Persistent low-grade inflammation, SARS-CoV-2 infection and mental health deterioration signs impact on systemic levels of aminoacid neurotransmitter precursors

Supplementary Material

PSY Team

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# Supplementary Methods

## Software

Study data were analyzed with R version 4.2.3. Import and general data transformation tasks were accomplished with *foreign* (1), *readxl* (2), the *tidyverse* package bundle (3), *rlang* (4) and the development package [*trafo*](https://github.com/PiotrTymoszuk/trafo). Text data was handled with *stringi* (5).

Descriptive statistics, statistical hypothesis testing and correlation analysis was done with the packages *rstatix* (6) and [*ExDA*](https://github.com/PiotrTymoszuk/ExDA). For linear modeling and robust linear modeling, base R functions, the packages *stats*, *MASS* (7), *caret* (8), [*lmqc*](https://github.com/PiotrTymoszuk/lmqc) and [*caretExtra*](https://github.com/PiotrTymoszuk/caretExtra) were employed. Correlation matrices were converted to force-directed graphs with *igraph* (9). For factor analysis and assessment of psychometric tool consistency, *stats*, *psych* (10) and [*clustTools*](https://github.com/PiotrTymoszuk/clustTools) were used.

Results were visualized with tools provided by the packages *ggplot2* (11) (ribbon and bubble plots), [*ExDA*](https://github.com/PiotrTymoszuk/ExDA) (scatter and box plots), [*lmqc*](https://github.com/PiotrTymoszuk/lmqc) (Forest plots) and *ggnetwork* (12) (force-directed graphs). Figures and tables were created with *cowplot* (13) and *flextable* (14), respectively. Parts of the manuscript and Supplementary Material were written in the *rmarkdown* environment (15) and rendered with the *knitr* (16) and *bookdown* (17) packages. Management of figures and tables in the rmarkdown documents was accomplished with [*figur*](https://github.com/PiotrTymoszuk/figur).

## SIMMUN cohort dataset

Individuals tested for SARS-CoV-2 via PCR at the University Hospital of Innsbruck Medical University (Innsbruck, Austria), as well as inpatients and outpatients of the University Clinic for Psychiatry I and II (Innsbruck, Austria) undergoing routine SARS-CoV-2 PCR screening were invited to participate in the SIMMUN study. The study enrollment was initiated on 10. June 2020. The inclusion criteria for the SIMMUN study were age of 18 - 70 years, proficiency in German language, residence in the study region (Tyrol, Austria), results of a SARS-CoV-2 PCR test conducted at the University Hospital of Innsbruck. The exclusion criteria were active SARS-CoV-2 infection (< 14 days following a positive test), pregnancy, active malignancies, organ transplantation, prior surgery in the past 3 months, or acute or chronic inflammatory illness and treatment with oral corticosteroids. The analysis inclusion criterion was the complete study variable dataset consisting of basic demographic and medical history variables, SARS-CoV-2 infection status and titer of anti-receptor-binding domain S12/S2 protein immunoglobulin gamma (anti-RBD IgG), psychometric scoring of depression, anxiety and stress as well as blood levels of inflammatory markers and metabolites related to serotonin and dopamine biosynthesis. A total of 165 SIMMUN study participants were analyzed here. The SIMMUN study variables with their transformation and stratification schemes are listed in **Supplementary Table S1**. The SIMMUN study data were gathered during in-person visits at the study center conducted between 17.June 2020 and 27.May 2021. The SIMMUN analysis inclusion scheme is presented in **Figure 1**. Significant differences between participants included in the analysis and excluded due to data missingness are shown in **Supplementary Table S2**. Characteristic of the analyzed SIMMUN collective split by the SARS-CoV-2 infection status is presented in **Table 1**.

Demographic and clinical history variables: age, sex, body mass index (BMI), professionally diagnosed mental illness, self-reported chronic somatic conditions, smoking and alcohol consumption history were surveyed during the study visit or extracted from electronic patient records. Body mass classes were defined as follows: normal for BMI < 25 kg/m2, overweight for BMI 25 - 30 kg/m2 and obesity for BMI > 30 kg/m2. Result and date of the SARS-CoV-2 PCR test were retrieved from electronic patient records.

The inflammatory markers included in the analysis: plasma concentration of neopterin (NEO) and neutrophil - lymphocyte ratio (NLR) were determined by the certified clinical routine laboratory at the University Hospital of Innsbruck. C-reactive protein (CRP) and interleukin-6 (IL6) were determined for the SIMMUN cohort as well but excluded from the analysis, since their levels were within the reference range in the great majority of participants. Elevated CRP > 0.5 mg/dL was detected in 10% and elevated IL6 > 7 pg/mL observed in 4.8% of the analyzed SIMMUN collective. Plasma concentrations of tryptophan (TRP), kynurenine (KYN), phenylalanine (PHE) and tyrosine (TYR) were determined by high-performance liquid chromatography, as described elsewhere (18,19). Kynurenine - tryptophan and phenylalanine - tyrosine ratios (KYN/TRP and PHE/TYR) serving as readouts of systemic indoleamine 2,3-dioxygenase (IDO) and phenylalanine hydroxylase (PAH) were computed (20). Plasma titer of anti-RBD IgG were quantified by ELISA as described before (21). Strongly non-normally distributed anti-RBD IgG levels were expressed as arbitrary units (AU) were stratified with the 1, 1 - 16.3 and > 16.3 AU cutpoints defined by the maximal titer in the uninfected participants (1 AU) and median titer in the SARS-CoV-2 subset (16.3 AU). Laboratory measurements at and below the lower detection limit were substituted with the lower detection limit. Laboratory measurements at and above the upper detection limit were substituted with the upper detection limit.

Mental stress was gauged with the 4-item perceived stress scale (PSS-4) (22). Anxiety and depression signs were scored with the hospital anxiety and depression scale (HADS) including 7 items for anxiety and 7 items for depression (23). The total possible score range for each subscale is 0 to 21, with higher scores indicating more intense symptoms of anxiety/depression. Clinically relevant signs of anxiety or depression were identified with the cutoff of 8 points at the respective subscale as proposed in the seminal HADS paper and a recent metaanalysis (23,24). PSS-4, HADS anxiety and depression scales displayed good-to-excellent internal consistency as measured by the McDonald statistic (25) (**Supplementary Figure S1**).

In order to improve normality of some numeric SIMMUN study variables prior to linear modeling and statistical hypothesis testing with parametric tools, logarithm (KYN, PHE, TYR, KYN/TRP, NEO, NLR) or square root transformations (PHE/TYR) were applied. Strongly non-normally distributed HADS scores and anti-RBD IgG were stratified with the cutoffs described above. Transformation and stratification schemes of study variable are provided in **Supplementary Table S1**.

SIMMUN cohort data were stored as SPSS files and imported in R with the read.spss() function (package *foreign*). Transformation, stratification of numeric study variables and coding of categorical features was done with an in-hose developed script.

## INCOV cohort dataset

Plasma proteome measurements in the INCOV cohort were obtained by proximity extension assay (PEA, Olink, Sweden). Plasma metabolome was quantified by ultra-high-performance liquid chromatography/tandem accurate mass spectrometry (Metabolon, USA) (26,27). Proteome and metabolome data in form of normalized, age- and sex-adjusted, log2-transformed plasma levels as well as clinical information (sex, SARS-CoV-2 infection status, COVID-19 severity, timepoint) for the INCOV cohort were extracted from [supplementary tables](https://data.mendeley.com/datasets/96v329bg7g/1) accompanying the report by Su and colleagues (26) (function read\_xlsx, package *readxl*) (2). In the current analysis, 354 INCOV study participants with the complete dataset of metabolites of interest (serotonin [5-HT], tryptophan [TRP], kynurenine [KYN], quinolinate [QUIN], phenylalanine [PHE], tyrosine [TYR] and dopamine 3-O-sulfate [DA sulfate]), inflammatory cytokines of interest (interleukin-6 [IL6], interleukin-10 [IL10], tumor necrosis factor-alpha [TNF] and interferon-gamma [IFNG]) and age at enrollment were included in the current analysis. The INCOV study variables are listed in **Supplementary Table S3**. The analysis inclusion scheme for the INCOV cohort is presented in **Figure 1**. Characteristic of the INCOV collective is shown in **Table 2**. Concerning the INCOV study design, plasma metabolites and proteome were determined in uninfected controls and SARS-CoV-2 individuals at three timepoints after diagnosis: acute (median: 10 days), sub-acute (median: 14) and recovery (median: 64 after diagnosis). The measurements were only partially matched by participant (i.e. multiple longitudinal measurements for an individual) hence precluding a classical repeated measurement analysis approach. Numbers of available INCOV cohort samples and the sampling timepoints are shown in **Supplementary Table S4**.

## Descriptive statistic and variable distribution

Numeric variables were presented in the text and tables as medians with interquartile ranges and ranges. Categorical variables are presented as percentages and counts within the complete observation set. Descriptive statistics were computed with the function explore() from the [*ExDA*](https://github.com/PiotrTymoszuk/ExDA) package.

Distribution normality and variance homogeneity was assessed by Shapiro-Wilk and Levene test, respectively (functions explore() and compare\_variables(), package [*ExDA*](https://github.com/PiotrTymoszuk/ExDA)). The distribution testing revealed substantial deviations from normality for some SIMMUN study parameters. For this reason, logarithm and square root transformations were applied prior to modeling and statistical hypothesis testing with parametric tools as specified in **Supplementary Table S1**. Most of the INCOV study variables were non-normally distributed. For this reason, non-parametric Spearman rank test was chosen for correlation analysis and robust linear regression was used as a modeling approach.

## Statistical significance, effect size

Except for results of multi-parameter linear modeling and multi-parameter robust linear regression, p values were corrected for multiple testing with the false discovery rate method (FDR) (28) separately for each analysis task. Effects with p < 0.05 were considered significant. The following effect size measures were used (29): Cramer’s V for comparison of distribution of categorical variables (weak: < 0.3, moderate: 0.3 - 0.5, large effect: 0.5), Cohen’s d for two group comparisons of normally distributed variables (weak: < 0.5, moderate: 0.5 - 0.8, large effect: 0.8), r for two group comparisons of non-normally distributed variables (weak: < 0.3, moderate: 0.3 - 0.5, large effect: 0.5), for correlation of non-normally distributed variables (weak: < 0.3, moderate: 0.3 - 0.5, strong correlation: 0.5), r for correlation of normally distributed variables (weak: < 0.3, moderate: 0.3 - 0.5, strong correlation: 0.5), as a measure of explained variance in modeling (weak: < 0.13, moderate: 0.13 - 0.26, substantial: 0.26).

## Statistical hypothesis testing

Differences in characteristic of SIMMUN participants included in the analysis and excluded due to data missingness were assessed by test with Cramer’s V effect size statistic and Mann-Whitney test with r effect size metric for numeric and categorical features, respectively (**Supplementary Table S2**). Differences in characteristic of SIMMUN participants or INCOV study participants stratified by the SARS-CoV-2 infection status were investigated by test with Cramer’s V effect size statistic and Mann-Whitney test with r effect size metric for numeric and categorical features, respectively (**Tables 1** and **2**). Correlation of metabolite concentrations with age, stress scoring (PSS-4) and inflammation measures (NEO and NLR) in the SIMMUN cohort was done by Pearson’s test (**Supplementary Table S6**). Differences in metabolite concentrations and metabolite ratios in the SIMMUN cohort stratified by the SARS-CoV-2 infection status or presence of depression symptoms (HADS) were assessed by tow-tailed T test with Cohen’s d effect size metric (**Supplementary Table S7**).

Statistical hypothesis testing was accomplished with the functions compare\_variables() and correlate\_variables() from the development package [*ExDA*](https://github.com/PiotrTymoszuk/ExDA) for comparison and correlation, respectively.

## Multi-parameter linear regression with backwards elimination in the SIMMUN cohort

Effects of age, sex, body mass class, somatic and metal conditions, body mass class, smoking and alcohol consumption history, the inflammation markers NEO and NLR, SARS-CoV-2 infection status, anti-RBD IgG titer, signs of depression and anxiety (HADS), mental stress scoring (PSS-4) on systemic levels of aminoacid neurotransmitter precursor and products of competitor pathways (TRP, KYN, KYN/TRP ratio, PHE, TYR and PHE/TYR ratio) was assessed by multi-parameter linear regression with backward elimination. Responses and numeric explanatory variables were transformed with log or squared root functions to improve normality as specified in **Supplementary Table S1** and normalized prior to modeling.

Full models including the complete set of explanatory variables listed above were constructed (function make\_lm(), package [*lmqc*](https://github.com/PiotrTymoszuk/lmqc)) and optimized by Bayesian information criterion (BIC) driven backwards elimination of non-significant terms (method step(), [*lmqc*](https://github.com/PiotrTymoszuk/lmqc), employing internally stepAIC() from the *MASS* package) (7). Normality and homogeneity of distribution of the model residuals was checked by Shapiro-Wilk and Levene test, respectively (method summary(type = 'assumptions'), package [*lmqc*](https://github.com/PiotrTymoszuk/lmqc)) and visually inspected in standard diagnostic plots of model residuals (residuals vs fitted, quantile-quantile plots, method plot() called for the model objects). Fit stats ( and root mean squared error [RMSE]) were retrieved from the model objects by calling summary(type = 'fit') (package [*lmqc*](https://github.com/PiotrTymoszuk/lmqc)). Validity of the optimized models was determined by likelihood-ratio test (LRT) versus the respective null models (method anova()). Reproducibility and proper parameterization of the optimized multi-parameter models was investigated by repeated cross-validation (10 folds, function train(method = 'lm'), package *caret*)(8) and by comparison of the RMSE and statistics obtained with in the training dataset and in cross-validation (method summary(), package *caretExtra*). As presented in **Supplementary Figure S2A**, similar values of error fit in the training and cross-validation data suggest good reproducibility of the optimized models and lack of over-parameterization.

Inference statistics for the model estimates (, expected values and 95% confidence intervals) were extracted with the summary() method. Significance of the estimates () was determined by two-tailed T test. Results of the linear modeling in the INCOV cohort are presented in **Figure 2** and **Supplementary Table S5**.

## Multi-paramater robust linear modeling in the INCOV cohort

Effects of the key explanatory factors identified by multi-parameter linear modeling in the SIMMUN cohort on systemic neurotransmitter availability were subsequently tested in the publicly available INCOV collective (26). To this end we modeled normalized plasma levels of serotonin (5-HT) as the end product of serotonin biosynthesis associated with mental disorders (30–35), and the major product of systemic dopamine decay dopamine 3-O-sulfate (DA-sulfate) (36,37). The explanatory variables were age, plasma levels of inflammatory cytokines (IL6, IL10, TNF, IFNG), plasma concentrations of neurotransmitter precursors and competitor pathway products (for 5-HT: TRP, KYN and quinolinate [QUIN], for DA sulfate: PHE and TYR) and timepoint of SARS-CoV-2 infection (acute, sub-acute, recovery versus uninfected control). Since most numeric features were non-normally distributed, MM algorithm robust linear regression with Huber’s psi function was chosen (7,38) as a modeling approach.

The robust linear models were built with the make\_lm() wrapper ([*lmqc*](https://github.com/PiotrTymoszuk/lmqc) package) around the MASS package rlm() function (7). The parameterization and reproducibility of the models were tested by 10-fold cross-validation with the *caret* package (8) as described for multi-parameter linear regression. As presented in **Figure 5A**, similar values of error fit in the training and cross-validation data suggest good reproducibility of the robust linear models and proper model parameterization. Inference statistics were extracted from the models with summary() method. Significance of the model estimates () was determined by two-tailed T test, 95% confidence intervals were computed based on theoretical t distribution. The robust linear modeling results are presented in **Figure 5** and **Supplementary Table S8**.

## Time course of inflammatory cytokines and metabolites in the INCOV cohort

Differences in normalized blood concentrations of inflammatory cytokines and metabolites between uninfected controls, acute and sub-acute SARS-CoV-2 infection and recovery were investigated with robust linear modeling with uninfected subset serving as baseline. Models were constructed with the make\_lm() function (package [*lmqc*](https://github.com/PiotrTymoszuk/lmqc)) employing internally the MMrlm() algorithm with Huber’s psi function (package *MASS*) (7,38). Inference statistics were computed as described for multi-parameter robust linear modeling. The time course modeling results are presented in **Figure 6** and **Supplementary Table S9**.

## Correlation and network analysis in the INCOV cohort

Pairwise Spearman’s correlation coefficients were calculated for plasma concentrations of inflammatory cytokines (IL6, IL10, TNF and IFNG), and metabolite involved in serotonin (5-HT, TRP, KYN and QUIN) and dopamine turnover (PHE, TYR and DA sulfate) with the cor() function from the *stats* package at each of the timepoints of SARS-CoV-2 infection (uninfected, acute, sub-acute and recovery). The correlation matrices were subsequently scaled into the [0, 1] range with the function and converted to undirected force directed graphs with the graph\_from\_adjacency\_matrix() function from the *igraph* package (9). The graphs were visualized with tools provided by the *ggnetwork* package (12) as two-dimensional network plots with the node proximity determined by the value and random repulsion, and edge color and width coding for the value and sign (**Figure 7**).

## 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>.

# Supplementary Tables

Table 1: Study variables in the SIMMUN cohort.

| **Variable type** | **Variable labela** | **Format** | **Unit** | **Transformation** | **Categories** |
| --- | --- | --- | --- | --- | --- |
| response | TRP | numeric | µmol/L | identity |  |
| KYN | numeric | µmol/L | logarithm |  |
| PHE | numeric | µmol/L | logarithm |  |
| TYR | numeric | µmol/L | logarithm |  |
| KYN/TRP | numeric |  | logarithm |  |
| PHE/TYR | numeric |  | square root |  |
| explanatory | age | numeric | years | identity |  |
| sex | categorical |  |  | female, male |
| mental illness | categorical |  |  | no, yes |
| somatic illness | categorical |  |  | no, yes |
| SARS-CoV2 | categorical |  |  | no, yes |
| body massb | categorical |  |  | normal, overweight, obesity |
| smoking | categorical |  |  | no, yes |
| alcohol | categorical |  |  | no, yes |
| HADS, anxiety score | categorical |  |  | < 8, ≥ 8 |
| HADS, depression score | categorical |  |  | < 8, ≥ 8 |
| PSS-4, stress score | numeric |  | identity |  |
| NEO | numeric | nmol/L | logarithm |  |
| NLR | numeric |  | logarithm |  |
| anti-RBD IgG | categorical |  |  | negative, 1 - 16.3 AU, > 16.3 AU |
| aTRP: tryptophan; KYN: kynurenine; PHE: phenylalanine; TYR: tyrosine; KYN/TRP: kynurenine - tryptophan ratio; PHE/TYR: phenylalanine/tyrosine ratio; HADS: hospital anxiety and depression score; PSS-4: perceived stress scale, 4 item; NEO: neopterin; NLR: neutrophil - lymphocyte ratio; anti-RBD IgG: anti-receptor-binding domain S1/S2 immunoglobulin. | | | | | |
| bnormal: body mass index (BMI) < 25 kg/m², overweight: BMI 25 - 30 kg/m², obesity: BMI > 30 kg/m². | | | | | |

Table 2: Significant differences between participant of the SIMMUN study included in the analysis and SIMMUN participants excluded due to data missingness. Numeric variables are presented as medians with interquartile ranges (IQR) and ranges. Categorical variables are presented as percentages and counts within the complete observation set.

| **Variablea** | **Analyzed** | **Excluded** | **Test** | **Significanceb** | **Effect size** |
| --- | --- | --- | --- | --- | --- |
| Psychiatric comorbidity | 41% (68) complete: n = 165 | 84% (42) complete: n = 50 | χ² | p < 0.001 | V = 0.36 |
| HADS anxiety score | < 8: 66% (109) ≥ 8: 34% (56) complete: n = 165 | < 8: 42% (21) ≥ 8: 58% (29) complete: n = 50 | χ² | p = 0.016 | V = 0.21 |
| HADS depression score | < 8: 75% (124) ≥ 8: 25% (41) complete: n = 165 | < 8: 40% (20) ≥ 8: 60% (30) complete: n = 50 | χ² | p < 0.001 | V = 0.32 |
| Depression or anxiety signs, HADS ≥ 8 | HADS-: 65% (107) HADS+: 35% (58) complete: n = 165 | HADS-: 38% (19) HADS+: 62% (31) complete: n = 50 | χ² | p = 0.0066 | V = 0.23 |
| PSS-4 stress score | 6 [IQR: 3 - 8] range: 0 - 14 complete: n = 165 | 9 [IQR: 6 - 12] range: 0 - 16 complete: n = 49 | Mann-Whitney | p < 0.001 | r = 0.26 |
| Infection | healthy: 61% (101) SARS-CoV-2: 39% (64) complete: n = 165 | healthy: 84% (42) SARS-CoV-2: 16% (8) complete: n = 50 | χ² | p = 0.016 | V = 0.2 |
| COVID-19 severity | healthy: 61% (101) ambulatory: 28% (47) hospitalized: 10% (17) complete: n = 165 | healthy: 84% (42) ambulatory: 12% (6) hospitalized: 4% (2) complete: n = 50 | χ² | p = 0.033 | V = 0.2 |
| aHADS: hospital anxiety and depression scale; PSS-4: perceived stress scale, 4 item. | | | | | |
| bCorrected for multiple testing with the false discovery rate method. | | | | | |

Table 3: Study variables in the INCOV cohort.

| **Variable type** | **Variable labela** | **Unit** |
| --- | --- | --- |
| explanatory | TNF | Z-score of log₂ concentration |
| IFNG | Z-score of log₂ concentration |
| IL6 | Z-score of log₂ concentration |
| IL10 | Z-score of log₂ concentration |
| QUIN | Z-score of log₂ concentration |
| PHE | Z-score of log₂ concentration |
| response | 5-HT | Z-score of log₂ concentration |
| explanatory | TRP | Z-score of log₂ concentration |
| TYR | Z-score of log₂ concentration |
| KYN | Z-score of log₂ concentration |
| response | DA sulfate | Z-score of log₂ concentration |
| explanatory | age | years |
| aTNF: tumor necrosis factor-alpha; IFNG: interferon gamma; IL6: interleukin-6; IL10: interleukin-10; QUIN: quinolinate; PHE: phenylalanine; 5-HT: serotonin; TRP: tryptophan; TYR: tyrosine; KYN: kynurenine; DA sulfate: dopamine 3-O-sulfate. | | |

Table 4: Number of available samples and sampling timepoints in the INCOV cohort.

| **Time point** | **Days post infection** | **Sample number** |
| --- | --- | --- |
| healthy |  | 27 |
| acute | 10 [6.1 - 13] | 140 |
| sub-acute | 14 [9.9 - 20] | 126 |
| recovery | 64 [51 - 90] | 61 |

Table 5: Results of multi-parameter linear modeling of plasma concentrations of tryptophan, kynurenine, tyrosine, and kynurenine - tryptophan, and phenylalanine - tyrosine ratios in the SIMMUN cohort.

| **Responsea** | **Explanatory variableb** | **Stratum** | **Observations/stratum** | **Estimate, 95% CI** | **Significance** |
| --- | --- | --- | --- | --- | --- |
| TRP | Intercept | baseline |  | 0.12 [-0.052 - 0.29] | ns (0.17) |
| log NEO |  | 165 | -0.19 [-0.34 - -0.04] | p = 0.013 |
| HADS, depression score | ≥ 8 | 41 | -0.48 [-0.83 - -0.14] | p = 0.0064 |
| log KYN | Intercept | baseline |  | -0.19 [-0.36 - -0.012] | p = 0.036 |
| SARS-CoV2 |  | 64 | 0.48 [0.2 - 0.76] | p = 0.001 |
| log NEO |  | 165 | 0.35 [0.2 - 0.49] | p = 4.9e-06 |
| log NLR |  | 165 | -0.19 [-0.32 - -0.051] | p = 0.0074 |
| age |  | 165 | 0.21 [0.067 - 0.35] | p = 0.0041 |
| log KYN/TRP | Intercept | baseline |  | -0.16 [-0.32 - -0.004] | p = 0.045 |
| SARS-CoV2 |  | 64 | 0.42 [0.16 - 0.68] | p = 0.0017 |
| log NEO |  | 165 | 0.47 [0.34 - 0.61] | p = 5.4e-11 |
| age |  | 165 | 0.26 [0.12 - 0.39] | p = 0.00022 |
| PSS-4, stress score |  | 165 | 0.18 [0.055 - 0.31] | p = 0.0052 |
| log TYR | Intercept | baseline |  | -4.5e-16 [-0.14 - 0.14] | ns (1) |
| log NEO |  | 165 | -0.2 [-0.35 - -0.048] | p = 0.01 |
| age |  | 165 | 0.37 [0.21 - 0.52] | p = 4.5e-06 |
| sqrt PHE/TYR | Intercept | baseline |  | 0.15 [-0.04 - 0.34] | ns (0.12) |
| SARS-CoV2 |  | 64 | -0.38 [-0.68 - -0.08] | p = 0.013 |
| age |  | 165 | -0.27 [-0.41 - -0.12] | p = 0.00047 |
| aTRP: tryptophan; KYN: kynurenine; KYN/TRP: kynurenine - tryptophan ratio; TYR: tyrosine; PHE/TYR: phenylalanine - tyrosine ratio. | | | | | |
| bNEO: neopterin; HADS: hospital anxiety and depression scale; NLR: neutrophil - lymphocyte ratio; PSS-4: perceived stress scale, 4 item. | | | | | |

Table 6: Correlation of age, stress scoring blood neopterin levels and neutrophils - leukocyte ratio with plasma metabolite levels in the SIMMUN cohort investigated by Pearson's test.

| **Metabolitea** | **Explanatory variableb** | **n** | **Correlation coefficient, 95% CI** | **Significancec** |
| --- | --- | --- | --- | --- |
| TRP | age | 165 | -0.025 [-0.18 - 0.13] | ns (p = 0.84) |
| log KYN | age | 165 | 0.3 [0.16 - 0.43] | p < 0.001 |
| log KYN/TRP | age | 165 | 0.36 [0.21 - 0.48] | p < 0.001 |
| TRP | PSS-4, stress score | 165 | -0.17 [-0.31 - -0.016] | ns (p = 0.062) |
| log KYN | PSS-4, stress score | 165 | -0.016 [-0.17 - 0.14] | ns (p = 0.85) |
| log KYN/TRP | PSS-4, stress score | 165 | 0.12 [-0.029 - 0.27] | ns (p = 0.16) |
| TRP | log NEO | 165 | -0.17 [-0.32 - -0.02] | ns (p = 0.061) |
| log KYN | log NEO | 165 | 0.35 [0.2 - 0.47] | p < 0.001 |
| log KYN/TRP | log NEO | 165 | 0.52 [0.4 - 0.62] | p < 0.001 |
| TRP | log NLR | 165 | -0.16 [-0.31 - -0.01] | ns (p = 0.066) |
| log KYN | log NLR | 165 | -0.18 [-0.32 - -0.023] | ns (p = 0.061) |
| log KYN/TRP | log NLR | 165 | -0.055 [-0.21 - 0.098] | ns (p = 0.58) |
| log PHE | age | 165 | 0.14 [-0.013 - 0.29] | ns (p = 0.16) |
| log TYR | age | 165 | 0.3 [0.16 - 0.44] | p < 0.001 |
| sqrt PHE/TYR | age | 165 | -0.25 [-0.39 - -0.11] | p = 0.0044 |
| log PHE | log NEO | 165 | -0.044 [-0.2 - 0.11] | ns (p = 0.58) |
| log TYR | log NEO | 165 | -0.085 [-0.23 - 0.069] | ns (p = 0.37) |
| sqrt PHE/TYR | log NEO | 165 | 0.083 [-0.071 - 0.23] | ns (p = 0.37) |
| aTRP: tryptophan; KYN: kynurenine; KYN/TRP: kynurenine - tryptophan ratio; PHE: phenylalanine; TYR: tyrosine; PHE/TYR: phenylalanine - tyrosine ratio. | | | | |
| bPSS-4: perceived stress scale, 4 item; NEO: neopterin; NLR: neutrophil - lymphocyte ratio. | | | | |
| cCorrected for multiple testing with the false discovery rate method. | | | | |

Table 7: Comparison of plasma metabolite levels in SIMMUN study participants stratified by depression signs and SARS-CoV-2 infection status with two-tailed T test and Cohen's d effect size statistic.

| **Metabolitea** | **Explanatory variableb** | **Stratumc** | **Observations/stratum** | **Statisticd** | **Significance** | **Effect size** |
| --- | --- | --- | --- | --- | --- | --- |
| TRP | HADS, depression score | < 8 | 124 | 62 [IQR: 55 - 68] range: 33 - 93 | ns (p = 0.059) | d = 0.45 |
| TRP | HADS, depression score | ≥ 8 | 41 | 53 [IQR: 48 - 65] range: 43 - 85 | ns (p = 0.059) | d = 0.45 |
| TRP | SARS-CoV2 | uninfected | 101 | 59 [IQR: 50 - 66] range: 33 - 88 | ns (p = 0.12) | d = 0.28 |
| TRP | SARS-CoV2 | recovery | 64 | 62 [IQR: 56 - 69] range: 42 - 93 | ns (p = 0.12) | d = 0.28 |
| log KYN | HADS, depression score | < 8 | 124 | 0.71 [IQR: 0.57 - 0.86] range: -0.13 - 1.4 | ns (p = 0.12) | d = 0.35 |
| log KYN | HADS, depression score | ≥ 8 | 41 | 0.59 [IQR: 0.41 - 0.72] range: -0.084 - 1.4 | ns (p = 0.12) | d = 0.35 |
| log KYN | SARS-CoV2 | uninfected | 101 | 0.67 [IQR: 0.48 - 0.79] range: -0.13 - 1.4 | ns (p = 0.061) | d = 0.36 |
| log KYN | SARS-CoV2 | recovery | 64 | 0.71 [IQR: 0.57 - 0.88] range: 0.18 - 1.4 | ns (p = 0.061) | d = 0.36 |
| log KYN/TRP | HADS, depression score | < 8 | 124 | -3.4 [IQR: -3.6 - -3.3] range: -4 - -2.8 | ns (p = 0.85) | d = 0.036 |
| log KYN/TRP | HADS, depression score | ≥ 8 | 41 | -3.4 [IQR: -3.6 - -3.3] range: -4 - -2.7 | ns (p = 0.85) | d = 0.036 |
| log KYN/TRP | SARS-CoV2 | uninfected | 101 | -3.4 [IQR: -3.6 - -3.3] range: -4 - -2.7 | ns (p = 0.45) | d = 0.15 |
| log KYN/TRP | SARS-CoV2 | recovery | 64 | -3.4 [IQR: -3.5 - -3.3] range: -3.8 - -2.8 | ns (p = 0.45) | d = 0.15 |
| log PHE | SARS-CoV2 | uninfected | 101 | 4.2 [IQR: 4.1 - 4.4] range: 3.6 - 4.9 | ns (p = 0.58) | d = 0.11 |
| log PHE | SARS-CoV2 | recovery | 64 | 4.2 [IQR: 4.1 - 4.3] range: 3.7 - 5.3 | ns (p = 0.58) | d = 0.11 |
| log TYR | SARS-CoV2 | uninfected | 101 | 4.2 [IQR: 4 - 4.4] range: 3.6 - 5 | ns (p = 0.37) | d = 0.2 |
| log TYR | SARS-CoV2 | recovery | 64 | 4.2 [IQR: 4 - 4.4] range: 3.7 - 5.1 | ns (p = 0.37) | d = 0.2 |
| sqrt PHE/TYR | SARS-CoV2 | uninfected | 101 | 1 [IQR: 0.96 - 1.1] range: 0.78 - 1.4 | ns (p = 0.092) | d = 0.35 |
| sqrt PHE/TYR | SARS-CoV2 | recovery | 64 | 0.98 [IQR: 0.9 - 1] range: 0.79 - 1.6 | ns (p = 0.092) | d = 0.35 |
| aTRP: tryptophan; KYN: kynurenine; KYN/TRP: kynurenine - tryptophan ratio; PHE: phenylalanine; TYR: tyrosine; PHE/TYR: phenylalanine - tyrosine ratio. | | | | | | |
| bHADS: hospital anxiety and depression scale. | | | | | | |
| cMedian with interquartile range (IQR) and range. | | | | | | |
| dCorrected for multiple testing with the false discovery rate method. | | | | | | |

Table 8: Results of multi-parameter robust linear modeling of plasma concentrations of serotonin, and dopamine sulfate in the INCOV cohort.

| **Responsea** | **Explanatory variableb** | **Stratum** | **Observations/stratum** | **Estimate, 95% CI** | **Significance** |
| --- | --- | --- | --- | --- | --- |
| 5-HT | Intercept | baseline |  | -0.27 [-0.64 - 0.11] | ns (0.16) |
| TRP |  | 354 | 0.17 [0.048 - 0.29] | p = 0.0061 |
| KYN |  | 354 | 0.1 [-0.086 - 0.3] | ns (0.28) |
| QUIN |  | 354 | -0.011 [-0.2 - 0.18] | ns (0.91) |
| SARS-CoV-2 | acute | 140 | 0.25 [-0.18 - 0.68] | ns (0.26) |
| SARS-CoV-2 | sub-acute | 126 | 0.3 [-0.12 - 0.72] | ns (0.16) |
| SARS-CoV-2 | recovery | 61 | 0.72 [0.3 - 1.1] | p = 0.00071 |
| Age |  | 354 | -0.15 [-0.25 - -0.041] | p = 0.0065 |
| IL6 |  | 354 | -0.24 [-0.4 - -0.089] | p = 0.002 |
| IL10 |  | 354 | -0.061 [-0.21 - 0.088] | ns (0.42) |
| TNF |  | 354 | 0.0055 [-0.12 - 0.13] | ns (0.93) |
| IFNG |  | 354 | 0.063 [-0.074 - 0.2] | ns (0.37) |
| DA sulfate | Intercept | baseline |  | -0.2 [-0.52 - 0.12] | ns (0.22) |
| PHE |  | 354 | 0.0028 [-0.1 - 0.11] | ns (0.96) |
| TYR |  | 354 | 0.036 [-0.059 - 0.13] | ns (0.46) |
| SARS-CoV-2 | acute | 140 | -0.1 [-0.48 - 0.27] | ns (0.58) |
| SARS-CoV-2 | sub-acute | 126 | 0.17 [-0.19 - 0.53] | ns (0.35) |
| SARS-CoV-2 | recovery | 61 | 0.64 [0.28 - 1] | p = 0.00047 |
| Age |  | 354 | -0.02 [-0.11 - 0.068] | ns (0.66) |
| IL6 |  | 354 | -0.1 [-0.23 - 0.025] | ns (0.12) |
| IL10 |  | 354 | 0.026 [-0.1 - 0.15] | ns (0.69) |
| TNF |  | 354 | 0.14 [0.032 - 0.24] | p = 0.0096 |
| IFNG |  | 354 | -0.13 [-0.24 - -0.014] | p = 0.027 |
| a5-HT: 5-hydroxy tryptophan/serotonin; DA sulfate: dopamine 3-O-sulfate. | | | | | |
| bTRP: tryptophan; KYN: kynurenine; QUIN: quinolinate; IL6: interleukin-6, IL10: interleukin-10; TNF: tumor necrosis factor-alpha; IFNG: interferon gamma; PHE: phenylalanine; TYR: tyrosine. | | | | | |

Table 9: Results of robust linear modeling of plasma levels of inflammatory cytokines, tryptophan, tyrosine and their metabolites as a function of SARS-CoV-2 infection status in the INCOV cohort.

| **Responsea** | **Timepoint** | **Estimate, 95% CI** | **Significanceb** |
| --- | --- | --- | --- |
| TNF | uninfected: baseline | -0.24 [-0.54 - 0.063] | ns (p = 0.24) |
| acute | 2 [1.6 - 2.3] | p < 0.001 |
| sub-acute | 1.8 [1.5 - 2.2] | p < 0.001 |
| recovery | 1.1 [0.74 - 1.5] | p < 0.001 |
| IFNG | uninfected: baseline | 0.00042 [-3.2 - 3.2] | ns (p = 1) |
| acute | 7.7 [4.3 - 11] | p < 0.001 |
| sub-acute | 2.8 [-0.76 - 6.3] | ns (p = 0.24) |
| recovery | 0.61 [-3.2 - 4.4] | ns (p = 0.83) |
| IL6 | uninfected: baseline | -0.011 [-1.1 - 1.1] | ns (p = 1) |
| acute | 4.8 [3.6 - 6] | p < 0.001 |
| sub-acute | 3.3 [2.1 - 4.5] | p < 0.001 |
| recovery | 0.71 [-0.6 - 2] | ns (p = 0.45) |
| IL10 | uninfected: baseline | 0.0065 [-0.79 - 0.81] | ns (p = 1) |
| acute | 4.4 [3.5 - 5.3] | p < 0.001 |
| sub-acute | 2.8 [1.9 - 3.7] | p < 0.001 |
| recovery | 1.1 [0.11 - 2] | ns (p = 0.065) |
| QUIN | uninfected: baseline | 0.14 [-0.36 - 0.63] | ns (p = 0.7) |
| acute | 1.8 [1.3 - 2.4] | p < 0.001 |
| sub-acute | 1.6 [1 - 2.1] | p < 0.001 |
| recovery | 0.48 [-0.12 - 1.1] | ns (p = 0.24) |
| PHE | uninfected: baseline | 0.37 [-0.056 - 0.79] | ns (p = 0.19) |
| acute | 1.4 [0.97 - 1.9] | p < 0.001 |
| sub-acute | 0.89 [0.42 - 1.4] | p < 0.001 |
| recovery | 0.27 [-0.24 - 0.78] | ns (p = 0.46) |
| 5-HT | uninfected: baseline | 0.25 [-0.25 - 0.76] | ns (p = 0.48) |
| acute | -0.31 [-0.86 - 0.24] | ns (p = 0.44) |
| sub-acute | -0.089 [-0.65 - 0.47] | ns (p = 0.83) |
| recovery | 0.91 [0.3 - 1.5] | p = 0.0089 |
| TRP | uninfected: baseline | 0.3 [-0.48 - 1.1] | ns (p = 0.56) |
| acute | -1.9 [-2.7 - -1] | p < 0.001 |
| sub-acute | -1.1 [-2 - -0.25] | p = 0.028 |
| recovery | 0.0047 [-0.93 - 0.94] | ns (p = 1) |
| TYR | uninfected: baseline | 0.21 [-0.33 - 0.74] | ns (p = 0.56) |
| acute | 0.1 [-0.48 - 0.69] | ns (p = 0.83) |
| sub-acute | 0.34 [-0.25 - 0.93] | ns (p = 0.44) |
| recovery | 0.24 [-0.41 - 0.88] | ns (p = 0.57) |
| KYN | uninfected: baseline | 0.21 [-0.25 - 0.66] | ns (p = 0.5) |
| acute | 1.5 [0.99 - 2] | p < 0.001 |
| sub-acute | 1.2 [0.67 - 1.7] | p < 0.001 |
| recovery | 0.22 [-0.32 - 0.77] | ns (p = 0.56) |
| DA sulfate | uninfected: baseline | 0.33 [-0.22 - 0.88] | ns (p = 0.41) |
| acute | -0.28 [-0.88 - 0.31] | ns (p = 0.49) |
| sub-acute | 0.4 [-0.2 - 1] | ns (p = 0.35) |
| recovery | 1.3 [0.6 - 1.9] | p < 0.001 |
| aTNF: tumor necrosis factor-alpha; IFNG: interferon gamma; IL6: interleukin-6; IL10: interleukin-10; QUIN: quinolinate; PHE: phenylalanine; 5-HT: serotonin; TRP: tryptophan; TYR: tyrosine; KYN: kynurenine; DA sulfate: dopamine 3-O-sulfate. | | | |
| bCorrected for multiple testing with the false discovery rate method. | | | |

# Supplementary Figures

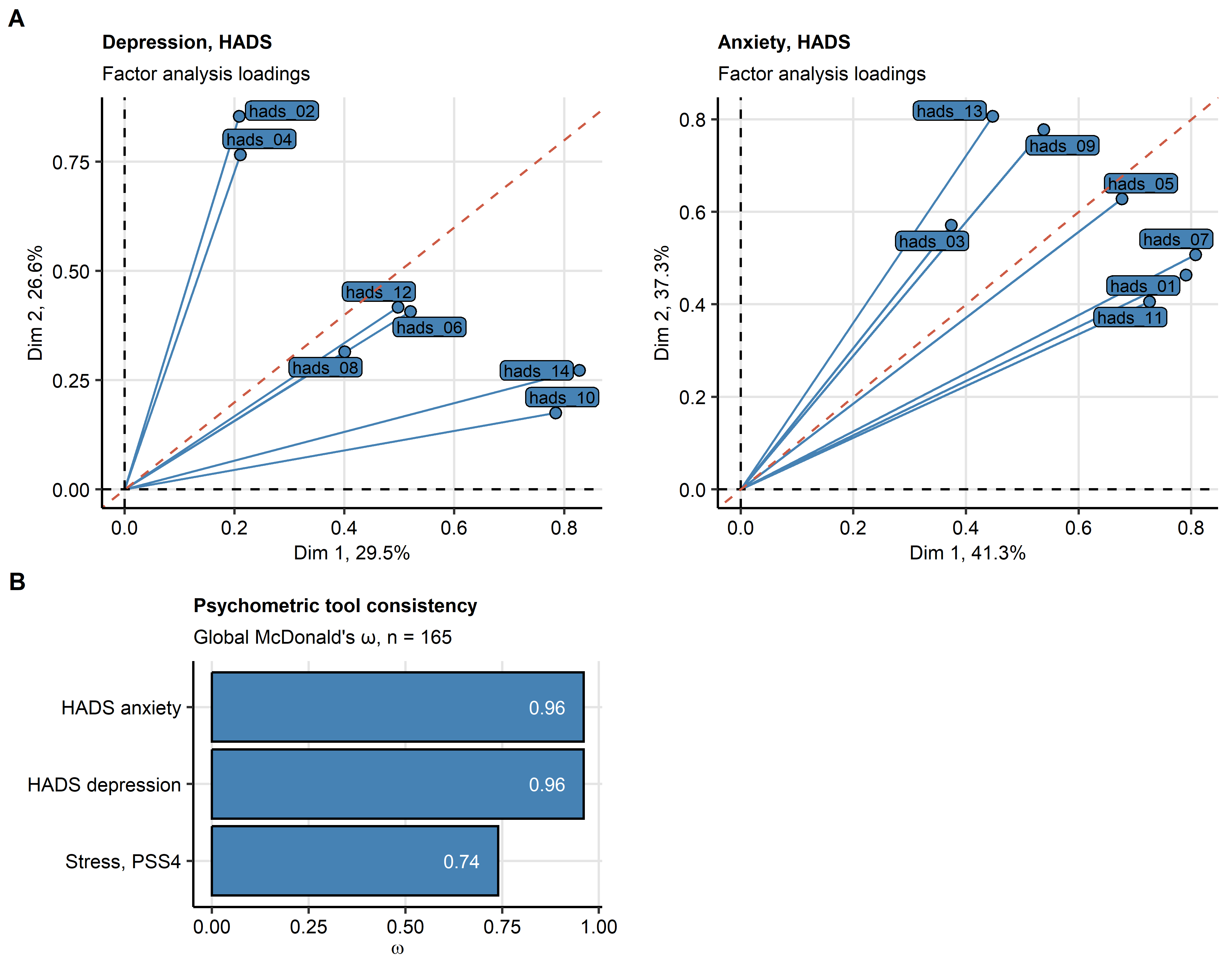


Figure 1: Consistency of the PSS4 stress HADS depression and HADS anxiety psychometric tools in the SIMMUN cohort.

**Supplementary Figure S1. Consistency of the PSS4 stress HADS depression and HADS anxiety psychometric tools in the SIMMUN cohort.**

*(A) Assessment of tau-equivalence of the HADS (hospital anxiety and depression scale) depression and anxiety tool by three-dimensional factor analysis. Loadings for the first two major factors are presented. Each point represents a single HADS item. Percentages of total dataset variance associated with the factors are indicated in the plot axes. Note: tau equivalence implicates equal loadings of each HADS items for all factors. In case of tau equivalence, proximity of the items to the diagonal of the plot (orange dashed line) is expected.*

*(B) Consistency of the HADS depression, HADS anxiety and PSS-4 (perceived stress scale, 4 item) psychometric tools determined by McDonald’s metric.*

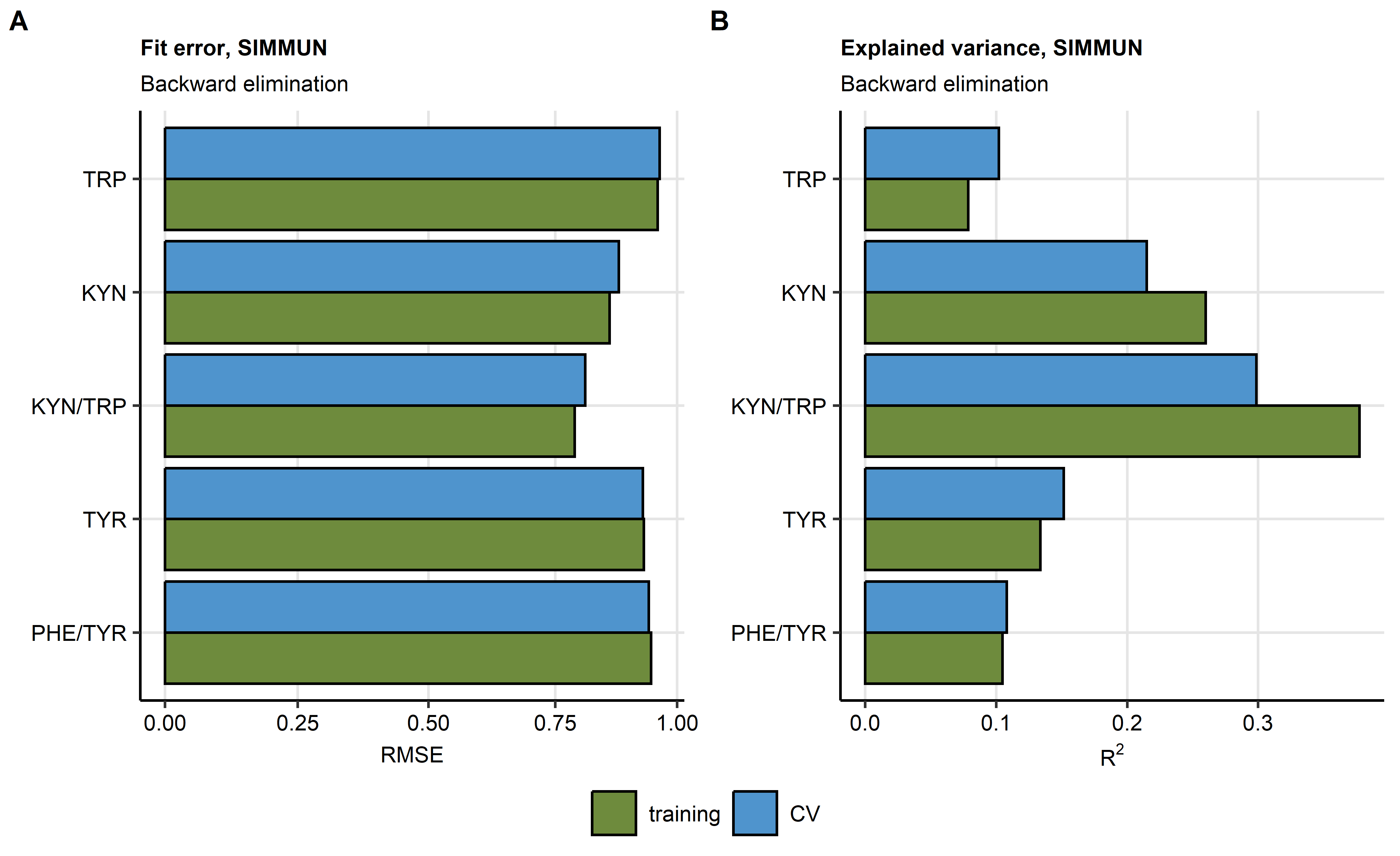


Figure 2: Root mean square error and R-squared statistics for multi-parameter linear models of tryptophan, tyrosine and their metabolites in the SIMMUN cohort.

**Supplementary Figure S2. Root mean square error and R-squared statistics for multi-parameter linear models of tryptophan, tyrosine and their metabolites in the SIMMUN cohort.**

*Multi-parameter linear regression models of plasma levels of tryptophan (TRP), kynurenine (KYN), kynurenine/tryptophan ratio (KYN/TRP), phenylalanine (PHE), tyrosine (TYR) and phenylalanine/tyrosine ratio (PHE/TRP) in the SIMMUN models were optimized by backwards elimination and their reproducibility was tested by repeated cross-validation (CV, 10 folds). Values of root mean square error (RMSE, A) and (B) in the training data set and CV are plotted.*

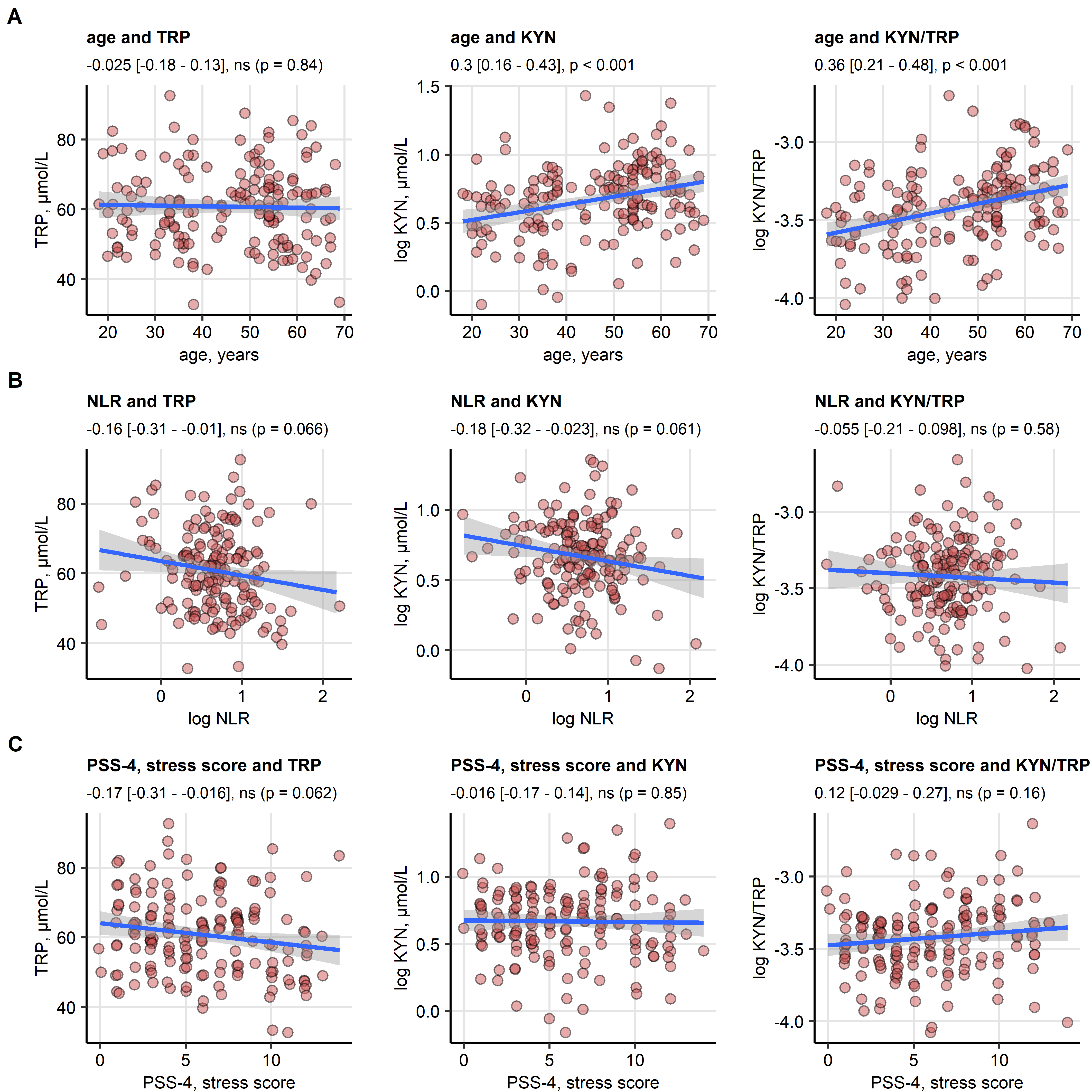


Figure 3: Correlation of tryptophan and its metabolites with age, plasma levels of neopterin, neutrophil - lymphocyte ratio, and mental stress scoring in the SIMMUN cohort.

**Supplementary Figure S3. Correlation of tryptophan and its metabolites with age, plasma levels of neopterin, neutrophil - lymphocyte ratio, and mental stress scoring in the SIMMUN cohort.**

*Correlation of age (A), the inflammatory marker neutrophil-leukocyte ratio (B, NLR), and stress scoring (C, perceived stress scale, 4-item [PSS-4]) with plasma concentrations of tryptophan (TRP), kynurenine (KYN) and kynurenine - tryptophan ratio (KYN/TRP) was assessed by Pearson’s test corrected for multiple testing with the false discovery rate method (n = 165. Single observations are depicted as points, fitted linear trends with 95% confidence intervals are visualized as blue lines with gray ribbons. Values of correlation coefficients (r) with 95% confidence intervals and p values are displayed in the plot captions.*

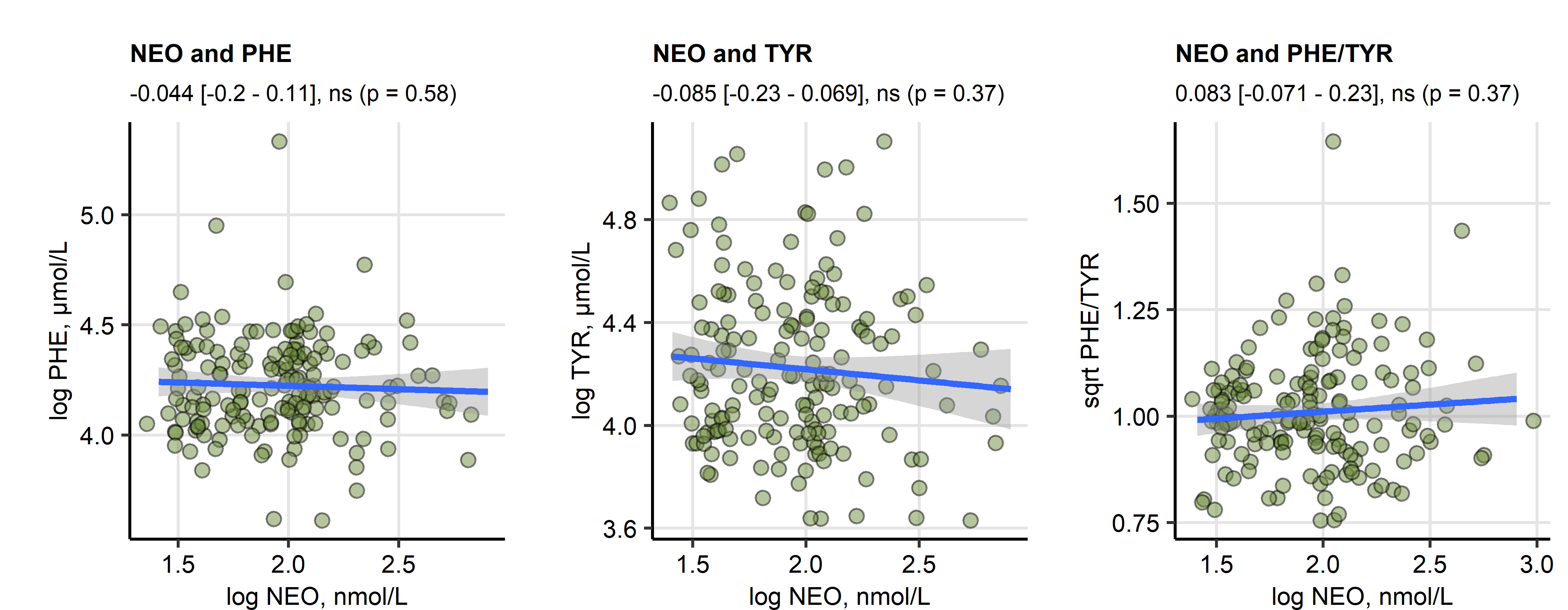


Figure 4: Correlation of phenylalanine and tyrosine with plasma levels of neopterin in the SIMMUN cohort.

**Supplementary Figure S4. Correlation of phenylalanine and tyrosine with plasma levels of neopterin in the SIMMUN cohort.**

*Correlation of the inflammatory marker neopterin (NEO) with plasma concentrations of phenylalanine (PHE), tyrosine (TYR) and phenylalanine - tyrosine ratio (PHE/TYR) was was assessed by Pearson’s test corrected for multiple testing with the false discovery rate method (n = 165. Single observations are depicted as points, fitted linear trends with 95% confidence intervals are visualized as blue lines with gray ribbons. Values of correlation coefficients (r) with 95% confidence intervals and p values are displayed in the plot captions.*

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