A Novel Way to Follow Triglyceride Metabolism using 13C MRS

D. K. Deelchand[1], J. E. M. Snaar[1], B. Ravikumar[2], R. Taylor[2], P. G. Morris[1]

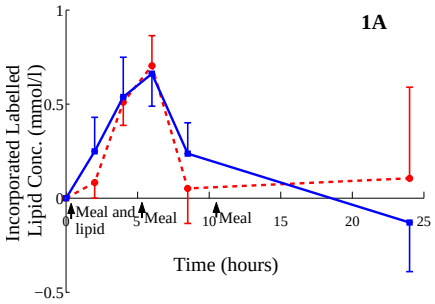
[1]Sir Peter Mansfield Magnetic Resonance Centre, School of Physics and Astronomy, University of Nottingham, Nottingham, United Kingdom, [2]Department of Diabetes and Metabolism, University of Newcatle-upon-Tyne, Newcatle-upon-Tyne, United Kingdom

Introduction: *In vivo* magnetic resonance spectroscopy (MRS) has been applied to living systems to analyse their triglyceride content and this approach has been compared to conventional methods such as morphometry and chemical analysis of tissues extracts[1]. However, there is a need to assess the metabolic turnover of the lipid stores, as well as their overall lipid content. In this study we have used ^{13}C MRS with a labelled lipid mixture ingested as one component of mixed meals to investigate triglyceride storage in the liver and calf muscle over a 24 hour period. Such studies will be of major importance in patients with lipid defects, in the study of diabetes mellitus and for fundamental studies of lipid metabolism in normal healthy subjects.

Methods: 8 healthy subjects were examined after an overnight fast. The following morning, baseline ^{13}C MRS spectra were acquired of the calf muscle and liver and fasting arterialised blood samples were drawn. Subjects were provided with a standard mixed meal (1230 kcal; 76% carbohydrate, 14% fat, 10% protein) together with 3 g of a 98+% ^{13}C labelled algal lipid mixture (45-55% palmitic, 10-15% palmitoleic, 20-30% oleic and 10-15% linoleic acids). Additional standard mixed meals were given after about 5 and 10 hours. Further MRS measurements of the liver were taken at 2, 4, 6, 8½ and 24 hours, while for the calf muscle spectra were acquired at 5, 8 and 24 hours. Blood and breath samples were taken at 2, 4, 6, 8 and 24 hours.
Subjects were examined in a supine position inside a 3T whole-body system. Two half-volume RF probes[2] were used (one leg and one liver), consisting of a circular ^{13}C surface coil and quadrature proton coil. For muscle spectroscopy, the calf was positioned on top of the leg probe while for liver MRS, the liver probe was placed over the subject's abdomen and held firmly in place with the help of a waist vest. In each case, a vacuum pillow was used to minimise movement and to ensure accurate repositioning.
Hard pulses of 100 µs duration with CYCLOPS phase cycling were used to excite ^{13}C resonances. During acquisition, broadband WALTZ-8 proton decoupling was used at a peak power of 68 ± 2 W. A repetition time of 720 ms was used to allow sufficient T_1 recovery of ^{13}C lipid magnetisation and to ensure that the SAR limits were not exceeded. 1500 scans were acquired over periods of 18 min for each 'time point'. Spectroscopic analysis was performed using the MRUI software package. Quantification of the labelled lipid was based on two phantoms, similar in shape to the liver and leg, and containing 5.33 mmol/l of labelled lipid solution (dissolved in deuterated chloroform).

Results: The change in concentration of the labelled lipid in the liver is shown as a function of time in Fig.1A. The unsaturated fatty acids (dashed line) are incorporated very slowly at first, 0.083 ± 0.083 mmol/l at 2 hours, before a significant increase in the next 2 hours, reaching a maximum of 0.704 ± 0.16 mmol/l at 6½ hours. Thereafter it decreases, until 24 hours later, there was only a small amount of labelled lipid present. For the methylene carbons (solid line), the lipid uptake was similar except that the maximum concentration was slightly less (0.662 ± 0.173 mmol/l at 6½ hours) and at the 24 hour time no labelled lipid was present. A smaller increase in the concentration of labelled lipid was observed in the calf muscle, reaching a maximum value after 5-10 hours. The time course of blood glucose concentration during the study period is shown in Fig. 1B. ^{13}C fractional enrichment in the breath and the change in very low density lipoprotein (VLDL) and chylomicron fractions with time are illustrated in Fig. 2A and B respectively.

Discussion: This study shows the feasibility of using ^{13}C MRS to follow the metabolic fate of labelled fatty acids and their distribution in key storage organs throughout the body. From the results, the maximum postprandial triglyceride level in the liver is reached at 6½ hours, while in the breath it is at 8 hours. This is the first time that a diurnal change in the hepatic fatty acids has been observed in normal subjects. This novel technique will help to advance further research in many areas where lipid storage is vital, for instance, in type 2 diabetes where recent evidence has suggested that insulin resistance in these patients is caused by their high level of lipid storage[3] (study currently under investigation in our laboratory).



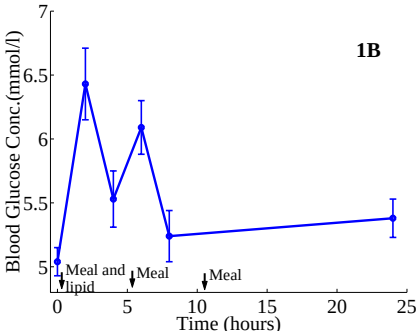
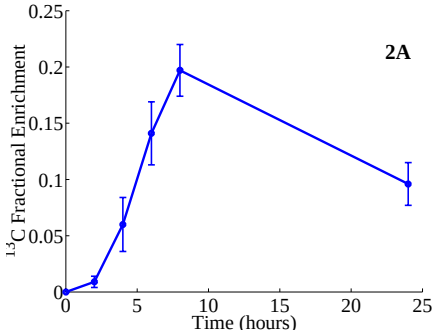


Fig. 1: (A) Time courses of incorporation of labelled algal into hepatic lipid. Dashed line represents the unsaturated carbon peaks at around 130 ppm and solid line the methylene carbons from 20 to 40 ppm region. (B) Blood glucose level during the study. Data are shown as mean ± SE.



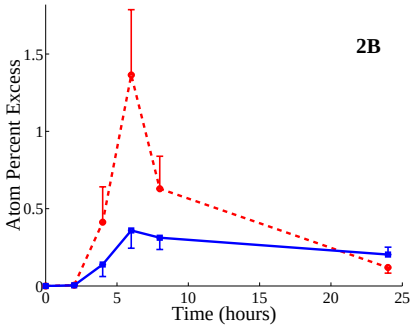


Fig. 2: (A) ^{13}C fractional enrichment in breath. (B) Change in VLDL fraction (solid line) and chylomicron fraction (dashed line) expressed as APE (Atom Percent Excess). Error bars represent SE.

References

1. Boesch C. et al, *Proc. Intl. Soc. Mag. Reson. Med.* 6: 1785, 1998.
2. Adriany G. et al, *J. Magn. Reson.* 125: 178, 1997.
3. Pan D.A. et al, *Diabetes* 46: 983, 1997.