Biology Methods & Protocols

Biology Methods and Protocols, 2023, bpad037
https://doi.org/10.1093/biomethods/bpad037
Advance Access Publication Date: 6 December 2023
Methods Article

Is recovery just the beginning? Persistent symptoms and health and performance deterioration in post-COVID-19, non-hospitalized university students—a cross-sectional study

Ashkan Latifi[1] and Jaroslav Flegr [1,*]

[1]Department of Philosophy and History of Sciences, Faculty of Science, Laboratory of Evolutionary Biology, Charles University, Viničná 7, Prague 128 00, Czechia

*Correspondence address. Department of Philosophy and History of Sciences, Faculty of Science, Laboratory of Evolutionary Biology, Charles University, Viničná 7, Prague 128 00, Czechia. E-mail: flegr@cesnet.cz

Abstract

Many individuals experience persistent symptoms such as deteriorated physical and mental health, increased fatigue, and reduced cognitive performance months after recovering from coronavirus disease 2019 (COVID-19). There is limited data on the long-term trajectory and prevalence of these symptoms, especially in milder cases. Our study aimed to assess the persistent effects of COVID-19 on physical and mental health, fatigue, and cognitive performance in a cohort of 214 students, averaging 21.8 years of age. Of these, 148 had contracted COVID-19 but were not hospitalized, with the time since infection ranging from 1 to 39 months. We utilized a comprehensive panel of cognitive tests to measure intelligence, memory, and psychomotor skills, and a detailed anamnestic questionnaire to evaluate physical and mental health. While contracting COVID-19 did not significantly impact overall health and performance, it was associated with increased reports of fatigue. However, the reported severity of the disease had a pronounced negative influence on physical health, mental well-being, fatigue, and reaction time. Trends of improvement in physical and mental health, as well as error rate, were observed within the first 2 years post-infection. However, fatigue and reaction time showed a trend of deterioration. Beyond the 2-year mark, physical health and error rate continued to improve, while mental health began to deteriorate. Fatigue and reaction time continued to decline. Overall, our findings suggest that some effects of contracting COVID-19 can persist or even deteriorate over time, even in younger individuals who had mild cases that did not require hospitalization.

Keywords: COVID-19; long-term effects; post-COVID sequelae; cognitive performance; physical health; mental health; fatigue; long COVID; SARS-CoV-2

Introduction

In December 2019, reports of a novel viral disease, COVID-19, emerging in China, shocked the world. Initially reported as primarily a respiratory infection, COVID-19 is now known to have a wide range of physiological, psychological, psychiatric, neurological, and anatomical impacts on patients and survivors. A growing body of research is pointing to the post-acute sequelae of COVID-19 in the survivors. For example, depression and cognitive impairment [1], fatigue [2], pain [3], psychiatric and neurological complications, see [4] and [5], and lower health-related quality of life (HRQoL) compared with controls [6] are among the symptoms that these survivors may suffer from.

Studies also show that COVID-19 post-infection effects can negatively affect patients' mental health. A systematic review and meta-analysis of COVID-19 survivors revealed that 50.1% of these survivors experienced at least one sequela for up to a year after being diagnosed with the infection. In addition, this meta-analysis reported that significant abnormalities on lung CT scan, abnormal pulmonary function, fatigue, psychiatric symptoms (primarily depression and PTSD), and neurological symptoms, for example, cognitive deficits and memory impairment were observed in these survivors. In addition, this meta-analysis found that the elderly (specifically males) with a history of more severe forms of the disease and those with an underlying health or mental condition exhibited a higher risk of these sequelae. In terms of disease severity, subjects who experienced a severe form of COVID-19 had complications such as Post-traumatic stress disorder (PTSD), cognitive deficits, concentration difficulties, sleep disturbance, and gustatory problems, whereas those with a history of mild COVID-19 developed high levels of anxiety and memory impairment [7].

Post-infection effects of COVID-19 have also been associated with cognitive impairment in patients [8]. In this regard, a systematic review pointed out that cognitive impairments were the most prominent in the domains of executive functions, attention, and episodic memory 6 months after disease onset in severe and moderate COVID-19 patients [9]. Another systematic review and meta-analysis reported that subjects with a history of COVID-19 infection experienced significant cognitive impairments compared with controls. However, these impairments were significant only in the sub-domains of processing speed and verbal

Received: October 30, 2023. **Revised:** November 18, 2023. **Editorial decision:** November 28, 2023. **Accepted:** December 04, 2023

memory, and not in attention, executive functions, fluency, visuospatial ability, and working memory [10]. The results of another recent meta-analysis and systematic review showed that COVID-19 patients exhibited significant cognitive deficits compared with controls, corroborating the findings of former studies pointing to cognitive impairments in executive functions, working memory, attention, and processing speed in these patients [11].

Regarding the studies reviewed so far, there are some points that need mentioning which justify the importance of future studies:

1. Few studies have considered the post-infection effects on those with mild-to-moderate COVID-19; in spite of the finding that even those with an asymptomatic COVID-19 progression may still develop cognitive impairments [12].
2. Few studies have investigated physical health, mental health, and cognitive functioning at once in the same sample, which is important because these domains are interconnected. For instance, cognitive impairment and depression are found to be pathophysiologically related [13], and cognitive impairments are more abundant in individuals suffering from anxiety, depression, and bipolar disorder [14].
3. Few studies have systematically studied the effects of "time elapsed since the onset of infection" on physical health, mental health, and cognitive functions over a span of at least 2 years. Most of the studies done have considered the COVID-19 post-infection effects unfolding in less than a year from infection onset [15–18].
4. The findings related to cognitive functioning, physical health complications, and mental health disorders associated with COVID-19 post-infection sequelae are varied and sometimes inconsistent. For example, although a study reported a prevalence of 82.3% for clinically significant levels of fatigue in the patients, another study found a prevalence of 11.5%; as for cognitive impairment/cognitive dysfunction, where a study reported a rate of 61.4%, another study put it at 23.5%; and as it regards depressive-anxiety symptoms, the observed rate in one study was 23.5% and in another 9.5% [19].

Accordingly, the aim of this study was to address the gaps identified in the existing literature concerning the post-infection consequences of COVID-19. The focus of our study was to explore the long-term effects of COVID-19. Specifically, we examined outcomes in cognitive functioning, physical health, and mental well-being over a period ranging from 1 to 39 months post-infection in individuals with mild-to-moderate cases of the disease. We concentrated on a cohort of 272 young, healthy university students. Data were collected through a medical history questionnaire, complemented by a broad panel of cognitive tests assessing aspects such as intelligence, memory, and psychomotor skills.

Materials and methods

Study participants

All students who took the online examination for an advanced course in evolutionary biology in 2022 and 2023 were invited to participate in an anonymous study "aimed at testing certain evolutionary psychology hypotheses and exploring the impact of various factors on exam performance." Participants were informed about the voluntary nature of their participation and the scientific use of their data when they began the electronic questionnaire. They were also reassured of their ability to withdraw from the study at any time by simply closing the survey page.

Both the examination and the subsequent survey were facilitated separately on the Qualtrics platform. Upon concluding the exam, students were notified of their performance, that is the number of correctly answered questions. During the anonymous survey, we asked them to report this figure.

While the primary focus of the research was to evaluate the influence of diverse biological and social aspects on life history strategies, the project's exploratory segment also aimed to scrutinize the impact of various factors on students' performance in the exam and a set of cognitive tests. This was explicitly mentioned in the pre-registration form (DOI 10.17605/OSF.IO/FGRWD).

The online survey comprised several questionnaires and performance tests, of which only a few pertained directly to the study at hand. The project, inclusive of the informed consent acquisition method (achieved by clicking the designated button onscreen), received approval from the Institutional Review Board of the Faculty of Science at Charles University (No. 2021/19). The study adhered strictly to the relevant ethical guidelines for human subject research.

Questionnaires and tests

In this study, we examined the impact of three primary independent variables—COVID-19 exposure, COVID-19 course, and time since COVID-19 infection—on 51 dependent variables. This included five indices derived from the 46 source variables, as detailed in Supplementary Table S1.

In the survey, we gaged the participants' *Intelligence* using the Cattel 16PF test (Variant A, Scale B) [20] and their memory with a modified Meili test [21, 22]. Initially, the Meili Memory Test involved presenting participants with a list of 12 distinct words (knife, handcuffs, pump, chain, tree, collar, ice, glasses, arrow, tank, bars, and rifle) for a period of 24 s. Approximately 5 min later, we prompted them for a *Free recall memory test*, asking them to recollect as many of these words as possible. Subsequently, we presented them with a list of 24 words and asked them to identify the original 12 words in a *Recognition memory test*.

We also evaluated the psychomotor skills of the participants, specifically their reaction time and precision, using two tests—the Choice test and the Stroop test. In the Choice test, participants were directed to swiftly click with a computer mouse on a particular letter (A, B, C, or D) displayed on the screen. These letters were each assigned to one of four horizontally arranged buttons at the center of the screen. The button sequence was randomized for each of the six trials. We recorded the number of accurately selected buttons throughout the six trials (*Choice test accuracy*), along with the mean reaction time for these six trials (*Choice test reaction time*).

Our variant of the Stroop test included three distinct sections. Each section began only after students had received instructions and had time to rest. They were directed to start "when they were prepared." In Part A, participants were required to select a specific word (e.g. "red") from a set of four options ("red," "green," "blue," and "brown"), which were displayed in the center of the screen in a randomized order. These words were presented in a color that did not correspond to their actual meaning. The command specifying which word to choose was positioned at the top of the screen, with participants instructed to disregard the font color. Part B mirrored the conditions of Part A, but in this section, participants were required to select a word displayed in a specific color, whilst ignoring the words' meanings. Part C was a slight variation of Part A, where the command specifying the word to be selected was consistently written in a contrasting color, not aligning with the meaning or color of the presented stimuli. Prior to each section, participants were provided with clear instructions, notified about the number of iterations (always five), and instructed to respond as rapidly as possible. Participants could initiate each section by clicking the "Start Test" button. We recorded the number of correct responses across all 15 attempts (*Stroop test accuracy*) and computed the average reaction time for all these attempts (*Stroop test reaction time*). Additionally, we calculated the average reaction times for each of the three sections of the test (*Stroop test reaction time* 1–3).

Participants were also tasked with solving three problems from the standard CRT [23]. These problems were slightly modified to deter participants from looking up solutions online. The problems were: "Duckweed grows on the surface of a pond, doubling its area every day. If it takes 48 days for the duckweed to cover the entire surface of the pond, how many days did it take to cover half the surface?", "Five workers can produce 5 parts in 5 minutes. How many minutes will it take for 100 workers to produce 100 parts?", and "A car with a doll costs 110 CZK. The car is 100 CZK more expensive than the doll. How much does the doll cost?". After solving each problem, participants were asked if they were already familiar with it. Approximately 18% of the participants recognized the problems, and their results were not included in the final assessment.

The "*Reading time*" variable was calculated as the mean Z-score of the time taken to read the instructions for all included tests. These instructions, concise in nature, were presented as short paragraphs on the webpage before each test. The "*Error rate score*" was derived from the mean of the inverted Z-scores obtained from the *Evolutionary biology test result*, *Intelligence*, *CRT*, *Choice test accuracy*, and the *Stroop accuracy*. The "*Reaction time score*" was determined as the mean Z-scores of *Reading time* and reaction times captured during the Choice test, and Stroop test.

In the anamnestic section of the questionnaire, participants were required to answer 19 questions related to their physical health, see Supplementary Table S1. These questions covered the frequency of various conditions, including allergies, skin disorders, digestive tract disorders, metabolic disorders, common infectious diseases, orthopedic disorders, neurological disorders, headaches, physical pains, and other chronic physical issues. Participants were also queried about their antibiotic usage over the past year and the preceding 3 years, their frequency of visits to a general practitioner, and any hospital stays that exceeded a week in the past 5 years. They provided responses using 6-point ordinal scales anchored by, e.g. "never" and "daily or more frequently."

Further, they reported the number of non-mental health medications prescribed by a doctor that they were currently taking, with options ranging from 0 to 6, where six indicated five or more medications. Questions about their current physical feeling, usual physical feeling, and a comparison of their physical condition to that of their peers were answered using 6-point scales. Finally, participants were asked to estimate their life expectancy, with six response options ranging from "more than 99 years" to "less than 60 years."

The responses to these questions were inverted when a higher value indicated better health and a lower value indicated poorer health. The index of *Physical sickness score* was then derived from the mean Z-scores of all these 19 questions [24]. Similarly, a *Mental sickness score* was derived from participants' responses to nine variables: frequencies of depression, anxiety, phobias, obsessions, other mental health problems, the number of prescribed mental health medications, and questions about their current mental state, usual mental feeling, and comparison of their mental condition to that of their peers, all of which were gaged using 6-point scales.

The *Fatigue score* was calculated based on the mean Z-scores of five variables: *Frequency of tiredness* (6-point scale, anchored by "never" and "daily or several times a day"), *Current level of tiredness*, *Fatigue after returning from work/school*, *Fatigue after several hours of bus travel*, and *Fatigue after several hours of train travel* (all using 6-point scales, anchored by "definitely not" and "definitely yes"). These variables were intended to reflect a continuum of fatigue (physical fatigue to mental fatigue). Frequency of tiredness and current level of tiredness mainly represented the physical fatigue pole, whereas fatigue after several hours of bus travel and fatigue after several hours of train travel mainly constituted the mental fatigue pole. And lastly, fatigue after returning from work/school stood in between.

In the anamnestic section of the survey, additional demographic and medical history data were gathered from participants. These included their age and official sex as stated on their birth certificate (with men coded as 1 and women coded as 0), and history of Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection (COVID-19). In coding the participants' COVID-19 infection status, we used a numerical system. Responses indicating no confirmed infection, such as "not yet" and "no but I was in quarantine," were coded as 0, representing COVID-19-negative status. A confirmed diagnosis of COVID-19, indicated by a response of "yes, I was diagnosed with COVID-19," was coded as 1, representing COVID-19-positive status. Responses that were uncertain about the infection status, such as "probably yes, but I was not diagnosed with COVID-19" and "I am waiting for the result of a diagnostic test," were coded as NA, indicating data not available. Participants who confirmed their COVID-19 diagnosis were further asked to specify the number of months since the onset of their illness and to rate its severity on a 6-point scale (1: No symptoms, 2: Like mild flu, 3: Like normal flu, 4: Like severe flu, 5: I was hospitalized, 6: I was in ICU).

For the precise wording of all questions, refer to the questionnaire text attached to the preregistration form (DOI 10.17605/OSF.IO/FGRWD) and Supplementary Table S1 containing a description of all dependent variables.

Data analysis

To address potential issues arising from an unbalanced dataset (with women outnumbering men two-to-one and infected subjects similarly outnumbering their non-infected counterparts), irregularities in data distribution, and potential confounding variables, we utilized a non-parametric multivariate method for the analyses of the impact of COVID-19 infection status, severity, duration, and time elapsed since onset on health and cognitive performance. Specifically, we employed a partial Kendall correlation test, controlled for age, sex, and the survey year, to investigate the effects of three COVID-19-related variables. These tests, as well as *t*-tests and chi squared tests used in the descriptive statistics section of the study, were conducted using the Explorer version 1.0 R script [25], which utilizes the ppcor R package [26]. In analyses of the mixed-sex sample, we controlled for age, sex, and the survey year (either 2022 or 2023). In the sex-specific analyses, we only controlled for age and year. The Kendall correlation test allows for the control of confounding variables and is robust against outliers and variable distribution shapes in general.

To adjust for multiple testing, we employed the Benjamini–Hochberg procedure, setting the false discovery rate (FDR) at 0.10 [27]. The dataset for this study is publicly accessible on Figshare 10.6084/m9.figshare.24032700.

Technical notes

The term "effect" is used throughout the article in a statistical context to denote an observed association—the difference between the actual population parameter and the null hypothesis value. Only in the "Discussion" section do we differentiate between cause and effect. As the main part of the study has an exploratory nature, we discuss not only statistically significant effects but also trends that did not achieve formal significance.

Results

Descriptive statistics

In total, 311 students participated in the evolutionary biology written examination, with over 95% of them consenting to partake in the subsequent anonymous study. Despite their initial agreement, some students either did not complete the questionnaire or hastily clicked through it, providing uniform answers to a majority of the questions. The finalized dataset encompassed information on COVID-19 experiences from 272 individuals, representing 87.5% of the students initially approached for the study. In total, 54 people reported no prior COVID-19 infection, 152 had experienced the infection, 53 people possibly had COVID-19 but had not received a formal diagnosis, and 13 had not had the virus but had undergone quarantine.

From the dataset, we excluded one notably older individual (a 43-year-old male who had COVID-19), as well as 53 individuals who reported possibly having COVID-19, but without a laboratory-confirmed diagnosis. The final dataset included 214 individuals, 66 who did not have COVID-19 and 148 (69.2%) who did. Among the 144 female students, 103 (71.5%) had experienced COVID-19, while among the 70 male students, 45 (64.3%) had; however, these differences were not statistically significant ($\chi^2 = 0.843$, df = 1, $P = .358$). The average age of all students, female students, and male students were 21.77 (SD = 1.61), 21.81 (SD = 1.71), and 21.70 (SD = 1.39), respectively (Fig. 1). The difference in age between men and women was non-significant ($t_{(165.23)} = 0.483$, $P = .630$), as was the difference in age between those who had (21.84) and had not (21.62) experienced COVID-19 ($t_{(161.78)} = -1.008$, $P = .315$).

Among the 148 subjects diagnosed with COVID-19, 15 (10.1%) reported "No symptoms," 52 (35.1%) described it as "Like mild flu," another 52 (35.1%) as "Like normal flu," and 29 (19.6%) as "Like severe flu"; none reported being "hospitalized" or "in ICU." Females reported a more severe course of COVID-19 than men (mean: 2.73 versus 2.44, median: 3 versus 2, Kruskal–Wallis $\chi^2_{(1)} = 4.04$, $P = .044$).

The average time since the beginning of COVID-19 was 13.41 months (SD = 8.47), 13.31 (SD = 8.24) in women and 13.63 (SD = 9.05) in men; this difference was not significant ($t_{74.172} = -0.197$, $P = .844$) (Fig. 1). The descriptive statistics for the dependent variables related to health and cognitive performance are presented in Supplementary Table S2.

The age of students did not exhibit any correlation with the likelihood of contracting COVID-19 (All: Tau = 0.024, $P = .600$, Women = 0.057, $P = .315$, Men: Tau = 0.058, $P = .478$) or the time elapsed since contracting the virus (All: Tau = 0.015, $P = .788$, Women: Tau = 0.010, $P = .881$, Men: Tau = 0.047, $P = .659$). On the other hand, it showed a positive correlation with the severity of

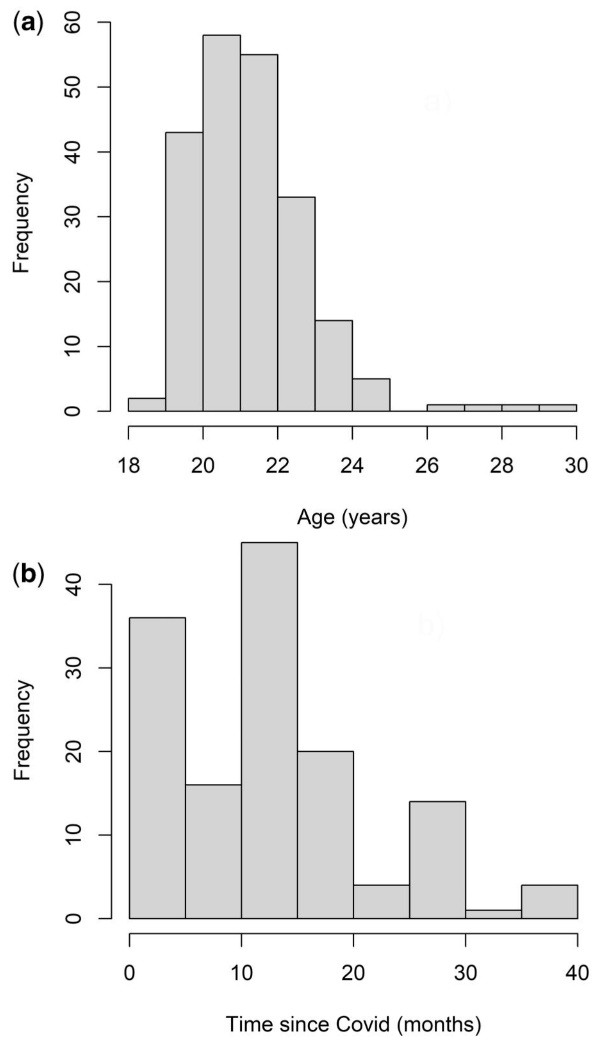

Figure 1. Distribution of age of participants and times since the beginning of COVID-19.

COVID-19 (All: Tau = 0.185, $P < .0001$, Women: Tau = 0.195, $P = .003$, Men: Tau = 0.131, $P = .212$). Furthermore, a negative correlation was found between the reported severity of COVID-19 and the time elapsed since the infection (All: Tau = −0.121, $P = .036$, Women: Tau = −0.086, $P = .215$, Men: Tau = −0.123, $P = .257$).

Effects of COVID-19 exposure, the severity of COVID-19, and time since contracting COVID-19 on health and performance

The associations between COVID-19 exposure, the severity of COVID-19, and time since contracting COVID-19 with health, performance, and personality metrics are presented in Table 1 (partial Kendall Taus) and Supplementary Table S3 (P-values). Merely contracting the infection significantly influenced the fatigue status of all participants (i.e. the mixed-sex group); however, this was not the case when participants were grouped by sex or when corrections for multiple tests were applied.

Figure 2 visualizes the impact of the severity of a COVID-19 infection on health, performance, and fatigue indices, broken down by sex. To provide context, the final two columns display these indices for individuals who have not contracted COVID-19. COVID-19 severity consistently demonstrated significant effects, impacting the physical health of all participants (i.e. the mixed-sex group) and each sex group separately. Additionally, it significantly influenced

Table 1. Effects of COVID-19 exposure, COVID-19 course, and time since COVID-19 on health, performance, and fatigue.

	Mixed sex			Women			Men		
	COVID N/Y	COVID Course	Since COVID	COVIDN/Y	COVID Course	Since COVID	COVID N/Y	COVID Course	Since COVID
Physical sickness score	0.056	**0.276***	−0.089	0.029	**0.257***	−0.030	0.107	**0.351***	**−0.227**
Mental sickness score	0.002	**0.158***	−0.024	−0.016	**0.206***	−0.006	0.027	−0.004	−0.060
Fatigue score	**0.103**	**0.177***	0.111	0.093	**0.175***	**0.143**	0.123	0.177	0.061
Error rate score	0.051	0.109	−0.086	0.000	0.100	−0.104	0.129	0.154	−0.067
Reaction time score	0.036	−0.077*	0.048	0.045	−0.126*	0.047	0.017	0.039	0.047
Source variables									
Cognitive performance									
Evolutionary biology test result	−0.043	0.102	0.090	0.043	0.101	0.094	**−0.177**	0.067	0.144
Intelligence	0.036	−0.020	0.074	**0.127**	−0.067	**0.144**	−0.096	0.043	−0.019
CRT	**−0.118**	−0.068	0.075	**−0.149**	−0.075	0.128	−0.043	−0.055	0.046
Recognition memory test	−0.009	−0.027	0.048	−0.026	0.018	0.066	0.015	−0.112	0.062
Free recall memory test	−0.020	−0.070	0.038	−0.048	−0.075	0.071	0.046	−0.068	0.014
Choice test accuracy	−0.076	**−0.123**	−0.076	NA	NA	NA	−0.145	**−0.244**	−0.148
Choice test reaction time	0.007	−0.013	−0.024	−0.001	−0.006	−0.058	0.016	0.027	−0.015
Stroop test accuracy	0.000	−0.082	0.070	−0.021	−0.034	0.045	0.021	**−0.256**	0.141
Stroop test reaction time	0.002	**−0.124**	0.062	−0.010	**−0.160**	0.055	0.025	−0.033	0.080
Stroop test reaction time first part	0.080	**−0.166**	0.078	**0.125**	**−0.170**	0.069	−0.009	−0.130	0.068
Stroop test reaction time second part	**−0.114**	−0.096	0.015	**−0.167**	**−0.144**	−0.012	−0.019	0.022	0.085
Stroop test reaction time third part	−0.020	−0.074	0.032	−0.060	−0.115	0.040	0.052	0.017	0.007
Reading time	**0.120**	−0.021	0.031	**0.187**	−0.110	0.031	−0.023	0.205	0.044
Physical health issues									
Allergies	0.044	**0.129**	−0.035	−0.028	0.080	0.003	**0.181**	**0.268**	−0.118
Skin disorders	0.009	**0.163**	**−0.155**	0.043	**0.196**	−0.078	−0.058	0.053	**−0.334**
Digestive tract disorders	0.009	**0.172**	−0.072	−0.033	**0.135**	−0.015	0.076	**0.277**	−0.191
Metabolic disorders	**0.114**	**0.137**	0.000	**0.166**	0.055	0.004	−0.042	**0.392**	0.008
Common infectious diseases	0.026	**0.322**	−0.068	0.091	**0.317**	−0.053	−0.127	**0.322**	−0.108
Orthopedic disorders	0.042	**0.143**	−0.098	−0.049	0.113	−0.092	**0.247**	0.197	−0.104
Neurological disorders	0.034	**0.155**	−0.013	−0.060	**0.169**	−0.023	**0.229**	0.084	−0.007
Headaches	0.083	**0.133**	−0.028	0.086	0.121	0.025	0.065	0.145	−0.152
Physical pains	0.021	0.107	−0.075	0.013	0.029	−0.001	0.037	**0.257**	−0.211
Chronic physical problems	0.081	**0.167**	−0.079	0.037	0.131	−0.031	**0.199**	**0.249**	−0.203
Antibiotics in the last year	0.035	**0.113**	−0.017	0.024	**0.154**	−0.014	0.061	0.015	−0.040
Antibiotics in the last 3 years	0.061	**0.117**	−0.044	0.062	**0.186**	−0.056	0.052	−0.074	−0.046
Doctor visits	−0.076	**0.196**	0.000	−0.088	**0.274**	−0.026	−0.090	0.068	0.052
Hospital visits in the past 5 years	−0.074	**0.141**	−0.070	**−0.139**	0.132	−0.083	0.097	0.202	−0.026
Prescribed drugs for physical health	0.027	0.031	0.003	0.087	0.022	0.044	−0.095	0.045	−0.121
Physical health disparity	**0.112**	0.100	−0.040	**0.219**	0.017	−0.015	−0.018	**0.241**	−0.094
Feeling physically unwell today	0.081	**0.166**	0.019	0.020	0.110	0.083	**0.214**	**0.257**	−0.110
Feeling physically unwell usually	0.042	**0.153**	−0.003	0.061	0.125	0.028	0.015	0.205	−0.063
Expected shorter lifespan	−0.001	0.088	0.035	−0.072	0.024	0.072	0.133	**0.254**	0.009

(continued)

Table 1. (continued)

	Mixed sex			Women			Men		
	COVID N/Y	COVID Course	Since COVID	COVIDN/Y	COVID Course	Since COVID	COVID N/Y	COVID Course	Since COVID
Mental health issues									
Depression	0.002	**0.131**	−0.026	0.006	**0.157**	0.009	−0.015	0.008	−0.088
Anxiety	−0.088	**0.206**	0.020	−0.088	**0.261**	0.051	−0.119	−0.002	−0.031
Phobia	−0.031	−0.045	−0.038	−0.056	−0.006	−0.058	0.021	−0.175	0.037
Obsession	0.025	0.027	**−0.144**	0.043	0.042	**−0.159**	−0.001	−0.033	−0.100
Other mental health problems	−0.075	**0.217**	0.027	−0.068	**0.271**	0.051	−0.102	0.011	−0.077
Prescribed drugs for mental health	0.062	**0.140**	−0.050	0.100	**0.149**	−0.087	−0.037	0.109	0.116
Mental health disparity	0.033	**0.188**	−0.019	0.034	**0.216**	0.001	0.013	0.115	−0.037
Feeling mentally unwell today	0.051	**0.199**	0.060	−0.015	**0.209**	0.046	**0.168**	0.105	0.097
Feeling mentally unwell usually	0.079	0.098	0.035	0.050	**0.151**	0.019	0.120	−0.061	0.077
Fatigue									
Tired usually	**0.102**	**0.191**	0.067	0.105	**0.180**	0.057	0.090	0.212	0.094
Tired now	0.067	**0.235**	0.055	0.026	**0.277**	0.055	**0.169**	0.115	0.041
Tired after work	0.082	0.092	**0.120**	**0.114**	0.059	**0.149**	0.017	0.174	0.050
Feeling tired after bus travel	0.082	0.109	0.068	0.064	0.083	0.108	0.107	0.170	−0.016
Feeling tired after train travel	**0.157**	**0.115**	0.105	0.105	0.132	**0.140**	**0.246**	0.074	0.039

This table presents the direction and strength of specific effects (Taus). A positive Tau indicates a positive association between the COVID-19-related variable (for instance, COVID-19 exposure coded as 0 for "no" and 1 for "yes") and worse health. Taus that are statistically significant ($P < .05$) are highlighted in bold. Results for indices (presented in the first five rows) that remained significant even after applying correction for multiple (five) tests are marked with asterisks, while, the results for the 46 source variables (as seen in the remainder of the table) that remained significant even after the correction for multiple (46) tests are underlined. *Note*: As all women scored 100% in the Choice test, statistical analysis was not applicable, and the corresponding cells are marked "NA" (Not Applicable).

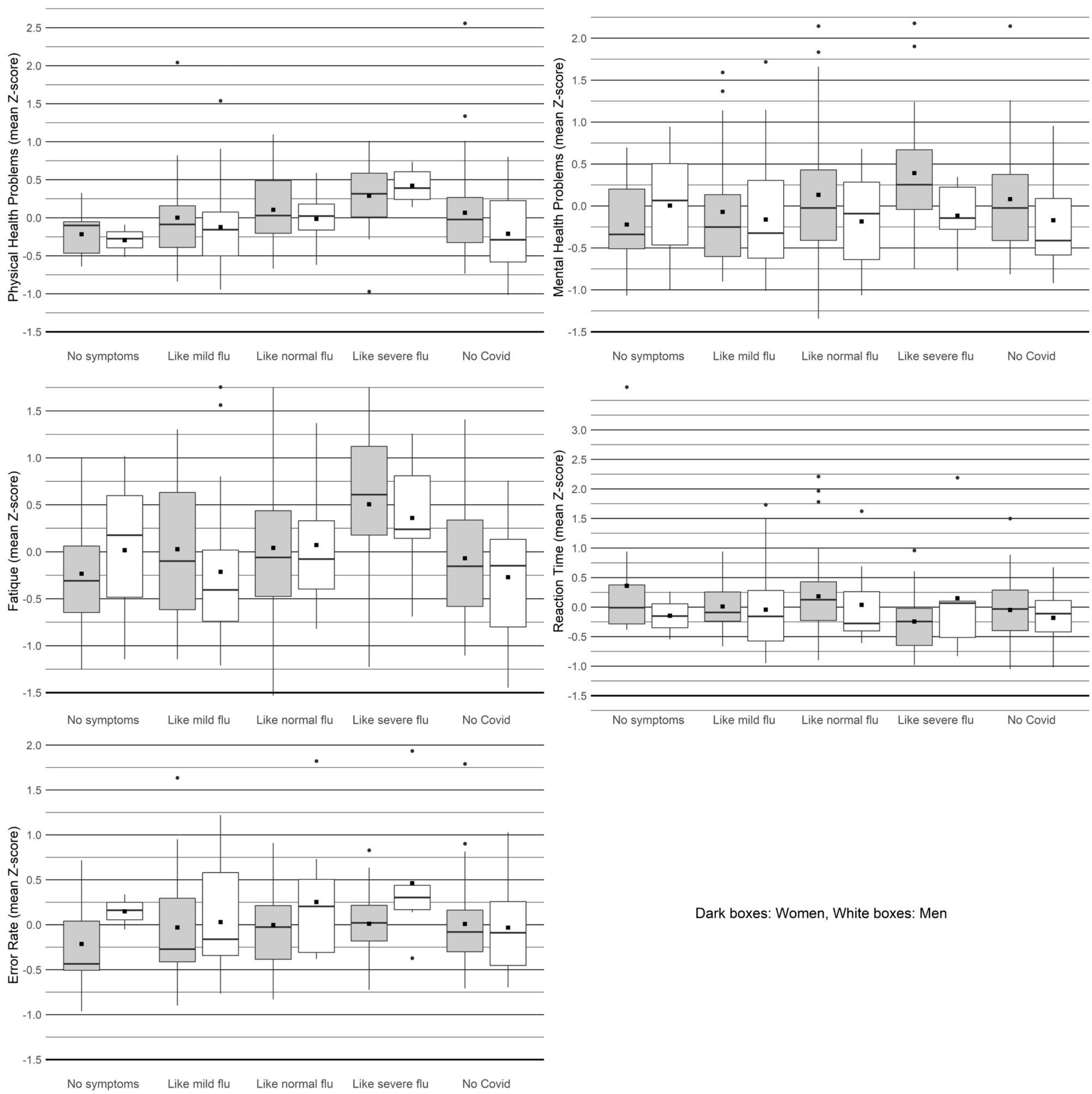

Figure 2. Effects of severity of COVID-19 (course) on five health and performance-related indices. The figure displays boxplots representing the distribution of five health and performance-related indices across various categories of COVID-19 severity for both men and women. Each box encompasses the interquartile range (IQR), with a line inside the box indicating the median. The whiskers extend beyond the box to illustrate the range of variability (95% Confidence Intervals [CIs]), and black squares denote the mean scores for each index.

mental health, fatigue status, and reaction times for all participants and females, but not for males.

In our study, the time elapsed since the onset of COVID-19 infection showed modest effects on both health and cognitive performance metrics. Specifically, we observed an improvement in physical health among males, while females experienced an exacerbation of fatigue levels. However, these changes were not statistically significant after applying the Benjamini–Hochberg correction for multiple testing. Consequently, during the 3-year follow-up period post-infection, we did not detect the anticipated recovery, defined as the disappearance or substantial reduction of COVID-19 symptoms. Fig. 3 and especially Supplementary Fig. S1 offer a potential explanation for these findings: the effects of COVID-19 appear to diminish in the first 2 years following infection, only to intensify subsequently. This pattern is particularly evident in the context of fatigue but is also noticeable in mental health outcomes and performance error rates. For an alternative interpretation of these observed trends, please refer to the "Discussion" section.

To further investigate the pattern suggested in Fig. 3 and Supplementary Fig. S1—that the outcomes related to COVID-19 first show improvement and then deterioration after 2 years—we conducted separate correlation analyses for two different time frames: those who contracted the virus less than 2 years ago and those who contracted it at least 2 years ago. For individuals who contracted the virus less than 2 years ago, we observed non-

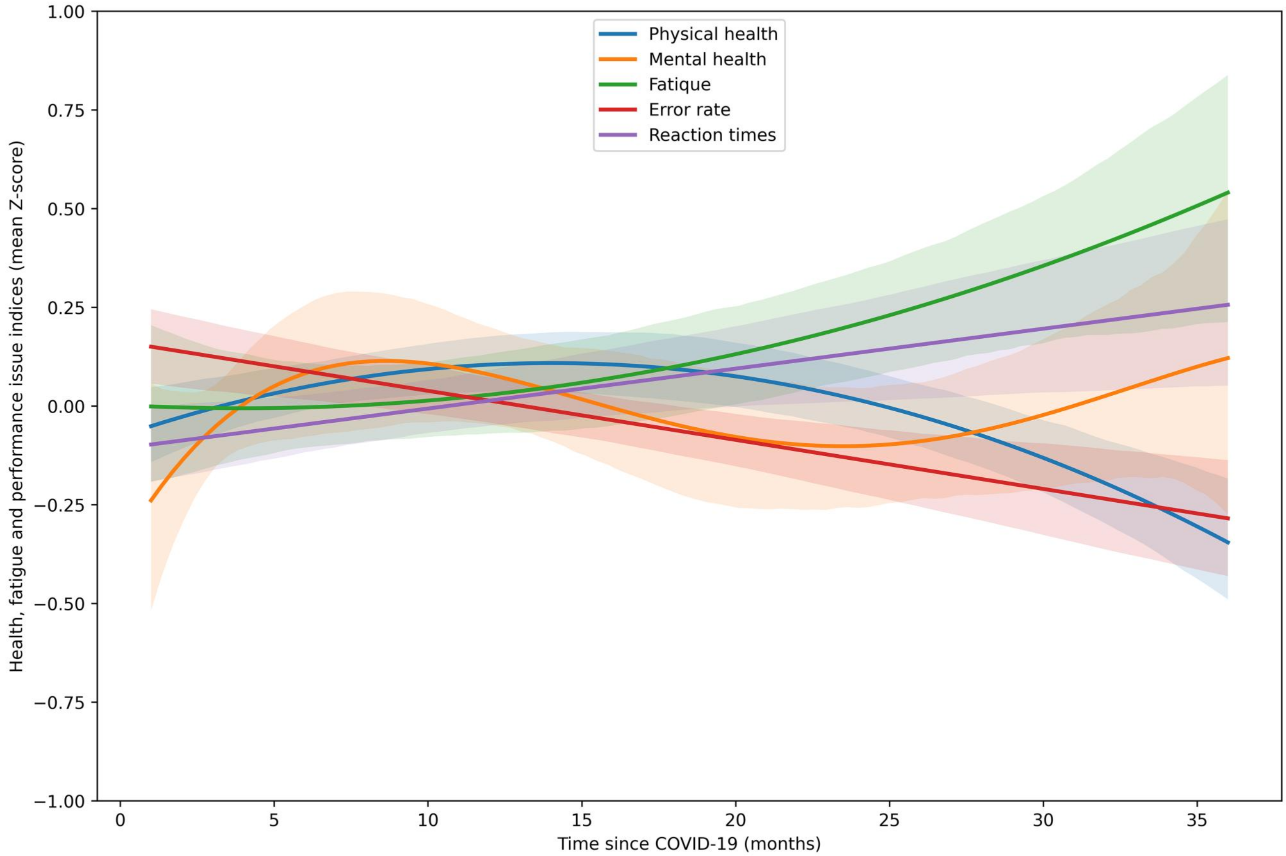

Figure 3. The correlation between the time since COVID-19 and five health and performance-related indices. This scatterplot illustrates the relationships between five health- and performance-related variables and the time elapsed since contracting COVID-19. To visualize the trends, the points for each variable are fitted by polynomial curves, chosen based on comparisons of Adjusted R-Squared values. Specifically, a second-degree polynomial curve is fitted for Physical Health, a fourth-degree polynomial curve for Mental Health, a second-degree polynomial curve for Fatigue, a first-degree polynomial curve for Error Rate, and a first-degree polynomial curve for Reaction Times. (For the figure where data for all variables were fitted with the third-degree polynomial curve, see Supplementary Fig. S1). Higher values on the y-axis indicate worse health and performance. The bands around the lines represent 80% CIs.

significant decreases in the impact of COVID-19 on physical health (Tau = −0.04, P = .52) and mental health (Tau = −0.04, P = .54), and error rates (Tau = −0.05, P = .41). Simultaneously, a non-significant increase was observed in fatigue (Tau = 0.07, P = .24) and reaction time (Tau = 0.43, P = .49). In women, positive trends between the time elapsed since COVID-19 infection onset (less than 2 years) and physical health (Tau = 0.050, P = .51), mental health (0.044, P = .56), and reaction time (0.035, P = .63), with fatigue being significantly affected (Tau = 0.158, P = .039), and also a negative trend with error rates (Tau = −0.055, P = .46) were observed. In men, the indices of physical health (Tau = −0.250, P = .036) and mental health (Tau = −0.250, P = .037) showed a significant decrease, fatigue, and error rates demonstrated a negative trend (Tau = −0.083, P = .49, Tau = −0.056, P = .63, respectively), and reaction time proved a positive trend (Tau = 0.058, P = .62).

For the 22 subjects who contracted COVID-19 at least 2 years prior, all indices—with the exception of physical health, showing a negative trend (Tau = −0.223, P = .16)—demonstrated positive trends over the duration since their initial contraction of the virus—mental health (Tau = 0.094, P = .56), fatigue (Tau = 0.240, P = .15), error rate (Tau = 0.099, P = .55), and reaction time (Tau = 0.069, P = .67). In this regard, female participants demonstrated negative trends in physical and mental health (Tau = −0.244, P = .22, Tau = −.030, P = .88, respectively). Furthermore, trends were positive for fatigue (Tau = 0.140, P = .48), error rates (Tau = 0.010, P = .95), and reaction time (0.214, P = .28) in this group. Taking into account the male participants, the trends were pronounced for the improvement of physical health (Tau = −0.382, P = .28) and reaction time (Tau = −0.22, P = .53), as well as the deterioration of mental health (Tau = 0.27, P = .43), fatigue (Tau = 0.475, P = .24), and error rate (Tau = 0.052, P = .88). Given the small sample size of only seven men infected for more than 2 years, the absence of statistical significance was not surprising.

Discussion

Our study explores the enduring impacts of COVID-19 on health, fatigue, and cognitive performance among university students. In this population, having had COVID-19 did not significantly impact health and performance; however, individuals who contracted COVID-19 reported increased fatigue. On the other hand, the severity of COVID-19 significantly and negatively influenced physical health (in all, women, and men), mental health (in all and women), fatigue (in all and women), and reaction time (in all and women). The progression of all five indices under study relative to the time elapsed since COVID-19 infection suggests that trends of improvement in physical health, mental health, and error rate are observed for all participants in the first 2 years post-infection; however, fatigue and reaction time demonstrate trends of deterioration. Thereafter, physical health and error rate continue their marginal trend of improvement, while mental health worsens. Fatigue intensifies and reaction times extend (deteriorate) marginally. In the case of women, during the first 2 years post-infection, their physical health, mental health, and reaction time show a trend of deterioration, but the error rate exhibits a marginal trend of improvement. It is noteworthy that fatigue significantly deteriorates for these participants in this period. Afterward, physical and mental health show a trend of improvement, fatigue, and reaction time keep declining, and the error rate marginally deteriorates. Nonetheless, for men during the first 2 years post-infection, mental and physical health significantly improve, fatigue and error rate demonstrate a trend of improvement, and reaction time deteriorates. Thereafter, physical health maintains a trend of improvement, but mental health, fatigue, and error rate show a trend of deterioration, and reaction time marginally improves.

The minimal or absent impact of merely contracting the infection on physical and mental health and cognitive performance, which contrasts with results of many already published studies, e.g. [1, 28–36], could be related to the fact that all students included in our study were younger than 31 years old (95% were 20–24 years old). As is often the case with such a young population, most students experienced a relatively mild form of COVID-19; only about 20% described it as “severe flu,” and none of the study participants was hospitalized due to COVID-19. However, it is noteworthy that even in our young sample, experiencing a non-hospitalized case of COVID-19 still resulted in elevated fatigue levels and non-significant trends of health and performance deterioration. As demonstrated in the previous study conducted using a nearly identical method [35], the impact of enduring a COVID-19 case on physical health is much more pronounced and significant in an older population (mean age >42 years).

Our results align with previous studies that have identified a correlation between COVID-19 severity and subsequent declines in physical health [35, 37–39], mental health challenges [7, 40, 41], and cognitive deficits [8, 42, 43]. Collectively, these findings suggest that individuals with a history of more severe COVID-19 symptoms typically face more pronounced post-infection complications.

In our partial correlation tests, we did not observe a consistent change in health and performance symptoms over time following infection. The exceptions were a decrease in symptoms of impaired physical health among men and an increase in fatigue among women. This finding appears to contrast with many anecdotal observations and numerous published studies, which suggest that symptoms generally lessen with time since infection for most individuals [44, 45], although in some cases they may stay constant or intensify [46–48]. Visual inspection of Fig. 3 and especially Supplementary Fig. S1, as well as separate analyses for our two groups of participants, namely, those who contracted the virus less than 2 years ago and those who contracted it at least 2 years ago suggest that these inconsistencies may be attributed to a non-monotonic trajectory of changes as time since infection progresses, as well as to insufficient follow-up time after the illness. Some symptoms likely only emerged in the weeks and months after infection (COVID-19 broke out in 16 individuals 3 or fewer months before the study began). Other symptoms could decrease and disappear in the following months, only to reappear or start worsening more than 23 months after infection. However, it is important to keep in mind that our study had a cross-sectional design. This means it may be subject to a cohort effect, where individuals infected longer were exposed to different virus variants than those infected more recently. Thus, the time since infection can overlap with the specific variant of the virus that caused the infection. It is also likely that, in Czechia, young individuals infected in 2020 and the first half of 2021 had not received the COVID-19 vaccine prior to contracting the virus. Therefore, stronger symptoms in individuals with a long time interval since infection may not be the result of gradual symptom intensification over time but rather the result of individuals being infected with different, more virulent, SARC-CoV-2 variants, or not being vaccinated before getting sick. However, our findings also show a negative relationship between the time elapsed since someone had COVID-19 and the severity of the illness they reported. This contradicts the assumption that the longer it has been since someone encountered COVID-19, the more virulent the strain of the virus they encountered and the more severe the course of the disease they experienced.

Post-hoc analysis on the effects of COVID-19 on specific variables used for index computation unveiled intriguing differences across sexes. Following infection, men tended to score lower on the evolutionary biology knowledge test, whereas women exhibited poorer performance on the CRT. A unique aspect of our research was the inclusion of the Reading Time test, which measured the speed at which students read the instructions for each test. This was the only test where students were not explicitly aware they were being tested. As such, factors like competitiveness were less likely to influence the results, while urgency or haste may have played a role. In this test, women who had contracted COVID-19 took noticeably longer to read the instructions compared to their non-infected counterparts. No such trend was observed in men. However, among men, an increase in reading time was associated with a more severe COVID-19 experience; this association approached statistical significance ($\mathrm{Tau} = 0.205$, $P = .053$). The observed patterns likely suggest that individuals who have recovered from COVID-19 may experience subtle cognitive impairments, which, however, might be compensated for by increased effort in scenarios where they are aware of being tested. Future research can specifically focus on such research designs that allow researchers to investigate this issue further and more thoroughly, particularly with an emphasis on the role of biological sex. No significant association was observed between memory test outcomes and the examined COVID-19-related variables for all participants, males, or females.

This result contrasts with anecdotal observations and the majority of published studies investigating the impact of COVID-19 on memory [9]. However, other studies also failed to demonstrate an effect of COVID-19 on memory test performance [8, 10]. In our recent study, which utilized the same memory test as employed in this current research, we observed that individuals who had experienced COVID-19 demonstrated significantly better memory test performance compared to those who had not contracted the virus [35]. Future research should focus on examining various memory components in the same individuals to better understand these discrepancies.

Interestingly, our data showed that women who experienced more severe COVID-19 symptoms demonstrated faster reaction times in the Stroop test. This effect did not appear even remotely in a simpler Choice Reaction Time test, where participants were only required to click a specific button on the screen out of four possible choices. Women who had recovered from an infection also scored higher in a concise 12-item intelligence test. While these effects were not observed in men, those who had contracted COVID-19 made fewer errors on the evolutionary biology test compared to men who had not been infected.

These intriguing results could possibly be linked to a “resilience effect,” where the process of overcoming a substantial health hurdle might unintentionally boost certain cognitive abilities [49]. Prior research has demonstrated, for instance, that mild stress can improve performance on non-declarative memory tests [50]. Additionally, stress, inclusive of chronic infection-related stress, can lead to a reduction in the personality trait of conscientiousness [51, 52]. This is relevant as high conscientiousness has been found to negatively influence performance on specific cognitive tests [53], possibly because of a tendency toward overcaution or overthinking. The observation in our study of the positive impact of having experienced COVID-19 on women's performance in an intelligence test and men's performance in an evolutionary biology examination necessitates further exploration, given that a variety of confounding factors could have potentially influenced this outcome.

Regarding health-related variables, women who had experienced COVID-19 reported a higher prevalence of metabolic diseases and perceived their physical health as inferior compared to their peers. At the same time, these women reported fewer hospital visits. This observation might be due to the artifact of conducting multiple tests. Setting the FDR to 0.1 implies that we expect 10% of the positive results to be false positives. Men who experienced COVID-19 recounted a more frequent occurrence of allergies, orthopedic issues, neurological problems, and other long-term physical conditions, and they also reported feeling unwell, both physically and mentally. Although these effects were all comparatively strong, only the impact of COVID-19 on orthopedic issues remained significant after adjusting for multiple testing. Accordingly, examining the source variables results corrected for type I errors, our findings regarding "orthopedic disorders" in males and "metabolic disorders" and "physical health disparity" in females were in agreement with those studies that observed deteriorated physical health in these areas in post-COVID19 patients (for reviews on orthopedic disorders, see [54] and [55] and for a review on metabolic disorders, see [56]).

With regard to cognitive functions, our study's findings on females' reading time scores are consistent with earlier studies that have found an association between COVID-19 infection and impairment in information processing speed [35, 57, 58]. However, in contrast to these studies, we discovered a significant negative correlation between COVID-19 infection and women's reaction times in the Stroop test. This suggests that infected women had improved reaction times compared to those who were not infected. The reason for this latter finding remains to be understood; nevertheless, there is a pattern in our findings that points to a hypothetical explanation. As our female participants progressed through the Stroop test, their reaction times showed notable improvement. This suggests that their performance was predominantly influenced by learning. In contrast, the male participants exhibited a slight but discernible trend toward slower reaction times. This indicates that their performance was more influenced by fatigue. Consequently, the magnitude and direction of COVID-19's impact on reaction times depend on the duration of the specific test employed in a study and the proportion of men and women in the sample examined.

Across the entire cohort, the severity of COVID-19 exhibited a significant correlation with almost all physical and mental health-related variables, even after adjusting for multiple tests. For women, a strong correlation of the severity of COVID-19 was observed with the incidence of common infectious diseases and the frequency of antibiotic use, both of which are proxies for immune deficiencies. Additionally, there were notable correlations with the number of visits to the general practitioner, the frequency of skin and neurological diseases, anxiety, depression, and other mental health issues. Women also reported a higher amount of medication use for mental disorders, rated their health lower compared to their peers, and felt more mentally unwell.

In men, the severity of COVID-19 showed a particularly robust correlation with the frequency of metabolic disorders (Tau = 0.392) and with the frequency of common infectious diseases (Tau = 0.322). However, it also significantly correlated with allergies, gastrointestinal diseases, the frequency of physical pain experiences, and the frequency of other long-term physical issues. Men with more severe COVID-19 cases rated their physical health as worse compared to that of their peers. They reported feeling physically unwell, both currently and typically, and believed their lifespan would be shorter. Although no correlation reached statistical significance for men's mental health, likely due to the small sample size of only 45 men who had contracted COVID-19, certain correlations were relatively strong, with Taus >0.1. Specifically, the severity of COVID-19 in men demonstrated a notable correlation with the number of various types of medications currently being taken for mental health issues, their comparison of mental health issues to those of their peers, and their present state of feeling mentally unwell.

Over time following infection, there was a general improvement in health status across nearly all variables. However, the correlations between health-related variables and the time elapsed since infection were relatively low and not statistically significant. For men, these negative correlations were stronger, particularly in relation to skin problems (Tau = –0.334), but also allergies, gastrointestinal complications, orthopedic issues, the frequency of common infectious diseases, headaches, other physical discomforts, and other long-term physical problems. The number of different types of medications currently taken for physical problems, how they rated their physical health compared to their peers, and how they felt physically unwell both today and usually, all declined with time since COVID-19. On the contrary, positive correlations emerged for some mental health-related variables, indicating a potential increase in issues over time following infection. This was the case for the number of types of prescribed medications currently taken for mental problems, feeling mentally unwell today, and usually feeling mentally unwell. Despite the relative strength of these trends, none reached statistical significance among men. Among women, these trends were weaker. For three variables, namely phobias, the number of different types of medications currently taken for mental problems, and especially the incidence of obsessions, the values even decreased over time since the COVID-19 infection. In this regard, our findings are aligned with studies that found a trend of improvement in the physical health of post-COVID-19 patients over time, for example [59]. Deterioration of mental health conditions over time in post-COVID-19 patients is also reported in earlier studies, for example [10, 60].

All the source variables for fatigue positively correlated with having had COVID-19, the severity of COVID-19 and the elapsed time since infection. The strongest correlation was observed with the severity of COVID-19, wherein the relationship was significant for four out of the five examined variables. The most prominent correlation was the response to the question of how tired the participant feels at the moment (Tau = 0.235, women: Tau = 0.277, men: Tau = 0.115). For men, the strongest relationship was observed between having had COVID-19 and experiencing fatigue after a long train journey (Tau = 0.246) and feeling tired at the present moment (Tau = 0.169). Traveling by train and taking a test in evolutionary biology are not physically demanding. This suggests that post-COVID fatigue may be more related to mental exertion than to strenuous physical activity. However, this is merely a post-hoc explanation of the observed pattern, and therefore, it needs to be verified in future studies.

Perhaps, the most concerning finding was that fatigue does not diminish but intensifies over time since having had COVID-19. This upward trend was statistically significant in the case of fatigue experienced after work (for both the entire cohort and women) and after a long train journey (for women). Our results diverged from those studies that reported decreasing levels of fatigue over time in COVID-19 patients [61–63]. However, they align with the findings of a study that observed a trend of increasing fatigue over months 1, 3, 6, and 12 following the onset of COVID-19 infection [64]. The discrepancies in findings could result from differences in study design and, importantly, variations in the duration over which the respective changes were monitored. For instance, one study noted an inverse trend in fatigue levels related to disease severity when comparing two assessments of the same COVID-19 patients conducted approximately 4 months apart. While the initial assessment showed a positive correlation between COVID-19 severity and fatigue, the follow-up indicated a negative correlation [65]. Another study documented a nonlinear progression of fatigue levels throughout the disease's trajectory: fatigue peaked during its acute phase, then decreased and stabilized around months 5 and 9, only to rise again at month 12 [66].

Students on the higher end of our age spectrum reported experiencing a more severe course of the disease. This was rather unexpected, considering the relatively young age of all students (all under 31 years old) and the narrow age range of the study participants. This correlation was stronger (Tau = 0.195) and significant among female students. The correlation was not significant among male students. Nevertheless, even in the case of men, the Kendall Tau value was observed to be 0.131, which corresponds to a Pearson's r value of 0.16. This is generally considered a moderate correlation in the context of biopsychological research, rather than a weak one.

Strengths and limitations

A key strength of our study is the comprehensive and representative sample of biology students in Prague. The high participation rate ensures that our findings accurately represent this specific group. Moreover, the homogeneity of the sample, which arises from the students' shared academic and likely socio-economic backgrounds, minimizes variability in potential confounding variables, thereby enhancing the study's analytical precision. This uniformity also allows even subtle effects to be more discernible. However, this same homogeneity does pose a limitation: it narrows the scope of our findings' applicability.

Importantly, participants were kept unaware of the study's focus on COVID-19 not only at the outset but also throughout the questionnaire's duration (which received IRB approval). This strategy of incorporating COVID-19-related questions into the survey without explicit disclosure reduced response bias tied to pre-existing attitudes or beliefs about the virus. Moreover, the wide range of topics covered in the study would have made it unlikely for participants to deduce that COVID-19 was a key focus. These measures ensure the accuracy and representativeness of our data, thereby enhancing the validity of our findings.

Indeed, this study also bears certain limitations. First, while the cognitive test performances were objectively measured, our reliance on self-reported data specifically for participants' health status could introduce inaccuracies due to recall bias or subjective perceptions. The participants' recollections of their symptoms and their personal assessments of health may not perfectly reflect their actual medical conditions, potentially skewing those aspects of our results.

Second, the cross-sectional design of this study makes it difficult to distinguish between the effects of time since contracting COVID-19 and potential cohort effects, which may arise from different cohorts being infected by various strains of the SARS-CoV-2 virus. Furthermore, this design challenges our understanding of causality. At first glance, the observed correlation between the time since infection and fatigue might appear primarily as a result of either the virus's cumulative impact or the cumulative effect of organ damage caused by the virus during the COVID-19 illness. However, it is also conceivable that both the time since infection and fatigue might be influenced by a third variable, such as the psychological impact of the pandemic or the evolution and succession of virus strains mentioned earlier. Hence, future longitudinal studies are an absolute necessity to conclusively establish any causal relationships.

We controlled for the effects of sex, age, and survey year. However, we did not control for many other confounding variables, including the time of day when the data were collected [67]. The results of Monte Carlo modeling showed that uncontrolled confounding variables introduce stochastic noise into the data, which may increase the risk of false-negative results but not false-positive results in statistical tests [68].

Conclusions

The primary insight from our study is the recognition that the consequences of contracting COVID-19 can persist for a prolonged period and may even worsen over time, including in younger individuals who are generally deemed more resistant to the virus. The likelihood and severity of these persistent effects are associated with the initial severity of the COVID-19 infection, even among individuals who experienced a mild case not requiring hospitalization. While the physical health sequelae of COVID-19 tend to diminish within the first 3 years following infection, this trend does not apply to all consequences of the virus. One of the most significant findings from our study is that fatigue levels progressively increase with time elapsed since infection during the first 3 years, that is across the entire period covered by our study. Consequently, it appears likely that fatigue is not just a result of general and transient health deterioration, but rather a specific and previously uncharacterized manifestation of COVID-19.

In summary, our study underscores that many critical aspects of the pandemic, especially the long-term effects of the disease, remain inadequately researched and should warrant far greater scientific focus than currently accorded.

Funding

This research was supported by the Czech Science Foundation, grant number 22-20785S. Our sponsor had no involvement in the study design, the collection, analysis, and interpretation of data, the writing of the report, or in the decision to submit the article for publication.

Authors' contributions

Ashkan Latifi (Formal analysis [equal], Investigation [equal], Methodology [equal], Validation [equal], Writing—review & editing [equal]), and Jaroslav Flegr (Conceptualization [equal], Formal analysis [equal], Investigation [equal], Methodology [equal], Project administration [equal], Supervision [equal], Validation [equal], Writing—original draft [equal], Writing—review & editing [equal])

Data availability statement

All data are available in the public repository figshare 10.6084/m9.figshare.24032700.v1.

Conflicts of interest statement

The authors declare no competing interests.

Supplementary data

Supplementary data is available at *Biology Methods and Protocols* online.

References

1. Lamontagne SJ, Winters MF, Pizzagalli DA, Olmstead MC. Post-acute sequelae of COVID-19: evidence of mood & cognitive impairment. *Brain Behav Immun Health* 2021;**17**:100347.
2. González-Hermosillo JA, Martínez-López JP, Carrillo-Lampón SA *et al.* Post-acute COVID-19 symptoms, a potential link with myalgic encephalomyelitis/chronic fatigue syndrome: a 6-month survey in a Mexican cohort. *Brain Sci* 2021;**11**:760.
3. Fernández-de-Las-Peñas C, de-la-Llave-Rincón AI, Ortega-Santiago R *et al.* Prevalence and risk factors of musculoskeletal pain symptoms as long-term post-COVID sequelae in hospitalized COVID-19 survivors: a multicenter study. *Pain* 2022;**163**:e989–e996.
4. Zawilska JB, Kuczyńska K. Psychiatric and neurological complications of long COVID. *J Psychiatr Res* 2022;**156**:349–60.
5. Santos RAG, Rodríguez MSR, Hernandez D. S. L. & Neurological manifestations in COVID-19 survivors: Observations in one of the largest only COVID-19 centers in México. (P5-10.003). *2023 AAN Annual Meeting Scientific Abstracts* P5-10.003, American Academy of Neurology, Minneapolis, Minnesota, 2023.
6. Tsuzuki S, Miyazato Y, Terada M *et al.* Impact of Long-COVID on health-related quality of life in Japanese COVID-19 patients. *Health Qual Life Outcomes* 2022;**20**:125.
7. Zeng N, Zhao YM, Yan W *et al.* A systematic review and meta-analysis of long term physical and mental sequelae of COVID-19 pandemic: call for research priority and action. *Mol Psychiatry* 2023;**28**:423–33.
8. Ariza M, Cano N, Segura B et al.; NAUTILUS Project Collaborative Group. COVID-19 severity is related to poor executive function in people with post-COVID conditions. *J Neurol* 2023;**270**:2392–408.
9. Tavares-Júnior JWL, de Souza ACC, Borges JWP *et al.* COVID-19 associated cognitive impairment: a systematic review. *Cortex* 2022;**152**:77–97.
10. Houben-Wilke S, Goërtz YM, Delbressine JM *et al.* The impact of Long COVID-19 on mental health: observational 6-month follow-up study. *JMIR Ment Health* 2022;**9**:e33704.
11. Sobrino-Relaño S, Balboa-Bandeira Y, Peña J *et al.* Neuropsychological deficits in patients with persistent COVID-19 symptoms: a systematic review and meta-analysis. *Sci Rep* 2023;**13**:10309.
12. Amalakanti S, Arepalli KVR, Jillella JP. Cognitive assessment in asymptomatic COVID-19 subjects. *VirusDisease* 2021;**32**:146–9.
13. Papazacharias A, Nardini M. & The relationship between depression and cognitive deficits. *Psychiatr Danub* 2012; **24**:S179–182.
14. Gualtieri CT, Morgan DW. The frequency of cognitive impairment in patients with anxiety, depression, and bipolar disorder: an unaccounted source of variance in clinical trials. *J Clin Psychiatry* 2008;**69**:1122–30.
15. Poletti S, Palladini M, Mazza MG *et al.*; COVID-19 BioB Outpatient Clinic Study group. Long-term consequences of COVID-19 on cognitive functioning up to 6 months after discharge: role of depression and impact on quality of life. *Eur Arch Psychiatry Clin Neurosci* 2022;**272**:773–82.
16. Malesevic S, Sievi NA, Baumgartner P *et al.* Impaired health-related quality of life in long-COVID syndrome after mild to moderate COVID-19. *Sci Rep* 2023;**13**:7717.
17. Méndez R, Balanzá-Martínez V, Luperdi SC *et al.* Long-term neuropsychiatric outcomes in COVID-19 survivors: a 1-year longitudinal study. *J Intern Med* 2022;**291**:247–51.
18. Fernández-de-Las-Peñas C, Martín-Guerrero JD, Cancela-Cilleruelo I *et al.* Trajectory curves of post-COVID anxiety/depressive symptoms and sleep quality in previously hospitalized COVID-19 survivors: the LONG-COVID-EXP-CM multicenter study. *Psychol Med* 2022;**53**:4298–9.
19. Sampogna G, Di Vincenzo M, Giallonardo V *et al.* The psychiatric consequences of Long-COVID: a scoping review. *J Pers Med* 2022; **12**:1767.
20. Cattell RB. *Handbook for the Sixteen Personality Factors Questionnaire* (16PF)**. Vol. 1** Carmel, IN: Institute for Personality and Ability Testing, 1970.
21. Meili R. *Lehrbuch Der Psychologischen Diagnostik*. Bern, Switzerland: Verlag Hans Huber, 1961.
22. Flegr J, Hampl R, Černochová D *et al.* The relation of cortisol and sex hormone levels to results of psychological, performance, IQ and memory tests in military men and women. *Neuroendocrinol Lett* 2012;**33**:224–35.
23. Frederick S. Cognitive reflection and decision making. *J Econ Perspect* 2005;**19**:25–42.
24. Flegr J. Toxoplasmosis is a risk factor for acquiring SARS-CoV-2 infection and a severe course of COVID-19 in the Czech and Slovak population: a preregistered exploratory internet cross-sectional study. *Parasit Vectors* 2021;**14**:508.
25. Flegr J, Flegr P. Doing exploratory analysis in R with a package Explorer v. 1.0. *Figshare*, Digital Science, London, 2021. 10.6084/m9.figshare.14685825.v1.
26. Kim S. ppcor: an R package for a fast calculation to semi-partial correlation coefficients. *Commun Stat Appl Methods* 2015; **22**:665–74.
27. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J Roy Stat Soc B Met* 1995;**57**:289–300.
28. Zhao YM, Shang YM, Song WB *et al.* Follow-up study of the pulmonary function and related physiological characteristics of COVID-19 survivors three months after recovery. *EClinicalMedicine* 2020; **25**:100463.
29. Lu Y, Li X, Geng D *et al.* Cerebral micro-structural changes in COVID-19 patients—an mri-based 3-month follow-up. *EClinicalMedicine* 2020;**25**:100484.
30. Havervall S, Rosell A, Phillipson M *et al.* Symptoms and functional impairment assessed 8 months after mild COVID-19 among health care workers. *JAMA* 2021;**325**:2015–6.
31. O'Mahoney LL, Routen A, Gillies C *et al.* The prevalence and long-term health effects of Long Covid among hospitalised and

non-hospitalised populations: a systematic review and meta-analysis. *EClinicalMedicine* 2023;**55**:101762.
32. Marchi M, Grenzi P, Serafini V *et al.* Psychiatric symptoms in Long-COVID patients: a systematic review. *Front Psychiatry* 2023;**14**:1138389.
33. Altuna M, Sánchez-Saudinós MB, Lleó A. Cognitive symptoms after COVID-19. *Neurol Perspect* 2021;**1**:S16–S24.
34. Delgado-Alonso C, Valles-Salgado M, Delgado-Álvarez A *et al.* Cognitive dysfunction associated with COVID-19: a comprehensive neuropsychological study. *J Psychiatr Res* 2022;**150**:40–6.
35. Flegr J, Latifi A. The lasting legacy of covid-19: exploring the long-term effects of infection, disease severity, and vaccination on health and cognitive function. *Biol Meth Prot* 2023. https://doi.org/10.1093/biomethods/bpad038.
36. Mazza MG, De Lorenzo R, Conte C et al.; COVID-19 BioB Outpatient Clinic Study group.Anxiety and depression in COVID-19 survivors: role of inflammatory and clinical predictors. *Brain Behav Immun* 2020;**89**:594–600.
37. Iqbal A, Iqbal K, Arshad Ali S *et al.* The COVID-19 sequelae: a cross-sectional evaluation of post-recovery symptoms and the need for rehabilitation of COVID-19 survivors. *Cureus* 2021;**13**:e13080.
38. Han Q, Zheng B, Daines L, Sheikh A. Long-term sequelae of COVID-19: a systematic review and meta-analysis of one-year follow-up studies on post-COVID symptoms. *Pathogens* 2022;**11**:269.
39. Mizrahi B, Sudry T, Flaks-Manov N *et al.* Long covid outcomes at one year after mild SARS-CoV-2 infection: nationwide cohort study. *BMJ* 2023;**380**:e072529.
40. Putri C, Arisa J, Hananto JE *et al.* Psychiatric sequelae in COVID-19 survivors: a narrative review. *World J Psychiatry* 2021;**11**:821–9.
41. Shanbehzadeh S, Tavahomi M, Zanjari N *et al.* Physical and mental health complications post-COVID-19: scoping review. *J Psychosom Res* 2021;**147**:110525.
42. Miskowiak KW, Johnsen S, Sattler SM *et al.* Cognitive impairments four months after COVID-19 hospital discharge: pattern, severity and association with illness variables. *Eur Neuropsychopharmacol* 2021;**46**:39–48.
43. Guo P, Benito Ballesteros A, Yeung SP *et al.* COVCOG 1: factors predicting physical, neurological and cognitive symptoms in long covid in a community sample. a first publication from the COVID and cognition study. *Front Aging Neurosci* 2022;**14**:804922.
44. Tassignon B, Radwan A, Blommaert J *et al.* Longitudinal changes in global structural brain connectivity and cognitive performance in former hospitalized COVID-19 survivors: an exploratory study. *Exp Brain Res* 2023;**241**:727–41.
45. Poole-Wright K, Guennouni I, Sterry O *et al.* Fatigue outcomes following COVID-19: a systematic review and meta-analysis. *BMJ Open* 2023;**13**:e063969.
46. Lucette AC, Jakabek D, Bracken SG *et al.* The kynurenine pathway relates to post-acute COVID-19 objective cognitive impairment and PASC. *Ann Clin Transl Neurol* 2023;**10**:1338–52.
47. Qin ES, Gold LS, Singh N *et al.* Physical function and fatigue recovery at 6 months after hospitalization for COVID-19. *PM R* 2023;**15**:314–24.
48. Carbone F, Zamarian L, Rass V *et al.* Cognitive dysfunction 1 year after COVID-19: evidence from eye tracking. *Ann Clin Transl Neurol* 2022;**9**:1826–31.
49. Flood A, Keegan RJ. Cognitive resilience to psychological stress in military personnel. *Front Psychol* 2022;**13**:809003.
50. Hidalgo V, Villada C, Almela M *et al.* Enhancing effects of acute psychosocial stress on priming of non-declarative memory in healthy young adults. *Stress* 2012;**15**:329–38.
51. Lindová J, Příplatová L, Flegr J. Higher extraversion and lower conscientiousness in humans infected with *Toxoplasma*. *Eur J Pers* 2012;**26**:285–91.
52. Zhu X, Yan W, Lin X *et al.* The effect of perceived stress on cognition is mediated by personality and the underlying neural mechanism. *Transl Psychiatry* 2022;**12**:199.
53. LePine JA, Colquitt JA, Erez A. Adaptability to changing task contexts: effects of general cognitive ability, conscientiousness, and openness to experience. *Pers Psychol* 2000;**53**:563–93.
54. Slouma M, Abbes M, Kharrat L *et al.* Post-COVID-19 reactive arthritis. *Clin Rheumatol* 2022;**41**:2609–10.
55. Slouma M, Abbes M, Mehmli T *et al.* Reactive arthritis occurring after COVID-19 infection: a narrative review. *Infection* 2023;**51**:37–45.
56. Steenblock C, Schwarz PEH, Ludwig B *et al.* COVID-19 and metabolic disease: mechanisms and clinical management. *Lancet Diabetes Endocrinol* 2021;**9**:786–98.
57. Ferrucci R, Dini M, Groppo E *et al.* Long-lasting cognitive abnormalities after COVID-19. *Brain Sci* 2021;**11**:235.
58. Almeria M, Cejudo JC, Sanz-Santos J *et al.* Impact of COVID-19 infection on cognition and its association with neurological symptoms. *Brain Behav* 2023;**13**:e2902.
59. Oliveira CR, Jason LA, Unutmaz D *et al.* Improvement of Long COVID symptoms over one year. *Front Med (Lausanne)* 2022;**9**:1065620.
60. Colizzi M, Peghin M, De Martino M *et al.* Mental health symptoms one year after acute COVID-19 infection: prevalence and risk factors. *Rev Psiquiatr Salud Ment* 2023;**16**:38–46.
61. Van Herck M, Goërtz YMJ, Houben-Wilke S *et al.* Severe fatigue in Long COVID: web-based quantitative follow-up study in members of online long COVID support groups. *J Med Internet Res* 2021;**23**:e30274.
62. Fumagalli C, Zocchi C, Tassetti L *et al.* Factors associated with persistence of symptoms 1 year after COVID-19: a longitudinal, prospective phone-based interview follow-up cohort study. *Eur J Intern Med* 2022;**97**:36–41.
63. Steinmetz A, Gross S, Lehnert K *et al.* Longitudinal clinical features of post-COVID-19 patients-symptoms, fatigue and physical function at 3- and 6-month follow-up. *J Clin Med* 2023;**12**:3966.
64. Mazza MG, Palladini M, Villa G *et al.* Prevalence, trajectory over time, and risk factor of post-COVID-19 fatigue. *J Psychiatr Res* 2022;**155**:112–9.
65. Peterson CJ, Sarangi A, Bangash F. Neurological sequelae of COVID-19: a review. *Egypt J Neurol Psychiatr Neurosurg* 2021;**57**:122.
66. Seeßle J, Waterboer T, Hippchen T *et al.* Persistent symptoms in adult patients 1 year after coronavirus disease 2019 (COVID-19): a prospective cohort study. *Clin Infect Dis* 2022;**74**:1191–8.
67. Adan A, Guàrdia J. Circadian variations of self-reported activation: a multidimensional approach. *Chronobiologia* 1993;**20**:233–44.
68. Flegr J, Horáček J. *Toxoplasma*-infected subjects report an obsessive-compulsive disorder diagnosis more often and score higher in obsessive-compulsive inventory. *Eur Psychiatry* 2017;**40**:82–7.