Siddiqui and Harvey, J Glycomics Lipidomics 2014, 4:1
DOI: 10.4172/2153-0637.1000e118

Journal of Glycomics & Lipidomics

Editorial Open Access

# Omega-3 Polyunsaturated Fatty Acids for Treatment of Nonalcoholic Fatty Liver Disease: A Possible Case for Personalized Therapy

**Rafat Siddiqui* and Kevin Harvey**

*Cellular Biochemistry Laboratory, Methodist Research Institute, Indiana University Health, Indianapolis, USA*

In a recent issue of PLOSone Depner et al. [1] reported an excellent study on omega-3 fatty acid-induced changes in lipid metabolomics that may be involved in attenuating western diet-induced nonalcoholic steatohepatitis (NASH). A western diet, which is typically rich in saturated and trans fats, that leads to fat accumulation in the liver. This condition resembles alcoholic fatty liver disease; however, it progresses in the absence of any alcohol intake and is known as nonalcoholic fatty liver disease (NAFLD) [2]. In most individuals, the liver is devoid of any symptoms or problems that will only culminate in mild steatosis. However, in some cases, especially individuals with obesity and metabolic syndrome, the fat accumulation is accompanied with inflammation and causes NASH. If untreated, NASH worsens and causes fibrosis resulting in scarring of the liver. The pathophysiologic pathway of NAFLD is not clearly elucidated. Day and James [3] in 1998 proposed the "two-hit phenomenon," where the "first hit" consists of triglyceride (TG) depositions in hepatocytes causing cellular dysfunction and death (lipotoxicity). The hyperinsulinemia and insulin resistance play a role in the first hit causing an alteration in uptake, synthesis, degradation, and secretion of free fatty acids leading to an accumulation of fat in hepatocytes, or steatosis [4,5]. Steatosis by itself is not detrimental, but the steatotic liver becomes more vulnerable to "second hits" by inflammatory cytokines, endotoxins, and oxidative stress causing mitochondrial dysfunction, which ultimately results in hepatocyte injury and fibrosis [6]. NAFLD is also associated with an increased risk of cardiovascular disease (CVD). To date there is no standardized optimal treatment, although several approaches to treat NAFLD have been adopted, including weight reduction, limiting dietary carbohydrate intake, inhibiting fat absorption by sibtramine and orlistat, lowering lipid profile by statins, improving insulin sensitivity by Metformin and thioglitazone and reducing oxidative stress by vitamin E [7-9].

Tissue biopsies from the liver of NAFLD/NASH patients show an accumulation of saturated and monounsaturated fatty acids (MUFAs) and a depletion of polyunsaturated fatty acids (PUFA) in TG and phospholipids (PL) [10]. The depletion of n-3 PUFA is typically more severe than for n-6 PUFA due to decreased elongation and desaturation of alpha-linoleic acid, the essential precursor fatty acids and because of decreased dietary intake [11]. In animal models, an association between n-3 PUFA deficiency and the development of hepatic steatosis was reported [12-14]. Several cross-sectional and case-control studies have also implicated the reduced dietary n-3 PUFA intake as a cause for NAFLD development [15-18]. Recent compelling evidence in the literature suggests that n-3 PUFA, including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), can be more beneficial than existing pharmaceutical agents for the treatment of NAFLD/NASH [17,19-21]. Studies have shown that n-3 PUFA are involved in the control of glucose homeostasis and specifically affect the development of IR and NAFLD progression [22]. Dietary n-3 PUFA affects hepatic lipid homeostasis through imparting both lipid synthesis and lipid oxidation. n-3 PUFA have been shown to suppress lipogenesis by downregulating the activity of SREBP-1c and of carbohydrate-responsive element-binding protein (ChREBP) [23,24], a glucose-responsive transcription factor that is linked to IR and hepatic steatosis. In addition, n-3 PUFA are natural ligands of PPAR-α receptors that increases hepatic mitochondrial β oxidation, and suppress *de novo* lipogenesis by increasing the transcription of fatty acid degradation genes [25]. Furthermore, EPA gives rise to E-resolvins and DHA gives rise to D-resolvins and protectins, which are inflammation-resolving cytokines and therefore play a critical role in suppressing oxidative stress and inflammation [26]. The suppressive effects of n-3 PUFA on inflammation, oxidative stress, and TG accumulation in the liver make them possible effective candidates for NAFLD/NASH. However, the molecular mechanism/s for n-3 PUFA to ameliorate NAFLD/NASH is still obscure. Although data presented by Depner et al. [1] do not establish a cause and effect relationship, the lipidomic data further establishes the potential of n-3 PUFA as a therapeutic agent for NAFLD/NASH.

Depner et al. [1] performed experiments on LDLR-/- mice and fed them western diet with olive oil (WD + O), EPA (WD + E), DHA (WD + D) or both (WD + E + D) and measured changes in metabolomics. The WD + O diet induced classical NASH markers of hepatic damage, including inflammation, oxidative stress, and fibrosis. This analysis identified 524 total metabolites; 320 known and 204 unknown identities. The known metabolites belonged to 8 major pathways including amino acid, carbohydrate, energy, lipid, nucleotide, peptide, vitamins and cofactors, and xenobiotics. The metabolites associated with lipid and amino acid pathways were most affected by diet. Dietary supplementation with EPA and/or DHA attenuated many of the WD + O-induced effects; however, WD + D was found to be better than WD + E at reversing WD + O effects on the liver, Feeding mice the WD + O led to an accumulation of palmitoyl-sphingomyelin, sphinganine, sphingosine, MUFA (18:1,n-9 and 18:1,n-7), n-6 PUFA (20:4, n-6), and alpha-tocopherol (vitamin E), whereas this diet reduced hepatic n-3 PUFAs (EPA, DHA) and oxidized lipids derived from n-3 PUFA (18-hydroxyeicosapentaenoic acid [18-HEPE] and 17,18-dihydroxyeicosatetraenoic acid [17,18-DiHETE]). WD + O diet was also associated with the loss of S-lactoylglutathione, a detoxification product of methylglyoxal (MG); MG is involved in forming advanced glycation end products (AGEP) [27] and promotes NASH [28]. The authors reported that these changes in hepatic metabolites were associated with hepatic damage (ATL and AST) and the induction of multiple gene expression markers of NASH, including MUFA synthesis (SCD1), inflammation (MCP1, CD68, and TLR4), oxidative stress (HMOX-1 & NOX2) and fibrosis (proCOL1A1). In contrast, WD + D feeding decreased hepatic MUFA, n-6 PUFA, n-6 PUFA

***Corresponding author:** Rafat Siddiqui, Cellular Biochemistry Laboratory, Methodist Research Institute, Indiana University Health, Indianapolis, IN 46202, USA, Tel: 317-962-6941; E-mail: RSiddiqu@IUHealth.org

**Received** February 27, 2014; **Accepted** February 28, 2014; **Published** March, 03, 2014

**Citation:** Siddiqui R, Harvey K (2014) Omega-3 Polyunsaturated Fatty Acids for Treatment of Nonalcoholic Fatty Liver Disease: A Possible Case for Personalized Therapy. J Glycomics Lipidomics 4: e118. doi:10.4172/2153-0637.1000e118

derived oxidized lipids, palmitoyl-sphingomyelin and α-Tocopherol. Changes in hepatic content of these metabolites were associated with a corresponding decline in the NASH gene expression markers (i.e., MCP1, CD68, ProCOL1A1, NOX2, SCD1 and TLR4). The authors conclude that DHA likely attenuated MUFA synthesis by decreasing fatty acid synthase (FASN), ATP-citrate lyase (ACL) and SCD1 expression. Their results suggest that DHA-containing diets regulate hepatic 16:0, 18:1,n-7, and 18:1,n-9 content by controlling multiple genes involved in DNL and MUFA synthesis as well as the availability of substrates required for DNL.

One interesting finding was the observed changes in lysophospholipids (lyso-PLs), revealing significant changes in oleic, linoleic, and arachidonic acid but not DHA-containing lyso-PLs. The metabolomic analysis identified a total of 42 lysophospholipids with 26 lyso-PLS with acyl chains in the sn-1 position, and 16 identified lyso-PLs with acyl chains in the sn-2 position. 1-oleylphosphoethanolamine, 1-arachidonylphosphocholine, and 1-arachidonylphosphoethanolamine were increased significantly by the WD + O diet, while diets containing EPA or DHA decreased these lysophospholipids. Hepatic 2-oleoylglyceroethanolamine was increased significantly while levels of 2-linolenoylphosphoethanolamine were suppressed by WD + O. DHA or EPA diet has no effect on these lyso-PLs. Levels of 2-arachidonylphosphocholine and 2-arachidonylphosphoethanolamine, were suppressed by DHA and EPA diets. As suggested by the authors, changes in lysoPLs composition represent either *de novo* phospholipid synthesis or remodeling of membrane phospholipids.

As reported by others, this study also presents the protective effects of n-3 PUFAs by modulating oxidative stress. The metabolomic analysis identified several oxidized PUFAs generated from 18:2,n-6, 20:4,n-6 and 20:5,n-3. The WD + D diet was the most effective diet at suppressing WD-mediated accumulation of proinflammatory oxidized lipids derived from n-6 PUFA. Interestingly, two n-3 PUFA-derived anti-inflamamtory oxidized lipids, 18-HEPE and 17,18-DiHETE, were detected. The data is consistent with the protective effects of oxidized n-3 PUFA metabolities in liver. Other studies have shown that n-3 PUFAs change cardiolipin content in liver mitochondrial membranes, thus affecting the fatty acid composition and PL class content of the membranes and influencing the activities of some of the respiratory chain complex enzymes [29]. Furthermore, livers from WD + n-3 PUFA groups had reduced vitamin E levels. We have also found similar results of vitamin E depletion on fish oil based emulsion infusion in guinea pigs (unpublished data). This may be due to utilization of hepatic Vitamin E by the abundance of EPA and DHA.

Although the authors describe several limitations to their studies, the data demonstrates that n-3 PUFA consumption changes carbohydrate and lipid metabolism, which is clearly linked to reduced TG deposits and hepatic inflammation. This work further provides evidence for the potential use of n-3 PUFAs for treating NALD/NASH. n-3 PUFAs are widely accepted as beneficial nutritional supplements, especially for their cardioprotective effects. It is becoming more convincing that patients at risk of obesity, metabolic syndrome, diabetes, and cardiovascular disease will greatly benefit from the regular use of n-3 PUFAs. EPA and DHA can be obtained either through diet (e.g., fatty fish) or through fish oil supplements. The American Heart Association , American Diabetes Association, and The World Health Organization recommend an average of 500 mg/day of EPA/DHA intake or at least two servings of fatty fish weekly [30,31]. Although these suggestions are based on various epidemiological studies for disease prevention, there is not enough data to suggest an optimal dose and duration for n-3 PUFA for therapeutic purposes for NAFLD/NASH. The amount of n-3 PUFAs and their chemical form (TG, PL, and free FA) varies greatly among marine animals and marine oil-based supplements. A number of pharmaceutical companies are now marketing standardized commercial preparations of n-3 PUFA products, eliminating batch-to-batch variation in n-3 PUFA content. Federal Drug Administration (FDA) has approved highly purified DHA and EPA ethyl ester (Lovaza) and EPA ethyl ester (vescepa) to reduce high TG levels in adults. In a recent study these n-3 PUFA preparations are compared; both n-3 PUFA products decreased TG but DHA-containing supplements have been associated with a significant increase in LDL-C [32]. However, these products do not show additional reduction in adverse cardiovascular events.

Often clinical studies are based on uniform doses. It is perhaps too early to recommend a uniform dose of n-3 PUFA to NAFLD/NASH patients. The effect of n-3 PUFAs may vary from individual to individual because of their metabolic and genetic variations. Furthermore, individuals with NAFLD/NASH may possibly have variable stages of disease and hepatic accumulation of TG. In addition, dietary habits also vary from person to person which can also influence the effect of n-3 PUFA. Some of these conditions are difficult to control in a clinical trial. It is possible that n-3 PUFA treatment in NAFLD patients can be individualized, depending on the clinical progression of disease. This low cost and relatively safer treatment may be an attractive solution to costly and toxic alternative that are currently available.

## References

1. Depner CM, Traber MG, Bobe G, Kensicki E, Bohren KM, et al. (2013) A metabolomic analysis of omega-3 fatty acid-mediated attenuation of western diet-induced nonalcoholic steatohepatitis in LDLR-/- mice. PLoS One 8: e83756.
2. Angulo P (2002) Nonalcoholic fatty liver disease. N Engl J Med 346: 1221-1231.
3. Day CP, James OF (1998) Steatohepatitis: a tale of two "hits"? Gastroenterology 114: 842-845.
4. Pagano G, Pacini G, Musso G, Gambino R, Mecca F, et al. (2002) Nonalcoholic steatohepatitis, insulin resistance, and metabolic syndrome: further evidence for an etiologic association. Hepatology 35: 367-372.
5. Chitturi S, Abeygunasekera S, Farrell GC, Holmes-Walker J, Hui JM, et al. (2002) NASH and insulin resistance: Insulin hypersecretion and specific association with the insulin resistance syndrome. Hepatology 35: 373-379.
6. Csak T, Ganz M, Pespisa J, Kodys K, Dolganiuc A, et al. (2011) Fatty acid and endotoxin activate inflammasomes in mouse hepatocytes that release danger signals to stimulate immune cells. Hepatology 54: 133-144.
7. Peng L, Wang J, Li F (2011) Weight reduction for non-alcoholic fatty liver disease. Cochrane Database Syst Rev : CD003619.
8. Loria P, Adinolfi LE, Bellentani S, Bugianesi E, Grieco A, et al. (2010) Practice guidelines for the diagnosis and management of nonalcoholic fatty liver disease. A decalogue from the Italian Association for the Study of the Liver (AISF) Expert Committee. Dig Liver Dis 42: 272-282.
9. Sanyal AJ, Chalasani N, Kowdley KV, McCullough A, Diehl AM, et al. (2010) Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. N Engl J Med 362: 1675-1685.
10. Araya J, Rodrigo R, Videla LA, Thielemann L, Orellana M, et al. (2004) Increase in long-chain polyunsaturated fatty acid n - 6/n - 3 ratio in relation to hepatic steatosis in patients with non-alcoholic fatty liver disease. ClinSci (Lond) 106: 635-643.
11. Baylin A, Kabagambe EK, Siles X, Campos H (2002) Adipose tissue biomarkers of fatty acid intake. Am J Clin Nutr 76: 750-757.
12. Pachikian BD, Essaghir A, Demoulin JB, Neyrinck AM, Catry E, et al. (2011) Hepatic n-3 polyunsaturated fatty acid depletion promotes steatosis and insulin resistance in mice: genomic analysis of cellular targets. PLoS One 6: e23365.
13. Werner A, Havinga R, Kuipers F, Verkade HJ (2004) Treatment of EFA deficiency with dietary triglycerides or phospholipids in a murine model of extrahepatic cholestasis. Am J Physiol Gastrointest Liver Physiol 286: G822-832.

14. Alfin-Slater RB, Bernick S (1958) Changes in tissue lipids and tissue histology resulting from essential fatty acid deficiency in rats. Am J Clin Nutr 6: 613-624.

15. Capanni M, Calella F, Biagini MR, Genise S, Raimondi L, et al. (2006) Prolonged n-3 polyunsaturated fatty acid supplementation ameliorates hepatic steatosis in patients with non-alcoholic fatty liver disease: a pilot study. Aliment Pharmacol Ther 23: 1143-1151.

16. Sofi F, Giangrandi I, Cesari F, Corsani I, Abbate R, et al. (2010) Effects of a 1-year dietary intervention with n-3 polyunsaturated fatty acid-enriched olive oil on non-alcoholic fatty liver disease patients: a preliminary study. Int J Food Sci Nutr 61: 792-802.

17. Zelber-Sagi S, Nitzan-Kaluski D, Goldsmith R, Webb M, Blendis L, et al. (2007) Long term nutritional intake and the risk for non-alcoholic fatty liver disease (NAFLD): a population based study. J Hepatol 47: 711-717.

18. Allard JP, Aghdassi E, Mohammed S, Raman M, Avand G, et al. (2008) Nutritional assessment and hepatic fatty acid composition in non-alcoholic fatty liver disease (NAFLD): a cross-sectional study. J Hepatol 48: 300-307.

19. Nobili V, Bedogni G, Alisi A, Pietrobattista A, Risé P, et al. (2011) Docosahexaenoic acid supplementation decreases liver fat content in children with non-alcoholic fatty liver disease: double-blind randomised controlled clinical trial. Arch Dis Child 96: 350-353.

20. Tanaka N, Sano K, Horiuchi A, Tanaka E, Kiyosawa K, et al. (2008) Highly purified eicosapentaenoic acid treatment improves nonalcoholic steatohepatitis. J Clin Gastroenterol 42: 413-418.

21. Zhu FS, Liu S, Chen XM, Huang ZG, Zhang DW (2008) Effects of n-3 polyunsaturated fatty acids from seal oils on nonalcoholic fatty liver disease associated with hyperlipidemia. World J Gastroenterol 14: 6395-6400.

22. Valenzuela R, Espinosa A, González-Mañán D, D'Espessailles A, Fernández V, et al. (2012) N-3 long-chain polyunsaturated fatty acid supplementation significantly reduces liver oxidative stress in high fat induced steatosis. PLoS One 7: e46400.

23. Jump DB, Botolin D, Wang Y, Xu J, Demeure O, et al. (2008) Docosahexaenoic acid (DHA) and hepatic gene transcription. Chem Phys Lipids 153: 3-13.

24. Takeuchi Y, Yahagi N, Izumida Y, Nishi M, Kubota M, et al. (2012) Polyunsaturated fatty acids selectively suppress sterol regulatory element-binding protein-1 through proteolytic processing and autoloop regulatory circuit. J Biol Chem 285:11681-11691.

25. Xu HE, Lambert MH, Montana VG, Parks DJ, Blanchard SG, et al. (1999) Molecular recognition of fatty acids by peroxisome proliferator-activated receptors. Mol Cell 3: 397-403.

26. Serhan CN, Petasis NA (2011) Resolvins and protectins in inflammation resolution. Chem Rev 111: 5922-5943.

27. Thornalley PJ (1996) Pharmacology of methylglyoxal: formation, modification of proteins and nucleic acids, and enzymatic detoxification--a role in pathogenesis and antiproliferative chemotherapy. Gen Pharmacol 27: 565-573.

28. Wei Y, Wang D, Moran G, Estrada A, Pagliassotti MJ (2013) Fructose-induced stress signaling in the liver involves methylglyoxal. Nutr Metab (Lond) 10: 32.

29. Aoun M, Fouret G, Michel F, Bonafos B, Ramos J, et al. (2010) Dietary fatty acids modulate liver mitochondrial cardiolipin content and its fatty acid composition in rats with non alcoholic fatty liver disease. J Bioenerg Biomembr 44: 439-452.

30. Kris-Etherton PM, Innis S, Ammerican Dietetic Assocition, Dietitians of Canada (2007) Position of the American Dietetic Association and Dietitians of Canada: dietary fatty acids. J Am Diet Assoc 107: 1599-1611.

31. Kris-Etherton PM, Harris WS, Appel LJ; American Heart Association Nutrition Committee. (2002) Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. Circulation 106: 2747-2757.

32. Bradberry JC, Hilleman DE (2013) Overview of omega-3 Fatty Acid therapies. PT 38: 681-691.