Structure and Quantitation of Lipids Accumulated in Human Skeletal Muscle Measured by Localized Proton Spectroscopy.

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Introduction:

Perturbations in the type as well as amount of intracellular lipids have been linked to changes in tissue whole body metabolism[1,2]. Biopsy studies of skeletal muscle are likely confounded by contamination from adiposite or extramyocellular lipids (EMCL) stores. We asked whether high resolution localized proton spectroscopy can quantitate the fatty acid composition of intramyocellular triglycerides in human subjects.

Theory:

NMR spectrum of pure triglyceride consists of ten resonance lines originating from different elements of acyl chain and glycerol backbone, as shown on Fig.1.

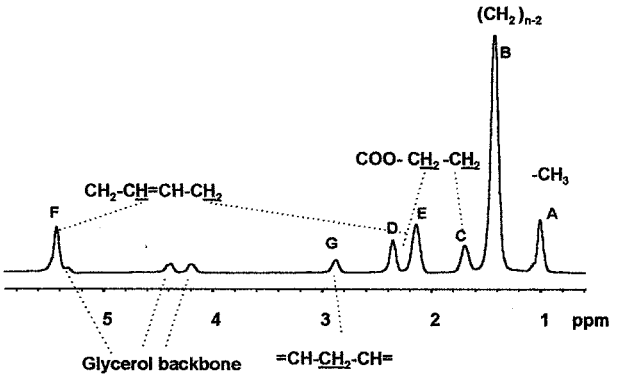

Fig.1 High resolution spectrum of triglycerols mixture of saturated, poly-unsaturated and mono-unsaturated acyl chains measured at 300 MHz (Varian, Inova).

The lines A,B,C and D in spectrum on Fig.1 originate from saturated (S), poly-unsaturated (P) and mono-unsaturated (M) fatty acids, E and F from poly- and mono-unsaturated fatty acids, and G exclusively from mono-unsaturated fatty acids. Assuming that

$$P + M + S = 1 \qquad (1)$$

one can calculate mono-unsaturated, poly-unsaturated and saturated fractions of triglycerols from NMR spectrum as follows :

$$G/A = P / (P+M + S)$$
$$F/A = (M+ P) / (P + M + S) \qquad (2)$$
$$S = 1 - P - M$$

or simply

$$P=G / A$$
$$M = (F - G) / A \qquad (3)$$
$$S = 1- P - M$$

Additionally, he ratio B/A describes the degree of proton density derived from the acyl chains and it is proportional to their length and degree of saturation. The dominant free fatty acid class can therefore be deduct from lines A, B, F and G in the NMR spectrum.

Results:

Proton spectra of intramyocellular lipids were measured in the gastrocnemius/soleus complex of 4 subjects with Generalized Lipodystrophy (GL) and analyzed using the above approach. GL is a condition associated with almost complete absence of adipose tissue in the interfacial and subcutaneous compartments[3].Therefore, their triacylglycerol pool appears in a single compartment, making these patients an excellent human model for testing this method. Fig.2 shows water suppressed proton spectrum from one of our GL patients. The areas from fat associated signals were calculated using NUTS (ACORNNMR) and equations (3) were applied to establish their relationship. Results are summarized in Table 1.

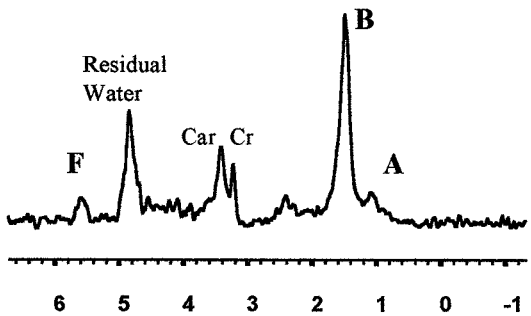

Fig.2 Localized ^{1}H spectrum of skeletal muscle from GL patient #1, (See Table1.) collected on a Gyroscan NT whole body system (Philips Medical System) VOI=15x15x15 mm3, PRESS sequence with Tr=5s, Te=33ms, 1024 data points over 1 kHz spectral width.

	FA fractions			
Patient	P	M	S	B/A
1	none	66.0%	34.0%	6.70
2	none	9.50%	90.50%	6.01
3	none	none	100.0%	7.33
4	none	none	100.0%	8.33

Table 1. The composition of fatty acids in skeletal muscle of patients with GL.

Discussion:

The IMCL in skeletal muscle of all patients with GL do not contain an NMR detectable level of poly-unsaturated fatty acids. Line G (2.9 ppm) is not detected in the NMR spectra for all four subjects studied. IMCL contains a mixture of saturated and mono-unsaturated lipids (patients 1and 2) or exclusively saturated lipids (patients 3 and 4). Whether the lack of IMCL poly-unsaturated fatty acids is a characteristic of lipid metabolism in GL patients or is due to inadequate sensitivity remains to be established.

Conclusions:

The average chain length and degree of saturation of intramyocellular lipids can be derived from high resolution proton spectra in vivo. This is a promising tool for the study of intracellular lipids metabolism.

References:

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2. D.T.Stein et al, Diabetes 46(1):23A(1997)
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