DIFFERENCES IN HEPATIC LIPID COMPOSITION LINKED TO HISTOLOGICAL EVIDENCE OF INFLAMMATION IN THE LIVER: A ^{1}H MRS STUDY INVESTIGATING OMEGA-3 FATTY ACID SUPPLEMENTATION

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Introduction: Non-Alcoholic Fatty Liver Disease (NAFLD) refers to a spectrum of liver damage ranging from steatosis (excess fat accumulation) and steatohepatitis (fat accumulation with inflammation) to fibrosis and cirrhosis. Previous studies investigating steatosis, and the effects of n-3 polyunsaturated fatty acid (PUFA) supplementation in rodents, showed that supplementation led to decreased lipid levels, as well as reduced inflammation following an ischemic insult[1]. Similarly, ultrasound and biochemical assessments of hepatic lipid profiles suggest that PUFA improves features of liver steatosis in NAFLD patients[2-5]. This study aims to use magnetic resonance imaging (MRI) and spectroscopy (MRS) to compare liver lipid profiles in patients with steatosis and steatohepatitis, and to investigate the effects of PUFA supplementation on the lipid content and composition.

Methods: 58 patients with NAFLD (21 with steatosis, BMI=29±3 kgm^{-2}, age=50±11years,15M; 37 with steatohepatitis, BMI=30±5 kgm^{-2}, age=51±9years, 28M), as confirmed by liver biopsy, attended two scanning sessions. NAFLD activity scores (NAS) and steatosis grades were obtained from the liver biopsy data. Scanning sessions consisted of MR measurements of liver volume, liver lipid content, and liver lipid composition. Following baseline scans, subjects were randomly assigned to one of two groups and underwent dietary intervention involving daily ingestion of 5g of either PUFA (N=29, BMI=30.3±4.5 kgm^{-2}, age= 49±7y, 22M) or oleic enriched sunflower oil (OESO, N=29 , BMI=29.1±3.8 kgm^{-2}, age53±11y, 21M) for 3 months before attending for a second scanning visit. All MR data were acquired on a Philips Achieva 3T system using a Q-Body coil for transmission and reception. Correlations were calculated using Pearson's correlation coefficients; significant differences were assessed using a t-test in SPSS 17.

Liver Volume Measurement: T_1-weighted 3D-TFE image, acquired with resolution=2.08x2.08x7.00mm^3, no. slices=36, no. voxels in-plane=180x182, TR=3.11ms with total scan time (equal to breath-hold time)=14.4s. Images were analyzed by region drawing in Analyze9 to calculate liver volume.

Liver Lipid % and T_2 measurement: ^{1}H MR spectra were acquired from a PRESS localized region with the following parameters: VOI=30x30x30mm^3, BW=2000Hz, samples=1024, TR=5000ms. 16 spectra were acquired with TE=40ms, 8 with TE=50ms, and 8 at TE=60ms. Spectra were individually realigned and phase corrected in jMRUI before averaging across each TE. Peak areas of water and CH_2 lipid peak were calculated using an in-house built Matlab script. Water and lipid CH_2 T_2 values were calculated from the peak areas at each TE, and the liver lipid content was calculated as described by Sczcpaniek *et al.*[6]

Liver Lipid Composition: ^{1}H MR spectra were acquired with a water-suppressed PRESS sequence and the following parameters: VOI=30x30x30mm^3, BW=2000Hz, samples=1024, TE/TR=40/5000ms, no. averages=32. Spectra were individually phase corrected and aligned before quantitation using the QUEST algorithm (Fig. 1) in jMRUI. Data are presented as peak area ratio's, uncorrected for T_2 relaxation.

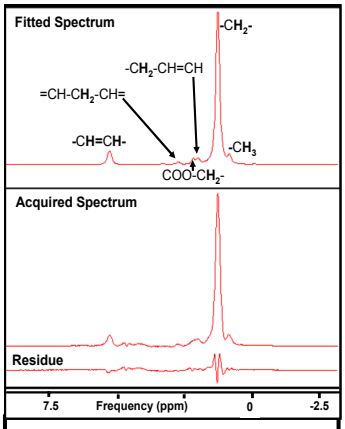

Figure 1: An example spectral fit for assessment of liver lipid composition.

Results and Discussion: Basal Data: In agreement with previous studies, liver lipid% and volume were significantly correlated with BMI across all subjects ($p<0.001$ and $p<0.001$ respectively). A highly significant correlation was found between liver volume and liver lipid% ($p<0.001$). Interestingly, the T_2 of water was found to be shorter in subjects with larger liver volumes ($p<0.001$) and higher lipid content ($p=0.002$), demonstrating the importance of individual T_2 calculations when assessing liver lipid levels, particularly at longer TE. In contrast, the lipid CH_2 T_2 was not significantly correlated with liver volume ($p=0.4$), but tended towards a positive correlation with liver lipid ($p=0.06$). It is possible that this blunted correlation may be due to the reduced SNR of lipid, as well as J-coupling interactions. Relationships to NAS: NAS was significantly correlated with liver volume ($p=0.02$) but was more strongly correlated with liver lipid% ($p<0.001$), which is unsurprising since a component of NAS relates to the severity of steatosis. No relationship was found between NAS and T_2 values implying that T_2 changes are not related to disease severity. Analysis of the liver lipid composition showed that the lipid chain length (measured from the total lipid peak area:methyl peak area) was significantly correlated with NAS ($p=0.003$, Fig. 2). This increase was seen in both the relative number of CH_2 groups (CH_2:CH_3 peak ratio, $p=0.003$) and the relative number of methene groups (-CH=CH-:CH_2 peak ratio, $p=0.008$). Differences between steatosis and steatohepatitis groups: Despite similar lipid% (29±4% vs 30±5%, $p=0.4$) and liver volumes (2.1±0.1 vs 2.2±0.1, $p=0.7$) in the steatosis and steatohepatitis groups respectively, the length of the lipid chain and the relative number of methylene and methene groups per molecule were significantly higher (mean ±SD peak area ratio, 40±20 vs 60±30, $p=0.04$, 40±20 vs 30±30, $p=0.05$ and 4±2 vs 5±2, $p=0.05$ respectively). This implies that the composition of liver lipid may be a factor in the generation of inflammation/ injury in seen in steatohepatitis, in agreement with El-Baldry *et al*[1]. The effect of n-3 PUFA supplementation on the liver: No changes were seen in liver lipid content following 3 months of supplementation with PUFA (9.6±5.2% vs 8.9±5.8%, $p=0.5$) or OESO (10.0±6.2% vs 10.3±6.2%, $p=0.9$). Similarly, no changes were seen in the lipid composition (PUFA: total lipid:CH_3 ratio = 55±26 vs 50±25, $p= 0.2$; CH_2:CH_3 ratio = 45±22 vs 42±22, $p=0.3$; -CH=CH-:CH_3 ratio=4.5±2.1 vs 4.1±1.9, $p=0.2$. OESO: total lipid:CH_3 ratio = 54±31 vs 54 ±34, $p=0.95$; CH_2:CH_3 ratio =45±27 vs 46±30 , $p=0.95$; -CH=CH-:CH_3 ratio=4.3±2.5 vs 4.4 vs 2.6, $p=0.8$). These findings are in contrast to Capanni *et al.*[2] and Sparado *et al*.[3] who observed improved echo texture using ultrasound techniques following 12 months of 1g/d PUFA supplementation and 6 months of 2g/d respectively, and Tanaka *et al.*[8] who observed reduced steatosis, inflammation and fibrosis (from biopsy) in 6/7 subjects following 2.7g/d eicosapentaenoic acid supplementation. However, these results agree with those of Vega *et al*[9], who measured liver triglyceride levels using MRS following 9g/d fish oil for 8 weeks, finding a significant decrease in plasma triglyceride levels with no change in liver levels. Differences in results are likely due to the different techniques used to assess liver lipid levels, as well as differences in fatty acid type, amount, and study duration.

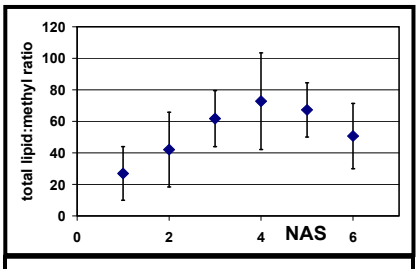

Figure 2: Plot of total lipid/methyl area with NAFLD Activity Scores. Data shown are mean ± SD.

Conclusions: Observations of a relationship between liver lipid% and liver volume on individual water T_2 values in the liver have implications for future studies assessing liver lipid content with MRI and MRS. MRS measurement of the liver lipid composition suggests an increase in lipid chain length in steatohepatitis (compared to steatosis) which is predictive of the NAFLD Activity Score. Finally, to our knowledge, this was the first study which has assessed the effects of n-3 PUFAs versus a control group in biopsy proven NAFLD patients. In contrast to previous studies, this study finds no evidence for omega-3 fatty acid supplementation reducing levels of steatosis in patients with NAFLD.

References: 1. El-Baldry, AM. *et al.,* (2007), *Hepatology*; **45** pp855-863. **2.** Capanni, M *et al.* (2006), *Aliment. Pharmacol. Ther.* 23, pp1143–115. **3.** Spadaro, L. *et al*., (2008), *Digestive and Liver Disease* 40, pp194–199. **4**. Zhu F, *et al.,* (2008*), WJ Gastroent.* 14(41) pp6395-6400. **5.** Sofi, F. *et al.* (2010), *International Journal of Food Sciences and Nutrition*, 61(8) pp792-802. **6.** Sczcpaniek LS *et al.*, (1999), *Am. J. Physiol. Endocrinol. Metab*., 276, pp977–989. **7.** Johnston, NA. *et al.* (2008) *Hepatology,* 47, pp1513-1523. **8.** Tanaka N *et al.* (2008), *J Clin Gastroenterol* 42, pp413–8. **9.** Vega GL. *et al.* (2008), *J Invest Med*. 56: 780–5.