REVIEW

Influence of Lipid Class Used for Omega-3 Fatty Acid Supplementation on Liver Fat Accumulation in MASLD

Isaiah SABINARI[1,2], Olga HORAKOVA[1], Tomas CAJKA[3], Veronika KLEINOVA[1,2], Mariusz R. WIECKOWSKI[4], Martin ROSSMEISL[1]

[1]Laboratory of Adipose Tissue Biology, Institute of Physiology of the Czech Academy of Sciences, Prague, Czech Republic, [2]Department of Physiology, Faculty of Science, Charles University, Prague, Czech Republic, [3]Laboratory of Translational Metabolism, Institute of Physiology of the Czech Academy of Sciences, Prague, Czech Republic, [4]Laboratory of Mitochondrial Biology and Metabolism, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

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Summary

Metabolic dysfunction-associated steatotic liver disease (MASLD) occurs in subjects with obesity and metabolic syndrome. MASLD may progress from simple steatosis (i.e., hepatic steatosis) to steatohepatitis, characterized by inflammatory changes and liver cell damage, substantially increasing mortality. Lifestyle measures associated with weight loss and/or appropriate diet help reduce liver fat accumulation, thereby potentially limiting progression to steatohepatitis. As for diet, both total energy and macronutrient composition significantly influence the liver's fat content. For example, the type of dietary fatty acids can affect the metabolism of lipids and hence their tissue accumulation, with saturated fatty acids having a greater ability to promote fat storage in the liver than polyunsaturated ones. In particular, polyunsaturated fatty acids of *n*-3 series (omega-3), such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), have been intensively studied for their antisteatotic effects, both in preclinical animal models of obesity and hepatic steatosis and in overweight/obese patients. Their effects may depend not only on the dose and duration of administration of omega-3, or DHA/EPA ratio, but also on the lipid class used for their supplementation. This review summarizes the available evidence from recent comparative studies using omega-3 supplementation *via* different lipid classes. Albeit the evidence is mainly limited to preclinical studies, it suggests that phospholipids and possibly wax esters could provide greater efficacy against MASLD compared to traditional chemical forms of omega-3 supplementation (i.e., triacylglycerols, ethyl esters). This cannot be attributed solely to improved EPA and/or DHA bioavailability, but other mechanisms may be involved.

Keywords

MASLD • Metabolic dysfunction-associated steatotic liver disease • NAFLD • Non-alcoholic fatty liver disease • n-3 polyunsaturated fatty acids

Corresponding author

Martin Rossmeisl, Laboratory of Adipose Tissue Biology, Institute of Physiology of the Czech Academy of Sciences, Vídeňská 1083 , 14200 Prague 4, Czech Republic. E-mail: martin.rossmeisl@fgu.cas.cz

Introduction

The increasing global prevalence of obesity goes hand in hand with an increased risk of metabolic disorders (i.e., metabolic syndrome), which are additionally associated with non-alcoholic fatty liver disease, currently affecting ~30 % of the global population [1]. In fact, the new term metabolic dysfunction-associated steatotic liver disease (MASLD) has recently been coined to reflect the important role of

cardiometabolic risk factors associated with the disease [2-4]. While increased intrahepatic fat accumulation (i.e., liver steatosis or fatty liver) is characteristic of the early stages of MASLD, the disease can progress to metabolic dysfunction-associated steatohepatitis (MASH; previously known as NASH), which is associated with hepatocellular damage and inflammatory changes that may be accompanied by some degree of fibrosis. MASH can further develop into serious conditions such as cirrhosis and hepatocellular carcinoma, but overall mortality is already increased at the stage of hepatic steatosis and progressively increases with worsening MASLD histology [5]. A number of promising drugs are currently being investigated for treating MASLD/MASH based on different mechanisms of action. These agents include, for example, long-acting fibroblast growth factor 21 analogs [6], peroxisome proliferator-activated receptor agonists [7], or glucagon-like peptide 1 receptor agonists [8].

Lifestyle measures leading to weight loss and/or appropriate dietary modifications can positively affect fat accumulation in the liver [9,10], thereby potentially limiting progression to steatohepatitis [11]. Regarding the influence of diet, both total energy content and macronutrient composition seem to play an important role in the above processes [12]. In particular, the type of fatty acids (FA) in the diet can affect lipid metabolism in the liver and thus their tissue deposition. Accordingly, saturated FA appear to have a greater ability to promote fat storage in the liver and also in visceral adipose tissue (AT) compared to polyunsaturated FA (PUFA; [13,14] and reviewed in [12,15]). In particular, long-chain PUFA of *n*-3 series (omega-3), such as docosahexaenoic acid (DHA; 22:6*n*-3) or eicosapentaenoic acid (EPA; 20:5*n*-3), which contain the first double bond between the 3rd and 4th carbon atoms, starting from the terminal methyl end of the molecule, have been extensively studied under a variety of MASLD-promoting conditions, primarily based on their well-documented hypolipidemic [16-20] and anti-inflammatory properties (reviewed in [21-23]). From the results of a number of MASLD studies conducted in both preclinical animal models (mainly laboratory mice or rats) and human subjects, a general conclusion can be drawn that dietary supplementation with DHA and/or EPA can reduce liver fat (see e.g. [24-26] for recent reviews), but the efficacy towards MASH appears to be limited ([27] and reviewed in [25,28-30]). On the other hand, as might be expected, the antisteatotic effects of omega-3 supplementation in the liver depend on various factors, such as dose, duration of administration, and the DHA/EPA ratio of the supplemented omega-3. Moreover, it is important to note that most published studies have used triacylglycerol (TG; i.e., the chemical form found in fish oil) - or ethyl ester (EE)-based concentrates for omega-3 supplementation. In contrast, there is much less evidence, particularly in human subjects with MASLD, of the effects of omega-3 when these PUFA are supplemented using other lipid classes such as phospholipids (PL) or wax esters (WE; [31,32]).

This article does not present an exhaustive review of the published literature regarding MASLD and obesity, but instead strongly focuses on the effects of omega-3 supplementation on liver steatosis, mainly studied in the context of obesity or weight gain. Emphasis is placed on more recent comparative studies in preclinical animal models or in humans where omega-3 bound in different lipid classes have been administered. Finally, we also provide a brief overview of possible mechanisms (including omega-3 bioavailability) that may be common or unique to the different lipid classes of omega-3 used for supplementation.

Lipid classes used for omega-3 supplementation and their common sources

Essential α-linolenic acid (18:3*n*-3) serves as a precursor for the synthesis of omega-3 in animals and humans, but its conversion to EPA and DHA in the body is relatively inefficient. On the other hand, marine phytoplankton, an integral part of the marine food chain, is the richest source of omega-3 [21,33]. Therefore, marine fatty fish, especially those that live in colder environments (e.g., herring, sardine, mackerel, salmon), represent a major source of omega-3 for human consumption. Oils from these marine sources provide omega-3 primarily in the chemical form of TG and typically contain ~12 % DHA and ~18 % EPA bound in the *sn*-2 position of TG molecules; however, these oils can be further processed, using an EE intermediate, to obtain re-esterified TGs (rTG) in which DHA or EPA can also be esterified in the *sn*-1/3 position [34]. In addition to TG-based marine oils or EE products, oils from Antarctic krill (*Euphausia superba*) and from the copepod *Calanus finmarchicus*, which contain EPA and DHA in the chemical forms of PL and WE, respectively, can also be an alternative source of omega-3 [31,32]. An overview of the most important sources of omega-3 in

which EPA and DHA are bound in different lipid classes, including TG, EE, WE and PL, is given in Figure 1. Existing data on the composition of marine oils in terms of the lipid classes contained and the distribution of DHA and EPA in these lipid types are limited [32,35]. In this regard, our recent LC-MS-based lipidomic analysis revealed five major lipid classes, i.e., diacylglycerols (DG), free FA, phosphatidylcholine (PC), TG and WE, which are present in varying amounts in marine oils of different origins (Table 1). As expected, TG-based oils (e.g. herring oil and the rTG product) contained largely TG molecules (~80 %), whereas WEs are the lipid class that predominates (~80 %) in Calanus oil; in contrast, the relative content of PC as the major PL species contained in krill oil is only ~50 % and other types of lipids, such as TG, contribute significantly (Table 1).

Regarding the distribution of omega-3 in the main lipid classes of the selected marine oils (Table 1, middle and lower part), in the rTG concentrate enriched with DHA, TG and DG are the main species that contained DHA (up to 85 %) and EPA (up to 45 %); however, herring oil, as an example of a natural fish oil, shows a more even distribution of omega-3 in all major lipid classes except WE. Calanus oil showed a significant representation of DHA and EPA in all five major lipid classes, but the biological significance of such a distribution is probably low due to the predominance of the WE class in this type of oil. In krill oil, a PL-based omega-3 concentrate representative, PC, DG and free FAs represent those lipid classes enriched primarily in EPA and less in DHA (Table 1, middle and lower part). Thus, in addition to the composition and position of omega-3 in a given lipid molecule, the distribution of omega-3 into different types of lipid classes likely affects bioavailability, especially when omega-3 are supplemented through complex products such as krill oil.

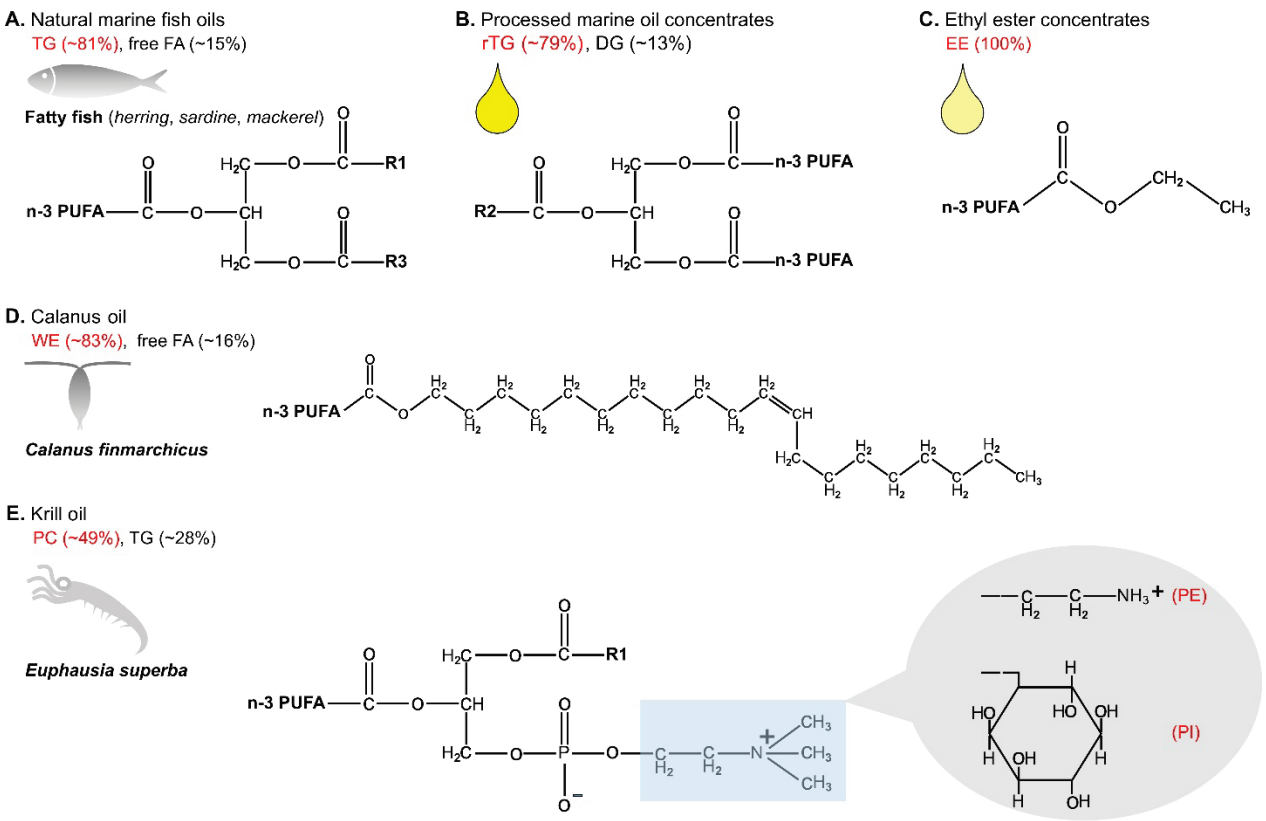

Fig. 1. Overview of marine sources of omega-3 with EPA and DHA bound in different lipid classes, including a schematic representation of the chemical formula of the main lipid class present in the respective product. Information on the relative abundance of the main lipid classes in the marine oils listed in A-E is based on the data given in Table 1. In the case of Calanus oil (D), a representative wax ester with fatty alcohol 20:1*n*-9 is shown, which together with 22:1*n*-11 are the main fatty alcohols contained in Calanus oil [32]. Krill oil (E) contains primarily phosphatidylcholine (PC) and triacylglycerols (TG), while other lipid classes including different types of phospholipids (e.g.; PE, PI,…) are only marginally present. DG, diacylglycerols; EE, ethyl esters; FA, fatty acids; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PUFA, polyunsaturated fatty acids; R1(2,3), other types of fatty acids in the *sn*-1, 2 and 3 position, respectively; TG, triacylglycerols; rTG, re-esterified triacylglycerols; WE, wax esters.

Table 1. Main lipid classes and their omega-3 occupancy in selected oils of marine origin

Lipid class composition distribution				
Lipid class (%)	Herring oil	Krill oil	Calanus oil	Epax 1050
DG	1.1	3.5	0.1	13.0
FA	15.0	4.9	15.5	1.7
PC	0.1	48.7	0.1	6.5
TG	80.5	28.3	0.8	78.7
WE	0.0	0.0	82.5	0.0
Distribution of omega-3 fatty acids in different lipid classes				
DHA (22:6n-3)				
DG	24.9	20.0	22.0	73.0
FA	35.8	24.2	34.2	25.4
PC	8.9	24.9	56.2	15.1
TG	13.6	5.3	19.3	85.4
WE	0.0	0.0	40.2	0.0
EPA (20:5n-3)				
DG	45.8	82.1	74.9	44.1
FA	10.3	35.2	28.7	13.8
PC	20.6	51.2	40.3	18.9
TG	17.4	12.9	34.5	44.8
WE	0.0	0.0	58.6	0.0

The results are based on LC-MS analysis of the oils listed. Relative content of the main lipid classes in oils (upper part) and the percentage of species containing DHA or EPA in the respective lipid fractions. The distribution of omega-3 (i.e., DHA and EPA) in lipid classes is determined by the cumulative sum of all distinct lipid species within a given lipid class, including DHA or EPA. These species may consist of identical omega-3 within their molecular structure (e.g., PC 22:6_22:6, TAG 22:6_22:6_22:6) or may be combined with other FA (e.g., PC 14:0_22:6, TAG 14:0_16:0_22:6). Abbreviations: DG, diacylglycerols; Epax 1050, concentrate of re-esterified triacylglycerols enriched with DHA from sources other than tuna (Epax Norway AS); FA, fatty acids; PC, phosphatidylcholines; TG, triacylglycerols; WE, wax esters.

Comparative studies using omega-3 concentrates based on different lipid classes

Original articles comparing the effects of omega-3 supplementation *via* different lipid classes on hepatic steatosis in MASLD were retrieved from the PubMed database using different keyword strings. While several existing review articles have summarized findings regarding the efficacy of omega-3 against MASLD [25,30], they do not specifically focus on studies using different lipid classes for omega-3 supplementation analyzed simultaneously in a single study. Our current review therefore focuses on comparative studies that included two or more lipid classes used for omega-3 administration, under conditions associated with weight gain and/or obesity. In addition, source articles were selected from those published in the last 15 years. We compiled specific search strings that combined keywords relevant to the focus of our review. These keywords thus encompassed the different ways in which (i) the disease of interest is described in the literature (e.g., NAFLD, MASLD, MAFLD), (ii) the primary phenotype (e.g., hepatic or liver steatosis, liver triacylglycerol(s)), and also (iii) the comparative nature of the retrieved studies involving different sources of omega-3 (e.g., fish oil, krill oil, Calanus oil) and different classes of lipids used for their supplementation (e.g., triacylglycerols, ethyl esters, phospholipids, wax esters). Regarding the retrieved studies, most of them were performed in rodents (38 in total), mainly in laboratory mice, but only 21 of these studies were included in the review because they fulfilled the condition where the liver phenotype is studied in the context of weight gain or obesity. In contrast, only five human studies that reported MASLD-related metabolic phenotypes were included in the review because comparative studies involving omega-3 supplementation *via* different lipid classes and direct quantification of liver fat content are lacking. In the reported studies, four lipid

classes, including TG, PL, WE and EE, were used for omega-3 supplementation, with the vast majority of studies comparing TG and PL forms of omega-3.

Based on a detailed examination of studies obtained in rodents, omega-3 PL from various marine sources (e.g., krill oil, herring meal extract, squid roe oil, algae oil) appear to be more effective in reducing liver fat compared to fish sources rich in omega-3 TG (see Table 2 for details). This was observed in both the preventive and reversal (i.e., treatment) experimental designs (e.g., [36-38]), using different types of high-fat diets (HFD; either based on corn oil or lard) and experimental conditions (e.g., 22 °C vs. 30 °C). Also, in KK-Ay mice, a genetic model of obesity and hyperglycemia, Sugimoto *et al.* [39] demonstrated greater efficacy of PL-rich scallop oil in reducing liver TG content than fish oil supplementation. Moreover, regarding different sources of omega-3 PL, a recent study on mice fed an HFD/high-cholesterol diet suggests that krill oil may have greater antisteatotic effects than squid oil extract [40]. Similarly, Wang *et al.* [41] show that omega-3 PL derived from Silver carp head is superior to Salmon head extract in reducing liver fat, suggesting that some other components of omega-3 PL concentrates are likely to contribute to antisteatotic effects in the liver ([37,38] and reviewed in [31]). On the other hand, there are studies where no significant reduction in liver fat was observed after administration of either form of omega-3 supplementation [42] or, alternatively, where there was a stronger effect when omega-3 was administered in the TG form compared to the PL form [43,44]. However, in a study by Botelho *et al.* [43], fish oil used for omega-3 supplementation in the form of TG contained both EPA and DHA, while algae oil as a source of omega-3 PLs was completely devoid of EPA. This certainly had a negative impact on the efficacy of omega-3 PL supplementation, which was reflected in significantly lower EPA content in the liver after algae oil supplementation compared to other forms of supplementation. Botelho *et al.* [43] and Gui *et al.* [44], moreover, did not accurately quantify liver fat after omega-3 supplementation (only histological images of the liver are presented), making it difficult to draw firm conclusions from these studies.

In terms of reducing liver TG content, other lipid classes used for omega-3 supplementation, such as highly purified EE or WE, have rarely been tested. Therefore, assessing their relative efficacies from the available studies is impossible, especially when compared to more commonly used classes such as TG or PL. One short-term prevention-type study compared the effects of DHA-rich omega-3 formulations based on EE, PL, free FA and TG forms in BALBc mice [45]; interestingly, at lower dietary fat levels (5 %), EE, PL and TG form reduced liver fat, but at higher dietary fat levels (22.5 %), all were ineffective. On the other hand, compared with omega-3 supplemented in the form of EE, Calanus oil-derived WE exhibited potent antisteatotic capabilities with respect to liver fat content while increasing adiponectin expression in AT [46].

Only five comparative human studies were found, where the effects of omega-3 supplementations on MASLD-related parameters (i.e., not directly on liver fat accumulation) were examined (Table 3). Compared to placebo-treated subjects, there was no difference in the effects of omega-3 supplemented for 14 weeks as PL (algae oil) or TG (fish oil) on reducing serum TG in statin-treated hypertriglyceridemic patients [60], and a similar effect was found in a 2-week crossover study using PLs from herring roe vs. fish oil in patients with hypertriglyceridemia [61]. When comparing omega-3 administered in the TG form with their EE form (i.e., using Omacor), a similar degree of reduction in plasma TG was observed with both forms of supplementation [62]. However, Schuchardt *et al.* [17,63] reported a reduction in serum TG in statin-treated dyslipidemic subjects who were given omega-3 in the form of rTG, whereas omega-3 EE had no effect. Comparisons of seal and tuna oils in subjects with hypertriglyceridemia suggest better effectiveness of tuna oil in reducing plasma TG [64]; however, the results are difficult to interpret due to the higher amounts of EPA and DHA supplemented *via* tuna oil.

In summary, based on an analysis of studies primarily in rodents, it can be concluded that in the context of obesity-related MASLD, PL-based omega-3 supplementation has stronger effects in terms of reducing liver fat compared with other lipid classes used for supplementation, with the possible exception of WE, whose relative efficacy to the PL form has not been tested in a comparative study. The absence of comparative human studies on the effects of omega-3 supplementation on liver fat precludes any conclusions regarding the greater efficacy of omega-3 PLs found in rodent studies. On the other hand, the very limited number of studies examining the effects of omega-3 supplementation on MASLD-related phenotypes, such as circulating TG levels, suggested similar efficacy of PL vs. TG or TG vs. EE.

Table 2. Comparative rodent studies on MASLD using omega-3 supplemented in different lipid classes

Omega-3 concentrates	EPA/DHA dose	Species	Age[a] (weeks)	Sex	Study design	Primary outcome (Liver TG/MASLD)	MASLD-related phenotypes	Reference
ω-3PL vs. ω-3TG	ω-3PL (Herring meal extract) EPA: 5.66 DHA: 24.34 ω-3TG (rTG) EPA: 6.78 DHA: 23.22 (g/kg diet)	Mouse C57BL/6J Mouse C57BL/6J	12 28	Male Male	<u>Prevention</u> (9 weeks) Groups: CON HFD HFD + ω-3PL HFD + ω-3TG <u>Reversal</u> HFD + metformin (16 weeks) then Treatments (9 weeks): HFD + metformin + ω-3PL HFD + metformin + ω-3TG	Liver TG: ω-3PL ↓ (40% of HFD) ω-3TG ↓ (20% of HFD) (ω-3PL > ω-3TG) Liver TG: ω-3PL ↓ (77% of HFD) ω-3TG ↓ (52% of HFD) (ω-3PL > ω-3TG)	Plasma TG: ↓ω-3PL Plasma NEFA: ↓ω-3PL Weight gain: ↓↓ω-3PL ↓ω-3TG FBG: ↓↓ω-3PL ↓ω-3TG Adiponectin: ↑ω-3PL Plasma Insulin: ↓ω-3PL Plasma TG: ↓ω-3PL ↓ω-3TG Plasma TC: ↓ω-3PL ↓ω-3TG Plasma NEFA: ↓ω-3PL ↓ω-3TG	Rossmeisl *et al*., 2012 [36]
	ω-3PL (Krill oil) EPA: 9.3 DHA: 4.1 ω-3TG (Fish oil) EPA: 17.5 DHA: 12.5 (g/kg diet)	Mouse C57BL/6J	9-10	Male	<u>Prevention</u> (6 weeks) Groups: HFD HFD + ω-3PL HFD + ω-3TG	Liver TG: ω-3PL ↑ (150% of HFD) ω-3TG ↑ (400% of HFD)	Body weight: ↑ω-3PL Plasma TG: ↓ ω-3PL ↓↓ω-3TG Plasma TC: ↓ω-3PL ↓↓ω-3TG Plasma NEFA: ↓ω-3PL ↓↓ω-3TG HDL: ↓↓ω-3TG Liver TC: ↑ω-3PL ↑ω-3TG	Tillander *et al*., 2014 [48]
	ω-3PL (Krill oil) EPA: N/A DHA: N/A ω-3TG (Fish oil) EPA: N/A DHA: N/A (g/kg diet)	Mouse C57BL/6J	9-10	Male	<u>Prevention</u> (6 weeks) Groups: HFD HFD + ω-3PL HFD + ω-3TG	Liver TG: ω-3PL ↑ (200% of HFD) ω-3TG ↑ (196% of HFD)	Liver DG: ↓ω-3PL	Skorve *et al*., 2015 [49]

	ω-3PL (Microalgae oil) EPA: N/A DHA: N/A ω-3TG (Fish oil) EPA: N/A DHA: N/A (g/kg diet)	Mouse C57BL/6J	14	Male	<u>Reversal</u> CON HFD (8 weeks) then Treatments (8 weeks): HFD + ω-3PL HFD + ω-3TG	Liver TG: ω-3PL ↓ (62% of HFD) ω-3TG ↓ (50% of HFD) (ω-3PL > ω-3TG)	Weight gain: ↓ω-3PL Serum TG: ↓↓ω-3PL↓ω-3TG Serum TC: ↓ω-3PL ↓↓ω-3TG	Yook *et al*., 2015 [50]
	ω-3PL-l (Algae oil-lower purity) EPA: 3.0 DHA: 97.0 ω-3PL-h (Algae oil-higher purity) EPA: 1.1 DHA: 98.1 ω-3TG (Fish oil) EPA: 52.0 DHA: 45.0 (mg/kg body weight by oral gavage)	Mouse C57BL/6J	3-4	Male	<u>Prevention</u> (16 weeks) Groups: CON HFD HFD + ω-3PL-l HFD + ω-3PL-h HFD + ω-3TG	Liver lipid droplets (HFD): ω-3PL-l (↓) ω-3PL-h (↓) ω-3TG (↓)	Weight gain: ↓ω-3PL-l ↓ω-3PL-h Serum TG: ↓ω-3PL-l ↓ω-3PL-h ↓ω-3TG Serum TC: ↓ω-3PL-l ↓ω-3PL-h ↓ω-3TG	Yu *et al*., 2017 [51]
	DHA (Algal oil) EPA: 13 DHA: 738 DHA/EPA-A [2:1 (Fish oil + Algal oil)] EPA: 252 DHA: 469 DHA/EPA-B [1:1 (Fish oil + Algal oil)] EPA: 358 DHA: 346	Mouse C57BL/6J	5	Male	<u>Prevention</u> (11 weeks) Groups: CON HFD HFD + DHA HFD + DHA/EPA-A HFD + DHA/EPA-B HFD + DHA/EPA-C	Liver TG: DHA ↓ (56% of HFD) DHA/EPA-A ↓ (31% of HFD) DHA/EPA-B ↓ (27% of HFD) DHA/EPA-C ↓ (27% of HFD) Liver lipid droplets (HFD): DHA (↓) DHA/EPA-A (↓↓) DHA/EPA-B (↓↓) DHA/EPA-C (↓↓)	Serum TG: ↓↓DHA ↓DHA/EPA-A ↓DHA/EPA-B ↓DHA/EPA-C Serum TC: ↓↓DHA ↓DHA/EPA-A ↓DHA/EPA-B ↓↓DHA/EPA-C Serum LDL.: ↓DHA ↓DHA/EPA-A ↓DHA/EPA-B ↓↓DHA/EPA-C Serum HDL: ↑DHA ↑DHA/EPA-A ↑DHA/EPA-B ↑↑DHA/EPA-C	Shang *et al*., 2017 [52]

	DHA/EPA-C [1:2 (Fish oil + Algal oil)] EPA: 460 DHA: 244 (mg/kg body weight by oral gavage)						Liver TC: ↓↓DHA ↓DHA/EPA-A ↓DHA/EPA-B ↓DHA/EPA-C	
	ω-3PL (Algae oil) DHA + EPA: 4.5 ω-3TG (Fish oil) DHA + EPA: 4.5 (% of total energy intake)	Mouse C57BL/6J	7	Male	<u>Prevention</u> (12 weeks) Groups: CON HFD HFD + ω-3PL HFD + ω-3TG	Liver lipid droplets (HFD): ω-3PL (↓) ω-3TG (↓↓) (ω-3TG > ω-3PL)	Body weight: ↓ω-3PL ↓ω-3TG Serum TG: ↓ω-3PL ↓↓ω-3TG Serum TC: ↓ω-3PL ↓ω-3TG Serum LDL-C: ↓ω-3PL ↓ω-3TG Serum AST: ↓ω-3PL ↓ω-3TG Serum ALT: ↓ω-3PL ↓ω-3TG	Gui *et al*., 2019 [44]
	SCO-PL (Scallop oil phospholipid fraction) EPA: 2.6 DHA: 2.1 SCO-TG (Scallop oil triglyceride fraction) EPA: 2.6 DHA: 2.1 (g/kg diet)	Mouse C57BL/6J	4	Male	<u>Prevention</u> (4 weeks) Groups: HFD + SOY-TG HFD + SOY-PL HFD + SCO-PL HFD + SCO-TG	Liver TG: SCO-TG ↓ (17% of SOY-TG) SCO-PL ≈ SOY-PL (ω-3TG > ω-3PL)	Liver weight: ↓ SCO-PL Serum TG: ↓ SCO-PL Serum TC: ↓↓SCO-PL Serum HDL: ↓ SCO-PL	Sugimoto *et al*., 2021 [53]
	ω-3PL-K (Krill oil) EPA: 28 DHA: 13 ω-3PL-S (Squid roe oil) EPA: 28 DHA: 11	Mouse C57BL/6J	16	Male	<u>Reversal</u> CON HFD (9 weeks) then Treatments (9 weeks): HFD + ω-3PL-K HFD + ω-3PL-S HFD + ω-3TG	Liver TG: ω-3PL-K ↓ (23% of HFD) ω-3PL-S ↓ (21% of HFD) ω-3TG ↓ (21% of HFD) Liver lipid droplets (HFD): ω-3PL-K (↓↓↓) ω-3PL-S (↓↓) ω-3TG (↓)	Liver weight: ↓ω-3PL-K ↓ω-3PL-S ↓ω-3TG Liver TC: ↓↓ω-3PL-K ↓ω-3PL-S ↓ω-3TG HOMA-IR: ↓ω-3PL-K ↓ω-3PL-S ↓ω-3TG Serum TG: ↓ω-3PL-K	Chen *et al*., 2024 [40]

	ω-3TG (Fish oil) EPA: 22 DHA: 15 (mg per day by oral gavage)					(ω-3PL > ω-3TG)	↓ω-3PL-S ↓ω-3TG Serum TC: ↓ω-3PL-K ↓ω-3PL-S ↓ω-3TG Serum ALT: ↓↓ω-3PL-K ↓ω-3PL-S ↓ω-3TG Serum AST: ↓↓ω-3PL-K ↓ω-3PL-S ↓ω-3TG	
	ω-3PL (Microalgae oil) EPA: 1.08 DHA: 99.2 ω-3TG (Fish oil) EPA: 51.92 DHA: 44.98 (mg/kg body weight by oral gavage)	Mouse C57BL/6J	20	Male	<u>Reversal</u> CON HFD (8 weeks) then Treatments (16 weeks): HFD + ω-3PL HFD + ω-3TG	Liver lipid droplets (HFD): ω-3PL (↓↓) ω-3TG (↓) (ω-3PL > ω-3TG)	Body weight: ↓ω-3PL ↓ω-3TG Blood glucose: ↓ω-3PL ↓ω-3TG Serum TG: ↓↓ω-3PL ↓ω-3TG	Ran *et al*., 2022 [54]
	ω-3PL (Krill oil) EPA: 21 DHA: 12 ω-3TG (rTG) EPA: 8 DHA: 25 (g/kg diet)	Mouse C57BL/6N	12	Male	<u>Prevention</u> (8 weeks) Groups: HFD HFD + ω-3PL HFD + ω-3TG	Liver TG: ω-3PL ↓ (60% of HFD) ω-3TG ≈ HFD (ω-3PL > ω-3TG)	Body weight: ↓ω-3PL ↑ω-3TG Liver weight: ↓ω-3PL Plasma insulin: ↓ω-3PL Plasma TG: ↑ω-3PL Plasma TC: ↓ω-3PL ↓ω-3TG Plasma NEFA: ↑ω-3PL	Kroupova *et al*., 2020 [55]
	ω-3PL (Krill oil) EPA+DHA: 25 ω-3TG (rTG) EPA+DHA: 25 (g/kg diet)	Mouse C57BL/6N	10	Male	<u>Prevention</u> (8 weeks) Groups: HFD HFD + ω-3PL HFD + ω-3TG	Liver TG: ω-3PL ↓ (68% of HFD) ω-3TG ↓ (32% of HFD) (ω-3PL > ω-3TG)	Weight gain: ↓ω-3PL HOMA-IR: ↓↓ω-3PL ↓ω-3TG Adiponectin: ↑ω-3PL	Rossmeisl *et al*., 2020 [37]

	ω-3PL (Krill oil) EPA: 19.9 DHA: 12.1 ω-3TG (rTG) EPA: 19.0 DHA: 12.2 (g/kg diet)	Mouse C57BL/6N	18	Male	<u>Reversal</u> HFD (8 weeks) then Treatments (16 weeks): HFD + ω-3PL HFD + ω-3TG	Liver TG: ω-3PL ↓ (50% of HFD) ω-3TG ↓ (15% of HFD) (ω-3PL > ω-3TG)	Body weight: ↓ω-3PL ↓ω-3TG Blood glucose: ↓↓ω-3PL Plasma insulin: ↑ω-3TG Plasma TC: ↓ω-3PL Plasma AST: ↓ω-3PL Plasma ALT: ↓ω-3PL ↓↓ω-3TG Plasma adiponectin: ↑↑ω-3PL ↑ω-3TG	Sistilli *et al*., 2021 [38]
	ω-3PL (Algae oil) EPA: 0.0 DHA: 0.77 ω-3TG (Fish oil) EPA: 0.44 DHA: 0.25 (mg per day by oral gavage)	Mouse LDLr KO C57BL/6	16 - 18	Male	<u>Prevention</u> (4 weeks) Groups: HFD HFD + ω-3PL HFD + ω-3TG	Liver lipid droplets (HFD): ω-3PL (≈) ω-3TG (↓↓) (ω-3TG > ω-3PL)	Weight gain: ↓ω-3TG Plasma TG: ↓↓ω-3PL ↓ω-3TG Plasma TC: ↓ω-3PL ↓↓ω-3TG	Botelho *et al*., 2013 [43]
	ω-3PL (Scallop oil-Mutsu) EPA: 25.9 DHA: 4.1 ω-3PL (Scallop oil-Uchiura) EPA: 17.5 DHA: 9.8 ω-3TG (Fish oil) EPA: 4.3 DHA: 19.6 (g/kg diet)	Mouse KK-A^{y}	5	Male	<u>Prevention</u> (7 weeks) Groups: HFD HFD + ω-3PL(SCO-M) HFD + ω-3PL (SCO-U) HFD + ω-3TG	Liver TG: ω-3PL (SCO-M) ↓ (12% of HFD) ω-3PL (SCO-U) ↓ (20% of HFD) ω-3TG ↑ (22% of HFD) (ω-3PL > ω-3TG)	Serum TG: ↓ω-3PL (SCO-M) ↓ω-3PL (SCO-U) ↓↓ω-3TG Serum TC: ↓↓ω-3PL (SCO-M) ↓ω-3PL (SCO-U) ↓ω-3TG	Sugimoto *et al*., 2020 [39]
	ω-3PL (Scallop oil) EPA: 21.4 DHA: 7.6 ω-3PL (Krill oil)	Mouse KK-A^{y}	5	Male	<u>Prevention</u> (6 weeks) Groups: CON CON + ω-3PL (SCO) CON + ω-3PL (KO) CON + ω-3TG	Liver TG: ω-3PL (SCO) ≈HFD ω-3PL (KO) ↑ (312% of HFD) ω-3TG ≈HFD	Serum TG: ↓↓ω-3PL (SCO) ↓↓ω-3PL (KO) ↓ω-3TG Serum TC: ↓ω-3PL (SCO) ↑ω-3PL (KO) Serum HDL: ↓↓ω-3PL (SCO) ↑ω-3PL (KO)	Sugimoto *et al*., 2021 [56]

	EPA: 11.8 DHA: 8.4 ω-3TG (Fish oil) EPA: 20.7 DHA: 8.8 (g/kg diet)						↓ω-3TG Liver weight: ↑ω-3PL (KO)	
	ω-3PL (Krill oil) EPA: N/A DHA: N/A ω-3TG (Fish oil) EPA: N/A DHA: N/A (oral gavage)	Mouse ICR	10	Male	<u>Prevention</u> (12 weeks) Groups: CON HFD HFD + ω-3PL HFD + ω-3TG	N/A	Body weight: ↓ω-3PL ↓ω-3TG Serum TG: ↓ω-3PL Serum TC: ↓↓ω-3PL ↓ω-3TG Serum LDL: ↓↓ω-3PL ↓ω-3TG Serum HDL: ↑↑ω-3PL ↑ω-3TG	Cui *et al*., 2017 [57]
	ω-3PL (Krill oil) EPA: 3.0 DHA: 1.7 ω-3TG (Fish oil) EPA: 2.0 DHA: 2.9 (g/kg diet)	Wistar rats		Male	<u>Prevention</u> (6 weeks) Groups: HFD HFD + ω-3PL HFD + ω-3TG	Liver TG: ω-3PL ↓ (22% of HFD) ω-3TG ↓ (10% of HFD) (ω-3PL > ω-3TG)	Plasma TG: ↓ω-3PL ↓ω-3TG Plasma TC: ↓ω-3PL ↓ω-3TG Liver TC: ↓↓ω-3PL ↓ω-3TG	Ferramosca *et al*., 2012 [47]
	ω-3PL (Squid meal) EPA: 6.9 DHA: 23.5 ω-3TG (Fish oil) EPA: 2.1 DHA: 7.7 (g/kg diet)	Wistar rats	4	Male	<u>Prevention</u> (6 weeks) Groups: CON + 7% SOY CON + 7% SOY + ω-3PH-PL CON + 7% SOY+ ω-3TG	Liver TG: ω-3PL ↓ (22% of CON) ω-3TG ↓ (35% of CON) (ω-3TG > ω-3PL)	Serum TG: ↓↓ω-3PL ↓ω-3TG Serum AST: ↓↓ω-3PL ↓ω-3TG Serum ALT: ↓↓ω-3PL ↓ω-3TG	Hosomi *et al*., 2019 [58]
	ω-3PL (Krill oil) EPA: 3.0 DHA: 1.8	Wistar rats	8-10	Male	<u>Prevention</u> (8 weeks) Groups: CON	Liver TG: ω-3PL ≈ HFD ω-3TG ≈ HFD	Weight gain: ↓↓ω-3PL ↓ω-3TG Serum TG: ↓↓ω-3PL ↓ω-3TG Serum TC: ↓ω-3PL ↓ω-3TG	Aydin Cil *et al*., 2021 [42]

	ω-3TG (Fish oil) EPA: 3.1 DHA: 2.9 (g/kg diet)				HFD HFD+ ω-3PL HFD+ ω-3TG			
	ω-3PL (Krill oil) EPA: 13.2 DHA: 4.6 ω-3TG-M (Menhaden oil) EPA: 5.5 DHA: 2.0 ω-3TG-S (Salmon oil) EPA: 10.0 DHA: 1.9 ω-3TG-T (Tuna oil) EPA: 2.6 DHA: 2.9 (g/kg diet)	Sprague-Dawley rats	4	Female	<u>Prevention</u> (8 weeks) Groups: HFD HFD + ω-3PL HFD + ω-3TG-M HFD + ω-3TG-S HFD + ω-3TG-T	N/A	Weight gain: ↑ω-3PL ↑ ↑ω-3TG-M ↑ω-3TG-S ↑↑ω-3TG-T Liver weight: ↑ω-3TG-S ↑ω-3TG-T	Tou *et al*., 2011 [59]
Salmon PL vs. Silver carp PL	ω-3PL-S (Salmon head extract) EPA: N/A DHA: N/A ω-3PL-SC (Silver carp head extract) EPA: N/A DHA: N/A (g/kg diet)	Mouse C57BL/6J	5	Male	<u>Prevention</u> (9 weeks) Groups: CON HFD HFD + ω-3PL-S HFD + ω-3PL-SC	Liver TG: ω-3PL-S ≈ HFD ω-3PL-SC ↓ (56% of HFD) Liver lipid droplets (HFD): ω-3PL-S (↓) ω-3PL-SC (↓) (ω-3PL-SC > ω-3PL-S)	Weight gain: ↓ω-3PL-S ↓↓ω-3PL-SC Serum TG: ↓ω-3PL-S ↓↓ω-3PL-SC Serum TC: ↓ω-3PL-S ↓↓ω-3PL-SC Liver TC: ↓ω-3PL-S ↓↓ω-3PL-SC	Wang *et al*., 2023 [41]
ω-3PL vs. ω-3EE vs. ω-3FFA vs. ω-3TG	ω-3PL (Squid roe oil) EPA: 1.95 DHA: 5.13 ω-3EE EPA: 1.76 DHA: 5.13	Mouse BALBc	N/A	Male	<u>Prevention</u> (1 week) Groups: LFD LFD + ω-3PL LFD + ω-3EE LFD + ω-3FFA LFD + ω-3TG	Liver TG: ω-3PL ↓ (53% of LFD) ω-3EE ↓ (64% of LFD) ω-3 FFA ↓(34% of LFD) ω-3TG ↓ (45% of LFD) ω-3PL ≈ HFD ω-3EE ≈ HFD	LFD: Serum TG: ↓ω-3PL ↓ω-3EE ↑ω-3FFA ↑ω-3TG Serum TC: ↓↓ω-3PL ↓↓ω-3EE ↓ω-3FFA ↓ω-3TG Liver TC: ↓ω-3PL ↓↓ω-3EE ↓ω-3FFA ↓↓ω-3TG HFD:	Tang *et al*., 2012 [45]

	ω-3FFA EPA: 1.86 DHA: 5.13 ω-3TG (Fish and algae oil mixture) EPA: 1.94 DHA: 5.13 (g/kg diet)				HFD HFD + ω-3PL HFD + ω-3EE HFD + ω-3FFA HFD + ω-3TG	ω-3FFA ≈ HFD ω-3TG ≈ HFD	Serum TG: ↑ω-3PL ↓↓ω-3EE ↑ω-3FFA Liver TC: ↓ω-3PL ↓ω-3EE	
ω-3WE vs. ω-3EE	ω-3WE (Calanus oil-derived) EPA: 3.5 DHA: 1.3 ω-3EE (Omacor) EPA+DHA: 4.7 (g/kg diet)	Mouse C57BL/6J	12	Male	Reversal HFD (7 weeks) then Treatments (20 weeks): HFD + ω-3WE HFD + ω-3EE	Liver TG: ω-3WE ↓ (55% of HFD) ω-3EE ≈ HFD (ω-3WE > ω-3EE)	Body weight: ↓ω-3WE AT adiponectin: ↑ω-3WE Plasma glucose: ↓ω-3WE ↓ω-3EE Plasma insulin: ↓↓ω-3WE ↓ω-3EE Plasma NEFA: ↓↓ω-3WE ↓ω-3EE	Hoper *et al*., 2014 [46]

Only studies (n = 24) published in the last 15 years were included in the table. The "MASLD-related phenotypes" section contains only information on parameters differentially affected by the lipid classes used for omega-3 supplementation in a given study. The direction of effect of a given omega-3 supplementation compared to the respective control: ↑, increase; ↓, decrease; ≈, no effect (the size of the effect is expressed by the number of arrows). [a]Age at the beginning of omega-3 interventions. Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; CON, control (usually low-fat) diet; DG, diacylglycerols; DHA, docosahexaenoic acid; EE, ethyl esters; EPA, eicosapentaenoic acid; FFA, free fatty acids; HDL, high-density lipoprotein; HFD, high-fat diet; HOMA-IR, homeostatic model assessment for insulin resistance; LDL, low-density lipoprotein; LDLr KO, Low-density lipoprotein receptor knock-out mice; LFD, low fat diet; N/A, not applicable or not assessed; NEFA, non-esterified fatty acids; PL, phospholipids; rTG, re-esterified triacylglycerols; TC, total cholesterol; TG, triacylglycerols; WE, wax esters.

Table 3. Comparative human studies on MASLD using omega-3 supplemented in different lipid classes.

Omega-3 concentrates	EPA/DHA dose	Target	Age of subjects (years)	Study design	MASLD-related phenotypes	Reference
ω-3PL vs. ω-3TG	ω-3PL (Algae oil) EPA: 2400 DHA: 6480 ω-3TG (Fish oil) EPA: 1400 DHA: 2000 (mg per day)	Hypertriglyceridemic statin-treated subjects	18 - 79	14 weeks Double-blind, randomized, parallel trial	Serum TG: ↓ ω-3PL (to placebo) ↓ ω-3TG (to placebo)	Maki *et al*., 2014 [60]
	EPA/DHA-PL (Herring roe + fish oil mixture) EPA: 628 DHA: 1810 EPA/DHA-TG (Fish oil) EPA: 1843 DHA: 178 (mg per day)	Mild hypertriglyceridemic subjects	43 - 48	2 weeks Randomized, single-blind, crossover trial (4 weeks washout)	Serum TG: ↓ EPA/DHA-PL (to baseline) ↓ EPA/DHA-TG (to baseline) Serum TC: ↓ EPA/DHA-PL (to baseline) ↓ EPA/DHA-TG (to baseline) Serum LDL: ↓ EPA/DHA-PL (to baseline) ↓ EPA/DHA-TG (to baseline)	Cook *et al*., 2016 [61]
Seal Oil vs. Fish Oil	EPA/DHA-Seal oil EPA: 340 DHA: 450 EPA/DHA-Fish oil EPA: 210 DHA: 810 (mg per day)	Hypertriglyceridemic subjects	42 - 73	6 weeks Double-blind randomized, parallel, placebo-controlled trial	Plasma TG: ↓ EPA/DHA-Fish oil (to placebo) Systolic BP: ↓ EPA/DHA- Fish oil (to placebo) Mean arterial BP: ↓ EPA/DHA- Fish oil (to placebo)	Meyer *et al*., 2009 [64]
ω-3rTG vs. ω-3EE	ω-3rTG (rTG) EPA: 1008 DHA: 672 ω-3EE (Ethyl	Dyslipidemic statin-treated subjects	30 - 75	6 months Double-blind, placebo-controlled trial rTG vs. EE	Serum TG: ↓ ω-3rTG (to placebo)	Schuchardt *et al*., 2011 & 2014 [69,63]

	esters) EPA: 1008 DHA: 672 (mg per day)					
	ω-3AG (rTG) EPA: 767 DHA: 1930 ω-3EE (Ethyl esters) EPA: 1702 DHA: 1382 (mg per day)	Hypertriglyceridemic subjects	≥ 18	8 ± 2 weeks Double-blind, randomized, placebo-controlled trial	Plasma TG/HDL: ↓ ω-3AG (to placebo) ↓ ω-3EE (to placebo) Plasma non-HDL/HDL: ↓ ω-3AG (to placebo)	Hedengran *et al.*, 2015 [62]

Only studies (n = 5) published in the last 15 years were included in the table. The primary outcome, liver fat content, was not assessed in these studies, while the section "MASLD-related phenotypes" contains only information on parameters differentially affected by the lipid classes used for omega-3 supplementation in a given study. Direction of effect of a given omega-3 supplementation compared to the respective control: ↑, increase; ↓, decrease; ≈, no effect. Abbreviations: AG: acylglycerols; BP, blood pressure; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PL, phospholipids; rTG, re-esterified triacylglycerols; TC, total cholesterol; TG, triacylglycerols.

Bioavailability and the mechanism of action of omega-3 in MASLD

The bioavailability of omega-3 from the diet, i.e., the rate of absorption and transport of EPA, DHA and other omega-3 into the circulation and/or site of action, is an important factor in determining the biological effects of these FA. The chemical binding form may influence the bioavailability of omega-3. However, other factors, such as the matrix effect, the galenic form, i.e., the method of preparing and compounding medicinal products, as well as inter-individual differences or age, also contribute to the final effect (discussed in detail in [34,65]). Interestingly, the liver already contains significant amounts of DHA in tissue PL under conditions when no extra EPA and DHA is supplemented *via* the diet; however, administration of varying amounts of omega-3 in the form of rTG (as part of HFD) resulted in saturable incorporation of DHA and, to a lesser extent, EPA into the hepatic PL fraction in C57BL/6J mice [65]. Regarding the assessment of omega-3 bioavailability, it should be noted that the gold standard for assessing omega-3 status in the body is the omega-3 index, i.e., the EPA+DHA content of erythrocytes expressed as a percentage of all FA analyzed [55,66]; however, studies investigating this topic have also used alternative and less accurate methods of assessment, which makes interpretation of published data significantly more difficult.

Results of preclinical mouse experiments on omega-3 supplementation under conditions of obesity/MASLD induced by HFD administration suggest improved bioavailability of EPA or DHA+EPA in plasma and liver (mainly at the level of hepatic PL fraction) when omega-3 are administered in the form of PLs from marine fish or krill oil compared to rTG [36-38,55]. No information is available on studies in rodents with dietary obesity examining the bioavailability of omega-3 from WE compared to other chemical forms. However, omega-3 from Calanus oil rich in WEs have been shown to be bioavailable in AT and liver of HFD-fed mice, despite some loss of free FA and fatty alcohols through feces [67]. Furthermore, despite the limitations resulting from the bioavailability analysis at the level of total plasma lipids, the study by Paluchova *et al.* [35] indicated improved plasma DHA bioavailability from Calanus oil compared to rTG when these oils were orally administered to C57BL/6N mice for 8 days and provided the same daily dose of 12 μg DHA.

In humans, the question of improved bio-availability when omega-3 are administered in a particular chemical form remains controversial. Earlier studies on healthy volunteers suggested better plasma bio-availability of omega-3 from PL (krill oil) compared to TG (fish oil) or EE form [68,69]. A 4-week study in obese subjects with insulin resistance suggested a greater increase in plasma EPA levels after omega-3 supplementation using krill oil compared to fish oil, where the EPA dose was very similar in both cases, i.e.,~0.21 g/day [70]. In addition, in a 6-month double-blind placebo-controlled trial in statin-treated hyperlipidemic subjects, the bioavailability of EPA+DHA, assessed as the omega-3 index, was better when omega-3 (~1.7 g dose) were administered *via* rTG compared to the EE concentrate [66]. This led to a significant reduction in fasting serum TG levels in the rTG but not in the EE group [17]. In contrast, a recent 12-week study on healthy volunteers found comparable increases in the omega-3 index (see above) after daily supplementation with ~250 mg of EPA+DHA either *via* fish oil (i.e., in the TG form), krill oil (PL) or Calanus oil (WE; [71]). This is consistent with the results of a previous randomized, two-period crossover study [72] that demonstrated similar increases in plasma EPA+DHA levels within 72 hours after a single administration of omega-3 *via* either Calanus oil (416 mg EPA+DHA) or omega-3 EE concentrate (Omacor/Lovaza; 840 mg EPA+DHA). Regardless of the controversy regarding the improved bioavailability of omega-3 in the circulation when these FAs are administered in a particular chemical form, the question of their tissue availability remains open, as there are no human studies investigating this phenomenon in the liver.

A number of mechanisms are involved in the accumulation of fat in the liver. Briefly, hepatocytes take up FAs from the diet and non-esterified FAs (NEFA) released from AT by lipolysis, while they can also synthesize FAs *via de novo* lipogenesis (DNL). It was found that 59 % of TG stored in the liver of obese MASLD patients originated from plasma NEFA, while DNL and diet contributed 26 and 15 %, respectively [73]. Thus, a therapeutic strategy based on affecting deregulated lipolysis in the hypertrophic AT of obese patients could favorably influence MASLD development, as demonstrated in HFD-fed mice with pharmacological inhibition of adipose triglyceride lipase [74]. In any case, hepatic steatosis may arise when the quantity of FAs taken up from plasma and/or DNL in the liver exceeds the ability of the tissue to oxidize FAs either *via* mitochondrial or

peroxisomal FA oxidation or to export TG *via* lipoprotein particles. Liver mitochondria exhibit increased rates of FA-driven respiration during the development of diet-induced MASLD, suggesting an adaptive response to overcome the FA load in the liver [75,76]. However, this appears to be related to the presence of an ER stress response and dysfunctional unfolded protein response [76] with subsequent activation of the transcription factor sterol regulatory element-binding protein 1c, leading to increased DNL [77]. Hepatic DNL may also be potentiated due to activation of CB1 receptors by endocannabinoids [78]. In addition, decreased autophagic flux associated with metabolic changes in hepatocytes may further contribute to the development of liver steatosis in MASLD [79,80]. In this regard, it appears that peroxisomes, not mitochondria, may be the major contributor to the production of reactive oxygen species that cause oxidative damage during MASLD development [75,81]. Given that peroxisomes seem to be crucial for inducing the oxidative insult necessary for the onset and/or progression of MASLD, a defective autophagy would not only impair the proper removal of oxidized molecules (lipids, proteins or DNA) but also the removal of damaged organelles involved in ROS production. Indeed, failed degradation of peroxisomes has been associated with defects in peroxisome dynamics and results in increased oxidative stress [82]. Moreover, a mechanism based on peroxisomal FA degradation with subsequent H_2O_2 production and peroxin PEX2 stabilization was identified that negatively modulates intracellular lipolysis *via* posttranslational modification of adipose triglyceride lipase, thereby contributing to the progression of steatosis [83]. Besides dysregulation of various lipid metabolism pathways, there are a number of other mechanisms that contribute to the development and progression of MASLD, such as changes in the gut microbiota and increased intestinal permeability, which are associated with increased energy extraction from food, metabolic endotoxemia (i.e., increased plasma lipopolysaccharide levels), and overproduction of ammonia [84-86]. However, a detailed description of these mechanisms is beyond the scope of this review article.

Dietary supplementation with omega-3 can positively affect intrahepatic TG accumulation in mice and humans with MASLD, as observed in a number of primary studies as well as meta-analyses (see e.g. [29,30,87] and the section “Comparative studies” in this review article). However, the magnitude of the reduction in liver fat induced by an omega-3 intervention may depend on a number of factors, including baseline % liver fat, change in omega-3 index, or weight loss in response to the omega-3 intervention [88,89]. In addition, predominantly preclinical studies suggest that the antisteatotic effects of omega-3 in the liver may also depend on the class of lipids used for their supplementation ([31] and this review). In terms of mechanisms, chronic omega-3 administration is associated with a whole-body metabolic adaptation that primarily involves a switch from glucose oxidation to FA oxidation, leading to inhibition of glucose utilization, especially in the postprandial state [18,20]. The involvement of FA oxidation in the antisteatotic effects of omega-3 supple-mentation was further demonstrated in carnitine-deficient mice with impaired mitochondrial β-oxidation of FA, in which EPA supplementation further exacerbated severe TG accumulation in the liver [90]. In this regard, a recent study in fat-1 transgenic mice with increased endogenous levels of omega-3 shows that specific DHA-derived lipid autacoids, such as resolvin D1 and maresin 1, can unblock TCA cycle flux and metabolic utilization of long-chain acyl-carnitines in hepatocytes [91], similar to the effect of combination therapy with omega-3 and 10 % caloric restriction on mitochondria of abdominal AT in HFD-fed mice [92]. On the other hand, omega-3 have been consistently shown to affect primarily peroxisomes, as evidenced by elevated hepatic markers of peroxisomal but not mitochondrial β-oxidation in mice fed omega-3-supplemented HFD [19,36,92,93]. Omega-3 can also inhibit the hepatic DNL pathway [20,94,95], although the degree of inhibition may vary depending on the lipid class used for their administration; indeed, omega-3 PL in the form of krill oil appear to be much more potent compared to omega-3 TG [38,95]. The detailed mechanisms of action of omega-3 supplementation, particularly in the form of PLs, on hepatic FA oxidation and DNL have recently been reviewed elsewhere [31]. Interestingly, in fat-1 transgenic mice fed HFD, pharmacological inhibition of soluble epoxide hydrolase stabilized hepatic levels of cytochrome P-450-derived omega-3 epoxides, which was associated with reduced ER stress and up-regulation of hepatic autophagy, along with more intense antisteatotic effects [96]. These data further suggest the involvement of ER stress and autophagy regulation in the effects of omega-3 interventions on liver fat accumulation in MASLD and potentially also on the transition from simple steatosis to MASH.

Regarding the involvement of extrahepatic tissues in the beneficial effects of omega-3 supplementation on hepatic steatosis, it is primarily changes in AT and the gut by which omega-3s may indirectly influence liver TG

content. Indeed, in the epididymal AT of mice fed a semisynthetic HFD based on α-linolenic acid and supplemented with EPA and DHA, increased expression of genes involved in mitochondrial biogenesis was observed along with an increase in β-oxidation of FA [92]; *in situ* catabolism of FA in abdominal fat could thus lead to their lower release and subsequent deposition in the liver. Furthermore, adiponectin, an adipokine with antilipotoxic and anti-inflammatory properties, may also contribute to the beneficial effects of omega-3 on liver fat accumulation and hepatic insulin sensitivity [97-99]; however, in patients with type 2 diabetes, the ability of omega-3 (in the form of rTG) to induce plasma adiponectin was relatively limited compared to the insulin sensitizer pioglitazone [18]. Nevertheless, omega-3 PLs had a greater potency to elevate circulating adiponectin levels when compared to similar doses of EPA/DHA supplemented *via* omega-3 TG [31,37,38]. Similarly, adiponectin expression was stimulated in the perirenal and epididymal AT of HFD-fed mice receiving omega-3 *via* Calanus oil (i.e., omega-3 in WE) but not in mice receiving an equivalent dose of EPA/DHA *via* EE (i.e., Omacor; [46]). In addition, AT is also a source of bioactive lipids that can be modulated in response to omega-3 administration and thus affect the immunometabolic properties of other tissues including the liver (reviewed in [100]). Accordingly, administration of an EPA/DHA concentrate based on rTG resulted in increased levels of 13-DHAHLA, an anti-inflammatory lipid from the family of fatty acid esters of hydroxy fatty acids, in both AT and circulation of HFD-fed mice [35,101]. We and others have also shown that AT levels of endocannabinoids such as anandamide and 2-arachidonoylglycerol were reduced in response to omega-3 PL supplementation in obese rodents [36,102,103]. Importantly, this effect of omega-3 PL was also seen in the circulation and was stronger compared to TG-based omega-3 [36,37]. Given the role of CB1 receptors in the potentiation of hepatic DNL (see above) and the impairment of mitochondrial function [104], the reduction of endocannabinoid levels in AT and plasma may contribute to the antisteatotic effects of omega-3 supplementation (especially in the form of PL) in MASLD.

Interestingly, the more potent effect of omega-3 PL (vs. TG-based omega-3 supplementation) in terms of reducing hepatic steatosis may also involve the induction of mitochondrial ß-oxidation in the small intestine, as evidenced by gene expression within this metabolic pathway, as well as palmitate oxidation, which were specifically increased in the proximal ileum of omega-3 PL-supplemented mice [55]. It is worth noting that the antisteatotic effects of alternative supplementation forms of omega-3, such as PL and WE, may be due in part to certain bioactive substances contained in complex preparations such as krill oil and Calanus oil, respectively. Examples are palmitoleic acid and plant alkaloids in the case of krill oil ([37,38] and reviewed in [31]) and omega-3 stearidonic acid in the case of Calanus oil, which is converted to EPA in humans [105].

Conclusions

Based on a majority of the comparative studies retrieved, mostly conducted in preclinical mouse models, it can be concluded that the class of lipids used for supplementation contributes largely to the efficacy with which dietary omega-3 can prevent or alleviate hepatic steatosis in MASLD. Regarding the efficacy of individual lipid classes used for omega-3 supplementation, in particular the PL class (e.g., in the form of fish meal extract, krill oil, algae oil) is associated with stronger antisteatotic effects in the liver compared to TG-based omega-3 supplementation (e.g., fish oil, rTG). In addition to improving the bioavailability of mainly EPA in the hepatic PL fraction, omega-3 PLs appear to more effectively reduce hepatic DNL and modulate the production of bioactive lipids in both AT and liver, which may contribute to enhancing mitochondrial function, stimulating autophagy and reducing ER stress. Considering the superior antisteatotic effects of marine omega-3 PL, the involvement of extrahepatic tissues such as AT and the gut with its microbiome cannot be excluded. On the other hand, conducting comparative studies based on the administration of similar amounts of omega-3 using different supplementation forms is often technically very challenging. This is due to differences in the omega-3 content of various formulations, but potentially also to differences in the length of dietary intervention required for the onset of action of a given formulation in the context of the chosen experimental model. This fact makes it difficult to draw strong conclusions when comparing the antisteatotic efficacy of the various omega-3 formulations.

Unfortunately, there are no studies in humans with MASLD where the effect of two or more lipid classes on fat accumulation in the liver has been compared as a primary outcome. Thus, randomized controlled trials of sufficient size and duration are needed

to test the efficacy of alternative lipid classes such as PL or WE used for omega-3 supplementation. Since omega-3 should ideally be part of our normal diet, identifying the optimal class of lipids for supplementation may also be important for their possible co-administration with different drugs to further enhance treatment efficacy.

Abbreviations

AG, acylglycerols; AT, adipose tissue; CON, control diet; DG, diacylglycerols; DHA, docosahexaenoic acid; DNL, *de novo* lipogenesis; EE, ethyl esters; EPA, eicosapentaenoic acid; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MASH, metabolic dysfunction-associated steatohepatitis; MASLD, metabolic dysfunction-associated steatotic liver disease; NAFLD, non-alcoholic fatty liver disease; NEFA, non-esterified fatty acids; PC, phosphatidylcholine; PL, phospholipids; PUFA, polyunsaturated FA; rTG, reesterified triacylglycerols; TC, total cholesterol; TG, triacylglycerols; WE, wax esters.

Conflict of Interest

There is no conflict of interest.

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