In vivo detection of *trans*-fatty acids by ^{13}C MRS at 7T

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INTRODUCTION

The severe health implications of *trans*-fatty acids are well-known. Increased consumption of these fats leads to increased risks of coronary heart disease, diabetes, cancer, liver dysfunction, and Alzheimer's disease (1). Accumulation of *trans*-fats results from consumption of partially hydrogenated oils and fats in dairy products and meat; *trans*-fats constitute 2-3 % of the total caloric intake in US (2). Characterization of *trans*-fats in *ex vivo* samples is typically done by gas chromatography or high resolution ^{13}C NMR (3) but non-invasive detection of *trans*-fats in humans or rodents has not been reported. Here, we report non-invasive detection of *trans*-fats in human subcutaneous fat by ^{13}C NMR at 7T.

METHODS

All spectra were acquired on a whole-body 7T scanner (Achieva, Philips Medical Systems, Cleveland, OH, USA) using a partial volume human calf coil operating in quadrature for both ^{1}H and ^{13}C. For the phantom experiments, oleic acid (*cis* 18:1, n-9), or elaidic acid (*trans* 18:1, n-9) (Nu-CheckPrep, MN, USA) were dissolved in chloroform (1.75 M) in a 10 mL glass vial. Proton spectra were acquired using a TE-averaged STEAM sequence with 6 TEs: 18 to 21 ms, TM 15 ms, TR 3 s. Carbon-13 spectra of phantoms were acquired by averaging 128 non-selective FIDs using WALTZ-16 decoupling with a 18 μT proton pulse centered at 1.3 ppm and NOE (10 μT at 5 % duty cycle and a mixing time of 1.5 s). To avoid power limitations while preserving spectral resolution, the decoupling was performed only during the first 20 % of the 630 ms acquisition time. All human experiments (n = 20) were performed using a protocol approved by the local IRB. For human ^{13}C studies, TR was 8 s, NSA 32, for a total scan time of 5 min. The average power experienced by each volunteer varied from 10-18 W depending on loading of the coil.

RESULTS AND DISCUSSION

The phantom proton spectra of *cis*- and *trans*- oleic acid are nearly identical (Fig.1) with the two biggest differences being in the methylene protons α to C=C (*cis* 2.03 vs. *trans* 1.98 ppm; δ = 15 Hz) and in the methine protons (*cis* 5.36 vs. *trans* 5.39 ppm; δ = 9 Hz). However, given a typical *in vivo* shimming of 20 Hz and given the expected percentage *trans*-to-*cis* of about 6 %, these δ differences are below the resolution detection limit. In contrast (Fig.2), the allylic carbons (alpha to C=C, $\alpha_{=}$) display substantially different chemical shifts (*cis* 27.18 vs. *trans* 32.59 ppm; δ = 406 Hz; 5.41 ppm). In addition, both the *cis* and *trans* allylic resonances do not overlap significantly with other resonances. The double-bonded carbons (130 ppm region) had a δ (*trans*-*cis*) = 26 Hz which can be resolved in phantoms (data not shown) but only appear as a small shoulder on *in vivo* spectra. ^{13}C spectra from two volunteers, one on a Mediterranean style diet vs. another on a Western style diet, are compared in Fig. 3. The *trans*-allylic peak at 32.78 ppm is easily detectable in the volunteer on a Western diet but could not be detected in the volunteer on a Mediterranean diet. A fit of the spectrum using a Voigt lineshape gave a *trans* : *cis* ratio = 4.4 %, consistent with *ex vivo* reports (4). The small difference in the chemical shift assignments *in vivo* and *in vitro* is probably due to differences in the "solvent", which is fat/water *in vivo* and chlorophorm *in vitro*. Note however that the δ (*trans* - *cis*) differs by only 0.01 ppm *in vivo* vs. *in vitro*. This method should prove useful for routine measures of *trans*-fats in humans on various diets.

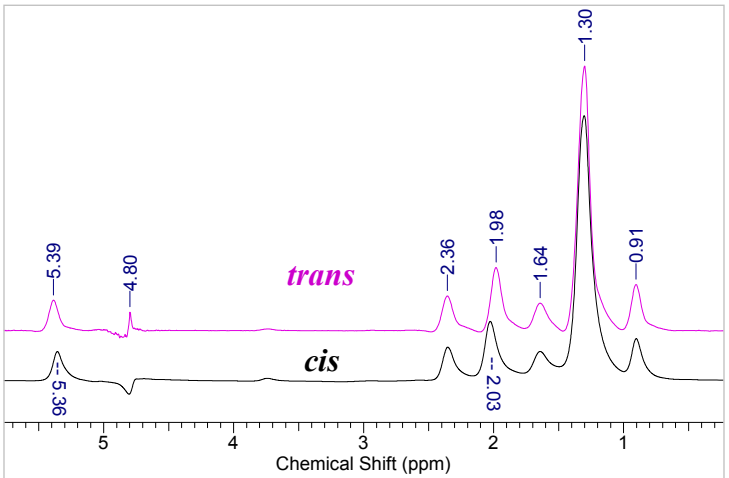

Fig.1. Nearly identical proton spectrum of *trans*- vs. *cis*- oleic acid.

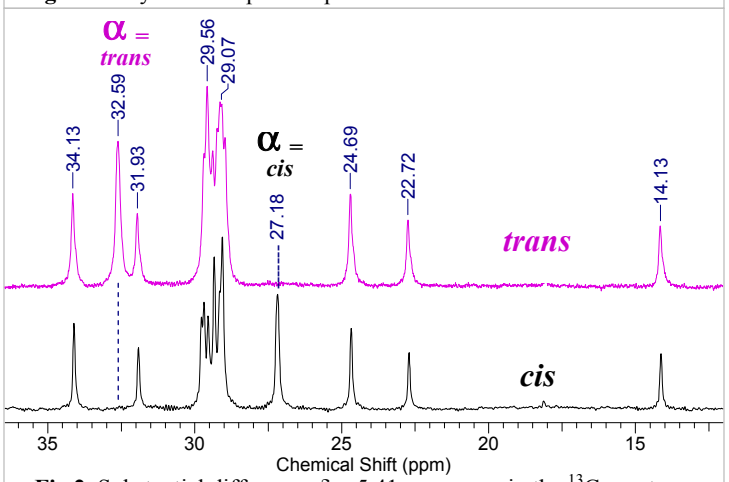

Fig.2. Substantial difference, δ = 5.41 ppm, seen in the ^{13}C spectrum of *trans*- vs. *cis*- oleic acid.

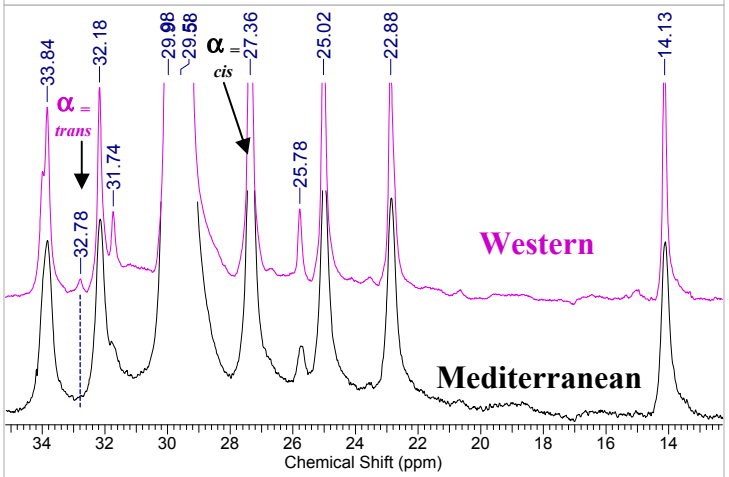

Fig.3. Assignment of signals from *trans* and *cis* carbons α to the double bond. An identifiable *trans* peak is seen in a healthy volunteer on a Western-type diet, whereas no *trans* peak can be seen in the volunteer on the Mediterranean diet.

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