Altered Plasma Fatty Acids Associate with Gut Microbial Composition in Common Variable Immunodeficiency

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Abstract

Purpose: Fatty acid (FA) abnormalities have been found in various inflammatory disorders and have been related to disturbed gut microbiota. Patients with Common variable immunodeficiency (CVID) have inflammatory complications associated with altered gut microbial composition. We hypothesized that there is an altered FA profile in CVID patients, related to gut microbial dysbiosis.

Methods: Plasma FAs were measured in 39 CVID patients and 30 healthy controls. Gut microbial profile, a food frequency questionnaire and the effect of the oral antibiotic rifaximin, were investigated in CVID patients.

Results: The *n-3* PUFAs**,** EPA (1.4 [1.0-1.8] vs 1.9 [1.2-2.5], median [IQR], P<0.05) and DHA (3.2 [2.4-3.9] vs 3.5 [2.9-4.3], P<0.05) were reduced in CVID compared to controls. Also, *n-6* PUFAs (34.3 ± 3.4, vs 37.1 ± 2.8, mean ± SD, P< 0.001) and linoleic acid (LA) (24.5 ± 3.3, vs 28.1 ± 2.7, P< 0.0001), and the FA anti-inflammatory index (98.9, [82.1-119.4], vs 117.0, [88.7-153.1], median [IQR]), P<0.05) were reduced in CVID. The microbial alpha diversity was positively associated with plasma *n-6* PUFAs, (r=0.41, P<0.001) and LA (r=0.51, P<0.001), but not *n-3* PUFAs (P= 0.78). Moreover, a 2-week course of rifaximin significantly reduced the proportion of *n-6* PUFAs (P=0.04, UNIANOVA). Overall, the altered proportions of *n-3* and *n-6* PUFAs did not seem to be related to dietary intake, intestinal malabsorption or presence of CVID enteropathy.

Conclusion: We found a potentially unfavourable FA profile in CVID, where plasma proportion of *n-6* PUFAs was related to gut microbial diversity and altered by microbial therapy.

Introduction

Common variable immunodeficiency (CVID) has a prevalence of 1:25 000 to 1:50 0000 in Caucasians and is the most common symptomatic primary immunodeficiency in adults [1]. Approximately 10 % of CVID patients have a monogenic cause [2], whereas the remaining patients have a more complex pattern of inheritance involving multiple genetic and environmental factors [3]. CVID comprises a heterogeneous group of patients, characterized by hypogammaglobulinemia resulting in recurrent infections with capsulated bacteria in the respiratory tract. In addition, a large proportion of the patients (70–80%) have non-infectious autoimmune and/or inflammatory complications, such as splenomegaly, lymphadenopathy, enteropathy, granulomas and lymphoid interstitial pneumonitis [3, 4]. The aetiology of these inflammatory and autoimmune complications is largely unknown, but we have previously shown an association with altered gut microbial composition and blood lipids [5, 6].

Fatty acids, both free and as part of complex lipids, play a number of key roles in metabolism and cellular functions. They constitute a major component of cell membranes, act as precursors of extracellular signalling molecules and influence membrane-derived intracellular signalling pathways. These factors are important for human health and disease risk, and numerous studies support a role for FAs in various autoimmune and inflammatory disorders [7, 8]. In general, the *n-3* polyunsaturated FAs (PUFAs) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) seem to have anti-inflammatory properties, whereas the *n-6* PUFA arachidonic acid (ARA) appears to mediate inflammatory reactions, at least partly by acting as precursors for various bioactive lipid mediators with opposing biological effects [9] (Fig. 1). Circulating FAs are also used as biomarkers of dietary FA intake in epidemiological studies, often in relation to inflammatory or cardiometabolic outcomes [10, 11]. However, accumulating evidence supports gut microbiota as a modifier of the dietary impact on the host metabolic state, including FA metabolism [12], linking gut microbiota to metabolic and inflammatory diseases.

Since a large proportion of patients with CVID have autoimmune and/or inflammatory complications, as well as a disturbed gut microbiota and intestinal pathology, we hypothesized that the plasma FA profile is altered in CVID patients and related to gut microbial dysbiosis rather than diet. In the present study, we therefore evaluated the plasma FA composition in CVID patients compared with healthy controls, and its relation to gut microbial composition and diet. We also analyzed the effect of a gut microbiota-targeted intervention on the plasma FA profile after a 2-week course of the non-absorbable antibiotic rifaximin.

Methods

Participants and Procedure

CVID was defined as decreased serum levels of IgG, in addition to IgA and/or IgM by a minimum of two standard deviations below the mean for age, and exclusion of other causes of hypogammaglobulinemia [13]. CVID subgroups were classified as “Infection only” or “Complications” based on previously defined criteria [14], and CVID enteropathy was defined as persistent diarrhoea after exclusion of gastrointestinal infection [5].

This study is a secondary data analysis of a randomized controlled trial that analysed the effect of a 2-week course oral rifaximin on systemic inflammation and gut microbiota composition [15]. The majority of the analyses was performed on plasma, diet and gut microbiota data from the CVID baseline cohort (before randomization), collected between Oct 8, 2013, and Oct 20, 2014. The effects of rifaximin on FAs are presented at the end of the paper. Details regarding the randomization, masking and procedures of the rifaximin trial have previously been described [15], but are also are given in Supplemental methods.

In the present study, 39 CVID participants were compared to 30 healthy controls (one patient from the original rifaximin study was not included because there was not enough plasma available for FA analyses). The overall exclusion criteria were: ongoing infection, antibiotics in the last 12 weeks, a history of allergic reaction to rifaximin, malignancy, impaired kidney function, pregnancy or lactation, use of probiotics in the last 6 months, immunosuppressive drugs, comorbidity that could influence with the patient’s safety or compromise the study results (e.g., cardiovascular disorders, alcoholism, psychiatric disease, HIV infection), and polypharmacy (patient with an extensive medication list i.e. ten drugs or more).

The Regional Committee for Medical and Research Ethics approved the study (protocol number 2013/1037). All study participants signed a written, informed consent. The work described herein has been carried out in accordance with the Declaration of Helsinki.

Blood sampling protocol

Non-fasting peripheral venous blood was drawn into sterile blood collection tubes with EDTA as anticoagulant (plasma). The tubes were immediately immersed in melting ice, centrifuged within 15 minutes at 2000*g* for 20 minutes to obtain platelet-poor plasma. Plasma was stored at − 80°C in aliquots and thawed only once.

Plasma FA composition

The total FA composition was analysed in EDTA-plasma, as previously described [16]. FA concentrations are expressed as percentages of total FAs by weight (wt%). The *omega 3* index was defined as the sum of EPA and DHA, expressed as a percentage of the total FA content. The anti-inflammatory index was defined as the sum of

EPA (C20:5*n-3*), DGLA (C20:3n-6), DHA (C22:6*n-3*) and DPA (C22:5*n-3*), divided on ARA (C20:4*n-6*), multiplied with 100 as previously described [17, 18].

Stool collection and analysis

Participants collected stool samples at home within 24 hours prior to their hospital visit, or alternatively at the hospital, with a standardized collection device [19]. The stool samples were then transferred by the participants to stool collection tubes with Stool DNA Stabilizer (Stratec Biomedical, Birkenfeld, Germany) [20]. Samples were stored at minimum − 20°C according to the manufacturer's recommendations until DNA extraction. Bacterial DNA was extracted using the PSP Spin Stool DNA Plus Kit (Stratec) before being subjected to amplification of the 16S ribosomal RNA gene with dual-indexed barcodes according to an established protocol [21], followed by sequencing on an Illumina MiSeq (San Diego, CA; Supplemental Methods).

Food Frequency Questionnaire

CVID patients were asked to complete a self-administrated, validated Norwegian food frequency questionnaire designed to reflect dietary habits over the past year [22, 23]. The questionnaire offers multiple-choice alternatives and the opportunity to provide supplemental information regarding specific dietary restrictions or habits. It covers 180 food items and has serving size alternatives specified in household units, which is then converted to grams per day using software developed at the Institute for Nutrition Research, University of Oslo [22]. Thirty-seven CVID patients completed the questionnaire.

Statistical analysis

Univariate analyses were performed using parametric (t-test) or non-parametric methods (Mann–Whitney U) for continuous variables, and Fisher's exact test for categorical variables, as appropriate. Correlation analyses were performed using parametric (Pearson) or non-parametric (Spearman) tests as appropriate. Univariate Repeated measures ANOVA (UNIANOVA) was used to assess the effect of treatment focusing on the interaction between time and treatment group for the different FAs, followed by paired samples t-test or Wilcoxon's Rank-Sum test for paired data if significant. For the longitudinal data, we compared datasets from three different time points using the ANOVA test (parametric) or Friedman's test (non-parametric). Calculations were performed in SPSS (version 24, IBM, NY).

Results

Reduced EPA and DHA in patients with CVID

Baseline characteristics of patients (n=39) and controls (n=30) are presented in Table 1, showing no significant differences in age, sex, BMI, statin-use or smoking. We found reduced proportions of the two most abundant marine *n-3* PUFAs**,** EPA (P<0.05) and DHA (P<0.05), in patients with CVID compared to controls (Table 2), resulting in a non-significant trend towards lower total proportion of *n-3* PUFAs in CVID patients (P=0.055, Fig. 2A). In contrast, the essential FA alpha-linolenic acid (ALA, C18:3*n-3*, P<0.05), as well as the downstream FA C18:4n-3 (stearidonic acid, SDA, P<0.05) were increased in CVID as compared to controls (Table 2).

Reduced n-6 PUFAs in CVID

Plasma proportion *of n-6* PUFAs was reduced in CVID compared to healthy controls (P< 0.001, Figure 2B). This observation was primarily caused by a lower percentage of C18:2*n-6* (linoleic acid, LA, P< 0.0001, Table 2), which is the main contributor of *n-6* PUFAs levels and constitute an essential FA not synthesized by humans (Figure 1). In contrast, the downstream *n-6* PUFA metabolism products were increased in CVID, with increased proportions of C20:3*n-6* (dihomo-gamma-linolenic acid, DGLA, P<0.01), C22:4*n-6* (P<0.0001) and C22:5*n-6* (P<0.01, Table 2).

MUFAs, but not SFAs and trans FAs, were increased in patients with CVID

MUFAs are either obtained from the diet or synthesized by elongase/ desaturase enzymes from SFAs. Plasma MUFAs were increased in CVID as compared to healthy controls (P<0.0001, Figure 2C), mainly due to increased C18:1*n-9* (P<0.0001), which is the major contributor to MUFA levels measured in humans, and increased C18:1*n-7* (P<0.01, Table 2).

Plasma FA proportions of total SFAs (P=0.76), free FAs (P=0.06) and trans FAs (P= 0.18) were not different between CVID patients and healthy controls (Supplemental Table S1).

FA composition is stable over time in CVID patients

To study if plasma FA proportions of *n-3* PUFAs, *n-6* PUFAs and MUFAs remained stable over time, we measured these FAs in 20 CVID patients (11 females, mean age 50.8 years, SD 6.9) at three different time points over eight weeks (0, 2 and 8 weeks). Sixteen patients completed all three measurements and we found no significant changes in plasma wt% of *n-3* PUFAs (p=0.27), *n-6* PUFAs (p=0.67) or MUFAs (p=0.53) during these measurements (Supplemental Fig. S1).

CVID patients have a potential unfavourable FA composition

To evaluate the potential net effects of the PUFAs pattern in the CVID patients we calculated the anti-inflammatory index based on the proportions of the potential anti-inflammatory FAs (DPA + DHA + DGLA + EPA), relative to the potential inflammatory FA ARA, showing a significant reduction in the anti-inflammatory index in CVID as compared to controls (P = 0.02, Figure 2D). Furthermore, the *omega 3* index (the sum of EPA and DHA, expressed as a percentage of the total FA content) was also reduced in patients with CVID (Figure 2E). Taken together, these results may suggest that CVID patients have an unfavourable FA composition with potential pro-inflammatory net effect.

Associations between dietary and plasma n-3 and n-6 PUFAs in CVID patients

We next examined the possible contribution of diet and FA gut absorption to the disturbed FA profile in CVID. Some plasma FAs are commonly used as objective indicators of dietary FA intake, e.g. ALA and LA, which are not biosynthesized at all, and EPA and DHA, which are synthesized endogenously in very limited amounts (Figure 1) [24, 25]. In the CVID patients (n=37), dietary intake of the marine FA EPA was positively correlated with plasma EPA (rho=0.41, P<0.05) and DHA (rho=0.45, P<0.01), respectively, and dietary DHA was also correlated with plasma DHA (rho=0.47, P<0.01). Contrary, dietary intake of ALA did not correlate with plasma ALA (rho=0.21, P=0.22) and there was no association between dietary intake and plasma FA proportion of LA (rho=0.30, P=0.07). Notably, however, the mean fish intake in this CVID cohort was 81 grams/day and according to National Dietary Survey [26], mean Norwegian dietary fish intake was 67 grams/day, suggesting that the reduced marine *n-3* PUFAs, EPA and DHA in CVID patients were not merely related to reduced fish intake from the diet

(Supplemental Fig. S2A). Overall, there were no differences in neither the dietary intake of fat, protein, carbohydrate, meat and egg, nor the dietary intake of SFAs, MUFAs and total PUFAs, between CVID patients and the general Norwegian population, according to Norkost 3 (Supplemental Fig. S2B-C), indicating a normal diet in the CVID patients compared to the general Norwegian population.

Gut microbiome and FA profile in CVID patients

We have previously reported reduced microbial diversity and gut microbial dysbiosis in CVID patients compared to controls [5]. When evaluating microbial diversity and its association to FAs in patients with CVID, multiple measures of intra-individual alpha diversity were positively associated with plasma *n-6* PUFAs (Faith's PD whole tree index, r=0.41, P<0.001 and Supplemental Table S2), but not plasma *n-3* PUFAs (Faith's PD whole tree, r=0.05, P=0.78 and Supplemental Table S2). Furthermore, LA, the main contributor of *n-6* PUFAs levels, also correlated positively with alpha diversity (Faith's PD whole tree, r=0.51, P<0.001 and Supplemental Table S2). In contrast, plasma MUFAs were negatively associated with microbial diversity (Faith's PD whole tree, r=-0.34, P=0.036 Supplemental Table S2).

Next, we examined if 15 specific bacterial taxa, previously found to differentiate between CVID patients and healthy controls [5, 27], were associated with plasma proportions of MUFAs, *n-3* and *n-6* PUFAs (Table 3). *Desulfovibrionaceae*, shown to be decreased in CVID patients, was negatively correlated with *n-3* PUFAs and EPA, and showed a positive association with MUFAs. No other significant correlations between the FA pattern and the 15 selected bacterial taxa were found (Table 3).

Rifaximin-induced alterations on plasma FAs

Rifaximin is a broad-spectrum oral antibiotic with negligible systemic absorption, making it suitable to study how changes in gut microbial composition affect systemic markers in blood. We have previously shown that a 2-week course of rifaximin led to significant alterations in gut microbial composition in CVID with a decrease in alpha diversity and alteration in 16 different bacterial taxa [15]. In the present study, we explored if rifaximin-induced alterations of gut microbial composition could influence plasma FA proportions. Briefly, 39 CVID patients, aged 21–69 years (62% women), were randomized to rifaximin 550 mg bid versus no treatment for two weeks, and followed up for an additional six weeks. When comparing the two groups (*rifaximin* versus *no treatment*), we found a significant change of the proportion of *n-6* PUFAs (P=0.04, UNIANOVA), due to a significant decrease from week 0 to week 2 in the patients receiving rifaximin (Table 4). This change was also significant for the major contributor of *n-6* PUFAs, LA (Table 4). However, there was no significant change in the plasma FA proportions of neither *n-3* PUFAs, nor EPA and DHA (Table 4). The proportion of plasma MUFAs increased significantly from week 0 to week 2 in the *rifaximin* arm compared to the *no treatment* arm, but the impact of rifaximin did not last to the end of the observation period (Table 4).

Based on these findings, we wanted to explore if there were any correlations between the 16 bacterial taxa that we have previously shown to be significantly changed by a 2-week course with rifaximin [15], and FAs altered by rifaximin (Supplemental Table S3). We found that the decrease in plasma FA proportion of *n-6* PUFAs, after rifaximin therapy, was positively correlated with two taxa from the Firmicutes phyla: *Ruminococcaceae UCG-002* (rho=0.44, P<0.01) and *Clostridiales. Family XIII.Family XIII UCG-001* (rho=0.41, P<0.01). These bacterial taxa also correlated with the major contributor to *n-6* PUFA, LA (Supplemental Table S3). Interestingly, the increase in

MUFAs from week 0 to week 2 could be related to a negative correlation with the same taxon that was positively correlated with *n-6* PUFAs, namely *Ruminococcaceae UCG-002* (rho=-0.488, P< 0.01).

To summarize the microbiota related findings: *n-6* PUFAs and LA correlated with alpha diversity, and all three measures were reduced by rifaximin therapy. Two bacteria that were decreased by rifaximin, also correlated with *n-6* PUFAs, suggesting a link between alpha diversity, *n-6* PUFAs and specific taxa.

FAs and clinical sub-groups in CVID

Lastly, we evaluated whether the FA profile in CVID could be related to different clinical subgroups of CVID patients. There were no significant differences in plasma FA proportions of *n-3* PUFAs, *n-6* PUFAs and MUFAs between CVID patients with non-infectious complications (n=32) compared to CVID patients with infection only (n=7) (Supplemental Fig.S3A). Furthermore, when categorizing CVID patients according to presence (n=13) or absence (n=26) of enteropathy, we found no differences in the proportions of plasma *n-3* PUFAs, *n-6* PUFAs and MUFAs between the two groups (Supplemental Fig. S3B), suggesting the disturbed proportions of these FAs do not merely reflect intestinal pathology.

Discussion

To the best of our knowledge, this is the first study investigating the FA profile in CVID patients. Our main findings were: (i) CVID patients have a potential unfavourable FA profile with reduced plasma FA proportions of EPA and DHA, and a reduced anti-inflammatory index; (ii) *n-6* PUFAs and LA were decreased in CVID patients, and were positively associated with gut microbial alpha diversity; (iii) a 2-week course of oral rifaximin known to reduce gut microbial diversity, led to a reduction of *n-6* PUFAs; (iv) The altered plasma FA proportions of *n-3* and *n-6* PUFAs did not seem to be related to altered dietary intake, intestinal absorption or the presence of CVID enteropathy.

There are numerous reports on the potential beneficial effects of increased *n-3* PUFAs on autoimmunity and metabolic disorders such as diabetes, obesity and cardiovascular diseases [17, 28, 29], supporting that reduced plasma proportions of EPA and DHA, and a reduced anti-inflammatory FA index in CVID patients may represent an unfavourable FA profile. The parallel findings of *reduced* plasma FA proportions of EPA and DHA, and *increased* ALA, are not contradictory, because the conversion of ALA to EPA, and in particular to DHA (Fig. 1), appears to be very inefficient in humans [30, 31].

CVID enteropathy did not influence plasma FA percentage in the CVID patients, suggesting that altered FA profile between controls and patients with CVID was not due to enteropathy-associated malabsorption. Furthermore, we found positive correlations between dietary sources of the marine *n-3* PUFAs, EPA and DHA, and their respective plasma proportions, suggesting a normal FAs absorption in CVID. The lack of correlation between dietary and plasma proportion of ALA is, however, in line with considerable discrepancies in previous published studies [24, 25, 32, 33]. The fact that dietary LA and plasma LA did not correlate in CVID, may be related to gut microbial modulation as discussed below.

Several studies, both in mice and humans, suggest that the gut microbiota could play a role in lipid metabolism and lipid levels in blood and tissues [34]. Although several intervention studies have been conducted to assess

the impact of dietary PUFAs, SFAs and MUFAs on gut microbiota composition [35–37], studies on the relationship between gut microbiota and blood PUFAs and MUFAs are rather scarce both in healthy subjects and in disease states [38]. Previously, Horigome *et al.* found a positive correlation between *Bifidobacterium* and levels of blood EPA and DHA in Japanese middle-aged breast cancer survivors (> 99% women, mean age 59 years and 46 % with history of chemotherapy) [39]. In contrast, Org *et al.* found a negative correlation between DHA and *Bifidobacterium* in a population-based cross-sectional study of Finnish men aged 45–70 (mean age 62, mean BMI 28) [38]. The latter study found associations between 19 different taxa and serum FAs, and relevant to CVID dysbiosis, a negative correlation with DHA, MUFAs, PUFAs and the genus *Blautia*. The conflicting findings in these two studies, and the lack of correlation between the CVID dysbiosis taxa *Bifidobacterium* and *Blautia* and FAs in our study, are likely due to major confounding factors such as age, sex, BMI, therapy, genetics and geography. In the present study, *Desulfovibrionaceae* in stools correlated negatively with plasma proportions of *n-3* PUFAs and EPA, and positively with MUFAs. This bacterial family has previously been shown to be reduced in CVID and is a part of the CVID dysbiosis index, and although the implication of this finding is at present unclear, the negative association between *n-3* PUFAs and positive association with MUFAs merit further investigation.

A major finding in the present study was a significant association between *n-6* PUFAs and LA and the gut microbial intra-individual alpha diversity in CVID patients. This association was further supported when microbial therapy with rifaximin both decreased *n-6* PUFAs and LA and, as previously shown, gut microbial alpha diversity. Interestingly, specific taxa, which were altered by rifaximin, correlated to *n-6* PUFAs, linking specific gut microbial bacterial taxa to plasma *n-6* PUFAs. Hence, the findings support an important 'proof-of-concept' that alteration of the gut microbial composition could affect FA measurements in blood. Whereas most studies on FAs and gut microbiota have been focusing on *n-3* PUFAs, our findings suggest that *n-6* PUFAs, and in particular LA, could be of relevance in relation to the interaction between gut microbiota and FAs. Moreover, whereas LA has been thought to promote inflammation, there are also some data that suggest the opposite effect [40], and our present study may support potential beneficial effects of FA on gut microbiota composition.

The strengths of this study are, compared to other FA studies, the inclusion of detailed dietary- and gut microbial data. In addition, we report results from a randomized control trial evaluating the effect of microbial therapy on plasma proportions of FAs in CVID patients. Finally, the longitudinal data on MUFA, *n-3* and *n-6* PUFAs in patients with CVID confirmed stable FA proportions over time, implying that FAs are relative stable markers in this cohort. The study also has some limitations in that relatively few patients and controls were examined. In particular, the low number of patients with non-infectious complications may have hampered the comparisons between the two clinical subgroups in CVID. Furthermore, we did not have diet data on the healthy controls and therefore used data from National Dietary Survey as a control cohort for dietary analyses. Lastly, many comparisons were performed and some of the findings could be by chance.

In conclusion, we found a potentially unfavourable FA profile in CVID patients with reduced plasma EPA, DHA and anti-inflammatory index, and decreased *n-6* PUFAs and LA. Plasma FA proportions of *n-6* PUFAs and LA were related to gut microbial diversity and altered by a 2-week course of rifaximin, suggesting an intriguing interaction between gut microbiota and these FAs, and in particular LA, in CVID. Future studies should be conducted to investigate the relationship between gut microbiota and the FA profile, as well as the potential clinical consequences of unfavourable FA composition in CVID.

Abbreviations

CVID: common variable immunodeficiency

FA: fatty acid

ALA: alpha-linolenic acid

EPA: eicosapentaenoic acid

DPA: docosapentaenoic acid

DHA: docosahexaenoic acid

LA: linoleic acid

GLA: gamma-linolenic acid

DGLA: dihomo-gamma-linolenic acid

ARA: arachidonic acid

SDA: stearidonic acid

MUFAs: monounsaturated fatty acids

PUFAs: polyunsaturated fatty acids

D5D: delta-5 desaturase

D6D: delta-6 desaturase

Declarations

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Conflicts of interest

The authors declare that they have no conflict of interest.

Availability of data and material

The datasets analyzed during the current study are not publicly available due to Norwegian legislation regarding general data protection regulation but are available from the corresponding author (TS), on reasonable request.

Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki. The studies involving human participants were reviewed and approved by The Regional Committee for Medical and Research Ethics, South

East Norway (protocol number 2013/1037).

Authorship Contributions

MEM, BF, PA and SFJ collected clinical data. TS, JRH, XYK, PB, BH RKB and SFJ performed analyses. TS and SFJ performed statistical analyses. BF, PA and SFJ contributed to the study conception. TS, PA and SFJ wrote the paper. All authors critically revised the manuscript for important intellectual content, approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

Consent to participate and to publish

Written informed consent to participate in the study was obtained from all participants. All patients signed informed consent regarding publishing their data.

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Tables

Table 1. Baseline characteristics of the study population

Baseline characteristics	CVID patients (n=39)	Controls (n=30)	P value
Age mean ± SD (range)	49.8 ± 12 (21-69)	49.7 ± 13 (25-69)	0.87[a]
Male, n (%)	15 (38)	11 (37)	0.99[b]
BMI mean ± SD (range)	26.0 ± 4.7 (16.9-38.3)	24.4 ± 3.2 (19.5-31.0)	0.20[a]
Smoking, n (%)	4 (10)	2 (7)	0.99[b]
Statins, n (%)	3 (0.08)	0	0.25[b]
Infection only, n (%)	7 (18)	-	-
Enteropathy, n (%)	13 (33)	-	-

BMI: Body mass index. Data were analysed using [a]Mann Whitney *U* test or [b]Fisher's exact test. Information on smoking habits was missing in three controls.

Table 2. Plasma proportions of *n-3* PUFAs, *n-6* PUFAs and MUFAs in CVID patients and healthy controls

	CVID patients (n=39)	Controls (n=30)
n-3 PUFAs		
C18:3*n-3* (ALA)*	**0.83 (0.73-0.92)**	0.74 (0.54-0.84)
C18:4*n-3* (SDA)*	**0.04 (0.03-0.06)**	0.03 (0.02-0.04)
C20:4*n-3*	0.17 (0.14-0.21)	0.16 (0.13-0.19)
C20:5*n-3* (EPA)*	1.40 (0.95-1.79)	**1.93 (1.21-2.48)**
C21:5*n-3*	0.010 (0.005-0.018)	0.017 (0.005-0.028)
C22:5*n-3* (DPA)	0.75 (0.63-0.83)	0.78 (0.65-0.90)
C22:6*n-3* (DHA)*	3.15 (2.42-3.92)	**3.52 (2.93-4.33)**
n-6 PUFAs		
C18:2*n-6* (LA)****	24.5 ± 3.30	**28.1 ± 2.70**
C18:3*n-6* (GLA)	0.39 (0.31-0.48)	0.33 (0.20-0.45)
C20:2*n-6*	0.21 ± 0.04	0.19 ± 0.03
C20:3*n-6* (DGLA)**	**1.68 ± 0.29**	1.49 ± 0.30
C20:4*n-6* (ARA)	7.15 ± 1.31	6.72 ± 1.41
C22:2n-6	0.01 (0.009-0.012)	0.01 (0.008-0.012)
C22:4*n-6******	**0.21 ± 0.04**	0.16 ± 0.05
C22:5*n-6**	**0.12 (0.10-0.15)**	0.10 (0.08-0.12)
MUFAs		
C14:1*n-5*	0.05 (0.03-0.08)	0.04 (0.04-0.07)
C16:1*n-7*	1.61 ± 0.54	1.57 ± 0.40
C16:1*n-9***	**0.27 ± 0.07**	0.22 ± 0.03
C18:1*n-7**	**1.66 ± 0.25**	1.49 ± 0.15
C18:1*n-9******	**22.2 ± 3.29**	18.4 ± 2.45
C20:1*n-7*	0.013 (0.011-0.017)	0.014 (0.011-0.015)
C20:1*n-9**	**0.19 (0.17-0.22)**	0.15 (0.13-0.18)
C20:1*n-11*	0.04 (0.03-0.05)	0.04 (0.03-0.05)
C22:1*n-7*	0.010 ± 0.003	0.010 ± 0.002
C22:1*n-9***	**0.04 (0.03-0.04)**	0.03 (0.03-0.04)

C22:1*n-11*	0.005 (0.002-0.020)	0.007 (0.003-0.015)
C24:1*n-9*	1.34 ± 0.31	1.31 ± 0.24

Values indicate plasma FA content by weight percent (wt%). FAs significantly changed are marked in bold, as well as the corresponding mean/median values that are increased in either CVID or controls.

Data were analyzed using Student's t-test or Mann-Whitney *U* test, as appropriate, and are presented as mean ± SD, or median (25–75 percentile), respectively. *p<0.05; **p<0.01; ***p<0.001 and ****p<0.0001.

CVID, common variable immunodeficiency; ALA, alpha-linolenic acid; SDA, stearidonic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; LA, linoleic acid; GLA, gamma-linolenic acid; DGLA, dihomo-gamma-linolenic acid; ARA, arachidonic acid; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids.

Table 3. Correlations between *n-3* PUFAs, *n-6* PUFAs and MUFAs, and bacterial taxa involved in gut microbial dysbiosis in CVID

Bacterial Taxa	Increased in:	Correlation with *n-3* PUFAs, Rho	Correlation with EPA Rho	Correlation with DHA Rho	Correlation with *n-6* PUFAs, Rho	Correlation with MUFAs, Rho
Bacilli[a]	CVID	-0.01	0.01	-0.04	0.07	-0.12
Dorea[a]	CVID	-0.10	-0.12	-0.12	0.22	0.01
Roseburia[a]	CVID	0.11	0.08	0.11	-0.31	0.10
Gammaproteobacteria[a]	CVID	-0.01	-0.10	0.03	-0.10	0.01
Hungatella[b]	CVID	0.21	0.13	0.26	0.013	-0.08
Flavonifractor[b]	CVID	-0.01	-0.03	-0.02	-0.15	0.23
Veillonella[b]	CVID	0.03	-0.02	0.03	-0.23	0.15
Escherichia-Shigella[b]	CVID	-0.11	-0.18	-0.10	-0.04	-0.04
Bifidobacterium[a]	Healthy	0.12	0.27	-0.04	0.08	0.03
Porphyromonadaceae[a,c]	Healthy	-0.07	-0.11	0.07	0.13	-0.04
Christensenellaceae[a]	Healthy	-0.17	-0.13	-0.16	0.30	-0.25
Blautia[a]	Healthy	0.11	0.07	0.15	0.00	0.08
Sutterella[a]	Healthy	0.17	0.16	0.23	-0.11	0.17
Desulfovibrionacea[a]	Healthy	**-0.34***	**-0.32***	-0.25	-0.18	**0.37***
Christensenellaceae R-7 group[b]	Healthy	-0.12	-0.16	-0.18	0.29	-0.26

CVID, common variable immunodeficiency; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids. For the individual plasma FAs, we only studied those that are synthesized endogenously in limited amounts/ not synthesized at all and were found to correlate with diet, i.e., EPA, and DHA. Correlations were calculated by the Spearman's rank correlation test and are presented by rho. Significant correlations are marked in bold. *P<0.05, **P<0.01, ***P< 0.001.

[a]Significant bacterial taxa from Jørgensen at *al.*, *Mucosal Immunol* 2016 [5]. [b]Significant taxa from Jørgensen *et al.*, *J Allergy Clin Immunol*, 2019 [27] . [c]Formally known as Odoribacteraceae.

Table 4: The effect of rifaximin on fatty acids

Fatty acids	Group	Baseline[a]	2 weeks[a]	8 weeks[a]	P value[b] interaction
***n-3* PUFAs**	No Int	6.4 (5.5-7.8)	6.0 (5.0-7.7)	6.2 (5.6-7.3)	
(wt%)	Rif	5.8 (5.1-7.1)	6.4 (4.8-7.2)	6.5 (5.3-7.7)	0.587
EPA	No Int	1.6 (1.1-1.9)	1.4 (0.7-1.9)	1.4 (1.2-1.8)	
(wt%)	Rif	1.2 (0.9-1.7)	1.2 (0.8-1.9)	1.5 (1.0-2.0)	0.403
DHA	No Int	3.2 (2.6-4.0)	3.3 (2.4-3.9)	3.0 (2.6-3.8)	
(wt%)	Rif	2.8 (2.0-3.6)	2.8 (2.1-3.7)	3.2 (2.4-3.7)	0.491
***n-6* PUFAs** (wt%)	No Int	33.3 (31.0-36.7)	34.8 (31.0-37.3)	35.0 (30.9-37.5)	
	Rif	35.3 (32.7-37.0)	33.2 (30.0-36.0)**	32.7 (29.6-35.7)	0.040
LA	No Int	22.9 (20.8-26.8)	24.4 (22.2-28.1)	25.8 (21.8-27.8)	
(wt%)	Rif	24.8 (23.4-27.1)	23.5 (20.9-26.0)**	23.6 (21.5-27.1)	0.035
MUFAs	No Int	27.8 (25.7-28.7)	26.8 (24.6-27.5)	28.3 (26.1-30.4)	
(wt%)	Rif	26.8 (25.8-30.3)	27.6 (25.8-30.7)*	28.2 (25.4-31.2)	0.087

Fatty acid proportions in plasma in the “rifaximin” (Rif, n = 19) and in the “no intervention” group (No Int, n = 20). [a]Data are given in median (25–75 percentile). [b] The P value reflects the interaction between time and group from UNIANOVA. *P <0.05 vs. baseline. **P ≤0.01 vs. baseline.

EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; LA, linoleic acid.

Figures

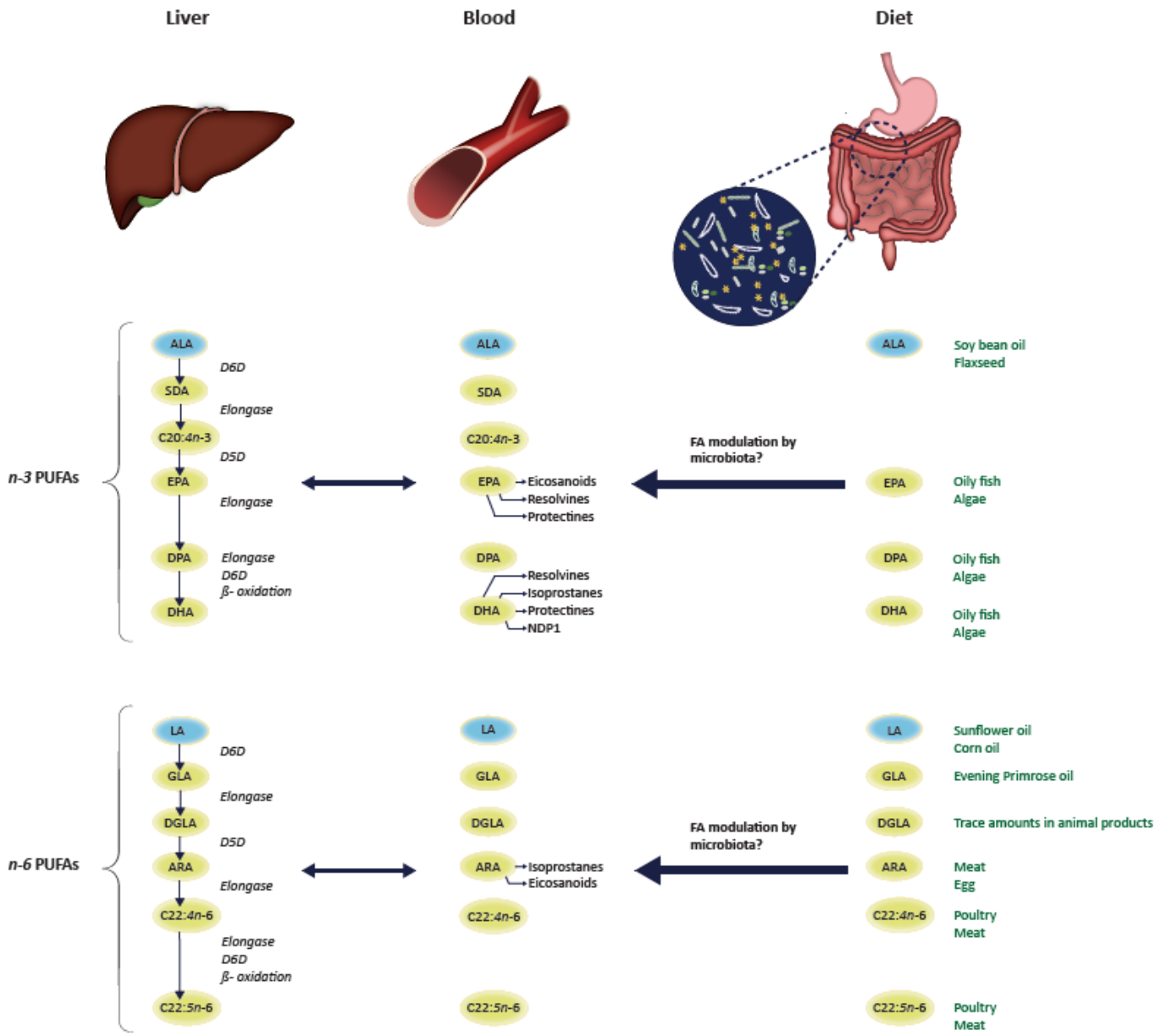

Figure 1

Metabolism and biosynthesis of n-3 and n-6 PUFAs in relation to diet and gut microbiota. This is a simplified illustration of factors influencing the most abundant plasma PUFAs, showing n-3 and n-6 PUFA biosynthesis and metabolism, as well as diet sources (green colour) and the possible role of gut microbiota modulation of dietary fatty acids. ALA and LA (blue colour) are essential fatty acids, obtained through diet only. The biosynthesis of non-essential PUFAs are mainly confined to the liver, but conversion rates are generally low. EPA, DHA and DGLA are precursors of bioactive anti-inflammatory compounds (small arrows) and ARA give rise to mostly inflammatory compounds (small arrows). ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; LA, linoleic acid; GLA, gamma-linolenic acid; DGLA, dihomo-gamma-linolenic acid; ARA, arachidonic acid; SDA, stearidonic acid; D5D, delta-5 desaturase; D6D, delta-6 desaturase. Illustration: Ine Eriksen, University of Oslo.

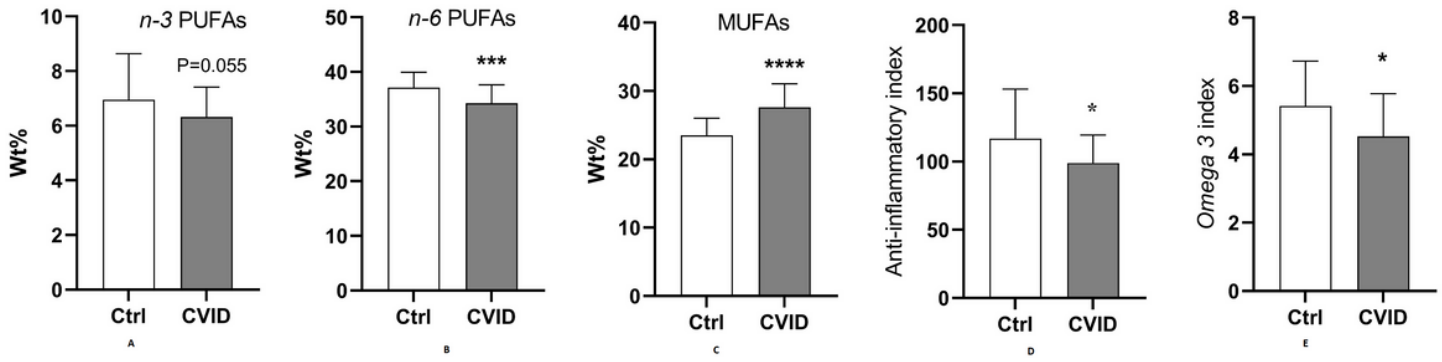

Figure 2

FA profile in CVID and healthy controls. Plasma FA proportions of (A) n-3 PUFAs, (B) n-6 PUFAs, (C) MUFAs, (D) anti-inflammatory index and (E) omega 3 index in controls (Ctrl, n=30) and patients with CVID (Common variable immunodeficiency, n=39). The anti-inflammatory index was defined as the sum of EPA (C20:5n-3), DGLA (C20:3n-6), DHA (C22:6n-3) and DPA (C22:5n-3), divided on ARA (C20:4n-6), multiplied with 100. The omega 3 index was defined as the sum of EPA and DHA, expressed as a percentage of the total FA content. PUFAs, polyunsaturated fatty acids; MUFAs monounsaturated fatty acids; Wt%, weight %. Data are presented with mean (SD) or median (25-75 percentile) and were analysed using Mann-Whitney U test or Student's t-test, as appropriate. *p< 0.05, **p< 0.01, ***p< 0.001 and ****p< 0.0001 versus controls.

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