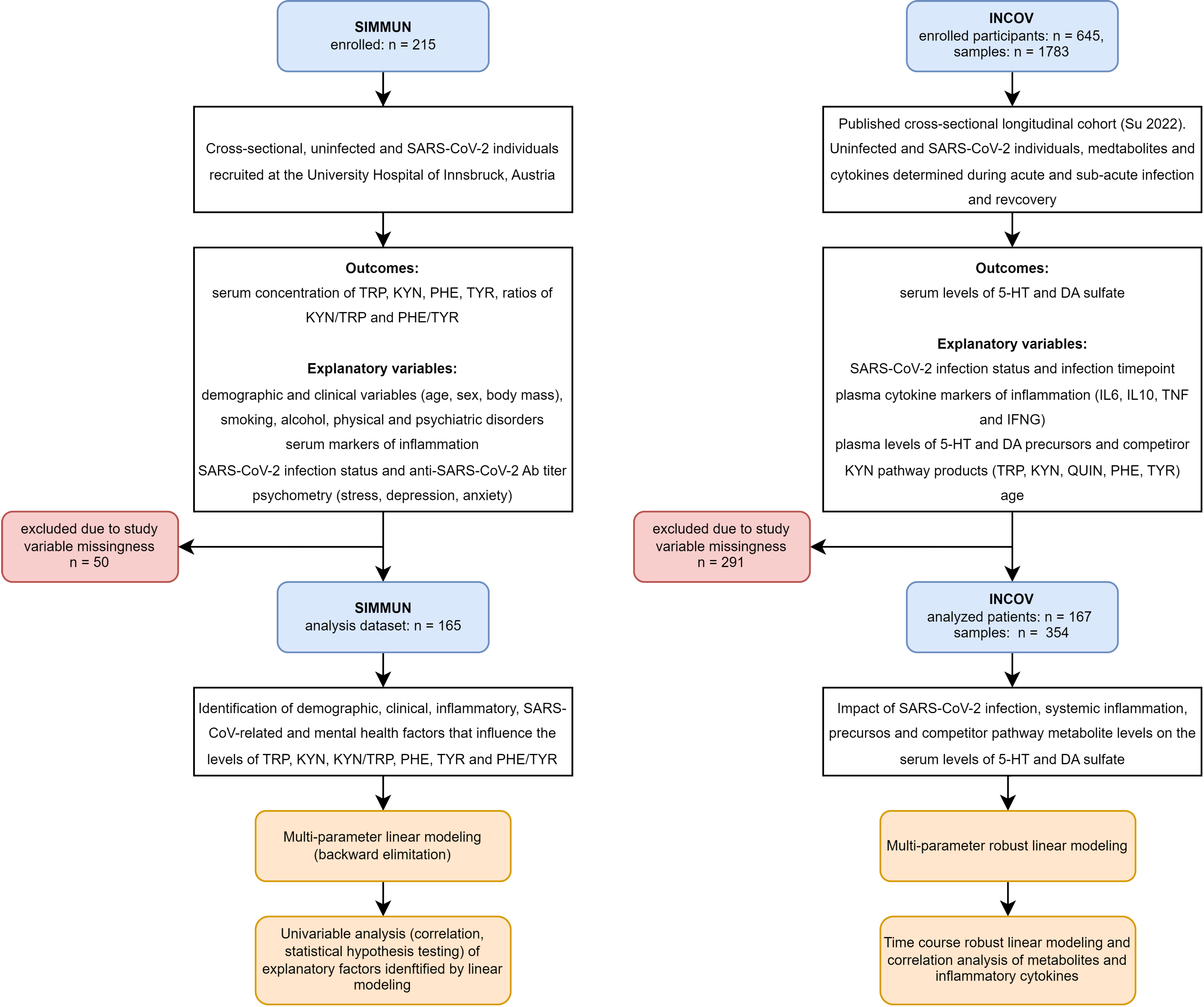


Figure 1: Inclusion scheme for the SIMMUN and INCOV cohorts, and analysis strategy.

**Figure 1. Inclusion scheme for the SIMMUN and INCOV cohorts, and analysis strategy.**

*Sampling timepoints in the INCOV cohort: acute: median 10 days, sub-acute: median 14 days, recovery: median 64 days after diagnosis of SARS-CoV-2 infection via PCR.*

*TRP: tryptophan; KYN: kynurenine; PHE: phenylalanine; TYR: tyrosine; KYN/TRP: kynurenine - tryptophan ratio; PHE/TYR: phenylalanine - tyrosine ratio; QUIN: quinolinate; 5-HT: serotonin; DA: dopamine; Ab: antibody; IL6: interleukin-6; IL10: interleukin-10; TNF: tumor-necrosis factor alpha; IFNG: interferon gamma.*

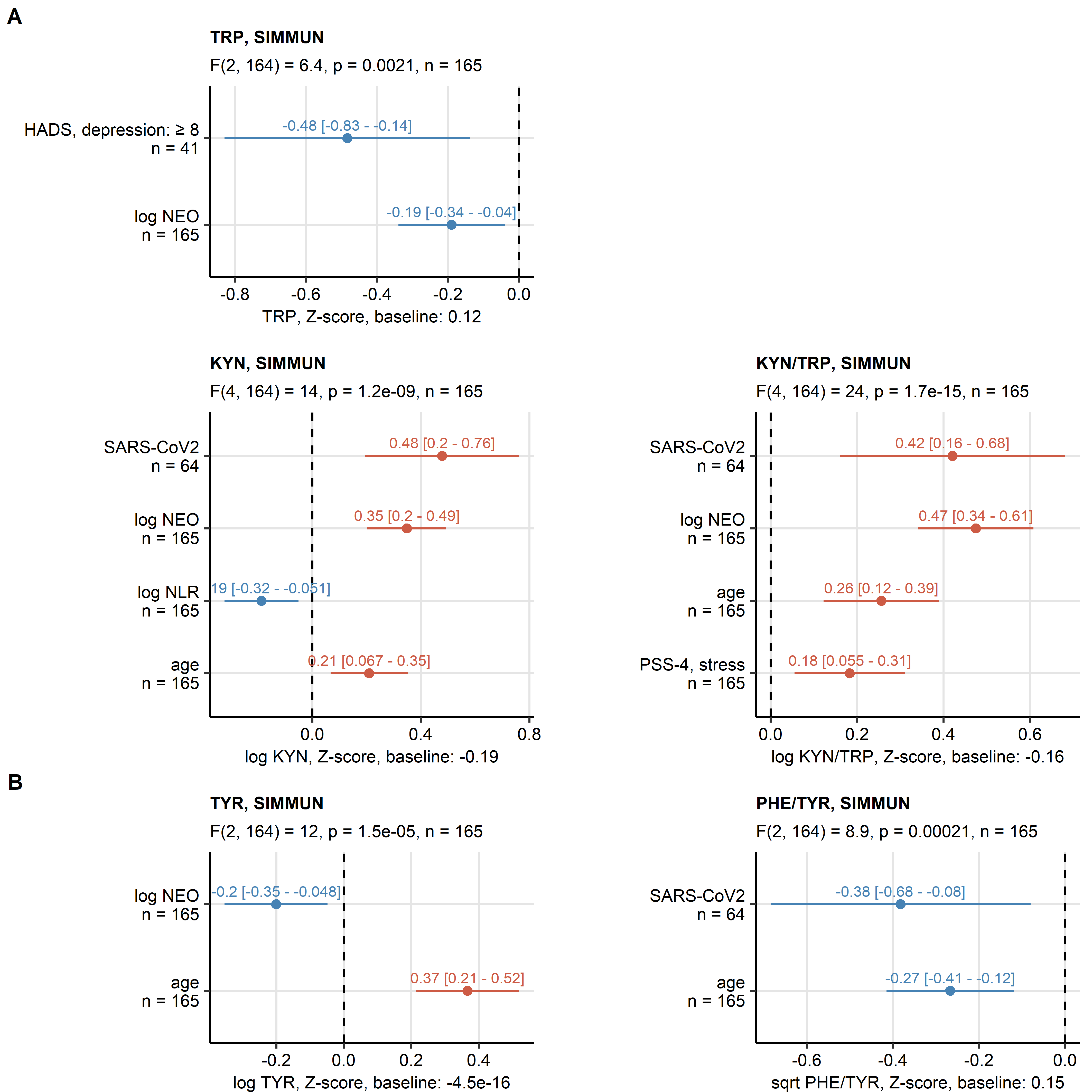


Figure 2: Results of multi-parameter linear modeling of tryptophan, kynurenine, kynurenine/tryptophan ratio, tyrosine and phenylalanine/tyrosine ratio in the SIMMUN cohort.

**Figure 2. Results of multi-parameter linear modeling of tryptophan, kynurenine, kynurenine/tryptophan ratio, tyrosine and phenylalanine/tyrosine ratio in the SIMMUN cohort.**

*Effects of systemic inflammation markers (neopterin [NEO], neutrophil-lymphocyte ratio [NLR]), SARS-CoV-2 infection status, titre of immunoglobulin gamma against the receptor binding domain of the S1/S2 SARS-CoV-2 protein (anti-RBD IgG), symptoms of anxiety and depression (hospital depression and anxiety scale [HADS] 8 points), intensity of mental stress (perceived stress scale, 4 item [PSS4]), age and sex on readouts of serotonin and dopamine availability and KYN pathway activity were investigated by multi-parameter linear regression with backward elimination of non-significant terms. Numeric variables were normalized prior to modeling. Overall model validity was assessed by likelihood-ratio test (LRT) as compared with the respective null models. Significant coefficient estimates () with 95% confidence intervals are presented in Forest plots. LRT results (F values, degrees of freedom and p values) and total numbers of complete observations are indicated in the plot captions. Model intercepts (baseline) are indicated in the X axis titles.*

*TRP: tryptophan; KYN: kynurenin; KYN/TRP: kynurenine - tryptophan ratio; PHE: phenylalanine; TYR: tyrosine; PHE/TYR: phenylalanine - tyrosine ratio.*

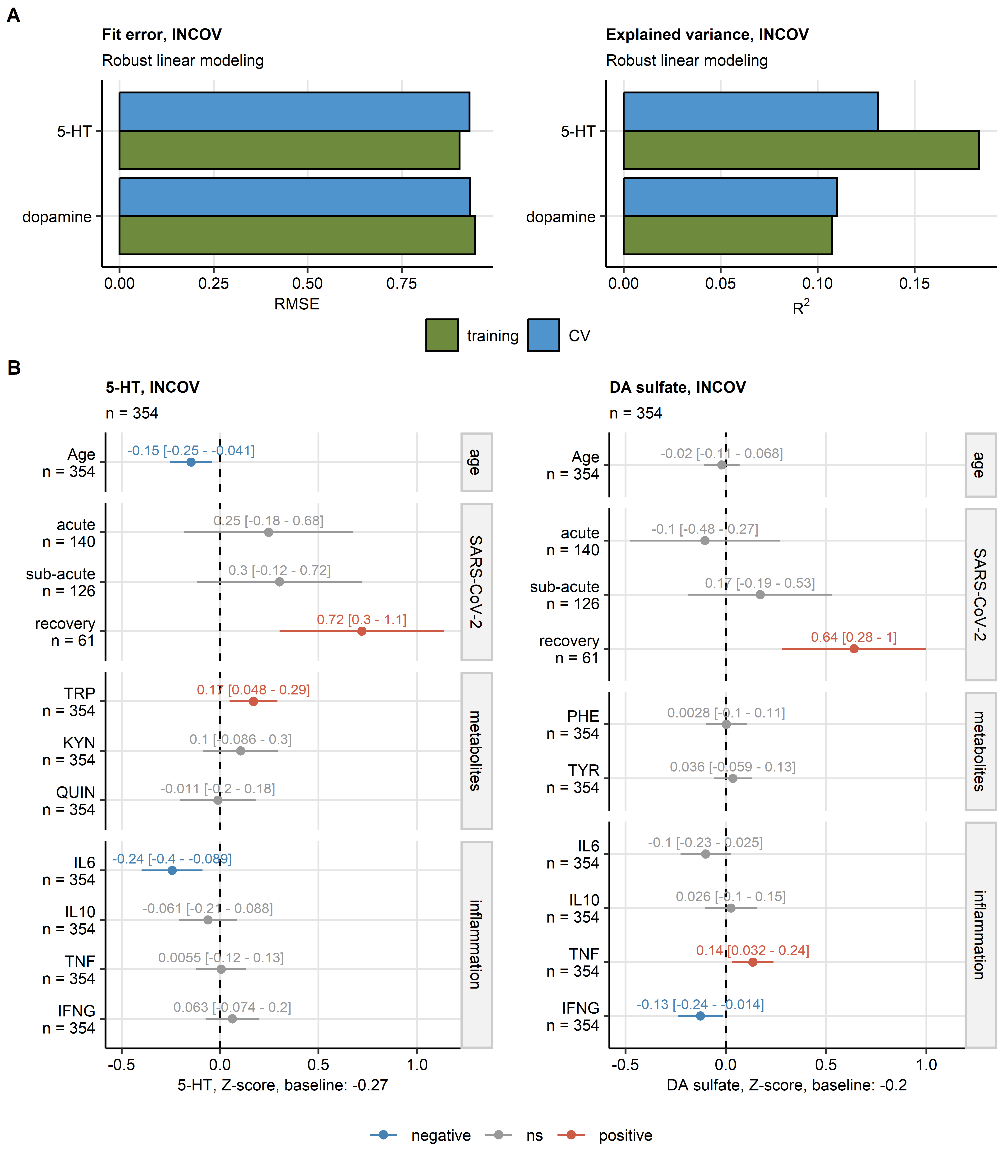


Figure 3: Results of multi-parameter robust linear modeling of plasma levels of serotonin and dopamine 3-O-sulfate in the INCOV cohort.

**Figure 3. Results of multi-parameter robust linear modeling of plasma levels of serotonin and dopamine 3-O-sulfate in the INCOV cohort.**

*Effects of patient’s age, timepoint after SARS-CoV-2 infection (acute: median 10 days, sub-acute: median 14 days, recovery: median 64 days after SARS-CoV-2 infection diagnosis via PCR as compared with healthy controls), plasma levels of metabolites related to neurotransmitter biosynthesis and KYN pathway activity (tryptophan [TRP], kynurenine [KYN], quinolinate [QUIN], phenylalanine [PHE] and tyrosine [TYR]), and plasma concentrations of cytokine markers of inflammation (interleukin-6 [IL6], interleukin-10 [IL10], tumor necrosis factor-alpha [TNF] and interferon-gamma [IFNG]) on plasma concentrations of serotonin (5-hydroxy tryptamine [5-HT]) and dopamine 3-O-sulfate (DA sulfate) were modeled by multi-parameter robust linear regression.*

*(A) Model fit error (root mean square error [RMSE]) and fraction of explained variances (R-squared) of the robust linear models assessed in the genuine training dataset and 10-fold cross-validation (CV).*

*(B) Estimates of model coefficients () with 95% confidence intervals presented in Forest plots. Numbers of complete observations are indicated in the plot captions. Model intercepts (baseline) are indicated in the X axis titles.*

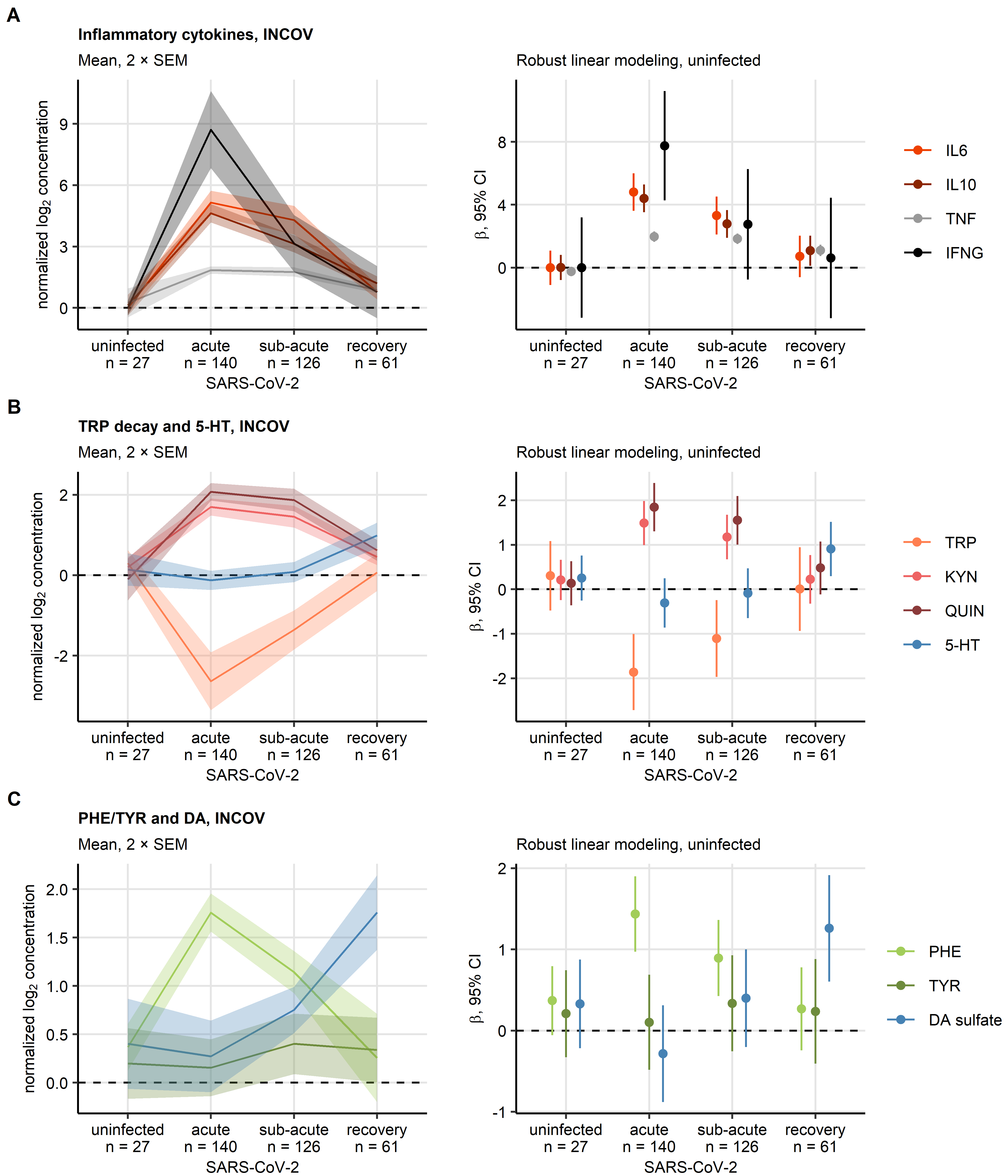


Figure 4: Time course of cytokine markers of inflammation, serotonin, dopamine 3-0-sulfate and the neurotransmitter biosynthesis precursors and kynurenine pathway products during SARS-CoV infection and recovery in the INCOV cohort.

**Figure 4. Time course of cytokine markers of inflammation, serotonin, dopamine 3-0-sulfate and the neurotransmitter biosynthesis precursors and kynurenine pathway products during SARS-CoV infection and recovery in the INCOV cohort.**

*Time courses of normalized log2-transformed plasma concentrations of cytokine makers of inflammation (A, interleukin-6 [IL6], interleukin-10 [IL-10], tumor necrosis factor-alpha [TNF] and interferon gamma [INFG]), metabolites implicated in serotonin synthesis (B, tryptophan [TRP], kynurenine [KYN], quinolinate [QUIN] and serotonin/5-hydroxy tryptamine [5-HT]) and metabolites implicated in dopamine turnover (C, phenylalanine [PHE], tyrosine [TYR] and dopamine 3-O-sulfate [DA sulfate]). Differences between uninfected controls and acute, sub-acute SARS-CoV-2 infection and recovery (median 10, 14 and 64 days after SARS-CoV-2 infection diagnosis via PCR, respectively) were modeled by robust linear regression with the uninfected controls as baseline. Mean normalized concentrations with 2 standard errors of the mean (2 SEM) are depicted as solid lines with tinted ribbons (plots on the left). Robust linear model coefficient estimates () with 95% confidence intervals are visualized in Forest plots (right). Numbers of complete observations per timepoint are indicated in the X axes.*

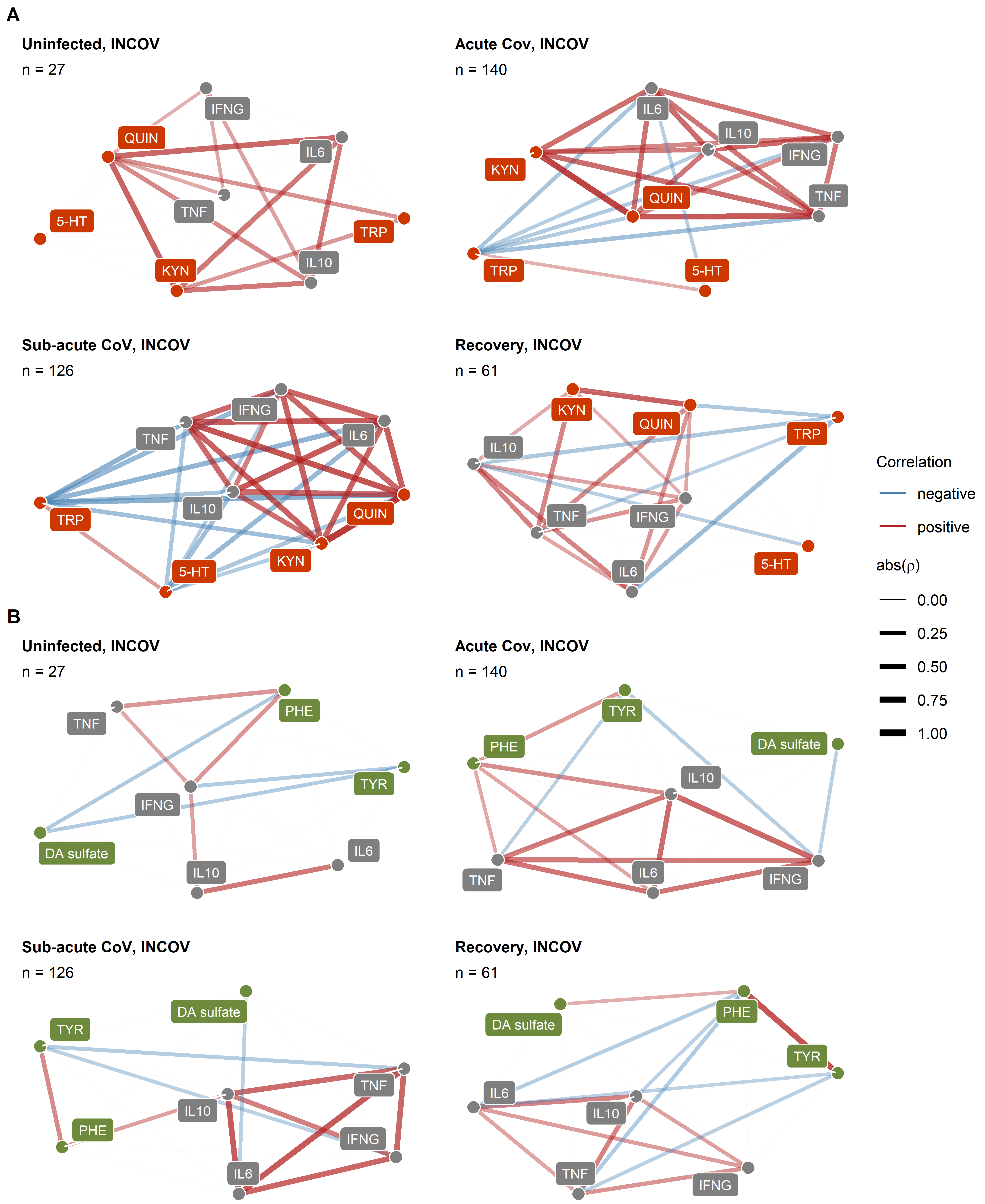


Figure 5: Correlation of plasma levels of cytokine markers of inflammation, serotonin and dopamine 3-O-sulfate, neurotransmitter precursors and kynurenine pathway products in healthy controls and SARS-CoV-2 patients of the INCOV cohort.

**Figure 5. Correlation of plasma levels of cytokine markers of inflammation, serotonin and dopamine 3-O-sulfate, neurotransmitter precursors and kynurenine pathway products in healthy controls and SARS-CoV-2 patients of the INCOV cohort.**

*Plasma levels of metabolites implicated in serotonin synthesis (tryptophan [TRP], serotonin [5-hydroxy tryptamine, 5-HT]), kynurenine pathway activity (kynurenine [KYN], quinolinic acid [QUIN]) and dopamine metabolism (phenylalanine [PHE], tyrosine [TYR], dopamine sulfate [DA sulfate]) and cytokine markers of inflammation (interleukin-6 [IL6], interleukin-10 [IL10], tumor necrosis factor-alpha [TNF], interferon-gamma [INFG]) were subjected to pairwise correlation analysis in uninfected and SARS-CoV-2-infected individuals (acute: median 10 days, sub-acute: median 14 days, recovery: median 64 days after SARS-CoV-2 infection diagnosis via PCR). Correlation coefficient matrices for correlation coefficients > 0.2 were visualized as force-directed network plots. Node color codes for the variable type (gray: inflammatory markers, orange: serotonin metabolism and KYN pathway, green: dopamine metabolism), edge width and color codes for the value and sign of the correlation coefficient.*

# References

1. Sorgdrager FJH, Naudé PJW, Kema IP, Nollen EA, De Deyn PP. Tryptophan metabolism in inflammaging: From biomarker to therapeutic target. *Frontiers in Immunology* (2019) 10:2565. doi: [10.3389/FIMMU.2019.02565/BIBTEX](https://doi.org/10.3389/FIMMU.2019.02565/BIBTEX)

2. Dantzer R, O’Connor JC, Freund GG, Johnson RW, Kelley KW. From inflammation to sickness and depression: when the immune system subjugates the brain. *Nature reviews Neuroscience* (2008) 9:46–56. doi: [10.1038/NRN2297](https://doi.org/10.1038/NRN2297)

3. Hüfner K, Fuchs D, Blauth M, Sperner-Unterweger B. How acute and chronic physical disease may influence mental health – An Analysis of neurotransmitter precursor amino acid levels. *Psychoneuroendocrinology* (2019) 106:95–101. doi: [10.1016/j.psyneuen.2019.03.028](https://doi.org/10.1016/j.psyneuen.2019.03.028)

4. Hüfner K, Galffy M, Egeter J, Giesinger JM, Arnhard K, Oberacher H, Gostner JM, Fuchs D, Sperner-Unterweger B. Acute and Chronic Mental Stress both Influence Levels of Neurotransmitter Precursor Amino Acids and Derived Biogenic Amines. *Brain Sciences* (2020) 10: doi: [10.3390/BRAINSCI10060322](https://doi.org/10.3390/BRAINSCI10060322)

5. Maes M, Berk M, Goehler L, Song C, Anderson G, Gałecki P, Leonard B. Depression and sickness behavior are Janus-faced responses to shared inflammatory pathways. *BMC Medicine* (2012) 10:1–19. doi: [10.1186/1741-7015-10-66/TABLES/1](https://doi.org/10.1186/1741-7015-10-66/TABLES/1)

6. Geisler S, Gostner JM, Becker K, Ueberall F, Fuchs D. Immune activation and inflammation increase the plasma phenylalanine-to- tyrosine ratio. *Pteridines* (2013) 24:27–31. doi: [10.1515/PTERID-2013-0001/MACHINEREADABLECITATION/RIS](https://doi.org/10.1515/PTERID-2013-0001/MACHINEREADABLECITATION/RIS)

7. Haroon E, Raison CL, Miller AH. Psychoneuroimmunology meets neuropsychopharmacology: Translational implications of the impact of inflammation on behavior. (2012) 37: doi: [10.1038/npp.2011.205](https://doi.org/10.1038/npp.2011.205)

8. Hüfner K, Oberguggenberger A, Kohl C, Geisler S, Gamper E, Meraner V, Egeter J, Hubalek M, Beer B, Fuchs D, et al. Levels in neurotransmitter precursor amino acids correlate with mental health in patients with breast cancer. *Psychoneuroendocrinology* (2015) 60:28–38. doi: [10.1016/J.PSYNEUEN.2015.06.001](https://doi.org/10.1016/J.PSYNEUEN.2015.06.001)

9. Brown SJ, Huang XF, Newell KA. The kynurenine pathway in major depression: What we know and where to next. *Neuroscience & Biobehavioral Reviews* (2021) 127:917–927. doi: [10.1016/J.NEUBIOREV.2021.05.018](https://doi.org/10.1016/J.NEUBIOREV.2021.05.018)

10. Schwarcz R, Bruno JP, Muchowski PJ, Wu HQ. Kynurenines in the mammalian brain: When physiology meets pathology. (2012) 13: doi: [10.1038/nrn3257](https://doi.org/10.1038/nrn3257)

11. Badawy AAB. Kynurenine pathway of tryptophan metabolism: Regulatory and functional aspects. *International Journal of Tryptophan Research* (2017) 10: doi: [10.1177/1178646917691938/ASSET/IMAGES/LARGE/10.1177\_1178646917691938-FIG2.JPEG](https://doi.org/10.1177/1178646917691938/ASSET/IMAGES/LARGE/10.1177_1178646917691938-FIG2.JPEG)

12. Lukić I, Ivković S, Mitić M, Adžić M. Tryptophan metabolites in depression: Modulation by gut microbiota. *Frontiers in Behavioral Neuroscience* (2022) 16:367. doi: [10.3389/FNBEH.2022.987697/BIBTEX](https://doi.org/10.3389/FNBEH.2022.987697/BIBTEX)

13. Robinson CM, Shirey KA, Carlin JM. Synergistic Transcriptional Activation of Indoleamine Dioxygenase by IFN- and Tumor Necrosis Factor-. *Journal of interferon & cytokine research : the official journal of the International Society for Interferon and Cytokine Research* (2003) 23:413. doi: [10.1089/107999003322277829](https://doi.org/10.1089/107999003322277829)

14. Dai W, Gupta SL. Regulation of indoleamine 2,3-dioxygenase gene expression in human fibroblasts by interferon-gamma. Upstream control region discriminates between interferon-gamma and interferon-alpha. *Journal of Biological Chemistry* (1990) 265:19871–19877. doi: [10.1016/S0021-9258(17)45453-6](https://doi.org/10.1016/S0021-9258(17)45453-6)

15. Litzenburger UM, Opitz CA, Sahm F, Rauschenbach KJ, Trump S, Winter M, Ott M, Ochs K, Lutz C, Liu X, et al. Constitutive IDO expression in human cancer is sustained by an autocrine signaling loop involving IL-6, STAT3 and the AHR. *Oncotarget* (2014) 5:1038–1051. doi: [10.18632/ONCOTARGET.1637](https://doi.org/10.18632/ONCOTARGET.1637)

16. Vancassel S, Capuron L, Castanon N. Brain Kynurenine and BH4 Pathways: Relevance to the Pathophysiology and Treatment of Inflammation-Driven Depressive Symptoms. (2018) 12:499. doi: [10.3389/fnins.2018.00499](https://doi.org/10.3389/fnins.2018.00499)

17. Marx W, McGuinness AJ, Rocks T, Ruusunen A, Cleminson J, Walker AJ, Gomes-da-Costa S, Lane M, Sanches M, Diaz AP, et al. The kynurenine pathway in major depressive disorder, bipolar disorder, and schizophrenia: a meta-analysis of 101 studies. *Molecular Psychiatry 2020 26:8* (2020) 26:4158–4178. doi: [10.1038/s41380-020-00951-9](https://doi.org/10.1038/s41380-020-00951-9)

18. Hunt C, Macedo e Cordeiro T, Suchting R, Dios C de, Cuellar Leal VA, Soares JC, Dantzer R, Teixeira AL, Selvaraj S. Effect of immune activation on the kynurenine pathway and depression symptoms – A systematic review and meta-analysis. (2020) 118: doi: [10.1016/j.neubiorev.2020.08.010](https://doi.org/10.1016/j.neubiorev.2020.08.010)

19. Fellendorf FT, Bonkat N, Dalkner N, Schönthaler EMD, Manchia M, Fuchs D, Reininghaus EZ. Indoleamine 2,3-dioxygenase (IDO)-activity in Severe Psychiatric Disorders: A Systemic Review. *Current Topics in Medicinal Chemistry* (2022) 22: doi: [10.2174/1568026622666220718155616](https://doi.org/10.2174/1568026622666220718155616)

20. Hüfner K, Giesinger JM, Gostner JM, Egeter J, Koudouovoh-Tripp P, Vill T, Fuchs D, Sperner-Unterweger B. Neurotransmitter Precursor Amino Acid Ratios Show Differential, Inverse Correlations with Depression Severity in the Low and High Depression Score Range. *International Journal of Tryptophan Research : IJTR* (2021) 14: doi: [10.1177/11786469211039220](https://doi.org/10.1177/11786469211039220)

21. Paul-Savoie É, Potvin S, Daigle K, Normand E, Corbin JF, Gagnon R, Marchand S. A deficit in peripheral serotonin levels in major depressive disorder but not in chronic widespread pain. *The Clinical journal of pain* (2011) 27:529–534. doi: [10.1097/AJP.0B013E31820DFEDE](https://doi.org/10.1097/AJP.0B013E31820DFEDE)

22. Capuron L, Schroecksnadel S, Féart C, Aubert A, Higueret D, Barberger-Gateau P, Layé S, Fuchs D. Chronic low-grade inflammation in elderly persons is associated with altered tryptophan and tyrosine metabolism: role in neuropsychiatric symptoms. *Biological psychiatry* (2011) 70:175–182. doi: [10.1016/J.BIOPSYCH.2010.12.006](https://doi.org/10.1016/J.BIOPSYCH.2010.12.006)

23. Meiser J, Weindl D, Hiller K. Complexity of dopamine metabolism. *Cell Communication and Signaling* (2013) 11:1–18. doi: [10.1186/1478-811X-11-34/FIGURES/5](https://doi.org/10.1186/1478-811X-11-34/FIGURES/5)

24. Fanet H, Capuron L, Castanon N, Calon F, Vancassel S. Tetrahydrobioterin (BH4) Pathway: From Metabolism to Neuropsychiatry. *Current neuropharmacology* (2021) 19:591–609. doi: [10.2174/1570159X18666200729103529](https://doi.org/10.2174/1570159X18666200729103529)

25. Neurauter G, Schrocksnadel K, Scholl-Burgi S, Sperner-Unterweger B, Schubert C, Ledochowski M, Fuchs D. Chronic immune stimulation correlates with reduced phenylalanine turnover. *Current drug metabolism* (2008) 9:622–627. doi: [10.2174/138920008785821738](https://doi.org/10.2174/138920008785821738)

26. Huang C, Huang L, Wang Y, Li X, Ren L, Gu X, Kang L, Guo L, Liu M, Zhou X, et al. 6-month consequences of COVID-19 in patients discharged from hospital: a cohort study. *The Lancet* (2021) 397:220–232. doi: [10.1016/S0140-6736(20)32656-8](https://doi.org/10.1016/S0140-6736(20)32656-8)

27. Al-Aly Z, Xie Y, Bowe B. High-dimensional characterization of post-acute sequelae of COVID-19. *Nature* (2021) 594:259–264. doi: [10.1038/s41586-021-03553-9](https://doi.org/10.1038/s41586-021-03553-9)

28. Hüfner K, Tymoszuk P, Ausserhofer D, Sahanic S, Pizzini A, Rass V, Galffy M, Böhm A, Kurz K, Sonnweber T, et al. Who Is at Risk of Poor Mental Health Following Coronavirus Disease-19 Outpatient Management? *Frontiers in Medicine* (2022) 9: doi: [10.3389/fmed.2022.792881](https://doi.org/10.3389/fmed.2022.792881)

29. Sahanic S, Tymoszuk P, Luger AK, Hüfner K, Boehm A, Pizzini A, Schwabl C, Koppelstätter S, Kurz K, Asshoff M, et al. COVID-19 and its continuing burden after 12 months: a longitudinal observational prospective multicentre trial. *ERJ open research* (2023) 9:00317–2022. doi: [10.1183/23120541.00317-2022](https://doi.org/10.1183/23120541.00317-2022)

30. Staudt A, Jörres RA, Hinterberger T, Lehnen N, Loew T, Budweiser S. Associations of Post-Acute COVID syndrome with physiological and clinical measures 10 months after hospitalization in patients of the first wave. *European Journal of Internal Medicine* (2022) 95: doi: [10.1016/j.ejim.2021.10.031](https://doi.org/10.1016/j.ejim.2021.10.031)

31. Gietl M, Burkert F, Seiwald S, Böhm A, Hofer S, Gostner JM, Piater T, Geisler S, Weiss G, Loeffler-Ragg J, et al. Interferon-gamma Mediated Metabolic Pathways in Hospitalized Patients During Acute and Reconvalescent COVID-19. *International Journal of Tryptophan Research : IJTR* (2023) 16: doi: [10.1177/11786469231154244](https://doi.org/10.1177/11786469231154244)

32. Lionetto L, Ulivieri M, Capi M, De Bernardini D, Fazio F, Petrucca A, Pomes LM, De Luca O, Gentile G, Casolla B, et al. Increased kynurenine-to-tryptophan ratio in the serum of patients infected with SARS-CoV2: An observational cohort study. *Biochimica et biophysica acta Molecular basis of disease* (2021) 1867: doi: [10.1016/J.BBADIS.2020.166042](https://doi.org/10.1016/J.BBADIS.2020.166042)

33. Santiago-Mujika E, Heinrich K, George S, Forton C, Madaj Z, Burmeister AR, Sims M, Pospisilik A, Brundin P, Graham SF, et al. Increased levels of circulating neurotoxic metabolites in patients with mild Covid19. *bioRxiv* (2022)2022.06.22.497189. doi: [10.1101/2022.06.22.497189](https://doi.org/10.1101/2022.06.22.497189)

34. Luporini RL, Pott-Junior H, Di Medeiros Leal MCB, Castro A, Ferreira AG, Cominetti MR, de Freitas Anibal F. Phenylalanine and COVID-19: Tracking disease severity markers. *International Immunopharmacology* (2021) 101:108313. doi: [10.1016/J.INTIMP.2021.108313](https://doi.org/10.1016/J.INTIMP.2021.108313)

35. Bizjak DA, Stangl M, Börner N, Bösch F, Durner J, Drunin G, Buhl JL, Abendroth D. Kynurenine serves as useful biomarker in acute, Long- and Post-COVID-19 diagnostics. *Frontiers in immunology* (2022) 13: doi: [10.3389/FIMMU.2022.1004545](https://doi.org/10.3389/FIMMU.2022.1004545)

36. Dewulf JP, Martin M, Marie S, Oguz F, Belkhir L, De Greef J, Yombi JC, Wittebole X, Laterre PF, Jadoul M, et al. Urine metabolomics links dysregulation of the tryptophan-kynurenine pathway to inflammation and severity of COVID-19. *Scientific reports* (2022) 12: doi: [10.1038/S41598-022-14292-W](https://doi.org/10.1038/S41598-022-14292-W)

37. Saito K, Ishikawa R, Kitamura I, Ogawa K, Arakawa N, Sun Y, Imai K, Maeda T, Saito Y, Hasegawa C. Characterization of serotonin as a candidate biomarker of severity and prognosis of COVID-19 using LC/MS analysis. *Journal of Pharmacological Sciences* (2022) 150:49–55. doi: [10.1016/J.JPHS.2022.06.005](https://doi.org/10.1016/J.JPHS.2022.06.005)

38. Bower JE, Radin A, Kuhlman KR. Psychoneuroimmunology in the time of COVID-19: Why neuro-immune interactions matter for mental and physical health. *Behaviour Research and Therapy* (2022) 154:104104. doi: [10.1016/J.BRAT.2022.104104](https://doi.org/10.1016/J.BRAT.2022.104104)

39. Kucukkarapinar M, Yay-Pence A, Yildiz Y, Buyukkoruk M, Yaz-Aydin G, Deveci-Bulut TS, Gulbahar O, Senol E, Candansayar S. Psychological outcomes of COVID-19 survivors at sixth months after diagnose: the role of kynurenine pathway metabolites in depression, anxiety, and stress. *Journal of Neural Transmission* (2022) 129: doi: [10.1007/s00702-022-02525-1](https://doi.org/10.1007/s00702-022-02525-1)

40. Matits L, Munk M, Bizjak DA, Kolassa IT, Karrasch S, Vollrath S, Jerg A, Steinacker JM. Inflammation and severity of depressive symptoms in physically active individuals after COVID-19 - An exploratory immunopsychological study investigating the effect of inflammation on depressive symptom severity. *Brain, behavior, & immunity - health* (2023) 30: doi: [10.1016/J.BBIH.2023.100614](https://doi.org/10.1016/J.BBIH.2023.100614)

41. Su Y, Yuan D, Chen DG, Ng RH, Wang K, Choi J, Li S, Hong S, Zhang R, Xie J, et al. Multiple early factors anticipate post-acute COVID-19 sequelae. *Cell* (2022) 185:881–895.e20. doi: [10.1016/J.CELL.2022.01.014](https://doi.org/10.1016/J.CELL.2022.01.014)

42. Widner B, Werner ER, Schennach H, Wachter H, Fuchs D. Simultaneous measurement of serum tryptophan and kynurenine by HPLC. *Clinical Chemistry* (1997) 43:2424–2426. doi: [10.1093/CLINCHEM/43.12.2424](https://doi.org/10.1093/CLINCHEM/43.12.2424)

43. Deisenhammer F, Bauer A, Kavelar C, Rudzki D, Rössler A, Kimpel J, Borena W, Reindl M. 12-month SARS-CoV-2 antibody persistency in a Tyrolean COVID-19 cohort. *Wiener klinische Wochenschrift* (2021) 133:1265–1271. doi: [10.1007/S00508-021-01985-X](https://doi.org/10.1007/S00508-021-01985-X)

44. Cohen S, Kamarck T, Mermelstein R. A global measure of perceived stress. *Journal of health and social behavior* (1983) 24:385–396. doi: [10.2307/2136404](https://doi.org/10.2307/2136404)

45. Zigmond AS, Snaith RP. The hospital anxiety and depression scale. *Acta psychiatrica Scandinavica* (1983) 67:361–370. doi: [10.1111/J.1600-0447.1983.TB09716.X](https://doi.org/10.1111/J.1600-0447.1983.TB09716.X)

46. Bjelland I, Dahl AA, Haug TT, Neckelmann D. The validity of the Hospital Anxiety and Depression Scale: An updated literature review. *Journal of Psychosomatic Research* (2002) 52:69–77. doi: [10.1016/S0022-3999(01)00296-3](https://doi.org/10.1016/S0022-3999(01)00296-3)

47. Su Y, Chen D, Yuan D, Lausted C, Choi J, Dai CL, Voillet V, Duvvuri VR, Scherler K, Troisch P, et al. Multi-Omics Resolves a Sharp Disease-State Shift between Mild and Moderate COVID-19. *Cell* (2020) 183:1479. doi: [10.1016/J.CELL.2020.10.037](https://doi.org/10.1016/J.CELL.2020.10.037)

48. Sun Y, Drevets W, Turecki G, Li QS. The relationship between plasma serotonin and kynurenine pathway metabolite levels and the treatment response to escitalopram and desvenlafaxine. *Brain, Behavior, and Immunity* (2020) 87:404–412. doi: [10.1016/J.BBI.2020.01.011](https://doi.org/10.1016/J.BBI.2020.01.011)

49. Tateishi H, Setoyama D, Kang D, Matsushima J, Kojima R, Fujii Y, Mawatari S, Kikuchi J, Sakemura Y, Fukuchi J, et al. The changes in kynurenine metabolites induced by rTMS in treatment-resistant depression: A pilot study. *Journal of psychiatric research* (2021) 138:194–199. doi: [10.1016/J.JPSYCHIRES.2021.04.009](https://doi.org/10.1016/J.JPSYCHIRES.2021.04.009)

50. Holck A, Wolkowitz OM, Mellon SH, Reus VI, Nelson JC, Westrin Å, Lindqvist D. Plasma serotonin levels are associated with antidepressant response to SSRIs. *Journal of affective disorders* (2019) 250:65–70. doi: [10.1016/J.JAD.2019.02.063](https://doi.org/10.1016/J.JAD.2019.02.063)

51. Hashizume K, Yamatodani A, Yamamoto T, Ogihara T, Kumahara Y, Wada H. Effects of oral and intravenous administrations of dopamine and L-dopa on plasma levels of two isomers of dopamine sulfate in man. *Life sciences* (1987) 41:2697–2704. doi: [10.1016/0024-3205(87)90462-0](https://doi.org/10.1016/0024-3205(87)90462-0)

52. Goldstein DS, Swoboda KJ, Miles JM, Coppack SW, Aneman A, Holmes C, Lamensdorf I, Eisenhofer G. Sources and physiological significance of plasma dopamine sulfate. *The Journal of clinical endocrinology and metabolism* (1999) 84:2523–2531. doi: [10.1210/JCEM.84.7.5864](https://doi.org/10.1210/JCEM.84.7.5864)

53. McDonald RP. *Test theory: A unified treatment*. 1st Editio. New Yor: Psychology Press (1999). doi: [10.4324/9781410601087](https://doi.org/10.4324/9781410601087)

54. BARTLETT MS. THE STATISTICAL CONCEPTION OF MENTAL FACTORS. *British Journal of Psychology General Section* (1937) 28:97–104. doi: [10.1111/j.2044-8295.1937.tb00863.x](https://doi.org/10.1111/j.2044-8295.1937.tb00863.x)

55. Revelle W. Package ’psych’ - Procedures for Psychological, Psychometric and Personality Research. *R Package* (2015)1–358. <https://cran.r-project.org/web/packages/psych/index.html http://personality-project.org/r/psych-manual.pdf>

56. Benjamini Y, Hochberg Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society: Series B (Methodological)* (1995) 57:289–300. doi: [10.1111/j.2517-6161.1995.tb02031.x](https://doi.org/10.1111/j.2517-6161.1995.tb02031.x)

57. Cohen J. Statistical Power Analysis for the Behavioral Sciences. *Statistical Power Analysis for the Behavioral Sciences* (2013) doi: [10.4324/9780203771587](https://doi.org/10.4324/9780203771587)

58. Kassambara A. rstatix: Pipe-Friendly Framework for Basic Statistical Tests. (2021) <https://cran.r-project.org/package=rstatix>

59. Huber PJ. Robust Statistics. *International Encyclopedia of Statistical Science* (2011)1248–1251. doi: [10.1007/978-3-642-04898-2\_594](https://doi.org/10.1007/978-3-642-04898-2_594)

60. Ripley B. MASS: Support Functions and Datasets for Venables and Ripley’s MASS. (2022) <https://cran.r-project.org/package=MASS>

61. Kuhn M. Building predictive models in R using the caret package. *Journal of Statistical Software* (2008) 28:1–26. doi: [10.18637/jss.v028.i05](https://doi.org/10.18637/jss.v028.i05)

62. Csardi G, Nepusz T. The igraph software package for complex network research. *InterJournal* (2006) Complex Sy:1695. <https://igraph.org>

63. Briatte F, Bojanowski M, Canouil M, Charlop-Powers Z, Fisher JC, Johnson K, Rinker T. ggnetwork: Geometries to Plot Networks with ’ggplot2’. (2021) <https://cran.r-project.org/package=ggnetwork>

64. Moncrieff J, Cooper RE, Stockmann T, Amendola S, Hengartner MP, Horowitz MA. The serotonin theory of depression: a systematic umbrella review of the evidence. *Molecular Psychiatry 2022* (2022)1–14. doi: [10.1038/s41380-022-01661-0](https://doi.org/10.1038/s41380-022-01661-0)

65. Lin H, Liu X, Sun H, Zhang J, Dong S, Liu M, Li L, Tian J, Guo Y, Gan J, et al. Sustained abnormality with recovery of COVID-19 convalescents: a 2-year follow-up study. *Science Bulletin* (2022) 67:1556. doi: [10.1016/J.SCIB.2022.06.025](https://doi.org/10.1016/J.SCIB.2022.06.025)

66. Sathyasaikumar KV, Notarangelo FM, Kelly DL, Rowland LM, Hare SM, Chen S, Mo C, Buchanan RW, Schwarcz R. Tryptophan Challenge in Healthy Controls and People with Schizophrenia: Acute Effects on Plasma Levels of Kynurenine, Kynurenic Acid and 5-Hydroxyindoleacetic Acid. *Pharmaceuticals 2022, Vol 15, Page 1003* (2022) 15:1003. doi: [10.3390/PH15081003](https://doi.org/10.3390/PH15081003)

67. Cysique LA, Jakabek D, Bracken SG, Allen-Davidian Y, Heng B, Chow S, Dehhaghi M, Pires AS, Darley DR, Byrne A, et al. Post-acute COVID-19 cognitive impairment and decline uniquely associate with kynurenine pathway activation: a longitudinal observational study. *medRxiv* (2022)2022.06.07.22276020. doi: [10.1101/2022.06.07.22276020](https://doi.org/10.1101/2022.06.07.22276020)

68. Werner ER, Werner-Felmayer G, Fuchs D, Hausen A, Reibnegger G, Yim JJ, Pfleiderer W, Wachter H. Tetrahydrobiopterin biosynthetic activities in human macrophages, fibroblasts, THP-1, and T 24 cells. GTP-cyclohydrolase I is stimulated by interferon-gamma, and 6-pyruvoyl tetrahydropterin synthase and sepiapterin reductase are constitutively present. *Journal of Biological Chemistry* (1990) 265:3189–3192. doi: [10.1016/S0021-9258(19)39752-2](https://doi.org/10.1016/S0021-9258(19)39752-2)

69. Werner-Felmayer G, Prast H, Werner ER, Philippu A, Wachter H. Induction of GTP cyclohydrolase I by bacterial lipopolysaccharide in the rat. *FEBS letters* (1993) 322:223–226. doi: [10.1016/0014-5793(93)81574-J](https://doi.org/10.1016/0014-5793(93)81574-J)

70. Huber C, Richard Batchelor J, Fuchs D, Hausen A, Lang A, Niederwieser D, Reibnegger G, Swetly P, Troppmair J, Wachter H. Immune response-associated production of neopterin. Release from macrophages primarily under control of interferon-gamma. *The Journal of experimental medicine* (1984) 160:310–316. doi: [10.1084/JEM.160.1.310](https://doi.org/10.1084/JEM.160.1.310)

71. Valdiglesias V, Marcos-Pérez D, Lorenzi M, Onder G, Gostner JM, Strasser B, Fuchs D, Bonassi S. Immunological alterations in frail older adults: A cross sectional study. *Experimental gerontology* (2018) 112:119–126. doi: [10.1016/J.EXGER.2018.09.010](https://doi.org/10.1016/J.EXGER.2018.09.010)

72. Marttila S, Jylhävä J, Eklund C, Hervonen A, Jylhä M, Hurme M. Aging-associated increase in indoleamine 2,3-dioxygenase (IDO) activity appears to be unrelated to the transcription of the IDO1 or IDO2 genes in peripheral blood mononuclear cells. *Immunity & Ageing : I & A* (2011) 8:9. doi: [10.1186/1742-4933-8-9](https://doi.org/10.1186/1742-4933-8-9)

73. Peters R. Ageing and the brain. *Postgraduate medical journal* (2006) 82:84–88. doi: [10.1136/PGMJ.2005.036665](https://doi.org/10.1136/PGMJ.2005.036665)

74. Ertugrul A, Ucar G, Basar K, Demir B, Yabanoglu S, Ulug B. Influence of clozapine on platelet serotonin, monoamine oxidase and plasma serotonin levels. *Psychiatry research* (2007) 149:49–57. doi: [10.1016/J.PSYCHRES.2005.12.009](https://doi.org/10.1016/J.PSYCHRES.2005.12.009)

75. Tyano S, Zalsman G, Ofek H, Blum I, Apter A, Wolovik L, Sher L, Sommerfeld E, Harell D, Weizman A. Plasma serotonin levels and suicidal behavior in adolescents. *European neuropsychopharmacology : the journal of the European College of Neuropsychopharmacology* (2006) 16:49–57. doi: [10.1016/J.EURONEURO.2005.05.005](https://doi.org/10.1016/J.EURONEURO.2005.05.005)

76. Miller AH, Raison CL. The role of inflammation in depression: from evolutionary imperative to modern treatment target. *Nature reviews Immunology* (2016) 16:22–34. doi: [10.1038/NRI.2015.5](https://doi.org/10.1038/NRI.2015.5)

77. Miller CL, Llenos IC, Cwik M, Walkup J, Weis S. Alterations in kynurenine precursor and product levels in schizophrenia and bipolar disorder. *Neurochemistry International* (2008) 52:1297–1303. doi: [10.1016/J.NEUINT.2008.01.013](https://doi.org/10.1016/J.NEUINT.2008.01.013)

78. Clark SM, Pocivavsek A, Nicholson JD, Notarangelo FM, Langenberg P, McMahon RP, Kleinman JE, Hyde TM, Stiller J, Postolache TT, et al. Reduced kynurenine pathway metabolism and cytokine expression in the prefrontal cortex of depressed individuals. *Journal of Psychiatry and Neuroscience* (2016) 41:386–394. doi: [10.1503/JPN.150226](https://doi.org/10.1503/JPN.150226)