Cholesterol detection in adipose tissue by natural abundance *in vivo* ^{13}C MRS at 7T

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Introduction

As a ubiquitous component of all animal tissues, cholesterol has vital structural roles in membranes and in lipid metabolism. Abnormal cholesterol levels are strongly associated with cardiovascular disease. Adipose tissue is a major site of cholesterol storage [1]. A variety of nutritional and metabolic alteration may influence the adipose tissue cholesterol level, such as age, dietary cholesterol load, which has been studied in rats by chemical analysis. Localized natural abundance ^{13}C magnetic resonance spectroscopy (MRS) has been shown to be a powerful noninvasive technique for the study of lipids *in vivo* [2]. ^{13}C MRS has been applied to study the major classes of unsaturated and saturated fatty acids, like mono- and polyunsaturated fatty acids, ω-1, ω-2, ω-3, C2, C3, allylic and CH_2 envelop [3]. **In this work**, detection of cholesterol is reported for the first time noninvasively in human calf adipose tissue by ^{13}C MRS at 7T, with J-refocused ^{1}H PRESS localized DEPT sequence combined with broadband decoupling.

Materials and Methods

Measurements were performed on a 7T Philips Achiena MRI system using a quadrature dual-tuned ^{13}C/^{1}H partial volume extremity coil. A J-refocused ^{1}H PRESS localized DEPT sequence as shown in Fig.3 right, with a 90° RF pulse inserted in the middle of the double echo PRESS localization part, was used. ^{1}H-^{1}H scalar couplings are completely refocused by the 90° pulse as long as TE1=TE2, while the chemical shifts are refocused by each echo pulse. The offset of the ^{13}C frequency was centered at 30ppm of the CH_2 envelop region and the offset of 1H frequency was centered at 1.3ppm for both PRESS localization and DEPT enhancement pulses. 32ms PRESS echo time was used where TE1=TE2=16ms. Polarization transfer echo time was optimized (τ 3.7 ms) corresponding to the ^{1}H-^{13}C coupling constants in lipids covering from 125 Hz to 145 Hz. Waltz16 decoupling with 18μT pulses was used to fulfil the SAR limit. ^{13}C spectra were acquired by averaging 192 FIDs with TR 10s for a total scan time of 32min. The sequence was applied to detect lipids in the calf adipose tissue (Fig.1 left) of two healthy subjects in a total of 5 measurements (twice on one subject and three times on the other subject).

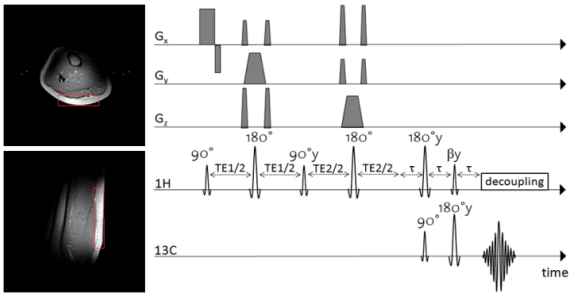


Figure 1 *localized voxel in adipose tissue with partial volume coil at 7T (left) and J-refocused ^{1}H PRESS DEPT sequence (right).*

Results and Discussion

Natural abundance ^{13}C MR spectra acquired with the proposed J-refocused ^{1}H PRESS localized DEPT sequence from the left calf of two healthy subjects are shown in Fig. 2. Eight well established peak assignments are labelled in green with their chemical shifts marked in black which are in agreement with literature values (Table 1). In addition, there are also five obvious peaks observed in both subjects consistently across all measurements, which have not been reported yet. The three peaks at 28.28ppm, 24.34ppm and 24.03ppm (Fig. 2; yellow) are tentatively assigned to cholesterol in accordance to literature values [7, 8]. The other two peaks at 33.24ppm and 30.90ppm (Fig.2; blue) are assigned to the allylic carbon atoms C13 *trans* and C18 respectively (Table 1) [4, 5 and 6]. The C13 *trans* peak at 33.24ppm has a slightly different chemical shift compared to a previous reported *trans* oleic acid peak (32.78ppm, [3]) and might stem from a isoprene unit or acetylenic fatty acids with two olefinic groups adjacent to it [6]. The diallylic peak at 25.78ppm [3], however, could be barely seen probably due to the echo timing parameters which are sensitive factors in PRESS localized DEPT enhancement.

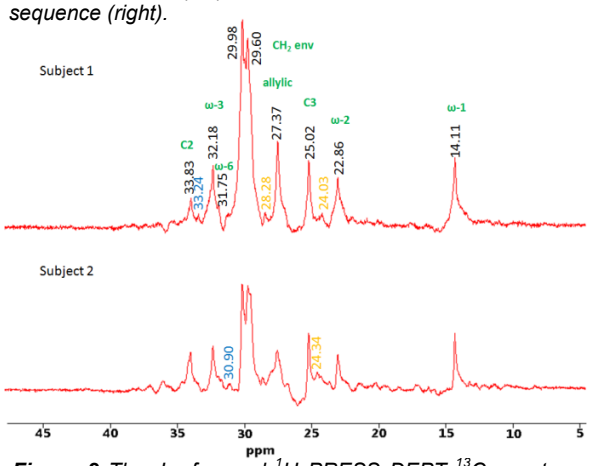
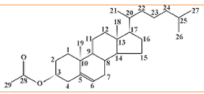


Figure 2 *The J-refocused ^{1}H PRESS DEPT ^{13}C spectra from adipose tissue of two healthy subjects at 7T, with cholesterol marked as yellow*

In conclusion, ^{13}C MRS has the potential of detecting cholesterol and other lipid components from adipose tissue and studying the interaction between cholesterol storage and whole body metabolism.

Name [a]	Structure	Chemical shift
ω-1	-CH_2-CH_3	14.11
ω-2	-CH_2-CH_2-CH_3	22.86
ω-3 (β-CH_3)	=CH-CH_2-CH_3	32.18
C2	COO-CH_2-CH_2-	33.83
C3	COO-CH_2-CH_2-	25.02
allylic	=CH-CH_2-	27.37
CH_2 envR	-CH_2-CH_2-CH_2-	29.98
CH_2 envL	-CH_2-CH_2-CH_2-	29.60
C16 (ω-6)	-CH_2-CH_2-CH_2-CH_3	31.75
C13 trans [4, 5]	=C-CH=CH-CH_2-CH_2-	33.24
C18 [6]	-CH_2-CH_2-CH_2-CH_3 (20:4) =CH-CH_2-CH=CH-CH_2-CH_3 (22:6)	30.90
C16 cholesterol [7, 8]		28.28
C23 cholesterol [7, 8]		24.03
C15 cholesterol [7, 8]		24.34

[a] most refer to AOCS lipid library and Ref. [3]
envR, envL = CH_2 envelop Right, Left

Table 1 *Peak assignments of J-refocused ^{1}H PRESS DEPT ^{13}C spectra at 7T*

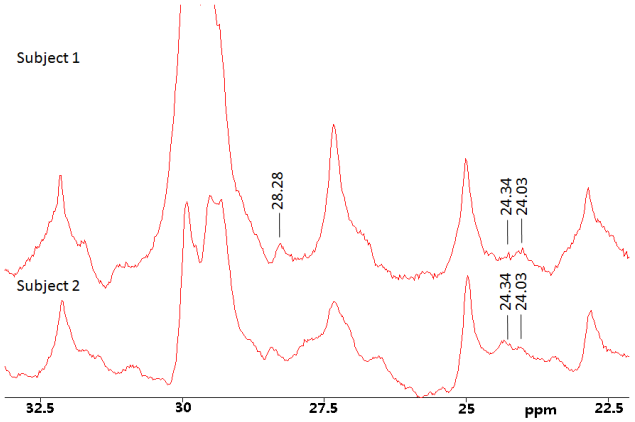


Figure 3 *The zoomed region from 22.5ppm to 32.5ppm of the spectra*

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