WHITE PAPER

The role of n3 long chain poly-unsaturated fatty acids in the nutritional management of pediatric Non-Alcoholic Fatty Liver Disease

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Description of non-alcoholic fatty liver disease

Non-alcoholic fatty liver disease (NAFLD) is a slowly progressive condition that begins with non-alcoholic fatty liver (steatosis) leading to non-alcoholic steatohepatitis (NASH). NAFLD can result in progressive fibrosis and lead to end-stage liver disease. Within the last decade, it has become one of the leading indications for liver transplantation in adults (Wong 2015).

Pediatric NAFLD is defined as chronic hepatic steatosis in children ≤18 years in the absence of significant alcohol intake, genetic diseases, or medications that cause steatosis. It is typically defined as the liver containing >5% fat, identified through imaging, direct quantification, or histologic estimation. Pediatric NASH is defined as hepatic steatosis and cellular ballooning with or without inflammation (Vos 2015).

Several metabolic risk factors are associated with pediatric NAFLD, i.e. abdominal obesity, hypertension, and insulin resistance. These come with a resultant increased risk of type 2 diabetes mellitus, metabolic syndrome, and cardiovascular disease in children (Alterio 2014).

Incidence and prevalence of Pediatric NAFLD

There are currently no studies describing the incidence of NAFLD in children. The prevalence of NAFLD have been described in the United States and internationally, and numbers vary by method of detection which usually includes screening by alanine aminotransferase (ALT), imaging or liver biopsy. In American studies, NAFLD prevalence (confirmed by autopsy) ranges from 0.7% in young children ages 2 to 4 years, to 13% in children and adolescents ages 2 to 19 years. The highest rate of fatty liver is seen in obese children with a prevalence of 38% in a US population (Schwimmer 2006), 36% in Germany and 44% in Italy (reviewed by Bellentani et al., 2017). Moreover, the prevalence of NAFLD increased 2.7-fold from the late 1980s to the current era (2007–2010), and at a more rapid rate than childhood obesity, based on analysis of ALT elevation in serial National Health and Nutrition Examination Survey cohorts (Welsh 2013). Consequently, NAFLD has become the most common liver disorder in children and adolescents in the United States (Vajro 2012). The rise in obesity observed in recent decades is considered to be one of the underlying reasons for increases in NAFLD numbers (Alisi 2009).

Detection and monitoring

Clinically, most pediatric patients with NAFLD/NASH have non-specific symptoms. Some complain of fatigue, malaise, or vague abdominal pain (42%–59% of cases) (Schwimmer 2006). NAFLD in children is clinically silent, and is often discovered by incidental findings of hepatic steatosis on ultrasonography and/or altered liver function test results made for other clinical reasons or during routine check-ups (Vajro 2012).

Liver function tests

In clinical practice, liver function tests (ALT, AST, GGT) are commonly used both in the diagnostic work-up of NAFLD and for monitoring the disease. Raised liver function tests indicate liver damage, but the degree of serum ALT elevation does not correlate with the presence or severity of histological findings on NAFLD in children (Nobili 2009) or adults (Mofrad 2003).

Despite the substantial limitations of liver function tests, the NASPHGAN US clinical guidelines currently suggest that screening with ALT should be considered for all obese and overweight children between the ages of 9 and 11 years with additional risk factors such as central adiposity, insulin resistance, diabetes, dyslipidemia, sleep apnea, or a family history of NAFLD/NASH) (see Figure 1).

Non-invasive blood tests

The use of blood tests as a risk stratification, diagnostic, prognostic or predictive tool, offers a patient-friendly alternative to biopsy and imaging. However, In NAFLD there is a paucity of such tests. The commercially available OWLiver test (www.owlmetabolomics.com) is a serum-based lipidomic analysis that diagnoses the presence of steatosis and additionally provides a tool for monitoring progression of NAFL to NASH. The test works by determining the lipid profile of a patient from a blood sample and mapping this to a database containing patterns from healthy and NAFLD patients. The test was developed using US and European samples which is important for the robustness of the test and its ability to predict outcomes in both populations. The accuracy of such tests is reported as a combination of sensitivity (ability to detect diseased patients) and specificity (ability to correctly detect healthy patients) in the term ROC-AUC. The ROC-AUC value for OWLiver is impressively high at 0.95 (the highest score being 1.0) and thus offers a potentially useful non-invasive alternative to biopsy.

Ultrasound and MRI

Ultrasound is cheap and easily available. However, the test has limitations with regards to resolution (the ability to differentiate between degrees of fat) and it is operator dependent. When compared with liver biopsy in the adult population, ultrasound has a sensitivity ranging from 60% to 96% and a specificity ranging from 84% to 100% (Joseph 1991, Dasarathy 2009). When the percentage of steatosis is ≥20%, sensitivity and specificity increase to 100% and 90%, respectively (Dasarathy 2009); however, lower sensitivity has been reported when fat content is <30% (Saadeh 2002). In children, its accuracy has been evaluated only in one study that reported comparable results (Shannon 2011). The US and EU clinical guidelines (NASPGHAN[1] and ESPGHAN[1]) advise that ultrasound should not be used for the determination or quantification of steatosis (Vos 2017 and Vajro 2012). This is due to poor sensitivity and specificity in children and adolescents. Thus, caution should be taken when depending on ultrasound as a single diagnostic modality.

Elastography is an advanced form of ultrasound used for determining the stiffness of liver tissue, and is therefore used as a non-invasive test for diagnosis of liver fibrosis. Controlled attenuation parameter (CAP) has been used together with elastography to provide quantitative measurements of liver fat and fibrosis, which has proven to have high accuracy (Stoopen-Rometti 2017, Mikolasevic 2016, Koplay 2015). The presence of machines with both elastography and CAP, such as Fibroscan (Echosens), are now entering GP practices and there are currently over 600 Fibroscan machines in use in the US. Fibroscan is approved by the FDA for use in adults and children. It is recommended in guidelines (eg AASLD, AGA EASL, WHO) for assessment of various adult liver diseases. In 2013 it was recommended by the UK National Institute for Health and Care Excellence (NICE) for use in liver disease monitoring in children (Diagnosis and Management of Chronic Hepatitis B in Children, Young People or Adults;

MRI has high accuracy for the detection of fatty liver in adults and advanced techniques exist to allow precise measurement of fatty infiltrate. MRI has been reported to detect liver fat as low as 3% in adults (Dulai 2016, Fishbein 2005, Cheruku 2005). This makes MRI the gold standard for clinical studies in adults where biopsy is not possible (Lin 2017). The NASPGHAN guidelines recommend that when available, MRI and MRS are highly accurate for estimating steatosis in children and adolescents, although further studies are needed in children to identify and validate cutoffs that have diagnostic accuracy for NAFLD (Vos 2017). However, the high cost and comprehensive patient involvement and resource use for an MRI examination normally excludes MRI as a standard clinical procedure.

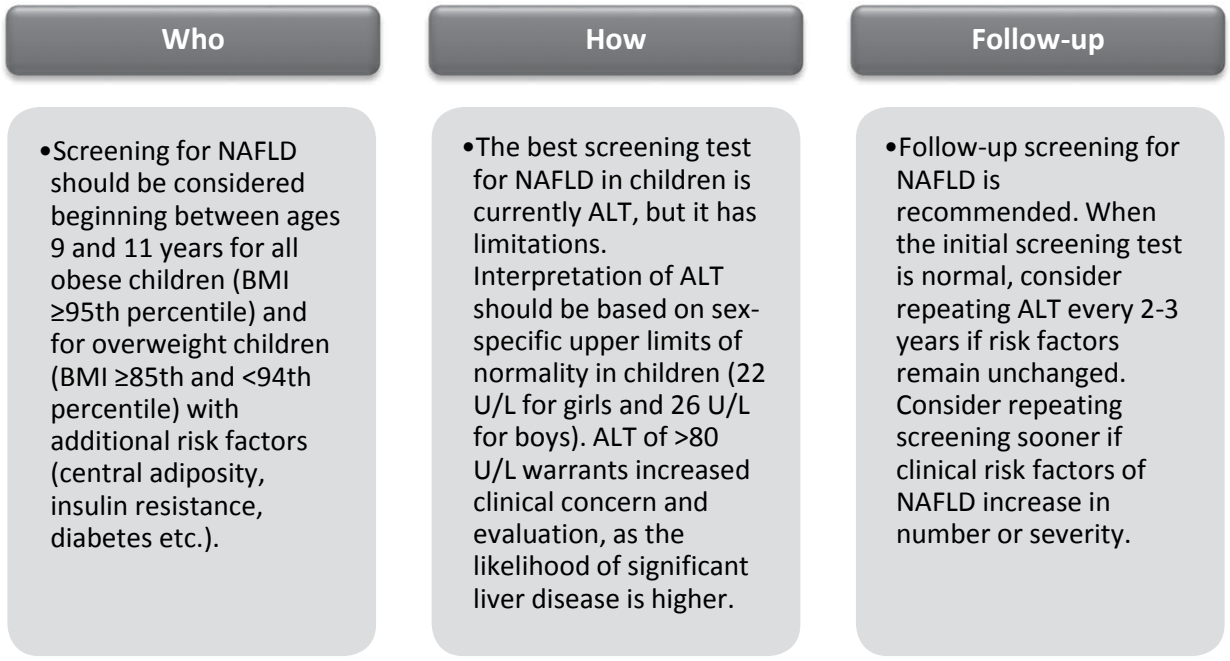


Figure 1 Recommendations from NASPHGAN[1] on screening of children who are considered to have increased risk of NAFLD.

1 NASPGHAN The North American Society of Pediatric Gastroenterology, Hepatology and Nutrition (Vos et al. 2017) ESPGHAN European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (Vajro et al. 2012)

Current treatment options

At present, dietary improvements and increased physical activity (lifestyle modifications) are the primary treatment for pediatric NAFLD.

The US guidelines issued by NASPGHAN[1] recommend that lifestyle modifications to improve diet and increase physical activity should be first-line treatment for all children with NAFLD. Metformin alone or in combination with vitamin E have been considered for treatment in pediatric NAFLD, but have so far not shown to be of significant value for the management of the disease. Bariatric surgery may be considered for selected adolescents with BMI ≥35 kg/m^2, who have non-cirrhotic NAFLD and other serious comorbidities (e.g., Type 2 diabetes mellitus, severe sleep apnea, idiopathic intracranial hypertension) that are likely to improve with weight loss surgery (Vos 2017). The treatment options in pediatric NAFLD is shown in Figure 2.

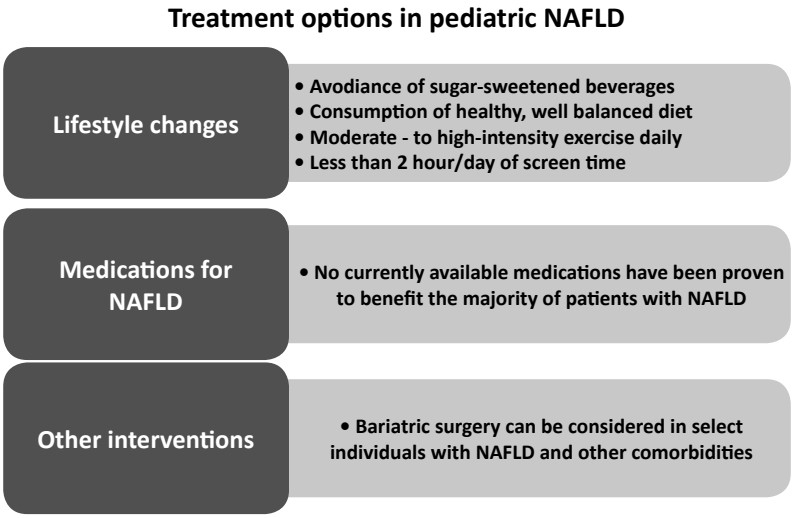


Figure 2 Treatment options in pediatric NAFLD according to the US NASPGHAN guidelines (Vos 2017).

Benefits of managing steatosis

Steatosis is now considered the initial step in a pathway that leads to advanced liver disease (Day 2015) with approximately 5- 10 % developing NASH during their lifetime (McPerson 2015). Development of NASH is therefore an important risk for these patients.

Pediatric NAFLD may be more severe compared to NAFLD identified in adulthood (Holterman 2013). The histology of NAFLD steatosis in children is different from adults. Focal zone 1 steatosis, although rare in adults with NAFLD, does occur in children with NAFLD. The focal zone 1 steatosis and focal zone 3 steatosis are distinct sub-phenotypes of pediatric NAFLD. Children with zone 1 steatosis are more likely to have advanced fibrosis and children with zone 3 steatosis are more likely to have steatohepatitis (Africa, 2017). There is concern that establishment of a chronic progressive disease at early age disposes the patient to a longer lifetime exposure to the disease. This is supported by the risk of progression from simple steatosis to NASH and liver fibrosis being higher in pediatric patients (Kelsey et al. 2014; Regnell et al. 2015). Moreover, limited data suggest that children diagnosed with NAFLD have high blood pressure, with a subsequent increased risk of developing cardiovascular disease (Schwimmer 2014). It is also believed that the liver is involved in the pathophysiology of cardiovascular disease possibly through hepatic and

systemic insulin resistance (Pisto 2014). Indeed, the majority of deaths in NAFLD patients is due to coronary heart disease, and overall mortality has been reported to increase in NAFLD patients by 57% (Musso 2011).

Intrahepatic fat content is significantly improved with exercise, (either aerobic or resistance), even in the absence of weight loss (Keating et al., 2015; Sullivan et al., 2012; Bacchi et al., 2013). Weight loss is also effective in improving NAFLD, with benefits dependent on the amount of weight lost (Hannah and Harrison, 2016; Marchesini et al., 2016). However, most patients have difficulty with long-term changes in exercise and diet and are unable to maintain a reduced body weight. In such patients, liver fat reverts to its original level of infiltration (Dudekula 2014, Pugh 2016)

Adjunctive treatments to exercise and weight loss are therefore needed for the management of NAFLD.

Summary of n-3 LC-PUFA studies in pediatric NAFLD

The efficacy of the n-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFAs), particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), in the dietary management of patients with NAFLD is actively being investigated in both adult and pediatric populations. A body of evidence suggests beneficial effects of n-3 LC-PUFAs in both populations through the reduction of steatosis. In addition some, but not all, studies show benefits on histological outcomes such as ballooning and inflammation (Nobili 2014, Li 2015). The current paper focuses on the studies performed in children below the age of 18.

The effect of n-3 LC-PUFA in children and adolescents with NAFLD has been studied in four randomized, double-blind, placebo controlled clinical trials (Nobili 2011, 2013 & 2014, Janczyk 2015, Pacifico 2015, Boyraz 2015). Most studies were large with the number of enrolled children ranging from 58 to 108, with mean ages between 11 and 14 years. All studies also had adequate durations from 6 to 24 months. Two of the studies investigated the effects of DHA alone (Nobili 2011, 2013 & 14 and Pacifico 2015), and the other two used a combination with EPA and DHA (Janczyk 2015 and Boyraz 2015).

The study by Pacifico et al. is particularly interesting as it uses MRI to quantify liver fat, in addition to collecting liver biopsies at baseline. They reported that the proportion of children diagnosed with definite NASH (based on liver biopsies) was as high as 65% at baseline. After 6 months, liver fat as measured with MRI was reduced with 53.4% in the DHA group, which was significant compared to placebo. DHA also significantly reduced abdominal visceral fat, insulin and triglyceride levels compared to placebo (Pacifico 2015). These results are of interest as they suggest that in addition to reducing liver fat, n-3 LC-PUFA may also contribute in reversing the metabolic milieu permissive of fat deposition in children with NAFLD. Also, when looking at the three other studies which use ultrasonography, the majority show a significant improvement in liver steatosis after using n-3 LC-PUFA (Boyraz 2015, Nobili 2011 & 2013), and improvements in liver enzymes, insulin and plasma triglyceride levels (Nobili 2011, Boyraz 2015).

In the study by Nobili, placebo controlled data shows that DHA reduced steatosis as measured with ultrasound. Compared to placebo, DHA treatment showed beneficial effects across all grades of steatosis, with reduced numbers of children with ultrasound grade 3, and increased numbers of children with liver cleared of fatty infiltrate (Nobili 2011 & 2013). In addition, the same study provided non-controlled data (i.e., without a placebo control) showing that amongst the 20 children with biopsy, of which 60% had NASH, there were significant improvements in histological outcomes including steatosis, ballooning, lobular inflammation and NAS (Nobili 2014). Consequently, this study provides a strong indication that DHA have benefits in ultrasound detected levels of steatosis and additionally may provide clinically relevant effects on hallmarks of NASH such as liver ballooning and inflammation (Nobili 2011, 2013, 2014).

In conclusion, the current scientific evidence in children suggests that supplementation with n-3 LC-PUFA improves liver steatosis and liver enzymes. It may also have beneficial effects on insulin/insulin sensitivity and triglyceride levels which are indicative of improved metabolic health.

Study:	Study size:	Study duration:	Improvement of liver fat:	Reduction in liver enzymes:	Change in insulin/insulin resistance:	Decrease in triglycerides:
Nobili V et al, 2011, 2013	N = 60	24 months	✓	✓	✓	✓
Pacifico L et al, 2015	N = 58	6 months	✓	✓	✓	✓
Boyraz M et al, 2015	N = 108	12 months	✓	✓	✓	✓
Janczyk W et al, 2015	N = 64	6 months	Not significant	✓	Not significant	Not significant

Table 1 Summary of clinical studies in children.

Mechanism of action for n-3 LC-PUFA

Importantly, n-3 LC-PUFA may exert cardio-protective properties both through the reduction of liver fat, and also through its well documented effect on triglyceride reduction.

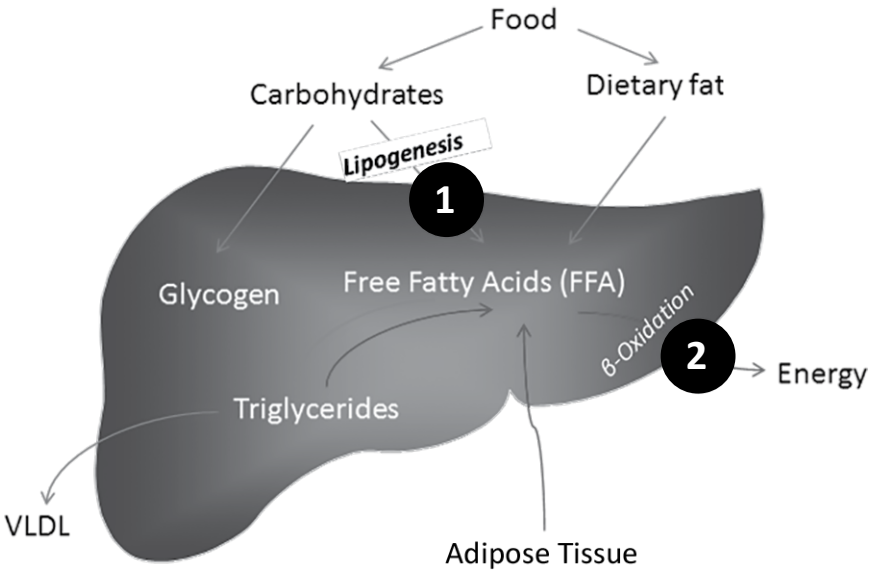
N-3 LC-PUFA has well-documented pleiotropic effects. In the case of NAFLD patients these multiple mechanisms lead to a health benefit by overall reduction of hepatic fat. N-3 LC-PUFA causes liver fat reduction by reducing the de novo lipogenesis (DNL) from carbohydrate via inhibition of the master transcriptional factors SREBP-1c and ChreBp-1c, and by increasing hepatic fatty acid oxidation via activation of the nuclear receptor PPARα. Long-term fructose over-consumption of high fructose corn syrup (HFCS) containing drinks and processed foods enhance hepatic DNL, which is thought to play a pivotal role in the development of pediatric NAFLD (Softic 2016). Inhibiting DNL by N-3 PUFA may offer an attractive approach to correct this abnormality.

N-3 LC-PUFAs are well known for their ability to reduce plasma triglycerides (up to 50% TG reduction (von Schacky 2006 which provides an important health benefit in dyslipidemic patients, a co-morbidity often associated with fatty liver.

N-3 LC-PUFAs also have a mild anti-inflammatory effect via multiple actions; For example, N-3 fatty acids compete with N-6 fatty acids as substrates within the same metabolic pathways, thus increased N-3 reduces the production of inflammatory omega-6 prostaglandins and leukotrienes (e.g., PGE_2, LTB_4); N-3 reduces levels of TNF-α and inflammatory interleukins by reducing the activity of inflammatory intra-cellular signaling molecules (PPARγ, NF

kappa B). The anti-inflammatory effect of N-3 LC-PUFAs is considered to be relevant for a number of chronic inflammatory disease states, but as yet this mechanism has not been shown to be clinically significant in NAFLD.

Figure 3: Effects of



1. Reduced *denovo* Lipogenesis
2. Increase fatty acid oxidation - via PPARα

Conclusions

- **NAFLD is the most common liver disorder in children and adolescents in the United States, and is associated with several metabolic risk factors and risk of cardiovascular disease.**
- **If left untreated, NAFLD can progress into fibrosis and lead to end-stage liver disease requiring liver transplantation in adulthood.**
- **There are currently no available medications, and guidelines only recommend body weight loss by exercise and dietary control for obese patients.**
- **Scientific evidence in children shows that supplementation with n-3 LC-PUFA improves liver steatosis and liver function tests.**
- **N-3 LC-PUFA may also improve metabolic health in children by reducing triglyceride levels.**

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