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RESEARCH ARTICLE

Life Expectancy and COVID-19 in Relation to Systemic Endotheliopathy (Systemic Endotheliopathy after COVID-19 as Life Expectancy Indicator: Development, Risk Factors, Prevention)

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ABSTRACT

Life expectancy is a key indicator for assessing the population health, as it includes an estimate of lifetime mortality rates from various causes. Since 2019, coronavirus disease 2019 (COVID-19) has become the main cause of increased mortality and decreased life expectancy. This is mainly due to unfavorable outcomes of COVID-19 in the acute period. Currently, more and more data is accumulating regarding the negative impact of an incurred COVID-19 on the aging rates and the development of cardiovascular complications, which persists for a long time after the pathogen is eliminated from the body. It is believed that the development of systemic endotheliopathy can be a key mechanism of such long-term outcomes. Despite the widespread distribution of this infection in the population and the large number of cases of prolonged COVID-19 syndrome, the results on the long-term impact of COVID-19 on the development and course of endotheliopathy are scattered and do not provide a comprehensive understanding of risk factors and possible methods of prevention of such endotheliopathies, that could be used for practical needs. Therefore, our review summarized data on the pathogenetic mechanism of development, course features and risk factors of endotheliopathies in relation to COVID-19. We also presented methods of prevention of systemic endotheliopathy in patients with a history of COVID-19 with an emphasis on the dietary component and included our own research results regarding this matter.

Keywords: Life expectancy; COVID-19; systemic endotheliopathy; prevention methods

Introduction

COVID-19 as the main cause of life expectancy reduction

Life expectancy is a key indicator for assessing the population health, as it includes an estimate of lifetime mortality rates from various causes. Since 1900, the average life expectancy worldwide has doubled and now exceeds 70 years. Since 1870, life expectancy has increased significantly around the world: from 34 to 79 years in Europe, from 35 to 77 years in America, from 27.5 to 73.6 years in Asia, and from 26 years to 63 years in Africa (https://ourworldindata.org/life-expectancy). The present improvement of diagnostic methods and treatment and preventive measures and their broad implementation among the population have become the main factors that constantly contribute to the increase in the life expectancy of people, and, accordingly, the increase in the average expected life expectancy. In the US, the leading causes of death among adults, the rate of which even increased between 2014 and 2018, were accidents, medications, firearms, alcohol, suicide, and chronic noncommunicable diseases (CNCDs), namely stroke and diabetes. Mortality from infectious diseases, chronic lung diseases, cardiovascular diseases and malignant neoplasms, on the contrary, decreased during this period [1].

Coronavirus disease 2019 (COVID-19) has become the main cause of increased mortality and decreased life expectancy in patients with COVID-19 since 2019. Thus, according to the results of assessment of life tables in 29 countries (Aburto J. M., 2022) COVID-19 led to a loss of life expectancy at birth in 27 countries from 2019 to 2020, the largest of which – more than 1 year – was found among women in 8 countries and men in 11 countries, mainly due to increased mortality rates in the elderly age groups. For these countries, it took an average of 5.6 years to increase their life expectancy by 1 year and this progress was wiped out during 2020 due to COVID-19. Losses in life expectancy in Central and Eastern Europe in 2020 exceeded those seen since the collapse of the Eastern Bloc (excluding Lithuania and Hungary), while similar losses in Western Europe were last seen during World War II. Compared to recent trends, women from 15 countries and men from 10 countries in 2020 had lower life expectancy at birth than in 2015, when life expectancy was negatively impacted by a particularly severe flu season [2].

Currently, the significant shortening of life expectancy due to COVID-19 is associated precisely with the acute course of the disease and advanced age. A retrospective review by Slater and colleagues [3] demonstrates that the cause of death in the vast majority of hospitalized patients with SARS-CoV-2 is a direct consequence of COVID-19 infection. A study of mortality in 8 countries with a wide range of life expectancies, around 20 years (between 60 and 80), showed that approximately 65% of deaths from COVID-19 occurred above life expectancy in these countries, that is, among the elderly, who usually suffer from comorbid pathology, among which CVDs occupy an important place [4].

The development of endotheliopathy after COVID-19 as a possible mechanism for accelerating aging rates

There is still not enough time to assess the long-term effects of an incurred COVID-19 on the reduction of life expectancy. However, the results of recent studies already confirm the impact of the incurred COVID-19 infection on the acceleration of aging, which in the future may lead to an increase in CNCDs and, consequently, a further increase in mortality. Thus, Mongelli and colleagues [5] found that COVID-19 survivors had a higher biological age delta, calculated on the basis of methylation results (10.45 $\pm$ 7.29 years vs 3.68 $\pm$ 8.17 years), more often had accelerated rates of aging (76.6% vs 48.9%) and shorter telomeres (3.03 $\pm$ 2.39 kb vs 10.67 $\pm$ 11.69 kb) compared to COVID-free patients. Patients <60 years of age were most affected, which may increase the risk of development of various pathological conditions in young and middle age, and therefore the risk of increased comorbidity in old age [5]. Similar results were obtained by Cao X. et al. (2022), namely patients with severe COVID-19 compared to patients with mild COVID-19 and patients with mild COVID-19 compared to healthy individuals had a significant acceleration of DNAm age according to the Horvath, Hannum, and GrimAge clocks and a significant DNAm telomere length attrition acceleration [6].

In addition, there is already evidence in favor of a future increase in the frequency of pathological conditions and their complications among COVID-19 convalescents, namely, an increase in cardiovascular complications in patients with acute COVID-19, the development and persistence over many years of metabolic disorders in SARS-CoV-1 convalescents and the development of long COVID-19 in COVID-19 convalescents. COVID-19 is a respiratory infectious disease, but it also has a wide range of identified extrapulmonary disorders and complications, which can persist for a long time even after the elimination of the infectious

agent from the patient's body. The most common extrapulmonary disorders are CVDs. It is believed that the development of **endotheliopathies** is one of the key links in the emergence of vascular complications in COVID-19, because the COVID-19 course is often associated with pulmonary and extrapulmonary vascular inflammation at both the macro- and microvascular level, and the subsequent development of endothelial disorders.

Vascular endothelium, consisting of a monolayer of endothelial cells (EC), plays an important role in the regulation of many important functions in the human body, such as maintaining normal rheological properties of blood, hemostasis, angiogenesis, and also participates in the development of inflammatory processes and immune responses. As a result, damage to the endothelium in patients with COVID-19 may be the main reason for the acceleration of vascular aging processes and the development of related systemic pathologies.

Endothelial damage in patients with COVID-19 is due to the fact that the virus, namely severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), infects people by binding through the angiotensin-converting enzyme 2 (ACE-2) receptor, which is expressed not only by type 2 alveolar cells and bronchial epithelial cells, but also significantly by EC. The direct effect of SARS-CoV-2 (SARS-CoV-2 replication) on EC is still not fully confirmed, but it remains likely that viral components stimulate the activation of EC [7], and the intracellular presence of viral bodies in EC partially confirms the participation of the virus in development of endotheliopathy [8]. On the other hand, development of endotheliopathies is the result of release of inflammatory mediators by infected cells and the activation of immune reactions. Such mechanisms of damage to the endothelium contribute to the frequent occurrence of endotheliopathies in patients with COVID-19, and the systemic nature of the process determines the high risk of damage to those systems and organs in which the endothelium is present, which today is considered as an organ weighing 1.5-1.8 kg (which can be compared with the mass of the liver) or a continuous monolayer of endothelial cells 7 km long; the surface area of the endothelium is about 600 m^2. That is, based on the evidence that endothelial dysfunction (ED) is associated with the risks of the development and progression of atherosclerosis, hypertension, coronary insufficiency, myocardial infarction, diabetes and insulin resistance, renal failure, dyslipidemia, endocrine disorders, etc., then it can be assumed that in the coming decades, patients with a history of COVID-19 will face the progression of all existing chronic diseases, in the pathogenesis of which ED plays a leading role, which will result in an increase in morbidity and mortality, and, consequently, an acceleration of the aging process, an increase in mortality due to systemic complications and reduced life expectancy.

Nowadays, plenty of results of studies investigating the development and course of endotheliopathies among COVID-19 patients are available [8- 10]. However, despite the wide distribution of this infection among the population and a large number of cases of long-Covid syndrome, the results regarding the impact of incurred COVID-19 on the development and course of endotheliopathies are quite limited.

The purpose of our review was to summarize the results of modern scientific research on pathogenetic mechanism of development, course features, risk factors and possible prevention methods of systemic endotheliopathy in patients with a history of COVID-19. The obtained results can become the basis for the future creation of effective algorithms with the aim of prolonging the life expectancy of patients with inccured COVID-19.

Endothelial changes in COVID-19 acute period

Persistent EC activation is a common phenomenon during 10 weeks of acute SARS-CoV-2 infection, so both pulmonary and extrapulmonary endotheliopathies are highly prevalent disorders in patients with COVID-19 [11,12]. Currently available studies show that SARS-CoV-2 can affect the function of the endothelium in two ways: 1) by directly affecting ECs and causing their damage, death, detachment; 2) indirectly – as a result of the induction of secretion of pro-inflammatory cytokines, immune cells, activated platelets and the development of type 3 hypersensitivity. Both mechanisms of effect on the endothelium are directly related to the severity of COVID-19, which links the course of endotheliopathies with the course of COVID-19 [7,13]. Damage to the endothelium during a severe course of COVID-19 is usually accompanied by disturbance of coagulation processes, which may be one of the main factors in increasing the frequency of thrombotic events in these patients and increasing mortality [14,15].

Among the consequences of the negative impact of COVID-19 on the endothelium, we can highlight a violation of the barrier function of the endothelium, the system of hemostasis and vascular tone. All of them are interrelated, because they have common causes: disruption of intercellular tight

junctions (presence and relative density along the vascular tree), damage to the EC glycocalyx, damage, apoptosis or detachment of EC, excessive release of endothelial extracellular vesicles (EVs). Disruption of tight junctions and glycocalyx is usually caused by direct exposure to SARS-CoV-2, which causes significant changes in endothelial permeability [16]. Damage to ECs and an increase in the speed of their exfoliation also occurs under the direct influence of the virus on ECs and reduces the efficiency of EC regeneration. The consequence of this, on the one hand, is a significant increase in levels of circulating ECs and endothelial extracellular vesicles (EVs) (exosomes, microvesicles, and apoptotic bodies) in the bloodstream, and on the other hand, a decrease in vascular reactivity and disruption of the synthesis of hemostasis factors, factors involved in the relaxation and contraction of blood vessels [8,17]. Apoptotic ECs detached from the intima, as well as apoptotic ECs in the intima, become procoagulants due to the increased expression of phosphatidylserine and the loss of anticoagulant components, which contributes to an increase in systemic inflammation, blood rheology disorders, and the emergence of endothelial dysfunction [8,18,19]. The proportion of circulating EVs secreted by ECs compared to EVs originating from platelets and erythrocytes is relatively low under physiological conditions, but there is a significant increase in large EVs (100–300 nm) and small EVs (30–100 nm) in patients with COVID-19. In addition, the type of EVs in patients with COVID-19 is strongly associated with D-dimer levels, and elevated EV levels of prothrombotic/endothelial damage factors and proteins associated with cardiovascular pathology (MB, PRSS8, REN, HGF), is related with the severity of a COVID-19 case, which suggests that EVs play an important role in the formation of thrombotic complications in patients with COVID-19 [10,20,21].

Increased cytokine synthesis during COVID-19 may participate in the development of all of the following consequences of endothelial damage. For example, TNF-α can change the composition and structure of tight junctions, reduce vasorelaxation, induce contraction of arterial segments, accelerate aging and induce oxidative stress in ECs by reducing the degradation of asymmetric dimethylarginine, and in high concentrations – reduce the level of eNOS and thereby shorten the half-life of NO; IL-6 affects the permeability of ECs due to its influence on tight junctions, as well as on remodeling of the structure and changes in actin contractility, it modulates vascular function by increasing the expression of angiotensin II type 1 receptor mRNA, which subsequently leads to greater vasoconstriction, greater formation of free oxygen radicals and the occurrence of ED; IL-1β inhibits EC proliferation. Activation of ECs by cytokines can activate NADPH oxidases, which generate superoxide anions, contributing to local oxidative stress, while under normal conditions, ECs, on the contrary, reduce local oxidative stress: they synthesize superoxide dismutases, which absorb important active forms of oxygen O^{-2}, glutathione peroxidases, heme oxygenases. In severe COVID-19 cases, the secretion of cytokines becomes excessive and is called a cytokine storm, which leads to an even greater violation of the integrity and function of the ECs [8,9,14,15,22,23].

In summary, the development of endotheliopathies during the acute period of COVID-19 occurs as a result of the direct (replication of the virus in the ECs) and indirect (development of a pro-inflammatory state, oxidative stress, immune reactions) negative impact of SARS-CoV-2. The consequences of such a lesion are disturbance of the endothelial barrier, homeostasis, an increase in apoptosis, the number of circulating ECs and EVs associated with impaired relaxation of the vessel wall, which collectively leads to the formation of intravascular microthrombi and an even greater deepening of the processes of inflammation and thrombus formation.

Endotheliopathies in COVID-19 convalescents or patients with prolonged COVID-19 syndrome

Endotheliopathy is very common among convalescent COVID-19 patients. Persistent activation of ECs and dysregulation of angiogenesis in COVID-19 convalescents (at least in 6 weeks after an acute COVID-19 disease) was observed in a recent study by Fogarty and colleagues [24]. And Sollini et al. [25] studied 10 patients with a mean age of 52 years, associated symptoms persisting after COVID-19 with the presence of vascular inflammation, the marker of which was an increase in the target-to-blood pool ratio in three vascular regions (thoracic aorta, right iliac artery and femoral artery) compared to the control. However, despite the emergence of the hypothesis that COVID-19, especially at late complicated stages, is an endothelial disease, the exact mechanism underlying the development of endotheliopathies and their persistence among patients who have recovered from SARS-CoV-2 is still unknown. It is assumed to be related to a gradual decrease in the inflammation level at late stages of the disease until complete remission, however, the available study results show that even after normalization of acute-phase markers, endotheliopathy can persist in a

patient for a long time after the disease resolution [11,13,23,26].

Endotheliopathy is believed to underlie the development of long-Covid syndrome, so it is not surprising that the risk factors for these two pathologies are the same. In addition to a number of authors who found a relationship between symptoms and markers of endotheliopathy, this is also supported by the results of Whitaker et al., who found that in patients who suffered from COVID-19 a year ago, the frequency of some systemic symptoms (diarrhea, skin redness and itching, nausea/vomiting, chest pain) even increased, which may be a consequence of the long-term persistence of endotheliopathy [27]. The presence of symptoms of long-Covid syndrome in patients after incurred COVID-19 can be considered not as a risk factor for endotheliopathy, but rather as an indicator of this pathology. In a study by Tong et al. [28], serum levels of VCAM-1, ICAM-1, P-selectin, and fractalkine were not significantly different between 345 individuals with COVID-19 in one year after hospital discharge and healthy controls. However, considering the proportion of symptomatic patients after an incurred COVID-19 (39% of those who recovered had post-Covid syndrome), it can be assumed that the obtained results are due to the low proportion of symptomatic patients among the convalescents.

Risk groups for the development of endotheliopathies among COVID-19 patients

Which patient categories are more prone to the risk of developing endotheliopathies after suffering from COVID-19? Based on conducted studies, these include:

- Female gender;
- Elderly age;
- Presence of cardiovascular risk factors (CVR), including increased body mass index, smoking, etc.;
- Presence of 2 or more comorbid pathologies;
- Hospitalization during the COVID-19 acute phase.

Thus, women have a higher probability of developing endotheliopathies and have 1.5 times more symptoms associated with long-Covid syndrome [11,27,29].

The relation of endotheliopathies with increasing age, especially >50 years, is probably due, on the one hand, to a decrease in compensatory mechanisms, the rate of damage elimination, regeneration, and on the other hand, to an increased intensity of the main mechanisms of aging, such as inflammation and violation of redox balance, which have already undergone significant changes as a result of COVID-19, that causes a broad range of further complications [11,27,29]. However, it cannot be said that age is a limiting factor in the development of endotheliopathies. Even in young patients, according to the results of a number of studies, systemic disorders of the endothelial functions developed. Ratchford et al. [30] conducted a cross-sectional analysis of healthy young adults and young adults (mean age 20.2 ± 1.1 years) who tested positive for SARS-CoV-2 3–4 weeks prior to testing to identify potential systemic effects of SARS-CoV-2 on the vascular system. The index of brachial artery flow-mediated vasodilatation (FMD) in the arm and single passive leg movement (sPLM) were lower in the SARS-CoV-2 group, suggesting the development of ED in these patients. Similar data were obtained by Nandadeva et al. [31] in a study of 16 young adults at least 4 weeks after a diagnosis of COVID-19: FMD and peak velocity after cuff removal were significantly reduced in young adults who were still symptomatic. Interestingly, the asymptomatic subjects had similar vascular function compared to the control group.

Such results further confirm that endotheliopathy is a key mechanism for the resolution and persistence of symptoms after recovery from COVID-19 [31]. Attention is also drawn to the fact that functional disorders were observed in young patients in the absence of detected prothrombotic changes, while the authors of studies involving older patients after incurred COVID-19 emphasize changes in coagulation processes and the risk of developing thrombotic complications. Thus, von Meijenfeldt et al. [32] associated vascular damage with systemic hypercoagulation and a hypofibrinolytic state. In 52 COVID-19 convalescents (mean age 59 years), persistent prothrombotic changes (increased ability to generate thrombin and decreased fibrinolytic potential of plasma) were observed during a 4-month follow-up compared to healthy controls, although PT, V factor, VWF, fibrinogen, D-dimer and thrombin-antithrombin levels normalized after 4 months of follow-up to levels similar to those found in healthy controls. The prothrombotic state was significantly worse (as measured by reduced thrombin generation time, increased endogenous thrombin potential, and peak thrombin level) in COVID-19 convalescents (n=50, mean age 50 $\pm$ 17 years) up to 10 weeks after acute SARS-CoV-2 infection (median follow-up 68 days), compared to controls in the study by Fogarty et al.[11] It is interesting that the authors of the study suggested

that prothrombotic changes may contribute to the further loss of normal EC rest [11]. In their subsequent study, these authors added that EC activation and plasma hypercoagulability, as assessed by thrombin generation assays, persisted up to 8 months after hospital discharge and normalized in 1 year, while the hyperfibrinolytic state persisted even after 12 months. That is, in patients >50 years of age, in one year after an incurred COVID-19, the fibrin level can be one of the effective indirect markers of endotheliopathy [33].

As for cardiovascular risk factors, it has been proven that they, along with cardio-metabolic factors, are significantly associated with the development, course and duration of systemic endotheliopathy. Thus, Chioh et al. [34] demonstrated that in patients who had incurred COVID-19, endothelial disorders increased with the growth of CVR, and in the presence of a similar CVR factor with patients in the control group, these disorders were more pronounced in patients with incurred COVID-19 and pre-existing diseases (arterial hypertension, diabetes mellitus).

Hospitalization during the acute phase of COVID-19 is another, but no less important, risk factor for endotheliopathy. Santoro et al. [35] followed up 658 patients with COVID-19 three months after a negative SARS-CoV-2 molecular test and found an association between the risk of ED and the severity of COVID-19. A significant decrease in FMD was observed with increasing severity of COVID-19, and ED was more common among patients who were hospitalized during the acute period of illness compared to those treated at home (78.3% vs 21.7%).

Hospitalization due to COVID-19 as a risk factor is likely due to the fact that such patients have a significantly increased pro-inflammatory state (including the development of a cytokine storm) and usually a significantly higher viral load. A significant number of viral particles and proteins causes, first of all, more pronounced damage to the glycocalyx and negatively affects the tight junctions of the ECs. Pronounced inflammation causes significant damage to the endothelium during the acute period of COVID-19 and adversely affects the state and function of the endothelium until these levels normalize. Chioh and colleagues [34] identified increased levels of pro-inflammatory cytokines such as IL-1β, IL-17A, IL-2, and RANTES in the early phase of recovery from COVID-19, which was associated with increased levels of circulating ECs. The obtained data indicate the development of cytokine-driven endothelial disorders in patients who have incurred COVID-19. Fan et al. [36] evaluated 39 patients (mean age 43 years) in 12.7 ± 3.6 months after recovery from COVID-19 and found that hypercoagulation, ED, and inflammation were still detectable in some patients approximately 1 year after recovery from COVID-19. Increased levels of D-dimer, factor VIII levels, IL-6, vWF:Ag and ICAM-1 levels were detected in 17.9%, 48.7%, 35.9%, 17.9% and 7.7% of patients, respectively, with higher mean levels compared to the control group. Thrombin generation (thromboscreening) showed higher mean endogenous thrombin potential (ETP) and higher mean peak height and delayed retention time compared to controls. Interestingly, the persistence of elevated D-dimer levels is not always accompanied by endothelial dysfunction among elderly patients. In a study by Townsend and colleagues [37], D-dimer levels (>500 ng/ml) were observed in 25.3% of 150 subjects (mean age 47.3 years) for up to 4 months after SARS-CoV-2 infection and were associated with a more severe course and elderly age. However, markers of coagulation and inflammation that may be associated with endothelial damage and dysfunction (namely prothrombin time, activated partial thromboplastin time, fibrinogen, platelet count, C-reactive protein, IL-6, and sCD25) normalized in 90% patients within 80.5 (range 44–155) days after initial diagnosis.

Also important is the fact that an elevated level of acute phase markers is not a mandatory phenomenon during screening in patients with a history of COVID-19. Disturbance of inflammatory processes in the acute period can cause the emergence of endotheliopathy, which will persist even after the normalization of inflammatory markers in COVID-19 convalescents. For example, in the above study, markers of endotheliopathy (anti-von Willebrand factor (VWF), VWF propeptide, factor VIII, plasma levels of soluble thrombomodulin) were significantly elevated in COVID-19 convalescents compared to controls, but long-term endotheliopathy in these convalescent COVID-19 patients did not depend on the acute phase response or active NETosis (this is a unique form of cell death characterized by the release of decondensed chromatin and granular contents into the extracellular space) [11]. Moreover, endothelial damage caused by COVID-19 may even increase in the post-infection period as measured by the logarithmic index of reactive hyperemia, an indicator of endothelium-mediated dilatation of peripheral arteries (FMD), which was confirmed in a study by Mejia-Renteria et al. [38].

It is still unclear whether there is a relationship between certain markers of endothelial damage or dysfunction at different time intervals after the

infection. In addition, no studies were found that compared patients with existing endotheliopathies with/without a history of COVID-19.

In summary, the development and progression of systemic endotheliopathies in patients after an incurred COVID-19 depends on a number of risk factors associated with both the acute period of COVID-19 and the recovery period. Gender, elderly age, the severity of COVID-19 acute period, and the presence of comorbid pathology can be distinguished among unmodified risk factors. Cardiovascular risk factors are among the only modifiable risk factors for the development of endotheliopathies. Therefore, their correction is an effective means of both the prevention of endotheliopathy in patients with COVID-19 and the treatment of convalescent patients. The best studied among the endotheliopathy markers in young patients are functional ones (flow-mediated dilation, single passive limb movement, peak blood velocity following cuff release), while in elderly patients, screening for markers of prothrombotic changes (thrombin-generating capacity, plasma fibrinolytic potential, endogenous thrombin potential, peak thrombin, fibrin level) prevails. An additional indicator of endotheliopathy can be the presence of long-Covid syndrome symptoms.

Prevention of endotheliopathies in COVID-19 patients

There is still no recommended list of prophylactic measures to prevent endotheliopathies not only in patients with COVID-19, but also in general cases. However, taking into account the above risk factors for the development and progression of endotheliopathies in patients with COVID-19 or COVID-19 convalescents, it can be argued that effective methods of prevention in this category of patients primarily include lifestyle correction and the prescription of "metabolic protectors" in order to eliminate or reduce cardiometabolic risks. Given the relationship between inflammatory markers and endothelial damage during the COVID-19 acute period, it can be assumed based on available studies that timely anti-inflammatory therapy can also be an effective prophylaxis of the development of endotheliopathies.

It can also be assumed that both in convalescents and in patients with acute COVID-19, nutritional correction with the prescription of vitamins and a certain number of amino acids can be extremely effective in preventing damage to the endothelium and its dysfunction even before the initial screening of endotheliopathies with patients' concomitant therapy. At the same time, the prescription of medicamental anti-inflammatory or antioxidant therapy, which is used to correct endothelial dysfunction, may be ineffective without prior evaluation.

There are many studies that support the role of deficiencies of vitamins D and B, as well as C and A with antioxidant properties in patients with COVID-19 in the development of ED. In addition, the prescription of vitamin therapy as a preventive measure is widespread both in healthy individuals and in patients with various infectious and non-infectious diseases, while the use of amino acids for this purpose is not common. There are currently no studies examining the effect of prescribing amino acids in COVID-19 patients or convalescents with the aim of influencing the development of endothelial dysfunction. Therefore, the efficacy of such therapy can be asserted on the basis of general data regarding the prescription of amino acids and data regarding the effect on the ED development in healthy individuals or patients with non-infectious diseases and elderly patients, because they belong to the risk category for the development of endotheliopathies.

Thus, for patients who are COVID-19 convalescents or who belong to the group with the long-term COVID-19 course and have existing risk factors for endotheliopathies, it is especially important to understand exactly which amino acid doses are recommended for healthy individuals, the decrease of which amino acid levels may be associated with the development of endotheliopathy, as well as which products and in what quantity can be useful for consumption in this case.

For a general assessment of the amount of amino acids necessary for the human body, three separate terms should be distinguished: 1) "metabolic need" is a magnitude of protein deposition, which uses dietary amino acids; 2) "dietary need" determines the amount of protein that must be taken to support metabolic needs; and 3) “recommended dietary intake (safe level)” corresponds to the dietary requirement plus 2SD, i.e., the intake that would meet the metabolic needs of 97.5% of the population.

Usually, the metabolic need for amino acids is determined based on the assessment of the magnitude of net protein synthesis, nitrogen balance and amino acid oxidation. The metabolic need for amino acids is not a constant value, it depends on age (which reflects the stage of development and reproductive state), gender and the state of the body itself. Therefore, the lifestyle (sedentary, active) and the presence of chronic

conditions directly affect changes in the body's metabolic needs.

The dietary need, which is necessary to support the metabolic need, is influenced by the digestibility of amino acids (due to the processes of digestion and absorption) and the amino acid pattern of the dietary protein. Therefore, aging processes, human genotype and various diseases that change the efficacy of absorption and the rate of metabolism of amino acids also affect the dietary need for amino acids. In order to eliminate significant differences in dietary and metabolic needs, the recommended level of daily acid consumption is established experimentally and usually among healthy individuals, while for all others it is necessary to independently take into account changes in the amino acid needs due to age, pathological processes and hereditary factors.

The best known recommendations regarding the required amount of amino acids in the diet are the recommendations of the Food and Nutrition Board (FNB) and the Institute of Medicine (IOM) [39].

Among amino acids, the purpose of which can have a positive effect on the endothelium, we can highlight arginine, betaine, glutamine, glycine, histidine, acetate, carnitine. Arginine and glutamine are the main amino acids that are necessary to support proliferative and vasodilatory functions of EC. Therefore, their additional consumption can improve the endothelial function and prevent the development of endotheliopathies. Arginine, a substrate of eNOS, is necessary for the production of vasoprotective NO [40,41]. One study found a low arginine to ornithine ratio in patients with Covid-19, further suggesting a possible benefit of arginine intake among patients suffering from Covid-19 [42]. The use of L-citrulline (substrate for recycling l-arginine) can contribute to the improvement of endothelial dysfunction due to the ability to increase the level of L-arginine, which indicates characteristics for the prevention of endotheliopathies [43].

One of the possible mechanisms of the development of endothelial dysfunction in CNCD may be an increase in the level of asymmetric dimethylarginine (ADMA), an endogenous inhibitor of NO-synthase. ADMA, being an endogenous structural analogue of L-arginine, inhibits the activity of NO synthase in vitro and in vivo, leading to a decrease in NO production and an increase in the risk of developing cardiovascular diseases. ADMA is released during proteolysis of proteins methylated on arginine residues. Methylation is catalyzed by the enzyme protein-arginine N-methyltransferase (PAMT) 1. ADMA can be excreted in an unchanged form by the kidneys, but the main route of ADMA catabolism is hydrolysis. About 80% of ADMA is hydrolyzed by the enzyme dimethylarginine dimethylaminohydrolase (DDAG) to form citrulline.

Elevated ADMA levels have been shown to be an independent risk factor for both cardiovascular events and all-cause mortality in patients with CNCD. Exogenous arginine can increase NO production even when NOS is oversaturated, a phenomenon known as the arginine paradox. This may be due to the presence of the NOS inhibitor, ADMA, which competitively binds NOS. Adding arginine further increases its concentration in the body due to the increased rate of arginine binding to NOS. Therefore, based on this phenomenon, arginine supplementation may be a potential therapeutic approach for the treatment of age-related vascular diseases.

However, the inconsistency of the existing data is probably related to heterogeneous cohorts of the study subjects in terms of age, the number of CNCDs they have, and the dosage of arginine. All this, of course, requires additional studies.

Today, there is indisputable evidence of the adverse effect of ADMA on a number of pathological conditions, which are associated with both blood vessels and age: elevated ADMA plasma levels are positively correlated with risk factors for the development of atherosclerosis; the positive correlation of ADMA with age in individuals without coronary or peripheral artery symptoms is confirmed [44].

It has been suggested that high ADMA levels may accelerate the loss of telomerase activity, enhance cellular senescence, and induce ED. This is a new mechanism to explain the association of ADMA and ED with an increased risk of cardiovascular events. In addition, Celermajer et al. [45] demonstrated that aging is associated with progressive ED in the absence of disease. Aging of the endothelium is associated with a decrease in the eNOS expression and activity, as well as with increased adhesion of the endothelium to monocytes. These effects can be prevented by stable transfection of endothelial cells of human telomerase.

Amino acids that can prevent damage to the endothelium can also include those that reduce the severity of inflammatory processes. It is known that endothelial dysfunction, as well as arterial stiffness, does not fully normalize in COVID-19 patients, but partially decreases in 48 weeks after the onset of the COVID-19 disease, and this decrease depends on the level of C-reactive protein at the time of hospitalization of COVID-19 patients [46]. Therefore, the impact on inflammatory markers in patients with

COVID-19, both in the acute period, in convalescent patients, and in long-term COVID-19, may be important for the prevention of the development of endotheliopathies.

Betaine is an amino acid that can be safely used for this purpose. It is known as trimethylglycine and is a stable and nontoxic natural substance that can be accumulated in large quantities in the cell without affecting its functions. The anti-inflammatory mechanism of betaine is realized through the inhibition of NF-κB and NLRP3 inflammasome activity [47]. In a study of 29 relatively healthy individuals, mean age 15.5 ± 0.3 years, 14 weeks of betaine intake prevented the increase of pro-inflammatory cytokines (TNF-α and IL-6) levels. This fact may be useful to consider this effect for patients with COVID-19 in terms of endotheliopathy prevention and life expectancy [48]. Carnitine can also be effective for the prevention of endotheliopathies due to its positive effect on the general functional and organic condition of patients, especially the elderly. This is supported by the results of studies on the reduced level of carnitine in elderly patients with frailty syndrome and the positive effect of carnitine administration on the course of this syndrome [49,50]. A study in men with frailty syndrome (asthenia) found that the frailty phenotype was characterized by lower concentrations of isovalerylcarnitine, higher levels of octanoyl-L-carnitine, decanoyl-L-carnitine, dodecanoyl-L-carnitine, and tetradecanoyl-L-carnitine. Metabolic profiles with measurement of glycine and tryptophan levels can distinguish elderly men with frailty from people without frailty [51]. Similar results on the increased risk of developing frailty syndrome in the event of carnitine metabolism disorders were obtained in another study [50]. A high level of carnitine had a beneficial effect on functional status and contributed to the treatment of frailty syndrome in elderly patients [49].

The presented studies confirm the relationship between endothelial dysfunction parameters and a number of amino acids. However, it is still unknown which doses of amino acids and during which time period can and should be prescribed for the purpose of prevention of endotheliopathies.

• For L-arginine, the dose is up to 20 g/day, and several short-term studies indicate no serious side effects even when taking higher doses. The longest duration of use for the purpose of ED correction in studies was 18 months with a dosage of 6.4 g/day, and the highest dosage administered within a long time was 20 g/day for 28 days (however, in this case, no results of a significant effect on endothelial function were found);

• L-glutamine – up to 20 g/day

• Betaine – there is no maximum and daily standard; usually, the dose of 0.324 to 3 g/day is used

• L-carnitine – up to 6 g/day.

The fact that the pace of life of modern people, their lifestyle with typically limited physical activity allows to compensate for the need for amino acids with food in order to maintain health and prevent vascular aging is doubtful.

It is very important to understand what causes the development of endotheliopathy – whether it is a result of an incurred COVID-19 infection, of or it has already formed in a patient with age, or as a result of existing CNVDs.

Therefore, we present our own data of a substudy evaluating the efficacy of using Betargin in patients with mild arterial hypertension who suffered a mild form of COVID-19 infection.

A total of 18 patients with mild hypertension and a history of COVID-19 (no more than 0.5 years since the disease resolution) were examined. The median age was 54.5 [47.5; 67] years, the patients, in addition to the diet and the use of one antihypertensive drug (perindopril or ramipril), took L-Betargin, 1 stick x 3 times/day for 6 weeks. Inflammatory markers C-reactive protein (CRP) and tumor necrosis factor-alpha (TNF-α) were measured and evaluated, as well as aging markers – percentage (%) of DNA methylation based on measurement of 5-methylcytosine (5-MC) and telomere length (TL) in the blood.

At the beginning of the study, the levels of evaluated parameters were as follows: CRP 5.91 [1.5; 6.77] mg/l versus the control 1.2 [0.8; 1.7] mg/l, $p<0.001$; TNF-α 4.26 [1.96; 8.03] pg/ml versus the control 2.10 [1.54; 2.63] pg/ml, $p<0.001$; 5-MC 1.36 [1.05; 2.66] % versus the control 3.10 [1.87; 4.62] %, $p<0.001$; TL 0.89 [0.64; 1.11] versus the control 0.95 [0.75; 1.48], $p<0.01$. That is, an increase was noted in 6 months after incurred COVID-19 in the absence of any somatic pathology except hypertension, and in the absence of complaints, except for general weakness, confirmed increase in inflammatory markers, the presence of hypomethylation processes and a reduction in TL – the latter may indicate the initiation of accelerated aging processes.

After 6 weeks of administration of the relevant medicines, CRP level decreased by 1.75 [-0.69; 5.69] mg/l, and TNF level decreased by 0.24 [-1.5; 0.92] pg/ml; 5-MC % and TL levels remained unchanged.

After 3 months from the start of taking the medicines, significant changes occurred: CRP level

decreased by -3.05 [-5.09; 0.95] mg/l, TNF decreased by -2.14 [-4.10; 0.78] pg/ml, 5-MC indicators, %, decreased by - 2.48 [1.12; 3.05] % versus 1.36 [1.05; 2.66]%), and TL level decreased by - 0.92 [0.78; 1.24] versus 0.89 [0.64; 1,11]) – a tendency to improvement was demonstrated, which indicates, in our opinion, the beginning of the formation of a "healthy phenotype".

Therefore, the obtained data allow us to assume that the addition of the amino acids betaine, arginine and carnitine due to anti-inflammatory, antihypoxic, and detoxifying effects reduces the severity of inflammatory processes in patients with COVID-19 and can probably prevent damage to the endothelium, which requires additional studies in the future with the involvement of the majority of patients with the assessment of biological age indicators, ED and vascular stiffness. The use of Betargin (arginine, betaine, carnitine) in patients who suffered from COVID-19 demonstrated an existing anti-inflammatory effect that was realized over time, which, in our opinion, is important for the prevention of the development of endotheliopathies, and changes in age indicators confirm its metabolic protective effect.

Conclusions:

1) An incurred COVID-19 infection increases the risk of various pathological conditions in young and middle age, as well as the risk of increased comorbidity in the elderly, separately affecting the acceleration of aging, which in the future may lead to an increase in CNCDs and, as a result, further increase in mortality rates.
2) The development of endotheliopathy can explain the development of complications among COVID-19 patients and manifest itself in blood rheology disorders, namely, changes in coagulation processes and the development of endothelial dysfunction, which implies the development and progression of pathological conditions, and on the other hand, directly affect the reduction of life expectancy among the population.
3) Female gender, elderly age, the presence of cardiovascular risks and comorbidities, hospitalization due to COVID-19 are risk factors for the development of endotheliopathy.
4) The presence of long-Covid syndrome symptoms can be one of the indicators of existing endotheliopathy.
5) Along with the correction of existing cardiometabolic risk factors in COVID-19 patients and convalescents, timely supplementation of vitamins (A, B, C, D) and amino acids (arginine, betaine, carnitine) is necessary for the prevention of endotheliopathies and probably can have a positive effect on increasing disease-free life expectancy, i.e. healthy longevity.

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References

1. Leonhardt D. Life Expectancy, Falling Covid is a terrible health crisis. It's not the country's only health crisis. The New York Times. 2021 July 22.
2. Aburto JM, Schöley J, Kashnitsky I, et al. Quantifying impacts of the COVID-19 pandemic through life-expectancy losses: a population-level study of 29 countries. *Int J Epidemiol*. 2022;51(1):63-74. doi: 10.3390/ijms23116196.
3. Slater TA, Straw S, Drozd M, Kamalathasan S, Cowley A, Witte KK. Dying 'due to'or 'with'COVID-19: a cause of death analysis in hospitalised patients. *Clin Med*. 2020;20(5):e189-e190. doi: 10.7861/clinmed.2020-0440.
4. Malik RJ. Across regions: Are most COVID-19 deaths above or below life expectancy? *Germs*. 2021;11(1):59. doi: 10.18683/germs.2021.1241.
5. Mongelli A, Barbi V, Gottardi Zamperla M, et al. Evidence for biological age acceleration and telomere shortening in COVID-19 survivors. *Int J Mol Sci*. 2021;22(11):6151. doi: 10.3390/ijms22116151.
6. Cao X, Li W, Wang T, et al. Accelerated biological aging in COVID-19 patients. *Nat Commun*. 2022;13(1):1-7. doi: 10.1038/s41467-022-29801-8.
7. Flaumenhaft R, Enjyoji K, Schmaier AA. Vasculopathy in COVID-19. *Blood*. 2022;140(3):222-235. doi: 10.1182/blood.2021012250.
8. Six I, Guillaume N, Jacob V, et al. The Endothelium and COVID-19: An Increasingly Clear Link Brief Title: Endotheliopathy in COVID-19. *Int J Mol Sci*. 2022;23(11):6196. doi: 10.3390/ijms23116196.
9. Varga Z, Flammer AJ, Steiger P, et al. Endothelial cell infection and endotheliitis in COVID-19. *Lancet*. 2020;395(10234):1417-1418. doi: 10.1016/S0140-6736(20)30937-5.
10. Krishnamachary B, Cook C, Spikes L, Chalise P, Dhillon NK. The potential role of extracellular vesicles in COVID-19 associated endothelial injury and pro-inflammation. *medRxiv*. 2020;2020.08.27.20182808. doi: 10.1101/2020.08.27.20182808. Preprint
11. Fogarty H, Townsend L, Morrin H, et al. Persistent endotheliopathy in the pathogenesis of long COVID syndrome. *J Thromb Haemost*. 2021;19(10):2546-2553. doi: 10.1111/jth.15490.
12. Roncati L, Ligabue G, Fabbiani L, et al. Type 3 hypersensitivity in COVID-19 vasculitis. *Clin Immunol*. 2020;217:108487. doi: 10.1016/j.clim.2020.108487.
13. Martínez-Salazar B, Holwerda M, Stüdle C, et al. COVID-19 and the vasculature: current aspects and long-term consequences. *Front Cell Dev Biol*. 2022;10. doi: 10.3389/fcell.2022.824851.
14. Goshua G, Pine AB, Meizlish ML, et al. Endotheliopathy in COVID-19-associated coagulopathy: evidence from a single-centre, cross-sectional study. *Lancet Haematol*. 2020;7(8):e575-582. doi: 10.1016/S2352-3026(20)30216-7.
15. Pine AB, Meizlish ML, Goshua G, et al. Circulating markers of angiogenesis and endotheliopathy in COVID-19. *Pulm Circ*. 2020;10(4):2045894020966547. doi: 10.1177/2045894020966547.
16. Rauti R, Shahoha M, Leichtmann-Bardoogo Y, et al. Effect of SARS-CoV-2 proteins on vascular permeability. *Elife*. 2021;10:e69314. doi: 10.7554/eLife.69314.
17. Bikdeli B, Madhavan MV, Jimenez D, et al. COVID-19 and thrombotic or thromboembolic disease: implications for prevention, antithrombotic therapy, and follow-up: JACC state-of-the-art review. *J Am Coll Cardiol*. 2020;75(23):2950-2973. doi: 10.1016/j.jacc.2020.04.031.
18. Yamaoka-Tojo M. Endothelial glycocalyx damage as a systemic inflammatory microvascular endotheliopathy in COVID-19. *Biomed J*. 2020;43(5):399-413. doi: 10.1016/j.bj.2020.08.007.
19. McConnell MJ, Kondo R, Kawaguchi N, Iwakiri Y. Covid-19 and Liver Injury: Role of Inflammatory Endotheliopathy, Platelet Dysfunction, and Thrombosis. *Hepatol Commun*. 2022;6(2):255-269. doi: 10.1002/hep4.1843.
20. Rosell A, Havervall S, Von Meijenfeldt F, et al. Patients with COVID-19 have elevated levels of circulating extracellular vesicle tissue factor activity that is associated with severity and mortality—brief report. *Arterioscler Thromb Vasc Biol*. 2021;41(2):878-882. Doi: 10.1161/ATVBAHA.120.315547.
21. Jing H, Zuo N, Novakovic VA, Shi J. The Central Role of Extracellular Vesicles in the Mechanisms of Thrombosis in COVID-19 Patients With Cancer and Therapeutic Strategies. *Fron Cell Dev Biol*. 2021;9. doi: 10.3389/fcell.2021.792335.

22. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*. 2020;395(10223):497-506. doi: 10.1016/S0140-6736(20)30183-5.
23. Libby P, Lüscher T. COVID-19 is, in the end, an endothelial disease. *Eur Heart J*. 2020;41(32):3038-3044. doi: 10.1093/eurheartj/ehaa623.
24. Fogarty H, Ward SE, Townsend L, et al.; Irish COVID-19 Vasculopathy Study (iCVS) Investigators. Sustained VWF-ADAMTS-13 axis imbalance and endotheliopathy in long COVID syndrome is related to immune dysfunction. *J Thromb Haemost*. 2022;10.1111/jth.15830. doi: 10.1111/jth.15830.
25. Sollini M, Ciccarelli M, Cecconi M, et al. Vasculitis changes in COVID-19 survivors with persistent symptoms: an [18F] FDG-PET/CT study. *Eur J Nucl Med Mol Imaging*. 2021;48(5):1460-1466. doi: 10.1007/s00259-020-05084-3.
26. Becker RC. COVID-19-associated vasculitis and vasculopathy. *J Thromb Thrombolysis*. 2020;50(3):499-511. doi: 10.1007/s11239-020-02230-4.
27. Whitaker M, Elliott J, Chadeau-Hyam M, et al. Persistent COVID-19 symptoms in a community study of 606,434 people in England. *Nat Commun*. 2022;13(1):1-0. doi: 10.1038/s41467-022-29521-z.
28. Tong M, Yan X, Jiang Y, et al. Endothelial biomarkers in patients recovered from COVID-19 one year after hospital discharge: A cross-sectional study. *Mediterr J Hematol Infect Dis*. 2022;14(1). doi: 10.4084/MJHID.2022.033.
29. Fernández-de-Las-Peñas C, Martín-Guerrero JD, Pellicer-Valero ÓJ, et al. Female sex is a risk factor associated with long-term post-COVID related-symptoms but not with COVID-19 symptoms: The LONG-COVID-EXP-CM multicenter study. *J Clin Med*. 2022;11(2):413. doi: 10.3390/jcm11020413.
30. Ratchford SM, Stickford JL, Province VM, et al. Vascular alterations among young adults with SARS-CoV-2. *Am J Physiol Heart Circ Physiol*. 2021;320(1):H404-H410. doi: 10.1152/ajpheart.00897.2020.
31. Nandadeva D, Young BE, Stephens BY, et al. Blunted peripheral but not cerebral vasodilator function in young otherwise healthy adults with persistent symptoms following COVID-19. *Am J Physiol Heart Circ Physiol*. 2021;321(3):H479-484. doi: 10.1152/ajpheart.00368.2021.
32. von Meijenfeldt FA, Havervall S, Adelmeijer J, et al. Sustained prothrombotic changes in COVID-19 patients 4 months after hospital discharge. *Blood Adv*. 2021;5(3):756-759. doi: 10.1182/bloodadvances.2020003968.
33. von Meijenfeldt FA, Havervall S, Adelmeijer J, Thalin C, Lisman T. Persistent endotheliopathy in the pathogenesis of long COVID syndrome: Comment from von Meijenfeldt et al. *J Thromb Haemost*. 2022;20(1):267-269. doi: 10.1111/jth.15580.
34. Chioh FW, Fong SW, Young BE, et al. Convalescent COVID-19 patients are susceptible to endothelial dysfunction due to persistent immune activation. *Elife*. 2021;10:e64909. doi: 10.7554/eLife.64909.
35. Santoro L, Falsetti L, Zaccone V, et al. Impaired Endothelial Function in Convalescent Phase of COVID-19: A 3 Month Follow Up Observational Prospective Study. *J Clin Med*. 2022;11(7):1774. doi: 10.3390/jcm11071774.
36. Fan BE, Wong SW, Sum CL, et al. Hypercoagulability, endotheliopathy, and inflammation approximating 1 year after recovery: Assessing the long-term outcomes in COVID-19 patients. *Am J Hematol*. 2022;97(7):915-923. doi: 10.1002/ajh.26575.
37. Townsend L, Fogarty H, Dyer A, et al. Prolonged elevation of D-dimer levels in convalescent COVID-19 patients is independent of the acute phase response. *J Thromb Haemost*. 2021;19(4):1064-1070. doi: 10.1111/jth.15267.
38. Mejia-Renteria H, Travieso A, Sagir A, et al. In-vivo evidence of systemic endothelial vascular dysfunction in COVID-19. *Int J Cardiol*. 2021;345:153-155. doi: 10.1016/j.ijcard.2021.10.140.
39. Trumbo P, Schlicker S, Yates AA, Poos M; Food and Nutrition Board of the Institute of Medicine, The National Academies. Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids. *J Am Diet Assoc*. 2002;102(11):1621-1630. doi: 10.1016/s0002-8223(02)90346-9.
40. Xu S, Ilyas I, Little PJ, et al. Endothelial dysfunction in atherosclerotic cardiovascular diseases and beyond: from mechanism to pharmacotherapies. *Pharmacol Rev*. 2021;73(3):924-967. doi: 10.1124/pharmrev.120.000096.
41. Gambardella J, Khondkar W, Morelli MB, Wang X, Santulli G, Trimarco V. Arginine and endothelial function. *Biomedicines*.

2020;8(8):277. doi: 10.3390/biomedicines8080277.

42. Rees CA, Rostad CA, Mantus G, et al. Altered amino acid profile in patients with SARS-CoV-2 infection. *Proc Natl Acad Sci*. 2021;118(25):e2101708118. doi: 10.1073/pnas.210170811.
43. Allerton TD, Proctor DN, Stephens JM, Dugas TR, Spielmann G, Irving BA. l-Citrulline supplementation: impact on cardiometabolic health. *Nutrients*. 2018;10(7):921. doi: 10.3390/nu10070921.
44. Miyazaki H, Matsuoka H, Cooke JP, et al. Endogenous nitric oxide synthase inhibitor: a novel marker of atherosclerosis. *Circulation*. 1999;99(9):1141-1146. doi: 10.1161/01.cir.99.9.1141.
45. Celermajer DS, Sorensen KE, Spiegelhalter DJ, Georgakopoulos D, Robinson J, Deanfield JE. Aging is associated with endothelial dysfunction in healthy men years before the age-related decline in women. *J Am Coll Cardiol*. 1994;24(2):471-476. doi: 10.1016/0735-1097(94)90305-0.
46. Zanoli L, Gaudio A, Mikhailidis DP, et al. Vascular dysfunction of COVID-19 is partially reverted in the long-term. *Circ Res*. 2022;130(9):1276-1285. doi: 10.1161/CIRCRESAHA.121.320460.
47. Zhao G, He F, Wu C, et al. Betaine in inflammation: mechanistic aspects and applications. *Front Immunol*. 2018;9:1070. doi: 10.3389/fimmu.2018.01070.
48. Nobari H, Cholewa JM, Pérez-Gómez J, Castillo-Rodríguez A. Effects of 14-weeks betaine supplementation on pro-inflammatory cytokines and hematology status in professional youth soccer players during a competition season: a double blind, randomized, placebo-controlled trial. *J Int Soc Sports Nutr*. 2021 Dec;18(1):1-0. doi: 10.1186/s12970-021-00441-5.
49. Malaguarnera G, Catania VE, Bonfiglio C, Bertino G, Vicari E, Malaguarnera M. Carnitine Serum Levels in Frail Older Subjects. Nutrients. 2020;12(12):3887. doi: 10.3390/nu12123887.
50. Rattray NJW, Trivedi DK, Xu Y, et al. Metabolic dysregulation in vitamin E and carnitine shuttle energy mechanisms associate with human frailty. *Nat Commun*. 2019;10(1):5027. doi: 10.1038/s41467-019-12716-2.
51. Meng L, Shi H, Wang DG, et al. Specific Metabolites Involved in Antioxidation and Mitochondrial Function Are Correlated With Frailty in Elderly Men. *Front Med* (Lausanne). 2022;9:816045. doi: 10.3389/fmed.2022.816045.