Omega-3 phospholipids and obesity-associated NAFLD: Potential mechanisms and therapeutic perspectives

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Abstract
Prevalence of non-alcoholic fatty liver disease (NAFLD) increases in line with obesity and type 2 diabetes, and there is no approved drug therapy. Polyunsaturated fatty acids of n-3 series (omega-3) are known for their hypolipidaemic and anti-inflammatory effects. Existing clinical trials suggest varying effectiveness of triacylglycerol- or ethyl ester-bound omega-3 in the treatment of NAFLD, without affecting advanced stages such as non-alcoholic steatohepatitis. Preclinical studies suggest that the lipid class used to supplement omega-3 may determine the extent and nature of their effects on metabolism. Phospholipids of marine origin represent an alternative source of omega-3. The aim of this review is to summarise the available evidence on the use of omega-3 phospholipids, primarily in obesity-related NAFLD, and to outline perspectives of their use in the prevention/treatment of NAFLD. A PubMed literature search was conducted in May 2021. In total, 1088 articles were identified, but based on selection criteria, 38 original papers were included in the review. Selected articles describing the potential mechanisms of action of omega-3 phospholipids have also been included. Preclinical evidence clearly indicates that omega-3 phospholipids have strong antisteatotic effects in the liver, which are stronger compared to omega-3 administered as triacylglycerols. Multiple mechanisms are likely involved in the overall antisteatotic effects, involving not only the liver but also adipose tissue and the gut. Robust preclinical evidence for strong antisteatotic effects of omega-3 phospholipids in the liver should be confirmed in clinical trials. Further research is needed on the possible effects of omega-3 phospholipids on advanced NAFLD.

KEYWORDS
krill oil, marine phospholipids, n-3 polyunsaturated fatty acids, non-alcoholic fatty liver disease, obesity

1 | INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) affects 25% of the world's population and it is predicted to become the main indication for liver transplantation by 2030. NAFLD represents a spectrum of conditions ranging from increased intrahepatic accumulation of triacylglycerols (TAGs), that is fatty liver aka hepatic steatosis, to non-alcoholic...
steatohepatitis (NASH), a state of hepatocellular inflammation and ballooning with possible collagen deposition, which can progress further to fibrosis, cirrhosis and hepatocellular carcinoma. It is estimated that 10%-30% of patients with steatosis develop NASH and 20%-30% of these subjects then develop cirrhosis. Although hepatic steatosis can also occur in lean people, the development of NAFLD is strongly associated with obesity, insulin resistance and their metabolic sequelae (i.e. metabolic syndrome), as well as type 2 diabetes. Hepatic steatosis and NASH in extremely obese subjects may be present in up to 85% and 40% of cases, respectively, and the presence of metabolic syndrome and/or type 2 diabetes is associated with a potentially progressive, severe liver disease. Due to its high prevalence, NAFLD has become a major health problem that currently has no approved pharmacotherapy, and lifestyle modifications and weight reduction are the main tools for treating the disease.

Polysaturated fatty acids (FAs) of n-3 series (omega-3) are characterised by the presence of a double bond between the third and fourth carbon atom, with the methyl terminal carbon counting as carbon number one. Long-chain omega-3 such as eicosapentaenoic acid (EPA; 20:5n-3), docosapentaenoic acid (DPA; 22:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) are found in marine fish oils and are known for their anti-inflammatory and hypolipidaemic effects. In this context, EPA and DHA supplementation may also be effective in the treatment of human NAFLD, especially when it comes to hepatic steatosis (recently reviewed). However, both primary studies and meta-analyses confirm low efficacy of omega-3 towards more advanced stages of NAFLD, such as NASH and/or fibrosis. The effectiveness of omega-3 supplementation in NAFLD may depend on omega-3 dose, EPA/DHA ratio, duration of treatment and patient-specific factors, that is age, baseline liver fat or the presence of comorbidities. The lipid class used to supplement omega-3 could also play an important role. Thus, in addition to TAG- or ethyl ester-bound omega-3, phospholipid (PL)-bound EPA and DHA from marine sources have recently become the subject of many, mostly preclinical, studies. This review aims to summarise preclinical and clinical evidence regarding the use and efficacy of omega-3 PLs primarily in obesity-related NAFLD and to discuss potential mechanisms of action and therapeutic perspectives.

2 | PHOSPHOLIPID CLASSES AND SOURCES OF OMEGA-3 PHOSPHOLIPIDS

Omega-3 are naturally esterified mainly in TAGs and PLs, or present in the free form. In contrast to TAGs, PLs have only two FAs esterified to a glycerol backbone and a hydrophilic ‘head’ containing a phosphate group, which can be further modified with molecules such as choline, ethanolamine, serine, glycerol or inositol. Glycerophospholipids, representing the most common class of PLs, can be divided into subgroups based on the type of modification of the head group in the PL molecule. For example, phosphatidylcholine (PC) is the most common type of PLs in various marine sources.

3 | THE EFFECTS OF OMEGA-3 PHOSPHOLIPIDS ON NAFLD AND RELATED METABOLIC PHENOTYPES: OVERVIEW OF STUDIES

PubMed was searched for existing animal and human studies that examined the effects of various forms of omega-3 PLs primarily on obesity-related NAFLD and/or metabolic parameters. See Figure 1 for a flow chart showing keywords and selection criteria. A total of 590 animal studies and 498 human studies were found. Due to the lack of studies investigating the effects of omega-3 PLs on advanced NAFLD, the main emphasis was on hepatic steatosis (animal studies) or overweight/obesity-related metabolic disorders (human studies). Thus, based on the selection criteria, only 26 and 12 of the original papers were included in Table 1 (animal studies) and Table 2 (human studies), respectively. Selected papers are commented below in this section.

3.1 | Animal studies

Most current models based on genetic or dietary manipulation fail to mimic the complex human features of NASH. Because
hepatic steatosis, the first stage of NAFLD, is associated with overnutrition and obesity, rodent models with a high susceptibility to an obesogenic diet are most commonly used in preclinical studies. A review of animal studies examining the effects of krill-based products on various phenotypes including obesity, inflammation or cardiovascular function was published in 2015, but provided only minimal information on NAFLD. In the current work, we focus primarily on NAFLD and related phenotypes in animals (Table 1), categorising studies according to whether they used EPA- or DHA-rich PLs or krill oil/powder as a source of omega-3 PLs. Since studies using krill products predominate, they are discussed separately.

3.1.1 | The effects on simple steatosis

A reduction in total lipid content in the liver was reported in 21 animal studies involving the use of omega-3 PLs regardless of the source and EPA and DHA content of PLs, the experimental model and the type of diet. Dietary supplementation with EPA-enriched PLs, as opposed to DHA-enriched PLs, also resulted in reduced plasma TAG levels in various mouse models. Dietary supplementation with DHA-rich PLs for one week reduced liver TAG content in Balb/c mice fed a low-fat diet, but not when fed a high-fat diet (HFD). When EPA- and DHA-rich PLs were directly compared in rats with NAFLD induced by 1% orotic acid, both reduced liver lipids, with EPA-PL showing a greater effect. Herring-derived omega-3 PLs administered to HFD-fed C57BL/6 mice reduced liver TAG content whilst lowering plasma TAG and cholesterol levels and inducing adiponectin. However, most studies investigating the effects of omega-3 PLs have used krill oil or powder. Krill powder decreased TAGs in both liver and plasma of tumour necrosis factor α (TNFα)-humanised mice fed HFD, which are otherwise characterised by adverse changes in lipid metabolism. Krill oil supplementation reduced hepatic TAG content whilst decreasing de novo lipogenesis (DNL) in HFD-fed mice and rats. Studies in HFD-fed C57BL/6 mice showed that krill oil reduced hepatic lipids in a dose-dependent manner. This lipid-lowering effect was not seen in mice fed a low-fat diet and in one study using HFD, despite reduced hepatic expression of genes involved in DNL.

3.1.2 | The effects on advanced stages of NAFLD: NASH and fibrosis

Krill powder reduced hepatic levels of proinflammatory cytokines (Interleukin-2, TNFα) in TNFα-humanised mice fed HFD. In mice with thermoneutral housing fed a HFD for 24 weeks, krill oil supplementation reduced exacerbated hepatic steatosis whilst decreasing plasma alanine transaminase (ALT) and aspartate transaminase (AST) levels. In experiments in rats with orotic acid-induced NAFLD, supplementation with EPA- or DHA-rich PLs reduced plasma AST and
**TABLE 1** Effects of omega-3 PLs on NAFLD-related parameters in preclinical studies

| Name of concentrate | EPA/DHA dose | Target | Type of study | Results (NAFLD) | Other results | Reference |
|---------------------|--------------|--------|---------------|-----------------|--------------|-----------|
| **Animal studies**  |              |        |               |                 |              |           |
| EPA-containing PLs  |              |        |               |                 |              |           |
| EPA-PL              | EPA: 2.03    | Male C57BL/6J mice | 4 weeks       | ↓ Liver TAG (44.1%) | ↓ Body weight | Liu et al²⁶ |
|                     | DHA: 0.08    | HHHF vs. HHF + EPA-PL |             | ↓ Liver TC (10.3%) | ↓ Glucose AUC (OGTT) |   |
|                     | % of FA composition | | | | ↑ Plasma adiponectin | |
|                     |              | | | | ↓ Plasma TAG, TC and glucose | |
|                     |              | | | | ↓ Plasma IL-6 and TNF-α | |
|                     |              | | | | ↓ Hepatic FAS and G6PDH enzyme activity | |
|                     |              | | | | ↑ CPT enzyme activity | |
|                     |              | | | | ↓ Peroxisomal FA oxidation | |
|                     |              | | | | Liver gene expression: | |
|                     |              | | | | ↓ FA synthesis | |
|                     |              | | | | ↓ FA oxidation | |
|                     |              | | | | ≈ Body weight | |
|                     |              | | | | Liver gene expression: | |
|                     |              | | | | ↓ Cholesterol synthesis (EPA-PC, high dose) | |
| EPA-PC and EPA-PS  | EPA-PC: 57.4 | SAMP8 male mice | 8 weeks       | ↓ Liver TAG (29% in EPA-PC and 26.2% in EPA-PS) | ↓ Plasma TAG (both EPA-PC and EPA-PS, 52.1% and 49.6% respectively) | Ding et al²⁷ |
|                     | EPA-PS: 58.4 | SD vs. SD + 2% EPA-PC or 2% EPA-PS | | ↓ Liver TC | ≈ Plasma TC (EPA-PC) | |
|                     | % of FA composition | | | | ↑ Plasma TC (EPA-PS) | |
|                     |              | | | | Liver gene expression: | |
|                     |              | | | | ↓ FA synthesis | |
|                     |              | | | | ↓ FA oxidation | |
|                     |              | | | | ≈ Body weight | |
|                     |              | | | | Liver gene expression: | |
|                     |              | | | | ↓ Cholesterol synthesis (EPA-PC, high dose) | |
| EPA-PC              | EPA-PC: 40 or 80 mg/kg of body weight | Male Wistar rats | 3 weeks | ↓ Liver TAG, TC | ≈ Body weight | Liu et al²⁶ |
|                     |               | SD + 1% OA vs. SD + 1% OA + EPA-PC at low or high dose | | | Liver gene expression: | |
|                     |              | | | | ↓ FA oxidation | |
|                     |              | | | | ≈ Body weight | |
|                     |              | | | | Liver gene expression: | |
|                     |              | | | | ↓ Cholesterol synthesis (EPA-PC, high dose) | |
| EPA-PL              | EPA: 1.98    | Male C57BL/6J ApoE−/− mice | 8 weeks | ↓ Liver TAG, TC | ≈ Body weight | Zhang et al²⁹ |
|                     | % of FA composition | HHHS vs. HHHS + EPA-PL | | | Liver gene expression: | |
|                     |              | | | | ↑ FA oxidation, bile acid synthesis, cholesterol efflux | |
|                     |              | | | | ↓ FA and cholesterol synthesis | |
|                     |              | | | | ↓ Body weight | |
|                     |              | | | | Liver gene expression: | |
|                     |              | | | | ↓ Plasma TAG, TC | |
|                     |              | | | | ↓ Adipocyte size | |
|                     |              | | | | ↓ FSP27 protein in liver and WAT | |
|                     |              | | | | ≈ PLIN1 protein in liver and WAT | |
| EPA-PL              | EPA: 9.49    | Male C57BL/6J mice | 8 weeks | ↓ Liver TAG | ≈ Body weight | Zhang et al³⁰ |
|                     | DHA: 1.32    | HS vs. HS + EPA-PL | | | Liver gene expression: | |
|                     | % of FA composition | | | | ↑ FA oxidation, bile acid synthesis, cholesterol efflux | |
|                     |              | | | | ↓ FA and cholesterol synthesis | |
|                     |              | | | | ↓ Body weight | |
|                     |              | | | | Liver gene expression: | |
|                     |              | | | | ↓ Plasma TAG, TC | |
|                     |              | | | | ↓ Adipocyte size | |
|                     |              | | | | ↓ FSP27 protein in liver and WAT | |
|                     |              | | | | ≈ PLIN1 protein in liver and WAT | |

(Continues)
| Name of concentrate | EPA/DHA dose | Target | Type of study | Results (NAFLD) | Other results | Reference |
|---------------------|-------------|--------|---------------|-----------------|--------------|-----------|
| **DHA-containing PLs** | | | | | | |
| DHA-PL | EPA: 1.95 DHA: 5.13 g/kg diet | Male Balb/c mice | 1 week SD vs. SD + EPA-PL or HF vs. HF + EPA-PL | ↓ Liver TAG, vs. SD ≈ Liver TAG, vs. HF ↓ Liver TC, vs. HF | ↓ Body weight, vs. SD ≈ Body weight, vs. HF ≈ Plasma TAG ↓ Plasma TC, vs. SD ≈ Plasma TC, vs. HF | Tang et al31 |
| DHA-LPC rich oil | EPA: 2.9 DHA: 17.0 mol% | Male Wistar rats | 28 days 7% soybean oil diet vs. 4.5% soybean oil + 2.5% DHA-LPC rich oil diet | ↓ Liver TAG, TC | ≈ Body weight | Hosomi et al32 |
| **EPA vs. DHA-containing PLs** | | | | | | |
| EPA/ DHA-PL forage and EPA/ DHA-PL liposomes | EPA: 1 DHA: 1% of the diet | Male Wistar rats | 10 days SD + 1% OA vs. SD + OA + EPA-PL or DHA-PL or lipo-EPA or lipo-DHA | ↓ Liver TAG, TC ↓ AST and ALT | ≈ Body weight ≈ Plasma TAG, TC and glucose | Chang et al33 |
| **EPA/DHA-containing PLs** | | | | | | |
| Prevention EPA + DHA: 10 or 30 g/kg diet | Male C57BL/6J mice | 9 weeks HF vs. HF + EPA/DHA-PL | ↓ Liver TAG ↓ Liver TAG | ↓ Plasma TAG ↓ Glucose AUC (IGTT) | Rossmeisl et al23 |
| Reversal EPA + DHA: 30 g/kg diet | | 4 months HF + 9 weeks HF vs. HF + EPA/DHA-PL (+2 g of metformin in both diets) | | | | |
| | | | | | | |
| **Herring-derived omega-3 PL** | | | | | | |
| EPA/DHA-containing PLs | EPA: 4.3 DHA: 10 g/kg diet | Male C57BL/6N mice | 7 weeks HF vs. HF + herring-derived omega-3 PL | ↓ Liver TAG, TC | ↓ Plasma TG, TC ↓ Plasma glucose, insulin ↓ HOMA-IR index ↓ Glucose AUC (IGTT) ↓ Plasma adiponectin | Rossmeisl et al25 |
| | | | | | | |
| **Herring-derived omega-3 PL** | | | | | | |
| | | | | | | |

(Continues)
| Name of concentrate | EPA/DHA dose | Target | Type of study | Results (NAFLD) | Other results | Reference |
|---------------------|--------------|--------|---------------|-----------------|---------------|-----------|
| Partial hydrolysate of phospholipids (PH-PL) | EPA: 6.9 | Male Wistar rats | 28 days | ↓ Liver TAG | ≈ Body weight | Hosomi et al\textsuperscript{32} |
| | DHA: 23.5 mmol/kg diet | SD + 7% soybean oil diet vs. SD + EPA/DHA- LIPC | ≈ Liver TC | ↓ Plasma TAG, TC | | |
| Omega-3 PUFA binding phosphatidylglycerol (Omega-3 PUFA-PG) | EPA: 2.14 | Diabetic/obese KK-A\textsuperscript{Y} male mice | 30 days | ↓ Liver total lipid | ≈ Body weight | Chen et al\textsuperscript{35} |
| | DHA: 2.63 g/kg diet | SoyPC diet (5% soybean oil + 2% SoyPC) vs. Omega-3 PUFA-PG (5% soybean oil + 2% omega-3 PUFA-PG) | ≈ AST and ALT | ↓ Plasma TC | | |
| Krill | Krill powder | Transgenic C57BL/6 hTNF\textalpha male mice | 6 weeks | ↓ Hepatic cytokines (IL-2, TNF\textalpha) | ≈ Body weight | Bjørndal et al\textsuperscript{36} |
| | EPA: 5.4 | HF vs. HF + krill powder | ↓ Liver TAG-44% | ↓ Plasma TAG (21%) | | |
| | DHA: 2.4 | | | ↓ Plasma NEFA (45%) | | |
| | % of FA composition | | | ↓ Plasma TC (22%) | | |
| | Krill oil | Male C57BL/6 mice | 8 weeks | ↓ Liver TAG | ≈ Body weight | Piscitelli et al\textsuperscript{37} |
| | EPA: 1.1, 1.6 or 2.6 | HF vs. HF + 1.25 or 2.5 or 5% wt of KO | | ↓ Body weight | | |
| | DHA: 0.5, 0.8 or 1.3 g/kg diet | | | ↓ Plasma TC, glucose | | |
| | | | | ↑ Plasma adiponectin (HF+5% KO) | | |
| | | | | ↑ Hepatic endocannabinoid levels (AEA, 2-AG, PEA) | | |
| | | | | Liver gene expression: ↓ FA synthesis, cholesterol and glucose metabolism | | |
| | | | | ↓ FA synthesis | | |
| | | | | ↓ Plasma TAG | | |
| | | | | ↓ Plasma TC, glucose | | |
| | | | | ↑ Plasma adiponectin (HF+5% KO) | | |
| | | | | ↑ Hepatic endocannabinoid levels (AEA, 2-AG, PEA) | | |
| | | | | Liver gene expression: ↓ FA synthesis | | |
| | | | | ↓ Plasma TAG, NEFA, TC | | |
| | | | | ≈ Plasma insulin, glucose | | |
| | | | | Liver gene expression: ↓ FA and cholesterol synthesis, glucose production, FA oxidation | | |
| Krill oil | EPA: 1.9 | Male CBA/J mice | 3 months | N/A | ≈ Plasma TAG, NEFA, TC | Burri et al\textsuperscript{44} |
| | DHA: 1.1 g/kg diet | SD vs. SD + KO | | ≈ Plasma insulin, glucose | | |
| Krill oil | EPA: 0.3 | Male Sprague-Dawley rats | 12 weeks | ↓ Liver TAG, TC | ↓ Body weight | Ferramosca et al\textsuperscript{38} |
| | DHA: 0.2 % of FA composition | HF vs. HF + KO | | ↓ Plasma TAG | |
| Name of concentrate | EPA/DHA dose | Target | Type of study | Results (NAFLD) | Other results | Reference |
|---------------------|--------------|--------|---------------|----------------|---------------|-----------|
| Krill oil           | EPA: 3       | Male Wistar rats | 6 weeks | ↓ Liver TAG (20%) | ≈ Body weight | Ferramosca, et al<sup>39</sup> |
|                     | DHA: 1.7     | SD vs. SD + KO               |          | ↓ Liver TC            | ↓ CIC activity |          |
|                     | g/kg diet    |                     |          | ≈ Body weight         | ↓ Cytosolic FAS and ACC activity |          |
|                     |             |                     |          | ≈ Body weight         | ↓ Plasma TAG   |          |
|                     |             |                     |          | ≈ Body weight         | ↓ Plasma cholesterol |          |
| Krill oil           | -            | Male C57BL/6 mice | 18 weeks | ≈ Liver TAG (compared to SD) | ↓ Body weight (vs. HF) | Sadzuka et al<sup>45</sup> |
|                     |             | SD vs. SD + KO or HF or HF + KO |          | ≈ Liver TAG (21% but not significant, compared to HF) | ↓ Plasma leptin (vs. HF) |          |
|                     |             |                     |          | ≈ Plasma TAG (both vs. SD and HF) | ≈ Plasma cholesterol (both vs. SD and HF) |          |
| Krill oil           | EPA: 5.39    | Male C57BL/6 hTNFα mice | 6 weeks | N/A | ↓ Plasma TAG (19%) | Vigerust et al<sup>32</sup> |
|                     | DHA: 2.36 wt% | HF vs. HF + KO |          | | ↓ Plasma TC |          |
|                     |             |                     |          | | ↑ Hepatic ACOX1 activity (peroxisomal β-oxidation) |          |
|                     |             |                     |          | | ↑ Mitochondrial FA oxidation |          |
|                     |             |                     |          | | ↑ Acylcarnitine classes in plasma |          |
|                     |             |                     |          | | Liver gene expression: |          |
|                     |             |                     |          | | ↓ FA and cholesterol synthesis |          |
|                     |             |                     |          | | ≈ Acox1 |          |
| Krill oil           | EPA: 5.23    | Male C57BL/6J mice | 6 weeks | ≈ Liver TAG | ≈ Body weight | Tillander et al<sup>86</sup> |
|                     | DHA: 2.28 % of FA composition | HF vs. HF + KO |          | | ≈ Plasma TAG and TC |          |
|                     |             |                     |          | | Liver gene expression: |          |
|                     |             |                     |          | | ↓ FA and cholesterol synthesis |          |
| Krill oil           | EPA: 135.6   | Male C57BL/6N mice | 10 weeks | ↓ Liver TAG (H– E and Oil Red O staining) | ↓ Body weight | Yang et al<sup>10</sup> |
|                     | DHA: 97.5 g/kg KO | HF vs. HF + KO |          | ↓ Liver mass/body weight (35%) | ↓ Plasma TAG |          |
|                     |             |                     |          | | ≈ Plasma TC |          |
|                     |             |                     |          | | ≈ Fasting plasma glucose |          |
|                     |             |                     |          | | ↑ Liver AMPK activation |          |
|                     |             |                     |          | | Liver gene expression: |          |
|                     |             |                     |          | | ↓ FA synthesis |          |
| Krill oil           | 4.86% of KO in the diet | ApoE-deficient female mice | 12 weeks | ↓ Liver TAG, TC | ≈ Body weight | Parolini et al<sup>41</sup> |
|                     |             | WD vs. WD + KO |          | | ≈ Plasma TAG |          |
|                     |             |                     |          | | ↓ Plasma TC |          |
|                     |             |                     |          | | ↑ Total antioxidant capacity in plasma |          |
|                     |             |                     |          | | Liver gene expression: |          |
|                     |             |                     |          | | ↓ Cholesterol synthesis |          |
| Name of concentrate | EPA/DHA dose | Target | Type of study | Results (NAFLD) | Other results | Reference |
|---------------------|--------------|--------|---------------|-----------------|--------------|-----------|
| Krill oil           | EPA + DHA: 10 or 30 g/kg diet | Male C57BL/6N mice | 8 weeks | ↓ Liver TAG, (KO, high dose) | ↓ Body weight (KO, high dose) | Kroupova et al<sup>42</sup> |
|                     |              |        |              |                 | ↓ Plasma TAG |           |
|                     |              |        |              |                 | ↓ Plasma TC, insulin (KO, high dose) |           |
|                     |              |        |              |                 | ↓ Glucose AUC (OGTT) |           |
|                     |              |        |              |                 | ↑ Intestinal FA oxidation |           |
| Krill oil           | EPA + DHA: 10 or 30 g/kg diet | Male C57BL/6N mice | 8 weeks | ↓ Liver TAG (69%, KO, high dose) | ↓ Body weight (KO, high dose) | Rossmeisl et al<sup>24</sup> |
|                     |              |        |              |                 | ↓ HOMA-IR index (KO, high dose) |           |
|                     |              |        |              |                 | ↓ Fasting plasma glucose (KO, high dose) |           |
|                     |              |        |              |                 | ↓ Plasma insulin (KO, high dose) |           |
|                     |              |        |              |                 | ↑ Insulin sensitivity |           |
|                     |              |        |              |                 | ↑ Plasma adiponectin |           |
| Krill oil           | -            | Male Sprague-Dawley rats | 8 weeks | ↓ AST, ALT | Hepatic oxidative stress | Helal and El-Kashef<sup>47</sup> |
|                     |              |        |              |                 | Hepatic nitrosative stress in liver |           |
| Krill oil           | Prevention  EPA + DHA: 30 mg/g diet | Male C57BL/6N mice | 24 weeks | ↓ Liver TAG | ↓ Body weight | Sistilli et al<sup>43</sup> |
|                     | Reversal EPA + DHA: 30 mg/g diet | | | ↓ ALT | ≈ Plasma TAG |           |
|                     |              |        |              |                 | ↓ Liver TAG |           |
|                     |              |        |              |                 | ≈ Plasma TC |           |
|                     |              |        |              |                 | ↓ HOMA-IR index |           |
|                     |              |        |              |                 | ↑ Plasma adiponectin |           |
|                     |              |        |              |                 | ↑ Insulin sensitivity |           |
|                     |              |        |              |                 | Liver gene expression: |           |
|                     |              |        |              |                 | ↓ FA and cholesterol synthesis |           |
|                     |              |        |              |                 | ≈ Body weight |           |
|                     |              |        |              |                 | ≈ Plasma TAG, TC |           |
|                     |              |        |              |                 | ↓ HOMA-IR index |           |
|                     |              |        |              |                 | ↑ Plasma adiponectin |           |
|                     |              |        |              |                 | Liver gene expression: |           |
|                     |              |        |              |                 | ↓ FA and cholesterol synthesis |           |

Note: The included animal studies (n = 26) have been published in the last 10 years. Unless otherwise stated, all results in the table relate to liver metabolism.

Abbreviations: 2-AG, 2-arachidonoyl glycerol; ACC, acetyl-CoA carboxylase; ACOX1, acyl-coenzyme A oxidase 1; AEA, anandamide; ALT, alanine transaminase; AMPK, AMP-activated protein kinase; ApoE, apolipoprotein E; AST, aspartate transaminase; AUC, area under curve; CPT, carnitine palmitoyltransferase; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FA, fatty acid(s); FAS, fatty acid synthase; G6PDH, glucose-6-phosphate dehydrogenase; HDL, high-density lipoprotein; HF, high-fat diet; HHHF, high-fat/high-fructose diet; HHSF, high-fat/high-sucrose diet; HOMA-IR, homeostatic model assessment for insulin resistance; hTNFα, human tumour necrosis factor α; IGTT, intraperitoneal glucose tolerance test; KO, krill oil; IL-2, interleukin-2; LDL, low-density lipoprotein; LPC, lysophosphatidylcholine; N/A, not applicable; NEFA, non-esterified fatty acid(s); OA, orotic acid; OGTT, oral glucose tolerance test; PC, phosphatidylcholine; PEA, palmitoylethanolamide; PG, phosphatidyglycerol; PL, phospholipid; PS, phosphatidylserine; SD, standard diet; TAG, triacylglycerols; TC, total cholesterol; WD, Western diet.
ALT levels compared to controls. In a rat model of iron overload–induced liver damage, krill oil supplementation reduced liver fibrosis. However, studies investigating the effects of omega-3 PLs in animal models of diet-induced obesity associated with advanced stages of NAFLD are lacking.

### 3.2 Human studies

There are currently no human studies examining the effects of omega-3 PLs directly on NAFLD. However, we identified 12 studies that examined changes in metabolic parameters and/or effects on cardiovascular risk factors following administration of krill oil or krill powder to overweight/obese patients with or without type 2 diabetes (Table 2).

Bunea et al reported a ~27% reduction in serum TAGs, ~18% and ~39% reductions in total and low-density lipoprotein (LDL) cholesterol, and ~60% increase in high-density lipoprotein (HDL) levels in a group of obese hyperlipidaemic subjects who received 3 g of krill oil daily for 12 weeks. In this study, fish oil given at the same dose of 3 g/day (i.e. ~900 mg EPA and DHA) did not significantly affect TAG and LDL cholesterol levels, whilst it induced a less pronounced reduction in total and an increase in HDL cholesterol. The reason for the differential effects of krill oil and fish oil on blood lipids in this study is not clear; however, differences in the hydrolysis of TAGs and PLs in the intestine, subsequent absorption of hydrolysis products and metabolism of lipoprotein particles probably play an important role. The ability of krill oil to reduce plasma TAG concentrations was later confirmed in overweight/obese subjects with borderline high or high serum TAG levels as well as in healthy young adults. In another study conducted in hypertriglyceridaemic and overweight patients, krill oil raised HDL and lowered non-HDL cholesterol levels whilst lowering plasma TAG levels comparably to omega-3 ethyl esters. In a short-term 4-week study in overweight/obese healthy subjects, administration of krill oil did not result in significant changes in lipid homeostasis, despite increases in plasma omega-3 concentrations. Nevertheless, administration of krill oil under similar conditions led to a reduction in plasma levels of the endocannabinoid 2-arachidonoylglycerol (2-AG) in healthy obese subjects, whereas omega-3 TAGs were ineffective. Since 2-AG levels were correlated with the n-6/n-3 long-chain polyunsaturated FA ratio in plasma PLs, it is likely that krill oil supplementation reduced 2-AG biosynthesis due to the replacement of arachidonic acid (20:4 n-6), a precursor of 2-AG, by omega-3. Animal studies have suggested that this process may be more efficient in the case of omega-3 PLs compared to omega-3 TAGs.

Given the established role of increased activity of the endocannabinoid system in the development of obesity-related metabolic disorders, including NAFLD (see 4.2), the superior ability of omega-3 PLs to inhibit endocannabinoid synthesis may contribute to their strong antisteatotic effects, possibly also in humans. Furthermore, a long-term 24-week administration of krill powder caused a time-dependent decrease in plasma levels of the endocannabinoid N-arachidonoylthetanolamine (anandamide; AEA) along with a decrease in TAG levels in obese men. However, the same daily amount of krill powder administered for just 8 weeks did not change plasma TAG levels in overweight subjects with elevated blood pressure. Krill oil intake reduced cardiovascular risk markers such as fasting blood glucose levels and down-regulated the expression of cholesterol biosynthesis genes in peripheral blood mononuclear cells in healthy subjects. Lobraco et al demonstrated that krill oil administration reduces insulin resistance as assessed by the HOMA-IR index in obese patients with type 2 diabetes. In contrast, insulin sensitivity unexpectedly decreased after supplementation with krill and salmon oil mixture in overweight men. The human studies analysed suggest that the effectiveness of krill-based products in alleviating metabolic disorders associated with overweight/obesity is inconsistent and likely depends on the dose of EPA and DHA, the duration of supplementation and/or the degree of metabolic impairment of the patients.

### 4 OMEGA-3 PHOSPHOLIPIDS AND NAFLD: MECHANISMS OF ACTION

Hepatic steatosis, occurs when the rate of FA uptake from the circulation and DNL are greater than the rate of FA oxidation and their export as TAGs via very-low-density lipoproteins (VLDLs); this imbalance is then associated with insulin resistance. Here, we describe the main mechanisms, including the metabolic aspects mentioned above, involved in the effects of omega-3 PLs on NAFLD, primarily hepatic steatosis. We also look at the effects of omega-3 PLs on organs such as adipose tissue (AT) and the gut, as these may contribute to antisteatotic effects in the liver (Figure 2).

#### 4.1 Liver

##### 4.1.1 Inhibition of de novo lipogenesis

Hepatic DNL and cholesterol biosynthesis are amongst the most affected metabolic pathways observed in obese rodents after administration of krill oil or fish-derived PLs. Their down-regulation significantly contributes to the antisteatotic effect of omega-3 PLs, as activation of DNL in obesity leads to TAG accumulation in the liver. Suppression of DNL may be caused, for example, by inhibition of hepatic lipogenic gene expression in obese animals.
| Name of concentrate | EPA/DHA dose | Target | Type of study | Results (NAFLD) | Other results | Reference |
|---------------------|--------------|--------|---------------|-----------------|---------------|-----------|
| Krill powder        | EPA: 268 DHA: 132 mg per day | Mildly obese men | Single-centre, open label, pilot study 24 weeks Comparison to baseline | N/A ≈ Body weight ↓ Plasma TAG (20.6%) ≈ Plasma TC, LDL, insulin ↓ Plasma endocannabinoids (AEA 84%, PEA and OEA); ≈ Plasma endocannabinoids (2-AG) | Berge et al54 |
| Krill powder        | EPA: 100 DHA: 60 mg per day | Overweight men and women with mildly or moderately elevated blood pressure | Prospective, randomised, double-blinded, placebo-controlled study 8 weeks Comparison to baseline and vs. placebo | N/A ≈ Plasma total TC, HDL, LDL, TAG ≈ Serum ALT, AST, glutamyl transferase, creatinine | Sarkkinen et al55 |
| Krill oil           | Not specified 1-1.5 or 2-3 g of KO per day | Patients with hyperlipidemia | Multi-centre, prospective, randomised study 16 weeks Comparison to baseline and vs. fish oil or placebo | N/A ↓ Plasma glucose, TC, TAG, LDL (both treatments vs. baseline, fish oil and placebo) ↑ Plasma HDL (both treatments vs. baseline, fish oil and placebo) | Bunea et al56 |
| Krill oil           | EPA: 216 DHA: 90 mg per day | Overweight and obese men and women | Randomised, double-blind, controlled, parallel clinical trial 4 weeks Comparison to baseline and vs. menhaden or olive oil | N/A ≈ Body weight ≈ Plasma glucose, insulin, lipoprotein lipids, TAG, albumin, creatinine, electrolytes and liver enzymes ≈ HOMA-IR | Maki et al52 |
| Krill oil           | EPA: 216 DHA: 90 mg per day | Normoweight, overweight and obese men and women | Randomised, double-blind, controlled, parallel clinical trial 4 weeks Comparison to baseline and vs. menhaden or olive oil | N/A ≈ BMI, waist circumference, plasma glucose and insulin ↓ Plasma 2-AG (obese subjects, vs. baseline, menhaden oil and olive oil) ≈ Plasma AEA | Banni et al53 |
| Krill oil           | EPA +DHA: 0, 100, 200, 400 or 800 mg per day | Men and women with borderline high or high TAG levels | Double-blind, randomised, multi-centre, placebo-controlled study 12 weeks Comparison to baseline and vs. olive oil | N/A ↑ Omega-3 index (vs. baseline and placebo) ↓ Plasma TAG (200, 400 and 800 mg per day vs. baseline and placebo) ≈ Plasma TC, LDL, HDL | Berge et al59 |
| Name of concentrate | EPA/DHA dose | Target | Type of study | Results (NAFLD) | Other results | Reference |
|---------------------|--------------|--------|---------------|----------------|---------------|-----------|
| Krill oil           | Not specified 1000 mg of KO per day | Patients with type 2 diabetes | Randomised, double-blind, controlled crossover trial 4 weeks + optional 17 weeks Comparison to baseline and vs. olive oil | N/A | ↑ Endothelial function (17 weeks, vs. baseline) ↓ HOMA-IR (vs. olive oil) ↓ Plasma C-peptide (vs. olive oil) | Lobraco et al⁵⁸ |
| Krill oil           | EPA: 230 DHA: 154 mg per day | Overweight, middle-aged men | Randomised, double-blind, controlled crossover trial 8 weeks Comparison to baseline and vs. canola oil | N/A | ↓ Insulin sensitivity (Matsuda index, HOMA-IR, vs. canola oil) ↑ Plasma TC, HDL, LDL, apolipoprotein B (vs. baseline) ↑ Carotid artery intima-media thickness (vs. baseline) | Albert et al⁵⁹ |
| Krill oil           | EPA +DHA: 832.5 mg per day | Healthy young adults | Intervention study 28 days Comparison to baseline | N/A | ≈ CRP, ALT, AST, ALP, bilirubin ↓ Plasma TAG, TAG/HDL ratio ↑ HDL, LDL | Berge et al⁶⁰ |
| Krill oil           | EPA: 150 DHA: 90 mg per day | Moderately hypertriglyceridaemic subjects | Randomised, crossover, clinical trial 4 weeks Comparison to baseline and vs. omega-3 ethyl esters | N/A | ≈ Plasma TC, LDL, apolipoprotein B, fasting plasma glucose, creatinine, liver transaminases and CPK ↓ Plasma TAG (vs. baseline) ↓ hs-CRP (vs. baseline and omega-3 ethyl esters) ↑ Plasma HDL (vs. baseline) | Cicero et al⁶¹ |
| Krill oil           | Omega-3 FA: 4654 mg per week | Healthy overweight men and women | Randomised controlled trial 8 weeks Comparison to baseline and vs. fish oil or control oil | N/A | ↓ Plasma glucose, TAG (1%) – not significant (overall difference between groups) ↑ Total lipids, PLs, cholesterol, cholesteryl esters and non-esterified cholesterol in XS-VLDL (overall difference between groups) ↑ HDL₃-C, LDL-TAG (overall difference between groups) | Rundblad et al⁶⁶ |
| Krill oil           | Omega-3 FA: 4654 mg per week | Healthy overweight men and women | Randomised controlled trial 8 weeks Comparison to baseline and vs. fish oil or control oil | N/A | PBMC mRNA expression: ↓ Gluconeogenesis, cholesterol synthesis, FA oxidation (vs. baseline) | Rundblad et al⁶⁷ |

Note: The included human studies (n = 12) have been published in the last 20 years.

Abbreviations: 2-AG, 2-arachidonoyl glycerol; AEA, anandamide; ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase; CPK, creatine kinase; CRP, C-reactive protein; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FA, fatty acid(s); HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment for insulin resistance; KO, krill oil; LDL, low-density lipoprotein; N/A, not applicable; OEA, oleoylethanolamide; PBMC, peripheral blood mononuclear cells; PEA, palmitoylethanolamide; TAG, triacylglycerols; TC, total cholesterol; XS-VLDL, very small, very-low-density lipoprotein.
supplemented with omega-3 PLs. Reduced DNL pathway activity in the liver was also confirmed by detecting reduced activity of lipogenic enzymes such as acetyl-CoA carboxylase (ACC) and fatty acid synthase, or by reduced levels of short/medium-chain TAGs containing 38-48 carbons and 0-3 double bonds, a lipidomic marker of hepatic DNL.

Transcriptional regulators of de novo lipogenesis

Down-regulation of genes involved in DNL induced by administration of omega-3 PLs may be attributed to an increased PC/phosphatidylethanolamine ratio, as a decrease in this ratio leads to activation of sterol regulatory element-binding protein 1c (SREBP-1c), thereby initiating DNL and lipid accumulation. Interestingly, antisteatotic effects and down-regulation of hepatic DNL genes were observed in HFD-fed mice only when animals received omega-3-rich PCs, but not when these PCs contained omega-6 FAs. Further, it has been observed that administration of EPA-rich PCs reduces SREBP-1c expression in the liver, whilst fish oil had no effect. Transcription of genes involved in glycolysis and DNL can also be activated by carbohydrate-responsive element-binding protein (ChREBP). Polysaturated FAs have been shown to inhibit nuclear translocation of ChREBP and to increase ChREBP mRNA decay, and krill oil, but not fish oil, decreased the expression of max-like factor X, a functional heterodimeric partner of ChREBP. Reduced expression of the transcription factor SREBP-2 may be responsible for the down-regulation of cholesterol biosynthesis observed in rodents given omega-3 PLs but not fish oil.

Citrate transport

Interestingly, decreased expression and activity of mitochondrial citrate carrier (CIC) was observed in HFD-fed rats supplemented with krill oil. Mechanistically, inhibition of CIC will reduce the efflux of mitochondrial citrate into the cytosol, thereby reducing the supply of acetyl-CoA available for DNL, whilst reducing allosteric activation of ACC by citrate. Inhibition of CIC will also contribute to a reduction in cholesterol biosynthesis because this also starts with acetyl-CoA. However, CIC gene expression was not significantly affected by omega-3 PLs from herring, so this may be an effect specific to krill oil.

AMP-activated protein kinase

Finally, omega-3 administration is associated with activation of AMP-activated protein kinase (AMPK) in the liver, presumably through the hormone adiponectin (see Section 4.2 for details). Activation of AMPK leads, amongst other effects, to inhibitory phosphorylation of ACC, a key enzyme of the DNL pathway, in hepatocytes.

The contribution of suppressed DNL to the overall antisteatotic effect of omega-3 PLs may also explain their superior efficacy against hepatic steatosis compared with omega-3 TAGs. Simultaneously, reduced DNL may underlie the suppressive effect of krill oil on hepatic diacylglycerol (DAG) levels. Since DAGs are involved in hepatic insulin resistance, their reduced levels may be related to the improvement in hepatic insulin sensitivity due to krill oil supplementation.

Stimulation of the oxidation of fatty acids

Increased FA catabolism is another way that omega-3 PLs may reduce hepatic steatosis. However, although omega-3 act as ligands of the peroxisome proliferator–activated receptor (PPAR)-α transcription factor, omega-3 PLs do not appear to consistently affect β-oxidation-related genes, known targets of PPAR-α. However, administration of krill oil increased the activity of carnitine palmitoyltransferase I (CPT1), the enzyme catalysing the essential step of β-oxidation in mitochondria, concomitantly with an increase in free carnitine levels in HFD-fed rats. For example, in TNFα-humanised mice, mitochondrial and peroxisomal β-oxidation was enhanced by the effect of krill oil on CPT2 and acyl-CoA oxidase 1 (ACOX1) activity. EPA-PLs, compared with DHA-PLs, showed stronger antisteatotic effects related to PPARα-mediated activation of β-oxidation in the liver. This may also be related to the fact that EPA, but not DHA, increases β-oxidation and inhibits esterification of 1,2-DAGs and TAG synthesis in hepatocytes. Moreover, dietary intake of EPA-PLs decreased FSP27 protein content in both liver and AT of C57BL/6J mice, suggesting changes in lipid droplet formation and stimulation of lipolysis.

Accordingly, the improved bioavailability of omega-3, especially EPA, in response to administration of omega-3 in the form of PLs, observed in both obese mice and humans (reviewed in ), may contribute to the superior antisteatotic effects of this lipid class. Similar to DNL inhibition, but perhaps even more significant, may be the involvement of the adiponectin-AMPK axis in the stimulatory effect of omega-3 on β-oxidation in hepatocytes and in maintaining hepatic insulin sensitivity.

No effect of omega-3 PLs on hepatic VLDL-TAG production or TAG content of VLDL particles was observed in HFD-fed mice. Thus, hepatic VLDL-TAG secretion does not appear to be significantly affected by administration of omega-3 PLs in the above experimental models.

Adipose tissue

In obese NAFLD patients, 59% of hepatic TAGs come from plasma non-esterified FAs (NEFA), which are strongly influenced by AT metabolism. AT also releases secretory...
factors (i.e. adipocytokines) that regulate processes elsewhere in the body, including the liver. As for NEFA levels, these were reduced in dietary obese mice receiving omega-3 PLs from either herring or krill oil. This effect does not appear to be stronger compared to omega-3 given as TAGs.

Regarding the secretory function of AT in relation to the antisteatotic effects of omega-3 PLs, the focal point here is adiponectin. This hormone is a potent antilipotoxic and anti-inflammatory agent with insulin-sensitising properties. Omega-3 are known to stimulate adiponectin expression and secretion from AT into the circulation. Administration of omega-3 PLs was associated with an increase in circulating adiponectin levels in obese mice. This effect was dose-dependent and generally stronger compared to omega-3 administered as TAGs. Because adiponectin secretion by adipocytes is markedly stimulated by the EPA-derived metabolite 15d-PGJ2, it is possible that the higher EPA content of some omega-3 PL products (e.g. krill oil) could more effectively promote adiponectin secretion.

Regarding AT inflammation, decreased tissue levels of proinflammatory cytokines and/or reduced macrophage accumulation were observed in obese mice treated with omega-3 PLs, which, together with improved insulin sensitivity, further suggests improved AT function.

Elevated circulating levels of endocannabinoids such as 2-AG or AEA may also be a causative factor in the development of obesity-related metabolic disorders. Omega-3 PLs decreased AEA and/or 2-AG levels in visceral AT and in the circulation of obese rodents, showing a stronger effect compared to TAG-based omega-3. Reduced endocannabinoid synthesis in visceral AT may have beneficial effects in the liver due to reduced stimulation of cannabinoid (CB) receptors, for example the CB1 receptor, whose activation potentiates DNL.

4.3 | Intestine

Besides its role in nutrient absorption, the intestine has endocrine functions which together with changes in the gut microbiota and intestinal barrier may influence the pathogenesis of NAFLD/NASH. In this context, overweight/obesity is associated with qualitative and quantitative modifications of the gut microbiota (i.e. dysbiosis), activation of intestinal inflammation and increased intestinal permeability. Omega-3 intake is associated with alleviation of inflammation, also in enterocytes. Krill oil supplementation increased the intestinal barrier-related gene expression in HFD-fed mice and reduced intestinal inflammation, and systemic endotoxin levels in rat and mouse models of induced colitis. Anti-inflammatory effects of krill oil in the gut may be mediated by inhibition of histidine metabolism in the microbiome and host, in addition to inhibition of anti-inflammatory eicosanoid production.

There is relatively little evidence of the effects of omega-3 supplementation on the gut microbiota. Omega-3 intake improved dysbiosis and reduced mucosal inflammation in ulcerative colitis patients and stimulated bacteria producing short-chain FAs (SCFA) in healthy subjects. Butyrate, a SCFA, positively affects intestinal integrity whilst protecting against the development of NASH. In mice with induced colitis, DHA-PLs from algal oil improved the intestinal barrier and increased amount of SCFA-producing bacteria. Similar effects were observed when omega-3 PLs from Porcellio trituberculatus eggs were administered to HFD-fed mice, which was associated with alleviation of insulin resistance. Krill oil supplementation partially restored intestinal dysbiosis in models of infection-induced colitis and in HFD-fed mice induced an increase in the genera Allobaculum and Enterobacter icraflora, shown to be negatively associated with hyperlipidaemia and inflammation.

Glucose and lipid metabolism may also be affected by intestinal hormones such as incretins, that is glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide; in fact, incretin-based therapies also have a beneficial effect on various parameters related to NAFLD (reviewed in detail in ). Increased glucose-stimulated GLP-1 secretion was observed when omega-3 TAGs were administered either acutely into the colon or chronically in conjunction with high-fat feeding. The potential added benefits of using omega-3 PLs to stimulate incretin secretion and its potential role in the metabolic effects associated with omega-3 PL supplementation remain unclear.

Intestinal metabolism affects the bioavailability of omega-3 depending on the lipid class used for their administration. Conversely, the lipid class may play a role in the direct effects of omega-3 on intestinal metabolism. For example, krill oil, but not omega-3 given as TAGs, stimulated FA oxidation in the small intestine of HFD-fed mice. This may further contribute to the potent antisteatotic effects of this lipid class in the liver.

4.4 | Potential contributors to the effect of omega-3 phospholipids administered as krill oil

The composition of omega-3 PL products is often very complex, so other constituents may also contribute to the pleiotropic effects of these products. For example, krill oil contains the antioxidant astaxanthin, but also POA, elevated concentrations of which may contribute to the beneficial effects of krill oil on insulin sensitivity in obese mice. POA can also stimulate FA catabolism in the liver via PPARδ-dependent AMPK activation. Furthermore, the plant alkaloids trigonelline and stachydrine were recently
identified by metabolomic analysis as potential contributors to the potent antisteatotic effects of krill oil in the livers of obese mice with exacerbated steatosis. In one study, where the trigonelline concentration was ~50 mg/kg of diet, it was still about 20 times less compared to the standard diet, use with autophagy stimulants could improve efficacy in addition of these combination therapy (Figure 2), and its concomitant protective effects against carbon tetrachloride-induced liver fibrosis. Thus, the use of krill oil can be considered as a type of combination therapy (Figure 2), and its concomitant use with autophagy stimulants could improve efficacy in advanced NAFLD.

5 | THERAPEUTIC PERSPECTIVES

In terms of health benefits of omega-3 PLs in humans, daily doses of around 1-4 g of krill oil (i.e. ~200-800 mg EPA + DHA) administered to hyperlipidaemic overweight/obese subjects for at least 1 month may reduce circulating TAG levels and possibly increase HDL cholesterol. These conclusions are also supported by a meta-analysis of randomised controlled trials using krill oil. Nevertheless, further human studies are needed to reveal the effect of omega-3 PLs directly on NAFLD, including its advanced stages such as NASH. Although several existing studies do not suggest that krill-based products could alter AST and ALT levels in overweight/obese people, due to the relatively short duration of these studies and the absence of pathologically elevated levels of liver enzymes in basal condition, the effect of omega-3 PLs on NAFLD in humans is still unclear. Furthermore, there are no human studies examining the effects of omega-3 PLs from sources other than krill oil, at least not in relation to obesity and related metabolic disorders. However, in terms of improving impaired glucose homeostasis and insulin sensitivity in obesity, preclinical animal studies have shown greater efficacy of omega-3 PLs compared to the TAG form, which is often ineffective. This is important because NAFLD is closely related to obesity and the combination of antisteatotic and insulin-sensitising effects of omega-3 PLs may thus be advantageous in this context.

6 | CONCLUDING REMARKS

Preclinical animal studies have provided compelling evidence for the potent antisteatotic effects of omega-3 PL supplementation in the context of NAFLD in obesity. PC is the dominant PL class in most natural sources of omega-3 PLs, and in
terms of effects on obesity-related liver steatosis, it appears that enrichment of PC with omega-3 is necessary to achieve optimal efficacy compared to PC from other sources that do not contain omega-3. The pronounced suppression of DNL in the liver may be one of the main reasons for the improved efficacy of omega-3 PLs on hepatic steatosis; however, the involvement of other tissues such as the intestine (e.g. induction of FA oxidation in situ, alleviation of dysbiosis and improvement of intestinal integrity) and AT (e.g. adiponectin secretion) seems to be important. Also, higher relative EPA content could be associated with higher efficacy of different forms of omega-3 PLs, as EPA appears to be more effective than DHA in stimulating hepatic FA oxidation and adiponectin secretion by adipocytes. Comparative studies also suggest that the antisteatotic effects of omega-3 PLs are often stronger and have a partially different mechanistic basis compared to omega-3 administered in the form of fish oil or TAG-based concentrates. These improved effects of omega-3 PLs are likely based on the unique combination of the PL molecule with omega-3, whereby the metabolic properties of the PLs themselves are exploited whilst ensuring better bioavailability of omega-3. Increased omega-3 bioavailability than translates into more effective regulation of a number of processes, including inhibition of endocannabinoid synthesis or adiponectin secretion (see above). Importantly, some forms of omega-3 PLs, such as krill oil, contain other bioactive substances (e.g. astaxanthin, plant alkaloids, POA) that may contribute to their antisteatotic and insulin-sensitising effects. Finally, preclinical evidence for strong antisteatotic effects of omega-3 PLs in the liver should be confirmed in clinical trials, whilst further research is needed on the possible effects of omega-3 PLs on advanced stages of NAFLD such as NASH and fibrosis.

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CONFLICT OF INTEREST
The authors declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

AUTHORS CONTRIBUTIONS
O. H. and M. R. conception and design; M. M., G. S., O. H. and M. R. writing and revision of the manuscript; M. M., G. S., O. H. and M. R. approved the final version of the manuscript.

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