New ^{13}C NMR Method for Lipid Synthesis Rates

*Derf A. Lewis, #Tayseer A. Aldaghlas, #Joanne K. Kelleher and *#Maren R. Laughlin
Dept. of *Surgery and #Physiology, The George Washington University, Washington, DC

Introduction. Many metabolic tracer studies are limited by inability to measure the isotope fractional enrichment (FE) of the direct precursor of the product of interest, especially when several substrates contribute to the precursor pool. In the Hep G2 cell, the common precursor for the lipid and sterol synthetic pathways, cytosolic acetyl-CoA, has contributions from mitochondrial citrate, cytosolic acetate and amino acids. ^{13}C NMR isotopomer analysis of cholesterol is introduced as a method to measure the FE of cytosolic acetyl-CoA which, when combined with a time-dependent NMR assay of ^{13}C-lipid, yields an accurate measurement of the net rate of fatty acid synthesis. Although precursor FE could not be measured with the same time resolution as the lipid product, the behavior of the ^{13}C-glutamate pool, which is in fast exchange with the TCA cycle intermediates, may be a rough indicator of the ^{13}C time course of two other TCA cycle products, cytosolic citrate and acetyl-CoA.

Methods. Hep G2 cells were embedded in agarose beads, incubated overnight in experiental media to reach steady state, then superfused in a 10 mm NMR tube with 95% air/5% CO_2 at 36° in the $^{13}C/^{31}P/^{1}H$ probe of an AC300 Bruker spetrometer. DMEM media was supplemented with 10% FCS, 25 mM glucose, 10 mM acetate, and 4 mM glutamine. After two 30-min ^{13}C control spectra (67° PW, 2 sec RD, full composite pulse decoupling and NOE development), the system was switched to similar media except one substrate was labeled ([2-^{13}C]acetate, [5-^{13}C]glutamine, [2- or [1-^{13}C]glucose), and spectra were acquired for 20 hours. The lipid signal at 29-29.9 ppm, (carbons C4-C13 of palmitate) was use for the ^{13}C-lipid time course. The cells were frozen in N_2 at the end, lipid was extracted in chloroform:methanol, and ^{13}C in the various carbons was quantitated in NMR spectra using the solvent $CDCl_3$ signal as internal standard. Each substrate yields singly-labeled acetyl-CoA, so new fatty acyl chains are labeled at every other carbon. The total lipid ^{13}C content (from extracts) was used to calibrate the signal at 29 ppm in the last spectrum of the time course. The number of new fatty acyl chains could also be estimated in extracts by the difference in ^{13}C between those unique fatty acid sites that receive ^{13}C enriched precursor carbon (C2,C14,C16 = A), and those which receive ^{13}C at its natural abundance of 1.1% (C1,C3,C15 = U).

Time-dependent method:

fatty acid synthesis = d/dt(^{13}C lipid (29ppm)) x
mol ^{13}C/unit signal x $1/FE_{acetyl\text{-}CoA}$

Extract method:

acyl chain synthesis = (A - U)/time x $1/\{FE_{acetyl\text{-}CoA} - 0.011\}$

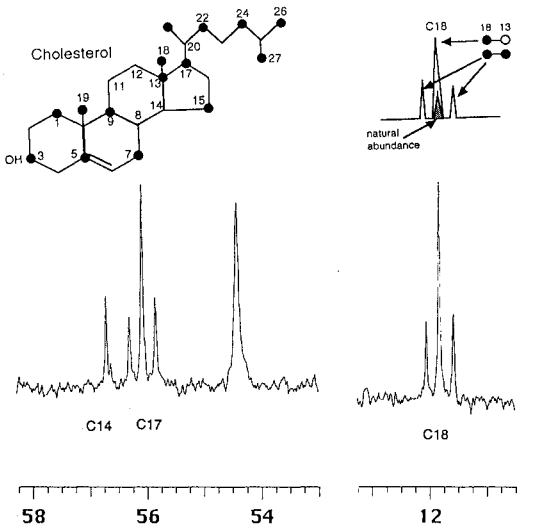
The FE of cytosolic acetyl-CoA was measured by isotopomer analysis of another of its products, cholesterol, which contains two bonds between enriched carbons (fig. 1), resulting in a doublet (C18D & C17D). ^{13}C bound to ^{12}C results in a singlet (C18S & C17S). The singlet must be corrected for the basal cholesterol, done using the several resonances that are not specifically enriched with ^{13}C (i.e., C14).

$$C18D / (18S + C18D - C14) = FE^2/(FE - 0.011)$$

Results and Discussion. The table shows the nominal substrate FE, and the measured cytosolic acetyl-CoA FE for the [2-^{13}C]acetate experiments. Due to low cholesterol synthesis in these cells, the FE was too low for the other substrates to measure, but could be estimated from the relative rates of lipid synthesis. The ^{13}C NMR signal at 29-29.9 ppm of Hep G2 cells was recorded. Lipid acetyl unit synthesis was calculated for the final 9 hrs using the time-dependent method, and fatty acyl chain synthesis from the extract method. Total synthesis was from [2-^{13}C]acetate data. Subtraction of the three measured rates from the total gave lipid from "other" sources. When using time-dependent data, exogenous glucose, acetate and glutamate accounted for all new lipid.

Lipid synthesis from glucose was much lower when using extracts than the time-dependent method. The extract method would be in error if the time to precursor FE steady state is long. The ^{13}C-glutamate signal, (thought to be in rapid exchange with TCA intermediates), yields exponential time constants of 0.38 hr for [2-^{13}C]acetate, 0.42 hr for [5-^{13}C]glutamine, and 8.13 hr for ^{13}C-glucose. Lactate accumulation was linear throughout. We cannot explain the difference in ^{13}C-glutamate time course between substrates. Perhaps the regulation of glucose oxidation changes over time with lactate accumulation in isolated Hep G2 cells.

Conclusion. This new, accurate ^{13}C-NMR assay for the absolute rate of fatty acid synthesis is dependent on the time-course of ^{13}C fatty acid, and the cytosolic acetyl-CoA FE measured by isotopomer analysis of extracted cholesterol. It shows promise for studies of the regulation of lipid synthesis in the liver.



	Nominal Substrate FE (%)	FE (%) cytosolic acetyl-CoA	Time-dep. Lipid Synth nmol acetyl hr.mg pro.	Extract Lipid Synth nmol chains hr.mg pro.
Total	-	-	85 ± 16	9.4 ± 2.5
[2-^{13}C]acetate	99	41 ± 4	35 ± 7	3.9 ± 1.2
^{13}C-]glucose	49.5	18*	34 ± 6	2.5 ± 0.8
[5-^{3}C]glutamine	99	18*	18 ± 3	1.8 ± 0.5
Other	-	1.1	-2*	1.3*

All shown with standard deviation
*calculated from relative lipid synthesis rates