A Proof-of-Concept Study Towards in vivo Triglyceride Composition Determination by MR Spectroscopy at 3T, with Validation Against High-Resolution NMR

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BACKGROUND/PURPOSE: Triglyceride (TG) is the predominant form of lipid storage in biological tissues, including adipose, bone marrow, and liver. TG is composed of three fatty acid (FA) esters attached to a glycerol molecule. Individual FA molecule may differ in the hydrocarbon chain length (CL, mostly 16C and 18C in animals), saturation index (saturated [0], mono-unsaturated [1], di-unsaturated [2], etc.), and the location of unsaturation (i.e. C=C double bond). Therefore tissue TG pool is generally a mixed population of various types of FAs and the composition can be characterized by the relative proportion of each type, for example, %-saturated, %-mono-unsaturated, etc. The importance of the FA types is increasingly recognized in many biological processes such as cancer, including hepatocellular carcinoma[1]. Earlier studies explored the feasibility of TG composition determination in humans using single-voxel *in vivo* MR spectroscopy (MRS)[1,2,3]. Proton spectra of TGs have multiple peaks of different chemical shift, whose relative peak areas reflect the proportions of different FAs. Previous MRS studies used known types of pure FAs[2] or published composition values[3] as the reference standard to validate the composition determination technique. High resolution nuclear magnetic resonance (NMR) is now recognized as a standard analytical chemistry technique (along with gas/liquid chromatography) for TG composition by American Oil Chemists' Society, and may be a more suitable reference standard for clinical MRS. In this proof of concept study, we selected 6 different fats/oils of different FA proportions, and compared single-voxel MR spectroscopy at 3T (128 MHz) against 400 MHz high-resolution NMR for TG composition determination. The hypothesis was that the measured TG compositions are similar, assessed by relative spectral peak sizes as well as by calculating % proportion of saturated FA (SFA), mono-unsaturated FA (MUFA) and poly-unsaturated FA (PUFA).

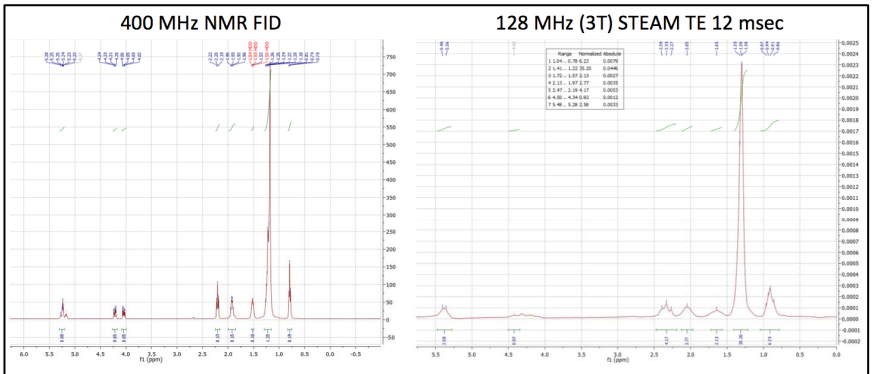

Figure 1: Beef tallow dissolved in deutro-chloroform. Six peaks at 0.9, 1.3, 1.6, 2.1, 2.3, and 2.8 ppm quantified. %SFA/MUFA/PUFA according to USDA 52/44/4, NMR 45/41/14, MRS 51/45/4.

MATERIALS/METHODS: Six different types of fats/oils were selected: cocoa butter and beef tallow rich in SFA, olive oil rich in MUFA (18:1), grapeseed oil rich in PUFA of saturation index 2 (18:2), flaxseed oil rich in PUFA of saturation index 3 (18:3), and peanut oil which is a more balanced admixture of SFA, MUFA, and PUFA. Cocoa butter and tallow are solid at room temperature and therefore were dissolved in deutrated chloroform. For the NMR experiment, 0.5 cc of liquefied/liquid fats/oils were examined using a Varian 400 MHz spectrometer (Agilent Technologies, Santa Clara, CA) and the free induction decay (FID) was recorded for each fat/oil type. For the MRS experiment, approximately 25 cc of liquefied/liquid fats/oils were poured into round-bottom flasks. Each flask was placed in the center of the magnet and 15x15x15 mm single-voxel localized MR spectroscopy was performed in the middle of the flask using a STEAM sequence on a 3T Philips Achieva mTX (Philips Healthcare, Best, Netherlands) using the Head T/R coil, TE 12 msec, TR/TM 5000/11 msec, number of signal average 4. The 400 MHz and 3T data were analyzed using MNOVA (Mestrelab Research, Santiago de Compostela, Spain). Autodetected peaks were integrated and recorded for the 0.9, 1.3, 1.3, 2.1, 2.3, and 2.8 ppm. When autodetection failed for a small peak (e.g. 2.8 ppm diacyl peak) in some fat/oil types, the same chemical shift range was integrated as other fat/oil types with detectable corresponding peak. The relative % of these 6 peaks were calculated and compared between 400 MHz and 3T. For each fat/oil, the TG composition was calculated in terms %SFA, MUFA, and PUFA, using method proposed by Ren et al[2], assuming that saturation index ≤2 for all FAs. Literature values of %SFA, MUFA, PUFA were also obtained from US Department of Agriculture National Nutrient Database for Standard Reference Release 26 (http://ndb.nal.usda.gov/ndb).

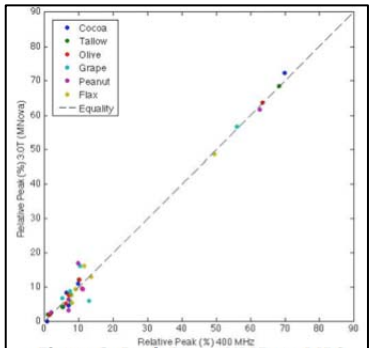

Figure 2: Peak Areas NMR vs. MRS

RESULTS/DISCUSSION: Example ^{1}H spectra (**Figure 1**) of beef tallow (animal fat) are very similar between 400 MHz NMR and 3T MRS, except for wider peak line-width (lower spectral resolution) at 3T. At clinical field strength of 3T, the spectral resolution is sufficient for FA peak quantification. There is an excellent 1:1 correlation of the peak areas between NMR and MRS (**Figure 2**) with Pearson's correlation r=0.99 and p-value < 0.001. Estimated %SFA, MUFA, PUFA calculated from spectral peak values are similar for each TG type across USDA reported values and estimated values from NMR and MRS data. The only exception is flaxseed oil, rich in tri-unsaturated fatty acids (18:3), in which TG composition estimates from NMR and MRS spectra returned nonsensical values. This is because the calculation method for % composition assumed all PUFA to be di-unsaturated; abundant tri-unsaturated FAs in flaxseed oil violate this assumption. As previously proposed, however, the contribution FAs with saturation index >2 are very small in animal fat[2,3], and may not be a significant problem *in vivo*. In summary, this proof-of-concept study showed that *in vivo* TG composition determination may be possible clinically using 3T MRS. Estimated TG composition at 3T is highly correlated to that by high-resolution NMR, and similar to reported values by USDA.

REFERENCES: [1] Griffitts 2009 J Lipid Res 50:611-22, [2] Ren et al. 2008 J Lipid Res. 49(9): 2055-62. [3] Bydder et al. 2011 Magn Reson Imaging 29(8):1041-6.

Table 1: Comparison of Calculated TG Composition

	Cocoa Butter			Beef Tallow			Olive Oil			Grapeseed Oil			Peanut Oil			Flaxseed Oil		
Source	USDA	NMR	MRS	USDA	NMR	MRS	USDA	NMR	MRS	USDA	NMR	MRS	USDA	NMR	MRS	USDA	NMR	MRS
%SFA	62.45	57.49	66.01	52.09	42.31	50.69	14.18	21.59	18.83	10.04	13.10	4.75	17.77	19.81	11.33	9.43	12.88	8.11
%MUFA	34.41	28.02	31.57	43.72	42.31	44.91	75.03	53.86	60.20	16.84	17.10	13.08	48.58	49.02	58.55	19.39	-27.09	-39.09
%PUFA	3.14	14.49	2.42	4.18	15.38	4.39	10.79	24.56	20.96	73.12	69.80	82.18	33.65	31.17	30.12	71.17	114.21	130.98