1H Decoupled 13C NMR at 7 Tesla in Humans: Composition of Adipose Tissue

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Introduction
A strong motivation for developing 7T MR for clinical research is the dual advantage of higher sensitivity and improved chemical shift dispersion for spectroscopy applications. One of the most attractive targets is direct detection of 13C which would allow kinetic studies of flux in specific metabolic pathways in tracer experiments. Proton decoupled 13C NMR spectroscopy of humans was introduced more than 20 years ago (1, 2) and the challenges of proton decoupling are well-known. Full decoupling of triglyceride protons would require irradiation across the bandwidth (in 1H) from about 0.8 to 5.8 ppm. The purpose of this study was to evaluate the feasibility of 1H WALTZ decoupled 13C spectroscopy in human subjects at 7T with an initial focus on triglycerides in subcutaneous tissue.

Methods
The protocol was approved by the Institutional Review Board. Informed consent was obtained from all participants (n=6) prior to the study. WALTZ proton decoupled 13C NMR spectra of subcutaneous fat were obtained at 7 Tesla (Achieva, Philips Medical Systems, Cleveland, OH). The coil was a half-cylinder dual 1H-13C coil with both channels in quadrature. SAR limits were observed. T2 weighted scout images were acquired followed by non-spatially localized 13C spectra. 13C data were acquired using a block pulse with flip angle 50°, applied every 25 s. Data were acquired with 16 kHz BW, 1024 points, and 16 averages were collected. Broadband WALTZ-16 decoupling was applied during signal acquisition centered at 2.56 ppm. Broadband NOE irradiation was applied for 10 s before the acquisition pulse. Total scan duration was 6:40 min. No muscle or subcutaneous fat heating was reported by the subjects. Pure triacylglycerols representing the 7 most abundant fatty acids in human adipose tissue (trimyristin, tripalmitin, tripalmitolein, tristearin, triolein, trilinolein and trilinolenin, ref 3 & 4) were purchased from Nu-Chek Prep, Inc. (Elysian, MN) and dissolved in CDCl3. Proton decoupled 13C NMR spectra of subcutaneous fat were obtained at 37° C and at 150 MHz. These spectra confirmed literature data (3) and were used to assign C16 of trilinolein and the sum of bisallylic carbons of trilinolein and trilinolenin.

Results
Consistent with earlier reports (1, 2, 4), five groups of resonances were easily identified: the methyl carbon at 14.1 ppm, a complex set of resonances between 20 and 36 ppm assigned to aliphatic carbons (see the inset between 12 and 38 ppm), glycerol carbons, unsaturated carbons between 128 and 131 ppm, and the carbonyl at about 172 ppm. In the inset, Carbon 2 and Carbon 3 methylene α and β to the carboxyl group. Two resonances observed in the oils under high resolution conditions were unique for the 7 examined fatty acids: the C17 of trilinolenin at 20.1 ppm and the C16 of trilinolein. Carbon 1 and 2 of trilinolenin, ref 3 & 4) were purchased from Nu-Chek Prep, Inc. (Elysian, MN) and dissolved in CDCl3. Proton decoupled 13C NMR spectra of subcutaneous fat were obtained at 37° C and at 150 MHz. These spectra confirmed literature data (3) and were used to assign C16 of trilinolein and the sum of bisallylic carbons of trilinolein and trilinolenin.

Discussion
13C NMR spectroscopy offers much improved chemical specificity because of chemical shift dispersion but it is limited by low gamma and 1% natural abundance. Although 1H decoupled 13C NMR spectroscopy is technically challenging in human subjects, 1H decoupled 13C NMR spectra from human adipose tissue were obtained routinely at 7T. Compared to 1H NMR spectra of adipose tissue (5), the improved chemical shift dispersion allowed distinction among unsaturated fatty acids with 2 or more unsaturated bonds. Since triglycerides turn over relatively slowly, chemical analysis of adipose tissue is widely used in nutritional studies to investigate the interactions among diet and numerous chronic illnesses such as diabetes, cardiovascular disease and cancer (4). 1H NMR spectroscopy provides only limited information about the composition of triglycerides (5).

References