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TITLE PAGE

Title

Systematic Review: Nutrition and Physical Activity in the Management of Paediatric Non-alcoholic Fatty Liver Disease

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Philippa S. Gibson: Contributed to study concept and design, undertook data acquisition and quality assessment, drafted the report.

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Michelle L Blumfield: Contributed to study concept and design and to the critical revision of the manuscript for intellectual content.

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STRUCTURED ABSTRACT

Objectives: To evaluate efficacy of nutrition and physical activity interventions in the clinical management of paediatric non-alcoholic fatty liver disease (NAFLD). The prevalence of paediatric NAFLD continues to rise alongside childhood obesity. Weight loss through lifestyle modification is currently first-line treatment, although supplementation of specific dietary components may be beneficial.

Methods: Medline, CINAHL, EMBASE, Scopus and Cochrane Libraries were systematically searched to identify randomised controlled trials (RCTs) assessing nutritional and physical activity interventions. Primary outcome measures were changes to liver biomarkers assessed by imaging, histology or serum liver function tests. Study quality was evaluated using the American Dietetic Association Quality Criteria Checklist.

Results: Fifteen articles met eligibility criteria investigating nutritional supplementation (vitamin E [n=6], probiotics [n=2], omega-3 fatty acids [n=5]), dietary modification (low glycaemic load [n=1] and reducing fructose intake [n=1]). No RCTs examining physical activity interventions were identified. Vitamin E was ineffective at improving alanine transaminase levels, while omega-3 fatty acids decreased hepatic fat content. Probiotics gave mixed results while reduced fructose consumption did not improve primary outcome measures. A low glycaemic load diet and a low fat diet appeared equally effective in decreasing hepatic fat content and transaminases. Most studies were deemed neutral as assessed by the American Dietetic Association Quality Criteria Checklist.

Conclusion: The limited evidence base inhibits the prescription of specific dietary and/or lifestyle strategies for clinical practice. General healthy eating and physical activity guidelines, promoting weight loss, should remain first-line treatment until high quality evidence emerges that support specific interventions that offer additional clinical benefit.

Key words

Vitamin E; Fructose; Omega 3 Fatty Acids; Probiotics.

SUMMARY BOX:

What is known:

- The prevalence of paediatric non-alcoholic fatty liver disease (NAFLD) continues to rise alongside childhood obesity.
- Weight loss through diet and lifestyle modification is currently first-line treatment, but difficult to achieve and sustain for many patients.

What is new:

- This review highlights the lack of high quality RCTs examining nutrition or physical activity interventions in paediatric NAFLD
- There is insufficient evidence to recommend dietary supplementation (vitamin E, probiotics, omega-3 fatty acids) for all paediatric NAFLD patients
- There is no evidence on which to base the type, frequency and duration of physical activity, which should be prescribed to children with NAFLD.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) describes a spectrum of hepatic injury associated with the accumulation of fat in the liver in the absence of an inherited metabolic defect or exposure to toxins including alcohol. Childhood obesity is on the rise, and in western obese paediatric populations, NAFLD prevalence is now estimated to range from 36% (1) up to 80% (2) . Due to a lack of pharmacological options for NAFLD treatment, lifestyle modification, targeting weight loss through reduced energy intake and increased physical activity, is currently recommended as the first line of treatment in both adults and children (3, 4). However, there are currently no specific guidelines for the type of dietary (e.g. supplementation and/or restriction of specific nutrients) or physical activity modifications (type, frequency, length of activity) that should be prescribed to children with NAFLD (5). Paediatric lifestyle interventions for NAFLD management have ranged from intensive 1 month lifestyle interventions (6) resulting in significantly improved alanine or aspartate transaminase (ALT and AST) levels, to 2-year interventions involving increased physical activity, nutrition education and behaviour therapy with supplementation (7-12) with varying impact on markers of liver function.

With lifestyle modification central to NAFLD management, it is essential that effective strategies be communicated to patients and their families, incorporating specific evidence-based nutrition and physical activity recommendations. Therefore the first aim of this systematic review was to determine the strength of evidence available for nutritional and lifestyle modifications in paediatric NAFLD subjects. Specifically, using stringent inclusion criteria, including only gold standard randomised controlled trials (RCTs), and critically appraising trial quality using the American Dietetic Association evidence analysis approach and quality criteria checklist (13). Then based on the current evidence, our second aim was to provide clinical guidance and insight to clinicians, dieticians and other health professionals as to whether specific dietary and physical activity guidelines should be advised for clinical management and/or prevention of paediatric NAFLD.

METHODS

This review was restricted to RCTs reporting the effects of nutrition and physical activity interventions on liver biomarkers in children diagnosed with NAFLD. We accepted any definition of NAFLD for the purpose of this review.

Eligible studies included publications written in English from any country with human participants (<19 years of age) with a diagnosis of NAFLD. Studies assessing food groups, nutrient intake, nutritional supplements, weight loss and/or physical activity were included. Only study arms where medications were used as part of standard treatment, without a reported increase in dose, and where participants were receiving medication prior to the intervention were included. There were no restrictions on the length of intervention or follow-up period.

Studies involving participants with late stage liver disease or conditions not commonly associated with NAFLD, and/or that influence dietary behaviour such as coeliac disease and severe malnutrition, were excluded. Studies involving participant's receiving enteral and/or parenteral nutrition were also excluded.

Literature Search:

Two independent researchers (SL, PSG) conducted separate literature searches to identify and appraise studies. Databases were searched from their inception until 18th January 2016. Databases include Medline, CINAHL, EMBASE, Scopus and The Cochrane Collaboration Libraries. Search results were verified and discrepancies resolved by group discussion (SL, PSG, JBM, KHH).

The selected search terms included: (NAFLD OR "non alcoholic fatty liver disease" OR "non-alcoholic fatty liver disease" OR NAFL OR "non alcoholic fatty liver" OR "non-alcoholic fatty liver" OR NASH OR "non alcoholic steatohepatitis" OR "non-alcoholic steatohepatitis") AND (diet* OR nutri* OR food OR weigh* OR overweight OR obes* OR BMI OR "Body Mass Index" OR "physical activit*" OR exercis* OR fitness) AND (Child* OR P#ediat* OR Adolescen* OR infan*). Related MeSH headings were used in Medline, CINAHL, Scopus and Cochrane libraries. Searches for EMBASE and Cochrane were modified to include the search terms pediat* OR paediat*. An example of specific search strategy details is shown in Table S1 (see Table S1, published online).

All articles were managed in a citation management software program (Endnote). All coding of articles for the first and second pass were completed using Endnote. Any study published only in abstract format or unpublished reports were excluded from the analysis. Titles and abstracts were evaluated against eligibility criteria. Studies appearing to be eligible based on abstract were read in full. Reference lists of eligible articles were hand-searched for additional articles that met the inclusion and exclusion criteria.

Data Extraction and Study Quality:

Data were extracted into Endnote independently by two researchers (SL and PSG) using an adaptation of the Cochrane Data Extraction Tool (14). Data extracted from the articles included: study setting, study population, participant demographics and baseline characteristics, inclusion and exclusion criteria, diagnosis criteria for NAFLD, details of the intervention and control conditions and any co-treatments, study methodology and blinding, recruitment and study completion rates, outcomes and times of measurement and effect size. The same two researchers (SL and PSG) independently assessed the risk of publication bias and study quality using the American Dietetic Association Quality Criteria Checklist (13). Any discrepancies were discussed and resolved by consensus. This checklist includes ten validity questions based on the Agency for Health and Research Quality, Important Elements for Research Studies. These elements identify sound study design and execution. If most of the answers to the validity questions (6 or more) were 'No', the study was deemed 'negative'; if the answers to questions 2, 3, 6 and 7 did not indicate that the study was exceptionally strong, the report was deemed 'neutral'; and if most questions, including the four core questions with at least one additional question were answered 'yes', then the study was deemed 'positive' (13).

Outcome Measures:

The primary outcome measures included changes; degree of histological change (fat, inflammation or fibrosis), change in ultrasonic echogenicity, change in liver fat as measured by MRI or change in transaminases (ALT, AST or gamma-glutamyl transpeptidase (GGT)).

Considering the role of dyslipidaemia, insulin resistance (IR) and adiposity in NAFLD pathogenesis (4), secondary outcome measures included changes to markers of cardiovascular risk [HDL, LDL, triglycerides, total cholesterol], insulin resistance [2-hour oral glucose tolerance

testing (2-h OGTT), fasting insulin levels (FI), fasting glucose levels (FSG), fasting glucose or insulin ratio's] and anthropometry [weight, BMI, BMI z-score and waist circumference (WC)]. Secondary outcome measures were only collected from eligible studies when available.

RESULTS

A total of 8441 records were derived from database searches. 1003 duplicates were removed, 3921 papers were excluded by title and 3402 by abstract. Of the 114 articles reviewed in full, 5 articles were conference abstracts, 82 articles were not in a population of interest and 12 did not report on a primary outcome of interest (Figure 1). Fifteen articles met the inclusion and exclusion criteria. Two of the eligible articles reported data from an extension of a RCT (9, 11). Results from the initial trial and extension have been combined for the purpose of this review (excluding ADA quality checks) (8-11).

Overall, 821 individuals participated in 13 RCTs ranging in duration from 4 weeks to 24 months (Table 1). Of the studies that met eligibility criteria, 11 trials investigated nutrient or nutritional supplementation (vitamin E (7-9, 15, 16), probiotics (17, 18) and omega-3 fatty acids (10, 11, 19-21)) and two assessed dietary modification (low glycaemic load diet (22) and a reduction of fructose intake (23)).

Study Population Characteristics

Inclusion and exclusion criteria varied between studies; nine studies required participants to be classified as obese with a BMI centile over the 85th (17, 20, 23) or 95th (6, 15, 16, 18, 21, 22) centile for gender and age. The mean/median BMI and BMI z-score of participants in each study arm at baseline ranged from 24.4 to 34.7kg/m^2 and 2.01 to 3.44 respectively. Jin et al. (23) and Alisi et al. (17) only included participants who identified as being of Hispanic (23) or Caucasian (17) descent respectively, while the remaining RCTs did not exclude participants based on ethnicity.

Diagnosis of NAFLD

To meet eligibility criteria of this review, all participants required a confirmed diagnosis of NAFLD; however diagnosis criteria varied between studies. Seven trials required a more robust method of diagnosing steatosis or fibrosis based on histology, with six of these trials also measuring transaminases (7-9, 11, 17, 20); whereas five trials included participants with diagnosis

of NAFLD via ultrasound (US) alongside elevated transaminases (6, 16, 18, 19, 21). Two studies (22, 23) made a NAFLD diagnosis using MRS whereas one study (15) based the NAFLD diagnosis on evidence of hepatic steatosis using ultrasound exclusively. Additionally, Alisi et al. (17) only included participants with diagnosed NASH based on histology.

Vitamin E

Our search identified six studies prescribing vitamin E supplementation (6-9, 15, 16). The formulation and dosage of vitamin E varied between studies with Ackam et al. (15) and Wang et al. (6) failing to specify the formulation of vitamin E prescribed. Nobili et al. (8, 9) prescribed alpha-tocopherol in conjunction with ascorbic acid while Vajro et al. (16) prescribed D-alpha-tocopherol acetate.

Five studies noted a decrease in ALT levels post-intervention (6-9, 16) with no significant difference in overall decrease between intervention and control [16, 18, 21, 22]. Conversely, Wang et al. (6) found that the decrease in ALT levels was greater in the intervention arm compared to the controls who received no lifestyle intervention. Three studies measured changes to liver imaging post-supplementation. Vajro et al. (16) and Akcam et al. (15) noted improvements to bright liver echogenicity whereas Wang et al. (6) noted no change in hepatic fat content measured with ultrasound after one month of supplementation. Two studies analysed histological changes post-intervention. Nobili et al. (8, 9) noted that improvements to NAFLD activity score were similar between intervention and control groups whereas Lavine et al. (7) found that resolution of NASH was significantly greater among children treated with Vitamin E compared to a placebo ($p=0.006$).

Probiotics

Two RCTs in children were identified, evaluating the influence of either single strain (Lactobacillus rhamnosus strain GG) (18) or multistrain VSL#3 (17) probiotic supplementation on hepatic biomarkers. Although Vajro et al. (18) reported no effect of lactobacillus rhamnosus strain GG on liver echogenicity, there was a significant decrease in average ALT variation in children treated with Lactobcaillus rhamnosus strain GG as compared to placebo. Conversely, Alisi et al. (17) found that VSL#3 supplementation reduced the severity of steatosis as assessed by ultrasound.

Omega-3 Fatty Acids

In total, five studies investigating the effect of omega-3 fatty acid supplementation in children with NAFLD were identified. The dosage and formulation of omega-3 fatty acids varied between the studies, with concentrations ranging between 250mg and 1300mg/day, while fish oil composition included DHA only (10, 11, 20) or a combination of DHA and eicosapentaenoic acid (EPA) (19). Three studies used linoleic acid as placebo (10, 11, 20) while one study used sunflower oil containing omega-6 LC-PUFA (19). Boyraz et al. did not describe either the PUFA composition or placebo content (21).

All five studies used liver enzyme levels as an assessment of NAFLD severity post-intervention. ALT (11, 19), AST (19) and GGT (19) levels were significantly reduced in intervention when compared to the placebo group while Boyraz et al. found no difference between intervention and placebo groups in ALT and AST reduction (21). All five studies used liver imaging as the method of detection post-intervention. Pacifico et al. was the only study to use MRS as a primary outcome measure. One study prescribing two different concentrations of omega-3 DHA (250mg vs. 500mg) alongside placebo indicated that after 6 months of DHA supplementation, the risk of severe steatosis was reduced (250mg = OR 0.01, $p<0.01$ / 500mg = OR 0.04, $p=0.01$) with no difference in dosage effectiveness as assessed by ultrasound (10, 11). Boyraz et al. showed decreased liver steatosis as assessed by ultrasound in PUFA group as compared to the control group after 12 months of treatment (21). Janczyk et al. did not observe any difference in degree of liver steatosis between omega-3 and placebo groups (19).

Dietary Modification

Two studies prescribing changes to dietary composition were identified with a total of 41 participants in the intervention and control arms (22, 23). Jin et al. (23) found that four weeks of reduced fructose consumption in an isocaloric diet did not improve hepatic fat content as measured by MRS. However, Ramon-Krauel et al. (22) found that a low GL diet in or a low fat diet improved hepatic fat content in both arms of the trial, with no significant differenced between the two groups after 6 months ($p=0.76$) as detected by MRS.

Both the low fat and low GI diets led to a decrease in BMI with no significant differences between the two groups (22), while Jin et al. reported no difference in body weight for either the fructose or glucose arms of the trial (23). However, the glucose arm of the Jin et al. study

demonstrated reduced fructose consumption significantly improved adipose IR (p=0.04) despite no change in overall BMI (23). Ramon-Krauel et al. showed both low fat and low GI diets showed improvements in ALT levels while the low fat group also showed significant improvements in insulin sensitivity (FI, HOMA). However, the low fat group also showed significant improvements in bot the waist circumference (p=0.02) and waist to hip ratio (p=0.01) after 6 months, which could contribute to these findings (22).

Lifestyle Modification

Notably, most trials recommended lifestyle modification as a co-intervention (7-11, 15-17, 19-22). The details and dietary targets guiding lifestyle modification were often limited, with three publications completely failing to outline the dietary and physical activity recommendations (7, 10, 19). Details of the interventions, their outcome measures and reported changes are in Table 1. No RCTs were identified which investigated the impact of exercise on hepatic biomarkers of children with diagnosed NAFLD. Four of the five studies reported reduced steatosis after supplementation with omega-3 fatty acids (10, 11, 20, 21) with the remaining study only reporting improvements in AST and GGT levels (19).

Study Quality

In utilising the ADA Quality Criteria, we identified four positive, ten neutral and one negative article (Table 2). The 11 articles deemed of 'neutral' or 'negative' quality were classified as such due to: unclear method of randomisation (6, 8, 15, 21-23); poor description of methodology (6, 8, 9, 15, 16, 19); unclear system of blinding (22); failing to measure effect size [excluding pilot studies] (6, 15, 16); failing to declare conflicts of interest (6, 8, 16) and no indication of funding source (9, 11). Ackam et al. (15) violated statistical assumptions and based conclusions on findings that were not statistically significant. Compliance to study protocols was poorly measured; pill count, pill diaries and/or patient interviews were only used in eight trials to measure participant adherence to supplementation regimes (8-11, 15, 19-21). In addition, only one vitamin E study (16) assessed compliance to vitamin E supplementation via serum levels of vitamin E. Similarly three studies assessed compliance by blood docosahexaenoic acid (DHA) levels pre- and post-intervention (10, 11, 20). Four trials assessed compliance with dietary recommendations using objective nutritional assessment tools (9, 15, 19, 22) with findings reported in two publications (19, 22). Weight loss

was used to assess dietary compliance in three studies (8, 9, 16). Compliance with supplementation recommendations or lifestyle modification was not measured by any means in two studies (6, 18).

DISCUSSION

The eligible RCTs for this systematic review include a variety of nutrition interventions for the management of paediatric NAFLD with considerable heterogeneity in study protocols and outcomes. Thirteen of the 15 eligible trials assessed the impact of various nutritional components or supplementation on NAFLD severity. Most notable were the differences in NAFLD diagnostic cirteria and primary outcome measures utilized between the studies. While diagnosis of NAFLD based on histology remains the gold standard, several of the studies included in this review were based on a NAFLD diagnosis from elevated transaminase levels, ultrasound or MRS. Abnormal liver function tests are a poor diagnostic tool, and have poor sensitivity and specificity for NAFLD with normal ALT levels commonly found in NAFLD patients and degree of elevation of transaminases poorly reflective of histological severity (24). Ultrasound is commonly used to assess for steatosis using echogenicity of the liver as it is time and cost effective, however, if levels of fat in the liver are low, or patients are morbidly obese (25), diagnosis may be missed. Additionally, MRS are more reliable non-invasive methods but are expensive, time consuming not commonly used in clinical practice (26). Furthermore, steatosis is not necessarily the problem at hand, but our inability to diagnose the more significant conditions NASH and fibrosis. Therefore, until more robust data based on histologically diagnosed paediatric NAFLD patients with dietary supplementation are produced, caution should be applied when prescribing nutritional supplementation for the treatment of NAFLD.

Vitamin E

While six of the RCTs evaluated the role of vitamin E supplementation in NAFLD treatment, it is difficult to directly compare trials as a result of: differing doses and/or unclear formulations of vitamin E; supplementation of vitamin E in conjunction with other antioxidants; variable intervention periods; and poor reporting of study results. The high quality TONIC trial by Lavine et al. (7) found no significant differences in ALT levels, their primary outcome measure, between the intervention

and control group after 96 weeks of supplementation. However, the authors noted significant improvements in liver histology among participants with biopsy-proven NASH.

Furthermore, vitamin E supplementation may be beneficial in children with poor adherence to lifestyle recommendations. Vajro et al. (16) stratified data based on compliance to lifestyle modification, using weight loss as an indicator of compliance. Normalisation of ALT was seen in control children who strictly adhered to lifestyle intervention and children who only complied with the supplementation regime with minimal adherence to dietary and physical activity recommendations. As poor compliance with weight reduction initiatives is a widespread issue among overweight and obese paediatric populations (27), the evidence base for the utility of vitamin E supplementation as a targeted strategy in children with NAFLD with poor compliance to lifestyle recommendations should be strengthened.

Probiotic Supplementation

There is increasing understanding of the role of the gut microbiota in NAFLD pathogenesis. However, only two RCTs in children were identified, evaluating the influence of either single strain (Lactobacillus rhamnosus strain GG) (18) or multistrain VSL#3 (17) probiotic supplementation on hepatic biomarkers. Whereas no effect of lactobacillus rhamnosus strain GG on liver echogenicity was found, in an ordinal logistic regression model, 4 months of VSL#3 supplementation reduced the risk of moderate and severe steatosis as assessed by ultrasound. In addition, participants supplemented with VSL#3 experienced a significant decrease in BMI from a mean of 27.1 to 24.9 however there was no improvement observed in mean ALT levels. The author's credit the positive effects reported on fatty liver and body weight to VSL#3-dependent reversal of dysbiosis, but this was not measured directly. Furthermore, the RCTs at 8 and 16 weeks were of short duration, preventing assessment of the long-term effects of probiotic supplementation. We note that long term follow up examining the effect of discontinuing probiotic supplementation is warranted in light of evidence showing resilience by the microbiome to short-term dietary interventions with rapid return to baseline composition observed (28).

Omega-3 Fatty Acid Supplementation

Intakes of PUFAs, in particular omega-3 fatty acids, have been implicated in NAFLD pathogenesis, with deficiency believed to contribute to hepatic fat accumulation and the

progression of steatosis to NASH. We identified five RCTs testing omega-3 fatty acid supplementation in paediatric NAFLD patients. These were well-designed trials with three out of the five studies (10, 11, 20) considered positive for overall quality rating based on the American Dietetic Association Quality Criteria Checklist. However, again direct comparison of the studies is difficult given differences in the concentration and composition of omega-3 fatty acid supplements used and the variable treatment periods. Four of the studies reported reduced steatosis after supplementation (10, 11, 20, 21) with the remaining study only reporting improvements in AST and GGT levels (19). Similar findings have been noted in a recent RCT conducted in adults, with a significant reduction in liver fat percentage in NAFLD patients supplemented with 4g DHA/EPA for 15 to 18 months (29). Interestingly, a study evaluating the consumption of fish and omega-3 fatty acids in children with NAFLD demonstrated very low intakes of fish, alongside low intakes of omega-3 fatty acids, which was associated with increased portal and lobular inflammation (30).

Dietary Modification

Although lifestyle and dietary modification aimed at weight loss is the first-line treatment for NAFLD, we only identified two RCTs evaluating diet-based interventions. Ramon-Krauel et al. compared a low-GL diet to a low fat diet with authors noting no significant differences in diet effectiveness. However, significant reductions in ALT levels were observed in both low-GL and low fat diets (22). Furthermore, despite the growing evidence implicating fructose in NAFLD pathogenesis, we only identified one study assessing fructose intake (31), based on high fructose beverages compared against equally high glucose beverages. Results ultimately noted no effect of reduced dietary fructose consumption on hepatic fat, as measured by MRS or liver enzymes. While eleven trials recommended a co-intervention involving dietary modification only two of these used objective measures to assess participant compliance. In general descriptions of the types of dietary modification recommendations made was poor and insufficient information regarding dietary measurement, participant compliance or the healthcare professionals involved was provided. Although there are clear challenges to measuring dietary intake, particularly in children and adolescents, reporting of dietary methods in line with recent recommendations for paediatric obesity interventions to assist with future meta-analysis should be encouraged for future studies (32).

Physical Activity

Although a significant body of evidence in adults suggests that physical activity may influence NAFLD severity (33), we did not identify any RCTs assessing the impact of exercise on hepatic biomarkers in children diagnosed with NAFLD. However, trials in obese paediatric populations (without NAFLD diagnosis per se) have demonstrated the positive effects of physical activity on steatosis as assessed by MRI (34, 35) or MRS (36, 37). One cohort study investigated lifestyle modifications in 84 Italian children with biopsy proven NAFLD and prescribed a balanced, low calorie diet with moderate aerobic activity (38). Results showed significant improvements in serum ALT and steatosis with a weight loss of over 20% body weight over a 1-year period. The same group then went on to show lifestyle interventions with diet and physical activity induced weight loss and were associated with significant improvements in liver histology (9). Twelve of the fifteen RCTs included in this review prescribed exercise recommendations as a co-intervention. However, the type and duration of physical activity prescribed to participants was poorly described with little or no rationale to justify recommendations. Furthermore, only Janczyk et al. used an objective assessment tool to evaluate adherence to physical activity recommendations (19). We therefore recommend that a high quality RCT should be undertaken to assess the influence of physical activity on relevant clinical markers, such as LFTs and imaging techniques, in children with diagnosed NAFLD.

Study Quality

While the subject of nutrition and physical activity management strategies in children with NAFLD was recently reviewed by Africa et al (39), the authors were were non-discriminant about the inclusion of non-randomised clinical trials and also included studies where children without a NAFLD diagnosis were included in the clinical population. In addition to our stringent inclusion criteria, one of the strengths of this systematic review was the use of the ADA Quality Criteria Checklist that provides an insight into the risk of bias for each study, and determines the quality rating for each study based on design. Overall, the majority of RCTs included in this review were evaluated as 'neutral' quality; therefore the results of these studies should be interpreted with caution (13). Based on our critique of the eligible studies it is apparent that future RCTs should aim to be more transparent in their reporting of diagnostic criteria and in the inclusion/ exclusion

criteria used to recruit study participants. Studies should complete a thorough assessment of participants' characteristics and behaviours that may contribute to NAFLD or confound study results. In addition, while dietary and physical activity may confound study results, adherence to lifestyle recommendations should be measured using an objective measure with results clearly reported.

Conclusions

This systematic review has identified the heterogeneity of studies assessing dietary and physical activity interventions in paediatric NAFLD patients. Findings from this systematic review suggest that vitamin E may be a possible treatment option in patients with biopsy proven NASH, or demonstrate poor compliance with lifestyle recommendations. Probiotic of omega-3 fatty acid supplementation may be a possible nutritional intervention to improved primary and secondary outcomes in children with NAFLD. Additionally, there is little evidence in children about the impact of physical activity on NAFLD management. Therefore, more high-quality RCTs should be completed to determine the type, frequency and duration of physical activity which should be prescribed to paediatric NAFLD population. At present, there is insufficient high quality evidence to prioritise a single approach for the effective management of paediatric NAFLD. Generalised lifestyle modification targeting weight loss through reduced energy intake and increased physical activity should remain the first line of treatment for paediatric NAFLD until additional high quality evidence is available.

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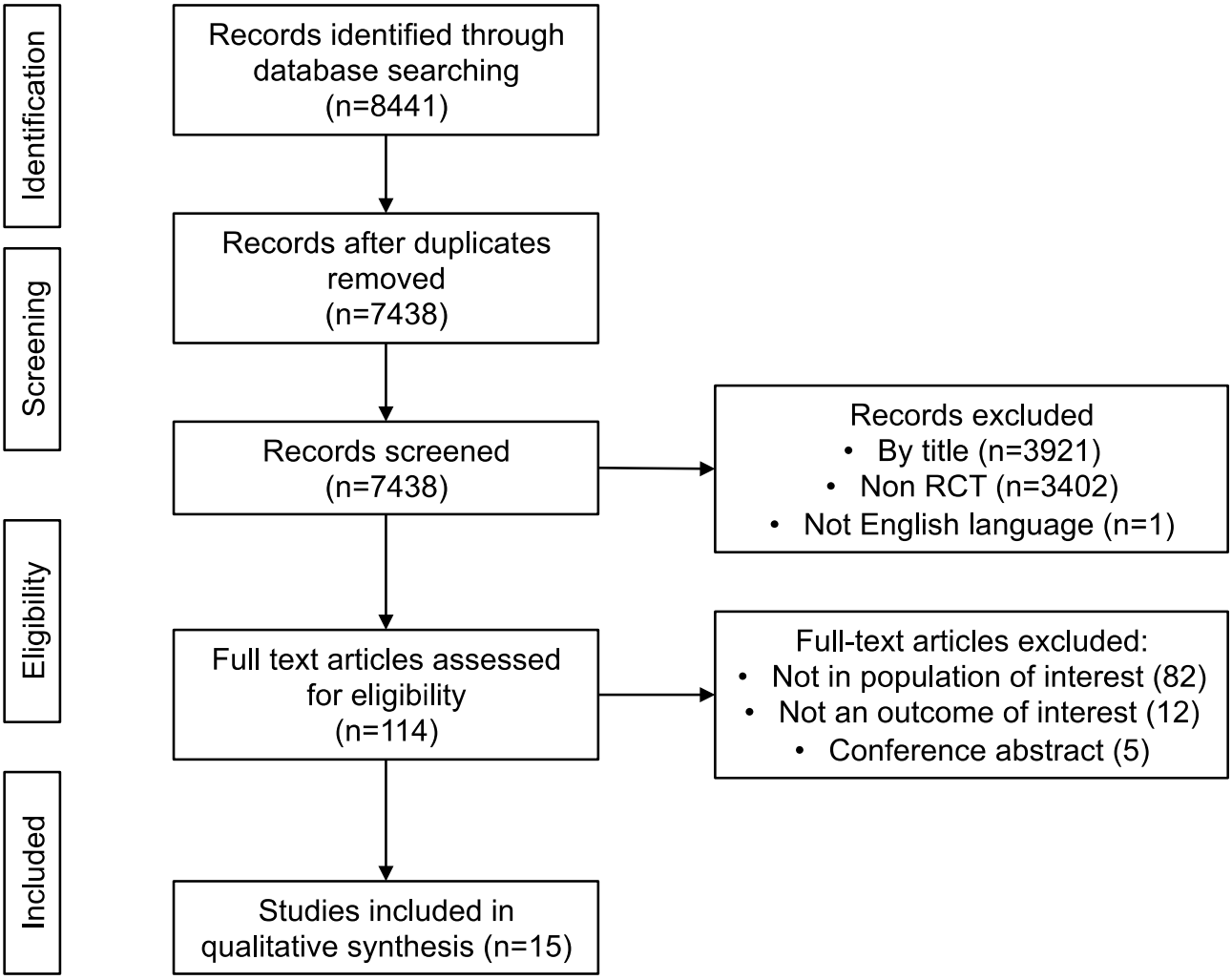

Figure 1: Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram of study selection process for systematic literature review

TABLE

Table 1: Summary of randomised control trials retrieved (n=15)

Author (Year); Country	Intervention [co-intervention and Control]	Age Range; Sample size (% male)	Study Duration [Control]	Outcome Measures	Changes to Outcome Measures Post-Intervention
Vitamin E					
Vajro et al. (2004); Italy	Oral α-acetatetocopherol (400mg/d 2 months, 100mg/d 3 months, 2 month washout) [Low calorie diet (Italian Recommended Dietary Allowanced) + exercise]	NR; 28 (75%)	7 months [Placebo]	Primary: US, ALT Secondary: BMI	Stratified results based on weight loss; 1. Decrease BMI + vitamin E: improved liver echogenicity, ↓ALT[a] 2. Decrease BMI + placebo: improved liver echogenicity, ↓ALT[b] 3. Stable BMI + vitamin E: improved liver echogenicity, ↓ALT[b] 4. Stable BMI + placebo; No change ALT or liver echogenicty[a]
Nobili et al. (2006, 2008); Italy	Alpha-tocopherol (600ID/d) + Ascorbic Acid (500mg/d) [1 hr monthly nutritional counselling. Hypo-/ Iso-caloric diet (Italian Recommended Dietary Allowance) + 45min/day aerobic exercise]	3-18; 90 (32%)	24 months [Placebo]	Primary: NAS[#], ALT, AST Secondary: Z-BMI, BMI, TG, TC, FSG, FI, HOMA-IR	Primary: ↓ NAFLD Activity Score, ↓ ALT, AST in intervention and control [b] Secondary: ↓ Z-BMI, BMI in intervention and control [b] ↑FSG, FI, TC, TG, HOMA-IR in intervention and control [b]
Wang et al. (2008); China	Vitamin E (100mg/d) + low calorie diet + excercise/ aerobic exercise + reduced caloric intake/ no intervention [NA]	10-17; 66 (64%)	1 month [Lifestyle control/ control]	Primary: US, ALT, AST Secondary: Z-BMI, TG, TC, FSG, FI, HOMA	Primary: No change to liver echogenicity [b]. ↓ ALT and AST in intervention group [a] Secondary: ↓ Z-BMI, TG, TC, FI, FSG and HOMA-IR in intervention group [a]
Akcam et	400IU/d Vitamin E	9-17; 67 (NR)	6 months	Primary: US	Primary: ↓ steatosis in intervention and control

al. (2011); Turkey	[Tailored dietary advice + 30 minutes aerobic PA/day]		[Control]	Secondary: BMI, TG, TC, FSG, FI, HOMA-IR	[a] Secondary: ↓ BMI in intervention [a] ↓ TC, LDL, TG, HOMA-IR in intervention and control [b] No change FSG, FI in intervention and control [b]
Lavine et al. (2011); USA	RRR-α-tocopherol (800IU/d) [Uniform standard-of-care advice on diet and exercise at each visit by physicians and dietitians]	8-17; 173 (81%)	120 weeks [Placebo]	Primary: NAS[#], ALT, AST, GGT, ALP Secondary: QOL, Z-BMI, BMI, BW, WC, TG, TC, FSG, HOMA-IR, HDL, LDL	Primary: Improved NAFLD Activity Score in intervention group [a], ↓ ALT, AST, GGT, ALP in intervention and control [b] Resolution of NASH was significantly greater in children treated with vitamin E than placebo [a] Secondary: ↑TG, BW, BMI, WC, QOL in intervention and control [b] No change Z-BMI, FSH, HOMA-IR in intervention and control [b] ↓ TC, HDL, LDL in intervention and control [b]
Probiotic					
Vajro et al. (2011); Italy	Oral Lactobacillus rhamnosus strain GG (12 billion CFU/day) [NA]	NR; 20 (90%)	8 weeks [Placebo]	Primary: US, ALT, AST Secondary: TG, TC, Z-BMI	Primary: No change in liver echogenicity, AST in both groups. ↓ALT in intervention group [a] Secondary: No change to secondary outcome measures in both groups
Alisi et al. (2014); Italy	2 sachets VSL#3/day [Low Calorie Diet (Italian Recommended Dietary Allowances) + aerobic exercise (3 x 30-40 mins/week)]	NR; 44 (55%)	4 months [Placebo]	Primary: US, ALT Secondary: TG, HOMA, BMI	Primary: ↓ risk of severe NASH (OR 0.001) [a]. ↓ ALT in intervention and control [b] Secondary: ↓ TG, HOMA in intervention and control [b] ↓ BMI in intervention group [a]
Omega-3 Fatty Acids					
Nobili et al. (2011, 2013); Italy	250mg vs. 500mg DHA/day [Low calorie diet]	<18 years; 60 (42%)	24 months [Placebo 290mg/day linolenic acid]	Primary: US, ALT Secondary: HOMA-IR, BMI, TG	Primary: ↓ risk of severe steatosis (250mg = OR 0.01/ 500mg = OR 0.04) [a], ↓ ALT with no difference between dosages [a] Secondary: ↓ TG, BMI, HOMA-IR with no difference between dosages [a], ↓ BMI in intervention and controls [b]
Bohraz et	1000mg/day omega-3	9-17; 108	12 months	Primary: US,	Primary: ↓ steatosis, ALT and AST in both

al. (2015);	[American Heart Association diet (50% CHO, 20% protein, 30% fat) with reduced caloric intake and PA 3 times a week with intention for weight loss]	(51%)	[Placebo]	ALT, AST Secondary: BMI, HDL-C, TG, TC, FI, FG, IS, HOMA-IR, SBP	intervention and control groups [a] Secondary: ↑ HDL-C [a],↓ TG, FI and HOMA-IR [a], ↓ BMI in intervention and control groups [b], ↓ SBP in intervention group [a]
Janczyk et al. (2015)	450-1300mg/day 3:2 DHA:EPA [Individually prescribed diet in conjunction with increased PA with intention of weight loss]	>5 and <19; 76 (86%)	6 months [Placebo omega-6 sunflower oil]	Primary: ALT, AST, GGT Secondary: FSG, FI, HOMA-IR, adiponectin, cholesterol, steatosis (US), BMI, WC	Primary: ↓ ALT levels in intervention and control [b] Secondary: ↓ AST and GGT [a] ↑ adiponectin in omega-3 group [a]. No changes in ALT, steatosis, FSG, FI, HOMA-IR, BMI z-score or WC z-score [b]
Pacifico et al. (2015)	250mg/day DHA [Low calorie diet (Italian Recommended Dietary Allowanced) + 60 mins PA 5 times a week]	<18 years; 51 (59%)	6 months [Placebo 290mg/day linoleic acid]	Primary: Hepatic fat (MRS), ALT Secondary: TG, Z-BMI, FI, HOMA-IR	Primary: ↓ Hepatic fat by MRS in intervention group [a] ↓ ALT in intervention group [b] Secondary: ↓ FI, TG [a], ↓ BMI, BMI-SDS, FI, HOMA-IR in intervention group [b]
Dietary Modification					
Ramon-Krauel et al. (2013); USA	Low Glycemic Load diet [nutritional education, exercise, grocery lists, food preparation demonstrations]	7-18; 17 (82%)	6 months [Conventional low fat diet]	Primary: Hepatic Fat (MRS), ALT, AST Secondary: BMI, HDL, LDL, TG, FI, FSG, HOMA-	Primary: ↓ hepatic fat (%), ALT, AST in intervention and control [b] Secondary: ↓ BMI, FI, FSG, HOMA-IR, LDL, HDL, TG in intervention and control [b]

				IR	
Jin et al. (2014); USA	Consumption of 3 x 8floz bottles/d (33g glucose/bottle) [no change to diet or physical activity]	11-18; 21 (52%)	4 weeks [Consumption of 3 x 8floz bottles/d (33g fructose/ bottle)]	Primary: Hepatic Fat (MRS), ALT, AST Secondary: BW, TG, FI, HOMA-IR, FFA, LDL	Primary: No change to hepatic fat (%), ALT, AST in intervention and control [b] Secondary: ↓ HOMA-IR, plasma FFA, LCL, FI in intervention group [a] No change in BW, TG in intervention and control [b]

[a]: statistically significantly difference when compared to the control
[b]: no statistical difference between intervention and control
[*]: VSL#3 is eight strains of probiotics (Streptococcus thermophilus, bifidobacteria [B. breve, B. infantis, B. longum], Lactobacillus acidophilus, L. plantarum, L. paracasei and L. delbrueckii subsp. bulgaricus)
[#]: NAFLD Activity Score is a numerical score of NAFLD severity [sum of separate scores for steatosis (0-3), hepatocellular ballooning (0-2) and lobular inflammation (0-2)]
Abbreviations: ALP = alkaline phosphatase; ALT = alanine transaminase; AST = aspartate transaminase; BMI = body mass index; CFU = colony forming units; CHO = carbohydrate; DHA = docosahexanoic acid; FI = fasting insulin; FSG = fasting serum glucose; GGT = gamma-glutamyl transpeptidase; HDL = high density lipoprotein; HOMA-IR = homeostatic model assessment of insulin resistance; IU = international units; LDL = low density lipoprotein; LV = left ventricular function; NA = not available; OR = odds ration; MRS = magnetic resonance spectroscopic imaging; NAS = NAFLD activity score; NR = not reported; NRT = non-randomised control trial; PA = physical activity; QOL = quality of life; RCT = randomised control trial; SBP = systolic blood pressure; TC = total cholesterol; TG = triglycerides; US = ultrasound; VAT = visceral adipose tissue; WC = waist circumference; Z-BMI = BMI Z-score.

Table 2: Quality rating and risk of bias determined using the American Dietetic Association Quality Criteria Checklist

Theme	Author	Validity Questions[1]										Overall Quality Rating
		1	2	3	4	5	6	7	8	9	10	
Vitamin E	Nobili (2006)											Neutral
	Nobili (2008)											Neutral
	Lavine(2011)											Positive
	Vajro (2004)											Neutral
	Wang (2008)											Negative
	Ackam (2011)											Neutral
Probiotics	Vajro (2011)											Neutral
	Alisi (2014)											Neutral
Omega-3	Nobili (2010)											Positive
	Nobili (2013)											Positive
	Boyraz (2015)											Neutral
	Janczyk (2015)											Neutral
	Pacifico (2015)											Positive
Low Glycaemic Load Diet	Ramon-Krauel (2013)											Neutral
Fructose	Jin (2014)											Neutral

Low Risk of Bias Risk of Bias

[1]Validity Questions: 1) Clear Research Question; 2) Unbiased Selection Of Participants; 3) Randomization/ Group Comparability; 4) Description Of Withdrawals; 5) Blinding; 6) Study Procedures Described; 7) Clearly Defined Outcomes; 8) Appropriate Statistical Analysis; 9) Results Support Conclusion; 10) Funding Or Sponsorship Bias Unlikely