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Long Chain Polyunsaturated Fatty Acids and Their Role in COVID 19-associated Inflammation and Dysbiosis

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ABSTRACT

The COVID-19 pandemic has been an on-going global health problem since it started more than three years ago. Efficient treatment and its emerging long-term effects remain unresolved. The primary cause of COVID-19 deaths is the excessive immune response resulting in a cytokine storm leading to eventual organ failure. Dysbiosis has been observed in patients infected with SARS-CoV-2, promoting further inflammation and increasing risks for long COVID. Most of the current treatments for COVID-19 including vaccines are directed against the virus and its replication. However, these treatments need to be administered before viral infection (in the case of vaccines) or early in the infection process. Moreover, these treatments do not address the inflammatory phase of the disease, where cytokine storm increases the risk of death or may be a significant contributor to long COVID. Omega-3 fatty acids which are components of cell membranes are known to be precursors of anti-inflammatory compounds and aid in the regulation of the human microbiome. This article presents a hypothesis that posits the role of long-chain polyunsaturated fatty acids in modulating COVID-19 inflammation via the regulation of the lung and gut microbiomes, which have been associated with the body's immune response to SARS-CoV-2 infection. With known safety and benefits of omega-3 fatty acids, supplementation may be effective in preventing severe COVID-19 symptoms, managing mild and moderate symptoms, and may even extend post-recovery to mitigate increased risk for cardiovascular diseases after SARS-CoV-2 infection and recovery.

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COVID-19, SARS-CoV-2, polyunsaturated fatty acids, omega-3, supplementation, microbiome, Dried Blood Spot, inflammation, dysbiosis

1. INTRODUCTION

Corona Virus Disease -19 (COVID-19) has been spreading globally since the end of 2019 and its infection rate continues to spike two years after the first case was identified. This acute atypical respiratory disease originated from Wuhan, China via zoonotic transmission [1]. This later led to a subsequent outbreak resulting in a pandemic. According to the World Health Organization's (WHO) weekly epidemiological report as of 5 February 2023, the number of global COVID-19 confirmed cases has reached 754 million, with over 6.8 million deaths documented. Europe, Western Pacific, and the Americas are the three regions with the most cases having more than 271 million, 200 million, and 189 million confirmed cases, respectively. With these confirmed cases, cumulative deaths in Europe are 32% of confirmed cases, while Western Pacific and America have 6% and 43%, respectively [2]. Many countries have experienced several infection waves that resulted in mutations on the original virus strain giving rise to several virus variants. The Omicron Variant, which is currently the most dominant variant [2], replaces other variants of concern such as the Alpha, Beta, and Delta Variant [3].

Various viral genome analyses have confirmed that the pandemic is caused by a novel virus that is an enveloped, positive-sense, single-stranded RNA virus with high homology to SARS-CoV, hence the viral name Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) [4]. The genome of coronaviruses encodes four structural proteins: spike, membrane, envelope, and nucleocapsid. Among these structural proteins, the spike protein is the determinant of diversity among the viral species. Moreover, this protein includes two subunits, namely the S1 subunit and the S2 subunit, which are responsible for host cell receptor binding and viral and cellular membrane fusion, respectively. The SARS-CoV-2 undergoes a general infection cycle: attachment, penetration, replication, maturation, and release (Figure 1) [4].

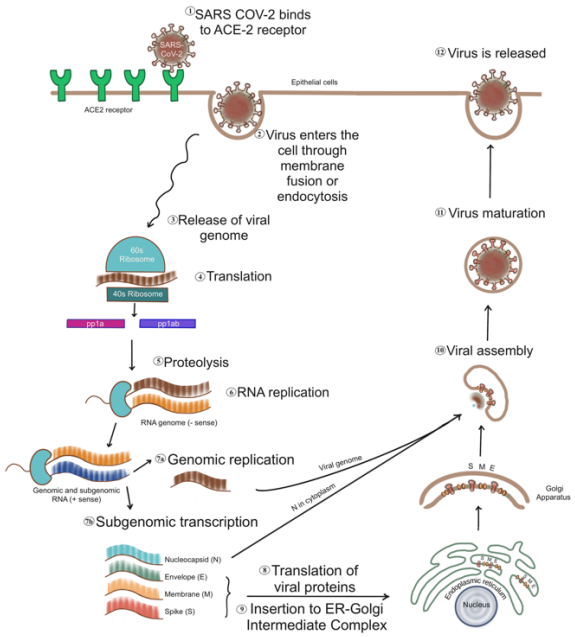

Figure 1: The infection cycle of SARS-CoV-2 inside the host. Once inside the host, (1) the virus will attach to the ACE-2 receptor and (2) enter the cell via membrane fusion or endocytosis. This will be followed by the (3) release of the viral genome and (4) translation of the viral RNA. Translation will then yield two protein products: pp1a and pp1ab which will then undergo (5) proteolysis and (6) replication through viral polymerase. (12) Before the virus is released from the cell, it will undergo stages 7-11 showing the replication of genomic RNA, translation of viral proteins, assembly, and maturation of the virus.

1.1 COVID-19 Pathogenesis

SARS-CoV-2 pathogenesis has three phases involving (1) the viral phase, (2) the inflammation phase, and (3) the hyper-inflammation or clotting phase [5,6]. The initial phase involves the rapid replication of the SARS-CoV-2 virus upon its penetration into the host cell. This phase can be characterized by symptoms such as fever, joint and muscle pain, sore throat, loss of taste and smell, as well as diarrhea. This phase is when the viral transmission is at its peak. The second phase occurs at the advent of the host's immune system response against the viral infection. Once the immune response exceeds the normal rate, this will transition into the third phase which is hyperinflammation that can result in more severe symptoms such as chest pain, blood clotting, and acute respiratory distress (Figure 2) [6].

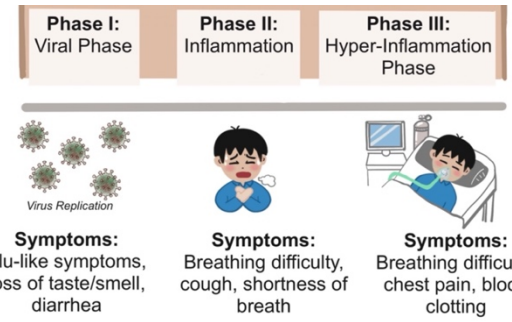

Figure 2: Phases of SARS-CoV-2 infection. Phase one is the viral phase that is characterized by rapid virus replication and is accompanied by flu-like symptoms, loss of taste or smell, and diarrhea. Inflammation is the second phase of infection and patients may show breathing difficulty, cough, and shortness of breath as symptoms. The critical and third phase of infection is hyper-inflammation which is manifested as breathing difficulty, chest pain, and blood clotting. When hyper-inflammation occurs, patients require hospitalization and oxygen support.

Disease severity in COVID-19 patients is not only determined by the extent of viral infection but also by the abnormality of the host response [5]. If there is an imbalanced immune response to SARS-CoV-2 [7], the immune system exhibits an abnormal inflammatory response promoting excessive production of pro-inflammatory cytokines and chemokines. Furthermore, eicosanoids are also observed to have increased amounts that lead to high levels of leukotrienes, prostaglandins, prostacyclins, and thromboxanes, all of which enhance the inflammatory response of the immune system [7,8]. In a study that compared the pro-inflammatory cytokines in the bronchoalveolar lavage fluid between COVID-19 patients and normal adults, high concentration of pro-inflammatory cytokines such as interleukins, tumor necrosis factors, macrophage inflammatory proteins, platelet-derived growth factor, and vascular endothelial growth factor along with others was observed. This confirms the exaggerated inflammatory response to COVID-19 [9,10]. This finding was inferred to be in direct relation to the cytopathic nature of the SARS-CoV-2 as viral replication in epithelial cells initiates an increase in the levels of pyroptosis that further leads to the hyperinflammatory response to SARS-CoV-2 infection [9,10,11].

1.2 Inflammation and polyunsaturated fatty acids (PUFAs)

Omega-3 and Omega-6 long-chain polyunsaturated fatty acids (PUFA) are important building blocks of cellular membranes, whose function is to provide the cell's structural integrity and are considered to be important parts of the membrane. Moreover, these molecules also perform crucial roles in cell signaling and inflammation control [12].

Alpha-linolenic acid (ALA) is an essential omega-3 fatty acid that functions as the precursor for eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), both act as anti-inflammatory agents [13]. However, the production of EPA and DHA in the human body does not suffice the anti-inflammatory demand, thus, are obtained through dietary sources (Figure 3) [14]. On the other hand, the essential omega-6 fatty acid, linoleic acid (LA), is converted to arachidonic acid (AA) via chain elongation and desaturation. AA is considered the most relevant omega-6 PUFA because in contrast to omega-3 PUFAs, omega-6 such as AA are generally pro-inflammatory. Furthermore, the cell membrane contains an abundant amount of AA as it is efficiently synthesized in the human body [15]. Though the mechanisms of how PUFAs regulate the innate and adaptive systems are diverse, it is known that PUFAs have roles in activating cells from these two types of immune systems [16]. Table 1 shows the different lipid mediators that can be derived from AA, EPA, and DHA and their modes of action in the immune response.

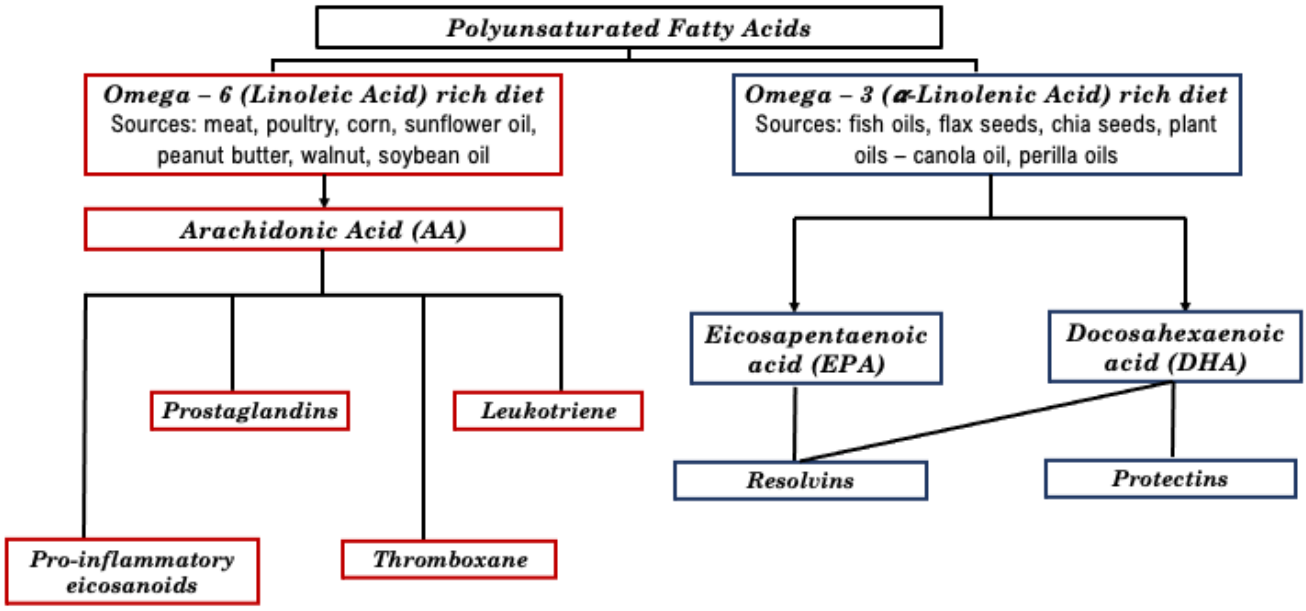

Figure 3: Dietary sources of Polyunsaturated fatty acids and their metabolic pathways.

During tissue injury, AA undergoes enzymatic release via phospholipase A2 enzyme and is utilized as a substrate for the formation of pro-inflammatory eicosanoids, which then activates the accumulation of inflammatory cytokines that emit pro-inflammatory signals toward the site of infection [14]. Simultaneously, DHA, EPA, and their metabolites negate the abnormal rate of inflammation caused by the pro-inflammatory agents. This then balances the inflammatory response during tissue injury or infection to preserve the host's immune system function by preventing the onset of a cytokine storm. Specifically, EPA and DHA are also released from the membrane through another enzymatic release which then forms anti-inflammatory eicosanoids and pro-resolving lipid mediators [14]. Inflammation resolution is then promoted by their derivatives such as epoxyeicosatrienoic acid (EET), an AA-derived molecule, and specialized pro-resolving mediators (SPM), derived from omega-3 fatty acids (FA) primarily EPA, DHA, and docosapentaenoic acid (DPA), and includes resolvins and protectins [14]. These mediators, particularly SPM, promote macrophage phagocytosis of cellular debris hence leading to the inhibition of pro-inflammatory cytokines. SPMs are generally utilized as a novel class of immunoresolvents mainly focused on the inhibition of acute responses and initiation of the clearance of dying cells, tissue pathogens, and remnants [14, 23]. Thus, it is posited that the optimal balance of inflammatory omega-6 and anti-inflammatory omega-3 FA is necessary to ensure normal immune function and prevent diseases or further infections [14].

Table 1: Lipid Mediators derived from Arachidonic Acid (AA), Eicosapentaenoic Acid (EPA), and Docosahexaenoic acid (DHA) and their modes of action in the immune response.

	LIPID MEDIATORS	MODE OF ACTION
AA (Omega-6 PUFAs) DERIVED LIPID MEDIATORS	*Pro-inflammatory eicosanoids*	Induces inflammation responses such as fever, redness, swelling, and pain [17]
	Prostaglandins	Increased synthesis in inflamed tissues and may contribute to the development of acute inflammation [18]
	Thromboxane	Constricts blood vessels which then promotes inflammation and apoptosis [19] Plays significant roles in myocardial infarction as it induces platelet aggregation and narrowing of blood vessels [19]
	Leukotriene	Tightens airway muscles and has roles in the excessive production of mucus in fluid thereby resulting in airway tissue edema [20]

EPA AND DHA DERIVED LIPID MEDIATORS (Omega-3 PUFAs)	***Resolvins***	Blocks production of pro-inflammatory mediators and regulates the movement of leukocytes to inflammation sites [21] Initiates anti-inflammatory and pro-resolving signals to stop further inflammation which may lead to chronic disease [21]
	Protectins	Anti-inflammatory and has protective actions in neural systems [22]

1.3 Inflammation and the Microbiome

Aside from PUFAs, the human microbiome also plays a role in inflammation. The human body is inhabited by a vast number of microbial species as part of the normal microflora, forming a mutualistic effect on each other. In a setting of dysbiosis, inflammation can be triggered. However, in recent studies, it was deduced that the optimal ratio of omega-3 and omega-6 PUFAs has a positive correlation in establishing the optimal microbiome [14]. In the study confirming the link between the immune system and the gut microbiome via long chain polyunsaturated fatty acids (LC-PUFAs), it was found that a decrease in pro-inflammatory bacterial species *Faecalibacterium,* while promoting the accumulation of probiotics such as *Bifidobacteria,* aid in strengthening the gut barrier by regulating the inflammatory response [14, 24-25]. Therefore, differential bacterial taxa may either induce the proinflammatory immune response or counter positive feedback by fostering anti-inflammatory roles, inferring that there is a significant relationship between the lung/gut microflora and LC-PUFA levels [25].

Recent treatments for COVID-19 include the prescription of Remdesivir as an antiviral drug and subjection to hemoperfusion of severely infected COVID-19 patients for cytokine level restoration [26]. Other drugs that are authorized by the FDA to treat mild to moderate COVID-19 patients include Paxlovid [27], Molnupiravir [28], and Casirivimab + Imdevimab [29]. Though these drugs are cost-effective, they can be expensive [30] and are only used when the individual is already infected with SARS-CoV-2 and are limited to control viral replication [26-29]. An adjunct and potentially less expensive measure is to nurture an optimal balance of a microbiome in eubiosis, and favourable levels of LC-PUFAs that will prevent complications from hyperinflammation [14, 25, 31-34]. At the eubiotic state, excessive immune response to SARS-CoV-2 will be prevented and potentially lead to a decline in severe COVID-19 cases and death. This article presents a hypothesis linking the roles of PUFAs on viral immune response and the maintenance of a eubiotic human microbiome as a means to regulate immune response to SARS-CoV-2 infection including a way of measuring such state.

1.4 Role of the immune system in inflammation

The immune system protects the body from agents that may cause harm to it. Without this, the human body would be defenseless against foreign objects that enter and cause harmful body changes. The immune system is composed of different organs, cells, and proteins. During the early stage of infection, cells of the innate immune system such as neutrophils and macrophages recognize the conserved characteristics of pathogens [35]. Neutrophils are short-lived but play a very important role in immune response as it is the first leukocyte to arrive at the site of injury, trying to eliminate the pathogens and initiate an inflammatory response [36]. Cytokines and chemokines are produced to mobilize neutrophils and macrophages in the body, thereby, activating local cellular response [37,38]. If the infection does not subside, the lymphocytes generate receptors with immunoglobulin-like structures which are highly adapted to the pathogen [35]. This also creates immunological memory to make specific responses against the pathogen and remember it [39]. Thus, in cases of reinfection, the immune system will have a faster and more efficient response.

Inflammation is induced by the immune system in response to different factors such as pathogens, damaged cells, and toxic compounds and is essential in maintaining homeostasis [40]. There are five well-known signs of inflammation and these are heat, redness, pain, swelling, and loss of function [41]. Inflammation comes in two forms - acute and chronic. Acute inflammation occurs immediately after the injury and may resolve in two weeks [42]. This type of inflammation will cause cytokines, leukotrienes, acute phase proteins, and chemokines to stimulate the migration of neutrophils and macrophages to where the inflammation occurs [38]. If this inflammation is not resolved in six weeks, this will result in subacute or chronic inflammation [42]. Chronic inflammation is a long-term reaction to an inflammatory stimulus that could last for weeks, months, or even a lifetime. This is characterized by the continuous recruitment of monocytes and lymphocytes accompanied by tissue injury due to the sustained inflammatory response [43]. The controlled inflammatory response is beneficial to us and protects against pathogens. However, if not resolved, this could lead to tissue damage, chronic inflammation which includes metabolic syndromes, and autoimmune diseases that could lead to loss of organ function [44].

When it comes to SARS-CoV-2 infection, the body reacts in two phases. The innate immune system provides the first line of defense in conjunction with the secretion of interferons and cytokines, activating the adaptive immune system. In this phase of infection, symptoms such as fever, muscle ache, and malaise may be experienced. The next phase involves the adaptive immune system comprised of the cellular and humoral immune responses. The CD8+ T cells target cells that are infected by the virus. Antibodies specific to the virus are generated by B cells that are triggered by CD4+ helper T cells [45]. The intensity of symptoms experienced by COVID-19 patients depends on the innate immune response. Infection control in asymptomatic patients or those with mild symptoms could be attributed to the innate immune response. Severe symptoms could be due to failure of the non-specific first line of defense of the body or the development of an acquired immune response [46]. When amplified, this could become pathogenic to the patient, especially those with comorbidities. For example, in old patients, late production of interferons could lead to increased recruitment of inflammatory cells [47]. This could then lead to enormous inflammation resulting in a cytokine storm or overproduction of cytokines. Cytokine storm is attributed to be one of the major causes of multiple organ failure which could be seen in severe COVID-19 cases [48]. Furthermore, too much penetration of inflammatory cells like monocytes and neutrophils could result in lung injury or even cytokine-induced apoptosis of lung epithelial cells [49]. Thus, severe cases of COVID-19 are a result

of uncontrolled viral infection and a dysregulated immune and inflammation response [46].

2. Hypothesis

In SARS-CoV-2 infection, having a regulated immune response is one of the most crucial aspects to prevent cytokine storm, severe patient symptoms, organ failure, and even death. The human microbiome is known to affect the host's immune response where dysbiosis may stimulate and lead to uncontrolled inflammation. Thus, we hypothesize that having optimal levels of LC- PUFAs, omega-3 index value of ≥ 8% [50], and its synergistic interactions with natural microflora in the human body is key towards regulating inflammation (Figure 4). The omega-3 Index is the relative percentage of EPA and DHA LC-PUFA to the total fatty acids found in red blood cells and an omega-3 Index of ≥ 8% has been shown to correlate with greatest cardioprotection [50]. This should lead to the prevention of complications (e.g. long COVID) arising from chronic inflammation induced by COVID-19 infection. A recent pilot study of 100 COVID-19 patients showed that patients with a higher omega-3 Index had a better chance of survival than those with a lower omega-3 Index [51]. Although there was no statistical significance observed between the groups, it should be noted that only one patient out of 25 died in one quartile with an omega-3 Index ≥ 5.7%, while 13 patients out of 75 died in the other 3 quartiles, which had lower omega-3 Indices. Larger clinical studies should confirm this finding. Furthermore, we present in this paper several studies that support our hypothesis that optimum levels of PUFAs in the body may be used as preventive measures in having severe COVID-19 symptoms.

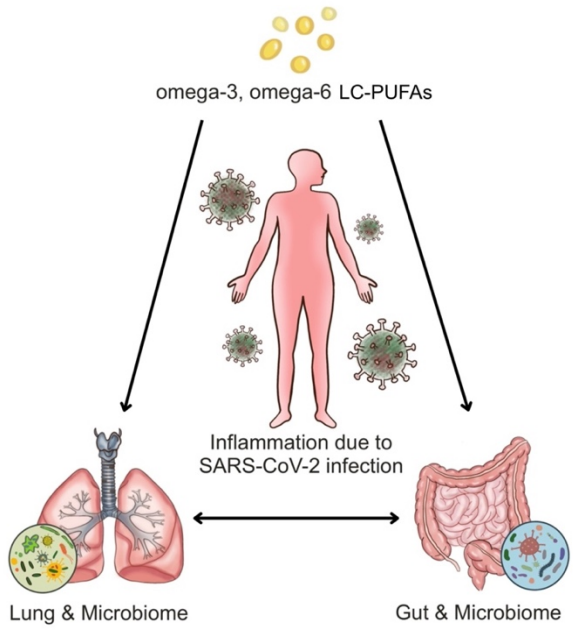

Figure 4: Synergistic relationship between LC-PUFAs and the microbiome in the gut-lung axis. Optimum levels of PUFAs play a crucial role in preventing severe COVID-19 symptoms as they aid in the maintenance of the natural human microflora which affects the host's immune response while dysbiosis leads to chronic inflammation.

3. Discussion

3.1 Role of the Gut and Lung Microbiome in COVID-19

COVID-19 is prominently recognized as a respiratory disease and SARS-CoV-2 infection of the respiratory tract happens in a background of diverse microbes. Thus, it is apparent that the microbiota of the upper and lower respiratory tract (URT and LRT) can affect COVID-19 initiation and development.

The lung microbiome has been demonstrated to help in the deployment of appropriate lung immune responses [52, 53], activation of lung immune cells [54], prevention of dysregulated immune responses [55], and secondary lung infections [56] during viral and non-viral lung diseases. Therefore, when the lung microbiome shifts away from its normal composition towards a pathogenic state, called lung dysbiosis, aggravation of respiratory diseases can be expected. Recent studies show that a dysbiotic lung flora can help develop severe COVID-19 complications such as acute respiratory distress syndrome (ARDS). The lung flora was shown to be altered in non-COVID-19 ARDS patients, having increased bacterial abundance, lower diversity, and enriched with gut-associated bacteria [57]. These alterations were associated with inflammation, specifically with pro-inflammatory cytokine IL-6, and mortality [57]. Interestingly, respiratory tract dysbiosis has been reported as a predictor of worsening conditions in COVID-19 patients [58].

Initially, it has been suggested that the airway microbiome can act as a barrier, preventing viral attachment to host cells [59]. Recently, one study has demonstrated the potential of the lung microbiome to directly interfere with the SARS-CoV-2 life cycle. In a cell culture setting, *Bacteroides ovatus* and *B. thetaiotaomicron* have been shown to modify lung heparan sulfate, a co-receptor for SARS-CoV-2 entry, blocking the virus from entering lung cells [60]. Normally, the lungs are colonized by bacteria belonging to the Bacteroidetes phylum [61], raising the importance of maintaining a healthy lung microbiome.

Apart from regulating host immunity, some studies also suggest that airway microbes are important in assisting the normal function of the lungs [62-63] which can be impaired during SARS-CoV-2 infection. The lung microbiome is regarded as a prerequisite for normal alveolar development since smaller lungs have been observed in germ-free (GF) rodents [62], and GF mice have been observed to have a reduced number of alveoli [63]. The latter study confirmed this lung function-lung microbe association when the reintroduction of *Lactobacillus* spp. lung isolates restored the number of fully developed alveoli [63].

Although fever and respiratory illnesses commonly plague COVID-19 patients, an increasing number of empirical studies have reported that a significant proportion of patients (as much as 5 to 33%) present gastrointestinal (GI) symptoms such as vomiting, diarrhea, nausea, and vomiting and abdominal pain [64-66]. A systematic review and meta-analysis reported that of all hospitalized COVID-19 patients, 15.74% manifest at least one GI impairment, with diarrhea as the most common predictor of worsening disease outcome [67]. Reviews of empirical studies revealed that the gut microbial composition is disfigured among COVID-19 patients and covaries with the severity of the disease [68,69]. With these studies, it is highly conceivable that gut flora could be intimately associated with COVID-19.

As with the lung microbiome, the gut microbiome is better studied and thus more established in providing host immunity against viral and non-viral diseases. The gut microbiome has also been known to regulate the deployment of early antiviral responses, training of immune cells, and prevention of pathogen colonization – all of which can be extended in COVID-19 [68-70]. Also, in parallel with the lung microbiome, the intestinal flora is intimately tied with the functional homeostasis of the GI tract which may be damaged during COVID-19 [68-70]. The association of the intestinal flora with intestinal function includes the establishment of gut architecture [71], metabolism

of indigestible food into short-chain fatty acids (SCFAs) which have activities for intestinal homeostasis [72], synthesis of nutrients such as vitamins [73], and intestinal angiogenesis [74]. In exploring the effects of the gut microbiome against SARS-CoV-2 infection, a murine study found that bacteria belonging to the Bacteroidetes phylum can downregulate ACE2 expression while species in the Firmicutes phylum exhibited variable effects [75]. This can be extended in COVID-19 since it has been observed that the fecal abundance of *Alistepes onderdonkii* and *Bacteroides ovatus* (both Bacteroidetes species) was inversely correlated with COVID-19 severity while the abundance of *B. dorei*, *B. thetaiotaomicron*, *B. massiliensis*, and *B. ovatus* showed an inverse association with fecal viral load [76,77], with *B. dorei* having been specifically demonstrated to reduce the expression in the colon of ACE2 [75] which is the main receptor for SARS-CoV-2 adhesion. The direct involvement of Bacteroidetes species with SARS-CoV-2 infection can find support in studies showing that COVID-19 patients with diabetes, hypertension, and obesity present a low abundance of Bacteroidetes species and had the highest morbidity and mortality from COVID-19 [78-80].

Also, multiple studies have shown that respiratory diseases, not only COVID-19, are often coupled with gut dysbiosis [81-83] and secondary GI tract dysfunctions [84, 85]. These observations demonstrate the concept of the gut-lung axis which is described as the crosstalk between the gut and lung flora (Figure 5). This microbiome-mediated crosstalk operates bidirectionally through the diffusion of microbes, microbial metabolites, endotoxins, inflammatory mediators, and microbiota-regulated immune cells into the blood, thus connecting the gut and the lungs and orchestrating immune responses, homeostasis, and microbial composition in both environments. Since gut-lung crosstalks occur during respiratory disorders, this bidirectional interaction may be crucial during SARS-CoV-2 infection and subsequent COVID-19 pathogenesis.

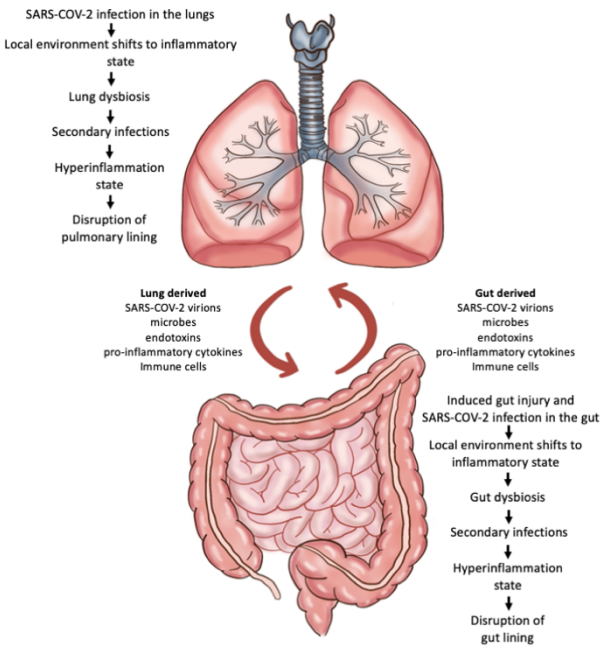

Figure 5: The Gut-Lung axis in SARS-COV-2 infection. The gut-lung axis, through blood circulation and lymph, is bidirectional. Lung dysbiosis during COVID-19 allows translocations of microbes as well as pro-inflammatory cytokines to the gut. This may then lead to subsequent infection and dysbiosis in the gut, which may later compromise the intestinal lining and allow translocations of gut microbes, endotoxins, and pro-inflammatory cytokines to the lungs – worsening the conditions present already in the lungs.

When SARS-CoV-2 enters and infects the respiratory tract towards the lungs, the local microenvironment changes towards an inflammatory state. If the infection is not resolved early, lung dysbiosis may occur due to the persistent inflammatory environment. Alterations in lung microbiota may foster secondary infections that may promote further inflammation resulting in hyperinflammation, inducing lung epithelial barrier disruption [70]. A damaged pulmonary lining coupled with a leaky vasculature due to excessive inflammation allows the translocation of microbes, overproduced pro-inflammatory cytokines, and SARS-CoV-2 towards the intestinal organ through the blood, demonstrating the gut-lung axis. The presence of high levels of pro-inflammatory cytokines and lung-derived microbes in the blood has been reported to induce gut dysbiosis and gut injury [70, 85]. Meanwhile, immune cells modulated by an altered lung flora can also induce microbial dysbiosis and injury in the intestines which can occur even before microbes, cytokines, and viral particles leak into the vasculature [70].

Upon spread of viral particles into the intestines via circulation, gut epithelial cells are infected and are followed by inflammation of the GI tract, subsequently inducing changes in the local gut flora. Aan et al. mentioned that gut dysbiosis coupled with local inflammation increases the gut expression of ACE2, thereby favoring further SARS-CoV-2 and inflammation [70]. The exponential increase of SARS-CoV-2 in this organ due to increased infection further attracts gut dysbiosis since viral particles would increasingly bind to ACE2, disrupting its activity. Gut ACE2 is known to be involved in dietary amino acid uptake which can regulate the gut microbiota [86]. Furthermore, impaired ACE2 activity in the gut due to SARS-CoV-2 infection can promote intestinal permeability due to enterocyte malabsorption [85].

Gut alterations in COVID-19 are characterized by the depletion of beneficial bacteria which are known producers of SCFAs [86]. SCFAs have a diverse set of functions for local and distal homeostasis especially in the lungs [70] A recent transcriptomics study on gut epithelial organoids from rats has found that SCFA butyrate, a histone deacetylase inhibitor, can downregulate genes crucial for successful SARS-CoV-2 infection while upregulating antiviral pathways [87]. Other SCFAs traverse the gut barrier and are carried by the blood toward body organs such as the lungs. They have been found to promote hematopoiesis in the bone marrow, producing immune cells that travel to the lungs and mediate proper immune responses [88]. Another consequence of gut dysbiosis is the failure in the production of appropriate immune responses in the lungs due to reduced stimulation of immune cells by gut microbes. Mice treated with broad-spectrum antibiotics have reduced B and T cell responses during influenza infection in the lungs along with an abrogated T cell influenza-specific response stimulated by inflammasome-mediated DC migration [89]. From the studies mentioned above, we can say that increased gut expression of ACE2 and gut dysbiosis-induced gut barrier disruption, combined with extended cycles of SARS-CoV-2 infection of gut epithelial cells severely compromises the intestinal lining thereby allowing the translocation of microbes, endotoxins, and gut pro-inflammatory cytokines. The translocated microbes, endotoxins, and pro-inflammatory cytokines can then migrate toward the lungs through the circulation, thus worsening the conditions already present in the lung environment.

These observations show how the gut-lung axis works during SARS-CoV-2 infection: lung dysbiosis may lead to gut dysbiosis, and vice versa, ultimately establishing a positive feedback loop of cytokine production, inflammation, and secondary infection which exacerbates the damage that both

organs sustain during COVID-19. Hence, intake of substances rich in omega-3 PUFAs may therefore not only modulate the gut flora towards an optimal state against SARS-CoV-2 infection but also indirectly regulate the respiratory microbiome since gut microbes have been established to have far-reaching immunomodulatory effects on the lungs via the gut-lung crosstalk.

3.2 Long COVID

'Long COVID' or what doctors refer to as post-acute sequelae of COVID-19 (PASC) is described as the condition seen in a significant number of COVID-19 patients which includes diarrhea, depression, anxiety, sleeping difficulties, fatigue, muscle weakness, and poor glycometabolic control that persist even after disease resolution [90-92]. This widely-known phenomenon can be attributed to gut dysbiosis since long-lasting alteration of the intestinal flora has been consistently observed among COVID-19-recovered patients [76, 93], and well-balanced gut microbiota has important implications for gut homeostasis. In one study, the gut microbiome profile of COVID-19 patients showed a significant decrease in species richness even after 6 months of hospital discharge [93]. Prolonged SARS-CoV-2 infection in the GI tract may be the reason gut dysbiosis persists in post-COVID-19 [68]. Studies have demonstrated that in some COVID-19 patients, shedding of viral RNA in fecal samples can continue for up to 42 days while the presence of SARS-CoV-2 can remain in the intestinal epithelium for up to 90 days post-disease resolution [94,95]. It has been suggested that gut dysbiosis is a product of the host immune response and altered gut environment during SARS-CoV-2 infection [68], thus supporting the claim that persistent viral infection of the GI tract would lead to persistent gut microbiome alteration. Another factor that can contribute to gut dysbiosis during long COVID is the enrichment of the fungus *Candida albicans* during and after COVID-19 resolution [96,97]. This fungus has been shown to disrupt gut microbiome re-assembly in humans and mice [98]. A study on the multi-kingdom microbiome of preterm infants further showed that *C. albicans* inhibited the genera *Escherichia* and *Klebsiella* along with other multiple dominant bacterial genera of the gut [99]. Further gut dysbiosis can be attributed to this fungus since gut enrichment of *C. albicans* has been known to exacerbate inflammation in the intestinal tissues and extra-intestinal tissues [100], thereby potentially anchoring a vicious cycle towards decreasing gut microbiome recovery and extending long COVID.

3.3 Role of Omega-3/Omega-6 PUFAs in COVID-19 associated inflammation

Given the need for an effective way to resolve COVID-19 associated inflammation, scientists have been focusing on devising a therapy involving the use of omega-3 PUFAs. For one, omega-3 fatty acids such as EPA and DHA are converted to SPMs in the body [101] and have been observed to have anti-inflammatory effects [102]. Omega-3 PUFAs have been shown to decrease the overall production of eicosanoids by intervening with AA in both cell membrane phospholipid integration, as well as COX and LOX enzyme metabolism [102]. Finally, an increase in the amount of omega-3 PUFAs is directly correlated with a significant decrease in the level of inflammatory markers such as cytokines in the body [102].

Various studies have been conducted with regard to using omega-3 fatty acids in treatments for COVID-19. A study was conducted in Iran that aimed to determine if administering EPA and DHA along with hydroxychloroquine as medication would alleviate the symptoms of COVID-19 patients. The results of their clinical trials have shown that the addition of omega-3 PUFAs resulted in a significant decrease in C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) levels. These parameters are important since high amounts of CRP in the body and elevated ESR are some of the markers of systemic inflammation. There was also a significant decrease in the amount of fatigue and body pain, as well as increased appetite in patients who were treated with EPA and DHA [103]. Doaei et al. (2021) conducted a study that involved determining the effect of omega-3 fatty acid supplementation on certain biochemical parameters of COVID-19 patients. Their findings showed that the arterial pH, bicarbonate, and base excess levels increased while lower levels of blood urea nitrogen, creatinine, and potassium were seen in patients taking omega-3 fatty acid supplementation. Moreover, patients with omega-3 supplementation had a significantly higher 1-month survival rate when compared to those without supplementation. When the mentioned parameters are in their opposite trends, this leads to microcirculation dysfunction, resulting in severe metabolic and respiratory acidosis in COVID-19 patients. Omega-3 supplementation also helped in mitigating the progression of renal impairment, which often leads to higher patient mortality, as well as improving overall kidney function [104]. In 2021, an app-based community survey was done by Louca et al. in which 445,850 subscribers would self-report information on SARS-CoV-2 infection in the populations of the UK, USA, and Sweden. Subscribers are also asked to input their regulated dietary supplements in the past three months and results of this study showed that those who are taking probiotics, omega-3, multivitamins, or Vitamin D had a lower risk of SARS-CoV-2 infection by 9-14%. Moreover, associations between the intake of these mentioned dietary supplements and a lower risk of SARS-CoV-2 infection were found to be significant among women but not in men [105]. In 2020, a study by Berger et al. showed that Icosapent ethyl (IPE), a highly purified form of EPA derived from fish oil, that has been used in lowering triglyceride levels and has anti-inflammatory effects [106], significantly reduced cardiovascular mortality and is described as a successful treatment for COVID-19 [107]. However, this study used only two patients – a single case study but hopes to be a starting point for research on the use of omega-3 FAs as COVID-19 treatment [110]. The use of IPE in treating COVID-19 patients was further supported by the study conducted by Kosmopoulos et al. (2021). In their randomized open-label trial, results showed that in comparison to patients subjected to usual COVID-19 care, IPE significantly reduced high sensitivity C-reactive protein, a protein that is linked to the overproduction of inflammatory cytokines [108], and improved symptoms of patients who were loaded with 8g IPE for three days, followed by 4g for eleven days [109]. Another study in 2021, showed that the omega-3 index has inverse associations with mechanical ventilation and death in severe COVID-19 even after factors such as sex and age have been adjusted [110]. Finally, a randomized clinical trial was conducted in 2020 that aimed to resolve inflammation storms in COVID-19 patients by introducing omega-3 PUFA supplementation through intravenous means [111]. The rationale of this trial is based on results from other studies showing that intravenous supplementation has been shown to significantly improve the conditions of the treated patients, such as those who were affected with acute respiratory distress syndrome (ARDS), while also minimizing the risk of having adverse effects on i.v. supplementation of omega-3 PUFA [111]. There have been several studies suggesting the therapeutic benefits of omega 3 PUFA against inflammation-associated pathologies also observed in COVID-19 patients. These studies have been extensively reviewed by Hathaway et al. [112] and will not be covered in this article.

The use of omega-3 supplements may also provide significance even after a SARS-CoV-2 infection. Several studies have shown that the risk for cardiovascular diseases (CVD) such as cerebrovascular disorders, dysrhythmias, ischaemic and non-ischaemic heart diseases, pericarditis, myocarditis, heart failure, and thromboembolic disease increase even after a year of SARS-COV-2 infection. These findings are not only true for patients with severe COVID-19 symptoms but also for those who experienced mild to moderate symptoms [113]. Supplementation with omega-3 has been found to decrease mortality rates in coronary heart diseases and sudden cardiac deaths [114]. Omega-3 fatty acids improve risk factors for cardiovascular diseases by decreasing serum levels of triacylglycerides and by boosting the breakdown of fatty acids, thereby accelerating triacylglyceride clearance from the plasma. Moreover, supplementation of omega-3 fatty acids has been shown to improve flow-mediated arterial dilation as well as the mechanical function of the heart [114,115].

3.4 Role of Omega3/Omega6 PUFAs in the regulation of gut and lung microbiome

As discussed in 3.1 of this paper, gut microbiota plays a significant role in the maintenance of intestinal health. Moreover, the intestinal microbiome acts as a signaling hub that communicates with the innate immune system of the host, and gut microbiota dysbiosis may result in complex disease [116] Microbes in the gut are known to be affected by the nutrients they receive. For one, fatty acids have been shown to affect the composition of the gut microbiota. According to the study by Watson et al., omega-3 PUFA supplementation causes an increase in the amount of beneficial bacterial species, including *Bifidobacterium*, *Lachnospira*, *Roseburia*, and *Lactobacillus* [24]. It has also been established that the gut microbiota is associated with the neurodevelopmental pathways in the body, and any detrimental changes in its composition may lead to brain dysfunction and behavioral impairments [117]. With that in mind, the introduction of omega-3 PUFAs would result in beneficial changes in both the gut microbiota, as well as preventing behavioral deficits and other neuropsychiatric disorders from happening [117]. In 2021, Robertson et al. showed sufficient evidence to suggest that administering omega-3 PUFA can help restore gut microbiota and that different doses would provide significantly different results. In mice treated with ceftriaxone, an antibacterial drug, around 60 mg to 90 mg of omega-3 PUFAs is enough to restore the natural composition of the gut microbiota. Moreover, the PUFAs were able to provide a more diverse microbiota, as well as enhance immunity through the secretion of intestinal mucosa immunoglobulin secretory IgA and increasing interleukin 10 (IL-10) concentration [118].

Omega-3 PUFAs are also known to regulate the gut microbiome by either inhibiting the production of proinflammatory mediators or promoting the production of anti-inflammatory mediators. Once endotoxins such as lipopolysaccharides (LPS) penetrate through the intestinal wall, they can damage the intestines and promote the accumulation of toxic bacterial products. Inflammatory responses can also be caused by trace amounts of LPS found within the systemic circulation. In response to this, the introduction of omega-3 PUFAs can inhibit proinflammatory cytokine production and LPS-associated MAPK kinase activity, thus decreasing the amount of proinflammatory mediators in the body. Along with this, the fatty acids also help in reducing gut inflammation by promoting the release of regulatory T cells and IL-10, inhibiting interleukin 17 (IL-17) production [119].

Finally, omega-3 PUFAs affect the gut microbiota by controlling the short chain fatty acid (SCFA) content. Among the bacteria found within the gut microbiota are butyric acid-generating bacteria, which convert non-fermentable dietary fibers to SCFAs like butyrate. These SCFAs are then used to control processes such as gene expression, inflammation, and apoptosis in host cells found within the colonic mucosa. With that, omega-3 PUFAs can increase the production of SCFAs such as butyrate, aside from its ability to restore microbiota composition. This apparent increase in SCFAs results in not only providing host resistance to pathogens, but also increasing the amount of SCFA-producing bacteria such as *Blautia*, *Bacteroides*, and *Coprococcus* [119, 120].

Aside from the gut microbiome, the lung microbiome has been recognized as a key component in providing resistance against potential pathogens, as well as ensuring the maturation and maintenance of the respiratory system. It has been established that the lung microbiota of healthy individuals would have *Provotella, Streptococcus, Veillonella, Neisseria, Haemophilus,* and *Fusobacterium* as the most abundant genera [121]. Many variables can affect the state of an individual's lung microbiota, such as the host immune response, diet, cigarette smoking, the use of certain medications such as antibiotics and corticosteroids, and–most importantly–nutrition [122].

Although there are only a few that focus on the lung microbiome, studies have already been conducted that sought to determine how PUFA supplementation affects the symptoms of various respiratory ailments. Given their beneficial qualities, a study was conducted that involved the administration of LC-PUFAs to BALB/c mice with ovalbumin-induced asthma to determine if the disease's symptoms can be mitigated. Results of their experiments showed that LC-PUFA supplementation prevented the occurrence of ovalbumin-induced airway hyperresponsiveness, reduced omega-6 PUFAs that minimized inflammation symptoms, reversed the imbalance of the gut microbiota, and improved the mice's overall airway pathological features [123]. The use of omega-3 fatty acids has also been shown to provide significant benefits to patients with acute lung injury such as improving respiratory function and prognosis, improving inflammatory factor levels, serving as an essential part of recovering respiratory function, and shortening the time for mechanical ventilation and ICU stay [124]. Despite these significant findings, it is important to note that there are still several factors to consider when administering PUFAs as part of disease treatment. Several studies have concluded that omega-3 PUFAs can be used in treating patients with ARDS; however, most of these are small-scale trials that have heterogeneous groups of patients with different backgrounds. Several aspects of clinical management have not been firmly established yet, and until large, homogenous, high-quality trials are conducted, it is not recommended to use omega-3 PUFAs as a universal treatment for ARDS [123].

3.5 Testing for Omega-3/Omega-6 levels in the body

Among the LC-PUFAs, EPA, DHA, and AA ratios are the most important when it comes to inflammation - a common body response in COVID-19 infection. High AA/EPA are linked to inflammation [124] and would have a greater risk for coronary heart diseases [125]. However, too low AA/EPA or too much omega-3 may reduce immune function and cause bleeding problems [126].

One challenge in measuring LC-PUFA levels is the process of obtaining samples from individuals, as the only steady and stable source of LC-PUFAs for testing exists in the red blood cells (RBCs) [128]. RBCs can be readily obtained from the blood and the LC-PUFAs within it exhibit high longevity. An effective sampling method that can be used is the dried blood spot (DBS) method, where a spot of blood extracted from an individual is

placed on a piece of filter paper, immediately dried, and stored in low temperatures [128,129]. The blood sample present in the DBS card is then isolated for RBCs and subjected to gas chromatography for fatty acid level measurement [128, 129]. Accomplished DBS cards can be used to determine polyunsaturated fatty acid levels, which indicates the composition of RBC membranes in terms of EPA and DHA. Moreover, the ratio of AAs and EPAs in RBCs is also used as a measure of fatty acid content in the blood, as both are essential for the regulation of inflammation [130]. Ideally, low AA/EPA ratios (should not be 1.5-3) should be seen as in most cases, AA, an omega-6 fatty acid, serves as a precursor for pro-inflammatory compounds while EPA, an omega-3 fatty acid, acts as a precursor for anti-inflammatory compounds. In comparison, an older method employs the direct isolation of RBCs from whole blood samples collected from subjects via several steps involving centrifugation and processing [130]. The isolated RBC samples are then stored at low temperatures before analysis via gas chromatography, similar to the DBS method. However, the DBS method is more advantageous than the centrifugation technique because DBS requires a low volume of blood, no further processing is required and the sample can be stored at ambient temperature for longer periods. In contrast, the centrifugation method involves the sampling of whole blood and requires several processing steps and larger volumes of blood. Furthermore, results from the DBS method are on the same level of accuracy as tests done on whole blood or directly on RBCs. Overall, this exhibits that the DBS method is an adequately accurate and more efficient method for the detection of LC-PUFA levels.

3.6 Determining microbiome diversity in the body

Since lung and gut dysbiosis plays an important role in the disease severity of COVID-19, it is important to maintain microbiota diversity in these aforementioned organs. The lung and gut microbiota of an individual can be determined through sputum and stool analysis, respectively. Direct collection of lung tissue can also be done to analyze lung microbiota as this method allows more specific characterization and examination of the microbiome, as well as the environment of the microbiome [131]. However, sampling of lung tissue is extremely invasive, non-reproducible, and time- and resource-consuming. Sputum samples, on the other hand, are easily accessible and obtained, and they also contain a higher microbial load than fluid obtained from the tissues [132]. Direct microscopic observation and production of cultures from sputum samples can be done to create a lung microbiome profile of an individual. However, it is important to note that this requires considerable time and effort. Metagenomics can also be performed to determine the identity of microorganisms in a sample. However, this method is more expensive and requires sophisticated laboratory equipment [133].

Stool samples that are used for gut microbiome profile identification can be measured for intestinal alkaline phosphatase (IAP) using colorimetric assay [133]. IAP serves an important role in maintaining balance in resident microbiota, as well as the presence of materials in the gut such as fatty acids. IAP in the intestine also dephosphorylates toxic microbial ligands, which allow it to protect the host from the effects of acute and chronic inflammation [134]. With this, IAP levels can then serve as an indicator of inflammation in the region. In the identification of the gut microbiome profile, endoscopies, mucosal biopsies, and sampling of intestinal fluid can be employed. However, the most ideal method is the use of fecal samples. These are collected from patients and transferred to the laboratory for analysis by making cultures, by qPCR, or by 16S rRNA sequencing. Stool samples can be collected repeatedly from subjects and are non-invasive, although they can be less accurate than other methods in representing the gut microbiota [135].

4. Conclusion

Cytokine storm due to the excessive immune response is the primary cause of COVID-19 death. Supplementation with omega-3 fatty acids may aid in immune response regulation as EPA and DHA are known to be precursors of anti-inflammatory compounds that decrease pro-inflammatory mediators, reducing the risk for a dysregulated immune response to SARS-COV-2 infection. Moreover, omega-3 fatty acids have been shown to assist in the maintenance of the ideal human microbiome which in turn influences the body on how to respond to infections. Recovered COVID-19 patients may also benefit from omega-3 supplementation as the risk for CVD increases upon COVID-19 infection and its severity. Several studies have shown that omega-3 fatty acids are effective in reducing sudden death caused by CVD. Levels of omega-3 fatty acids may then be measured through the DBS method, a simple yet efficient technique for determining the omega-3 index.

We have presented a hypothesis on how modulation of the microbiome with polyunsaturated fatty acids can prevent complications arising from SARS-CoV-2 viral infections. With the anti-inflammatory, pro-eubiosis of the human microbiome, and other beneficial effects of omega-3 PUFAs, supplementation with this fatty acid may be an important consideration pre- and post-infection to prevent long COVID.

The presented hypothesis has similarities to a previous hypothesis on the role of PUFAs and the microbiome in modulating immune checkpoint inhibitors and cancer [8]. This suggests that a general model based on PUFAs and the microbiome can be exploited in managing other inflammatory diseases. Randomized clinical trials will be needed to test this hypothesis on the synergistic effects of omega-3 fatty acids and the human microbiome on preventing complications arising from uncontrolled inflammation due to COVID-19 infection.

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