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ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии

საქართველოს სამედიცინო სიახლენი

GEORGIAN MEDICAL NEWS

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GMN: Georgian Medical News is peer-reviewed, published monthly journal committed to promoting the science and art of medicine and the betterment of public health, published by the GMN Editorial Board since 1994. GMN carries original scientific articles on medicine, biology and pharmacy, which are of experimental, theoretical and practical character; publishes original research, reviews, commentaries, editorials, essays, medical news, and correspondence in English and Russian.

GMN is indexed in MEDLINE, SCOPUS, PubMed and VINITI Russian Academy of Sciences. The full text content is available through EBSCO databases.

GMN: Медицинские новости Грузии - ежемесячный рецензируемый научный журнал, издаётся Редакционной коллегией с 1994 года на русском и английском языках в целях поддержки медицинской науки и улучшения здравоохранения. В журнале публикуются оригинальные научные статьи в области медицины, биологии и фармации, статьи обзорного характера, научные сообщения, новости медицины и здравоохранения. Журнал индексируется в MEDLINE, отражён в базе данных SCOPUS, PubMed и ВИНИТИ РАН. Полнотекстовые статьи журнала доступны через БД EBSCO.

GMN: Georgian Medical News – საქართველოს სამედიცინო სიახლენი – არის ყოველთვიური სამეცნიერო სამედიცინო რეცენზირებადი ჟურნალი, გამოიცემა 1994 წლიდან, წარმოადგენს სარედაქციო კოლეგიისა და აშშ-ის მეცნიერების, განათლების, ინდუსტრიის, ხელოვნებისა და ბუნებისმეტყველების საერთაშორისო აკადემიის ერთობლივ გამოცემას. GMN-ში რუსულ და ინგლისურ ენებზე ქვეყნდება ექსპერიმენტული, თეორიული და პრაქტიკული ხასიათის ორიგინალური სამეცნიერო სტატიები მედიცინის, ბიოლოგიისა და ფარმაციის სფეროში, მიმოხილვითი ხასიათის სტატიები.

ჟურნალი ინდექსირებულია MEDLINE-ის საერთაშორისო სისტემაში, ასახულია SCOPUS-ის, PubMed-ის და ВИНИТИ РАН-ის მონაცემთა ბაზებში. სტატიების სრული ტექსტი ხელმისაწვდომია EBSCO-ს მონაცემთა ბაზებიდან.

WEBSITE

www.geomednews.com

К СВЕДЕНИЮ АВТОРОВ!

При направлении статьи в редакцию необходимо соблюдать следующие правила:

1. Статья должна быть представлена в двух экземплярах, на русском или английском языках, напечатанная через **полтора интервала на одной стороне стандартного листа с шириной левого поля в три сантиметра**. Используемый компьютерный шрифт для текста на русском и английском языках - **Times New Roman** (**Кириллица**), для текста на грузинском языке следует использовать **AcadNusx.** Размер шрифта - **12**. К рукописи, напечатанной на компьютере, должен быть приложен CD со статьей.

2. Размер статьи должен быть не менее десяти и не более двадцати страниц машинописи, включая указатель литературы и резюме на английском, русском и грузинском языках.

3. В статье должны быть освещены актуальность данного материала, методы и результаты исследования и их обсуждение.

При представлении в печать научных экспериментальных работ авторы должны указывать вид и количество экспериментальных животных, применявшиеся методы обезболивания и усыпления (в ходе острых опытов).

4. К статье должны быть приложены краткое (на полстраницы) резюме на английском, русском и грузинском языках (включающее следующие разделы: цель исследования, материал и методы, результаты и заключение) и список ключевых слов (key words).

5. Таблицы необходимо представлять в печатной форме. Фотокопии не принимаются. **Все цифровые, итоговые и процентные данные в таблицах должны соответствовать таковым в тексте статьи**. Таблицы и графики должны быть озаглавлены.

6. Фотографии должны быть контрастными, фотокопии с рентгенограмм - в позитивном изображении. Рисунки, чертежи и диаграммы следует озаглавить, пронумеровать и вставить в соответствующее место текста **в tiff формате**.

В подписях к микрофотографиям следует указывать степень увеличения через окуляр или объектив и метод окраски или импрегнации срезов.

7. Фамилии отечественных авторов приводятся в оригинальной транскрипции.

8. При оформлении и направлении статей в журнал МНГ просим авторов соблюдать правила, изложенные в «Единых требованиях к рукописям, представляемым в биомедицинские журналы», принятых Международным комитетом редакторов медицинских журналов - http://www.spinesurgery.ru/files/publish.pdf и http://www.nlm.nih.gov/bsd/uniform_requirements.html
В конце каждой оригинальной статьи приводится библиографический список. В список литературы включаются все материалы, на которые имеются ссылки в тексте. Список составляется в алфавитном порядке и нумеруется. Литературный источник приводится на языке оригинала. В списке литературы сначала приводятся работы, написанные знаками грузинского алфавита, затем кириллицей и латиницей. Ссылки на цитируемые работы в тексте статьи даются в квадратных скобках в виде номера, соответствующего номеру данной работы в списке литературы. Большинство цитированных источников должны быть за последние 5-7 лет.

9. Для получения права на публикацию статья должна иметь от руководителя работы или учреждения визу и сопроводительное отношение, написанные или напечатанные на бланке и заверенные подписью и печатью.

10. В конце статьи должны быть подписи всех авторов, полностью приведены их фамилии, имена и отчества, указаны служебный и домашний номера телефонов и адреса или иные координаты. Количество авторов (соавторов) не должно превышать пяти человек.

11. Редакция оставляет за собой право сокращать и исправлять статьи. Корректура авторам не высылается, вся работа и сверка проводится по авторскому оригиналу.

12. Недопустимо направление в редакцию работ, представленных к печати в иных издательствах или опубликованных в других изданиях.

При нарушении указанных правил статьи не рассматриваются.

REQUIREMENTS

Please note, materials submitted to the Editorial Office Staff are supposed to meet the following requirements:

1. Articles must be provided with a double copy, in English or Russian languages and typed or compu-ter-printed on a single side of standard typing paper, with the left margin of **3** centimeters width, and **1.5** spacing between the lines, typeface - **Times New Roman** (**Cyrillic**), print size - **12** (referring to Georgian and Russian materials). With computer-printed texts please enclose a CD carrying the same file titled with Latin symbols.

2. Size of the article, including index and resume in English, Russian and Georgian languages must be at least 10 pages and not exceed the limit of 20 pages of typed or computer-printed text.

3. Submitted material must include a coverage of a topical subject, research methods, results, and review.

Authors of the scientific-research works must indicate the number of experimental biological species drawn in, list the employed methods of anesthetization and soporific means used during acute tests.

4. Articles must have a short (half page) abstract in English, Russian and Georgian (including the following sections: aim of study, material and methods, results and conclusions) and a list of key words.

5. Tables must be presented in an original typed or computer-printed form, instead of a photocopied version. **Numbers, totals, percentile data on the tables must coincide with those in the texts of the articles.** Tables and graphs must be headed.

6. Photographs are required to be contrasted and must be submitted with doubles. Please number each photograph with a pencil on its back, indicate author's name, title of the article (short version), and mark out its top and bottom parts. Drawings must be accurate, drafts and diagrams drawn in Indian ink (or black ink). Photocopies of the X-ray photographs must be presented in a positive image in **tiff format**.

Accurately numbered subtitles for each illustration must be listed on a separate sheet of paper. In the subtitles for the microphotographs please indicate the ocular and objective lens magnification power, method of coloring or impregnation of the microscopic sections (preparations).

7. Please indicate last names, first and middle initials of the native authors, present names and initials of the foreign authors in the transcription of the original language, enclose in parenthesis corresponding number under which the author is listed in the reference materials.

8. Please follow guidance offered to authors by The International Committee of Medical Journal Editors guidance in its Uniform Requirements for Manuscripts Submitted to Biomedical Journals publication available online at: http://www.nlm.nih.gov/bsd/uniform_requirements.html
http://www.icmje.org/urm_full.pdf
In GMN style for each work cited in the text, a bibliographic reference is given, and this is located at the end of the article under the title "References". All references cited in the text must be listed. The list of references should be arranged alphabetically and then numbered. References are numbered in the text [numbers in square brackets] and in the reference list and numbers are repeated throughout the text as needed. The bibliographic description is given in the language of publication (citations in Georgian script are followed by Cyrillic and Latin).

9. To obtain the rights of publication articles must be accompanied by a visa from the project instructor or the establishment, where the work has been performed, and a reference letter, both written or typed on a special signed form, certified by a stamp or a seal.

10. Articles must be signed by all of the authors at the end, and they must be provided with a list of full names, office and home phone numbers and addresses or other non-office locations where the authors could be reached. The number of the authors (co-authors) must not exceed the limit of 5 people.

11. Editorial Staff reserves the rights to cut down in size and correct the articles. Proof-sheets are not sent out to the authors. The entire editorial and collation work is performed according to the author's original text.

12. Sending in the works that have already been assigned to the press by other Editorial Staffs or have been printed by other publishers is not permissible.

Articles that Fail to Meet the Aforementioned Requirements are not Assigned to be Reviewed.

ავტორთა საყურადღებოდ!

რედაქციაში სტატიის წარმოდგენისას საჭიროა დავიცვათ შემდეგი წესები:

1. სტატია უნდა წარმოადგინოთ 2 ცალად, რუსულ ან ინგლისურ ენებზე,დაბეჭდილი სტანდარტული ფურცლის 1 გვერდზე, 3 სმ სიგანის მარცხენა ველისა და სტრიქონებს შორის 1,5 ინტერვალის დაცვით. გამოყენებული კომპიუტერული შრიფტი რუსულ და ინგლისურენოვან ტექსტებში - **Times New Roman (Кириллица)**, ხოლო ქართულენოვან ტექსტში საჭიროა გამოვიყენოთ **AcadNusx.** შრიფტის ზომა – 12. სტატიას თან უნდა ახლდეს CD სტატიით.

2. სტატიის მოცულობა არ უნდა შეადგენდეს 10 გვერდზე ნაკლებს და 20 გვერდზე მეტს ლიტერატურის სიის და რეზიუმეების (ინგლისურ, რუსულ და ქართულ ენებზე) ჩათვლით.

3. სტატიაში საჭიროა გაშუქდეს: საკითხის აქტუალობა; კვლევის მიზანი; საკვლევი მასალა და გამოყენებული მეთოდები; მიღებული შედეგები და მათი განსჯა. ექსპერიმენტული ხასიათის სტატიების წარმოდგენისას ავტორებმა უნდა მიუთითონ საექსპერიმენტო ცხოველების სახეობა და რაოდენობა; გაუტკივარებისა და დაძინების მეთოდები (მწვავე ცდების პირობებში).

4. სტატიას თან უნდა ახლდეს რეზიუმე ინგლისურ, რუსულ და ქართულ ენებზე არანაკლებ ნახევარი გვერდის მოცულობისა (სათაურის, ავტორების, დაწესებულების მითითებით და უნდა შეიცავდეს შემდეგ განყოფილებებს: მიზანი, მასალა და მეთოდები, შედეგები და დასკვნები; ტექსტუალური ნაწილი არ უნდა იყოს 15 სტრიქონზე ნაკლები) და საკვანძო სიტყვების ჩამონათვალი (key words).

5. ცხრილები საჭიროა წარმოადგინოთ ნაბეჭდი სახით. ყველა ციფრული, შემაჯამებელი და პროცენტული მონაცემები უნდა შეესაბამებოდეს ტექსტში მოყვანილს.

6. ფოტოსურათები უნდა იყოს კონტრასტული; სურათები, ნახაზები, დიაგრამები - დასათაურებული, დანომრილი და სათანადო ადგილას ჩასმული. რენტგენოგრამების ფოტოასლები წარმოადგინეთ პოზიტიური გამოსახულებით **tiff** ფორმატში. მიკროფოტოსურათების წარწერებში საჭიროა მიუთითოთ ოკულარის ან ობიექტივის საშუალებით გადიდების ხარისხი, ანათალების შეღებვის ან იმპრეგნაციის მეთოდი და აღნიშნოთ სურათის ზედა და ქვედა ნაწილები.

7. სამამულო ავტორების გვარები სტატიაში აღინიშნება ინიციალების თანდართვით, უცხოურისა – უცხოური ტრანსკრიპციით.

8. სტატიას თან უნდა ახლდეს ავტორის მიერ გამოყენებული სამამულო და უცხოური შრომების ბიბლიოგრაფიული სია (ბოლო 5-8 წლის სიღრმით). ანბანური წყობით წარმოდგენილ ბიბლიოგრაფიულ სიაში მიუთითეთ ჯერ სამამულო, შემდეგ უცხოელი ავტორები (გვარი, ინიციალები, სტატიის სათაური, ჟურნალის დასახელება, გამოცემის ადგილი, წელი, ჟურნალის №, პირველი და ბოლო გვერდები). მონოგრაფიის შემთხვევაში მიუთითეთ გამოცემის წელი, ადგილი და გვერდების საერთო რაოდენობა. ტექსტში კვადრატულ ფჩხილებში უნდა მიუთითოთ ავტორის შესაბამისი N ლიტერატურის სიის მიხედვით. მიზანშეწონილია, რომ ციტირებული წყაროების უმეტესი ნაწილი იყოს 5-6 წლის სიღრმის.

9. სტატიას თან უნდა ახლდეს: ა) დაწესებულების ან სამეცნიერო ხელმძღვანელის წარდგინება, დამოწმებული ხელმოწერითა და ბეჭდით; ბ) დარგის სპეციალისტის დამოწმებული რეცენზია, რომელშიც მითითებული იქნება საკითხის აქტუალობა, მასალის საკმაობა, მეთოდის სანდოობა, შედეგების სამეცნიერო-პრაქტიკული მნიშვნელობა.

10. სტატიის ბოლოს საჭიროა ყველა ავტორის ხელმოწერა, რომელთა რაოდენობა არ უნდა აღემატებოდეს 5-ს.

11. რედაქცია იტოვებს უფლებას შეასწოროს სტატია. ტექსტზე მუშაობა და შეჯერება ხდება საავტორო ორიგინალის მიხედვით.

12. დაუშვებელია რედაქციაში ისეთი სტატიის წარდგენა, რომელიც დასაბეჭდად წარდგენილი იყო სხვა რედაქციაში ან გამოქვეყნებული იყო სხვა გამოცემებში.

აღნიშნული წესების დარღვევის შემთხვევაში სტატიები არ განიხილება.

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FATIGUE IN PATIENTS WITH LONG COVID

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Abstract.

Purpose of the study: To characterize the metabolomic profile in patients with fatigue developing within the Long COVID, during dynamic observation.

Materials and methods: 24 patients diagnosed with U09.9 "Condition after COVID-19 unspecified" were included in a prospective study. Patients were recommended to engage in physical activity, which included moderate aerobic activity such as walking for 45 minutes a day, three days a week. Clinical assessment by scales (Modified Medical Research Council dyspnea scale; 6-minute walk test; Multidimensional fatigue inventory scale; Barthel index), and determination of metabolomic parameters were performed on days 1 and 14-18 of the study.

Results: During the observation period, lactate, fumaric acid, symmetrical dimethylarginine, asymmetric dimethylarginine remained above the reference values. The level of adipic acid returns to normal values. As a result of performing physical activity, such as walking, results on the Modified Medical Research Council scale dyspnea scale, Multidimensional fatigue inventory scale, 6 Minutes Walking Test and Barthel Index improve ($p<0,001$).

Conclusion: Metabolic profile of patients with Long COVID demonstrates the complex of abnormalities at 60 days after the onset of the disease. These metabolic changes are point to possible therapeutic targets for specific pathogenetic pharmacotherapy.

Key words. Long covid-19, metabolomic, rehabilitation, fatigue, mitochondrial function.

Introduction.

A new coronavirus infection, SARS-COV-2 (COVID-19), caused by the SARS-CoV-2 acute severe respiratory syndrome virus, has rapidly become a global pandemic. The majority of patients who have recovered from the acute stage of infection retained symptoms or developed new ones, such as general fatigue, cognitive disorders, pulmonary fibrosis, cough, shortness of breath, tachycardia, sense of smell, and others. Studies have shown that up to 87% of patients continue to experience at least one symptom even after the acute stage of the process is over. The most common symptoms are general fatigue, impaired concentration, and memory, and "Brain fog" [1,2]. The pathogenesis of delayed symptoms remains unclear so far. Among the most likely hypotheses are direct damage of central, peripheral and autonomic nervous system cells by the virus, immune dysregulation, chronic neuroinflammation, metabolomic disorders, coagulation disorders, residual lung damage and some others [3]. Changes in metabolomic profile in various viral infections such as SARS, H1N1, respiratory syncytial virus, Ebola virus and Dengue fever have been previously reported. To date, there are several studies indicating metabolic abnormalities in the acute phase of SARS-COV-2 infection [4-7]. In addition, it has been reported that only some metabolites were restored after discharge, and some metabolites continued to be abnormal in convalescent patients with COVID-19 [8]. We assumed that metabolic abnormalities originating in the acute stage of infection may persist after recovery, along with contributing to further changes in the metabolomics profile, which occupies an important place in the pathogenesis of post-COVID syndrome. Thus, understanding the metabolic changes in convalescent patients with COVID-19 may help to uncover clues to the pathophysiological processes underlying Long COVID and identify promising therapeutic targets.

In this article, we present the results of a prospective study of 24 patients with Long COVID.

Purpose of the study.

To characterize the metabolomic profile in patients with fatigue developing within the Long COVID, during dynamic observation.

Ethics.

This study was approved by the local ethical committee of the Sechenov First Moscow State Medical University, Ministry of Health of the Russian Federation (Sechenov University).

Materials and Methods.

Twenty-four patients were included in the study.

Patients were included in the study with diagnosis U09.9 "Condition after COVID-19 unspecified".

Patients were included for a follow-up period of 14-18 days, during which time their clinical and metabolomic parameters were monitored at two points, the first day of study inclusion and the 14th-18th day of study inclusion.

Inclusion criteria:

1. Presence of documented previous COVID-19 infection.
2. At least 4 weeks and no more than 12 weeks since onset of new COVID-19 coronavirus infection (positive SARS-COV-2 PCR test).
3. Age 18-69 years.
4. Symptoms associated with asthenic syndrome, increased fatigue, and no other neurological abnormalities.
5. Asthenic syndrome as assessed by the MFI-20 scale (total score > 20).

Exclusion criteria:

1. Age under 18 years of age, over 69 years of age.
2. Pregnancy, breastfeeding.
3. The presence of concomitant pathology: cancer diseases; systemic connective tissue diseases (systemic lupus

erythematosus, rheumatoid arthritis, dermatomyositis, systemic scleroderma, etc.), and systemic vasculitis (thrombohemorrhagic vasculitis, thrombocytopenic purpura, Wegener's disease, Goodpasture syndrome, etc.).

4. The presence of any other non-infectious chronic diseases in the stage of exacerbation.
5. The presence of an acute or chronic infectious process.
6. A score on the mMRC scale >2
7. SaPO2 at rest and after exercise < 95%.

Several clinical assessment methods used to determine a patient's level of activity and cognitive function: Modified Medical Research Council Dyspnea Scale (mMRC); 6 Minutes Walking Test (6MWT); Barthel Activities of Daily Living Index (ADL Barthel Index); Multidimensional fatigue inventory (MFI-20).

During the period of observation, patients were given two recommendations. The first recommendation was to engage in physical activity, which included moderate aerobic activity for 45 minutes a day, three days a week, with a self-monitoring diary. The second recommendation was not to take drugs, dietary supplements, or vitamins that can affect the metabolomic profile. These recommendations were given to all patients regardless of group allocation.

Several laboratory diagnostic methods used to determine the patient's condition. These methods include the examination of plasma for methylated arginine derivatives using high-performance liquid chromatography and tandem mass spectrometry, the examination of single urine analysis for organic acids using gas chromatography-mass spectrometry, and the examination of blood plasma for amino acids using high-performance liquid chromatography and tandem mass spectrometry. Additionally, blood plasma analysis is examined for coenzyme Q10 total (ubiquinone) using high-performance liquid chromatography with UV detection.

Statistical processing of the results was performed using several statistical methods. Quantitative values that had a normal distribution were described using arithmetic mean (M) and standard deviations (SD), 95% confidence interval (95% CI) limits. In the absence of a normal distribution, quantitative data were described with median (Me) and lower and upper quartiles (Q1 to Q3). Paired Student's t-test was used when comparing normally distributed quantitative measures calculated for two related samples. Wilcoxon's criterion was used when comparing quantitative indicators, whose distribution differed from normal, in the two related groups.

Results.

The observation included 24 patients, 12 women (50%) 12 men (50%). The age of the patients was Me 59 (55-62 Q1-Q3) years.

On average, patients were included on 60 ± 6 (57-63 95% CI) days after a negative PCR result for COVID-19.

All participants adhered to the recommendations for physical activity, which included walking for at least 45 minutes, at least three times a week. No deterioration in well-being was noted in any patient during the observation period.

An analysis of the metabolomics profile is shown below. We took into account parameters that were outside the reference values or in values close to the limit of normal (+20% of min, -20% of max) at the first or second point of the study, as well as parameters that changed statistically significantly during the period of observation.

Results of metabolomic screening.

Carbohydrate metabolic indices:

Lactate levels increased from Me 30,606 (Q1-Q3 19,734 - 31,613) to Me 33,815 (Q1-Q3 30,848 - 35,828) over the observation period (Figure 1).

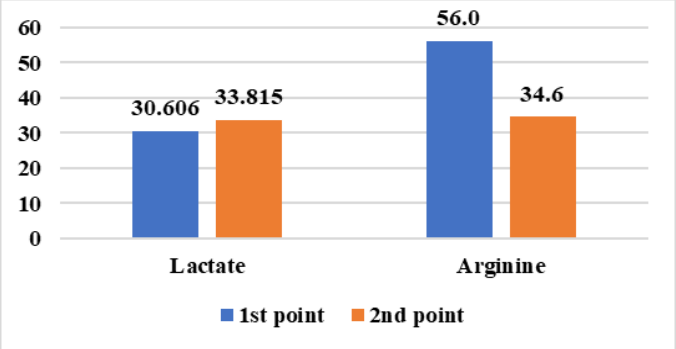

Figure 1. *Dynamics of lactate, arginine.*
Reference values: lactate (lactic acid) - 4.08-28.79 mmol/mol creatinine. MMA - 94.2-316.5 ng/mL.

Fat metabolism:

Adipic acid had decreased in the control group from Me 3,711 (Q1-Q3 2,330 – 4,184) to Me 2,076 (Q1-Q3 1,618 - 2,652) by the second point (Figure 2).

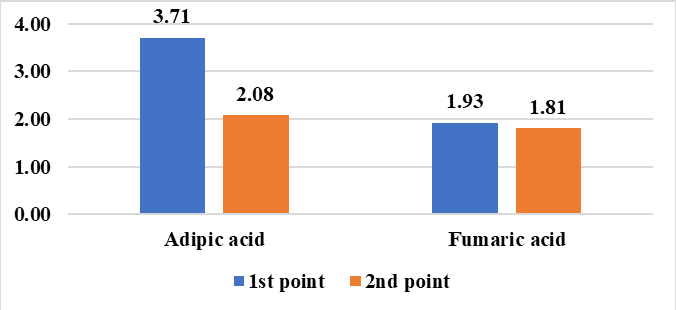

Figure 2. *Dynamics of adipic acid, fumaric acid.*
Reference values: adipic acid 0.525- 3.743 mmol/mol creatinine; fumaric acid 0.153-1.312 mmol/mol creatinine.

Protein and amino acid metabolism

The amino acid **histidine** had decreased in the control group from Me 61.80 (Q1-Q3 57.62 - 83.10), to Me 48,90 (Q1-Q3 37,35 - 62,35) by the second point ($p < 0,001$, method used: Wilcoxon test) (Reference values: 46-95 µmol/L).

Indicators responsible for mitochondrial function, cell energy supply.

Citric acid had decreased from Me 57.174 (Q1-Q3 34.935 - 151.571) to the lower limits of normal concentration by the second point Me 48.876 (Q1-Q3 17.523 - 280.523) (Reference values: 46.76-368.01 mmol/mol creatinine).

Indicators of the severity of oxidative stress, functioning of ammonia detoxification systems (ornithine cycle).

Fumaric acid was initially in values higher than normal Me 1,849 (Q1-Q3 1,318 - 3,373), during observation showed a slight increase to Me 1,995 (Q1-Q3 1,716 - 2,618), remaining above normal (Figure 2).

8-Hydroxydeoxyguanosine (8-OhdG) in the control group is Me 1.33 (Q1-Q3 1.08 - 1.70) at the first point, increased slightly to Me 1.81 (Q1-Q3 1.47 - 2.87) at the second point ($p < 0.001$, method used: Wilcoxon test) (Reference values: 0.85-3.6 nmol/mol creatinine).

Coenzyme Q10 in the control group had decreased from a Me value of 1104 (Q1-Q3 900 - 1373) at the first point, to a Me value of 809 (Q1-Q3 583 - 886) by the second point ($p <$ 0,001, method used: Wilcoxon test) (Reference values: 400-1900 μg/L).

Equilibrium of neurotransmitter systems.

Kynurenic acid initially in values below normal, Me 0.238 (Q1-Q3 0.066 - 0.378) at the first point, and continued to decrease by the second point Me 0.203 (Q1-Q3 0.051 - 0.407) (Reference values: 0.599-2.177 mmol/mol creatinine).

Quinolinic acid in the control group increased over the observation period from Me 2,016 (Q1-Q3 0,880 – 4,798) at the first point, to Me 2,208 (Q1-Q3 1,721 - 3,535) by the second point (Reference values: 0.761-2.374 mmol/mol creatinine).

Glutamic acid levels were in values closer to the lower limits, Me 51.60 (Q1-Q3 42.55 - 61.55) at the first point, had risen to Me 60.75 (Q1-Q3 45.22 - 70.08) by the second point (Reference values 40-159.7 μmol/L).

Vascular endothelial function, NO balance.

The **ADMA** (**Asymmetrical dimethylarginine**) remained above normal at both points. The index had risen from Me 256 (Q1-Q3 214 - 318) at the first point, to Me 259 (Q1-Q3 182 - 236) by the second point (Reference values: <100 - low, 100-123 - intermediate, >123 – high, ng/mL).

The **SDMA** (**Symmetrical Dimethylarginine)** decreased in the control group from Me 295 (Q1-Q3 254 - 358), to Me 270 (Q1-Q3 237 - 312) (Reference values: <73 - low, 73-135 - intermediate, >135 - high, ng/mL)

Arginine decreased in the control group over the observation period from Me 56.00 (Q1-Q3 40.59 to 69.20), to Me 34.55 (Q1-Q3 24.68 to 46.30) ($p < 0.001$, method used: Wilcoxon test) (Figure 1).

Scale scores.

On the **mMRC** dyspnea scale, the score at the first point was Me 2 (Q_1-Q_3 2-2), by the second point there was a slight decrease in the score ($p = 0.014$, method used: Wilcoxon test), but median remained at Me 2 (Q_1-Q_3 1-2) points (Table 1).

When evaluating **the 6MWT,** the result was Me 362 (Q1-Q3 354 to 409) meters at the first point, and Me 408 (Q1-Q3 375 to 445) meters at the second point ($p < 0.001$, method used: Wilcoxon criterion) (Table 1).

On the **MFI-20**, the score was Me 76 (Q1-Q3 74 to 77) at the first point, at the second point Me 66 (Q1-Q3 64 to 66) ($p <$ 0.001, method used: Wilcoxon criterion) (Table 1).

Table 1. *Dynamics of the mMRC scale (points), 6-minute walk test 6MWT (meters), measured asthenia scale MFI-20 (points), Barthel functional activity assessment scale (points).*

Scales	1st point (1st day of study)	2nd point (14-18 days of study)
Modified Medical Research Council (points)	Me 2 (Q_1–Q_3 2–2)	Me 2 (Q_1–Q_3 1–2)*
6 Minutes Walking Test (meters)	M±SD 372±61	M±SD 404±57 *
Multidimensional fatigue inventory (points)	M±SD 75±3	M±SD 65±3 *
Barthel Activities of daily living Index (points)	Me 70 (Q_1–$Q_3$66–73)	Me 77 (Q_1–$Q_3$73–79) *

Notes: *differences are statistically significant from baseline: *(p<0.001, method used: Wilcoxon test).*

On **the Bartel index** scored Me 78 (Q1-Q3 75 to 80) at the first point, Me 84 (Q1-Q3 82 to 86) at the second point ($p <$ 0.001, method used: Wilcoxon criterion) (Table 1).

Discussion.

Energy metabolism (metabolism of carbohydrates, fats, proteins).

Lactic acid has an important role in normal human physiology, being a signaling molecule involved in energy supply and pH regulation. Optimally, pyruvic acid is oxidized to form acetyl-CoA, which is used aerobically in the citric acid cycle to produce energy. In the anaerobic state, lactic acid is formed instead of pyruvic acid. Several studies have noted that serum lactate is a marker for assessing the severity of the inflammatory process, in various conditions [9,10]. A marked increase in lactate, with an excess of the upper limit of normal in the 2nd point, during the period of physical activation of patients indicate the persistence, or even increase of inflammatory status, hypoxia, metabolic stress and mitochondrial dysfunction.

Adipic acid is a dicarboxylic acid, a byproduct of omega-oxidation of fatty acids. Normally, fatty acids are metabolized to acyl-CoA by beta-oxidation. Conversion of fatty acids to acetyl-CoA requires transport across the mitochondrial membrane by carnitine transport. When beta-oxidation is impaired, fats are activated via an alternative metabolic pathway called omega-oxidation. Omega-oxidation leads to increased levels of dicarboxylic acids such as adipic acid and cork acid [11]. Increased levels of dicarboxylic acids in patients with Long COVID can lead to further mitochondrial dysfunction as well as free radical damage to the cell membrane.

Histidine is an amino acid mainly involved in the construction of regulatory proteins and peptides, as well as in copper transport [12,13]. Thus, the decrease of the amino acid in the control group characterizes the increase of catabolic processes, mediated regulatory disorders and, possibly, deterioration of copper transport systems, a microelement, which in turn is a co-factor in the regulation of inflammatory processes.

Indicators responsible for mitochondrial function, the energy supply of the cell.

Citric acid is the first metabolite in the energy production cycle, known as the Krebs cycle, which occurs in the mitochondria.

Disruption of fatty acid beta-oxidation, which is likely to occur in patients with Long COVID, can reduce acetyl-CoA and citrate levels. This situation, together with increased lactate levels and reduced beta-oxidation rate, indicates a disturbance of the Krebs cycle, resulting in mitochondrial dysfunction and reduced ATP production.

Indicators of oxidative stress, the functioning of ammonia detoxification systems (ornithine cycle).

Fumaric acid is one of the main metabolites of the Krebs cycle. Elevated levels of this compound in the urine indicate inefficient energy production. Another reason could be the intensity of the ammonia detoxification cycle [14,15]. In addition, increased levels of lactate contribute to a secondary increase in fumaric acid levels.

8-OhdG is another marker of oxidative stress, including oxidative DNA damage [16]. Thus, an increase of this index may indicate increased apoptosis.

Coenzyme Q10 plays an active role in the electron transport chain of the mitochondrial respiratory chain and ATP synthesis [17]. A statistically significant decrease in the control group indicates the persistence of high levels of oxidative stress, energy crisis and mitochondrial dysfunction.

Equilibrium of neurotransmitter systems.

Low concentration of **kynurenic acid** and high level of **quinolinic acid** can be caused by the expression of kynurenine-3-monooxygenase (QMO), which is induced by proinflammatory cytokines [18]. Therefore, the formation of 3-hydroxykinurenine increases much faster than that of kynurenic acid and the balance between 3-hydroxykinurenine and kynurenic acid formation shifts towards 3-HK. Quinolinic acid is a strong agonist of N-methyl-D-aspartate glutamate receptors (NMDA-R), excessive stimulation of which causes entry of calcium ions into the cell, activation of intracellular proteases and generation of reactive oxygen species and nitrogen. Ultimately, this situation may contribute to neuronal damage and increasing dysfunction of the nervous system [19,20].

Glutamic acid is a substitutable amino acid obtained from food and from the breakdown of intestinal proteins. Glutamate is the main excitatory neurotransmitter in the brain. It plays a role in the differentiation, migration, and survival of neurons in the developing brain. It is also involved in the maintenance of synapses, neuroplasticity, learning and memory. Glutamic acid is also a precursor of arginine, glutamine, proline, GABA, and polyamines [21]. Low levels of this amino acid may manifest as nonspecific disorders of the central nervous system.

Vascular endothelial function, NO balance.

ADMA is higher than normal at both sites. The conversion of arginine to nitric oxide (NO) is inhibited by ADMA. When NO synthesis is blocked, there is a narrowing of the blood vessel lumen, increased platelet aggregation and monocyte adhesion. Increased baseline levels of ADMA in human plasma are one of the main causes of endothelial dysfunction and high risk of cardiovascular complications in patients suffering from various pathologies [22].

SDMA is a highly sensitive marker of renal dysfunction, much more sensitive than creatinine and glomerular filtration rate. High values may indicate an increased risk of renal disease [23].

Arginine is a conditionally essential amino acid that is crucial for cardiovascular health and detoxification function (including ammonia/ornithine cycle detoxification). In the body, this amino acid is a precursor of nitric oxide. Nitric oxide is crucial for relaxing the endothelium, the layer of cells that lines the inside of blood vessels. Thus, arginine deficiency has widespread effects on the cardiovascular system. Reduced levels of this amino acid may mediate muscle weakness and fatigue [24]. Decreased arginine may indicate dysfunction of the urea cycle (ornithine cycle) as well as increasing endothelial dysfunction.

Although a number of metabolic indicators show a negative trend over the follow-up period, the clinical assessment scales (mMRC, 6MWT, MFI-20, Barthel index) show some improvement. This is probably due to the recommendation of moderate physical activity. Exercise tolerance, mainly as assessed by the 6MWT, increases with regular aerobic exercise. At the same time, symptoms of asthenia (as assessed by the MFI-20 scale) decrease to some extent, but the scores remain high, suggesting that asthenia persists. It is possible that worsening of the metabolomic profile contributes to a reduction in rehabilitation potential, and suggests that increased physical activity, without measures aimed at correcting metabolic changes, may lead to a lack of further positive dynamics, or even contribute to a worsening of well-being.

Conclusion.

The study of the metabolomic profile in patients with Long COVID in dynamics revealed certain metabolomic changes, namely:

1. Changes in energy metabolism: impaired glycolysis, beta-oxidation, and activation of catabolic processes (based on the dynamics of lactate, adipic acid, histidine).
2. Increasing mitochondrial dysfunction (based on the dynamics of citric acid, lactate, adipic acid).
3. Increasing oxidative stress, impaired ammonia detoxification system (based on fumaric acid, 8-OhdG, coenzyme Q10)
4. Neurotransmitter system disequilibrium (based on kynurenic acid, quinolinic acid, glutamic acid)
5. Vascular endothelial dysfunction, NO imbalance (based on ADMA, SDMA, arginine values).

These metabolic changes not only demonstrate the complex of abnormalities that develop in patients with Long COVID, but also point to possible therapeutic targets for specific pathogenetic pharmacotherapy. Additionally, monitoring these parameters over time can serve as a method to assess the effectiveness of the ongoing therapeutic measures.

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REFERENCES

1. Carfì A, Bernabei R, Landi F, et al. Persistent Symptoms in Patients After Acute COVID-19. JAMA. 2020;324:603-605.
2. Premraj L, Kannapadi NV, Briggs J, et al. Mid and long-

term neurological and neuropsychiatric manifestations of post-COVID-19 syndrome: A meta-analysis. J Neurol Sci. 2022;434:120162.
3. Castanares-Zapatero D, Chalon P, Kohn L, et al. Pathophysiology and mechanism of long COVID: a comprehensive review. Ann Med. 2022;54:1473-1487.
4. Cui L, Pang J, Lee YH, et al. Serum metabolome changes in adult patients with severe dengue in the critical and recovery phases of dengue infection. PLoS Negl Trop Dis. 2018;12:e0006217.
5. Wu J, Zhao M, Li C, et al. The SARS-CoV-2 induced targeted amino acid profiling in patients at hospitalized and convalescent stage. Biosci Rep. 2021;41:BSR20204201.
6. Thomas T, Stefanoni D, Reisz JA, et al. COVID-19 infection alters kynurenine and fatty acid metabolism, correlating with IL-6 levels and renal status. JCI Insight. 2020;5:e140327.
7. Danlos FX, Grajeda-Iglesias C, Durand S, et al. Metabolomic analyses of COVID-19 patients unravel stage-dependent and prognostic biomarkers. Cell Death Dis. 2021;12:258.
8. Zhang S, Luo P, Xu J, et al. Plasma Metabolomic Profiles in Recovered COVID-19 Patients without Previous Underlying Diseases 3 Months After Discharge. J Inflamm Res. 2021;14:4485-4501.
9. Lopez Krol A, Nehring HP, Krause FF, et al. Lactate induces metabolic and epigenetic reprogramming of pro-inflammatory Th17 cells. EMBO Rep. 2022;23:e54685.
10. Gupta GS. The Lactate and the Lactate Dehydrogenase in Inflammatory Diseases and Major Risk Factors in COVID-19 Patients. Inflammation. 2022:1-33.
11. Talley JT, Mohiuddin SS. Biochemistry, Fatty Acid Oxidation. 2023.
12. Sasaki C, Hiraishi T, Oku T, et al. Metabolomic approach to the exploration of biomarkers associated with disease activity in rheumatoid arthritis. PloS one. 2019;14:e0219400.
13. Feng RN, Niu YC, Sun XW, et al. Histidine supplementation improves insulin resistance through suppressed inflammation in obese women with the metabolic syndrome: a randomised controlled trial. Diabetologia. 2013;56:985-994.
14. Shambaugh GE 3rd. Urea biosynthesis I. The urea cycle and relationships to the citric acid cycle. Am J Clin Nutr. 1977;30:2083-7.
15. Ueno Y, Maeda T, Okamoto S, et al. Evaluation of Urea Cycle Activity by Metabolic Flux Analysis Using Mass Spectrometry. Methods Mol Biol. 2022;2544:129-144.
16. Jelic MD, Mandic AD, Maricic SM, et al. Oxidative stress and its role in cancer. J Cancer Res Ther. 2021;17:22-28.
17. Garrido-Maraver J, Cordero MD, Oropesa-Avila M, et al. Clinical applications of coenzyme Q10. Front Biosci (Landmark Ed). 2014;19:619-33.
18. Mellor A.L, Munn D.H. Tryptophan catabolism and T-cell tolerance: immunosuppression by starvation? Immunol Today. 1999;20:469-473.
19. Guillemin G.J. Quinolinic Acid: Neurotoxicity. FEBS J. 2012;279:1355.
20. Chiarugi A, Calvani M, Meli E, et al. Synthesis and release of neurotoxic kynurenine metabolites by human monocyte-derived macrophages. J Neuroimmunol. 2001;120:190-198.
21. Briguglio M, Dell'Osso B, Panzica G, et al. Dietary Neurotransmitters: A Narrative Review on Current Knowledge. Nutrients. 2018;10:591.
22. Wieczór AM, Wieczór R, Kulwas A, et al. Asymmetric dimethylarginine and angiogenesis: biological significance. Int Angiol. 2018;37:431-436.
23. Michael H, Szlosek D, Clements C, et al. Symmetrical Dimethylarginine: Evaluating Chronic Kidney Disease in the Era of Multiple Kidney Biomarkers. Vet Clin North Am Small Anim Pract. 2022;52:609-629.
24. Morris SM Jr. Arginine Metabolism Revisited. J Nutr. 2016;146:2579s-2586s.