Fatty Acid Speciation by NMR Spectroscopy and Potential Methods for Liver cancer Diagnaosis

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There is growing evidence from various models of hepatocarcinogenesis that the detected alterations in lipid/phospholipid metabolism reflects a decrease in the expression of desaturase enzymes, resulting in changes in the concentration and conjugation state of fatty acids. In our preliminary studies (*1*) and toward a robust in-vivo method we used high resolution NMR spectroscopy to evaluate the changes in conjugation state of fatty acids (the substrates to the desaturase enzymes in the fatty acid biosynthesis pathway) by calculating the area ratio, *R,* of the bis-allyl (2.8ppm) and vinyl (5.3ppm) resonances. These studies showed a predominance of compounds containing one to two double bonds during hepatocarcinogenesis. To determine the double bond species, e.g. oleic, linoleic, linolenic or arachidonic we used a HSQC pulse sequence modified with a *J* – pulse and narrowband sech/tanh inversion pulses to increase the spectral resolution such that, resonances from the four fatty acid species could be positively identified and quantified. The results of this year long study are presented here for the first time and summarized in Fig. 1.

Briefly, we show that using the modified HSQC pulse sequence, at least one peak from the projections of the indirect dimension (^{13}C) can be unambiguously assigned to a fatty acid compound. Using these we were able to quantify fatty acid species accurately and we show that during early stages of diet induced hepatocarcinogenesis, compounds containing fatty acids linolenic and linoleic accumulate in the rat liver and correlate with tumour formation. We were also able to detect the signature signal of arachidonic acid very early on. The advantages and disadvantages of the new HSQC pulse sequence are discussed and potential new methods are suggested.

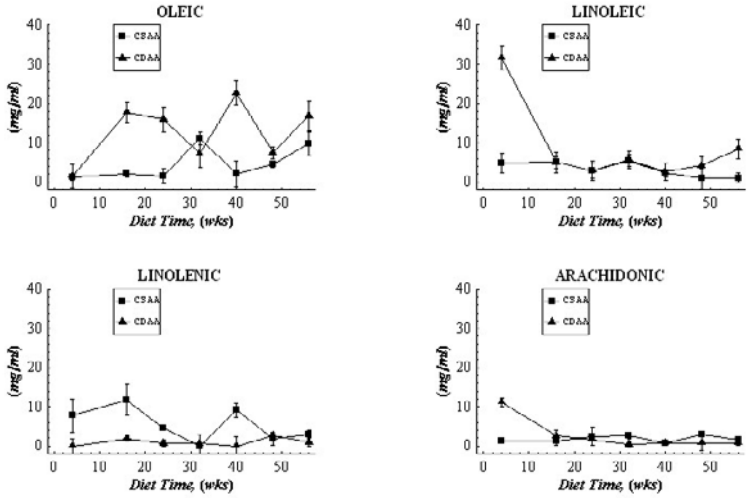
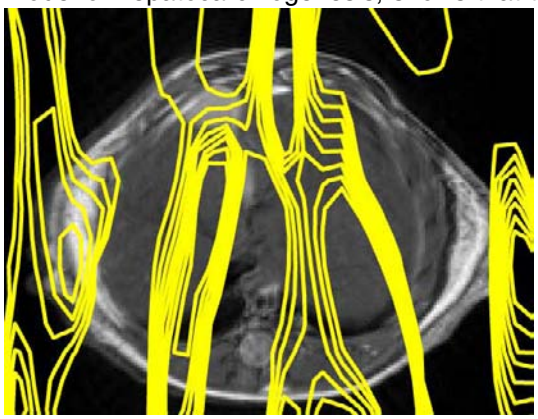


Fig. 1. Concentrations of compounds containing the fatty acid species with time.

A major advantage of the HSQC method is positive identification and the relatively fast acquisition times (~ 2 – 5 minutes) from extracts of about 200 mg of liver. A major disadvantage is that the liver has to be sampled invasively. The translation to in vivo application of the method has some disadvantages as well. Firstly, since the method relies on polarization transfer from ^{13}C to ^{1}H, there is the usual loss in detected signal because of the low natural abundance of ^{13}C. Thus, to fully exploit the observation of altered lipid metabolism by us and many others, new pulse sequence methods need to be developed and validated.

Of potential are methods such as CSI and quantum spectroscopy of the ^{1}H nucleus. Here we propose a CSI method where the ratio *R* is calculated from each voxel of the CSI data set. Our preliminary results, shown in Fig.2, from a transgenic mouse model of hepatocarcinogenesis, shows that the distribution of compounds containing fatty acids with a number of double bonds can be detected and mapped over the liver. The contours in Fig. 2 are when $0.2 < R < 0.6$ corresponding to compounds containing 1 ½ to 2 ½ double bonds and are overlaid on the morphological T2 weighted image of the liver. Obviously further refinement of the method and chemical shift registration method is needed for accuracy. Preliminary results on phantoms, by incorporating gradient shimming and a user controllable CSI weighting scheme shows excellent linewidths and chemical shift stability of a chosen resonant signal.



With further refinement, using selective RF pulses on the bis-ally and vinyl resonances we expect that this will be a new and convenient in vivo method for the detection of fatty acid species distribution in the liver during hepatocarcinogenesis.

Fig. 2. Contour map of R determined by CSI.

References

1. Y.A. Tesiram, D. Saunders and R.A. Towner, Application of proton NMR spectroscopy in the study of lipid metabolites in a rat hepatocarcinogenesis model, *BBA.,* 1737:61-68, 2005