THE EUROPEAN SOCIETY OF MEDICINE
Medical Research Archives, Volume 12 Issue 11

REVIEW ARTICLE

Natural health products for treatment of metabolism dysfunction-associated steatotic liver disease

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OPEN ACCESS

PUBLISHED
30 November 2024

CITATION
Chan, CB., 2024. Natural health products for treatment of metabolism dysfunction-associated steatotic liver disease. Medical Research Archives, [online] 12(11). https://doi.org/10.18103/mra.v12i11.6100

DOI
https://doi.org/10.18103/mra.v12i11.6100

ISSN
2375-1924

ABSTRACT

Metabolism dysfunction-associated steatotic liver disease affects approximately 30% of the world's population, yet there is only one approved treatment option applicable to more advanced disease. Many individuals consume natural health products for general health and a variety of medical conditions but none are recommended for metabolism dysfunction-associated steatotic liver disease in current European or American guidelines. Nevertheless, human trials indicate that some of these products may be efficacious for treatment of metabolism dysfunction-associated steatotic liver disease and these are supported by mechanistic studies using animal models. This narrative review aims to highlight recent research in human and animal trials on selected natural health products. So far, neither probiotics nor omega-3 polyunsaturated fatty acids have produced convincing, consistent benefits in human randomized controlled trials although studies in mouse models suggest that they have actions can lead to reduction of hepatic steatosis or other markers, such as liver enzymes. Two of the many polyphenols that have been studied were also reviewed here. Trials with resveratrol in humans have not yielded significant results whereas curcumin, the active ingredient in turmeric, appeared to consistently lower steatosis or liver enzymes. Both compounds reduced steatosis in rodent models of MASLD, involving a variety of mechanisms including anti-oxidant, anti-inflammatory and metabolic effects. More, better-designed and powered human trials are required to provide convincing evidence of efficacy of most natural health products.

1. Introduction

Many people eat specific foods or use dietary supplements because they believe they confer preventative or therapeutic properties for diseases, or will boost immunity. The United States (U.S.) National Center for Health Statistics estimates nearly 60% of adults used dietary supplements in the month prior to the survey[1], most commonly a multi-vitamin followed by vitamin D and omega-3 fatty acids. Uptake is even greater in Europe, with 93% reporting use within the previous year (predominantly vitamin D, vitamin C and magnesium, followed by multi-vitamins)[2]. Metabolism dysfunction-associated steatotic liver disease (MASLD) is considered to be the liver manifestation of the metabolic syndrome and affects about 30% of the global population[3]. As such, it has become the leading cause of liver transplant, yet to date only one treatment, resmitirom, has received Federal Drug Administration approval in the U.S. and it is specific for people with advanced disease causing liver scarring (fibrosis)[4]. (Resmitirom is currently under consideration in the European Union (E.U.)). In the absence of approved pharmacotherapies, patients may turn to natural health products or complementary health approaches. Although the current European Association for the Study of the Liver-European Association for the Study of Diabetes-European Association for the Study of Obesity (EASL-EASD-EASO) Clinical Practice Guidelines state that nutraceuticals cannot be recommended due to lack of efficacy and safety evidence[5], physician knowledge of these products can assist their patients in the decision to use them. In this article, a snapshot of 3 classes of natural health products that have been studied in human clinical trials as well as animal studies designed to elucidate mechanism of action, will be critically reviewed. The purpose is to present the current evidence for probiotics, 2 of the many polyphenolic natural health products and omega-3 fatty acids as documented in systematic reviews with meta-analyses, or other systematic reviews for treatment of MASLD. Supporting evidence from animal trials utilizing the corresponding compounds is presented along with possible future directions for human therapies based on the data from animal models.

2. Metabolism dysfunction-associated steatotic liver disease

2.1 DEFINITION AND PREVALENCE

Metabolism dysfunction-associated steatotic liver disease was previously known as non-alcoholic fatty liver disease (NAFLD)[6] and is a metabolic, chronic hepatic condition characterized by progressive hepatosteatosis, fibrosis, cirrhosis, and hepatocellular carcinoma. The newly proposed nomenclature and definition include the presence of hepatic steatosis detected by imaging or biopsy and the existence of one of the five distinct components of metabolic syndrome, including abdominal obesity, elevated blood pressure, high triglycerides, elevated blood glucose (prediabetes or diabetes), and low high density lipoprotein (HDL-C) cholesterol[6]. In this article, the terminology used in the original citation will be used; for general discussion, the updated nomenclature will be used. Population-based estimates of NAFLD prevalence average 35% in both North and South America, followed by Europe and Asia at 30% but the risk is increased by 3.5-fold in people with obesity[7]. In persons with type 2 diabetes and obesity, 56%[8] and 70%[9], respectively, are also diagnosed with NAFLD. Comparisons of prevalence using the definition for MASLD suggest that it is similar[10].

2.2 HEPATIC LIPID METABOLISM

The liver is central to regulation of substrate metabolism and dictates the fate of fat and carbohydrate. Abnormal steatosis occurs when hepatocyte fat accumulation exceeds its oxidation or export. Dysregulation of 4 major pathways may contribute to a metabolic imbalance of lipids that results in triglyceride accumulation: (i) increased influx of non-esterified fatty acids from the blood (sourced from diet intake or adipose tissue lipolysis); (ii) increased hepatic fatty acid synthesis through

the de novo lipogenesis pathway; (iii) decreased fatty acid oxidation; (iv) decreased very low-density lipoprotein-mediated release of triglyceride into the circulation. The regulation of all these pathways is controlled through a highly networked, intricate interplay among hormones that activate or dampen these metabolic pathways[11,12].

2.2.1 Pathophysiology of metabolism dysfunction-associated steatotic liver disease

Many factors contribute to MASLD development, suggesting a need for multiple 'hits' on a background of genetic susceptibility. Visceral obesity is one of the starting points for the pathologic progression of MASLD because it often initiates systemic insulin resistance[13,14]. The consequent hyperglycemia and hyperlipidemia results in increased delivery of these substrates to the liver, among other tissues, upregulating de novo lipogenesis and triglyceride accretion[15,16]. Accumulation of toxic lipids such as diacylglycerols, in concert with systemic inflammation may trigger mitochondrial dysfunction, oxidative and endoplasmic reticulum stress, along with hepatic inflammation and fibrogenesis[15,17]. Westernized diets also remodel the gut microbiome[14], with microbial metabolites causing an additional source of inflammation, altered lipid metabolism and increased gut permeability[15]. Steatohepatitis is associated with specific changes in the gut microbiome structure[14]. Although the majority of individuals may exist with simple steatosis, perhaps 5-10% will progress to steatohepatitis (MASH) and fibrosis, which are more intractable to treatment[15]. Without proper management, steatohepatitis can progress to cirrhosis and hepatocellular carcinoma, which are leading causes of liver transplantation globally[18]. However, it should be noted that most hepatic steatosis is 'silent' and its diagnosis may be an incidental finding requiring further investigation[5]. Given the contribution of poor diet to obesity and microbiome dysbiosis, it is reasonable to think that dietary modification could ameliorate MASLD.

2.2.2. Current approved treatment goals and options

Current diagnosis and treatment guides for MASLD indicate lifestyle interventions with the goal of weight loss as the first line of treatment and management for early-stage MASLD[5,19]. Like for type 2 diabetes, weight loss prompted by lifestyle changes can be helpful but is not successful for the majority of patients[20]. The EASL-EASD-EASO guidelines indicate that interventions to reduce steatosis, steatohepatitis and fibrosis are warranted to reduce risk of liver-related outcomes but note many unanswered questions in developing their recommendations[21]. The European guidelines indicate that, if approved locally, resmiratron should be considered for adults with stage ≥2 fibrosis without cirrhosis and for other high-risk individuals[5]. The U.S. guidelines recommend pharmacotherapy for patients with biopsy-proven non-alcoholic steatohepatitis (NASH/MASH) and fibrosis[19]. However, MASLD pharmacotherapies are currently underdeveloped with resmitirom the only approved drug that targets MASH and liver fibrosis[4]. Multiple drug types such as de novo lipogenesis inhibitors, glucagon like peptide-1 (GLP-1) receptor agonists, sodium-glucose linked transporter-2 inhibitors, and peroxisome proliferator-associated receptor agonists used to treat the associated comorbidities, such as glycemic control and obesity, can improve clinical markers of MASLD[5]. In particular, several trials indicate that the anti-obesity GLP1 receptor agonist drugs effectively resolve steatosis and fibrosis[17]. A review of emerging therapies has been published recently[22].

3. Natural health products

In Canada, "Natural Health Products" is an official designation by Health Canada governed by the Natural Health Products Regulations[23]. Under the Regulations, natural health products are defined as: Probiotics, herbal remedies, vitamins and minerals, homeopathic medicines, traditional medicines such as traditional Chinese medicines, other products like amino acids and essential fatty acids. They must be deemed safe and available without a prescription from a healthcare professional. The term is useful for this review, in which we shall

review the evidence for probiotics, essential fatty acids and some herbal remedies.

The E.U. designates herbal medicinal products as "any medicinal product, exclusively containing as active ingredients one or more herbal substances, one or more herbal preparations, or a combination of the two." The registration of these products should have a history of use in the E.U. of at least 15 years but that does not fulfill requirements for supporting scientific evidence for marketing authorization[24]. Regarding probiotics, there is currently no regulating framework for products intended for human consumption[25] despite many calls from industry and others. The E.U. Ombudsman has opened a case to review a formal complain[26], which may resolve the issue.

Recently there has been an attempt at international alignment of regulations governing use of natural health products. It outlines the similarities and differences in their classification in countries around the world, including the E.U. and United Kingdom, the U.S. and others and highlights barriers to harmonization[27]. For the purpose of this report, natural health products includes nutritional supplements and products used in traditional healing practices as defined in Canada[23]. Currently, vitamin E is mentioned in the U.S. guidelines published in 2018 and would be classified as an natural health products. The summarized evidence suggest that in both adults and children, vitamin E significantly resolves steatohepatitis but not fibrosis. The recommendation is that vitamin E may be considered for adults with biopsy-proven MASH after due consideration of benefits and risks but not for patients with diabetes, in the absence of a biopsy, or in patients with cirrhosis or cryptogenic cirrhosis[19]. Omega-3 fatty acids were not recommended due to lack of evidence[19]. In the recently-released European guidelines, nutraceuticals in general were not recommended due to lack of evidence[21].

4. Results

4.1 PROBIOTICS

Probiotics are foods and other formulations that contain live micro-organisms and have a health benefit on the host when an adequate dose is consumed[28]. Probiotics may be present in fermented foods such as yogurt and kefir. The most common organisms are from the genera *Lactobacillus*, *Bifidobacterium*, *Saccharomyces*, *Streptococcus*, *Enterococcus*, *Escherichia*, and *Bacillus*. Probiotics are also available as supplements. The rationale for their use in MASLD is to address the microbiome dysbiosis, as discussed in Section 2.2.1. Users are cautioned that not all products are backed by high-quality evidence of their efficacy to alter the gut microbiome or exert other beneficial effects[28].

4.1.1 Probiotics results in human randomized controlled trials

Overall, probiotics demonstrated benefits on a number of MASLD outcomes but the overall evidence is weak based on small study populations, short follow-up, high risk of bias, heterogeneity and use of surrogate markers in many studies, rather than direct measures of steatosis and fibrosis[5]. The European guidelines cited 3 relevant meta-analyses[21]. Komolafe et al focused on the safety and efficacy of nutritional supplements in a network meta-analysis published in 2021[29]. Efficacy of probiotic trials against most of the specified endpoints (e.g. mortality, cirrhosis) could not be estimated. The odds ratio for any adverse event was 0.67 (95% confidence interval 0.3, 1.64), estimated from 5 trials suggesting that probiotics are safe but more studies that report safety issues are required[29]. Moreover, the analysis also included prebiotics and synbiotics in the same grouping as well as many different formulations of probiotic, making interpretation rather generic. Rong et al.[30] reported on 18 randomized controlled trials (13 in adults) that included probiotics with or without adjunct therapy, the most common being lifestyle intervention in 8 studies. These were appropriately compared with control groups that also received lifestyle therapy + placebo. Probiotics improved ultrasound grading, NAS (an unweighted composite of steatosis, lobular

inflammation, and ballooning scores), and liver enzymes but not CAP score (controlled attenuation parameter, a measure of steatosis)[30]. In the 5 trials showing reductions in steatosis by ultrasound, all used various combinations of *Lactobacillus* and *Bifidobacteria* strains (with 2 studies also adding *Streptococcus thermophilus* BT01) as capsules or, in 1 study, provided in yogurt, which are considered to be traditional probiotic strains. Similarly, Zhu et al.[31] identified 20 studies that reported a primary outcome of alanine transaminase (ALT). Traditional probiotics significantly reduced ALT but results were highly heterogenous. Significant benefits of traditional probiotics were also seen for aspartate transaminase (AST), gamma-glutamyl transferase (GGT), and CAP score. A minimal benefit on body weight was also detected but effects on fasting blood glucose were not significant. Sub-analyses did not detect differences in outcomes associated with the probiotic formulations used. High heterogeneity was detected for many of these outcomes, which may be related to age and baseline liver health status[31]. Moreover, liver enzymes are non-specific markers of liver damage. A systematic review without meta-analysis concluded that probiotics improved inflammation, blood glucose and lipids, induced weight loss, and ameliorated liver injury (variously assessed by liver enzyme measurements, steatosis and fibrosis). However, measures of magnitude of these effects were not presented and the study sample size was frequently less than 100 participants[32]. In a network meta-analysis of studies in pediatric populations, probiotics plus lifestyle interventions were not significantly superior to placebo on lowering ALT, the primary outcome. However, probiotics (with and/or without lifestyle interventions) were effective in reducing AST, GGT, total cholesterol and triglyceride in children[33]. Inability to provide conclusive guidance may be, in part, due to use of different probiotic formulations, doses, underpowered studies and patient populations in trials. Moreover, trials with objective measurement of MASLD-specific outcomes are required.

4.1.2 Probiotic results in animal trials

Focusing on studies of traditional formulations of probiotics provided to animal models of MASLD confirms the many mechanisms that may be affected by such treatment to improve liver steatosis and other outcomes. In various diet-induced obesity mouse models, treatment with single organisms including *Bacteroides ovatus*[34], certain strains of *Lactobacillus* species[35], *Bifidobacterium bifidum*[36] ameliorates hepatic steatosis and associated biomarkers. Mechanisms include increased production of short-chain fatty acids[34] and improved gut barrier function as evidenced by reduced circulating lipopolysaccharide[34,37] or increased tight junction proteins[36]. Downstream, down-regulation of enzymes involved in de novo lipogenesis[34,35] and up-regulation of enzymes involved in fatty acid oxidation[34,35] may be responsible for reduced steatosis. Some studies also report reduced fibrosis[37].

Interestingly, animal trials suggest that new-generation probiotics may have more potent effects on MASLD outcomes than existing formulations. New-generation probiotics are identified by specific properties targeting an outcome of interest and frequently include novel probiotic strains. The hope is that by selecting strains with tailored microbial properties, treatment of individuals can be more precisely selected for efficacy[31] but obstacles to providing these formulations as treatment for MASLD remain. Recent reviews treat this topic in greater depth[38,39]. Moreover, rodent trials have many limitations, including most being conducted only in males, several background diets (most of which do not result in fibrosis) and 100-fold differences in probiotic dose over 4-28 week durations[31] that may reduce translation of the results to humans.

4.2 POLYPHENOLS

Polyphenolic compounds are secondary metabolites produced by plants, with a huge variety of chemical structures and properties but all contain at least one hydroxylated aromatic ring structure. Many of them have antioxidant, anti-

inflammatory and metabolic activities that are believed to confer their nutritional benefits in humans[40]. However, the mechanisms of action of many of these compounds are unclear, as their bioavailability as intact species is likely low and undocumented for most compounds. Some activities are likely secondary to their effects on the gut microbiome[41]. Interest in polyphenols is high as many traditional medicines contain polyphenolic compounds that may explain their effects. In this review, curcumin and resveratrol are considered as 2 representatives of the many polyphenols that have been studied in the context of MASLD.

4.2.1 Curcumin

The active compounds in turmeric (*Curcuma longa*), used as a spice in many cuisines, is curcumin.

Curcumin is acknowledged as a powerful antioxidant and anti-inflammatory agent and has been used in traditional medicine formulations[42]. Over 300 clinical trials have been registered on ClinicalTrials.gov for conditions from cardiovascular disease to various cancers.

4.2.1.1. Curcumin effects in human randomized controlled trials

In a recent meta-analysis that included 11 studies (all conducted in Iran), curcumin administered to adults with NAFLD had significant benefits on liver markers including AST and ALT, while also reducing body mass index, triglycerides, total cholesterol and homeostatic model assessment for insulin resistance (HOMA-IR). There was no significant effect on low density lipoprotein (LDL)- or HDL-C[43]. The largest study had less than 90 participants. Two trials used turmeric (2-3 g/day), 4 used curcumin (0.25-1.5 g/day) and 3 used curcumin in combination with another natural health product. One trial combined curcumin with resistance training and compared with placebo or resistance training alone, while the others used appropriate placebo controls. The interventions ranged from 8-12 weeks and adverse events in excess of control groups were not noted[43].

A second meta-analysis[44] examined steatosis and fibrosis related outcomes in 16 randomized controlled trials ranging in length from 8-24 weeks. A strength of this analysis is that it included trials that utilized ultrasonography to diagnosis hepatosteatosis and interventions with curcumin or turmeric (2 trials, 0.5 and 2 g/day) were compared with placebo or no treatment controls. Curcumin doses differed by at least 4-fold between studies, with several formulations mentioned. Curcumin/turmeric reduced NAFLD severity and was 4-fold more likely to resolve steatosis than placebo. Effects on liver scarring were not significant. Other significant markers included body mass index, ALT, AST and total cholesterol but LDL- and HDL-C and triglyceride were not affected. However, all studies had a small number of participants and overall study quality was low to moderate. In 3 studies that evaluated adverse gastrointestinal symptoms there was no difference between intervention and control[44].

Overall, curcumin consistently lowered liver enzymes and total cholesterol. Hepatic outcomes such as NAFLD severity score and steatosis were also improved. There were small cardiometabolic benefits identified in some studies. Safety issues were not identified. However, the evidence is generally graded low to moderate and the majority of trials are conducted in a single country. Additional well-structured trials with a greater number of participants of varying ethnicity are required.

4.2.1.2 Curcumin effects in animal experiments

In animal models, curcumin appears to activate a variety of mechanisms to reduce steatosis in the liver as well as having indirect effects via the adipose tissue and pancreatic islets. There is some evidence that curcumin treatment can alleviate fibrosis as well as steatosis in mouse and other animal models.

Treatment of mouse models with native curcumin suggests that it may influence a number of hepatic metabolic pathways including those associated with negative regulation of fatty acid uptake and de novo lipogenesis and induction of fatty acid oxidation[45]. In addition, modulation of nuclear factor kappa-B (NF-κB), a master regulator of inflammation, has also been reported in a model of

NASH in which treatment attenuated steatosis and fibrosis while lowering liver enzymes[46]. These results are intriguing because many compounds reduce steatosis but are ineffective on fibrosis. Benefits of curcumin on fibrosis were also reported by other investigators[47]. Another study reports that curcumin reduces inflammation by modulating macrophage phenotype in both adipose and liver tissues[48]. Consistently, curcumin down-regulates proteins involved in the regulation of lipogenesis[49]. However, curcumin may also target hepatic stellate cells reduce fibrosis, notably and somewhat paradoxically by increasing their capacity to store lipid droplets[50], highlighting the complexity of curcumin's effects on MASH pathology.

Non-absorbed curcumin reaches the colon and evidence suggests that some of its therapeutic effects are exerted by microbial metabolites. Curcumin treatment in mice with steatosis attenuated liver fat accumulation, along with increased proteins associated with improved gut barrier function, reduced circulating lipopolysaccharide, lower hepatic NF-κB and reduced inflammatory markers in the liver and circulation[51]. Curcumin favorably altered the *Firmicutes*/*Bacteroidetes* ratio, increased *Akkermansia* and short-chain fatty acid-producing genera, all of which have been associated with improved metabolic profiles in mice fed high fat diets[52].

The effects of curcumin in a model of hepatocellular carcinoma have been reported recently[53]. Provision of curcumin attenuated steatosis and improved the metabolic phenotype as in other studies. Seven of 8 MASH-hepatocellular carcinoma model mice developed 8-10 hepatic tumors, which were reduced in number to 0-2 by curcumin concomitant with lower hepatic inflammatory markers, macrophage infiltration and fibrosis[53].

Limitations of curcumin bioavailability are being addressed through novel formulations such as liposomes and nanoparticles[54]. Several studies in mice show that strategies such as synthetic formulations with increased half-life, improved stability at physiological pH or enhanced gut absorption have better efficacy than the native molecule to reduce hepatic steatosis[55-57]. Liposomal curcumin preparations injected intravenously into mice with NASH ameliorated hepatic steatosis, inflammation and insulin resistance. Some of the pathways modulated by the treatment were associated with fibrosis and immune activation[58].

A main limitation of these studies is that curcumin induces reduced weight gain in most models. Therefore, it is difficult to ascertain whether the effects of curcumin on liver endpoints is direct or mediated via changes in the visceral adipose tissue, which then alter hepatic function (see Section 2.2.1). Studies that control for changes in weight are required to fully understand this issue. Overall, the results are generally consistent with human trials showing benefits on steatosis and liver enzymes but effects on advanced endpoints like fibrosis and hepatocellular carcinoma shown in some animal studies have not yet been replicated in humans.

4.2.2. Resveratrol

Resveratrol is classified as a stilbene polyphenol and is found in berries, nuts, grapes and, notably, red wine[59]. It initially received considerable research attention as a potential mediator of the "French Paradox" effect[60].

4.2.2.1 Resveratrol effects in human randomized controlled trials

Yang et al.[43] reported on 6 placebo-controlled studies conducted in 4 countries. The study duration was 8-24 weeks, and the dose of resveratrol varied by 20-fold, from 0.15 to 3 g/day. Resveratrol administered to adults with NAFLD had no significant benefits on any outcome included in the systematic review including liver enzymes, BMI, lipids or HOMA-IR. The largest study had 105 participants. The meta-analysis had limitations, including only 2 studies with evaluable data for HOMA-IR. Scrutiny of individual studies indicates that almost none of the endpoints were statistically significant, thus the findings of null effects in the meta-analysis is unsurprising.

4.2.2.2. Resveratrol effects in animal experiments

A review of older trials in mice or rats found 9 studies in which hepatic steatosis was induced by diet manipulation or in genetically susceptible strains. In all of them, indicators that resveratrol treatment (ranging from 10-400 mg/kg/day dose and 4-10 weeks duration) improved steatosis were consistently observed. Most studies identified mechanisms including down-regulation of lipogenesis enzymes or induction of fatty acid oxidation[59]. This is consistent with resveratrol's known action through sirtuin-1 (Sirt1)[61]. Physiologically, the Sirt1 pathway is activated by energy depletion e.g. during fasting, which would serve to limit lipogenesis and increase fatty acid oxidation[62]. Sirt1 silencing effectively blocked the benefits of resveratrol on lipid accumulation in hepatocytes[63].

More recent animal experiments have examined other pathways. Reduction of steatosis and liver enzymes are consistent findings[64-68] with mechanisms identified including antioxidant[64,69] and anti-inflammatory effects[67,68,70] involving NF-κB down-regulation[64-66]. Some studies in NASH models also report anti-fibrotic effects of resveratrol treatment, even in the absence of improved steatosis[70].

Studies of resveratrol in cancer models identified DNA methylation as a target. Hosseini et al.[71] reported that high fat diet-fed mice have increased methylation of the Nrf2 promoter in liver tissue, which was reversed by resveratrol treatment. This was associated with down-regulation of lipogenic enzymes. Resveratrol also modulates the gut microbiome in mice with hepatic steatosis, improving gut integrity and increasing short chain fatty acid-producing genera[72].

Autophagy refers to lysosomal degradation of cell components that may be dysfunctional, thereby re-establishing cellular homeostasis. Autophagy was identified as an important process in restoring the health of the liver in steatotic mice[66]. Zhang et al.[73] and Ji et al.[74] also reported induction of autophagy in cell-based studies and in mice[73,74].

As for curcumin, tissue delivery in efficacious doses can be improved using technology. Nanoparticles specially constructed to be taken up by the liver were used to deliver resveratrol in a mouse model of NAFLD. The system reduced steatosis and activated NF-κB, consistent with resveratrol reaching its target[75]. Another group, using a similar nanocarrier strategy to target the liver, demonstrating increased uptake, activation of Sirt1-related pathways to reduce lipogenic potential and reversal of NAFLD endpoints[76].

In rodent models, resveratrol consistently reduces hepatosteatosis, likely through multiple mechanisms including suppression of lipogenesis, anti-inflammation and anti-oxidation with induction of autophagy. There is some limited evidence for benefits on fibrosis and even tumour formation. However, given the lack of effect in human trials, the future of resveratrol for treating MASLD is uncertain.

4.3 OMEGA-3 FATTY ACIDS

Omega-3 fatty acids belong to the polyunsaturated long-chain class of fatty acids and are essential for human health. As bioconversion from the precursor alpha-linolenic acid is poor in humans, dietary consumption of the bioactive molecules eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) should be encouraged. Common sources of EPA and DHA include fatty fish (e.g. salmon, mackerel, tuna) and other marine organisms (e.g. krill). Alpha-linolenic acid is present in certain plant oils (e.g. flaxseed, canola, soy oils), chia seeds and walnuts[77]. As noted in Section 1, many people consume omega-3 fatty acids as fish oil supplements. Doses up to 5 g/day for adults are deemed safe by the European Food Safety Authority. Although there is much research on the potential for increased omega-3 fatty acid consumption in the prevention or treatment of several chronic diseases, including cardiovascular disease, cancers and Alzheimer's Disease, it remains inconclusive[77]. Fatty acids of this class have decreased abundance in livers of people with NAFLD[78]; therefore, there is a rationale

for studying whether increasing consumption of omega-3 fatty acids can have a benefit on steatosis and other endpoints.

4.3.1 Omega-3 fatty acids effects in human randomized controlled trials

In the network meta-analysis of Theodoridis et al.[33], administration of omega-3 fatty acids in combination with lifestyle intervention to children with NAFLD was the most effective intervention in improving the primary outcome, ALT. The comparators in the network included combination therapies of omega-3 fatty acids with vitamin D or vitamin E ± choline, metformin, lifestyle alone, probiotics and others. Regarding AST and GGT, omega-3 fatty acids + lifestyle was effective, and also for HDL-cholesterol and triglycerides. A strength of this review is that the selected studies used histology or ultrasound to diagnose NAFLD but the outcomes in the analysis did not include a measure of steatosis. The interventions were variously recorded as omega-3 long-chain polyunsaturated fatty acids (1 study), docosahexaenoic acid (DHA, 4 studies) or DHA+ eicosapentaenoic acid (1 study). The results are consistent with other meta-analyses[79,80].

A systemic review of 13 studies examined the effects of omega-3 fatty acids on oxidative stress markers in adult populations with NAFLD/NASH in the absence or concomitantly with type 2 diabetes and/or cardiovascular disease[81]. Diagnosis in the included studies was confirmed by biopsy/ histology or ultrasound. Interventions included fish oil (1 study, 3 months duration), EPA (2 studies, 12 months duration) or a combination (8 studies, 6-48 months duration) or unspecified omega-3 polyunsaturated fatty acids (2 studies, 6-24 months duration). Some trials also included specific dietary modification, e.g. American Heart Association diet with caloric restriction. Narrative synthesis arrived at a conclusion of inconsistent results. For example, only 5 of 8 studies found benefits on any of liver enzymes, liver fat or other metabolic markers in participants with NAFLD. Two of the longest studies found no effect. In studies of people with NASH, only 2 of 6 reported beneficial outcomes. The review authors noted difficulty in reaching conclusions, given the wide range of formulations, dosing and duration of studies[81].

An umbrella review published in 2023 attempted to address the inconsistent findings regarding omega-3 fatty acid treatment of MASLD in 8 previous meta-analyses, which encompassed 6561 participants[82]. The analysis found significant benefit of omega-3 fatty acid treatment on ALT (6 studies), AST (6 studies), GGT (5 studies) and liver fat (2 studies). Clearly, more studies with objective measures of steatosis are required to resolve whether omega-3 fatty acids are useful in MASLD treatment.

Another group conducted a systematic review and Bayesian network meta-analysis that included 3 trials of omega-3 fatty acids among the treatment options[83]. The studies analysed were of NAFLD concurrent with type 2 diabetes. However, none of the omega-3 fatty acid trials reported liver fat content. Moreover, omega-3 fatty acid effects were not different from controls for glycated hemoglobin, body mass index, waist circumference, insulin resistance or ALT[83].

Several other reviews have been published in the last 5 years. In particular, de Castro and Calder highlight hepatic metabolic pathways that, in theory, could be influenced by omega-3 fatty acids to prevent or treat MASLD, including NF-κB and regulators of lipogenesis, which were discussed in the context of curcumin and resveratrol (Sections 4.2.1.2 and 4.2.2.2), as well as enzymes that regulate fatty acid oxidation or lipid transport[84]. They conclude that null effects of omega-3 fatty acids in 5 out of 17 studies reviewed could be a result of trial design, patient compliance or other factors, and methods used to measure outcomes.

Current U.S. guidelines concur that the evidence for omega-3 fatty acids in the treatment of MASLD is unconvincing; however, approved health claims that they may be useful for hypertriglyceridemia are included in the guidance statements[19]. The EASL-EASD-EASO guidelines do not recommend omega-3 fatty acids[21].

4.3.2. Omega-3 fatty acids effects in animal experiments

Investigations of liver steatosis find that providing fish oil at 2% of energy to mice fed high fat diet ameliorated steatosis, inflammation and fibrosis, along with liver enzymes; this effect did not involve a reduction in oxidative stress[85]. Other studies have similar findings for a combination of DHA and EPA[86-88], which partially resolved steatosis. Whereas high fat diets upregulated genes associated with inflammation and lipogenesis, the omega-3 fatty acid treatment reduced abundance of those genes, including NF-κB while increasing peroxisome proliferator-associated receptor-α to promote fatty acid oxidation [86]. Omega-3 fatty acids also induce Nrf2, a master controller of antioxidant responses[89].

Regarding NASH, mice fed a combination of high fat diet and dextran sodium sulfate (HF-DSS), a polysaccharide that induces gut inflammation, develop hepatic steatosis and inflammation with fibrosis. That gut inflammation induces liver inflammation when high fat diet alone does not is consistent with multiple hits being needed to develop NASH. Although studies of omega-3 fatty acids in the HF-DSS model have not been published, it is speculated that omega-3 fatty acid could be protective, given their ability to reduce gut permeability, systemic lipopolysaccharide and gut inflammation scores[90].

Combinations of omega-3 fatty acids with other fats may also be efficacious as shown by studies combining DHA with extra virgin olive oil [91] or EPA with hydroxytyrosol, a phenolic compound extracted from olive tree leaves[92,93]. Finally, the source of omega-3 fatty acids may dictate their efficacy. For example, omega-3 fatty acids sourced from marine algal oils have a higher abundance of phospholipids relative to triglycerides, in comparison with omega-3 fatty acids sourced from fish oil, and this is associated with greater anti-steatotic activity in direct comparisons[94].

Thus, for omega-3 fatty acids the animal data appears more consistent than the human data regarding resolution of MASLD outcomes. It has been suggested that for humans, omega-3 fatty acids may play a more important role in MASLD prevention and maintaining liver health[90]. In addition, better designed trials of adequate length may help resolve inconsistencies.

5. Limitations

While the approval of a drug to treat MASH is an important step in more specific treatment for MASLD, there is still room for more therapies, particularly those that are well-tolerated and have good safety profiles and that target earlier disease stages to prevent progression to MASH. Many natural health products, like probiotics and omega-3 fatty acids are already used by millions of people as dietary supplements for general health and are generally safe.

In Canada, most trials must be conducted under the authorization of the Natural and Non-prescription Health Products Directorate to ensure that safety and efficacy are appropriately evaluated and that the trial is supported by preclinical data. However, trials of natural health products are often conducted with low budgets as they are not supported by large pharmaceutical companies, and therefore tend to have small enrolment in a single experimental centre, which limits gathering definitive data.

With respect to MASLD, mouse and rat models of steatosis can be readily induced with high fat diets or by feeding methionine-deficient diets[95] but inflammation and fibrosis are not reliably identified. Treatment of mice with CCl_4, DDS or other hepatic toxins in combination with high fat diet exacerbates the inflammatory/fibrotic profile; thus, these types of models are needed for study of MASH-related outcomes[90,95].

6. Conclusions

In conclusion, efficacy against MASLD outcomes has yet to be clearly shown in human trials for either probiotics or omega-3 fatty acids, although some studies do suggest a benefit, necessitating larger

trials using adequate doses and duration. Curcumin is also popular as a supplement with anti-oxidant and anti-inflammatory properties, and several studies of safety have not raised serious concerns[43,44]; with additional data it may be found acceptable as a therapy for MASLD. The promising findings for resveratrol in animal trials have not replicated in humans.

Conflict of Interest:

None.

Funding Statement:

None.

Acknowledgements:

None.

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