DIFFERENCES IN HEPATIC LIPID COMPOSITION LINKED TO HISTOLOGICAL EVIDENCE OF INFLAMMATION IN THE LIVER: A 1H 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. Similarly, ultrasound and biochemical assessments of hepatic lipid profiles suggest that PUFA improves features of liver steatosis in NAFLD patients. 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. Scan 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 (OSEO, N=29, BMI=29±1.3 kgm⁻², age=53±11y, 21M) for 3 months before attending for a second attending scan 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: T₁-weighted 3D-TFE image, acquired with resolution=2.08x2.08x7.00mm³, 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 T₂ Measurement: 1H MR spectra were acquired from a PRESS localized region with the following parameters: VOI=30x30x30mm³, BW=2000Hz, samples=1024, TE/TR=40/5000ms, no. averages=32. Spectra were individually phase corrected and aligned before quantification using the QUEST algorithm (Fig. 1) in jMRUI. Data are presented as peak area ratios, uncorrected for T₁ relaxation.

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%, and BMI (r=0.96, p<0.001). Interestingly, the T₂ 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 T₂ calculations when assessing liver lipid levels, particularly at longer TE. In contrast, the lipid CH₂ T₂ 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 T₂ values implying that T₂ 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 (r=0.003, Fig. 2). This increase was seen in both the relative number of CH₂ groups (CH₂:CH₃ peak ratio, r=0.003) and the relative number of methene groups (-CH=CH₂:CH₂-CH₃ peak ratio, r=0.008). Differences between steatosis and steatohepatitis groups were subtle and required long TE to discern. Despite similar lipid% and liver volumes (29±11% vs 22±11%, p=0.4), the steatohepatitis groups had significantly higher (mean ±SD peak area ratio, 40±20 vs 60±30, p=0.04, 40±20 vs 30±30, p=0.05 and 42±2 vs 52±2, p=0.05 respectively). This implies that the composition of liver lipid may be a factor in the generation of inflammation/injury seen in steatohepatitis, in agreement with El-Baldry et al. 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 OSEO (10.0±6.2% vs 10.3±6.2%, p=0.9). Similarly, no changes were seen in the lipid composition (PUFA: total lipid:CH₂:CH₃ ratio = 55±26 vs 50±25, p=0.2; CH₂:CH₃ ratio = 45±22 vs 42±22, p=0.3; CH₃:CH₂:CH₃ ratio = 45±21 vs 41±19, p=0.2). OSEO: total lipid:CH₂:CH₃ ratio = 54±31 vs 54±34, p=0.95; CH₂:CH₃ ratio = 45±27 vs 46±30, p=0.95; CH₃:CH₂:CH₃ ratio = 43±25 vs 4.4±2.6, p=0.8). These findings are in contrast to Cappanni et al. and Sparado et al. 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. 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., 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 volume differences. Differences in liver lipid levels, as well as differences in fatty acid type, amount, and study duration.

Conclusions: The improvements of a relationship between liver lipid% and liver volume on individual water T₂ 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 liver 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.