2D localized COSY for the quantification of omega-3 PUFA content in oil phantoms and *in vivo* in rat liver

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Target audience: This work is relevant to the field of liver lipid metabolism and the application of 2D MRS.

Introduction: Non-alcoholic fatty liver disease (NAFLD) is the most common liver disorder in the Western world and is characterized by excessive lipid accumulation in the liver. In addition, NAFLD is associated with marked changes in liver lipid composition. In obese patients with NAFLD, hepatic saturated fatty acids are significantly increased at the expense of polyunsaturated fatty acids (PUFA), in particular omega-3 (n-3) PUFA[1,2]. N-3 PUFA are central regulators of lipid metabolism, activating fatty acid oxidation and inhibiting lipogenesis[3]. Therefore a decrease in n-3 PUFA in the liver may contribute to liver lipid accumulation and the assessment of liver lipid composition is important to better characterize the pathophysiology of NAFLD and to study the effects of treatments. Currently available methods to determine liver lipid composition require *ex vivo* tissue samples, but liver biopsy is an invasive procedure, which is associated with a clinically significant risk of morbidity. The **aim** of this study was to develop a two-dimensional (2D) MRS method for the *in vivo* determination of liver lipid composition, in particular the content of n-3 PUFA. We exploit the specific chemical structure of n-3 PUFA, i.e. the double bond starting after the third carbon atom from the methyl end of the fatty acyl chain (Figure 2A), to specifically detect n-3 PUFA by applying 2D correlation spectroscopy (COSY). Here we demonstrate the application of 2D localized COSY (L-COSY)[4](Figure 1) to quantify n-3 PUFA content in oil phantoms and *in vivo* in rat liver.

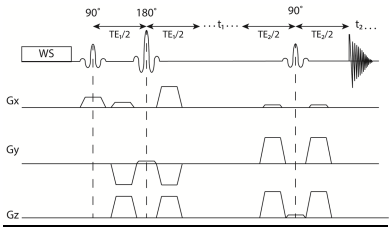

Figure 1: L-COSY sequence with VAPOR water suppression (WS). TE_1 = 5.7 ms, TE_2 = 3.6 ms, total TE = 9.3 ms.

Methods: All experiments were performed on a 7 T horizontal Bruker MR system with a 86 mm-diameter quadrature volume coil for excitation and a 30 mm surface coil for signal reception (Bruker BioSpin). **Phantom study:** Various emulsions of combinations of linseed oil (60% n-3 PUFA) and sunflower oil (0% n-3 PUFA) in water (20% oil) were prepared to make phantoms with n-3 PUFA contents ranging from 0% to 45% with respect to the total fatty acid content. Localized 1D ^{1}H MR spectra were obtained using a PRESS sequence in a 6x6x6 mm^3 voxel with TR/TE = 2000/9.3 ms and 64 averages. 2D L-COSY experiments (Figure 1) were performed on the same voxel with TR/TE = 1500/9.3 ms. 2D data were acquired by sampling 2048 complex points along F2 and 128 points along F1, with a spectral width of 4000 Hz for both dimensions and 8 averages per t_1 increment. ***In vivo* study:** *In vivo* measurements were performed on a Zucker Diabetic Fatty (ZDF) rat (11 weeks of age; 345 g) fed with a methionine-choline deficient (MCD) diet for 5 weeks. A 6x6x6 mm^3 voxel was positioned in the median lobe of the liver (Figure 4A) and 1D ^{1}H MRS was performed as in the phantom study. A 2D L-COSY spectrum was measured in the same voxel with the same parameters as in the phantom study, but with 16 averages per t_1 increment, yielding a total acquisition time of around 51 min.

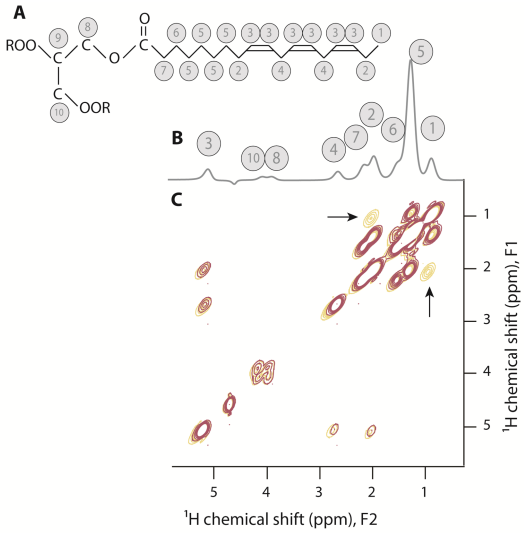

Figure 2: Triglyceride molecule with n-3 fatty acyl chain (A) with corresponding peak assignments in a 1D ^{1}H MR spectrum (B). (C) 2D spectra of oil phantoms with 0% n-3 PUFA (red) and 45% n-3 PUFA (yellow). The cross peaks resulting from the correlation between the methyl protons and allylic protons in n-3 PUFA is indicated with an arrow.

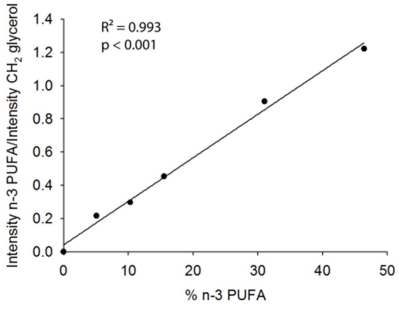

Figure 3: Normalized intensities of the cross peak between methyl and allylic protons (specific for n-3 PUFA) measured in various oil phantoms as a function of the percentage of n-3 PUFA in the phantoms.

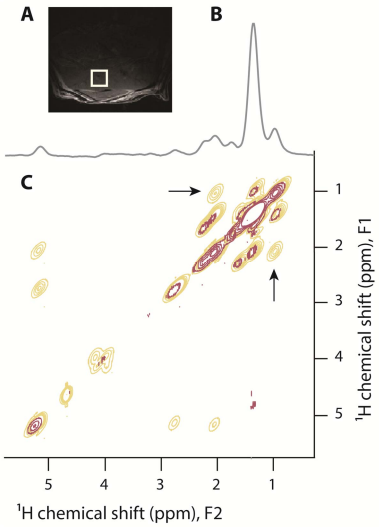

Figure 4: *In vivo* 1D ^{1}H MR spectrum (B) and 2D L-COSY spectrum (C) obtained from a 6x6x6 mm^3 voxel in the liver (A) of a ZDF rat. The *in vivo* 2D spectrum (red) is overlaid on a 2D L-COSY spectrum from an oil phantom with 45% n-3 PUFA (yellow).

Results: **Phantom study:** In the 1D ^{1}H MR spectrum of an oil phantom with 45% n-3 PUFA, next to the large signal from the lipid methylene protons (peak number 5), smaller peaks from other lipid protons can be distinguished, i.e. methyl protons (1), α-methylene protons (7), β-methylene protons (6), allylic protons (2), diallylic protons (4), and olefinic protons (3) (Figure 2B). The signal from diallylic protons predominantly originates from n-3 and n-6 PUFA and can therefore be used to quantify PUFA, but it lacks the chemical specificity to differentiate between n-3 and n-6 PUFA. In the 2D L-COSY spectrum, correlations between methyl protons and allylic protons are unique to n-3 PUFA, whereas other fatty acyl chains, including n-6 PUFA, yield correlations between methyl protons and methylene protons (Figure 2C). The intensity of the cross peak between methyl and allylic protons below the diagonal was quantified in different oil phantoms with n-3 PUFA contents ranging from 0% to 45% and was normalized relative to the intensity of one of the diagonal peaks of the glycerol CH_2 protons (peak 10). The normalized n-3 PUFA cross peak intensity correlated significantly with the percentage of n-3 PUFA (R^2= 0.993, p<0.001; Figure 3). ***In vivo* study:** An *in vivo* 2D L-COSY spectrum was obtained in rat liver within an acquisition time of 51 min. The *in vivo* 2D L-COSY spectrum contained all major lipid peaks, but the cross peak between methyl and allylic protons specific for n-3 PUFA was not visible (Figure 4).

Discussion and Conclusion: 2D L-COSY MRS allows specific and quantitative detection of n-3 PUFA and is a promising technique for liver lipid profiling in NAFLD. However, in this study, the n-3 PUFA cross peak could not be detected *in vivo* in the liver of a rat model of NAFLD. NAFLD is associated with decreased n-3 PUFA content in the liver, which might explain the lack of the n-3 PUFA cross peak. In future experiments we will determine the detection limit of n-3 PUFA with 2D L-COSY and investigate whether it is feasible to detect n-3 PUFA in the liver *in vivo*.

References: 1. Araya J, et al., Clinical Science 106: 635-643, 2004; 2. Puri P, et al., Hepatology 46(4): 1081-90, 2007; 3. El-Badry AM, et al., J Hepatol 47(5): 718-25, 2007; 4. Thomas MA, et al., Magn Reson Med 46: 58-67, 2001.