WHITE PAPER

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

by Derek Tobin, Merethe Brevik-Andersen, Laila Norrheim Larsen

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 (NAFL) leading to non-alcoholic steatohepatitis (NASH). Typically, patients with NAFLD are asymptomatic, or present with ambiguous symptoms such as fatigue and abdominal discomfort (Hassan, 2014).

NAFL is defined as >5% of liver weight being composed of intrahepatic triglycerides, or >5% of cells showing histological presence of hepatic fat deposition. NASH is defined as hepatic steatosis, cellular ballooning and hepatic inflammation. NASH patients may also develop liver fibrosis, cirrhosis and hepatocellular carcinoma. The development of NAFL to NASH occurs in 5-10% of NAFL patients and requires multiple "hits" such as insulin resistance, mitochondrial dysfunction and ER stress. This model is known as the multiple hit model and is gaining recognition as the etiological description of disease progression (Buzetti 2016).

NAFLD is the most common liver disorder in Western countries with a prevalence of 27-34% (WGO Guidelines 2012) Vernon 2011, Blachier 2013.). Whilst NAFLD parallels components of metabolic syndrome, approximately 7% of NAFLD patients have normal weight and normal liver function tests. In these subjects, the disease may still be progressive (Fracanzani 2008).

Several metabolic risk factors are associated with NAFLD (*i.e.* abdominal obesity, elevated blood pressure, diabetes mellitus and dyslipidemia), and the incidence of fatty liver amongst these subjects is high (e.g., $\geq$70% in diabetes type 2 patients) (Blachier 2013, Byrne and Targher 2015). Interestingly, NAFL may also progress to more serious liver disorders when the body is exposed to infections, either of the liver itself or distant sites.

NAFLD is also associated with considerable comorbidities, and NAFLD is considered an independent risk factor for cardiovascular disease (CVD). Indeed CVD is the main cause of death in NALFD patients (38% of all causes is due to CVD; Angulo 2015). The risk of developing type 2 diabetes is also increased in NAFLD patients and has been shown to be up to 5 times increased risk (Hazelhurst 2016). There is clearly a link between metabolic syndrome, CVD, type 2 diabetes and NAFLD. Although NAFLD has been identified as an independent risk factor for CVD, there is probably considerable cross-talk between metabolic morbidities. This cross-talk can potentially lead to a worsening loop of dyslipidemia, inflammation and insulin resistance exacerbating co-morbidities in what has been described as a "vicious circle" (Francque 2016).

Detection and Monitoring

Initial identification of subjects with NAFLD often occurs due incidental findings since there is no screening program for this condition. Typically, patients have findings from imaging performed for other reasons, or imaging is performed as part of a differential diagnosis following detection of raised liver enzymes. Once other causes of steatosis are disregarded (e.g., high alcohol intake, use of steatogenic drugs, chronic liver disease and infection), the patient is then diagnosed with NAFLD.

The prevalence of NAFLD is high in diabetics and in obese subjects and therefore a suspicion of this condition should be raised particularly for patients with type 2 diabetes (Chalasani 2018; Practice Guidelines AASLD).

Below is a list of validated and clinically useful tests used in the identification of NAFLD, although at present there is no general consensus algorithms describing specific diagnostic work-ups.

Liver function tests and standard blood 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 (Mofrad 2003). As such, liver enzymes have poor accuracy for the identification and monitoring of NAFLD but are cheap and available tools and therefore commonly used. The interpretation of results from liver enzymes should be made with caution.

Novel non-invasive blood test (OWLiver)

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 distinguishes between NAFL and 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 its robustness and its ability to predict outcomes in both populations. The accuracy of this test is impressively high with an ROC-AUC of 0.95 (the highest score being 1.0) and thus offers a potentially useful non-invasive alternative to biopsy.

Ultrasound, MRI and novel imaging (Fibroscan)

Ultrasound

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, 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).

Elastography

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 American Gastroenterological Association, European Federation of Societies for Ultrasound in Medicine and Biology, European Association for the Study of the Liver, American Association for the Study of Liver diseases) for assessment of liver diseases.

MRI

MRI has high accuracy for the detection of fatty liver and advanced techniques exist to allow precise measurement of fatty infiltrate. MRI has been reported to detect liver fat as low as 3% (Dulai 2016, Fishbein 2005, Cheruku 2005). This makes MRI the gold standard for clinical studies where biopsy is not possible (Lin 2017).

NAFLD scores

A variety of scores have been developed and validated to determine the presence of NAFLD. These scores are predominantly focused on identifying patients with NASH. The AASLD practice guidelines for "The diagnosis and management of non-alcoholic fatty liver disease" (Chalasani 2018) provides a description of the more common algorithms and recommends use of the NAFLD Fibrosis score (Fib 4) to identify patients with fibrosis (stage 3) or cirrhosis (stage 4). The NAFLD fibrosis score is based on age, BMI, hyperglycemia, platelet count, albumin, and AST/ALT ratio.

Current Treatment Options

The US guidelines issued by the AASLD (Chalasani 2018) recommend 3-5% body weight reduction in order to improve steatosis. Lifestyle modifications are the first-line recommendation for all patients regardless of disease severity. Current medications on the market mostly aim at treating NASH patients and include pioglitazone, Vitamin E in non-diabetic patients, statins and omega-3 for lipid control. The current European clinical practice guidelines from the EASL-EASD-EASO for the management of NAFLD is that overweight patients should aim to reduce their weight by 7-10% through structured programs aimed at lifestyle changes in diet and physical activity.

Benefits of Managing Steatosis

NAFLD is an independent risk factor for cardiovascular disease (coronary heart disease, abnormal cardiac function and structure, valvular heart disease and arrhythmia). Indeed, the majority of deaths in NAFLD patients is due to coronary heart disease and cardiovascular complications. Overall mortality has been reported to increase in NAFL patients by 57% due to the aforementioned liver and cardiac pathologies (Musso 2011). It is believed that the liver is involved in the pathophysiology of cardiovascular disease possibly through hepatic and systemic insulin resistance (Pisto 2014). As NAFLD progresses, the risk of CVD increases which may in part be due to the onset of dyslipidemia. For two comprehensive reviews of NAFLD as a risk factor for cardiac disease and type 2 diabetes see (Ballestri 2014, Byrne 2015). Interestingly, PUFA may exert cardio-protective properties both through the reduction of liver fat, and also through its well documented effect on triglyceride reduction.

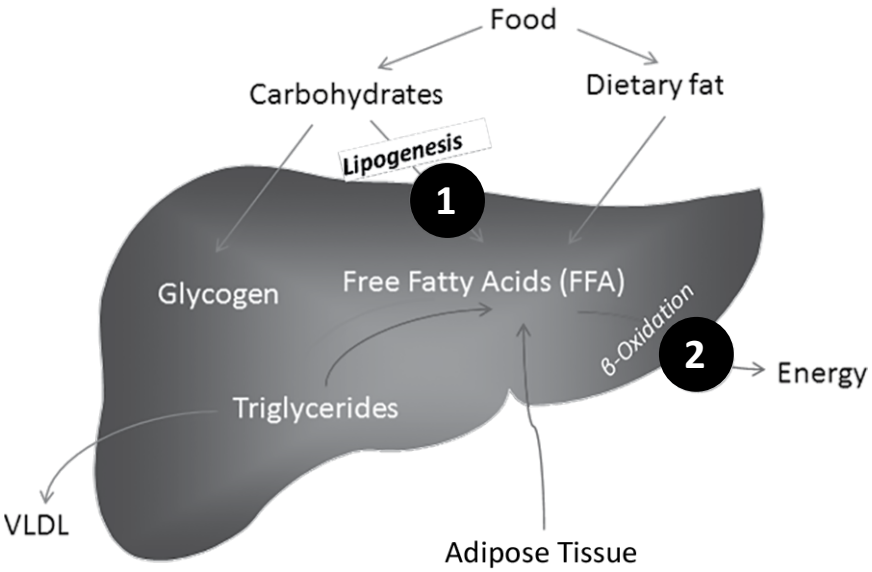
Steatosis is now considered the initial step, and a major driver (Jump 2018) in a pathway that leads to advanced liver disease (McPerson 2015). The associated risks of severe liver disease, development of insulin resistance and type 2 diabetes, and increased risk of cardiovascular disease is a powerful driver for the early treatment of this condition.

Mechanism of Action for PUFA

PUFA has well-documented pleiotropic effects which are beneficial to general health. EPA and DHA are essential for optimal fetal development and healthy aging (Swanson), are precursors to the neuroprotective metabolites NPD1 and synaptamide, constitute phospholipids of most biological membranes with a relevant role in structure and function (Abedi), have anti-inflammatory properties and modulate viscosity of cell membranes [22], and contribute to membrane fluidity, which can influence the function of membrane receptors (Abedi 2014). Series D resolvins and protectins, two active metabolites derived from DHA, may modulate the inflammatory response by decreasing cytokine production and promoting the resolution of inflammation (Serhan 2008). These metabolites could have a potential and important role in metabolic syndrome since a low-grade inflammation characterizes this condition Monteiro 2010). It has been suggested that by reducing the ratio of n-6/n-3 PUFA in diet, the risk factors of metabolic syndrome could be reduced (Poudyal 2011).

In the case of NAFL patients, PUFA causes reduced hepatic lipogenesis and increased use of fat as an energy source (fatty acid oxidation). These effects are mediated by PUFA binding to nuclear receptors such as PPARα to promote lipid oxidation and by inhibiting SREBP-1, ChREB resulting in reduced lipogenesis. PUFA is well known for their ability to reduce plasma triglycerides (up to 50% TG reduction (von Schacky 2006)) which also plays a role in dyslipidemic NAFLD patients.

Figure 1: PUFA effects on the liver.



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

Summary of PUFA Studies in NAFL

Marine omega-3 is rich in EPA and DHA, these particular forms of PUFA provide the health benefits described throughout this paper.

The effect of PUFA on fatty liver has been studied in a plethora of human clinical studies. There is significant variation in the dosage, source and type of PUFA, duration of treatment and means of monitoring, which leads to variation in study results and difficulty in summarizing data. When looking at studies which use imaging to detect fat content of the liver, the majority show a positive, statistically significant benefit of using omega-3. These studies are placebo controlled studies and provide solid support for a beneficial effect of PUFA in reducing the fat content of the liver. A summary of studies of PUFA RCT trials is provided in Table 1.

Study	Size	Duration (months)	Liver fat improvement	Liver enzyme improvement	TG improvement
Capanni, 2006	46	12	✓	✓	✓
Zhu, 2008	86	2-6	✓	✓	✓
Spadaro, 2008	50	12	✓	✓	✓
Cussons, 2009	12	6	✓	-	✓
Scorletti, 2014	103	15-18	✓	-	✓
Sanyal, 2014	162	12	Not significant	Not significant	Not significant
Argo, 2015	34	12	✓	-	-
Li, 2015	78	6	✓	✓	✓
Dasarthy, 2015	37	12	Not significant	Not significant	Not significant
Qin 2015	123	12	-	✓	✓

Table 1: RCT studies of PUFA in NAFL and outcomes

The findings from individual studies shows predominantly positive findings for liver fat content and liver injury. This is also reflected in review articles which support an overall beneficial effect of PUFA treatment of NAFL.

In a meta-analysis by Lu, 10 randomized controlled trials were analyzed with 577 cases of NAFL/NASH and demonstrated a statistically significant effect of PUFA in decreasing hepatic liver fat and GGT but not other liver function tests (Lu 2016). This meta-analysis included studies that used ultrasound to evaluate hepatic fat content, which is known to have poor resolution (ability to detect small changes) and precision (repeatability).

Castro and Calder (Castro 2017) performed a meta-analysis of 17 studies of which 12 studies reported a significant decrease in hepatic fat content after PUFA intake. The authors conclude that a minimum of 2,6 g EPA+DHA and 6 months of supplementation is required to demonstrate changes in liver fat.

NAFLD and metabolic syndrome are associated metabolic disorders that benefit from PUFA supplementation due to the demonstrated pleiotropic effects of these fatty acids. General support for this can be seen from population studies where fish intake and health outcomes are measured. For example, in a Japanese cohort of 90,296 subjects an inverse association was noted between n-3 PUFA intake and hepatocellular carcinoma cases, irrespective of HCV or Hepatitis B status (Sawada 2016). These results are indicative of n-3 PUFAs support of liver health.

Conclusions

- **Liver steatosis (NAFL) affects up to 30% of the US population and is associated with progressive liver disease, insulin resistance and cardiovascular disease**
- **Currently, there are limited options for treatment of patients with NAFLD, with guidelines recommending 3-10% body weight loss for obese patients by exercise and dietary control.**
- **PUFA has multiple activities as an anti-inflammatory agent, triglyceride reducing agent, and cardio-protective agent.**
- **There is substantial evidence that PUFA has a beneficial effect on hepatic fat accumulation and hepatic injury.**

References

Abedi E., Sahari M.A. Long-chain polyunsaturated fatty acid sources and evaluation of their nutritional and functional properties. Food Sci. Nutr. 2014;2:443–463. doi: 10.1002/fsn3.121

Angulo P, Kleiner DE, Dam-Larsen S, Adams LA, Bjornsson ES, Charatcharoenwitthaya P, 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.

Ballestri, S. Lonardo, A., Bonapace, S., Byrne, CD., Loria, P., Targher, G. "Risk of cardiovascular, cardiac and arrhythmic complications in patients with non-alcoholic fatty liver disease" World J Gastroenterol 2014 February 21; 20(7): 1724-1745.

Buzzetti, E., Pinzani, M., Tsochatzis, EA. "The multiple-hit pathogenesis of non-alcoholic fatty liver disease (NAFLD)». Metabolism clinical and experimental 65 (2016) 1038-1048

Byrne CD, TargherG: NAFLD: A multisystem disease (review).J Hepatol 2015.

Castro, GSd. And Philip C. Calder. "Non-alcoholic fatty liver disease and its treatment with n-3 polyunsaturated fatty acids". Clinical Nutrition xxx (2017) 1-19 DOI:http://dx.doi.org/10.1016/j.clnu.2017.01.006

Chalasani 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 Jan;67(1):328-357. doi: 10.1002/hep.29367. Epub 2017 Sep 29

Cheruku S, Jain S, Webb B, Gleason T,Stevens WR. Hepatic MRI for fat quantitation: its relationship to fat morphology, diagnosis, and ultrasound. J Clin Gastroenterol 2005; 39: 619-625

Dasarathy S, Dasarathy J, Khiyami A, Joseph R, Lopez R, McCullough AJ. Validity of real time ultrasound in the diagnosis of hepatic steatosis: a prospective study. J Hepatol 2009, 51: 1061-1067

Dulai PS., Sirlin CB., Loomba, R."MRI and MRE for non-invasive quantitative assessment of hepatic steatosis and fibrosis in NAFLD and NASH: Clinical trials to clinical practice." Journal of Hepatology 2016 vol. 65 j 1006–1016

Fishbein M, Castro F, Cheruku S, Jain S, Webb B, Hepatic MRI for fat quantitation: its relationship to fat morphology, diagnosis, and ultrasound." J Clin Gastroenterol. 2005 Aug;39(7):619-25.

Fracanzani AL, Valenti L, Bugianesi E, Andreoletti M, Colli A, Vanni E, et al. Risk of severe liver disease in NAFLD with normal aminotransferase levels: a role for insulin resistance and diabetes. Hepatology 2008;48:792–798

Francque, van der Graaff, Kwanten: Non-alcoholic fatty liver disease and cardiovascular risk: Pathophysiological mechanisms and implications Journal of Hepatology 2016 vol. 65 j 425–443

Hassan K., Bhalla, V. Regal, MEE., A-Kader, HH. "Nonalcoholic fatty liver disease: A comprehensive review of a growing epidemic". World J Gastroenterol 2014; 20(34): 12082-12101

Hazlehurst, Woods, Thomas, Cobbold and Tomlinson. Non-alcoholic fatty liver disease and diabetes Metabolism. 2016 Aug; 65(8): 1096–1108.

Jump DB, Lytle KA, Depner CM, Tripathy S. Omega-3 polyunsaturated fatty acids as a treatment strategy for nonalcoholic fatty liver disease. Pharmacol Ther. 2018 Jan;181:108-125. doi: 10.1016/j.pharmthera.2017.07.007. Epub 2017 Jul 16.

Koplay, M., Sivri, M., Erdogan, H., Nayman, A. “Importance of imaging and recent developments in diagnosis of nonalcoholic fatty liver disease” World J Hepatol 2015 April 18; 7(5): 769-776

Lin, SC., Heba, E., Bettencourt, R., LinGY., Valasek, MA., Lunde, O., Hamilton, G., Sirlin, CB., Loomba, R. “Assessment of treatment response in non-alcoholic steatohepatitis using advanced magnetic resonance imaging.” Aliment Pharmacol Ther. 2017 Jan 24. doi: 10.1111/apt.13951.

Lu,W., Li, S., Li, J., Wang, J., Zhang, R., Zhou,Y., Yin, Q., Zheng, Y., Wang,F., Xia,Y., Chen,K., Liu,T., Lu,J., Zhou,Y. and Chuanyong Guo “Research Article Effects of Omega-3 Fatty Acid in Nonalcoholic Fatty Liver Disease: A Meta-Analysis.» Gastroenterology Research and Practice Volume 2016, Article ID 1459790, 11 pages

McPherson S, Hardy T, Henderson E, Burt AD, Day CP, Anstee QM. Evidence of NAFLD progression from steatosis to fibrosing-steatohepatitis using paired biopsies: implications for prognosis and clinical management. J Hepatol. 2015 May;62(5):1148-55. doi: 10.1016/j.jhep.2014.11.034. Epub 2014 Dec 1.

Mikolasevic I, Orlic L, Franjic N, Hauser G, Stimac D, Milic S. “Transient elastography (FibroScan(®)) with controlled attenuation parameter in the assessment of liver steatosis and fibrosis in patients with nonalcoholic fatty liver disease - Where do we stand?” World J Gastroenterol. 2016 Aug 28;22(32):7236-51. doi: 10.3748/wjg.v22.i32.7236.

Mofrad PS, Sanyal AJ. “Nonalcoholic fatty liver disease.” MedGenMed. 2003 Apr 16;5(2):14.

Mofrad P, Contos MJ, Haque M, Sargeant C, Fisher RA, Luketic VA, Sterling RK, Shiffman ML, Stravitz RT, Sanyal AJ. “Clinical and histologic spectrum of nonalcoholic fatty liver disease associated with normal ALT values.” Hepatology. 2003 Jun;37(6):1286-92.

Monteiro R., Azevedo I. Chronic inflammation in obesity and the metabolic syndrome. Mediat. Inflamm. 2010; 289645:1–289645:10. doi: 10.1155/2010/289645

Musso G, Gambino R, Cassader M, Pagano G. “Meta-analysis: natural history of non-alcoholic fatty liver disease (NAFLD) and diagnostic accuracy of noninvasive tests for liver disease severity.” Ann Med 2011;43:617–649

Palmentieri B, de Sio I, La Mura V, et al. The role of bright liver echo pattern on ultrasound B-mode examination in the diagnosis of liver steatosis. Dig Liver Dis. 2006, 38: 485-489

Poudyal H., Panchal S.K., Diwan V., Brown L. ω-3 fatty acids and metabolic syndrome: Effects and emerging mechanisms of action. Prog. Lipid Res. 2011;50:372–387. doi: 10.1016/j.plipres.2011.06.003.

Von Schacky, C. “A review of omega-3 ethyl esters for cardiovascular prevention and treatment of increased blood triglyceride levels” Vascular Health and Risk Management 2006:2(3) 251–262

Sawada et al Consumption of n-3 fatty acids and fish reduces risk of hepatocellular carcinoma Gastroenterology. 2012 Jun;142(7):1468-75. doi: 10.1053/j.gastro.2012.02.018. Epub 2012 Feb

Serhan C.N., Chiang N., van Dyke T.E. Resolving inflammation: Dual anti-inflammatory and pro-resolution lipid mediators. Nat. Rev. Immunol. 2008;8:349–361. doi: 10.1038/nri2294.

Stoopen-Rometti M, Encinas-Escobar ER, Ramirez-Carmona CR, Wolpert-Barraza E, Kimura-Hayama E, Sosa-Lozano LA, Favila R, Kimura-Fujikami Y, Saavedra-Abril JA, Loaeza-Del Castillo A. «Diagnosis and quantification of fibrosis, steatosis, and hepatic siderosis through multiparametric magnetic resonance imaging.” Rev Gastroenterol Mex. 2017 Jan - Mar;82(1):32-45. doi: 10.1016/j.rgmx.2016.06.001. Epub 2017 Jan 12.

Sun, Q., Ma, J., Campos, H., Hankinson, SE. and Frank B Hu. “Comparison between plasma and erythrocyte fatty acid content as biomarkers of fatty acid intake in US women.” Am J Clin Nutr 2007;86:74–81.

Sven M. Francque1,2,, Denise van der Graaff1, Wilhelmus J. Kwanten1,2Non-alcoholic fatty liver disease and cardiovascular risk: Pathophysiological mechanisms and implications Journal of Hepatology 2016 vol. 65 j 425–443

Swanson D., Block R., Mousa S.A. ω-3 fatty acids EPA and DHA: Health benefits throughout life. Adv. Nutr. 2012;3:1–7. doi: 10.3945/an.111.000893

World Gastroenterology Organisation Global Guidelines: Nonalcoholic Fatty Liver Disease and Nonalcoholic Steatohepatitis 2012. http://www.worldgastroenterology.org/guidelines/global-guidelines/nafld-nash/nafld-nash-english