Evaluations of Lifestyle, Dietary, and Pharmacologic Treatments for Pediatric Non-Alcoholic Fatty Liver Disease—a Systematic Review

Short title: Systematic review of pediatric NAFLD

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GYT, MJA, and JPM were all involved in the review design, data collection, data analysis, manuscript drafting, and review of the final manuscript. VN was involved in review design, data analysis, manuscript drafting, and review of the final manuscript.

Abstract:
Background & Aims: There are no approved treatments for pediatric non-alcoholic fatty liver disease (NAFLD) and there is a lack of consensus on the best outcome measure for randomized controlled trials. We performed a systematic review of treatments tested for pediatric NAFLD, the degree of heterogeneity in trial design, and endpoints analyzed in these studies.

Methods: We searched publication databases and clinical trial registries through January 7, 2018 for randomized controlled trials (published and underway) of children (<18 years) with NAFLD. We assessed improvements in histologic features, radiologic and biochemical markers of reduced fibrosis, metabolic syndrome parameters, and adverse events. The quality of the trials was assessed using a modified version of the Cochrane risk of bias tool.

Results: Our final analysis included 21 randomized controlled trials, comprising 1307 participants (mean age, 12.6 years; 63% male; mean duration of intervention, 8 months). Most studies evaluated weight loss with lifestyle intervention (n=8), oral polyunsaturated fatty acid treatment (PUFAs, n=6), or oral antioxidant treatment (n=7). Biomarkers of NAFLD decreased with weight loss, but most studies did not include histologic data. Trials of antioxidants were heterogeneous; some reported reduced histologic features of steatohepatitis with no effect on triglycerides or insulin resistance. PUFAs and probiotics reduced radiologic markers of steatosis, insulin resistance, and levels of triglycerides. Only 38% of the trials had biopsy-proven NAFLD as an inclusion criterion. There was heterogeneity in trial primary endpoints; 10 studies (48%) used levels of aminotransferases or ultrasonography findings as a primary endpoint and only 3 trials (14%) used histologic features as the primary endpoint. We identified 13 randomized controlled trials that are underway in children with NAFLD. None of the protocols include collection of liver biopsies; 9 trials (69%) will use magnetic resonance imaging quantification of steatosis as a primary outcome.

Conclusion: In a systematic review of published and active randomized controlled trials of children with NAFLD, we found a large amount of heterogeneity in study endpoints and inclusion criteria. Few trials included histologic analyses. Antioxidants appear to reduce some features of steatohepatitis. Effects of treatment with lifestyle modification, PUFAs, or probiotics have not been validated with histologic analysis. Trials that are underway quantify steatosis magnetic resonance imaging—outcomes are anticipated.

KEY WORDS: MRI, NASH, clinical trial design, progression, response

Introduction

Pediatric non-alcoholic fatty liver disease (NAFLD) refers to the spectrum of hepatic steatosis ('simple steatosis' or non-alcoholic fatty liver) and non-alcoholic steatohepatitis (NASH), with or without fibrosis[1]. NAFLD affects 5.5-10.3% children world-wide[2] and is closely related to the metabolic syndrome[3]; 34% of obese children have evidence of NAFLD[2]. With the acceleration in rates of pediatric obesity, the burden of pediatric NAFLD will increase similarly to that of adult NAFLD[4]. There is evidence that pediatric NAFLD is associated with increased mortality[5] and it is generally accepted that fibrosis is likely to be the major predictor of liver-related outcomes, as it is in adults with NAFLD[6], however the main long-term burden of NAFLD for patients is cardiovascular disease[7]. These clinical events will mainly manifest in adulthood, therefore altering the disease course of pediatric NAFLD may improve outcomes in later life.

NAFLD is identified either through screening of obese children or incidentally on imaging or with abnormal LFTs, but it cannot be formally diagnosed or staged without liver biopsy[8,9]. Current management focuses on weight loss through a combination of caloric restriction and physical exercise[10]. There are no licensed or uniformly recommended pharmacological therapies and bariatric surgery is only recommended if there are additional comorbidities[11]. The conclusions of other systematic reviews in the field have been limited to specific treatment modalities[12–14],pharmacological options[15–17], or have no distinguished children from adults[18].

One of the biggest challenges in designing a robust RCT in pediatric NAFLD is the lack of societal consensus on appropriate primary outcome measures. Liver biopsy is the gold standard for diagnosis and assessment[8]. There are no non-invasive markers or imaging modalities that are considered an equivalent for diagnosis or assessing response to treatment[19]. However, there can be reluctance from parents and ethical review boards for paired biopsies in pediatric clinical trials.

Our systematic review aimed to determine the most effective treatment for pediatric NAFLD and assess the degree of heterogeneity in trial design, and whether it may limit the conclusions drawn from RCTs. To address these issues, we performed a systematic review of randomized controlled trials (RCTs) in pediatric NAFLD, including a comprehensive assessment of trial quality and design. In addition, we assessed future RCTs based on data

from clinical trials registries. By inclusion of all treatment modalities and ongoing trials we aimed to achieve a broader view than had been covered in systematic reviews to date.

Materials and Methods

The systematic review was prospectively registered on PROSPERO (registration number CRD42016048084) and can be accessed on http://www.crd.york.ac.uk/PROSPERO.

Data sources and extraction:

The databases searched included MEDLINE OvidSP, Cochrane CENTRAL Register of Controlled Trials, PubMed, clinicaltrials.gov, and World Health Organization International Clinical Trials Registry. Search terms included: “child”, "pediatric”, "fatty liver", and “clinical trials” (see Supplementary Methods for full details of databases and search terms). Finalised searches were performed on January 7th 2018 and independently verified by two individuals (JPM, GYT). Six primary authors were contacted directly to verify results and methodology of their manuscripts.

Potentially relevant titles and abstracts were screened, and then full papers were reviewed for inclusion, independently in duplicate by two authors (GYT and JPM). Data extraction was also independently performed by two authors in duplicate (GYT and JPM). Differences were resolved by discussion with VN and MJA.

Selection of published studies:

The inclusion criteria were published RCTs of children given any intervention that aimed to improve NAFLD (NASH, Fibrosis), which included participants $\leqslant$18 years of age and with a diagnosis of NAFLD, either by radiological or histological modalities. The definition of NAFLD included the presence of hepatic steatosis with a negative viral (hepatitis B and C virus), metabolic (ceruloplasmin, ferritin, alpha-1-antitrypsin) and auto-immune screen (immunoglobulins, anti-nuclear antibody, anti-smooth muscle antibody, and anti-liver kidney microsomal antibody). The exclusion criteria were articles not written in English, non-randomised trials, those with secondary causes of hepatic steatosis (i.e. monogenic disorders, lipodystrophy, steroid-induced) and post liver transplantation. Reviews, letters, case reports/series and editorials were excluded. There were no restrictions on gender, ethnicity, numbers of participants or year of publication.

Selection of active/ongoing registered clinical trials:

Clinical trials registries were searched, including clinicaltrials.gov, IRCTN etc. The inclusion and exclusion criteria were as described above, in addition to trial status as active, recruiting, not completed, or not reported.

Outcome measures:

The primary outcome assessed was mean difference in Kleiner's[20] histological fibrosis. Secondary histological outcomes included steatosis, (lobular/portal) inflammation, and the combined NAFLD Activity Score (NAS)[20]. If pre- and post-treatment liver histology was not performed, then biochemical response (alanine aminotransferase (ALT)) or radiological improvement (improvement on ultrasound (US), magnetic resonance imaging (MRI), magnetic resonance spectroscopy (MRS)) were assessed. The effect on metabolic syndrome parameters was evaluated using insulin sensitivity (homeostatic model of assessment of insulin resistance (HOMA-IR)) and triglycerides (TG). Age-/sex-standardised weight loss and adverse events were also assessed. Primary outcome (or end-point) method was assessed for published and active, registered RCTs.

Quality assessment:

The quality of RCTs was assessed using a modified version of the Cochrane risk of bias tool[21], attributing 1 point to each item, with a total score of 7, as for previous meta-analyses in the field[22]. In addition, calculation of sample size *a priori* was attributed 1 point, giving a total quality score of 8. Trials with a score 6, 7, or 8 were defined as high-quality (HQ). Compliance with CONSORT 2010 publication checklist[23] was assessed, attributing 1 point to each item, or 0.5 to each half-item (total score 25). A bibliometric analysis was performed to indicate the impact of each RCT, including number of citations per month since publication.

Data synthesis and analysis:

Analysis was performed using RevMan 5.3[24] and in accordance with the Cochrane Handbook of Systematic Reviews[25]. Inter-assessor agreement on study inclusion and quality was measured using intraclass correlation coefficients (ICC).

Results

Study characteristics and quality assessment:

Database searches identified 10,115 published articles. 9,793 were excluded at screening stage and 299 were excluded on full-text review (Supplementary figure 1). The main reasons for exclusion on full-text review were lack of relevance (152/299) and non-randomised or non-controlled trials (130/299). Two pairs of studies (refs 11 & 12 and refs 13 & 14) reported the results of the same trial. Agreement between the two assessors was 0.86 (95% CI 0.74-0.98) for study inclusion and 0.90 (95% CI 0.76-0.96) for quality assessment. Of the 21 studies included 11/21 (52%) were of high-quality with a score of 6 or more (low risk of bias) and 8/21 (38%) were of low quality, with a score below 5 (Tables 1 & 3).

A total of 1307 participants were studied, with a mean age 12.6 years, 63% male, and a mean intervention duration of 8 months (range 1-24 months). Quality assessment found random sequence generation and allocation concealment were adequate in 48% (10/21). Blinding was adequate in 62% (13/21) and sample size was calculated *a priori* in 48% (10/21) of studies (Supplementary figure 2). Median CONSORT Reporting score was 20.5 (range 12.5-24.5) with high quality reporting (score 23 or greater) in 33% (7/21, Supplementary table 1). The most frequent areas of insufficient reporting were details of randomisation, trial registration, availability of full protocol, and identification as an RCT in the title (Supplementary figure 3). 4 studies did not define a primary outcome and 1 did not report findings of their primary outcome. 7/18 (39%) of RCTs did not meet their primary outcome (when it was defined), including 4 where sample size was calculated *a priori*.

Pre and post-treatment histology was available for 5 studies[27,29–32], however in 2[29,32] of these 5 a post-treatment biopsy was only performed for the intervention group, not for the control group.

Study design and selection of end-points

Three out of 21 (14%) RCTs used liver biopsy as their primary end-point[27,31,33] (Supplementary table 1, and Figure 1), namely improvements in NAS and fibrosis stage; with two further studies using histology as a secondary outcome measure.

Of the remaining studies, primary end-points included change in ALT or AST (5/21; 24%) and ultrasonographic echogenicity (5/21; 24%). No studies used novel biomarkers of NASH or non-invasive scoring systems for NASH or fibrosis in their primary outcome.

8/21 (38%) of studies used biopsy-proven NAFLD as an inclusion criteria, with the remainder diagnosed non-invasively.

Study interventions:

Dietary intervention (Tables 1 and 2)

Three RCTs[34–36] were included (total 47 participants; 1/3 high quality) with radiologically diagnosed NAFLD that assessed low fructose (or low glycaemic load) diets for 4 weeks to 6 months. Vos et al.[34] performed a pilot study of low fructose diet by exclusion of sugar-sweetened beverages (SSB) and high fructose corn syrup (HFCS) for 6 months, compared to a low-fat diet, without caloric restriction. Ramon-Krauel et al.[35] restricted carbohydrate (CHO) to 40% in the intervention group *ab libitum*, compared to a standard 'low fat' diet, and Jin et al[36] gave participants 3 glucose-containing beverages per day instead of fructose-containing beverages. Outcome measures used were change in liver enzymes (in all 3/3) and change in hepatic fat on MRS (2/3; 66%); none had post-treatment histology. In both of the 6 month studies, there was similar reduction in ALT, and improvement in hepatic fat on MRS in both low-fat and low-fructose treatment arms. 4 weeks of low-fructose had no effect on ALT or MRS. Therefore these RCT do not provide any evidence for benefit of low-fructose diet over low-fat diet on non-invasive markers of NAFLD, however they are small and have no untreated control group for comparison.

Weight loss by lifestyle intervention (Tables 1, 2, and 3)

Eight RCTs[27,32,37–42] were included (453 participants; 2/8 high quality), of which 3 had a baseline biopsy to describe the cohort and 1 study was in exclusively NASH patients. Duration ranged from 1 – 24 months, with 6/8 of 6 months or longer. Only one study compared lifestyle intervention to no treatment (i.e. a true control) with the remaining 6 RCT using lifestyle plus placebo against lifestyle plus drug.

6/8 studies used a low-fat diet (23-30%), caloric restricted (25-30 kcal/kg/day) diet with and exercise (from 30min 3x per week to 45min per day).

1 study gave education and advice, whilst Wang et al.[38] reported results of a 3-arm RCT, which included a strict 1-month lifestyle camp (3 hrs/day aerobic exercise, -250 kcal/day) versus unstructured lifestyle plus 100mg/day Vitamin E versus no intervention (standard of care).

Outcome measures used were change in liver enzymes (7/8; 88%) and change in ultrasonographic echogenicity (7/8; 88%), and post-treatment histology in 1/8 (13%).

All studies found an improvement in ALT and/or US echogenicity, with or without weight loss. Wang et al found 2.5 kg/m^2 loss of BMI was achieved in the strict lifestyle group, compared to 1.4 kg/m^2 in the unstructured lifestyle and vitamin E group. This study was found to be at higher risk of bias.

Significant weight loss was also achieved in Nobili et al.[27], with 2.9 kg/m^2 BMI loss at 24 months. This group was found to have improvement in histological steatosis, lobular inflammation, and hepatocyte ballooning, but fibrosis stage was unchanged.

Vajro et al.[37] commented on only 33% compliance with diet and found that diet-compliers had improvement in ALT whereas non-compliers had no improvement. Across all studies, greater BMI loss was associated with greater improvement in ALT, triglycerides (TG), and HOMA-IR.

Drop-out varied between 0-24% with no adverse effects.

These RCT suggest that lifestyle modification is safe and when investigated can improve histological features of NASH and that greater weight loss is associated with larger improvements in ALT as well as markers of metabolic dysfunction. The benefit of weight loss over no treatment has not been well described. It is also unclear whether more or sustained weight loss is required to improve fibrosis. These conclusions are limited by the lack of true control groups, low compliance, and only a single study with post-treatment histology.

Insulin sensitisers: metformin (Tables 3 and 4)

Four RCTs[39,40,43,44] (396 participants; 1 high quality) assessed metformin in non-diabetic children. Total daily doses of 500mg-1.5g were used for 4-24 months.

Three RCTs used change of steatosis on ultrasound as their primary outcome and the TONIC trial[43] used sustained reduction in ALT as primary outcome with histology as secondary outcomes.

The TONIC trial[43] was a 3-armed study that compared placebo, metformin, and vitamin E (800IU daily) in children with biopsy-proven. Metformin was no better than placebo in achieving sustained reduction of ALT though use was associated with an improvement in hepatocyte ballooning compared to placebo (44% vs 21%; p=0.02). The results of the vitamin E arm will be discussed under the antioxidants section.

2/4 (50%) studies found greater improvement in ultrasound echogenicity in with metformin use compared to placebo, however statistical significance was not reported. Shiasi Arani et al[44] also found improvement of radiological steatosis however did not include a placebo arm. Across all studies metformin was associated with an improvement in markers of insulin resistance and was weight-neutral.

Drop-out rate on metformin was similar to other assessed interventions (0-24% across studies) though mild adverse events (abdominal pain, nausea, diarrhoea) were reported in 29% vs 22% placebo in one study[39] and resulted in 9 discontinuations in another[44].

These studies indicate that metformin improves insulin resistance and may be associated with reduced steatosis on ultrasound; it also has potential for histological improvement of ballooning degeneration in NASH. It is associated with mild adverse events but its

discontinuation rate is no different to lifestyle. These conclusions are limited by the variation in outcome measures, lack of placebo control, and low reporting of statistical significance.

Antioxidants: vitamin E

Six RCTs[27,31,37,38,40,43,44] (516 participants; 2 high quality) were included. 3/6 used variable dosing of vitamin E compared to placebo (duration 1 – 24 months), 1/6 compared vitamin E with different lifestyle interventions (without a direct comparator), 1/6 used vitamin E compared to metformin with no placebo; finally, 1/6 used vitamin E plus vitamin C compared to placebo. Outcomes used by these studies were: change in liver enzymes (4/6; 66%), change in ultrasound steatosis (4/6; 66%), and histology (2/6; 33%), where histology was a secondary outcome for the TONIC trial.

Vitamin E use was associated with improvement in ALT and ultrasound echogenicity but was no better than placebo. The vitamin E arm of the TONIC trial[43] had improved ballooning and NAS compared to control, but, similar to metformin, did not meet its primary end-point of sustained ALT reduction. Nobili et al.[27] found that vitamin E and vitamin C in combination improved NAS, inflammation, and steatosis, but again was no different to control.

Overall antioxidants were safe, with only 1 withdrawal due to high ALT in a vitamin E group.

These data suggest that vitamin E shows no significant benefit over placebo on ALT or ultrasound though may improve features of NASH. These conclusions are limited by study heterogeneity, incomplete reporting, and lack of true placebo controls.

Antioxidants: cysteamine bitartrate

One high quality RCT[31] by Schwimmer et al. assessed use of cysteamine bitartrate delayed release (CBDR) 169 in children with NAFLD and NAS ≥4 on biopsy for 24-months. The primary outcome was improvement in liver histology. CBDR had no benefit on fibrosis or NAS compared to placebo, but improved lobular inflammation and ALT, without change in BMI SD. Greater magnitude of effect was found in children weighing <65kg. Adverse events were reported in 70% CBDR and 67% of placebo but dropout rate was 14% CBDR, compared to 4% in placebo. These data suggest that CBDR may be of benefit in improving NASH, particularly in younger adolescents. These conclusions are limited by the inclusion of only a single study.

Polyunsaturated fatty acids (PUFA, Tables 3 and 4)

Six RCTs[29,32,41,45–47] (397 participants; 3 high quality) compared PUFA formulations to placebo for 6-24 months. 5/6 studies used various combinations of docosahexaenoic acid

(DHA) and eicosapentanoeic acid (EPA) and 1/6 used a combination of DHA, choline, and vitamin E (DHA-CHO-VE), including a low dose of vitamin E (39 IU). Outcome measures were: liver enzymes in 5/6 (83%), ultrasound echogenicity in 4/6 (66%), one study used MRI hepatic fat fraction, and one study used change in plasma fatty acids.

4/6 (66%) studies found hepatic fat (on MRI) or ultrasound echogenicity was improved with PUFA treatment compared to placebo. Whereas only one study found an improvement in ALT over placebo. There was improved NAS (steatosis and ballooning) in the DHA-CHO-VE arm but this could not be compared to a control group which lacked histological assessment. Biopsy was not performed in the control group due to ethical reasons, based on guidance from the European Society for Pediatric Gastroenterology, Hepatology and Nutrition[8].

PUFA were safe and well tolerated with drop-out rates comparable to other interventions.

These data suggest that PUFA improves radiological steatosis but not ALT. It is not possible to draw conclusions on improvement of NASH or the optimum dosing/formulation of PUFA.

Polyunsaturated fatty acids with vitamin D (Tables 3 and 4)

One high-quality study[33] (41 participants) compared DHA plus vitamin D for 12 months in vitamin D deficient (<20ng/dl) children with biopsy-proven NAFLD. Primary outcome was change in liver enzymes with post-treatment biopsy was performed in the intervention group only, however a retrospective comparison to data from Nobili et al.[28] (who received DHA 500mg alone) was used for assessment of the impact of vitamin D on histology.

Treatment improved ALT greater than placebo and biopsy suggested that vitamin D improved NAS and each of its components without change in fibrosis stage. It should be noted that this comparison is retrospective and participants from Nobili et al. were not vitamin D deficient.

No adverse reactions were reported.

These data suggest that treatment with vitamin D and PUFA in vitamin D-deficient children improves ALT but it is not possible to conclude the effect on histology without a direct comparator.

Probiotics

Three RCTs[42,48,49] (128 participants, 2 high quality) compared probiotics against placebo. Three different probiotic formulations were used for 2 – 4 months. Outcomes used were liver enzymes and ultrasound echogenicity for all 3 studies.

2/3 (66%) found an improvement in ALT over placebo and 2/3 (66%) found improvement in ultrasound echogenicity. There was also improvement in insulin resistance and serum lipids. Probiotics were well tolerated with low drop-out, where reported.
These data suggest that probiotics improve ALT and ultrasound appearances of steatosis, in addition to markers of the metabolic syndrome. These data are limited by variation in formulation of probiotics and lack of histological data, so it is not possible to comment upon fibrosis or NASH.

Active, ongoing clinical trials (Table 5)

Database search identified 100 potentially relevant ongoing clinical trials (Supplementary Figure 4): 66 were not related to pediatric NAFLD and 21 were not RCTs.

13 trials were included (10 in NAFLD, 3 in NASH), involving 6 dietary interventions, 1 lifestyle intervention, 3 anti-oxidants and 3 others (including losartan) (Table 5). None of the identified trials plan to use liver histology as a primary end-point. 9/13 (69%) will use quantification of hepatic steatosis on MRI as primary end-point, 4/13 (31%) will use change in ALT/AST, and 1/13 (8%) will use novel biomarkers of NASH (cathepsin-D, lipopolysaccharide).

One relevant, non-randomised controlled trial in bariatric surgery was identified (NCT02412540). Participants undergoing vertical sleeve gastrectomy will be compared to those having comprehensive lifestyle intervention with a biopsy at 12-months and the primary outcome includes correlation of histological changes in NASH with MR and CK-18.

Discussion

This systematic review of RCTs investigates all therapeutic interventions in pediatric NAFLD. Of the 21 RCTs identified consisting of 1307 patients, there was marked heterogeneity in study design quality, sample size, duration, outcome measures, and therapeutic intervention. Lifestyle interventions and anti-oxidants were the most studied, with an emergence of probiotic and PUFA studies in the last 5 years. In contrast to adult RCTs, only 14% of pediatric studies included paired histology as outcome measure, with the remainder relying on non-invasive markers of NAFLD. 62% of RCTs included children without a biopsy-diagnosis of NAFLD. On review of both published and active RCTs there has been a general

shift from the use of ALT and ultrasound as end-points to MRI quantification of hepatic steatosis.

Assimilation of the data provides suggestions antioxidants may improve lobular inflammation, PUFA improve radiological steatosis, and probiotics improve ALT as well as ultrasound steatosis. However the optimum formulation, dosing, and duration is unclear due to study heterogeneity. Evidence suggests further study of PUFA and probiotics is warranted. Metformin, PUFA, and probiotics offer greatest benefit on weight loss, insulin resistance, and dyslipidaemia, which are pivotal targets in pediatric NAFLD to reduce their future cardiovascular risk.

Weight loss through lifestyle intervention is the mainstay of treatment for pediatric and adult NAFLD[9]. It is difficult to assess the magnitude of effect from lifestyle interventions due to lack of control groups. In keeping with the findings by Gibson et al.[13], there were no RCTs that specifically assessed the role of physical exercise on pediatric NAFLD. There was a trend towards greater reduction in ALT with greater weight loss, consistent with adult data[22].

Lifestyle intervention should remain the primary treatment for pediatric NAFLD, but there is insufficient data to recommend any particular dietary or exercise regimen. This review found no evidence to support a low-fructose diet, however these were only pilot studies and there are several larger RCTs in process. It is also not clear what degree of weight loss is required to improve NAFLD, whereby studies with less than 0.4 BMI SD reduction failed to achieve any improvement in ALT or ultrasound steatosis. Evidence from bariatric surgery suggests that reversal of fibrosis is possible with substantial weight loss[50]. Further studies of bariatric surgery in NAFLD are ongoing.

Our conclusions on diet & lifestyle are similar to those reached by other systematic reviews[13,14], including Africa et al., who also assessed non-randomised trials. We have suggested that antioxidants (as a class) improve lobular inflammation, following results of CBDR, which were not available to Sarkhy et al.[15] This review found the evidence on PUFA to be encouraging but due to variation in formulation and dosing used in trials, our conclusions are more measured than those from Chen et al.[16]

This analysis has highlighted the limitations of the current data for the treatment of pediatric NAFLD. Aminotransferases and hepatic steatosis fluctuate during the course of NAFLD[22,51], do not correlate with histology[52], and are not predictive of clinical outcomes in adults. There is some recent encouraging data correlating MRI PDFF with fibrosis in adults[53] however it

has not yet been shown to correlate with liver-related clinical outcomes in adults or children. However, histological primary end-points were no more likely to be met than non-invasive outcomes.

There are many challenges in RCT design for pediatric NAFLD. Liver biopsy is an invasive procedure with potential risks though data also suggests that liver biopsy in obese children is safe, with serious complications being rare[54]. An international consensus should be reached on whether quantification of steatosis is a suitable alternative for post-treatment histology in RCTs. It is also worthy of consideration that with improved research in serum biomarkers and non-invasive imaging techniques, biopsies will be used less frequently in clinical practice.

Conclusion

Antioxidants improve some histological features of NASH. There is encouraging non-invasive data for lifestyle modification, PUFA, and probiotics but there is a lack of histological data for correlation. RCTs have moved to use of MR quantification of steatosis as a primary outcome measure, but the predictive accuracy of MR for clinical outcomes in children requires future study.

References

1. Schwimmer JB, Behling C, Newbury R, et al. Histopathology of pediatric nonalcoholic fatty liver disease. Hepatology 2005;42:641–649.
2. Anderson EL, Howe LD, Jones HE, et al. The prevalence of non-alcoholic fatty liver disease in children and adolescents: A systematic review and meta-analysis. PLoS One 2015;10.
3. Mann JP, Vito R De, Mosca A, et al. Portal inflammation is independently associated with fibrosis and metabolic syndrome in pediatric nonalcoholic fatty liver disease. Hepatology 2016;63:745–753.
4. Younossi ZM, Blissett D, Blissett R, et al. The economic and clinical burden of nonalcoholic fatty liver disease in the United States and Europe. Hepatology 2016;64:1577–1586.
5. Feldstein AE, Charatcharoenwitthaya P, Treeprasertsuk S, et al. The natural history of nonalcholic fatty liver disease in children: a follow-up study for up to 20-years. Gut 2010;58:1538–1544.
6. Dulai PS, Singh S, Patel J, et al. Increased risk of mortality by fibrosis stage in nonalcoholic fatty liver disease: Systematic review and meta-analysis. Hepatology

2017;65:1557–1565.
7. Angulo P, Kleiner DE, Dam-Larsen S, et al. Liver Fibrosis, but No Other Histologic Features, Is Associated With Long-term Outcomes of Patients With Nonalcoholic Fatty Liver Disease. Gastroenterology 2015;149:389–397.e10.
8. Vajro P, Lenta S, Socha P, et al. Diagnosis of nonalcoholic fatty liver disease in children and adolescents: position paper of the ESPGHAN Hepatology Committee. J Pediatr Gastroenterol Nutr 2012;54:700–713.
9. Chalasani N, Younossi Z, Lavine JE, et al. The Diagnosis and Management of Nonalcoholic Fatty Liver Disease: Practice Guidance from the American Association for the Study of Liver Diseases. Hepatology 2018;67:328–357.
10. Vos MB, Abrams SH, Barlow SE, et al. NASPGHAN Clinical Practice Guideline for the Diagnosis and Treatment of Nonalcoholic Fatty Liver Disease in Children. J Pediatr Gastroenterol Nutr 2016;64:1.
11. Nobili V, Vajro P, Dezsofi A, et al. Indications and Limitations of Bariatric Intervention in Severely Obese Children and Adolescents With and Without Nonalcoholic Steatohepatitis: ESPGHAN Hepatology Committee Position Statement. J Pediatr Gastroenterol Nutr 2015;60:550–561.
12. González-Ruiz K, Ramírez-Vélez R, Correa-Bautista JE, et al. The Effects of Exercise on Abdominal Fat and Liver Enzymes in Pediatric Obesity: A Systematic Review and Meta-Analysis. Child Obes 2017;13:272–282.
13. Gibson PS, Lang S, Dhawan A, et al. Systematic Review: Nutrition and Physical Activity in the Management of Paediatric Nonalcoholic Fatty Liver Disease. J Pediatr Gastroenterol Nutr 2017;65:141–149.
14. Africa JA, Newton KP, Schwimmer JB. Lifestyle Interventions including Nutrition, Exercise, and Supplements for Nonalcoholic Fatty Liver Disease in Children. Dig Dis Sci 2016;61:1375–1386.
15. Sarkhy A, Nobili V, Al-Hussaini A. Does vitamin E improve the outcomes of pediatric nonalcoholic fatty liver disease? A systematic review and meta-analysis. Saudi J Gastroenterol 2014;20:143.
16. Chen L, Wang Y, Xu Q, et al. Omega-3 fatty acids as a treatment for non-alcoholic fatty liver disease in children: A systematic review and meta-analysis of randomized controlled trials. Clin Nutr 2016:6–11.
17. Socha P, Horvath A, Vajro P, et al. Pharmacological interventions for nonalcoholic fatty liver disease in adults and in children: a systematic review. J Pediatr Gastroenterol Nutr 2009;48:587–596.
18. Orci LA, Gariani K, Oldani G, et al. Exercise-based Interventions for Nonalcoholic Fatty Liver Disease: A Meta-analysis and Meta-regression. Clin Gastroenterol Hepatol

2016;14:1398–1411.
19. Schwimmer JB. Clinical advances in pediatric nonalcoholic fatty liver disease. Hepatology 2016;63:1718–1725.
20. Kleiner DE, Brunt EM, Van Natta M, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 2005;41:1313–1321.
21. Higgins JPT, Altman DG, Gøtzsche PC, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. . Br Med J 2011;343:889–893.
22. Musso G, Gambino R, Cassader M, et al. A Meta-Analysis of Randomized Trials for the Treatment of Nonalcoholic Fatty Liver Disease. 2010.
23. Schulz K, Altman D, Moher D, et al. CONSORT 2010 Statement: updated guidelines for reporting parallel group randomized trials. Lancet 2010;375:1136.
24. Copenhagen: The Nordic Cochrane Centre TCC. Review Manager (RevMan) Version 5.3. 2014.
25. Higgins J, Green S (editors). Cochrane Handbook for Systematic Reviews of Interventions. Version 5. The Cochrane Collaboration, 2011.
26. Nobili V, Manco M, Devito R, et al. Effect of vitamin E on aminotransferase levels and insulin resistance in children with non-alcoholic fatty liver disease. Aliment Pharmacol Ther 2006;24:1553–1561.
27. Nobili V, Manco M, Devito R, et al. Lifestyle intervention and antioxidant therapy in children with nonalcoholic fatty liver disease: A randomized, controlled trial. Hepatology 2008;48:119–128.
28. Nobili V, Bedogni G, Alisi A, et al. Docosahexaenoic acid supplementation decreases liver fat content in children with non-alcoholic fatty liver disease: double-blind randomised controlled clinical trial. Arch Dis Child 2011;96:350–353.
29. Nobili V, Alisi A, Della Corte C, et al. Docosahexaenoic acid for the treatment of fatty liver: randomised controlled trial in children. Nutr Metab Cardiovasc Dis 2013;23:1066–1070.
30. Lavine JE, Schwimmer JB, Van Natta ML, et al. Effect of vitamin E or metformin for treatment of nonalcoholic fatty liver disease in children and adolescents: the TONIC randomized controlled trial. JAMA 2011;305:1659–1668.
31. Schwimmer JB, Lavine JE, Wilson LA, et al. In Children With Nonalcoholic Fatty Liver Disease, Cysteamine Bitartrate Delayed Release Improves Liver Enzymes but Does Not Reduce Disease Activity Scores. Gastroenterology 2016;151:1141–1154.e9.
32. Zöhrer E, Alisi A, Jahnel J, et al. Efficacy of docosahexaenoic acid–choline–vitamin E in paediatric NASH: a randomized controlled clinical trial. Appl Physiol Nutr Metab 2017:1–7.
33. Della Corte C, Carpino G, De Vito R, et al. Docosahexanoic Acid Plus Vitamin D

Treatment Improves Features of NAFLD in Children with Serum Vitamin D Deficiency: Results from a Single Centre Trial. PLoS One 2016;11:e0168216.

34. Vos M, Weber M, Welsh J, et al. Fructose and oxidized low-density lipoprotein in pediatric nonalcoholic fatty liver disease: a pilot study. Arch Pediatr Adolesc Med 2009;163:674–675.
35. Ramon-Krauel M, Salsberg SL, Ebbeling CB, et al. A low-glycemic-load versus low-fat diet in the treatment of fatty liver in obese children. Child Obes 2013;9:252–260.
36. Jin R, Welsh J a, Le N-A, et al. Dietary fructose reduction improves markers of cardiovascular disease risk in Hispanic-American adolescents with NAFLD. Nutrients 2014;6:3187–3201.
37. Vajro P, Mandato C, Franzese A, et al. Vitamin E treatment in pediatric obesity-related liver disease: a randomized study. J Pediatr Gastroenterol Nutr 2004;38:48–55.
38. Wang C-L, Liang L, Fu J-F, et al. Effect of lifestyle intervention on non-alcoholic fatty liver disease in Chinese obese children. World J Gastroenterol 2008;14:1598–1602.
39. Nadeau KJ, Ehlers LB, Zeitler PS, et al. Treatment of non-alcoholic fatty liver disease with metformin versus lifestyle intervention in insulin-resistant adolescents. Pediatr Diabetes 2009;10:5–13.
40. Akcam M, Boyaci A, Pirgon O, et al. Therapeutic Effect of Metformin and Vitamin E Versus Prescriptive Diet in Obese Adolescents with Fatty Liver. Int J Vitam Nutr Res 2011;81:398–406.
41. Boyraz M, Pirgon O, Dundar B, et al. Long-Term Treatment with n-3 Polyunsaturated Fatty Acids as a Monotherapy in Children with Nonalcoholic Fatty Liver Disease. J Clin Res Pediatr Endocrinol 2015;7:121–127.
42. Alisi A, Bedogni G, Baviera G, et al. Randomised clinical trial: The beneficial effects of VSL#3 in obese children with non-alcoholic steatohepatitis. Aliment Pharmacol Ther 2014;39:1276–1285.
43. Lavine JE, Schwimmer JB, Van Natta ML, et al. Effect of Vitamin E or Metformin for Treatment of Nonalcoholic Fatty Liver Disease in Children and Adolescents: The TONIC Randomized Controlled Trial. JAMA J Am Med Assoc 2011;305:1659–1668.
44. Shiasi Arani K, Taghavi Ardakani A, Moazami Goudarzi R, et al. Effect of Vitamin E and Metformin on Fatty Liver Disease in Obese Children- Randomized Clinical Trial. Iran J Public Health 2014;43:1417–1423.
45. Janczyk W, Lebensztejn D, Wierzbicka-Rucińska A, et al. Omega-3 Fatty acids therapy in children with nonalcoholic Fatty liver disease: a randomized controlled trial. J Pediatr 2015;166:1358–1363.
46. Pacifico L, Bonci E, Di Martino M, et al. A double-blind, placebo-controlled

randomized trial to evaluate the efficacy of docosahexaenoic acid supplementation on hepatic fat and associated cardiovascular risk factors in overweight children with nonalcoholic fatty liver disease. Nutr Metab Cardiovasc Dis 2015;25:734–741.
47. Spahis S, Alvarez F, Dubois J, et al. Plasma fatty acid composition in French-Canadian children with non-alcoholic fatty liver disease: Effect of n-3 PUFA supplementation. Prostaglandins, Leukot Essent Fat Acids 2015;99:25–34.
48. Famouri F, Shariat Z, Hashemipour M, et al. Effects of Probiotics on Nonalcoholic Fatty Liver Disease in Obese Children and Adolescents. J Pediatr Gastroenterol Nutr 2017;64:413–417.
49. Vajro P, Mandato C, Licenziati MR, et al. Effects of Lactobacillus rhamnosus strain GG in pediatric obesity-related liver disease. J Pediatr Gastroenterol Nutr 2011;52:740–743.
50. Manco M, Mosca A, De Peppo F, et al. The Benefit of Sleeve Gastrectomy in Obese Adolescents on Nonalcoholic Steatohepatitis and Hepatic Fibrosis. J Pediatr 2017;180:31–37.e2.
51. Belfort R, Harrison SA, Brown K, et al. A Placebo-Controlled Trial of Pioglitazone in Subjects with Nonalcoholic Steatohepatitis. N Engl J Med 2006;355:2297–2307.
52. Molleston JP, Schwimmer JB, Yates KP, et al. Histologic abnormalities in children with nonalcoholic fatty liver disease and normal or mildly elevated alanine aminotransferase levels. J Pediatr 2014;164:707–713.
53. Ajmera V, Park CC, Caussy C, et al. Magnetic Resonance Imaging Proton Density Fat Fraction Associates With Progression of Fibrosis in Patients With Nonalcoholic Fatty Liver Disease. Gastroenterology 2018;Epub ahead.
54. Harwood J, Bishop P, Liu H, et al. Safety of blind percutaneous liver biopsy in obese children: a retrospective analysis. J Clin Gastroenterol 2010;44:e253–e255.

Figure legends

Figure 1. Proportion of RCTs using each outcome measure, by class of intervention.

Supplementary Figure 1. Flow chart for identification of completed, published randomised controlled trials in pediatric NAFLD suitable for inclusion.

Supplementary Figure 2. Cochrane Risk of Bias scores of RCTs.

Supplementary Figure 3. CONSORT scores of RCTs.

Supplementary Figure 4. Flow chart for identification of ongoing randomised controlled trials in pediatric NAFLD suitable for inclusion.

Table legends

Table 1. Characteristics of published randomised controlled trials of dietary or lifestyle intervention for pediatric NAFLD. Maximum Cochrane score is 8, where ≥6 is of high quality (HQ). ALT, alanine aminotransferase; BMI, body mass index; DHA, docosahexaenoic acid; MRS, magnetic resonance spectroscopy; NA, not assessed; PLA, placebo; PUFA, polyunsaturated fatty acids; SD, standard deviations (of BMI, age-/sex-corrected); US, ultrasound scan; VSL#3, a probiotic mixture of eight bacterial strains.
Cochrane Risk-of-Bias Tool (0-8):
A: Random sequence generation.
B: Allocation concealment.
C: Blinding of participants and personnel.
D: Blinding of outcome assessment.
E: Incomplete outcome data.
F: Selective reporting.
G: Other bias
H: Sample size calculated *a priori*.

Table 2. Findings of randomised controlled trials of dietary or lifestyle intervention for pediatric NAFLD. ALT, alanine aminotransferase; BMI, body mass index; DHA, docosahexaenoic acid; GL, glycemic load; HOMA-IR, homeostatic model of assessment of insulin resistance; MRS, magnetic resonance spectroscopy; NA, not assessed; NS, no significant difference between control and intervention groups; PUFA, polyunsaturated fatty acids; oxLDL, oxidised low-density lipoprotein; SD, standard deviations (of BMI, age-/sex-corrected); TG, triglycerides; US, ultrasound scan.

Table 3. Characteristics of published randomised controlled trials of pharmacological interventions for pediatric NAFLD. Maximum Cochrane score is 8, where ≥6 is of high quality (HQ). ALT, alanine aminotransferase; BMI, body mass index; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; IQR, interquartile range; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; NA, not assessed; PLA, placebo; PUFA, polyunsaturated fatty acids; oxLDL, oxidised low-density lipoprotein; SD, standard deviations (of BMI, age-/sex-corrected); US, ultrasound scan; VSL#3, a probiotic mixture of eight bacterial strains.

Cochrane Risk-of-Bias Tool (0-8):

A: Random sequence generation.

B: Allocation concealment.

C: Blinding of participants and personnel.

D: Blinding of outcome assessment.

E: Incomplete outcome data.

F: Selective reporting.

G: Other bias

H: Sample size calculated *a priori*.

†Mixture of: *Streptococcus thermophilus*, bifidobacteria [*B. breve*, *B. infantis*, *B. longum*], *Lactobacillus acidophilus*, *L. plantarum*, *L. paracasei*, and *L. delbrueckii* subsp. *bulgaricus*.

‡Prokid probiotic comprises Lactobacillus acidophilus $3x10^9$ colony forming units (CFU), Bifidobacterium lactis $6x10^9$ CFU, Bifidobacterium bifidum $2x10^9$ CFU, and Lactobacillus $2x10^9$ CFU per capsule.

Table 4. Findings of randomised controlled trials of pharmacological intervention for pediatric NAFLD. ALT, alanine aminotransferase; BMI, body mass index; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HDL, high-density lipoprotein; HFF, hepatic fat fraction; HOMA-IR, homeostatic model of assessment of insulin resistance; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; NA, not assessed; NAS, NASH activity score; NS, no significant difference between control and intervention groups; PUFA, polyunsaturated fatty acids; oxLDL, oxidised low-density lipoprotein; SD, standard deviations (of BMI, age-/sex-corrected); TG, triglycerides; US, ultrasound scan.

Table 5. Protocol primary end-points and interventions for ongoing randomised controlled trials in pediatric NAFLD. Adipo-IR, adipose insulin resistance index; alb, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; C', cholesterol; Cath-D, cathepsin-D; CK-18, cytokeratin-18; CRP, C-reactive protein; CVD, cardiovascular disease; DXA, dual-energy X-ray absorptiometry; ESR, erythrocyte sedimentation rate; FG, fasting glucose; FI, fasting insulin; Hb, hemoglobin; HOMA-IR, homeostatic method of assessment of insulin resistance; LPS, lipopolysaccharide; MRE, magnetic resonance elastography; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; NS, not stated; PDFF, proton density fat fraction; plt, platelets; TNFα, tumour necrosis factor alpha; ULN, upper limit of normal.

$^{+}$ Patient-Reported Outcomes Measurement Information System (PROMIS) questionnaire assesses anxiety

‡Chronic Liver Disease Questionnaire (CLDQ) assesses quality of life and University Rhode Island Change Assessment Scale (URICA) assesses readiness to change.

Supplementary Table 1. Details of end-points, CONSORT checklist reporting, and bibliometric data for included studies. Maximum CONSORT score is 25. Numbered reporting areas correspond to item numbers on the CONSORT 2010 Checklist (available at http://www.consort-statement.org/consort- 2010).

*No primary outcome was defined. ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FFA, free fatty acids; FGF19, fibroblast growth factor 19; FSH, follicle-stimulating hormone; GGT, gamma-glutamyl transferase; GLP-1, glucagon-like peptide-1; HDL, high-density lipoprotein; HOMA-IR, homeostatic model of assessment of insulin resistance; hsCRP, high-sensitivity C-reactive protein; ISI, insulin sensitivity index; JAMA, Journal of the American Medical Association; LDL, low-density lipoprotein; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; N/A, not applicable; NAS, NAFLD activity score; NASH, non-alcoholic steatohepatitis; oxLDL, oxidised low-density lipoprotein; PAI1, plasminogen activator inhibitor-1; SD, standard deviations (of BMI, age-/sex-corrected); TG, triglycerides; QoL, quality of life; ULN, upper limit of normal; US, ultrasound

scan; VLDL, very low-density lipoprotein; WC, waist circumference

$^{+}$ HOMA-IR was lower in DHA250 group than placebo but not DHA500 group.

‡ Number of participants with a BMI reduction $\geqslant$5% was significantly different between

placebo and control groups, but BMI change (as a continuous variable), was not.

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	3
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	5
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	6
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	6
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	7
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	7 (& Sup Methods)
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	6
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	7

Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	7
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	7
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	7
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	7
Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	7
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	-
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	8, & SupFig 1 & 4
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	8-13 & Tables 1 & 3 & SupTable 1
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	8-13 & Tables 1 & 3

Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	9-13 & Tables 2 & 4 SupTable 1
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	-
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	SupFig2
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	-
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	14-15
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	9-13
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	15
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	1