Assessment of concentrations of multidirectional omega-3 fatty acids in inborn errors of immunity with predominantly antibody defects: a pilot study

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Summary

Background. Omega-3 fatty acids are involved in many processes in the human body. Their beneficial effects were documented mainly in relation to cardiovascular and immune systems. Patients with immunodeficiencies with predominantly antibody defects due to their reduced immunoglobulin levels should have factors adversely affecting the course of the disease eliminated. **Methods.** Nineteen primary immunodeficient patients with predominant antibody defects (out of which fourteen with CVID) and eighteen immunocompetent participants had their blood tested in order to determine the concentration of EPA, DHA and omega-3 index values. The Mann–Whitney U tests were used to determine statistical significance. **Results.** Immunodeficient participants, especially with CVID, overall tend to have a slightly lower mean concentration of omega-3 fatty acids such as DHA and in particular EPA (CVID: 0.86% ± 0.28% *vs* 1.06% ± 0.31%, p = 0.095) as compared with the control group and the differences were most evident among patients aged 30-39 (0.67 ± 0.16% *vs* 1.12 ± 0.12%, p = 0.025). 63% of patients with immunodeficiency had an omega-3 index value between 4-8, compared to 39% in the control group. 37% of participants with predominantly antibody defects had an omega-3 index value > 8% (29% of all CVID group) compared with 61% of the control group. None of the participants achieved a result of 4% or lower. People without immunodeficiency consumed products rich in omega-3 acids more often. **Conclusions.** These findings suggest that primary immunodeficient patients with predominantly antibody defects tend to have lower omega-3 index values, albeit not significantly and seem to have higher cardiovascular risk than the control group. Research has also shown that education is needed

regarding the effects and necessity of consuming products rich in omega-3 fatty acids, especially in patients with immunodeficiency.

Key words

Omega-3 fatty acids; inborn errors of immunity; primary immunodeficiency; primary antibody deficiency; common variable immunodeficiency;

Impact statement: The identification of immunodeficiencies associated with altered levels of omega-3 fatty acids will facilitate the introduction of improved diagnostic techniques and therapies for these disorders in the future.

Introduction

Over the past several years in published clinical and epidemiological studies from around the world, an increased interest of the scientific community on the subject of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) is observed, as representatives of omega-3 fatty acids, in the context of their beneficial effects on human health. Both of these acids are involved in various processes in the human body, including regulation of the immune system, reduction in risk of cardiovascular events and an anti-inflammatory effect (1-3). Due to the fact that omega-3 acids are found in large concentrations in the brain tissue and in the human retina, it is believed that their insufficient supply within the diet may contribute to impaired fetal development and even be a risk factor for the development of Alzheimer's disease (1,4). It has also been proven that these acids can prevent the development of atherosclerosis by inhibiting inflammation and rupture of atherosclerotic plaque, hence by increasing the consumption of products containing DHA, it is possible to reduce the risk of mortality due to coronary artery disease by as much as 7% (5,6,7).

Structure and occurrence of DHA and EPA

Eicosapentaenoic and docosahexaenoic acids are considered, in terms of the human diet, to be the main representatives of long-chain n-3 polyunsaturated fatty acids (LC-PUFA), which means that in their molecular structure, the first double bond is located at the third carbon atom counting from the end of the methyl group, the so-called carbon Ω-3. In its 19-membered hydrocarbon chain, EPA contains 5 double bonds connected by methylene bridges, and DHA, consisting of a 21-membered chain, has as many as 6 of these bonds. Due to the lack of appropriate enzyme mechanisms that introduce multiple bonds at the appropriate

carbon atom, these acids are not synthesized *de novo* in the human body and therefore they must be supplied with food (1,8). The main source of omega-3 fatty acids is primarily the fatty tissue of marine animals and fish such as tuna, mackerel and salmon, as well as marine algae, which these fish feed on.

In addition, these acids can also be found in products such as meat (beef, lamb, pork, and poultry), milk and dairy products, chicken egg yolk and seafood. In the case of products of plant origin, sources of omega-3 fatty acids include oils obtained from basil, chia, flax, soybean and rapeseed seeds (1).

Effect of omega-3 fatty acids on the immune system

Despite such a wide spectrum of action of omega-3 acids and the existence of many studies and scientific papers indicating the beneficial effect of these acids on the human body, still little is known about the exact mechanisms in which they affect the human immune system *in vivo*. The literature contains extensive studies (9,10), summarizing the knowledge gathered so far on the impact of omega-3 fatty acids on individual components of innate and acquired immunity mechanisms, however, many of the articles quoted there refer to conclusions based on *in vitro* studies on animals or in people with immunocompetence. Therefore, there is significant evidence that EPA and DHA may affect a number of processes taking place during inflammation through various mechanisms, including inhibition of leukocyte chemotaxis and adhesion molecule expression, signal transmission regulation by affecting both surface and intracellular receptors, the reactivity of B and T lymphocytes modulation, and modification of the composition of fatty acids in cell membranes (9,10). Due to the small number of studies conducted and the absence of unequivocal conclusions, it would therefore be interesting to check whether omega-3 acids play an important role in people with impaired function of the immune system, i.e., in people with immunodeficiencies, and if such a relationship exists, to see whether supplementation with these acids could bring any benefits for them.

Primary antibody deficiencies

Primary antibody deficiencies (PADs) are the most common inborn errors of immunity (IEIs) and account for more than 50% of all cases. In symptomatic patients, there is an increased risk of not only infections but also of selected cancers, autoimmune diseases, and allergies (11).

The recognition of IEI is systematically improving, which is associated not only with extending the life of patients but also with the occurrence of new complications and

accompanying diseases. Therefore, prevention should become a priority, also in the field of non-communicable diseases. IEI patients develop immune dysregulation, which not only causes immunodeficiency, but may also affect the course of co-occurring chronic diseases. In order to provide patients with IEI with the best possible care, it is necessary to know whether it is possible to interfere with modifiable factors affecting health. Determination of the concentration of multidirectional omega-3 acids seems to be an important parameter, which, in case of deficiency, can be supplemented.

Common variable immunodeficiency (CVID) is one of the most common symptomatic primary humoral immunodeficiencies, characterized by decreased serum levels of IgG, IgA and in some patients IgM, among others. It is estimated that the disease affects, on average, 1:25,000 of the population. Its incidence in men and women is equal, although boys predominate among children. The age of onset of the disease is variable. Some authors speculate that there might be two peaks of incidence - in early childhood and around the third decade of life (12,13). Patients require immunoglobulin substitution for the rest of their lives. The etiology of CVID is unknown, although it is believed that certain environmental and genetic factors may predispose to the development of this disease. Due to the heterogeneous clinical picture, the diagnosis of this disease is a substantial diagnostic problem and, in many cases, results in a delay of many years between the diagnosis and the onset of the first symptoms. This translates not only into long-term, incorrectly used treatment, but also into an increased risk of complications, which mainly consists of pulmonary complications, autoimmunity and an increased, from 4 to 20%, risk of developing cancer (including non-Hodgkin's lymphoma and gastric cancer) (14,15). It seems that patients with CVID are a group of patients in whom unfavorable factors influencing the course of immunodeficiency and the occurrence of chronic diseases should be eliminated. However, this requires additional research and comparison of this group with a control group of unaffected individuals. The aim of this study is to evaluate whether there is a difference in omega-3 fatty acid concentration in blood between people with primary immunodeficiency with predominantly antibody defects and immunocompetent.

Materials and Methods

This pilot study was approved by the Bioethics Committee of the Nicolaus Copernicus University in Toruń (KB 215/2022). All participants have given informed consent to participate in the study.

Study population

Participants (n = 50) were divided between two groups based on the presence of humoral immunodeficiency. Patients with humoral immunodeficiency (n = 25) were recruited from a group under the supervision of Allergology, Clinical Immunology and Internal Diseases Clinic in Jan Biziel University Hospital No. 2 in Bydgoszcz, Poland. Inborn errors of immunity were diagnosed according to the European Society for Immunodeficiency (ESID) criteria (16).

Immunocompetent participants (n = 25) were selected from a population inhabiting an area of north-central Poland. During the study, four immunodeficient and two immunocompetent patients failed to attend blood testing. Additionally, two participants with immunological disorders and five without such disorders, were rejected during data analysis as a consequence of not meeting inclusion criteria. Ultimately 18 immunocompetent and 19 immunodeficient patients were included in the study (Figure 1), out of which 14 (74%) were previously diagnosed with common variable immunodeficiency (CVID), 3 (16%) with selective deficiency of immunoglobulin G (IgG) subclasses, 1 (5%) with hereditary agammaglobulinemia and 1 (5%) with non familial agammaglobulinemia. Additional information regarding comorbidities in immunodeficient participants is included in Table S1.

Considering participants with humoral immunodeficiency, the mean age (±SD) was 37.6 ± 10.6 years and BMI 22.63 ± 2.97 kg/m². In the immunocompetent group, the mean age was 34.9 ± 10.6 years and BMI 24.09 ± 2.99 kg/m², which resulted in p-values of 0.447 and 0.146, respectively. In the subgroup of immunodeficient patients, a large subset of participants suffering from common variable immunodeficiency may be distinguished. The mean age of the CVID group was 36.0 ± 8.0 years and BMI 23.10 ± 3.08 kg/m², which, compared to the control group, gave p-values of 0.747 for age and 0.367 for body mass index (Table 1).

Inclusion criteria

Participants were eligible for the study if they met the following criteria:

- Aged $\geqslant$ 18 years and < 60 years
- Body mass index $\geqslant$ 18.5 kg/m² and <30 kg/m²
- Have given informed consent

Additionally, in order to be included into the immunodeficiency group, participants must have been diagnosed as primary immunodeficiency with predominantly antibody defects according to the European Society for Immunodeficiency (ESID) criteria (16).

Questionnaire

Participants were asked to complete the questionnaire regarding their health status, age, weight, height, sex and dietary habits, including variable sources of omega-3 fatty acids. Closed questions with five possible answers were used to measure the frequency of most commonly consumed food ingredients containing omega-3 fatty acids, such as fish, olive oil, canola oil, linseed oil, walnuts, chia seeds and chicken eggs. The options from which participants could have chosen an answer were as follows: everyday, few times a week, once a week, 1-3 times a month, I do not consume. In order to estimate the mean monthly frequency of goods consumption containing omega-3 fatty acids, for each answer a corresponding average monthly value was assigned: for the everyday answer the value was set as 30, few times a week as 16, once a week as 4, 1-3 times a month as 2 and I do not consume as 0. The questionnaire template is attached in the Supplementary Material.

Blood sample collection

In order to determine the concentration of EPA and DHA, venous blood samples from median cubital veins were collected using 6ml vacuum tubes with EDTA. Samples were obtained in the morning, on an empty stomach, after the patients had rested overnight. Prior to the collection of blood samples, patients were instructed to rest for approximately 15 minutes. Furthermore, it was imperative that no food or drink had been consumed for a minimum of eight to twelve hours. Until laboratory testing, the material was stored at a temperature of 2-8 degrees Celsius. Samples were analyzed using gas chromatography in cooperation with certified external commercial laboratories. The results were available to investigators and participants individually. The omega-3 fatty acid testing involved assessing the concentration of polyunsaturated fatty acids EPA and DHA in venous blood and summarizing their percentage values (omega-3 index). Omega-3 Index was defined as the sum of EPA % and DHA % as measured in whole blood and derived by validated calculations to yield the equivalent sum of EPA % and DHA % in red blood cell membranes.

Omega-3 index <4 indicated low cardioprotective effect (supplementation and dietary changes recommended). An index of 4-8 indicated a moderate cardioprotective effect (changes in diet recommended, supplementation to be considered). An index >8 indicated a high cardioprotective effect (continuation of current activities recommended).

Statistical analysis

Data analysis included demographic characteristics of immunocompetent and immunodeficient participants with CVID subpopulation, such as age or BMI and data obtained from blood testing: EPA, DHA and Omega-3 index. Determination of p-values in demographic analysis was conducted using independent t-tests, which were preceded by Kolmogorov-Smirnov tests for normal distribution and two sample F-tests for variances. Parameters determined from blood were compared using the Mann–Whitney U test. Statistical analysis was performed in Microsoft Excel 2019 with the addition of the Analysis ToolPak.

Results

Omega-3 fatty acids concentration

In general, there were no statistically significant differences in EPA and DHA concentration or omega-3 index values between the immunodeficient group and immunocompetent group, as well as between the CVID group and immunocompetent group, except the 30-39 age group, which indicated a greater levels of EPA in immunodeficient participants as compared to immunocompetent ($p = 0.025$). However, groups with immunological disorders, especially with CVID, tend to have slightly lower mean concentrations of omega-3 fatty acids such as DHA and, in particular, EPA when compared with the group without immunological disorders (Table 2 and 3).

Interestingly, males were found to have higher EPA values, both among those with and without immune deficiencies, than females. On the other hand, such a trend did not occur in the context of DHA. In immunocompetent individuals, males had a higher percentage of DHA and, consequently, of the entire omega-3 index, whereas in immunodeficient individuals, including those with CVID, females had greater levels of those indices.

The similar tendency may be observed in terms of BMI, where overweight participants had higher mean EPA concentrations compared to normal weight subjects. Immunocompetent individuals of normal weight had lower average DHA and omega-3 index values than overweight participants, while immunocompetent individuals showed higher values than those with increased BMI. A comparison of EPA, DHA and omega-3 index values by sex, BMI and age in individuals with different levels of immune function are presented in Figure 2 and 3.

Omega-3 as a cardioprotective marker

Sixty-three percent of patients with immunodeficiency (n = 12) had an omega-3 index value between 4-8 compared to 39% of people in the control group (n = 7). 37% of participants with prevalent antibody defects (n = 7) had an omega-3 index value > 8% (29% of the whole CVID group, n = 4) compared with 61% of the control group (n = 11). None of the participants achieved a result of 4% or less. Omega-3 index results and patient characteristics are detailed in Table S2.

Sources of omega-3 fatty acids amongst participants

The most common source of omega-3 fatty acids among immunodeficient patients, as well as participants without immunological disorders, were eggs, which were consumed on average 9.5 and 13.3 times per month, respectively. The second most common omega-3 source was canola oil, which was used for food preparation 7.5 times per month in the immunodeficiency group and 10.1 times per month in the immunocompetent group. Other significant sources of omega-3 fatty acids included fish, which were consumed by participants with immunological disorders 2.7 times per month and 4.8 times per month by immunocompetent participants and additionally olive oil, which was added 2.3 and 6.2 times per month, respectively. Gathered data suggests that, in general, people without immunodeficiency were more likely to consume products rich in omega-3 acids.

Discussion and conclusions

The findings from this study suggest that people with humoral immunodeficiencies and with common variable immunodeficiency (CVID) in particular, might have decreased levels of omega-3 fatty acids: EPA and DHA compared to immunocompetent people.

There was no evidence of a statistically significant difference in concentration of EPA and DHA between compared groups, except from one age group in terms of EPA. Nevertheless, there was a trend for the immunodeficient patients to have reduced EPA concentration in comparison with the control group (Figure 2), especially pronounced within the CVID group.

Those findings are similar to the results of the study performed by Skarpengland et al. (17). In the study, 39 CVID patients and 30 healthy controls had plasma fatty acids measured and gut microbial profile defined. The researchers observed potentially unfavorable fatty acid profiles in CVID patients with decreased levels of EPA, DHA and anti-inflammatory index in blood plasma. Those changes were linked with gut microbial composition and were alternated by a 2-week course of treatment with rifaximin, suggesting a potential correlation between gut microbiota and polyunsaturated fatty acid levels in blood plasma. Analyzing currently

available literature, no other research focused on the topic of omega-3 fatty acids in patients with primary immunodeficiencies with predominantly antibody defects was found. Considering the promising results of research on omega-3 fatty acids concentration in CVID, it might be worth conducting further research concentrating on the mentioned disorders. Considering the omega-3 index, a value >8% is commonly associated with the greatest cardioprotection, whereas an index of <4% is associated with the mean cardioprotective effect (18). In our study, only seven immunodeficient participants (37%) had a good level of omega-3 index cardioprotection (>8%), including 4 participants with CVID (29% of all CVID patients). Out of immunocompetent participants, 11 (61%) achieved an omega-3 index of > 8%. This difference suggests an increased risk of cardiovascular disease development in primary immunodeficient participants with predominantly antibody defects, however such conclusions on this topic require further research.

It is important to note that this study has some limitations. Most importantly, initially recruited 50 participants resulted ultimately in only 37 of them being included in data analysis. A relatively small number of participants might have resulted in the p-value being statistically insignificant, although visible trends (Figure 3) may suggest otherwise. Moreover, rigorous inclusion criteria reduced the number of participants, however the criteria were supposed to eliminate nutritive disorders, children and elders. Unlike Skarpenglad et al. research (17), inclusion criteria included BMI values, which only allowed people falling within the normal or overweight category ($\geqslant$ 18.5 kg/m^2 and < 30 kg/m^2) to participate and age ($\geqslant$ 18 years and < 60 years). Another factor affecting the number of participants was the lack of widespread access to the omega-3 index value determination and its high cost. Furthermore, the questionnaire, despite the fact that it was primarily intended to gather demographic data and allow estimation of mean monthly consumption of omega-3 fatty acids, eventually only enabled the evaluation of the consumption frequency focused on products included within the questionnaire. Estimation of monthly omega-3 consumption was not possible due to discrepancies between products available on the market belonging to one category and the lack of average portion size consumed by each participant.

In conclusion, this pilot study suggests that the concentration of omega-3 fatty acids in people with primary immunodeficiencies with predominantly antibody defects and CVID, in particular, are slightly decreased, albeit not significantly, and mostly expressed in EPA concentration. However, further research on a larger scale is needed to determine potential discrepancies and implications of omega-3 concentration in humoral immunodeficiency before generalized conclusions may be drawn. This research has also shown that education is

needed regarding the effects and necessity of consuming products rich in omega-3 fatty acids, especially in patients with immunodeficiency.

Conflict of interest

None declared.

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Author Contributions:

All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this article, take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

KNB: Conceptualization, Data curation, Investigation, Funding acquisition, Supervision, Writing – original draft, Writing – review & editing. BS: Conceptualization, Data curation, Investigation, Formal Analysis, Funding acquisition, Visualization, Writing – original draft, Writing – review & editing. JL: Conceptualization, Investigation, Funding acquisition, Writing – original draft. ZB: Supervision.

Informed consent statement:

All the patients had given their written consent to participate in the study. The study protocol was approved by the Ethics Committee at Collegium Medicum in Bydgoszcz (KB 215/2022).

Data Availability Statement:

The data presented in this study are available in manuscript. Other data are available on request from the corresponding author. Some data is not publicly available due to the Personal Data Protection Regulation.

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Figure 1. Initial and final number of participants.

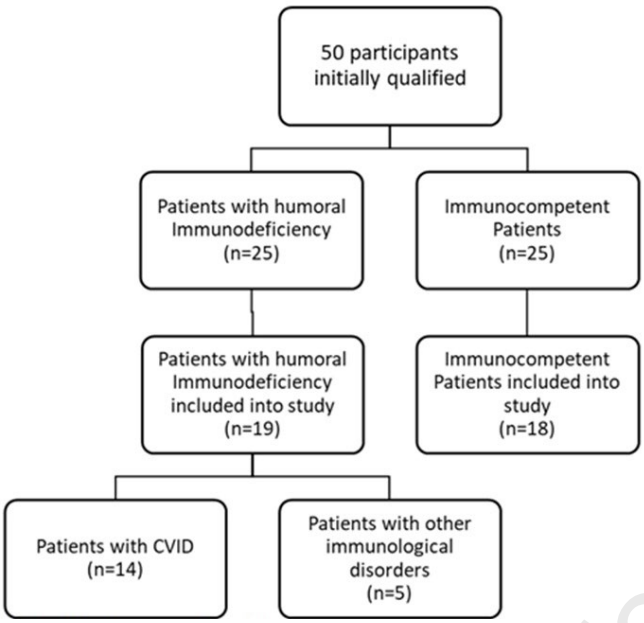


Figure 2. Overall results of EPA, DHA and omega-3 index measurements with standard deviation error bars. (a) Comparison of overall EPA, DHA concentrations and omega-3 index between immunocompetent participants, patients with humoral immunodeficiencies and patients with CVID. Scale adjusted to show the difference between EPA and DHA levels and their effect on omega-3 index values. (b) Zoomed-in comparison of EPA results of immunocompetent participants, patients with humoral immunodeficiencies and patients with CVID.

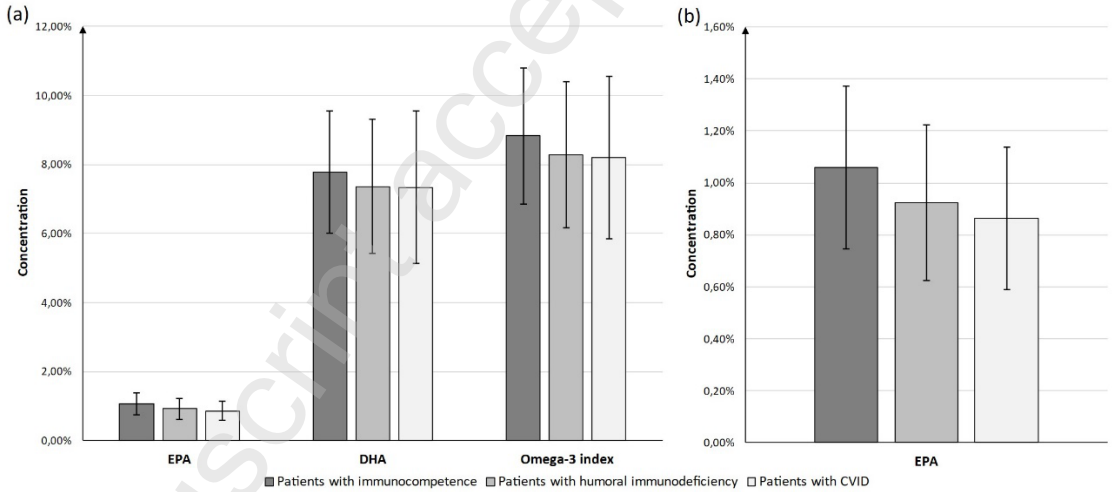
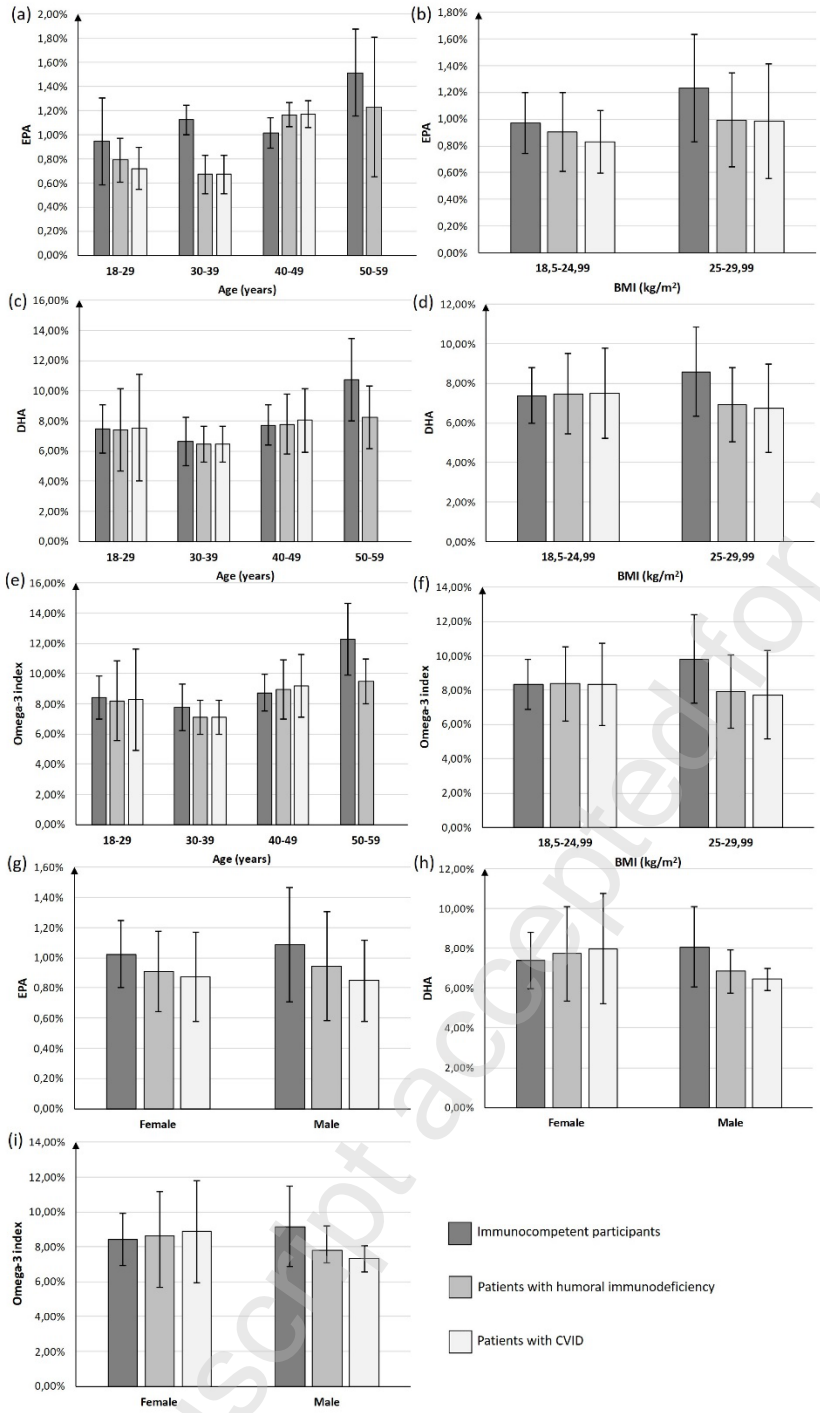


Figure 3. Differences in blood omega-3 fatty acid concentrations, namely EPA, DHA and omega-3 index between immunocompetent individuals, patients with humoral immunodeficiencies and those with CVID depending on age, sex and BMI with marked standard deviation error bars. (a) EPA concentrations in different age groups of patients. (b) EPA concentrations in patients of normal weight and overweight. (c) DHA concentrations in different age groups of patients. (d) DHA concentrations in patients of normal weight and overweight. (e) Omega-3 index values in different age groups of patients. (f) Omega-3 index values in patients of normal weight and overweight. (g) Sex differences in EPA

concentration. (h) Sex differences in DHA concentration. (i) Sex differences in omega-3 values.



Parameter:	Patients				
	Immunocompetent:	Immunodeficient:			
		Overall		CVID	
n	18	19	-	14	-
Sex (F/M)	8 / 10	11 / 8	-	8 / 6	-
Age	34.9 σ = 10.6	37.6 σ = 10.6	p = 0.447	36.0 σ = 8.0	p = 0.747
BMI	24.09 σ = 2.99	22.63 σ = 2.97	p = 0.146	23.10 σ = 3.08	p =0.367

Table 1. Comparison of demographic parameters between patients with humoral immunodeficiency, a subgroup of common variable immunodeficiency (CVID) patients and immunocompetent participants. Age and BMI are expressed as mean values with standard deviation. A p-value <0.05 was considered statistically significant. The letter sigma (σ) denotes the standard deviation calculated for each result.

Parameter	n (c)	n (def)	EPA [%] (c)	EPA [%] (def)	EPA p-value	DHA [%] (c)	DHA [%] (def)	DHA p-value	Omega-3 index [%] (c)	Omega-3 index [%] (def)	Omega-3 index p-value
Age											
18-29	8	6	0.95 σ = 0.36	0.79 σ = 0.18	0.439	7.48 σ = 1.43	7.4 σ = 2.63	0.439	8.43 σ = 1.61	8.19 σ = 2.75	0.333
30-39	3	5	1.12 σ = 0.12	0.67 σ = 0.16	**0.025**	6.65 σ = 1.54	6.45 σ = 1.13	0.881	7.77 σ = 1.59	7.12 σ = 1.19	0.881
40-49	5	6	1.02 σ = 0.13	1.17 σ = 0.10	0.100	7.73 σ = 1.25	7.79 σ = 1.96	1.000	8.74 σ = 1.31	8.96 σ = 1.99	0.855
50-59	2	2	1.52 σ = 0.36	1.23 σ = 0.58	0.439	10.75 σ = 2.38	8.25 σ = 1.48	0.439	12.26 σ = 2.74	9.48 σ = 2.06	0.439
BMI											
18.5-24.99	12	15	0.97 σ = 0.23	0.91 σ = 0.30	0.272	7.37 σ = 1.41	7.48 σ = 2.01	0.661	8.34 σ = 1.46	8.38 σ = 2.16	0.626
25-29.99	6	4	1.23 σ = 0.40	0.99 σ = 0.35	0.667	8.59 σ = 2.25	6.93 σ = 1.86	0.394	9.82 σ = 2.59	7.92 σ = 2.14	0.394
Sex											
Female	8	11	1.02 σ = 0.22	0.91 σ = 0.27	0.265	7.4 σ = 1.41	7.74 σ = 2.37	0.901	8.42 σ = 1.48	8.65 σ = 2.50	0.934
Male	10	8	1.09 σ = 0.38	0.95 σ = 0.36	0.399	8.08 σ = 2.03	6.85 σ = 1.10	0.155	9.17 σ = 2.31	7.79 σ = 1.41	0.143
Overall											
	18	19	1.06 σ = 0.31	0.92 σ = 0.30	0.171	7.77 σ = 1.77	7.36 σ = 1.94	0.354	8.83 σ = 1.97	8.29 σ = 2.11	0.302

Table 2. Summary of arithmetic mean concentrations of EPA and DHA, as well as omega-3 index, stratified by age, BMI and sex, for immunocompetent (c) and immunodeficient (def) patients. A p-value <0.05 was considered statistically significant. The letter sigma (σ) denotes the standard deviation calculated for each result.

Parameter	n (c)	n (CVID)	EPA [%] (c)	EPA [%] (CVID)	EPA p-value	DHA [%] (c)	DHA [%] (CVID)	DHA p-value	Omega-3 index [%] (c)	Omega-3 index [%] (CVID)	Omega-3 index p-value
Age											
18-29	8	4	0.95 σ = 0.36	0.72 σ = 0.18	0.234	7.48 σ = 1.43	7.55 σ = 3.337	0.610	8.43 σ = 1.61	8.27 σ = 3.52	0.445
30-39	3	5	1.12 σ = 0.12	0.67 σ = 0.16	**0.025**	6.65 σ = 1.54	6.45 σ = 1.13	0.881	7.77 σ = 1.59	7.12 σ = 1.19	0.881
40-49	5	5	1.02 σ = 0.13	1.17 σ = 0.11	0.117	7.73 σ = 1.25	8.05 σ = 2.08	0.754	8.74 σ = 1.31	9.22 σ = 2.11	0.602
50-59	2	0	1.52 σ = 0.36	-	-	10.75 σ = 2.38	-	-	12.26 σ = 2.74	-	-
BMI											
18.5-24.99	12	11	0.97 σ = 0.23	0.83 σ = 0.24	0.132	7.37 σ = 1.41	7.50 σ = 2.28	0.538	8.34 σ = 1.46	8.33 σ = 2.40	0.498
25-29.99	6	3	1.23 σ = 0.40	0.98 σ = 0.43	0.606	8.59 σ = 2.25	6.75 σ = 2.24	0.439	9.82 σ = 2.59	7.73 σ = 2.58	0.439
Sex											
Female	8	8	1.02 σ = 0.22	0.87 σ = 0.30	0.189	7.4 σ = 1.41	7.99 σ = 2.77	0.793	8.42 σ = 1.48	8.87 σ = 2.95	0.834
Male	10	6	1.09 σ = 0.38	0.85 σ = 0.27	0.303	8.08 σ = 2.03	6.46 σ = 0.56	0.065	9.17 σ = 2.31	7.31 σ = 0.73	0.058
Overall											
	18	14	1.06 σ = 0.31	0.86 σ = 0.28	0.095	7.77 σ = 1.77	7.34 σ = 2.20	0.314	8.83 σ = 1.97	8.20 σ = 2.36	0.254

Table 3. Summary of arithmetic mean concentrations of EPA and DHA, as well as omega-3 index, stratified by age, BMI and sex, for immunocompetent participants (c) and patients with common variable immunodeficiency (CVID). A p-value <0.05 was considered statistically significant. The letter sigma (σ) denotes the standard deviation calculated for each result.

Supplementary Material:

Table S1. Comorbidities of immunodeficient patients. Abbreviations: CVID - common variable immunodeficiency; IgRT - immunoglobulin replacement therapy; fSCIg - facilitated subcutaneous immunoglobulin; NK cell - Natural killer cell; SCIg - subcutaneous immunoglobulin.

Patient number	Sex	IEI	Comorbidities	Clinical phenotype	IgRT
1	M	Hereditary agammaglobulinemia	History of invasive aspergillosis. Covid-19 infection in the past. Anemia. Asperger's syndrome. Hyperkinetic disorder.	Infectious phenotype	fSCIg
2	M	IgG subclasses deficiency (IgG1, IgG2)	Behcet's disease. Gastritis. Hypertension. Nodular goiter. Post-steroidal diabetes mellitus. Cataracts. Osteoporosis. Cysts of the right kidney. Diverticulosis of the sigmoid colon.	mixed phenotype (infectious and autoimmune)	fSCIg
3	F	IgG deficiency (IgG1 subclass), deficiency of complement component C3 and NK cells	Chronic laryngitis. Allergic rhinitis. Bronchial asthma. History of bacterial overgrowth of the small intestine. Tricuspid valve regurgitation. Mitral valve regurgitation.	Infectious phenotype	fSCIg
4	F	Nonfamilial agammaglobulinemia	Bronchiectasis. Chronic respiratory failure - history of home oxygen therapy. Chronic sinusitis with polyps. Hypertension. Anemia. Hyperuricemia. History of vitamin D deficiency. Lipid metabolism disorders.	Infectious phenotype	fSCIg
5	F	CVID	Bronchiectasis. History of vitamin D3 deficiency. Chronic sinusitis. Post left lower lobectomy status.	Infectious phenotype	fSCIg
6	F	CVID	Diffuse nodular lesions of the lungs with mediastinal lymphadenopathy. History of vitamin D3 deficiency. Condition after wedge resection of the right lung. Esophagitis. Chronic gastritis. Esophageal varix I/II degree according to OMED classification. History of gastric ulcer. Post cholecystectomy status. Pigmented nevi.	mixed phenotype (Infectious phenotype and polyclonal lymphocytic infiltration)	fSCIg
7	F	CVID	Pancytopenia. Interstitial lung disease. History of folic acid and vitamin D deficiency. Hyperuricemia. Hypoalbuminemia. Inflammation of the esophagus. Diaphragmatic hernia. Gastritis and duodenitis. Hypothyroidism during pharmacological hyperthyroidism. Chronic urticaria. Chronic sinusitis. Hemangioma of the liver. Gallbladder stones. Kidney cysts. Mixed hyperlipidemia.	Infectious phenotype	fSCIg
8	F	CVID	Gastritis and duodenitis. Chronic sinusitis. Hypoechoic focal lesion of the thyroid	Infectious phenotype	SCIg

			gland. History of folic acid and vitamin D3 deficiency. History of Covid-19 infection.		
9	M	CVID	Deviated nasal septum. Bronchial asthma. Inflammation of the esophagus, stomach and duodenum. Irritable bowel syndrome. Normocytic anemia. History of folic acid deficiency. Disorders of lipid metabolism. Pigmented nevi. Caries.	Infectious phenotype	fSCIg
10	F	CVID	Splenomegaly. Accessory spleen. Reflux esophagitis. Inflammation of the stomach. Cysts of the liver. Hemangioma of the liver. Chronic sinusitis.	Infectious phenotype	fSCIg
11	M	CVID	Gastritis and duodenitis. Celiac disease. Left epididymal cyst.	mixed phenotype (infectious and autoimmune)	fSCIg
12	F	CVID	Interstitial lung disease. Leukopenia with neutropenia. Inflammation of the esophagus. Gastritis and duodenitis. Deviated nasal septum. Chronic inflammation of the nasal mucosa. History of vitamin D3 deficiency. History of an anaphylactic shock (after the HyQvia, Kiovig, Octagam drugs).	Infectious phenotype	SCIg
13	F	CVID	Leukopenia in the form of neutropenia. Chronic sinusitis. Status post decortication of the left lung in the course of lung abscess. Hypercholesterolemia. Inflammation of the esophagus. Gastritis and duodenitis. Microscopic colitis. Folic acid deficiency.	Infectious phenotype	fSCIg
14	M	CVID	History of hemolytic anemia. Bronchiectasis. Gastritis. Agenesis of the right kidney. Seborrheic dermatitis. Viral warts on the skin of the left hand.	Infectious phenotype	fSCIg
15	M	CVID	Chronic sinusitis. History of COVID-19 with respiratory failure.	Infectious phenotype	fSCIg
16	F	CVID	Pancytopenia. Mitral valve regurgitation. Esophageal varices grade II/III. Portal gastropathy. Hepatomegaly. Splenomegaly. Chronic colitis. Hypocalcemia. Hypoalbuminemia. History of folic acid, iron and vitamin D3 deficiency.	mixed phenotype (infectious and autoimmune)	fSCIg
17	M	CVID	Antiphospholipid syndrome. History of thrombocytopenia. History of lower extremity deep vein thrombosis. Status after appendectomy for acute suppurative appendicitis. Folic acid deficiency. Chronic rhinosinusitis.	mixed phenotype (infectious and autoimmune)	fSCIg

18	M	CVID with non-cancerous lymphoproliferation.	Radiosensitivity. Interstitial lung disease. Nontoxic nodular goiter. Pulmonary granulomas. Gastritis and duodenitis. Atopic dermatitis. History of folic acid and vitamin D3 deficiency.	mixed (Infectious and polyclonal lymphocytic infiltration	SCIg
19	F	Selective deficiency of IgG subclasses	Status post resection of the middle lobe of the right lung due to bronchiectasis and cirrhosis. Slight mitral valve regurgitation. Slight tricuspid regurgitation. Esophagitis. Hernia of the esophageal foramen of the diaphragm. Inflammation of the stomach and duodenum. Chronic sinusitis. Status post bilateral maxillary sinus surgery with widening of their natural orifices. Epilepsy.	Infectious phenotype	fSCIg
20	M	Immunodeficiency associated with other major defects	Vitiligo. Condition after removal of melanoma of the groin (In situ).	mixed phenotype (infectious and lymphoid malignancy)	fSCIg
21	M	CVID	Plaque psoriasis. Chronic urticaria. Pigmented nevi. Acquired vitiligo. Gastritis. Hernia of the esophageal hiatus of the diaphragm. Liver cysts. History of acute idiopathic pericarditis. A history of acute pancreatitis.	mixed phenotype (infectious and autoimmune)	fSCIg

Table S2. EPA, DHA and omega-3 index results and patient characteristics

Patient number	Sex	IEI	EPA	DHA	Omega-3 index	Age	Weight (kg)	Height (cm)	BMI
1	M	Hereditary agammaglobulinemia	0,84%	6,72%	7,56%	23	67	183	20,01
2	M	IgG subclasses deficiency (IgG1, IgG2)	1,64%	9,29%	10,93%	55	60	171	20,52
3	F	IgG deficiency (IgG1 subclass), deficiency of complement component C3 and NK cells	1,15%	6,50%	7,65%	49	54	163	20,32
4	F	Nonfamilial agammaglobulinemia	0,82%	7,20%	8,02%	57	54	164	20,08
5	F	CVID	1,18%	9,06%	10,24%	46	73	169	25,56
6	F	CVID	0,54%	7,55%	8,09%	39	58	170	20,07
7	F	CVID	0,49%	4,59%	5,08%	32	73	156	29,99
8	F	CVID	1,17%	6,38%	7,55%	47	71	174	23,45
9	M	CVID	0,99%	6,96%	7,95%	40	73	175	23,84
10	F	CVID	0,72%	6,52%	7,24%	39	55	170	19,03
11	M	CVID	0,71%	5,87%	6,58%	27	65	178	20,52
12	F	CVID	0,72%	6,00%	6,72%	27	60	164	22,31
13	F	CVID	0,94%	12,61%	13,55%	29	63	171	21,55
14	M	CVID	0,70%	7,09%	7,79%	37	80	180	24,69
15	M	CVID	0,90%	6,51%	7,41%	32	78	180	24,07
16	F	CVID	1,23%	11,24%	12,47%	47	59	172	19,94
17	M	CVID	1,28%	6,60%	7,88%	40	86	178	27,14
18	M	CVID with non-cancerous lymphoproliferation.	0,51%	5,73%	6,24%	22	65	175	21,22
19	F	Selectiv edeficiency of IgG subclasses	1,02%	7,47%	8,49%	26	70	165	25,71

20	M	Immunodeficiency associated with other major defects	1,03%	10,26%	11,29%	21	46	164	17,10
21	M	CVID	1,85%	8,56%	10,41%	40	99	181	30,22
22	F		1,19%	5,50%	6,69%	33	68	175	22,20
23	F		1,26%	9,06%	10,32%	51	60	165	22,04
24	M		0,66%	8,59%	9,25%	23	84	189	23,52
25	F		0,98%	6,04%	7,02%	35	70	172	23,66
26	F		1,00%	6,07%	7,07%	46	74	177	23,62
27	M		1,77%	12,43%	14,20%	57	87	186	25,15
28	F		0,56%	7,10%	7,66%	20	57	158	22,83
29	M		1,20%	7,48%	8,68%	21	85	188	24,05
30	F		0,98%	8,77%	9,75%	27	48	160	18,75
31	F		0,98%	7,87%	8,85%	40	60	174	19,82
32	M		0,76%	5,00%	5,76%	28	79	183	23,59
33	M		0,99%	9,04%	10,03%	42	79	172	26,70
34	F		1,23%	8,75%	9,98%	45	65	165	23,88
35	M		0,85%	8,20%	9,05%	27	66	180	20,37
36	M		1,20%	8,40%	9,60%	32	85	174	28,08
37	M		1,69%	8,88%	10,57%	29	96	184	28,36
38	M		0,88%	6,91%	7,79%	43	98	185	28,63
39	M		0,87%	5,85%	6,72%	29	80	168	28,34
40	M		1,25%	11,01%	12,26%	54	115	175	37,55
41	M		0,70%	4,98%	5,68%	26	110	186	31,80
42	F		0,68%	7,05%	7,73%	38	49,5	165	18,18
43	F		0,68%	6,78%	7,46%	44	84	163	31,62
44	M		0,60%	5,18%	5,78%	24	115	181	35,10

Omega-3 Fatty Acids Questionnaire

First Name and Surname:		Sex*: M / F *underline the correct word
Age(years):	Weight (kg):	Height (cm):
Contact number:		

Please answer the following question by marking an "X" next to the correct answer.	**YES**	**NO**
1. Have you been diagnosed with humoral immunodeficiency?		
2. Are you on a vegetarian or a vegan diet?		
3. Have you taken any dietary supplements in the past month? 3a. If yes, please write the name and dosage of the supplement: _____		

Please answer the following question by marking an "X" next to the correct	**YES**	**NO**

answer.		
4. Have you been diagnosed with hyperlipidemia?		
5. Have you been diagnosed with hypercholesterolemia?		
6. Have you been diagnosed with hypertension?		
7. Have you been diagnosed with diabetes melitus?		
8. Have you been diagnosed with autoimmune diseases? (e.g., Hashimoto's disease, rheumatoid arthritis, psoriasis, celiac disease)		
9. Have you had a heart attack and/or been diagnosed with coronary artery disease?		
10. Do you smoke cigarettes? 10a. If yes, please indicate how many packets per day: _____		

Please answer the following question by marking an "X" next to the correct answer indicating the frequency of the product consumption.		
11. Do you take omega-3 fatty acid supplements and how often?	Every day	
	Few times a week	
	Once a week	
	1-3 times a month	
	I do not consume	
11a. If you consumer supplements containing omega-3 fatty acids, please write the names and dosages: _____		
12. How often do you consume fish?	Every day	
	Few times a week	
	Once a week	
	1-3 times a month	
	I do not consume	
13. How often do you consume fish oil?	Every day	
	Few times a week	
	Once a week	
	1-3 times a month	
	I do not consume	
13a. If you consume fish oil, please write the name of the supplement and dosage: _____		
14. How often do you consume canola oil?	Every day	
	Few times a week	
	Once a week	
	1-3 times a month	
	I do not consume	
15. How often do you consume linseed oil?	Every day	
	Few times a week	
	Once a week	
	1-3 times a month	
	I do not consume	
16. How often do you consume olive oil?	Every day	
	Few times a week	
	Once a week	
	1-3 times a month	
	I do not consume	
17. If you consume other types of oil, please write the type and indicate the frequency of consumption: _____	Every day	
	Few times a week	
	Once a week	
	1-3 times a month	
	I do not consume	
18. How often do you consume walnuts?	Every day	
	Few times a week	

	Once a week	
	1-3 times a month	
	I do not consume	

19. If you consume <u>other types of nuts</u>, please write the type and indicate the frequency of consumption: _____	Every day	
	Few times a week	
	Once a week	
	1-3 times a month	
	I do not consume	
20. How often do you consume <u>chia seeds</u> or <u>linseed</u>?	Every day	
	Few times a week	
	Once a week	
	1-3 times a month	
	I do not consume	
21. If you consume <u>other types of seeds</u>, please write their type and indicate the frequency of consumption: _____	Every day	
	Few times a week	
	Once a week	
	1-3 times a month	
	I do not consume	
22. How often do you consume <u>eggs</u>?	Every day	
	Few times a week	
	Once a week	
	1-3 times a month	
	I do not consume	
23. Please specify how many infectious diseases have you had <u>in the past year</u>?	0 (I have not been ill)	
	1-2	
	3-4	
	5-6	
	> 6	