DISSERTATION

Omega-3-Fettsäuren und Oxylipin-Profile: Bedeutung für Diabetes mellitus Typ 2 und chronisches Darmversagen

Omega-3 Fatty Acids and Oxylipin Profiles: Implications for Type 2 Diabetes Mellitus and Chronic Intestinal Failure

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Abbreviations

AA	Arachidonic Acid
ALA	Alpha-Linolenic Acid
BMI	Body Mass Index
CAD	Coronary Artery Disease
CAP	Controlled Attenuation Parameter
COX	Cyclooxygenase
CRP	C-reactive Protein
CVD	Cardiovascular Disease
CI	Confidence Interval
CIF	Chronic Intestinal Failure
CYP	Cytochrome P450
DHET	Dihydroxy-Eicosatetraenoic Acid
DHA	Docosapentaenoic Acid
DiHDPA	Dihydroxy-Docosapentaenoic Acids
DiHETE	Dihydroxy-Octadecenoic Acid
EDP	Epoxydocosapentaenoic Acids
EEQ	Epoxyeicosatetraenoic Acids
EET	Epoxyeicosatrienoic Acid
EPA	Eicosapentaenoic Acid
FOLE	Fish Oil-Based Fat Emulsions
FAME	Fatty Acid Methyl Ester
FBS	Fasting Blood Sugar
GC	Gas Chromatography
HbA1c	Hemoglobin A1c
HDHA	Hydroxyeicosapentaenoic Acid
HEPE	Hydroxyeicosapentaenoic Acid
HETE	Hydroxyeicosatetraenoic Acid
HOTrE	Hydroxyoctadecatrienoic Acid
HDL-C	High-Density Lipoprotein Cholesterol

HOMA-IR	Homeostatic Model Assessment of Insulin Resistance
HTG	Hypertriglyceridemia
IDOs	Inflammation Dampening Oxylipins
IFALD	Intestinal Failure-Associated Liver Disease
IL-6	Interleukin-6
LA	Linoleic Acid
LC/ESI-MS/MS	Liquid Chromatography-Electrospray Lonization-Tandem Mass Spectrometry
LDL-C	Low-Density Lipoprotein Cholesterol
LOX	Lipoxygenase
LSM	Liver Stiffness
LTB4	Leukotriene B4
NAFLD	Non-Alcoholic Fatty Liver Disease
NASH	Non-Alcoholic Steatohepatitis
MetS	Metabolic Syndrome
MUFA	Monounsaturated Fatty Acid
PDA	Pentadecanoic Acid
PN	Parenteral Nutrition
PNALD	Parenteral Nutrition-Associated Liver Disease
PGE2	Prostaglandin E2
PUFA	Polyunsaturated Fatty Acids
RCT	Randomized Controlled Trial
SBS	Short Bowel Syndrome
SMD	Standardized Mean Difference
SPM	Specialized Pro-Resolving Mediator
TC	Total Cholesterol
TE	Transient Elastography
T2DM	Type 2 Diabetes Mellitus
TG	Triglyceride
TNF-α	Tumor Necrosis Factor-Alpha
TXB2	Thromboxane B2
sEH	Soluble Epoxide Hydrolase

VLDL-C	Very Low-Density Lipoprotein Cholesterol
WMD	Weighted Mean Difference

Abstract (English)

Background: Omega-3 polyunsaturated fatty acids (n-3 PUFAs) are widely considered to play a crucial role in human health and disease. N-3 PUFAs and their derived metabolites are thought to offer numerous benefits, including triglyceride (TG) reduction, antiarrhythmic, antithrombotic, and anti-inflammatory effects. However, the evidence supporting these advantages in humans remains a subject of ongoing debate.

In the first study of this dissertation, a meta-analysis synthesized data from numerous investigations on the effects of n-3 PUFA in type 2 diabetes mellitus (T2DM). The second study explored the associations between hepatic steatosis, as well as n-3 PUFA and oxylipin profiles in T2DM patients with and without hypertriglyceridemia (HTG). The third study examined the effect of parenteral n-3 PUFA supplementation on oxylipin formation in individuals with chronic intestinal failure (CIF).

Methods: In the meta-analysis, databases were searched to collect eligible randomized controlled trials (RCTs). Using a random effects model for pooled assessment, standardized mean difference (SMD) and 95% confidence interval (95% CI) were presented as the results. For the second study, forty patients with T2DM were categorized into a high TG group (TG $\geqslant$ 1.7mmol/l) and a normal TG group with TGs within the reference range (TG < 1.7mmol/l). Lastly, in a study assessing defined n-3 PUFA supplementation, eight CIF patients initially received no fish oil parenteral nutrition (PN) for 8 weeks and then continued fish oil containing parenteral nutrition for an additional 8 weeks.

Results: Study 1 showed that, in comparison to controls, n-3 PUFA supplementation in T2DM significantly lowered the levels of TG, total cholesterol (TC), hemoglobin A1c (HbA1c) and C-reactive protein (CRP), while high-density lipoprotein cholesterol (HDL-C) was elevated. In study 2, T2DM patients with HTG were associated with higher controlled attenuation

parameter (CAP) values, indicating hepatic steatosis, as well as more abundant cytochrome P450 (CYP) and lipoxygenase (LOX) metabolites. In study 3, CIF patients receiving fish oil showed a high omega-3 Index (11.96%) at baseline which decreased to 9.57% without fish oil supplementation, and then rose to 12.75% upon resuming fish oil. Use of fish oil in PN resulted in a substantial increase in CYP- and LOX-metabolites produced by eicosapentaenoic acid (EPA) and docosapentaenoic acid (DHA).

Conclusions: The findings from this dissertation improve our understanding of the role that n-3 PUFAs play in reducing cardiovascular risk factors among individuals with T2DM. Furthermore, it also sheds light on the associations between both n-3 and n-6 PUFAs and their metabolites in T2DM with HTG and CIF patients.

Abstract (Deutsch)

Hintergrund: Mehrfach ungesättigten Omega-3-Fettsäuren (n-3 PUFAs) wird eine wichtige Rolle für die menschliche Gesundheit und Krankheit zugeschrieben. N-3-PUFAs und ihren abgeleiteten Metaboliten werden zahlreiche Vorteile zugeschrieben, darunter die Senkung des Triglyceridspiegels (TG), sowie antiarrhythmische, antithrombotische und entzündungshemmende Wirkungen. Die Belege für diese Vorteile in menschlichen Populationen sind jedoch nach wie vor Gegenstand von Diskussionen.

In der ersten Studie dieser Dissertation wurden in einer Meta-Analyse Daten aus zahlreichen Untersuchungen zu den Auswirkungen von n-3-PUFA bei Diabetes mellitus Typ 2 (T2DM) zusammengefasst. Die zweite Studie untersuchte die Zusammenhänge zwischen Lebersteatose sowie n-3-PUFA und Oxylipinprofilen bei T2DM-Patienten mit und ohne Hypertriglyceridämie (HTG). Die dritte Studie untersuchte den Effekt einer parenteralen n-3-PUFA-Supplementierung auf die Oxylipin-Bildung bei Personen mit chronischem Darmversagen (CIF).

Methode: Für die Meta-Analyse wurden Datenbanken durchsucht, um geeignete randomisierte kontrollierte Studien (RCTs) zu sammeln. Unter Verwendung eines Modells mit zufälligen Effekten für die gepoolte Bewertung wurden die standardisierte mittlere Differenz (SMD) und das 95 %-Konfidenzintervall (95 % CI) als Ergebnisse präsentiert. Für die zweite Studie wurden Patienten mit T2DM (n=40) in eine Gruppe mit erhöhten Triglyceriden (TG ≥ 1,7mmol/l) und eine Gruppe mit TG-Werten innerhalb des Referenzbereichs (TG < 1,7mmol/l) eingeteilt. In dritten Studie schließlich erhielten CIF-Patienten (n=8) zunächst 8 Wochen lang eine parenterale Ernährung ohne Fischöl und dann weitere 8 Wochen lang eine fischölhaltige parenterale Ernährung.

Ergebnisse: Studie 1 zeigte, dass eine n-3-PUFA-Supplementierung bei T2DM im Vergleich zu Kontrollen die Werte von TG, Gesamtcholesterin, Hämoglobin A1c und C-reaktivem Protein deutlich senkte, während das High-Density-Lipoprotein-Cholesterin anstieg. In Studie 2 fanden sich bei T2DM-Patienten mit HTG höhere Werte des „controlled attenuation parameter“ (CAP) als Zeichen der Steatosis hepatis sowie höhere Spiegel von Cytochrom P450 (CYP)- und Lipoxygenase (LOX)-Metaboliten. In Studie 3 zeigte sich bei CIF-Patienten, die Fischöl erhielten, bei Studienbeginn ein hoher Omega-3-Index (11,96 %), der nach Absetzen des Fischöls auf 9,57 % sank und nach Wiederaufnahme der Fischölbehandlung auf 12,75 % anstieg. Die Verwendung von Fischöl in der parenteralen Ernährung führte zu einem erheblichen Anstieg der CYP- und LOX-Metaboliten, die aus Eicosapentaensäure (EPA) und Docosapentaensäure (DHA) gebildet werden.

Schlussfolgerung: Die Ergebnisse dieser Dissertation verbessern unser Verständnis der Rolle, die n-3-PUFAs bei der Verringerung kardiovaskulärer Risikofaktoren bei Personen mit T2DM spielen. Darüber hinaus beleuchten sie die Zusammenhänge zwischen n-3- und n-6-PUFAs und ihren Metaboliten bei T2DM mit HTG und CIF-Patienten.

1. Introduction

Omega-3 polyunsaturated fatty acids (n-3 PUFAs) are essential nutrients for human growth and health (Meyer, Mann et al., 2003). These FAs belong to a family of long-chain PUFAs with 18 to 24 carbon atoms (Calder & Yaqoob, 2009a). They are classified into n-3 and n-6 series based on the position of the first double bond from the omega-end of the fatty acid. n-3 PUFAs in humans mainly include eicosapentaenoic acid (EPA) and docosapentaenoic acid (DHA), which are the found in cell membrane phospholipids (Calder & Yaqoob, 2009b).

The importance of n-3 PUFAs was first reported in 1976 through a study in Eskimos, who had a low prevalence of cardiovascular diseases despite consuming a high-fat diet (Bang, Dyerberg et al., 1976). Since then, n-3 PUFAs have been extensively studied in both animal models and humans. Research has demonstrated that n-3 PUFAs can protect the cardiovascular system by regulating lipids, exerting antithrombotic and antiplatelet effects, and reducing inflammatory markers (Kinsella, Lokesh et al., 1990, Weylandt, Serini et al., 2015).

N-3 and n-6 PUFA- derived oxylipins are a class of lipid mediators with potent biological activity. Key pathways in oxylipin metabolism encompass cytochrome P450 (CYP), cyclooxygenase (COX), and lipoxygenase (LOX) enzyme systems, as well as non-enzymatic autoxidation (Capdevila, Falck et al., 2000, Fischer, Konkel et al., 2014). Oxylipins from n-3 PUFAs are generally anti-inflammatory and might help resolve inflammation. For instance, resolvins and protectins from EPA and DHA were described to inhibit neutrophil and macrophage recruitment and T-cell migration, while reducing pro-inflammatory cytokines(Ariel & Serhan, 2007, Liu, Gong et al., 2018). Conversely, oxylipins from the n-6 PUFA arachidonic acid (AA) often display pro-inflammatory effects that exacerbate inflammation, vasodilation, and bronchoconstriction (Khanapure, Garvey et al., 2007).

Thus, achieving a balance between n-3 and n-6 PUFAs and their corresponding oxylipins might be crucial for maintaining health and preventing disease

1.1. Omega-3 Fatty Acid and Type 2 Diabetes Mellitus

The incidence and prevalence of type 2 diabetes mellitus (T2DM) are escalating at a concerning pace across the globe, resulting in a substantial increase in cardiovascular and metabolic morbidity and mortality (Khan, Hashim et al., 2020). Dyslipidemia, characterized by elevated triglyceride (TG) and low-density lipoprotein cholesterol (LDL-C) along with reduced high-density lipoprotein cholesterol (HDL-C), is an important pathophysiological factor connecting T2DM to increased cardiovascular risk (Assmann, Schulte et al., 1998, Kastelein, Maki et al., 2014). A meta-analysis suggests that severe HTG contributes to a heightened risk of adverse cardiovascular outcomes in T2DM patients (Ye, Kong et al., 2019). To reduce this risk, it is important to maintain TG levels below 1.7mmol/l (150 mg/dl) (Chapman, Ginsberg et al., 2011).

N-3 PUFAs are recognized for their beneficial effects on blood lipids, particularly in reducing triglyceride (TG) levels. Two large clinical trials, ORIGIN (Outcomes Reduction with an Initial Glargine Intervention) and STRENGTH (Statin Residual Risk with Epanova in High Cardiovascular Risk Patients with Hypertriglyceridemia), have shown that the consumption of n-3 PUFA results in a reduction of blood TG levels. (Nicholls, Lincoff et al., 2020) (Investigators, 2012). Although the GISSI (Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico)-Prevenzione and JELIS (Japan EPA Lipid Intervention Study) trials were the first important interventional studies to show cardiovascular benefits, they did not exhibit significant effects on TG levels (Investigators, 1999, Yokoyama, Origasa et al., 2007). And while the STRENGTH study did not show cardiovascular benefits of n-3 PUFA supplementation in high-risk patients with hypertriglyceridemia, the REDUCE-IT (Reduction of Cardiovascular Events with Icosapent Ethyl–Intervention Trial)

study, using a pure EPA medication previously also studied in the JELIS trial, found significant cardiovascular protection (Bhatt, Steg et al., 2019).

Oxylipins derived from n-3 PUFAs, such as resolvins, protectins, and maresins are considered specialized pro-resolving mediators (SPMs) (Weylandt, Chiu et al., 2012), Studies have demonstrated their ability to reduce inflammation in adipose tissue, inhibit macrophage infiltration, and enhance insulin signalling (Schwab, Chiang et al., 2007), although their biological significance is currently still under investigation (Schebb, Kuhn et al., 2022). Arachidonic acid (AA), the most important n-6 PUFA, can be transformed into inflammatory oxylipins called eicosanoids, and there is a large body of research indicating that these AA-derived eicosanoids contribute to the development of chronic inflammation and metabolic disturbances (Burns, Nakamura et al., 2018, Patterson, Wall et al., 2012). Therefore, measuring the levels of eicosanoids and SPMs in different phases of dyslipidemia could potentially provide valuable insight into the inflammatory status of T2DM.

1.2. Omega-3 Fatty Acid and Chronic Intestinal Failure

Parenteral nutrition (PN) serves as the primary therapeutic option for patients with chronic intestinal failure (CIF) who are unable to absorb adequate enteral nutrition(Pironi, Arends et al., 2016). CIF commonly develops secondary to short bowel syndrome (SBS) resulting from congenital or acquired gastrointestinal abnormalities. SBS leads to diminished intestinal length and gut function, which impairs the ability to absorb enough nutrients through normal digestion and gut absorption (Seetharam & Rodrigues, 2011). For these patients undergoing long-term PN, intestinal failure associated liver disease (IFALD) is a significant concern, affecting 15-75% of adults (Kelly, 2006).

Conventional lipid emulsions often contain excessive amounts of n-6 PUFAs, such as linoleic acid (LA) and AA, which have been found to potentially contribute to pro-inflammatory and immunosuppressive effects. In light of this

evidence, researchers have developed fish oil-based fat emulsions (FOLE) that are rich in n-3 FAs (De Meijer, Gura et al., 2009). These are presumed to prevent fat build-up in the liver and reduce fatty transformation and liver damage from intravenous nutrition in an animal model (Meisel, Le et al., 2011). Clinical studies have demonstrated the effectiveness of FOLE in both preventing and reversing parenteral nutrition-associated liver disease (PNALD) particularly in the pediatric SBS population. The use of FOLE in these patients has led to a significant reduction in PNALD-related liver transplantation rates and overall mortality (Puder, Valim et al., 2009). A comparative study of different PN lipid emulsions in mice identified that fish oil-based emulsion elicited an increase in oxylipins derived from EPA, concomitant with a decrease in pro-inflammatory oxylipins originating from AA (Noureddine, Hartling et al., 2022).

Therefore, provision of omega-3 fatty acids in PN could augment levels of inflammation-dampening n-3 PUFA-derived oxylipins, thereby mitigating complications such as PNALD (Burdge & Calder, 2015). However, the precise impact of n-3 PUFAs and their therapeutic potential in CIF is not yet fully understood.

2. Objectives

The three studies that are the basis of this dissertation all aim at the better understanding of the metabolic effects of n-3 PUFA and n-6 PUFA and their oxylipins in metabolic disease.

Study 1 (Xiao, Zhang et al., 2022) was a pooled analysis investigating the impact of n-3 PUFA consumption on cardiometabolic risk factors, inflammatory markers, and glucose control parameters in individuals with T2DM as described in published studies.

Study 2 (Xiao, Pietzner et al.) analysed the effect of hypertriglyceridemia in T2DM patients with regard to hepatic steatosis, n-3 and n-6 PUFAs and their derived oxylipins with and without HTG.

Study 3 (Weylandt, Karber et al., 2023) explored the alterations in n-3 and n-6 PUFAs and their derived oxylipins due to fish oil containing PN in patients with CIF.

3. Methods

The meta-analysis was conducted in accordance with the Preferred Reporting Items for systematic reviews and meta-analyses (PRISMA) guidelines and registered at prospective register of systematic reviews (PROSPERO) (CRD： 42021275554) (Moher, Shamseer et al., 2015). The human studies were approved by the local ethics committees (Medical School Brandenburg and Charité - Universitätsmedizin Berlin), and followed the Declaration of Helsinki. All subjects provided written informed consent to participate in the respective studies.

3.1 Study 1

An extensive search approach, employing a variety of keywords and related terms, was executed across the PubMed, Embase, Web of Science, and Cochrane databases until the end of August 2022.

Selection procedure and quality evaluation

The inclusion criteria for studies were as follows: 1) patients with T2DM; 2) omega-3 fatty acid intervention treatment; 3) randomized controlled trials (RCT); 4) evaluation of cardiovascular disease (CVD) and T2DM outcomes. For the chosen articles, data extraction and quality appraisal were performed. To ensure a comprehensive review, the risk of bias in each study was also evaluated (Egger, Smith et al., 1997, Higgins, Altman et al., 2011).

Statistical analysis

The meta-analysis was performed using Review Manager 5.3 software. Heterogeneity between studies was tested using the Cochran Q test ($p < 0.1$) and the I^2 statistic ($I^2 > 50\%$), and subgroup analyses were conducted based on omega-3 fatty acid intake dosage to explore sources of heterogeneity.

3.2 Study 2

Subjects

Forty T2DM cases were enlisted from Brandenburg Medical School, University Hospital Ruppin-Brandenburg between June 2020 to October 2021. The study participants were categorized into a hypertriglyceridemia group consisting of individuals with triglyceride levels greater than or equal to 1.7 mmol/l (n = 22) and a control group included those with triglyceride levels lower than 1.7 mmol/l (n = 18). Basic information, relevant medication and diabetic complication were collected.

Laboratory and transient elastography

Clinical laboratory tests included lipid analysis (TG, total cholesterol (TC), LDL-C, HDL-C) and glycated hemoglobin A1c (HbA1c). Transient elastography (TE) was used to quantify liver stiffness (LSM) and hepatic steatosis (controlled attenuation parameter, CAP) (FibroScan, EchoSens).

FA examination

A simplified methylation method, as described previously (Kang & Wang, 2005), was used for lipid extraction. Gas chromatography (GC) (Agilent Technologies, US), equipped with an HP88 column and nitrogen as the carrier gas, was utilized to measure fatty acid concentrations. An internal standard, pentadecanoic acid (PDA), was also added to each sample in a known amount before GC injection. By measuring the peak area of each sample and comparing their retention times to those of fatty acid methyl ester (FAME) standards of known composition that were run at the same time, the relative fatty acid levels were determined. Absolute amount of each fatty acid in a sample was then calculated based on the ratio of its peak area to that of the internal standard.

Oxylipin examination

Sample analysis was performed based on previously optimized protocols (Fischer et al., 2014). Liquid chromatography-electrospray ionization-tandem mass spectrometry (LC/ESI-MS/MS) was employed to determine the oxylipins. For detection and quantification an Agilent 1290/1200 HPLC system (Zorbax Eclipse Plus C-18, 2.1 x 150 mm, 1.8 µm column) was coupled with an Agilent 6495/6460 Triple Quad mass spectrometer equipped with a JetStream ion source (Agilent Technologies, Santa Clara, USA). A mobile phase of acetonitrile and 0.1% formic acid in water was utilized during gradient chromatography. Metabolite measurements were generated from the relative peak areas in response to various target compound/internal standard-level ratios.

3.3 Study 3

Subjects

The study involved eight patients with CIF receiving standard PN support. All patients were routinely maintained on a fish-oil containing PN. For the study they received Lipovenös MCT 20% during an eight week wash-out period, and were then given 75 % Lipovenös MCT 20% plus 25 % Omegaven (containing 100% fish oil) for an additional eight weeks.

Laboratory and indirect calorimetry

Clinical laboratory tests included lipid analysis (TG, TC, LDL-C, HDL-C), HbA1c, as well as inflammation and liver parameters. Carbon dioxide production and oxygen intake were used to estimate the respiratory quotient and resting energy expenditure (Quark RMR, COSMED) (Matarese, 1997).

FA and oxylipin examination

Omega-3 Index was calculated as EPA+DHA of total red blood cell identified PUFAs. FAs were analysed using the method developed by Harris and v. Schacky (Harris, Poston et al.,2007, Pottala, Talley et al., 2012 and oxylipin

profiles were analyzed using the methods outlined in Study 2 (refer to the previous description).

Statistical analysis（study 2 and 3）

Statistical analyses were conducted using the IBM SPSS statistics and GraphPad Prism software. Independent t-test (normally distributed data) and Mann-Whitney U-test (non-normally distributed data) were employed for analysis between the two data sets. One-way analysis of variance (ANOVA) was used for multiple data comparisons. Categorical data was compared by Chi-square test. The relationship between the two group of variables was examined using Pearson/Spearman correlation. Statistical significance was established at $p < 0.05$.

4. Results

Study 1: In total, 46 eligible studies involved 4,991 subjects, with n-3 PUFA supplementation doses ranging from 0.26 to 12 g/day (one study utilized 100g of sardines) in the intervention group. The analysis showed significant reductions in TC and TG after n-3 PUFA intervention compared to controls (weighted mean difference (WMD) = -0.22, 95% CI: -0.32 to -0.11, I^2 = 26%; WMD = -0.36, 95% CI: -0.48 to -0.25, I^2 = 72%; $p < 0.05$, respectively), and a slight increase in HDL-C (WMD = 0.05, 95% CI: 0.02 to 0.08, I^2 = 34%, $p < 0.05$). No difference was found for LDL-C. The result of adiponectin had an upward trend, while leptin declined modestly, though neither reached significance. In addition, interventions involving n-3 PUFA reduced an average of 0.19% in HbA1c (95% CI: -0.31 to -0.06, I^2 = 0%, $p < 0.05$) than those without n-3 PUFAs, but not with fasting blood sugar (FBS) and homeostatic model assessment of insulin resistance (HOMA-IR). Also, n-3 PUFA supplementation decreased C-reactive protein (CRP) by an average of 0.40 mg/l (95% CI: -0. 74 to -0.07; I^2 = 73%, $p < 0.05$). However, there was no clear effect on interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α).

A subgroup analysis was carried out to investigate the effects of different doses of n-3 PUFA (cut-off 2g/day). The results demonstrated that there was no significant impact of high versus low doses of supplements on the observed heterogeneity. The results of Egger's test and the funnel plots did not reveal any obvious signs of publication bias or asymmetry among the analysed studies.

Study 2: This study assessed the effect of hypertriglyceridemia as an additional risk component in T2DM with regard to hepatic steatosis and oxylipin formation. Body mass index (BMI) was significantly higher in T2DM patients with HTG compared to individuals with normal TG. Similarly, clinical results showed that the HTG group had higher average TG (3.54 vs 1.37 mmol/l) and

CAP levels (345.7 vs 299.2 dB/m), whereas no significance was observed for TC, LDL-C, HDL-C, HbA1c, and LSM.

In the plasma FA and oxylipin analysis, the HTG group exhibited higher concentrations of C16:0, C18:1n-9c, and monounsaturated fatty acid (MUFA), whereas lower concentrations of C20:1n-9, C20:2n-6, and C22:5n-6 were found. Metabolites of the LOX- and CYP- were generally, and often significantly, more abundant in patients with HTG versus those with normal TGs. Plasma PUFA-derived CYP450 and LOX metabolite levels exhibited a positive association with TG concentrations.

Study 3: Eight patients who were dependent on PN obtained an average of 1553 ± 223 kcal/day. At baseline, under the patients' standard PN treatment, the mean HS-omega-3 index was 12.21 ± 1.81% which decreased to 9.54 ± 0.82% after an 8-week washout period. Upon resuming fish oil in PN for 8 weeks, the HS-omega-3 index increased to 13.28 ± 1.09% at week 16. Similar changes were seen in the levels of EPA, DPA and DHA. In contrast, levels of LA and AA increased without fish oil and decreased with its resumption ($p < 0.05$).

The omega-3 epoxyeicosanoid index changed from 1.51% at baseline to 0.86% after the washout period and then increased to 2.19% after 8 weeks of fish oil administration. The ratios of AA, EPA, and DHA metabolites via the P450 epoxygenase and sEH pathways during weeks 0, 8, and 16 were as follows: EETs+DHETs (32:37:30), EEQs+DiHETEs (36:16:49), and EDPs+DiHDPAs (34:26:40). EPA and DHA-derived 5-LOX and 12-LOX metabolites increased significantly with fish oil in PN, while HETEs showed minimal change. Additionally, the content of 18-HEPE increased significantly, being 3.2-fold higher after fish oil PN, while the levels of TXB2 declined. A positive linear relationship between the ratios of EPA- and DHA -derived metabolites and their respective precursor PUFAs was found. No significant alterations were observed in liver function or inflammatory markers.

5. Discussion

Study 1: This systematic review and meta-analysis found that n-3 PUFA supplementation may favourably modulate blood lipids, decrease HbA1c, and mitigate inflammation as indicated by reduced CRP levels in T2DM. The results support the beneficial effects of n-3 PUFAs on cardiovascular and diabetes risk factors. In addition, a subgroup analysis by n-3 PUFA dose and test for publication bias provided further validity for these findings.

Dyslipidemia is a critical factor in T2DM patients contributing to cardiovascular events (Lorber, 2014). Previous research has suggested that n-3 PUFAs promote fatty acid oxidation and inhibit the synthesis and secretion of very-low-density lipoprotein cholesterol (VLDL-C) and apolipoprotein B in the liver, expediting the removal of VLDL-C remnant. This process contributes to a reduction in blood lipids, particularly TGs (Shearer, Savinova et al., 2012). Moreover, it can also block endogenous cholesterol synthesis and increase the metabolism of exogenous cholesterol (Pizzini, Lunger et al., 2017). The findings of this meta-analysis align with previous analyses that n-3 PUFAs can lower the levels of TG and TC (O'Mahoney, Matu et al., 2018). However, our report did not show any significance on LDL-C levels.

Our study also lends support to the positive effect of n-3 PUFAs on HbA1c levels, along with a minor yet statistically insignificant decrease in FBG and HOMA-IR. HbA1c serves as a comprehensive indicator of long-term glycemic control, potentially offering a more accurate evaluation of the impact of n-3 PUFAs on human glycemic control. Furthermore, n-3 PUFAs have been reported to enhance insulin sensitivity and promote glucose uptake by modulating cellular signalling and gene expression (Clarke, 2000, Kalupahana, Claycombe et al., 2011). Thus, our meta-analysis supports the notion that increased consumption of n-3 PUFAs helps to improve glucose management in T2DM.

A limited number of RCTs have explored the effects of n-3 PUFAs intake on in-inflammatory biomarkers in T2DM. As is known, T2DM is a state of amplified inflammation, which contributes to a pro-thrombotic environment and an increased risk of atherosclerosis (Galicia-Garcia, Benito-Vicente et al., 2020). We observed that n-3 PUFA intake was linked to a decrease in CRP, indicating its potential anti-inflammatory effects.

Study 2： In the second study, in comparison to normal TG, we found that in T2DM patients with high TG there were higher BMI and hepatic steatosis (CAP) scores, as well as increased plasma palmitic and oleic acid levels. Most notably, plasma oxylipin profiles were profoundly altered between the two groups with PUFA-derived oxylipins showing marked increases in HTG patients.

Our data support previous research outcomes. Leite et al. reported that blood HTG and obesity were strongly associated with hepatic steatosis by abdominal ultrasound in T2DM (Leite, Salles et al., 2009). In a large recent study by de Lédinghen et al., involving 4451 patients, the researchers discovered a considerable elevation in CAP scores as the severity of metabolic syndrome (MetS) components intensified (de Lédinghen, Vergniol et al., 2014). Therefore, these pertinent studies indicate that liver steatosis assessment using CAP may play an important role in the initial screening, identification and stratification of MetS risk in the T2DM populations.

In our study, we did not identify changes in the levels of n-3 and n-6 PUFAs in plasma, but we found activation of oxylipin formation in patients with high TG. Thus, not only of those metabolites derived from AA but also those derived from EPA and DHA were significantly higher in participants belonging to the HTG group. This is in agreement with data from Dalle et al., showing that levels of PUFA-derived epoxide metabolites were higher in subjects with MetS (Dalle, Tournayre et al., 2022). Other studies also assessed oxylipins in the context of metabolic disturbances (Moeller, Ostermann et al., 2016) (Schuchardt, Schmidt

et al., 2013), in order to establish mechanisms and markers of metabolic disease. Interestingly, analysis of lipid profiles in non-alcoholic fatty liver disease (NAFLD) showed a significant increase in LOX pathway metabolites 5-/8-/15-HETE and the non-enzymatic metabolites 11-HETE of AA as markers of the progression of NAFLD towards non-alcoholic steatohepatitis (NASH) (Puri, Wiest et al., 2009).

These findings suggest that the levels of lipid mediators might offer valuable insights into the body's inflammatory and immune status. Consequently, examining the oxylipin profile can be instrumental in assessing metabolic health and disease risk, particularly in the context of obesity-related diseases, such as NAFLD and T2DM.

Study 3: This study confirms that endogenous n-3 PUFA levels can be obviously modulated by fish oil PN. PN enriched with fish oil led to highly significant increases in the HS-Omega-3 Index, EPA, and DHA levels, while levels of n-6 PUFAs like LA and AA declined.

Dietary patterns in Western countries are imbalanced, with high intakes of n-6 PUFA and insufficient consumption of n-3 PUFA (Simopoulos, 2002). In a clinical report exploring n-3 PUFA treatment for intestinal failure-associated liver disease (IFALD), pediatric patients underwent switching PN from Intralipid to fish-oil based Omegaven, which changed the FA composition and improved prognosis and prevented IFALD (Diamond, Pencharz et al., 2009). In adult PN, patients receiving SMOFlipid (containing n-3 PUFAs) had significantly altered FA profiles compared to the original Intralipid (containing soybean oil) and the n-6 to n-3 PUFA ratio was substantially reduced with SMOFlipid administration. However, SMOFlipid does not appear to significantly change conventional markers of liver function or inflammation (Osowska, Kunecki et al., 2019).

Our data also indicate that administering high levels of n-3 PUFA via fish oil with PN leads to an increased production of CYP- and LOX-dependent

metabolites from EPA and DHA. This finding implies that as the availability of EPA and DHA increases relative to AA following fish oil administration, CYP and LOX enzymes abundantly use EPA and DHA for oxylipin formation, as described in the context of oral n-3 PUFA administration before (Fischer et al., 2014). Other studies have also demonstrated similar patterns of these PUFA-derived LOX- and CYP-metabolites (Arnold, Markovic et al., 2010) (Schmöcker, Zhang et al., 2018).

Particularly noteworthy is the pronounced formation of EPA- and DHA derived epoxy-metabolites, EEQs and EDPs respectively, as compared to AA-derived EETs in the context of n-3 PUFA supplementation: CYP450 derived n-3 PUFAs eicosanoids may thus have a particularly important role in physiological processes.

We found increased levels of n-3 PUFA derived inflammation-dampening oxylipins, such as 17-HDHA and 18-HEPE, following fish oil supplementation. In a mouse model of colitis, the administration of 17-HDHA led to decreased neutrophil infiltration and necrotic cell death, contributing to the overall improvement of inflammation and tissue damage(Chiu, Gomolka et al., 2012). Similarly, a study by Rodriguez-Echevarria et al. showed that 18-HEPE and 17-HDHA reduced liver inflammation in obese mice by regulating the serum adipokine profiles (Rodriguez-Echevarria, Macias-Barragan et al., 2018).

Our findings thus provide evidence that PN enriched with fish oil generates a lipidome characterized by both cytochrome P450 (CYP) and lipoxygenase (LOX) pathway oxylipins, leading to elevated n-3 PUFA derived inflammation-dampening oxylipins (n-3 IDOs), which may serve an important function in mitigating IFALD.

Our research has several limitations that necessitate careful evaluation. The meta-analysis (study 1) showed significant heterogeneity that could be attributed to differences in population attributes, measurement techniques, n-3

PUFA dosing and duration across studies. Future research should strive to better define doses and target levels of n-3 PUFA, in order to provide more accurate and reliable conclusions.

The number of patients in study 2 is small. Consequently, the patients might not accurately reflect the T2DM population. Analyses in larger T2DM populations, as well as comparisons between patients with high and normal TG without T2DM are now required to further validate these results.

In both studies (2 and 3) oxylipin analyses were performed, but methodology of oxylipin lipidomics is currently still under development and scientific discussion, so that further advances in analytical approaches might change and expand the detection of potentially formed lipid metabolites. In addition to this, the biological effects of the detected oxylipins are still mostly unclear, so that a more complete picture of involved signalling pathways is warranted.

6. Conclusions

In conclusion, this comprehensive dissertation, encompassing three publications, provides additional insights into the implications of n-3 PUFAs - and their oxylipins - in T2DM and CIF. The research demonstrates the favourable effects of n-3 PUFAs on glycemic control, triglyceride levels, and inflammation in T2DM patients in a pooled analysis. The study also emphasizes the complex interplay between TG, hepatic steatosis (CAP), and oxylipins in T2DM patients, particularly the pro-inflammatory tendencies of high TG levels. Furthermore, it elucidates potential mechanisms for the prevention of intestinal failure associated liver disease due to fish oil-containing parenteral nutrition and n-3 PUFA derived inflammation-dampening oxylipins. This serves to refine our understanding of the potential health benefits of n-3 PUFAs in these populations.

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Statutory Declaration

"I, Yanan Xiao, by personally signing this document in lieu of an oath, hereby affirm that I prepared the submitted dissertation on the topic "Omega-3-Fettsäuren und Oxylipin-Profile: Bedeutung für Diabetes mellitus Typ 2 und chronisches Darmversagen" (Omega-3 Fatty Acids and Oxylipin Profiles: Implications for Type 2 Diabetes Mellitus and Chronic Intestinal Failure) independently and without the support of third parties, and that I used no other sources and aids than those stated.

All parts which are based on the publications or presentations of other authors, either in letter or in spirit, are specified as such in accordance with the citing guidelines. The sections on methodology (in particular regarding practical work, laboratory regulations, statistical processing) and results (in particular regarding figures, charts, and tables) are exclusively my responsibility.

Furthermore, I declare that I have correctly marked all of the data, the analyses, and the conclusions generated from data obtained in contributions of other persons (cf. declaration of contribution). I have correctly marked all texts or parts of texts that were generated in collaboration with other persons

My contributions to any publications to this dissertation correspond to those stated in the below joint declaration made together with the supervisor. All publications created within the scope of the dissertation comply with the guidelines of the ICMJE (International Committee of Medical Journal Editors; http://www.icmje.org) on authorship. In addition, I declare that I shall comply with the regulations of Charité – Universitätsmedizin Berlin on ensuring good scientific practice.

I declare that I have not yet submitted this dissertation in identical or similar form to another Faculty.

The significance of this statutory declaration and the consequences of a false statutory declaration under criminal law (Sections 156, 161 of the German Criminal Code) are known to me."

Date Signature

Declaration of your own contribution to the publications

Yanan Xiao contributed the following to the below listed publications:

Publication 1: **Xiao, Y.**, Zhang, Q., Liao, X., Elbelt, U., and Weylandt, K.H. (2022). The effects of omega-3 fatty acids in type 2 diabetes: A systematic review and meta-analysis. Prostaglandins, Leukotrienes and Essential Fatty Acids, 102456.
Impact factor: 4.006

Contribution: I was responsible for the research design and conducted comprehensive literature searches, and selected relevant sources. Further, I synthesized and summarized large datasets. I also performed the data analysis using appropriate statistical methods, and was responsible for interpreting the results, drawing meaningful conclusions, and discussing the implications of our findings. Additionally, I prepared all the tables and figures. Finally, I wrote the manuscript, integrating all the above elements in this research.

Publication 2: **Xiao Y.**, Pietzner A., Rohwer N., Jung A., Rothe M., Elbelt U., Weylandt, K.H. (2023). Bioactive Oxylipins in Patients with Type 2 Diabetes mellitus and Hypertriglyceridemia. Frontiers in Endocrinology 14: 1195247.
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Contribution: My responsibilities included collecting and pre-processing samples, along with executing the analysis of fatty acid profiles using Gas chromatography. I was also responsible for gathering the data. The statistical analysis of the data was carried out by using the SPSS/Graphpad Prism statistics software, which was subsequently followed by an in-depth interpretation of the results. I created all of the tables and figures. I also conducted a comprehensive literature search, interpreted the findings, and drafted the initial version of the manuscript.

Publication 3: Weylandt, K.H., Karber, M., **Xiao, Y.**, Zhang, I.W., Pevny, S., Blüthner, E., et al. (2023). Impact of intravenous fish oil on omega‐3 fatty acids and their derived lipid metabolites in patients with parenteral nutrition. Journal of Parenteral and Enteral Nutrition 47(2), 287-300.
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Contribution: I conducted all statistical analyses, interpreted the results, and generated all the figures and tables presented in the publications. I wrote the first draft of this manuscript.

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Signature, date and stamp of first supervising university professor / lecturer

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Signature of doctoral candidate

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Bioactive oxylipins in type 2 diabetes mellitus patients with and without hypertriglyceridemia

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Objective: Dyslipidemia, in particular elevated triglycerides (TGs) contribute to increased cardiovascular risk in type 2 diabetes mellitus (T2DM). In this pilot study we aimed to assess how increased TGs affect hepatic fat as well as polyunsaturated fatty acid (PUFA) metabolism and oxylipin formation in T2DM patients.

Methods: 40 patients with T2DM were characterized analyzing routine lipid blood parameters, as well as medical history and clinical characteristics. Patients were divided into a hypertriglyceridemia (HTG) group (TG ≥ 1.7mmol/l) and a normal TG group with TGs within the reference range (TG < 1.7mmol/l). Profiles of PUFAs and their oxylipins in plasma were measured by gas chromatography and liquid chromatography/tandem mass spectrometry. Transient elastography (TE) was used to assess hepatic fat content measured as controlled attenuation parameter (CAP) (in dB/m) and the degree of liver fibrosis measured as stiffness (in kPa).

Results: Mean value of hepatic fat content measured as CAP as well as body mass index (BMI) were significantly higher in patients with high TGs as compared to those with normal TGs, and correlation analysis showed higher concentrations of TGs with increasing CAP and BMI scores in patients with T2DM. There were profound differences in plasma oxylipin levels between these two groups. Cytochrome P450 (CYP) and lipoxygenase (LOX) metabolites were generally more abundant in the HTG group, especially those derived from arachidonic acid (AA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), γ-linolenic acid (γ-LA), and α-linolenic acid (α-LA), and a strong correlation between TG levels and plasma metabolites from different pathways was observed.

Conclusions: In adult patients with T2DM, elevated TGs were associated with increased liver fat and BMI. Furthermore, these patients also had significantly higher plasma levels of CYP- and LOX- oxylipins, which could be a novel indicator of increased inflammatory pathway activity, as well as a novel target to dampen this activity.

KEYWORDS

oxylipins, type 2 diabetes mellitus, polyunsaturated fatty acids, controlled attenuation parameter, hypertriglyceridemia

1 Introduction

Incidence and prevalence of type 2 diabetes mellitus (T2DM) are rising worldwide with an alarming speed (1), leading to increased cardiovascular and metabolic morbidity and mortality. T2DM is a chronic condition characterized by hyperglycemia due to inadequate insulin secretion and/or defective insulin action. In 2001, McGarry proposed a new concept of glycolipid metabolism in diabetes mellitus, suggesting that excessive deposition of circulating free fatty acids (FFAs) and triglycerides (TGs) are key factors for the regulation of insulin action (2, 3). In this context, TG levels are an important biomarker of dyslipidemia, with adverse outcomes in patients with increasing hypertriglyceridemia (HTG) levels above 2 mmol/l (4).

Elevated TGs are common in T2DM and contribute to a two-fold increase in atherosclerotic cardiovascular disease (ASCVD) in T2DM. However, recommendations for dyslipidemia control in T2DM focus mostly on lowering low-density lipoprotein cholesterol (LDL-C) (5). The role of TG might be underappreciated though, as a recent study demonstrated that even an increase in TGs within the normal range confers an increased risk of T2DM in healthy subjects without metabolic syndrome (6). Furthermore, recent data confirm that elevated fasting TG (above 2.25 mmol/l) were associated with an increased risk of cardiovascular disease (CVD) mortality in patients with T2DM (7). Patients with T2DM and HTG also seem to have a more atherogenic lipoprotein phenotype than patients with elevated TG without T2DM (8)

The involvement of omega-3 (n-3) and omega-6 (n-6) polyunsaturated fatty acids (PUFAs) in the cardiovascular system and diabetes has been a subject of considerable interest. (9, 10). It has been shown that n-3 PUFAs can mediate biological effects in human cardiovascular, neurological, inflammatory diseases and cancer through different mechanisms such as alteration of membrane composition and function, gene expression and signaling molecules (11, 12). Notably, n-3 PUFA is also well-known for lowering TG levels (13) and liver fat content (14). However, the results of several recent large studies have been inconsistent in terms of cardiovascular benefit. The ORIGIN and STRENGTH trials observed moderate TG reductions but no impact on cardiovascular outcomes by n-3 PUFA supplementation (15, 16). A cardiovascular benefit was described with albeit minimal changes of TG in the GISSI-Prevenzione and JELIS trials (17, 18), and a significant 18% TG-decrease in the REDUCE-IT trial (19).

N-3 and n-6 PUFA derived oxylipins also have potent biological activity, with their metabolism relying on three enzymatic pathways including cytochrome P450 (CYP), cyclooxygenase (COX) and lipoxygenase (LOX), as well as non-enzymatic autoxidation (20, 21). The role of these oxylipins is multifaceted. Epoxidation products from n-3 PUFA are involved in regulating cardiac function, as well as reduce TG levels by suppressing hepatic lipogenesis and increasing fatty acid oxidation. (22, 23). In addition, evidence suggests that specialized pro-resolving mediators (SPM), derived from n-3 PUFA via LOX action, could regulate inflammation resolution (4). This supports the concept of n-3 PUFA derived inflammation-dampening oxylipins (n-3 IDOs) (25–27). In contrast, n-6 PUFA metabolites may have opposite or neutral effects (28). In a study involving 123 Caucasian men, researchers found that plasma concentrations of 5-hydroxyeicosatetraeonic acid (5-HETE) and 11-HETE were significantly higher, in obese individuals (29). This finding was further supported by evidence from the obese zucker mouse model (30), which elevated levels of 5-HETE, 12-HETE, and leukotriene B4 (LTB4) were discovered in adipocytes. Moreover, several studies revealed that 12- and 20-HETE may promote vasoconstriction, endothelial dysfunction, and platelet aggregation effects, which contribute to a higher risk of CVD (31, 32).

A scientific statement from the American Heart Association emphasized the need to lower triglycerides in order to lower CVD risk, with an optimal goal of < 1.1 mmol/l for fasting TG and a screening threshold of < 2.25 mmol/l (33). The proposed interventions focus on lifestyle to optimize diet, reduce weight and increase aerobic exercise (33) plus aggressive LDL-lowering therapies and control of hypertension, optimal glycemic control in individuals with T2DM and antithrombotic therapies for secondary prevention of ASCVD. In addition, icosapent ethyl is promoted in eligible patients (34).

On this background, we set out to analyze patients with T2DM to identify lipidomic changes that might modify liver and/or cardiovascular risk in patients with T2DM depending on their TG levels.

2 Methods

2.1 Subjects

Adult patients with T2DM were recruited from the gastrointestinal and endocrine clinics of Brandenburg Medical School, University Hospital Ruppin-Brandenburg from August 2020 to August 2021. Based on the normal range of TG values, the cohort was divided into the hypertriglyceridemia (HTG) group with TG ≥ 1.7 mmol/l (n = 22) and the control group with TG < 1.7 mmol/l (n =18).

The baseline characteristics including age, gender, and body mass index (BMI) were assessed, and the lifestyle of enrolled patients was obtained by standardized self-assessment and validated questionnaires. In addition, medical history and current medication were documented in the hospital records. Finally, we excluded individuals who were under 18 years of age and/or diagnosed with type 1, gestational or other specific types of diabetes. All patients gave written informed consent. The inclusion criteria for diabetic nephropathy (DN) were as follows: 1) diabetes history: the presence of type 2 diabetes with a duration of at least several years; 2) urinary markers and kidney function (at least one of the following conditions: urine albumin-to-creatinine ratio ≥ 30 mg/g or urine albumin excretion rate ≥ 30 mg/24 h (≥ 20 μg/min) on at least 2 out of 3 tests within 3-6 months; estimated glomerular filtration rate < 60/ml/1.73 m^2 for more than 3 months; 3) exclusion of other causes of chronic kidney disease. The study was approved by the local ethic committee of the Medical School Brandenburg (Number: Z-02-20170508), and the study was

conducted in accordance with the principles of the Declaration of Helsinki.

2.2 Laboratory assessment

Venous blood was withdrawn from patients in the fasting state. Biochemistry parameters such as total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), TG, LDL-C, and glycated hemoglobin (HbA1c) were measured in a standard clinical setting by the central laboratory of the hospital.

2.3 Transient elastography

All patients underwent assessment of controlled attenuation parameter (CAP) (in dB/m) and liver stiffness measurement (LSM) (in kPa) by transient elastography (TE) (FibroScan, EchoSens). During performance of TE, patients were placed in a supine position, with the right arm raised behind the head, and then an ultrasound probe was used for site selection. Measurements were done from uniform liver parenchyma under the centerline of the probe, keeping the probe perpendicular to the skin. The TE testing procedure was performed by a skilled physician and was defined as a median of at least 13 valid measurements.

2.4 Fatty acid analysis

Blood samples were collected in EDTA tubes, centrifuged at 3500 rpm for 10 min at 4°C and the plasma samples were stored at -80°C until FAs analysis. Plasma samples for fatty acids composition analysis were prepared for gas chromatography (GC) according to established protocols for methylation and extraction of FAs (7, 35). For sample preparation, 100 µl of plasma was mixed with 50 µl pentadecanoic acid (PDA, 1 mg/mL in ethanol, Merck Schuchardt OHG, Hohenbrunn, Germany) as internal standard, 500 µl boron trifluoride (BF3, Sigma-Aldrich Chemie GmbH, Germany) in 14% methanol (Merck KGaA, Germany) and 500 µl n-hexane (Merck KGaA, Germany) in glass vials which were tightly closed. After vortexing, all samples were incubated at 100°C for 60 min in a preheated block. Then the mixture was added to 750 µl of water and vortexed for 4 minutes. After centrifugation, 100 µl of supernatant were transferred into a microinsert placed in a GC vial.

FAs analysis was performed using a 7890B gas chromatograph (Agilent Technologies, US) equipped with an HP88 column (112/8867, 60 m x 0.25 mm x 0.2 µm, Agilent Technologies, US) with the following temperature gradient: 50°C to 150°C with 20°C/min, 150°C to 240°C with 6°C/min, and 240°C for 10 min (total run time 30 min). Nitrogen was used as carrier gas at a flow rate of 1 ml/min. 1 µl of each sample was injected by splitless injection (injector 280°C). FID detection was performed at 250°C with the following flows: hydrogen at 20 ml/min, air at 400 ml/min, and make up at 25 ml/min. Methylated FA were identified by comparison of retention times with those of the Supelco® 37 FAME mix (CRM47885, Sigma Aldrich, US) and a mix of single FAME standards [DPA, C22:5 n-3, AdA, C22:4 n-6 (Cayman Chemicals, Ann Arbor, MI, United States)]. Analysis and integration of peaks were carried out with OpenLAB CDS ChemStation Edition (Agilent Technologies, Santa Clara, CA, USA). Finally, the peak area of each group of FAs was calculated and, considering the individual response factors, the areas of the internal standard (PDA) were used to calculate the absolute levels of FA (µg/ml).

2.5 Oxylipin analysis

To analyze total (free and esterified) LOX- and CYP-derived metabolites, the sample preparation was performed based on a further developed protocol (20, 21), as follows. Plasma samples (500 µl) were spiked with a mixture of antioxidants and 100 pg each of deuterated internal standards 14,15-dihydroxyeicosatetraenoic acid (DHET)-D11, 15- HETE-d8, 20-HETE-d6, 8,9-epoxyeicosatrienoic acid (EET)-d11, 9,10-dihydroxy-octadecenoic acid (DiHOME)-d4, d4-12(13)-EpOME, d4-13- hydroxyoctadecadienoic acid (HODE), d4-prostaglandin E2 (PGE2) and LTB4-D4 (Cayman Chemical, Ann Arbor, MI). Methanol and sodium hydroxide were then added and alkaline hydrolysis was carried out at 60°C for 30 min. Following centrifugation and pH adjustment, the obtained supernatants were added to Bond Elute Certify II columns (Agilent Technologies, Santa Clara, USA) for solid phase extraction. The eluates were evaporated on a heating block at 40°C under a stream of nitrogen and the residues were dissolved in 100 µl methanol/water.

LC/ESI-MS/MS analysis was performed using an Agilent 1290 HPLC system with binary pump, multisampler and column thermostat equipped with a Zorbax Eclipse plus C-18, 2.1 x 150 mm, 1.8 µm column using a gradient solvent system of aqueous acetic acid (0.05%) and acetonitrile/methanol 50:50. The flow rate was set at 0.3 mL/min, the injection volume was 20 µL. The HPLC was coupled with an Agilent 6495 Triplequad mass spectrometer (Agilent Technologies, Santa Clara, USA) with electrospray ionization source. Analysis was performed with Multiple Reaction Monitoring in negative mode. One patient was excluded from the FA and oxylipin analysis due to incomplete blood components.

2.6 Statistical analysis

The collected data were analyzed with GraphPad Prism 9 (Graph Software, La Jolla, CA, USA). Data were tested for normal distribution and presented as mean with standard errors of the mean (SEM), and percentages (%). For comparisons between the two groups, the t-test was used for normally distributed data, the Mann-Whitney U-test for skewed distributed data, and the Chi-square test for categorical variables. Pearson/Spearman correlation was used to investigate the association between two groups of variables. $p < 0.05$ was considered as statistically significant.

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3 Results

3.1 Study participants and biochemical parameters

A total of forty patients were included in the present study. The baseline characteristics, T2DM related complications and concomitant medication of the research participants are outlined in Table 1. Of these individuals, 20 were male and 20 were female, the mean age was 60.6 ± 1.13 years, and the mean BMI was 33.0 ± 1.07 kg/m^2. However, patients with high TGs had significantly higher BMI values (35.0 ± 1.65 kg/m^2) than those with normal TGs (30.7 ± 1.12 kg/m^2). With a prevalence of up to 20%, CAD and DN were the most prevalent complications. In addition, eight patients had liver cirrhosis and two patients were diagnosed with chronic pancreatitis. Metformin (58%), insulin (50%) and aspirin (ASS) (43%) were the most commonly used drugs in this cohort of patients with T2DM. There was no statistical difference in the percentages of complications between the two groups. However, the proportion of prescribed ASS was significantly higher in the HTG group (61%).

The clinical and laboratory features of patients are summarized in Table 2. We found that TC and LDL-C concentrations were higher in the HTG group than in the normal TG group whereas the levels of HDL-C were slightly lower, but these differences were not of statistical significance. The mean TG level in the HTG group was 3.54 ± 0.42 mmol/l, which was significantly higher than in the control group with 1.37 ± 0.07 mmol ($P > 0.05$). The mean CAP value in the HTG group was 345.7 ± 9.20 dB/m and thus significantly higher than in the normal TG group (299.2 ± 13.11 dB/m), whereas LSM values as indicator of fibrosis did not differ significantly. Furthermore, TG levels were positively correlated with BMI (r = 0.37, $P < 0.05$) as well as with CAP values (r = 0.47, $P < 0.05$), as shown in Figure 1.

3.2 Plasma fatty acid levels

Given that particularly n-3 PUFA have been shown to be able to affect TG levels we measured twenty-two FAs in plasma samples from the cohort assessed here (Table 3). FA content in plasma was dominated by saturated fatty acids (SFAs), followed by PUFAs and monounsaturated fatty acids (MUFAs) and the n-6 PUFAs constituted the largest fraction of total PUFAs. Compared with normal TG subjects, levels of C20:1n-9, C20:2n-6, and C22:5n-6 were significantly lower in HTG diabetics, whereas levels of C16:0, C18:1n-9c and MUFA were significantly higher in the HTG group. (Figure 2, Table 3).

3.3 Oxylipin levels

We next assessed the plasma oxylipin profiles between T2DM subjects with and without HTG. The linoleic acid (LA) metabolites were the most abundant, followed by those of arachidonic acid (AA). Compared to the normal TG group, products of the LOX pathway were generally higher in patients with HTG. Our results show that 5-/15-HETE, 5-/15-hydroxyeicosapentaenoic acid (HEPE), and 4-/7-/11-/17-hydroxyeicosapentaenoic acid (HDHA) are substantially increased in HTG patients, as well as the 8-/10-/13-/16-HDHA metabolites. Moreover, LA, γ-linolenic acid (γ-LA) and α-linolenic acid (α-LA) produced oxylipins HODE, hydroxyeicosatrienoic acid (HeTrE) and hydroxyoctadecatrienoic acid (HOTrE), were all higher, and the 12-HeTrE and 9-HOTrE reaching significance, in the HTG group (Figures 3A–F, Supplementary Table 1). AA 5-LOX -derived LTB4 also showed an increasing trend, and the non-enzymatic hydrolysis LTB4-6-trans and LTB4-6-trans-epi levels were significantly higher in the HTG group than in the normal TG group. (Figure 3G, Supplementary Table 1).

Furthermore, non-enzymatic monohydroxy products were increased in the HTG group, with significant differences for AA and EPA-derived 8-/9-/11-HETE and 8-/9-/11-HEPE. The largest increases were observed in 9-HETE. Additionally, 18-HEPE (described as resolvin E precursor) was significantly more abundant in the HTG group (Figure 4, Supplementary Table 1).

We also evaluated the CYP and sEH pathway products in plasma from both groups focusing here on AA-, LA-, EPA-, DHA-derived epoxy-metabolites, the epoxyeicosatrienoic acids (EETs), epoxyoctadecamonoenoic acids (EpOMEs), epoxyeicosatetraenoic acids (EEQs) and epoxydocosapentaenoic acids (EDPs), which can be further converted to DHETs, DiHOMEs, dihydroxy-octadecenoic acids (DiHETEs) and dihydroxy-docosapentaenoic acids (DiHDPAs) by soluble epoxide hydrolase (sEH). The levels of 5,6-/8,9-EEQ, 8,9-/11,12-DiHETE, 7,8-/10,11-/13,14-/16,17-/19,20-EDP and 10,11-/13,14-/16,17-DiHDPA were significantly higher in HTG group. Similar changes were seen by summarizing the CYP-derived epoxy and sEH-derived dihydroxy products as shown in Figures 5A–C. Among the ω-hydroxylase metabolites, 20-HETE, 20-HEPE, 20-/22-HDHA derived from AA, EPA, and DHA were significantly increased in the HTG group. However, there were no significant differences in LA-CYP450 products between the two groups (Figure 5D, Supplementary Table 1).

Lastly, we investigated the relationship between levels of plasma oxylipins and TGs. Significantly positive correlations were found for the CYP epoxygenase and their corresponding sEH products with an r of 0.71 for EEQs+DiHETEs. In addition, a total of LOX- and CYP450 ω-hydroxylase products derived from AA, EPA, DHA, LA, GLA and ALA were also positively correlated with TG levels (Figure 6).

4 Discussion

In this study, we found significant differences in BMI and CAP values for patients with T2DM and high TGs (TG ≥ 1.7mmol/l) as compared to those with TGs within the reference range (TG < 1.7mmol/l), but no increase in liver fibrosis parameters. Hypertriglyceridemia was strongly associated with an increased BMI, as well as increased CAP values in patients with T2DM. Furthermore, total amount of palmitic and oleic acid was significantly increased in blood plasma from diabetics with

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TABLE 1 Clinical characteristics of the study.

Variables	Total (n=40)	TG (<1.7) n=18	TG (≥1.7) n=22	p
N (Female/Male)	20/20	10/8	10/12	0.53
Age (years)	60.6 ± 1.13	59.8 ± 1.04	61.3 ± 1.24	0.69
Weight (kg)	95.5 ± 2.91	90.1 ± 3.83	100.9 ± 4.00	0.06
Height (cm)	170.9 ± 4.5	169.7 ± 5.4	170.9 ± 5.2	0.72
BMI (kg/m^2)	33.0 ± 1.07	30.7 ± 1.12	35.0 ± 1.65	0.04
Diabetic Complications, N (%)				
CAD	8 (20)	5 (28)	3 (14)	0.13
Stroke	1 (3)	1 (6)	0 (0)	–
PAD	3 (8)	1 (6)	2 (9)	0.86
Diabetic nephropathy	8 (20)	2 (11)	6 (27)	0.17
Diabetic neuropathy	2 (5)	1 (6)	1 (5)	0.73
Diabetic retinopathy	3 (7)	1 (6)	2 (9)	0.49
Diabetic foot syndrome	1 (3)	0 (0)	1 (5)	–
Hepatic and pancreatic, N (%)				
Liver cirrhosis	8 (20)	4 (22)	4 (18)	0.95
Chronic pancreatitis	2 (5)	1(6)	1 (5)	-
Medications, N (%)				
Metformin	23 (58)	11 (61)	12 (55)	0.83
DPP-IV-Inhibitor	12 (30)	6 (33)	6 (27)	0.68
SGLT-2-Inhibitor	7 (18)	4 (22)	3 (14)	0.77
Sulfonylurea	1(3)	1(6)	0 (0)	–
Acarbse	0 (0)	0 (0)	0 (0)	–
Glitazone	0 (0)	0 (0)	0 (0)	–
GLP-1-Analoga	4 (10)	1 (6)	3 (14)	0.75
Insulin	20 (50)	8 (44)	12 (55)	0.53
Statin	11 (28)	6 (33)	5 (23)	0.70
Fibrate	0 (0)	0 (0)	0 (0)	–
Ezetimib	3 (8)	2 (11)	1 (5)	0.86
Evolocumab	1 (3)	0 (0)	1 (5)	–
ACE-Hemmer	6 (15)	4 (22)	2 (9)	0.48
AT1-Receptor-Antagonist	16 (40)	7 (39)	9 (41)	0.90
Ca-Channel-Blocker	7 (18)	3 (17)	4 (18.2)	0.77
Beta-Blocker	13 (33)	4 (22)	9 (41)	0.21
Diuretics	11 (28)	5 (28)	5 (23)	0.75
ASS	17 (43)	11 (61)	6 (27)	0.03

Data are presented as mean ± SEM and number (percentage); p values are based on t-test or Chi-square test, and $p < 0.05$ was considered statistically significant; TG, triglyceride; BMI, body mass index; CAD, coronary artery disease; PAD, peripheral artery disease; ASS, aspirin; '-': missing data.

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TABLE 2 Laboratory and ultrasound characteristics of the study population.

Parameters	Total (n=40)	TG(<1.7) n=18	TG(≥1.7) n=22	p
HbA1c (%)	7.72 ± 0.27	7.88 ± 0.45	7.59 ± 0.37	0.67
TC (mmol/l)	5.00 ± 0.36	4.59 ± 0.29	5.35 ± 0.60	0.32
TG (mmol/l)	2.52 ± 0.29	1.37 ± 0.07	3.54 ± 0.42	<0.01
HDL-C (mmol/l)	1.13 ± 0.04	1.15± 0.06	1.11 ± 0.06	0.70
LDL-C (mmol/l)	3.08 ± 0.14	2.95 ± 0.22	3.19 ± 0.17	0.24
CAP (dB/m)	328.1 ± 8.74	299.2 ± 13.11	345.7 ± 9.20	<0.01
LSM (kPa)	10.44 ± 1.26	10.42 ± 2.21	10.45 ± 1.47	0.82

Data are presented as mean ± SEM; p values are based on t-test or Mann-Whitney test, and $p < 0.05$ was considered statistically significant; HbA1c, glycated hemoglobin; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; CAP, controlled attenuation parameter; LSM, liver stiffness measurement.

increased TGs. We also observed significant changes in plasma oxylipin profiles between these two groups. Metabolites tended to be higher in hypertriglyceridemic patients, possibly indicating a (subclinical) pro-inflammatory environment arising from the hypertriglyceridemic metabolic dysregulation.

Our findings regarding HTG and liver fat content are consistent with other findings. In a multivariate analysis of nearly 300 patients with and without NAFLD, TGs were strongly associated with liver fat content (36). In addition, this research also indicated that CAP values of patients with T2DM progressively increased with rising

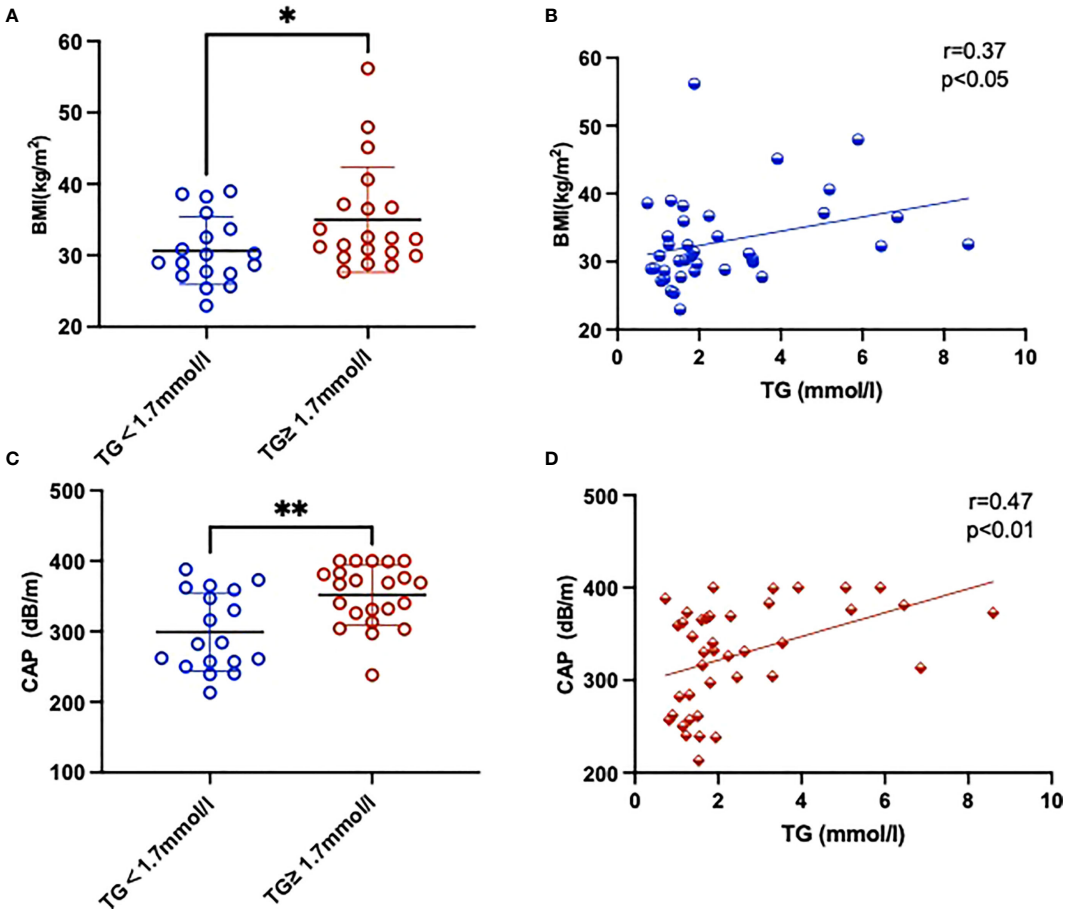

FIGURE 1
Comparison of BMI **(A)** and CAP **(C)** parameters between two groups with and without high TGs. Also shown is the correlation between BMI **(B)**, CAP **(D)** and TGs of the study. Results are shown as mean ± SEM. Statistics: **(A, C)** unpaired t-test/Mann-Whitney test, **(B, D)** Pearson/Spearman correlation. Significant changes are indicated as: *$p < 0.05$; **$p < 0.01$.

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TABLE 3 Absolute plasma fatty acids of the study.

Fatty Acid (μg/ml)	Total	TG (<1.7)	TG (≥1.7)	p
	n=40	n=18	n=22	
14:0	33.29 ± 2.56	28.93 ± 2.21	37.02 ± 2.85	0.21
16:0	585.78 ± 33.03	528.7 ± 22.14	634.7 ± 42.36	0.04
16:1n-7	49.12 ± 5.18	41.01 ± 4.16	56.07 ± 6.05	0.05
18:0	144.22 ± 7.89	136.3 ± 5.74	151 ± 9.73	0.22
18:1n-9	326.61 ± 13.61	304.1 ± 10.43	345.9 ± 16.33	0.04
C18:1n-7	31.54 ± 2.28	30.11 ± 2.11	32.76 ± 2.43	0.42
18:2n-6 (LA)	315.04 ± 19.48	311 ± 12.86	318.5 ± 25.16	0.80
20:0	5.14 ± 0.32	5.62 ± 0.36	4.73 ± 0.29	0.06
18:3n-6	7.09 ± 0.5	7.33 ± 0.5	6.89 ± 0.5	0.54
18:3n-3	8.26 ± 0.64	7.69 ± 0.49	8.75 ± 0.77	0.27
20:1n-9	3.48 ± 0.17	4.02 ± 0.2	3.03 ± 0.15	< 0.01
20:2n-6	3.53 ± 0.17	3.85 ± 0.18	3.25 ± 0.16	0.02
22:0	7.52 ± 0.38	7.86 ± 0.33	7.22 ± 0.41	0.24
20:3n-6	23.55 ± 1.34	21.99 ± 1.53	24.89 ± 1.19	0.14
20:4n-6 (AA)	98.75 ± 5.95	95.3 ± 5.99	101.7 ± 5.91	0.45
20:5n-3 (EPA)	11.5 ± 2	9.67 ± 0.91	13.08 ± 2.95	0.31
24:0	4.02 ± 0.29	4.27 ± 0.35	3.8 ± 0.24	0.35
24:1n-9	7.52 ± 0.49	8.08 ± 0.53	7.04 ± 0.46	0.15
22:4n-6	4.69 ± 0.32	5.02 ± 0.44	4.41 ± 0.22	0.21
C22:5n-6	3.07 ± 0.15	3.37 ± 0.17	2.81 ± 0.14	0.01
22:5n-3 (DPA)	6.45 ± 0.54	7.29 ± 0.44	5.72 ± 0.62	0.05
22:6n-3 (DHA)	22.22 ± 2.9	17.89 ± 1.27	25.93 ± 4.31	0.10
SFA	779.98 ± 41.43	711.7 ± 28.49	838.5 ± 52.52	0.53
MUFA	418.26 ± 18.44	387.3 ± 14.46	444.8 ± 21.86	0.04
PUFA	504.18 ± 25.8	490.4 ± 14.82	516 ± 35.22	0.53
n-6 PUFA	455.76 ± 22.05	447.9 ± 14.01	462.5 ± 28.94	0.67
n-3 PUFA	48.43 ± 5.14	42.53 ± 1.45	53.49 ± 8.3	0.24
n-6/n-3	10.23 ± 0.49	10.63 ± 0.34	9.89 ± 0.62	0.32
EPA + DHA	33.72 ± 4.52	27.55 ± 1.4	39.01 ± 7.19	0.11

Data are presented as mean ± SEM; p values are based on t-test or Mann-Whitney test, and $p < 0.05$ was considered statistically significant; LA, linoleic acid; AA, arachidonic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; SFA, saturated fatty acids; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

TG levels. A recent large trial by de Lédinghen et al. analyzed patients with suspected chronic liver disease (37) and demonstrated that CAP values significantly increased with an increasing pathology of metabolic syndrome (MetS) components.

Pathophysiological mechanisms of MetS are complex and manifold. FFAs and lipid toxicity lead to dysfunction of cell membrane structure, inhibition of glycogen synthesis, and an increase of IR (38). Moreover, fat accumulation promotes inflammation and a thrombotic state that exacerbates atherosclerosis (39). The relationship between elastography measurements and NAFLD has been extensively documented in clinical trials (40, 41). In summary, our data confirm that CAP levels are closely related to MetS components. In clinical practice, determination of MetS patients relies on clinical examination and blood tests. However, based on the non-invasive, rapid, simple, and reproducible characteristics of CAP, adding it to the clinical assessment of MetS development might improve detection and risk stratification. Even inclusion of NAFLD into the definition of the metabolic syndrome could be a step to better identify the population with metabolic risk (42).

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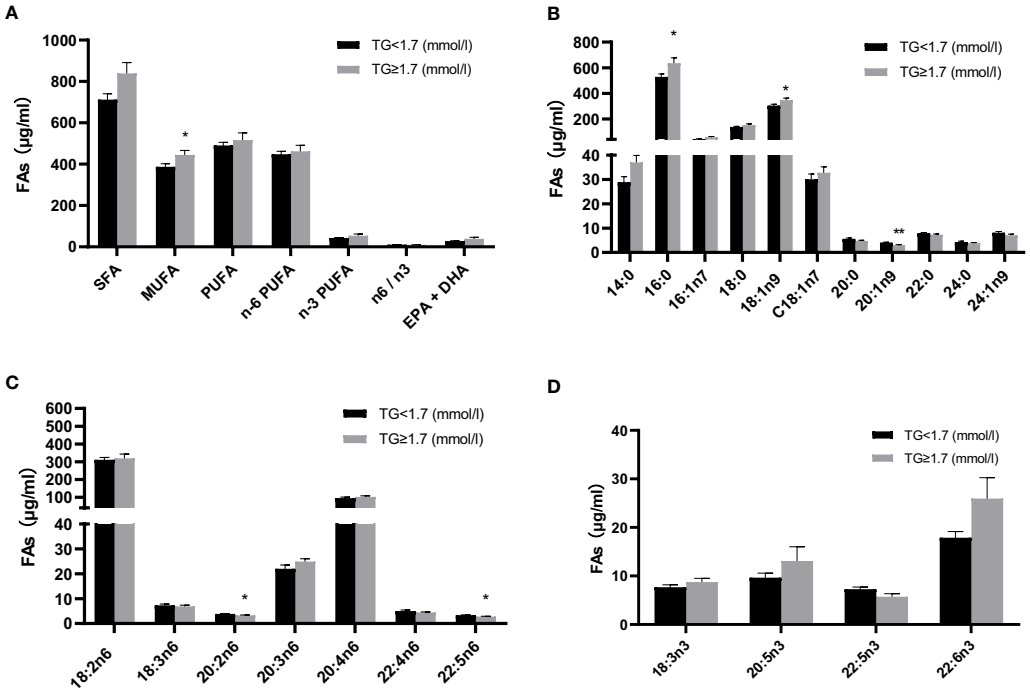

FIGURE 2
Comparison of absolute fatty acid levels (µg/ml) in plasma between two groups with and without high TGs. **(A)** Total fatty acids **(B)** individual fatty acids, with **(C)** n-6 fatty acids and **(D)** n-3 fatty acids. Results are shown as mean ± SEM. Statistics: unpaired t-test/Mann-Whitney test. Significant changes are indicated as: *p < 0.05; **p < 0.01. TG, triglyceride; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

As the n-3 PUFA have been proven to lower TGs and reduce cardiovascular risk (19), studies have shown that n-6 and n-3 PUFA serum levels are negatively correlated with TGs [e.g. (43)] and that n-3 PUFA administration lowers triglycerides also in healthy volunteers (20), as well as in patients (44). Both higher serum n-6 and n-3 PUFA were associated with a lower risk of NAFLD (45). However, our data do not show these inverse relationships between n-3 and n-6 PUFAs observed elsewhere. This might be due to the fact that we measured plasma and not serum samples. Indeed, except for significantly higher levels of palmitic acid, which is known to promote insulin resistance in T2DM patients (46), oleic acid, and lower levels of γ-LA, and 11-eicosenoic acid, we did not find significant differences, and not even a trend toward higher levels of n-3 and n-6 PUFAs in the high TG (and high CAP) group. Further investigation may be needed to consider not only different types of samples but also other potential factors contributing to the observed differences, such as variations in study populations, dietary habits, or methodology. This could help clarify the relationship between n-3 and n-6 PUFAs and TG levels.

As shown in the present study, monohydroxy metabolites of PUFAs were significantly higher in those patients with high TGs than in those with normal TGs, with a broad range of AA- as well as EPA- and DHA- metabolites displaying significant increases. In contrast, in the study by Schuchard et al. there were only slightly higher serum concentrations of 5-HEPE, a lower concentration of 12-HETE in hyperlipidemic patients compared to normolipidemic patients (47). In this population diabetes was not studied, though.

Increased TG levels can provide more FAs substrates for oxylipin production. Therefore, as TGs are broken down, they release FAs that can be converted into oxylipins through enzymatic oxidation (26). Recent research has further supported this observation, indicating a linear relationship between the generation of oxylipins and triglyceride oxidation (48). Meanwhile, gene expression also appears to influence the route of PUFA metabolism. Several studies have indicated that high TGs activate PPAR-alpha, leading to increased expression of genes involved in beta-oxidation and promoting the expression of inflammatory oxylipins (49). Moreover, excess TGs and their FFAs can cause accumulation of lipids, and then trigger the activation of stress-responsive signaling pathways. The activation of these pathways can lead to a comprehensive increase in the metabolites of PUFAs (50).

Oxylipins are bioactive lipids, and changes in their levels reflect underlying inflammation or metabolic disturbances. There have been recent attempts to use oxylipins for stratification of MetS patients, showing that levels of a number of mono- and dihydroxy as well as epoxy metabolites were higher in MetS (51). In another study, it was shown that 5-/8-/and 12-HETE were increased in obese subjects with low-grade inflammation. However, weight reduction over an 8-week intervention led to a significant reduction of these oxylipins (52). There is also work ongoing to use oxylipin for liver disease risk evaluation [reviewed in (53)]. A comprehensive study was conducted to assess oxylipins as progression indicators from NAFLD to NASH and observed increases in various oxylipin classes with advancing disease stage (54). Another study has previously demonstrated in a lipidomic analysis of NAFLD/NASH that significantly increased levels of AA-products such as 5-HETE, 8-HETE and 15-HETE and 11-HETE

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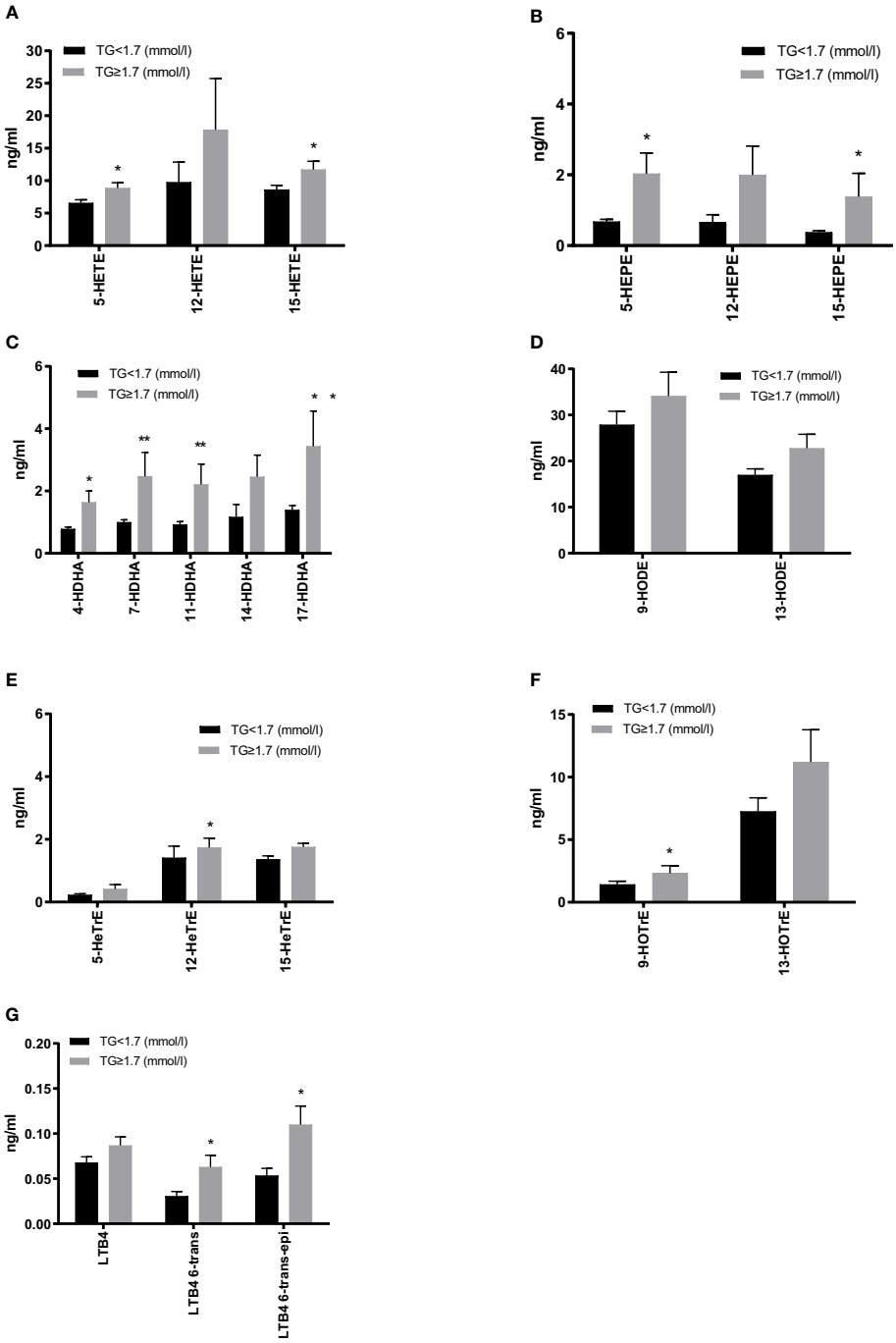

FIGURE 3
Comparison of plasma 5-lipoxygenase (LOX), 12-LOX and 15-LOX metabolites derived from arachidonic acid (AA) **(A)**, EPA **(B)**, DHA **(C)**, γ-linolenic acid (γ-LA) **(D)** and the LOX metabolites level derived from LA **(E)** and α-LA **(F)**, as well as leukotrienes derived from AA **(G)**. All results are shown as the mean ± SEM, p values are based on t-test or Mann-Whitney test; Significant changes are indicated as: *p < 0.05; **p < 0.01; HETE, hydroxyeicosatetraenoic acid; HEPE, hydroxyeicosapentaenoic acid; HDHA, hydroxydocosahexaenoic acid; HODE, hydroxyoctadecadienoic acid; HETrE, hydroxyeicosatrienoic acid; HOTrE, hydroxyoctadecatrienoic acid; LTB4, leukotriene B4.

indicate progression towards NASH (55). In a study of children with NAFLD, hepatic epoxyeicosanoids significantly increased with higher grades of steatosis (56). Therefore, our lipidomic analysis may also provide valuable insights into the metabolic risk stratification of T2DM.

Additionally, it is well-established that elevated levels of CYP- and LOX-metabolites, such as HETEs and LTs leads to inflammation, oxidative stress, endothelial dysfunction, and peripheral vascular resistance, and are generally associated with increased CVD risk. (57). 20-HETE has been reported to be associated with vascular inflammation and injury, angiogenesis, which are crucial factors in the development and progression of CVD (58). 9-HETE as a marker of oxidative stress was elevated in patients with coronary artery disease (57). In contrast, EETs help dilate arteries and reduce

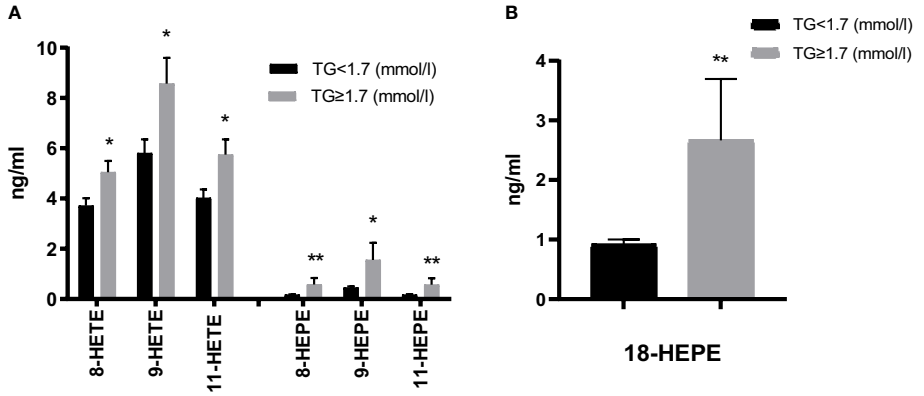

FIGURE 4
Comparison of non-enzymatic oxidation (AA, EPA) products **(A)** and the EPA-derived E-resolvin precursor 18-HEPE **(B)**. Results are shown as mean ± SEM. Statistics: unpaired t-test/Mann-Whitney test. Significant changes are indicated as: *p < 0.05; **p < 0.01.

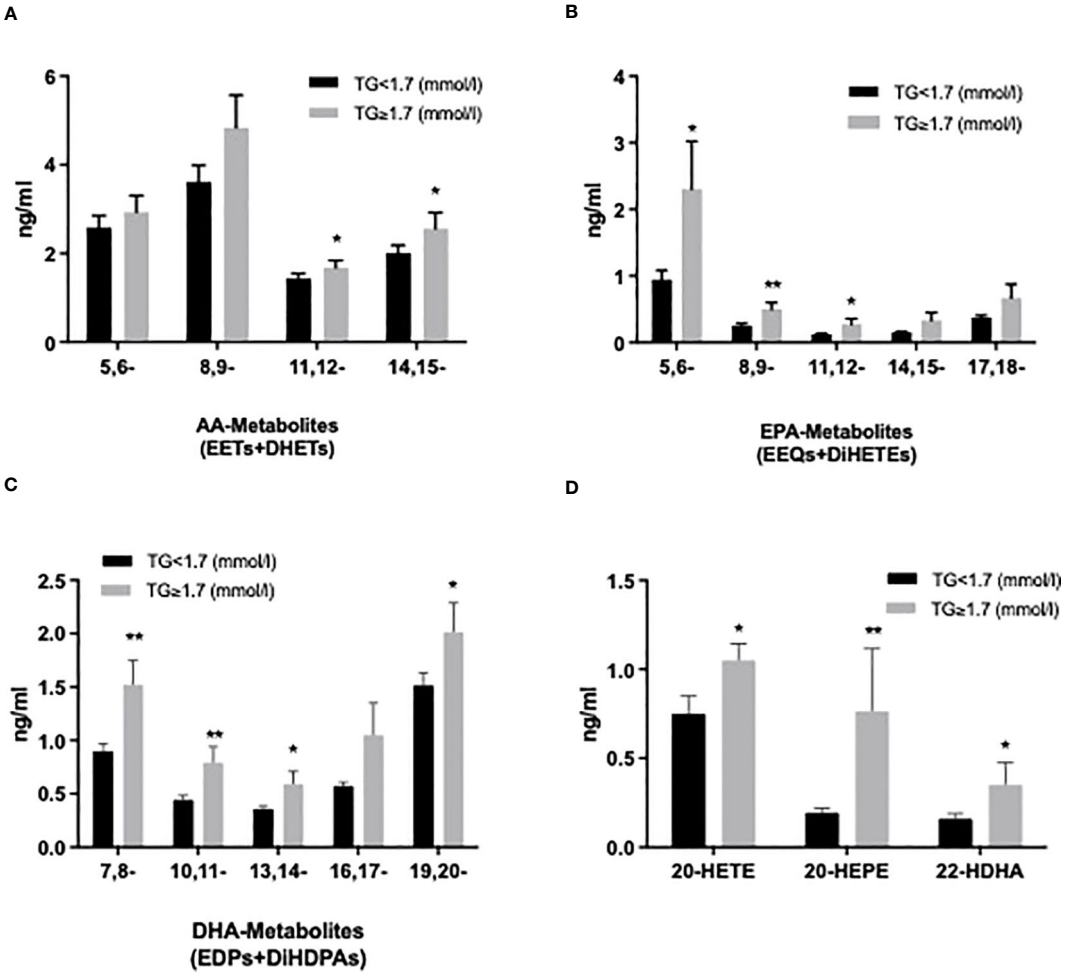

FIGURE 5
Comparison of plasma cytochrome P450 (CYP) epoxygenase metabolites level derived from AA **(A)**, EPA **(B)**, DHA **(C)**, as well as CYP450 ω-hydroxylase metabolites level of AA (20-HETE), EPA (20-HEPE), and DHA (22-HDHA) **(D)**. All results are shown as the mean ± SEM, P values are based on t-test or Mann-Whitney test; Significant changes are indicated as: *p < 0.05; **p < 0.01; EET: epoxyeicosatrienoic acid; DHET: dihydroxy-eicosatetraenoic acid; EEQ: epoxyeicosatetraenoic acid; DiHETE: dihydroxy-eicosatetraenoic acid; EDP: epoxydocosapentaenoic acid; DiHDAP: dihydroxy-docosapentaenoic acid; EpOME: epoxyoctadecamonoenoic acid; DiHOME: dihydroxy-octadecenoic acid.

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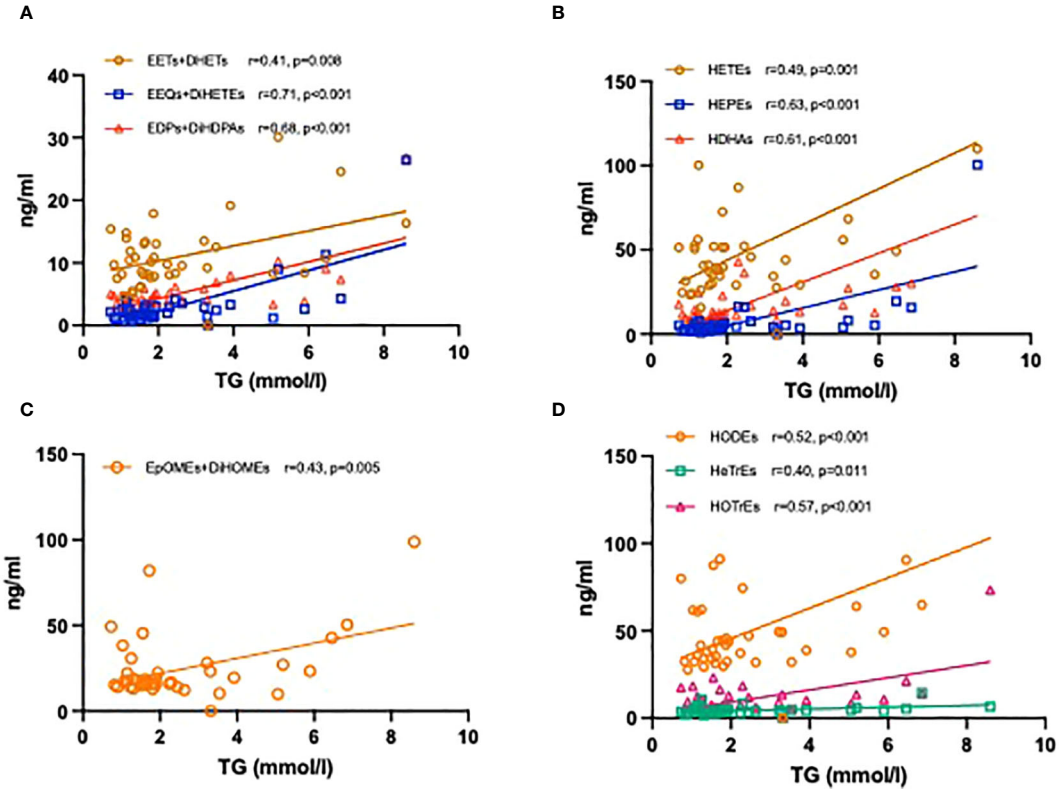

FIGURE 6
Correlation coefficients between the levels of TG and plasma oxylipins. CYP450 epoxygenase metabolites derived from AA, EPA, and DHA with TG levels **(A)**; LOX- and CYP450 ω-hydroxylase metabolites derived from AA, EPA, and DHA with TG levels **(B)**; CYP450 epoxygenase metabolites derived from LA with TG levels **(C)**; LOX- metabolites derived from LA, GLA and ALA with TG levels **(D)**.

inflammation, but their sEH products may negate the protective effects (59). Given the experimental data implicating n-3 PUFA derived inflammation-dampening oxylipins in the alleviation of metabolic liver disease (25–27, 60), our data presented here support a concept of optimizing n-3 PUFA levels by identifying individuals with fatty acid imbalance and evidence of excess inflammation, rebalancing oxylipin profiles could significantly impact outcomes. This could alleviate metabolic disease-associated inflammation due to increased n-3 inflammation dampening oxylipin (n-3 IDO) formation with increased TGs, and liver fat, in patients with T2DM. Our study has several limitations. The majority of patients were enrolled during presentation to our gastroenterology and endocrinology unit, ranging from routine to presentations due to comorbidities (see Table 1). Although in multivariate linear regression, the positive effect indicators for two groups of HTG and non-HTG patients with TG levels showed no significant between-group associations (Supplementary Table 2), there may still be some confounding factors in our results, which should be interpreted with caution. Specifically, first, our patients with a higher BMI and higher triglycerides showed slightly better blood glucose control, which may be contrary to the experience in clinical practice. Second, there was probably some undertreatment with regard to guideline-based therapies, as evidenced by low prescription rates of statins. Last, our focus on diabetic patients in this study was based on the well-established association between diabetes and HTG, as well as a higher risk of cardiovascular disease. Future studies with more resources could expand the scope to investigate non-diabetic individuals as well.

5 Conclusion

The findings presented here indicate a clear association between TGs, BMI, and hepatic steatosis as measured by CAP in patients with T2DM. Compared to normal TG subjects, the unique feature of high TG subjects was the upregulation of CYP450 and LOX activity, accompanied by elevated oxylipin levels, potentially contributing to an inflammatory metabolic state.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Ethics statement

The study involving human participants was reviewed and approved by Ethics Committee of Brandenburg Medical School. The patients/participants provided their written informed consent to participate in this study.

Author contributions

Conceptualization: UE, YX, and KW. Methodology: AJ, MR, AP, NR, and KW. Analysis: YX, UE, and KW. Investigation: YX, UE, AJ, AP, and KW. Data curation: AJ, AP, and YX. Writing—original draft preparation: YX and KW. Writing—review and editing: YX, UE, and KW. Visualization: YX, and KW. Supervision: UE and KW. Project administration: UE and KW. All authors have read and agreed to the published version of the manuscript. All authors contributed to the article.

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Conflict of interest

Author MR was employed by Lipidomix.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1195247/full#supplementary-material

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ORIGINAL RESEARCH

Impact of intravenous fish oil on omega-3 fatty acids and their derived lipid metabolites in patients with parenteral nutrition

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Abstract

Background: Long-term parenteral nutrition (PN) can lead to intestinal failure–associated liver disease (IFALD). Omega-3 (n-3) polyunsaturated fatty acids (PUFAs) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were shown to prevent IFALD. EPA-derived and DHA-derived oxylipins could contribute to this protective effect.

Methods: We analyzed the effect of parenteral fish oil on oxylipins in patients with chronic intestinal failure receiving PN ($n = 8$). Patients first received no fish oil for 8 weeks and then switched to PN with 25% of fat as fish oil for another 8 weeks. Fatty acid profiles of red blood cells, PUFA-derived oxylipins generated by cyclooxygenase, lipoxygenase (LOX), and cytochrome P450 (CYP) pathways, inflammatory markers, and liver function were assessed before and during fish-oil PN.

Results: EPA plus DHA in erythrocytes (the Omega-3 Index) was high with a median of 11.96% at baseline and decreased to 9.57% without fish oil in PN. Addition of fish oil in

Karsten H. Weylandt and Mirjam Karber contributed equally to this study.

PN increased the median Omega-3-Index to 12.75%. EPA-derived and DHA-derived CYP-dependent and LOX-dependent metabolites increased significantly with fish oil in PN, with less pronounced changes in arachidonic acid and its oxylipins. There were no significant changes of inflammation and liver function parameters.

Conclusions: This study shows that fish oil–containing PN leads to primarily CYP- and LOX-dependent n-3 PUFA–derived inflammation-dampening oxylipins arising from EPA and DHA. Within this short (16-week) study, there were no significant changes in inflammation and clinical readout parameters.

KEYWORDS

cytochrome P450, omega-3 polyunsaturated fatty acids, oxylipins, parenteral nutrition–associated liver disease

CLINICAL RELEVANCY STATEMENT

This study demonstrates that parenteral fish oil leads to very high levels of omega-3 (n-3) polyunsaturated fatty acids (PUFAs) in the blood and demonstrates the formation particularly of potentially beneficial n-3 PUFA epoxy and monohydroxy metabolites with high underlying substrate levels of docosahexaenoic acid and eicosapentaenoic acid.

INTRODUCTION

Intestinal failure–associated liver disease (IFALD) is a problem often complicating the treatment of patients with a reduction of gut function (intestinal failure) and subsequent dependence on intravenous nutrient supplementation. Depending on the criteria used, signs of IFALD affect 2%–90% of adults receiving long-term parenteral nutrition (PN).[1] Clinically, IFALD can manifest as hepatic steatosis and cholestasis. When not resolved, it can result in hepatic fibrosis and/or biliary cirrhosis and liver failure in a small number of cases. One strategy in the prevention and treatment of IFALD is to encourage enteral feeding and to reduce the duration and amount of PN, but this is not feasible for patients with intestinal failure.

In children with intestinal failure and subsequent need for exclusive PN, omega-3 (n-3) polyunsaturated fatty acids (PUFAs) were shown to protect from IFALD.[2–5] Fish oil as a parenteral fat source was able to improve lipid profiles, with decreased levels of serum low-density lipoprotein (LDL), very low-density lipoprotein, total cholesterol, and triglycerides.[6]

Lipids containing essential fatty acids (FAs) are a critical PN component, and in recent years, recommendations have been published to include n-3 PUFA–containing fish oil in PN in a variety of clinical contexts.[7] A range of experimental studies support the notion that an n-3 PUFA as well as an omega-6 (n-6) PUFA supply can be maintained on the basis of arachidonic acid (AA) and docosahexaenoic acid (DHA) with no special need for linoleic acid (LA) and alpha-linolenic acid.[8]

There is a large amount of experimental and clinical data showing anti-inflammatory and immune-modulating effects of n-3 PUFAs, particularly with regard to n-3 PUFA–derived so-called specialized pro-resolving lipid mediators (SPMs).[9,10]

Data from animal models indicate that fish oil protects the liver from PN-induced injury via peroxisome proliferator-activated receptor-gamma signaling mediated by the n-3 PUFA receptor GPR120.[11] Furthermore, n-3 PUFAs protect from acute liver injury and liver tumors through the suppression of cytokine formation.[12,13] Fat-1 transgenic mice, which are capable of endogenous production of n-3 PUFAs from n-6 PUFAs, have an increase in metabolites associated with a pro-resolving lipidome such as 17-hydroxydocosahexaenoic acid (HDHA).[14,15] Antisteatotic effects have been established for cytochrome P450 (CYP)–derived n-3 PUFA epoxides,[16] arguing toward anti-inflammatory protective effects of this class of mediators in the context of liver pathologies.[17] In humans, oral supplementation with n-3 PUFAs is associated with a decrease of liver fat content in patients with nonalcoholic fatty liver disease[18,19] and with an increase in circulating n-3 PUFA–derived lipid mediators in healthy volunteers,[20] as well as in patients with cardiovascular disease and severe hyperlipidemia.[21]

In human infants, a pro-resolving lipidome was found after shifting the lipid source of their PN to fish oil–containing preparations, with formation of monohydroxy metabolites of n-3 PUFAs.[22] However, the effect of parenteral administration of fish oil on the n-3 PUFA–derived inflammation-dampening oxylipins (IDOs) and potentially SPM generation in adults has not been comprehensively assessed yet.

In this study, we assessed the effect of fish oil–enhanced PN on the formation of AA-derived, eicosapentaenoic acid (EPA)–derived, and DHA-derived metabolites. To this end, we conducted a longitudinal study investigating the effect of fish-oil administration in patients with chronic intestinal failure (CIF). We found that fish-oil administration in PN leads to sustained, very high levels of n-3 PUFAs in the blood erythrocyte fraction, as indicated by stable high Omega-3 Index levels. These high levels of n-3 PUFAs were accompanied by high levels of CYP-derived and lipoxygenase (LOX)–derived n-3 PUFA oxylipins implicated in inflammation dampening in other studies.

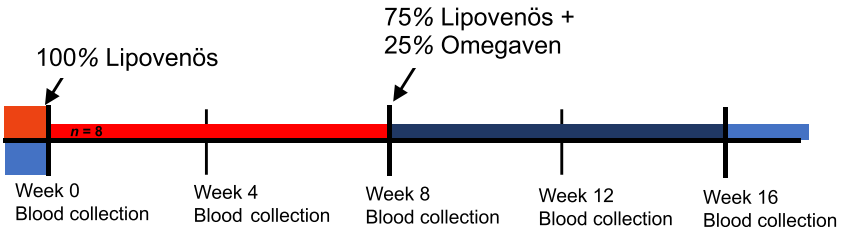

FIGURE 1 Studydesign. Regardless of preceding parenteral nutrition (PN) regimen, patients after inclusion received Lipovenös medium-chain triglyceride (MCT) 20% (Fresenius Kabi AG) during a washout phase of 8 weeks. This was followed by 8 weeks of 75% Lipovenös MCT 20% plus 25% Omegaven (Fresenius Kabi AG) as the lipid component of the PN administered

METHODS

Study population/design

In this prospective longitudinal pilot study, eight patients receiving long-term PN owing to intestinal failure underwent a defined change of their PN FA component according to the protocol shown in Figure 1: Regardless of preceding PN regiment, patients after inclusion received Lipovenös medium-chain triglyceride (MCT) 20% (Fresenius Kabi AG) during a washout phase of 8 weeks. This was followed by 8 weeks of 75% Lipovenös MCT 20% plus 25% Omegaven (Fresenius Kabi AG) as lipid compound. Lipovenös MCT 20% lipid content consists of 50% soybean oil and 50% MCT. Omegaven lipid content consists of 100% fish oil, which is rich in EPA and DHA. The FA composition of Omegaven and Lipovenös MCT 20% is shown in Supporting Information (Supplementary Table S1), with the batch of Omegaven used throughout containing an amount of 15.69 mg/ml EPA and 13.69 mg/ml DHA, as well as small (nanogram) amounts of some oxylipins (Supporting Information: Table S2).

Owing to variations in FA compositions between different batches of these lipid emulsions, lipid components used in this study were from the same batch. The overall quantitative composition of the respective original parenteral support solution—that is, all other nutrients (total energy, amino acids, glucose, micronutrients) were left unaltered.

The following inclusion criteria were applied: patients age ≥18 years, ≥6-month duration of home PN caused by CIF, and PN at least 3 days/week. Exclusion criteria were underlying liver disease not related to PN, malignant disease, chronic ongoing inflammation, and any contraindication to receive Lipovenös MCT 20% or Omegaven lipid. Patients underwent clinical examination, nutrition status assessment, and comprehensive blood tests collected after at least 6 h of fasting, including fasting from PN, every 4–8 weeks (as available in clinical routine). The first time point (baseline) for this study was before switching to 100% Lipovenös MCT 20% (week 0). Blood samples were directly analyzed for routine clinical chemistry parameters. Separate aliquots were stored in liquid nitrogen until further lipidomics analysis, all as described in the following sections. All participants gave written consent. The study was approved by the local ethics committee of Charité – University Medicine, Berlin (EA1/110/14 and EA2/034/13), and was performed in accordance with the ethical standards of the 1964 Declaration of Helsinki in its last revision of 2013.

FA analysis

Red blood cell (RBC) FA compositions were analyzed according to HS-Omega-3 Index methodology as described previously.[23,24] In short, RBCs, after being washed twice with phosphate-buffered saline, were stored at −80°C. FA methyl esters generated by acid transesterification were analyzed by gas chromatography using a GC-2010 gas chromatograph (Shimadzu) equipped with an SP-2560 100 m column (Supelco) with hydrogen as the carrier gas. Identification of FAs was done using a standard mix of FAs. The HS-Omega-3 Index is given as EPA plus DHA expressed as a percentage of total identified PUFAs. The coefficient of variation for EPA plus DHA was 5%. Analyses were quality controlled according to DINISO 15189.

Oxylipin analysis

To measure total (free and esterified) LOX-derived and CYP-derived metabolites, plasma samples (500 µl) were subjected to alkaline hydrolysis, and subsequent solid-phase extraction (SPE) was performed exactly as described previously.[20] As described before, free metabolites, including alkaline-sensitive prostaglandins (PGs) and thromboxanes (TXs), were directly extracted via SPE without prior alkaline hydrolysis.[20] In short, liquid chromatography–tandem mass spectrometry analysis was performed using an Agilent 6460 Triple Quad mass spectrometer with JetStream ion source (Agilent Technologies) coupled with an Agilent 1200 HPLC System (degasser, binary pump, well plate sampler, thermostatted column compartment) equipped with a Kinetex column (150 mm × 2.1 mm, 2.6 µm; Phenomenex). For exact conditions of chromatography analysis, please refer to Fischer et al.[20] Internal standards added to the samples before extraction included 10 ng each of 20-hydroxyeicosatetraenoic acid (HETE)-*d6*; 14,15-epoxyeicosatrienoic acid (EET)-*d8*; 14,15-dihydroxyeicosatrienoic acid (14,15-DHET)-*d11*; PGE2-d4; leukotriene B4-d5; and 15-HETE-*d8* (Cayman Chemical) and served for the quantification of groups of similar metabolites. Calibration curves for the quantification of individual metabolites

were established based on the changes in the relative peak area in response to different target compound/internal standard concentration ratios. Linearity was $r^2 > 0.99$ over a range from 1 to 20 ng absolute for any compound. 5,6-EET, 5,6-epoxyeicosatetraenoic acid (EEQ), and 4,5-epoxydocosapentaenoic acid (EDP) were undetectable in the samples, below the limit of validation, or instable. One patient was excluded from oxylipin analysis because of thrombocyte activation before analysis at week 0.

Clinical chemical parameter analysis

Clinical chemistry analysis included parameters of liver function, kidney function, lipid and glucose metabolism, hematology, inflammatory parameters, and electrolytes. Analysis was performed in a standardized setting in Labor Berlin – Charité Vivantes GmbH using clinical routine analysis facilities.

Indirect calorimetry

Resting energy expenditure (REE) was evaluated by means of indirect calorimetry in fasting conditions after a measurement stabilization period of about 10 min and an actual measurement of at least 30 min in a resting horizontal position. Patients were placed under a canopy hood that collected the expired air. Respiratory quotient and REE were assessed based on the measurement of carbon dioxide production and oxygen consumption via a proprietary device (Quark RMR, COSMED).[25]

Statistical analysis

Statistical analysis was performed with IBM SPSS statistics 22 and GraphPad Prism 6 (GraphPad Software). Data were tested for normal distribution and are given as mean ± SEM and median (interquartile range [IQR]). A general linear model with repeated measures was used to analyze the differences between groups. Normally distributed data were evaluated using Pearson correlation coefficient, whereas Spearman correlation coefficient was used for nonnormally distributed data. *P* values <0.05 were considered significant.

RESULTS

Patient characteristics

Eight patients with CIF were included in this study. Mean age of the group was 49 ± 24 years, with a mean body mass index of 22.8 ± 2.9 kg/m^2. Patients had received PN for a mean duration of 48 ± 33 months before inclusion, with a weekly PN energy intake of 7715 ± 3297 kcal. REE, as measured by indirect calorimetry, was 1553 ± 223 kcal/day, and PN covered 100% ± 22% of the REE (Table 1).

Impact of parenteral fish oil on FA composition of erythrocyte membranes

FA composition of erythrocyte membranes was determined at baseline. Median Omega-3 Index was 11.96% (IQR, 9.74%–14.38%), reflecting widespread use of fish oil–containing PN in standard care. Patients then entered a washout period of 8 weeks, during which they received only minimal amounts n-3 PUFA present in Lipovenös MCT 20% as part of their PN. The incorporation of FAs in erythrocyte membranes was a dynamic process and directly influenced by parenteral fish oil. After these 8 weeks, the Omega-3 Index had decreased to 9.57% (IQR, 8.25%–10.61%) at week 8. From week 9 to week 16, the eight patients received intravenous EPA plus DHA by administering 25% of FAs as fish-oil preparation; this led to the Omega-3 Index rising to 12.75% (IQR, 12.22%–15.02%) (Figure 2A).

TABLE 1 Patients' characteristics

Descriptive data	Baseline
Demographic, anthropometric, and disease characteristics	
Number of patients, *n* (f/m)	8 (7/1)
Age, mean ± SEM, years	49 ± 24
BMI, mean ± SEM, kg/m^2	22.8 ± 2.9
Anatomical SBS type, I/II/III, *n* (%)[a]	4/4/0 (50/50/50)
Underlying primary disease as cause of CIF, *n* (%)	
Mesenteric infarction	3 (37.5)
Inflammatory bowel disease	2 (25)
Bowel obstruction	1 (12.5)
Trauma	2 (25)
Home PN	
Duration, month, mean ± SEM, months	48 ± 33
Median (range), months	33 (9–122)
PN, mean ± SEM, days/week	5 ± 2
PN, mean ± SEM, kcal/infusion	1538 ± 275
PN, mean ± SEM, kcal/week	7715 ± 3297
Composition of PN, mean ± SEM	
Lipid, g/week	334 ± 146
Fish oil, g/week	47 ± 21
Glucose, g/week	747 ± 358
Amino acids, g/week	350 ± 138
REE, kcal	1553 ± 223 (*n* = 4)
REE covered by PN, mean ± SEM, %	100 ± 22 (*n* = 4)

Note: Composition of PN in g/week takes PN-days/week into account.
Abbreviations: BMI, body mass index; CIF, chronic intestinal failure; f, female; m, male; PN, parenteral nutrition; REE, resting energy expenditure measured with indirect calorimetry; SBS, short bowel syndrome.
[a]According to Messing et al.[26]

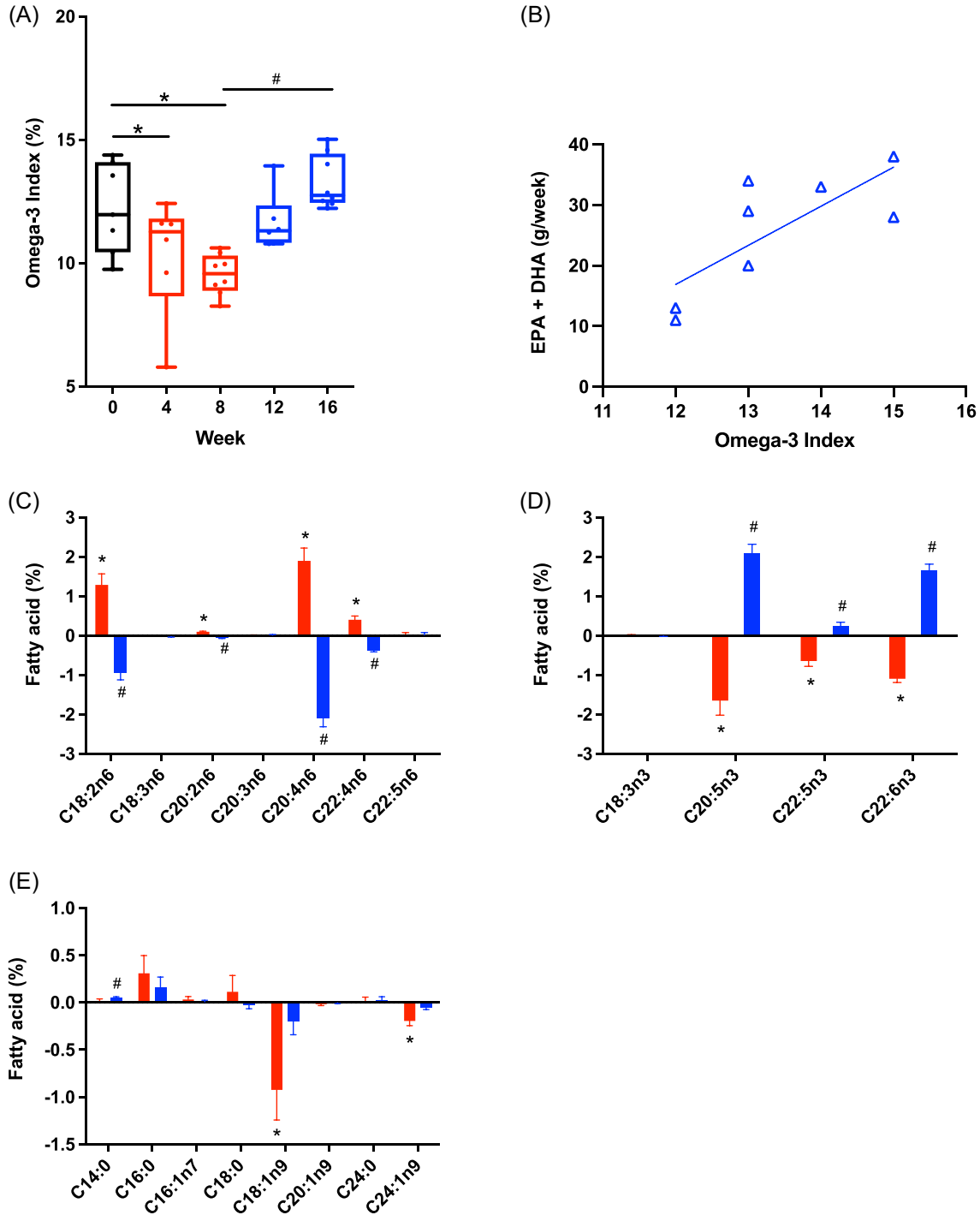

FIGURE 2 Effect of fish-oil supplementation on HS-Omega-3 Index and fatty acid composition. (A) Time-dependent changes of the Omega-3 Index during the fish oil–free washout period of 8 weeks and the fish-oil administration phase of 8 weeks from week 9 to week 16. (B) Omega-3 Index at the end of week 16 as a function of weekly supplementation of eicosapentaenoic acid (EPA) plus docosahexaenoic acid (DHA) in grams during weeks 9–16. (C–E) Percentage change of omega-6 (n-6) polyunsaturated fatty acids (PUFAs), omega-3 (n-3) PUFAs, and other individual fatty acids in the red blood cell fraction at two points of the study (week 8 vs week 0; week 16 vs week 8). Data are given as mean ± SEM or median (range). A general linear model for repeated measurements was used for analysis. *Significant ($P < 0.05$) change vs baseline (week 0). #Significant ($P < 0.05$) change vs week 8

EPA plus DHA doses given per week ranged from 11.13 to 38.43 g, depending on the individual patient's PN requirements (Figure 2B). Fish oil–containing PN was able to modulate the FA composition of erythrocyte membranes: LA (18:2n-6), eicosadienoic acid (EDA; 20:2n-6), AA (20:4n-6), and adrenic acid (AdA; 22:4n-6) levels were significantly higher (Figure 2C), whereas EPA (C20:5n-3), docosapentaenoic acid (DPA; C22:5n-3), DHA (C22:6n-3) (Figure 2D), and oleic acid (OA) (C18:1n-9) were significantly lower (Figure 2E) during the absence of n-3 PUFA. By contrast, under fish-oil administration, the relative amounts of LA, AdA, AA, and EDA were significantly lower (Figure 2C), whereas those of myristic acid (C14:0), EPA, DPA, and DHA in erythrocyte membranes were significantly higher (Figure 2D,E).

Impact of parenteral fish oil on circulating CYP-derived oxylipin levels

Blood content of n-3 PUFAs modified the amounts of CYP-derived epoxy metabolites of essential FAs. We calculated the Omega-3–Epoxymetabolite Index,[20] which reflects the relative abundance of CYP-epoxygenase–derived metabolites from n-3 PUFAs (DHA and EPA) in relation to those synthesized from AA. Without n-3 PUFAs in the PN, the median Omega-3–Epoxymetabolite Index was 0.86% at the end of the washout period (after week 8) and increased to 2.19% after week 16 under defined fish-oil administration in the PN (Figure 3G). As shown in Figure 3, 14,15-EET was the most abundant isomeric form among the EETs measured and was the only EET determined as having significantly higher levels during n-3 PUFA starvation (Figure 3A). Among the DHETs (5,6-DHET, 8,9-DHET, 11,12-DHET, and 14,15-DHET), 5,6-DHET was the most abundant isoform (Figure 3B). EEQs (8,9-EEQ, 11,12-EEQ, 14,15-EEQ, and 17,18-EEQ) and dihydroxyeicosatetraenoic acids (5,6-DiHETE, 8,9-DiHETE, 11,12-DiHETE, 14,15-DiHETE, and 17,18-DiHETE) all increased significantly with fish oil in the PN (Figure 3C,D). Similarly, EDPs and dihydroxy docosapentaenoic acids (DiHDPAs) also had the highest concentration at week 16, and 16,17-EDP and 7,8-DiHDPA were the dominating components (Figure 3E,F).

Regarding the omega-hydroxylase products, AA-derived 20-HETE showed almost no variation at the end of the washout period and decreased after readministration of fish oil in the PN, whereas EPA-derived 20-hydroxyeicosapentaenoic acid (20-HEPE) was significantly decreased at the end of the washout period and increased with administration of fish oil. On the other hand, the level of DHA-derived 22-HDHA was not affected by the washout period but significantly increased with fish-oil administration (Figure 3H). The ratios of 20-HETE:20-HEPE:22-HDHA changed dynamically from 32:21:47 (week 0) to 34:15:51 (week 8) to 27:28:45 (week 16).

Impact of parenteral fish oil on LOX-dependent and cyclooxygenase-dependent oxylipins

5-LOX catalyzes the formation of 5-HETE from AA, 5-HEPE from EPA, and the metabolites 4-HDHA and 7-HDHA from DHA as parenteral FA. 5-HEPE and 7-HDHA were significantly reduced at the end of the washout period when compared with baseline levels, which was not the case for 4-HDHA. After introduction of PN with fish oil for 8 weeks, EPA-derived and DHA-derived 5-LOX products increased significantly when compared with their levels in week 8. By contrast, we observed a significant reduction in 5-HETE formation 8 weeks after the introduction of fish oil (Figure 4A).

12-LOX converts AA to 12-HETE, whereas it converts EPA and DHA to 12-HEPE and 14-HDHA, respectively. 14-HDHA is of special biological interest, as it is the precursor of pro-resolving mediator maresin-1.[27] The level of 12-HETE remained unaffected during the whole study period, whereas the levels of 12-HEPE and 14-HDHA were lower during the washout period. Upon administration of parenteral fish oil, 12-HEPE and 14-HDHA increased again (Figure 4B). Similar results could be observed with 15-LOX–dependent metabolites 15-HETE, 15-HEPE, and 17-HDHA, which are derived from their corresponding FAs: AA, EPA, and DHA. The level of 15-HETE did not change throughout the study period, whereas fish oil in PN significantly increased plasma levels of 15-HEPE and 17-HDHA (Figure 4C).

18-HEPE, precursor of the anti-inflammatory E-resolvin family generated from EPA, is possibly formed through several mechanisms, including autoxidation and cyclooxygenase-2 (COX-2) activity,[26] and alternatively through microbial CYP monooxygenases.[28] The plasma level of 18-HEPE was highly responsive to parenteral fish-oil administration, being significantly reduced at the end of the very low n-3 PUFA period and significantly increased after 8 weeks of parenteral fish-oil administration, exhibiting a threefold increase after week 16 compared with week 8 (Figure 4D).

However, within the limitations of our analytical setup, we were not able to detect dihydroxy and trihydroxy SPMs such as maresin-1 or resolvin D1, D3, D5, or E1. We found small amounts of EPA-derived lipoxin A5, but only in a few samples, and there were no differences between the different time points (Figure 4E).

PGs and TXs are products of the action of COX enzymes on PUFAs. PGE_3 and TXB_3 levels at week 0 were below the limit of detection. The concentration of TXB_2 did not change during the washout period but decreased significantly at week 16. PGE_2—a paradigmatic lipid mediator with context-dependent, mostly proinflammatory actions—was not affected by fish-oil administration (Figure 4E).

9-HETE and 9-HEPE are produced via nonenzymatic oxidation from AA and EPA. The plasma level of 9-HETE remained unaltered during the washout period but showed a significant reduction with parenteral fish-oil administration. The level of 9-HEPE showed a significant decrease to the minimum values after the washout period but increased threefold after the introduction of fish oil compared with that at week 8 ($P < 0.01$) (Figure 4F).

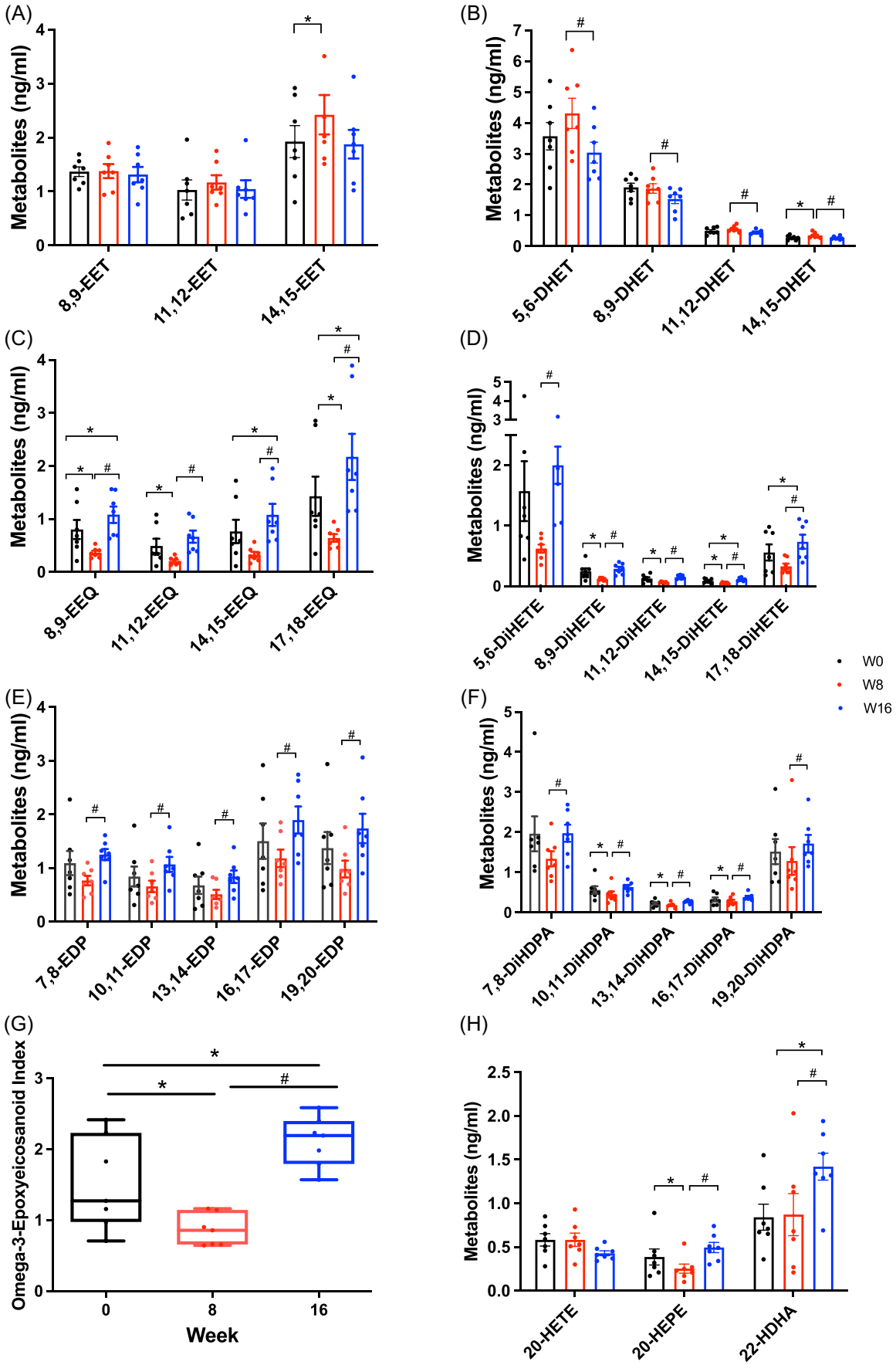

FIGURE 3 (See caption on next page)

Association of n-3, n-6 PUFAs with their metabolites and HS-Omega-3 Index

In our study, n-3 PUFA–derived LOX-dependent and CYP-dependent metabolites were strongly associated with their respective precursors under fish oil–containing PN. We plotted the ratios of EPA-derived/AA-derived and DHA-derived/AA-derived metabolites from the LOX- and CYP-epoxygenase/soluble epoxide hydrolase pathways against the precursor ratios (EPA/AA and DHA/AA, respectively) (Figure 5). The slopes of the correlation lines indicate that among the LOX enzymes, 5-LOX–derived metabolites displayed the highest susceptibility toward parenteral EPA and DHA administration (Figure 5A,B). Efficiency for lipid mediator conversion for EPA-derived and DHA-derived epoxy metabolites was similar (Figure 5C,D). Likewise, we observed a positive correlation between the HS-Omega-3 Index and the n-3 PUFAs EPA and DHA, with EPA contributing higher amounts at higher HS-Omega-3 Indices, whereas the n-6 PUFAs LA and AA were inversely correlated with a more pronounced decrease of AA with higher HS-Omega-3 Indices (Figure 5E,F).

Impact of parenteral fish oil on clinical parameters

We also analyzed the correlation between parenteral fish oil and clinical parameters of liver function and inflammatory markers (Table 2). Liver synthesis parameters such as pseudocholinesterase and serum albumin level or lipid parameters such as total cholesterol, high-density lipoprotein, LDL, and triglycerides were not affected. The mean value of alanine transaminase decreased from 41.13 U/L in week 8 to 33.63 U/L in week 16, albeit not by a significant level. Indicators of inflammation such as leukocyte count and high-sensitivity C-reactive protein remained unchanged.

DISCUSSION

This longitudinal study investigated the effect of fish oil–containing PN in patients with CIF. Patients in our study were characterized by a high Omega-3 Index of 11.96% at the start of the study, which decreased to 9.57% after a period of 8 weeks without any fish oil in PN and increased to 12.75% upon giving 25% of fat as a fish-oil component in PN for 8 weeks.

The high values of n-3 PUFAs in the blood at baseline already reflect the paradigm shift that occurred in recent years, with the use of fish oil laid out in practice guidelines for PN,[7] thereby modifying essential FA composition in PN.[8] Studies in children with IFALD in whom PN containing n-3 PUFAs improved prognosis and prevented IFALD led to Omegaven, a parenteral lipid component manufactured from fish oil, being approved for use as a drug in this context in the United States since 2018.[29]

Fish oil–containing lipid emulsions in long-term PN were shown to be adequate regarding nutrition requirements.[30] Studies using soybean oil/MCT/olive oil (OO)/fish oil[31] or soybean/MCT/OO/fish oil–based lipid emulsions[32] established changes in FA composition toward n-3 PUFAs.

However, there are few data about the clinical effects of fish oil in PN. Comparing fish oil–containing (SMOFlipid) baseline PN against pure fish oil–containing (Omegaven) PN found complex changes in lipopolysaccharide-induced cytokine profiles.[33] SMOFlipid did not alter liver function markers or inflammation, whereas OO-based ClinOleic (without fish oil) decreased some markers of liver function and inflammation in another study.[34] In acutely ill adult patients, SMOFlipid, as compared with a 100% soybean oil–based lipid emulsion, decreased the n-6:n-3 PUFA ratio, but there were no significant differences in biochemical measurements, Sequential Organ Failure Assessment score, length of intensive care unit stay, and mortality.[35] A comparison of four different lipid emulsion substitutions (long-chain triglycerides [LCTs], MCTs/LCTs, OO/LCTs, and a mix of LCTs/MCTs/OO/fish oil) in adult patients with intestinal failure receiving long-term PN showed that OO/LCT, but not the fish-oil lipid emulsion, led to a significant decrease in total bilirubin concentration and gamma-glutamyl transferase after 12 months.[36]

As outlined in the Introduction, a previous study showed a shift to a pro-resolving lipidome with fish oil–containing PN in infants,[22] and with this study, we aimed to assess this also in adult patients with short bowel syndrome. The Omega-3 Index in our patients was markedly higher than in healthy individuals receiving an oral administration of EPA and DHA using capsules of prescription-grade fish oil.[20] To precisely define the amounts of n-3 PUFAs given in the PN, we used a defined batch of n-3 PUFA–containing fish-oil lipid emulsion (Supporting Information: Table S1). This was done to control for the high natural variation of n-3 PUFA content in fish oil–containing parenteral lipid emulsions: For Omegaven (used in this study), the specification gives a broad range of possible n-3 PUFA content, ranging from 1.25 to 2.82 g EPA and from 1.44 to 3.09 g

FIGURE 3 Effect of fish-oil supplementation on cytochrome P450 (CYP)–epoxygenase and CYP-hydroxylase metabolites derived from arachidonic acid (AA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). (A–F) Dynamic changes of AA-derived, EPA-derived, and DHA-derived regioisomeric epoxy metabolites and their corresponding vicinal diols under parenteral fish-oil supplementation. (G) Changes of the Omega-3–Epoxyeicosanoid Index under parenteral fish-oil supplementation. Modulation of the Omega-3–Epoxyeicosanoid Index was calculated as follows: [(EEQs + DiHETEs) + (EDPs + DiHDPAs)]/(EETs + DHETs). (H) Changes on CYP-epoxygenase and CYP-hydroxylase monohydroxy products under parenteral fish-oil supplementation. Data are given as mean ± SEM. A general linear model for repeated measurements was used for analysis. *Significant ($P < 0.05$) change vs baseline level (W0). #Significant ($P < 0.05$) change vs W8. DiHDPA, dihydroxy docosapentaenoic acid; DiHETE, dihydroxyeicosatetraenoic acid; DHET, dihydroxyeicosatrienoic acid; EDP, epoxydocosapentaenoic acid; EEQ, epoxyeicosatetraenoic acid; EET, epoxyeicosatrienoic acid; HDHA, hydroxydocosahexaenoic acid; W, week

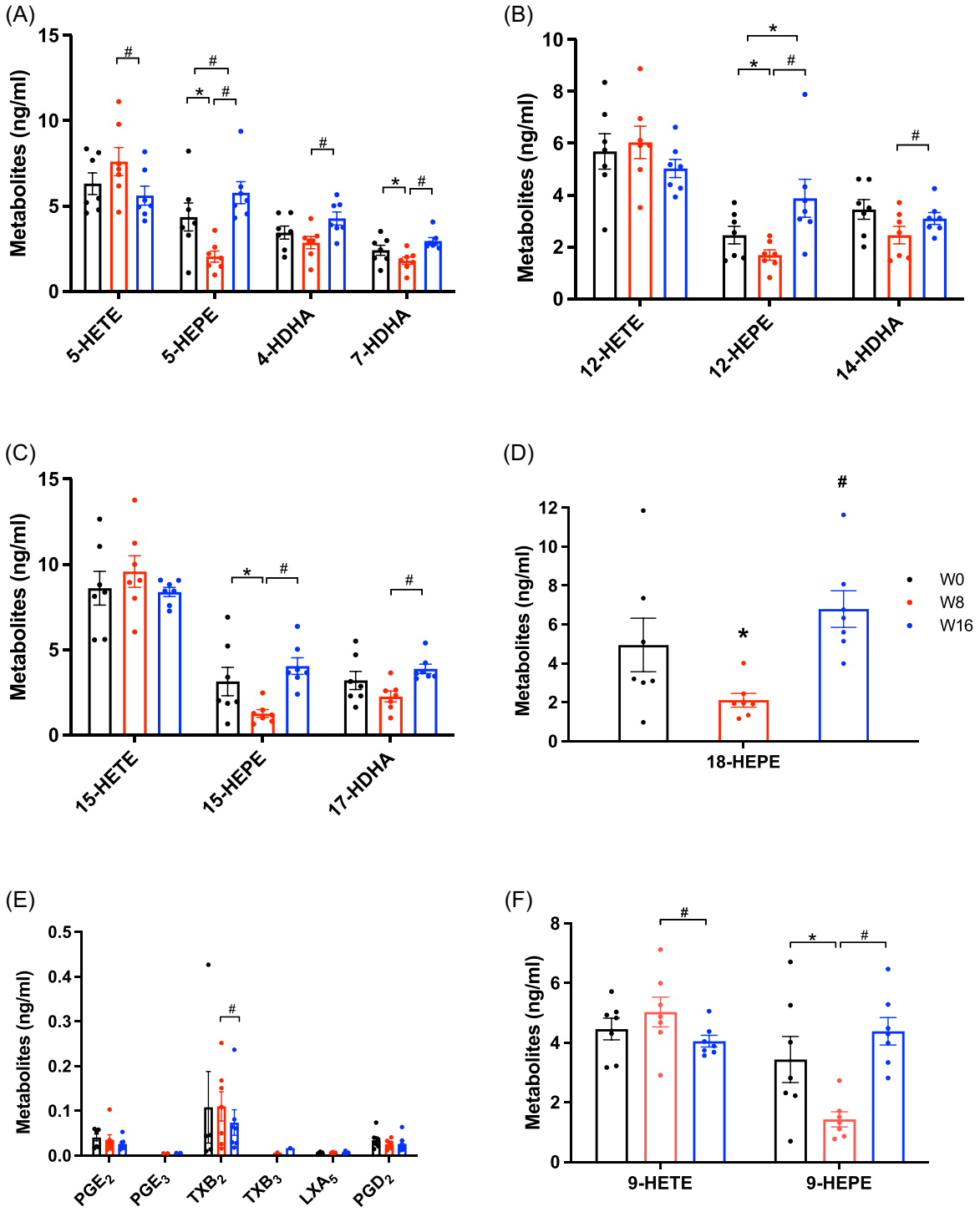

FIGURE 4 Effect of fish-oil supplementation on lipoxygenase (LOX)–dependent and cyclooxygenase (COX)–dependent monohydroxy and nonenzymatic oxidation metabolites derived from arachidonic acid (AA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). (A–C) Dynamic changes of AA-derived, EPA-derived, and DHA-derived 5-LOX, 12-LOX, and 15-LOX metabolites under parenteral fish oil. (D) Change of 18-HEPE concentration. (E) Changes on COX-dependent metabolites and lipoxin A5 (LXA_5) due to fish-oil administration. (F) Nonenzymatic oxidation metabolites derived from AA and EPA. A general linear model for repeated measurements was used for analysis. *Significant ($P < 0.05$) change vs basal level (W0). #Significant ($P < 0.05$) change vs W8. HDHA, hydroxydocosahexaenoic acid; HEPE, hydroxyeicosapentaenoic acid; HETE, hydroxyeicosatetraenoic acid; PGE, prostaglandin E; TXB, thromboxane B; W, week

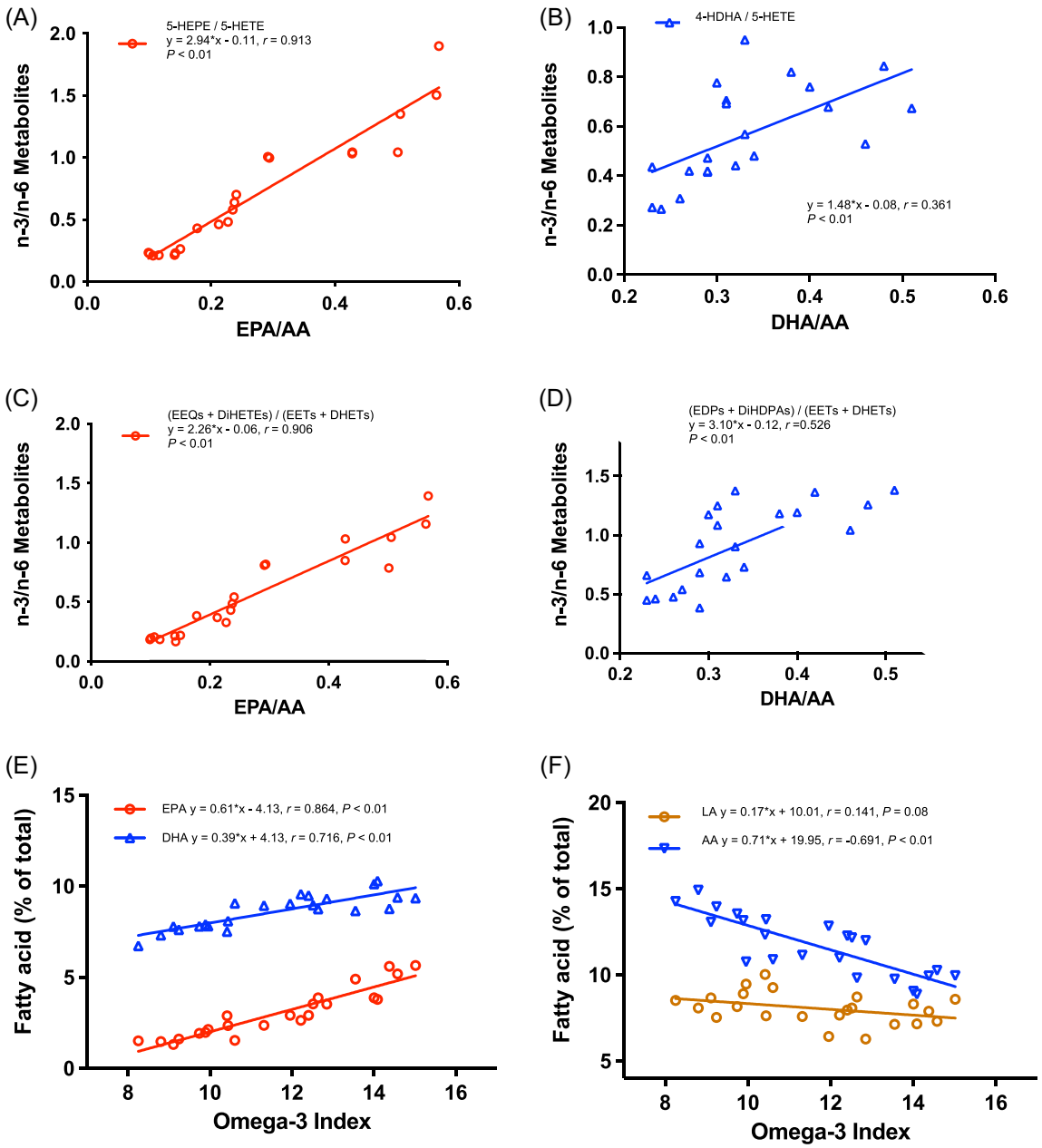

FIGURE 5 Relationship between omega-3, omega-6 polyunsaturated fatty acids (PUFAs) and some of their oxylipins and the HS-Omega-3 Index. (A–D) Correlation of omega-3, omega-6 PUFA–derived oxylipins with their respective precursor fatty acids. (E and F) Correlation of some omega-3, omega-6 PUFAs with HS-Omega-3 Index. For relative efficiencies, Pearson (B, D–F) or Spearman (A, C) coefficient correlation was performed, depending on whether the data were normally distributed. AA, arachidonic acid; DiHDPA, dihydroxy docosapentaenoic acid; DiHETE, dihydroxyeicosatetraenoic acid; DHA, docosahexaenoic acid; DHET, dihydroxyeicosatrienoic acid; EDP, epoxydocosapentaenoic acid; EEQ, epoxyeicosatetraenoic acid; EET, epoxyeicosatrienoic acid; EPA, eicosapentaenoic acid; HDHA, hydroxydocosahexaenoic acid; HEPE, hydroxyeicosapentaenoic acid; HETE, hydroxyeicosatetraenoic acid; LA, linoleic acid

DHA per 100 ml of lipid emulsion. For SMOFlipid, only the amount of n-3 PUFA–rich fish oil is given (as 30 g per 1000 ml lipid emulsion), without further specification of the EPA and DHA content.

Based on the high level of the Omega-3 Index, our data indicate that the administration of PN with fish oil is accompanied by a predominant accumulation of EPA-derived and DHA-derived LOX-epoxygenase–dependent and CYP-epoxygenase–dependent oxylipins. In contrast, AA-derived, CYP- and LOX-metabolites were not significantly affected by fish oil in the PN. Furthermore, we observed a positive correlation between the ratios of EPA-derived and DHA-derived to AA-derived metabolites and the ratio of their precursor PUFAs (Figure 5). By contrast, we could only observe small or no changes of plasma PGs and TXs in response to fish oil–containing PN. Only plasma TXB_2 (the stable intermediate of proinflammatory TXA_2) was reduced by the reintroduction of fish oil.

TABLE 2 Clinical parameters of patients with chronic intestinal failure

Clinical parameter	Week 0	Week 8	Week 16
Sodium, mmol/L	140.75 ± 3.06	140.3 ± 1.35	140.1 ± 0.69
Potassium, mmol/L	4.14 ± 0.47	4.16 ± 0.15	4.18 ± 0.12
Calcium, mmol/L	2.27 ± 0.12	2.27 ± 0.06	2.30 ± 0.04
Phosphate, mmol/L	1.14 ± 0.17	1.18 ± 0.06	1.12 ± 0.07
Magnesium, mmol/L	0.79 ± 0.08	0.76 ± 0.06	0.80 ± 0.03
Creatinine, mg/dl	0.87 ± 0.26	0.75 ± 0.06	0.82 ± 0.08
Estimated GFR, ml/min	75.00 ± 18.42	81.50 ± 5.20	75.75 ± 6.56
Urea, mg/dl	37.50 ± 19.77	35.38 ± 2.80	32.38 ± 2.67
Bilirubin, total, mg/dl	0.84 ± 0.75	0.93 ± 0.30	0.81 ± 0.18
Bilirubin, direct, mg/dl	0.28 ± 0.15	0.28 ± 0.07	0.29 ± 0.04
Serum albumin level, g/L	40.23 ± 4.51	41.53 ± 1.16	40.65 ± 1.27
Protein, g/L	74.29 ± 6.21	72.50 ± 2.47	74.43 ± 1.27
Total cholesterol, mg/dl	149.57 ± 50.22	140.40 ± 15.03	136.4 ± 13.39
HDL cholesterol, mg/dl	50.71 ± 27.16	43.75 ± 4.86	40.38 ± 6.35
Non-HDL cholesterol, mg/dl	106.71 ± 46.08	96.75 ± 13.02	95.88 ± 12.04
LDL cholesterol, mg/dl	84.57 ± 37.50	74.13 ± 10.50	71.50 ± 8.97
Triglycerides, mg/dl	133.86 ± 96.57	107.8 ± 30.40	114.0 ± 37.11
ALT, U/L	38.50 ± 22.17	41.13 ± 9.00	33.63 ± 7.39
AST, U/L	34.00 ± 7.45	36.5 ± 4.68	33.5 ± 2.76
AP, U/L	134.25 ± 68.10	123.80 ± 24.18	110.9 ± 20.62
Pseudocholinesterase, kU/L	6.61 ± 2.07	6.77 ± 0.67	6.71 ± 0.71
GGT, U/L	57.63 ± 51.89	51.38 ± 12.95	48.88 ± 14.70
Insulin, mU/L	15.19 ± 15.29	13.23 ± 5.88	8.41 ± 1.36
C-peptid, µg/L	4.18 ± 2.96	2.97 ± 0.88	2.31 ± 0.33
hs-CRP, mg/L	2.93 ± 2.82	1.16 ± 0.41	3.13 ± 0.75
Transferrin, g/L	2.87 ± 0.36	3.17 ± 0.29	3.04 ± 0.26
Hemoglobin, g/dl	12.58 ± 1.38	12.88 ± 0.49	12.26 ± 0.38
Hematocrit, %	0.38 ± 0.04	0.39 ± 0.01	0.37 ± 0.01
Erythrocyte count, per pl	4.49 ± 0.65	4.53 ± 0.24	4.33 ± 0.18
Leukocyte count, per nl	6.00 ± 1.59	6.73 ± 0.96	6.01 ± 0.72
Thrombocyte count, per nl	206.25 ± 104.01	189.9 ± 24.47	173.1 ± 22.83
MCV, fl	85.75 ± 8.03	86.13 ± 2.25	86.13 ± 1.73
MCH, pg	28.44 ± 3.13	28.64 ± 0.98	28.55 ± 0.77
MCHC, g/dl	32.81 ± 1.12	33.21 ± 0.48	33.18 ± 0.52
MPV, fl	11.34 ± 1.33	11.33 ± 0.48	11.14 ± 0.34
RDW-CV, %	15.21 ± 1.71	14.65 ± 0.74	14.66 ± 0.79
INR, %	1.11 ± 0.09	1.25 ± 0.12	1.12 ± 0.08

(Continues)

TABLE 2 (Continued)

Clinical parameter	Week 0	Week 8	Week 16
aPPT, s	38.66 ± 6.32	40.56 ± 3.80	43.58 ± 7.44
HbA_{1c}, %	5.08 ± 0.85	5.09 ± 0.16	5.03 ± 0.18

Note: A general linear model for repeated measurements was used for analysis. Data are mean ± SEM.

Abbreviations: ALT, alanine transaminase; AP, alkaline phospatase; aPPT, activated partial thromboplastin time; AST, aspartate aminotransferase; GFR, glomerular filtration rate; GGT, gamma-glutamyl transferase; HbA_{1c}, hemoglobin A_{1c}; HDL, high-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; INR, international normalized ratio; LDL, low-density lipoprotein; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; MPV, mean platelet volume; s, second.

*Significant ($P < 0.05$) change compared with baseline week 0.

#Significant ($P < 0.05$) change compared with week 8.

These results regarding LOX-epoxygenase–dependent and CYP-epoxygenase–dependent oxylipins are consistent with previous studies. Several CYP enzymes are obviously more efficient in the formation of EEQs from EPA compared with the generation of EETs from AA.[37] Moreover, DHA was shown to significantly inhibit CYP2J2-mediated AA metabolism.[38] As seen in Figure 3, all isoforms of EEQ and EDP measured were significantly increased, whereas the levels of EET remained mostly stable. The shift toward EPA/DHA-derived metabolites might have a beneficial effect on endothelial cells, vascular smooth muscle cells, and the humoral regulation system.[39] Furthermore, these oxylipins were shown in experimental models to protect from steatosis and steatohepatitis.[16] The average Omega-3–Epoxymetabolite Index reached 2.1 after fish-oil administration among our participants, which is, in general, consistent with that found by Fischer et al. (2.1 ± 0.15).[20]

Additionally, 14-HDHA, 17-HDHA, 15-HEPE, and 18-HEPE, which could be the precursors and/or pathway indicators[10] of the resolvin and protectin families of SPMs, were also significantly increased after the introduction of fish oil in PN, raising the possibility that the enhanced formation of 17-HDHA and 18-HEPE may improve liver function and dampen inflammation in our patients receiving PN, similar to their protective role in animal studies.[40,41] 18-HEPE was recently shown to be metabolized by different human LOX isoforms to different resolving products.[42] However, within the limitations of our analysis, we were not able to detect dihydroxy and trihydroxy SPMs such as maresin-1, resolvin D1 (RvD1), RvD3, RvD5, and RvE1, which might reflect differences in methodology, as discussed in a recent review in this field.[43]

Our data show that fish oil–containing PN leads to a CYP-based and LOX-based n-3 inflammation-dampening lipidome (rather than just a pro-resolving lipidome based on SPMs) that could account for many of the inflammation-limiting mechanisms that have been described for n-3 PUFA–derived CYP-oxylipins and LOX-oxylipins and suggest that these n-3 IDOs can contribute to the prevention of IFALD.

AUTHOR CONTRIBUTIONS

Ulrich F. Pape, Wolf H. Schunck, and Karsten H. Weylandt contributed to the conception and design of the research. Mirjam Karber contributed to the design of the research. Ulrich F. Pape, Mirjam Karber, Elisabeth Blüthner, and Sophie Pevny performed the acquisition of the data. Michael Rothe and Clemens von Schacky contributed to the analysis of the data. Ingrid W. Zhang, Yanan Xiao, and Karsten H. Weylandt contributed to the analysis and interpretation of the data and the writing of the manuscript. All authors critically revised the manuscript, agree to be fully accountable for ensuring the integrity and accuracy of the work, and read and approved the final manuscript.

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CONFLICT OF INTEREST

None declared.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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Curriculum Vitae

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Publication list

Publication

Xiao, Y., Zhang, Q., Liao, X., Elbelt, U. Weylandt, K.H. (2022). The effects of omega-3 fatty acids in type 2 diabetes: A systematic review and meta-analysis. Prostaglandins, Leukotrienes and Essential Fatty Acids, 102456. Impact factor: 4.006

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Poster

Assessment of plasma patterns of n-6 and n-3 PUFA oxylipins in type 2 diabetes mellitus patients with and without hypertriglyceridemia 11th International Singapore Lipid Symposium (iSLS11), 2023, Singapore

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