

# 13C and 31P NMR Spectroscopy in Perfused Mouse Liver: A novel method for investigating effectors of hepatic fatty acid metabolism

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## Introduction

The transition from *in vitro* studies to *in vivo* demonstration of compound efficacy is often confounded by animal dosing issues and/or less than optimal pharmacokinetic parameters. The perfused liver technique<sup>1</sup> in combination with the introduction of a <sup>13</sup>C-labelled metabolic substrate and <sup>13</sup>C NMR spectroscopy offers a real time, ex vivo probe of the major metabolic pathways (glucose metabolism, TCA cycle, fatty acid metabolism) in excised, functional livers. Here this technique is applied to perfused livers from db/+ mice fed a high fructose diet (a known model of increased hepatic lipogenesis) to develop a valuable assay for assessing the efficacy of novel small molecule inhibitors of fatty acid synthesis (FAS).

## Methods

Experiments were performed on a Bruker 11.7T (500MHz proton resonance frequency) wide bore NMR spectrometer using a 20mm TXO probe tuned for <sup>13</sup>C and <sup>31</sup>P observation. Db/+ mice fed a high fructose diet were anesthetized and, following a portal vein cannulation, livers were excised and placed in a custom 20mm NMR tube as part of the apparatus shown in Figure 1. ATP levels, measured via <sup>31</sup>P NMR spectroscopy, were used to monitor hepatic viability over the course of the studies. In this setting ATP levels were unchanged (<5%) for approximately three hours. The biosynthesis of fatty acids in the perfused liver was monitored by infusing [2-<sup>13</sup>C]Pyruvate and following the <sup>13</sup>C enrichment at the fatty acid -(CH<sub>2</sub>)<sub>n</sub>- position by <sup>13</sup>C NMR spectroscopy<sup>2</sup>. Subsequent treatment with novel FAS inhibitors reduced FAS in a dose dependent manner and gave a direct measurement of efficacy for those inhibitors.

## Results

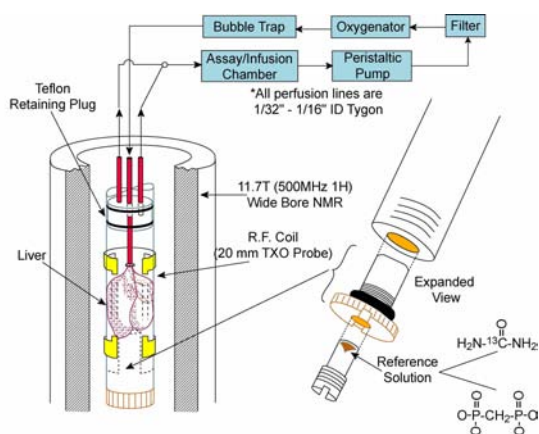
Figure 2 displays the time course of the integral of the -(<sup>13</sup>CH<sub>2</sub>)<sub>n</sub>- fatty acid peak for vehicle (DMSO) treated livers and livers treated with Cpd 1, a novel inhibitor of FAS. Cpd 1 inhibits FAS in a dose dependent manner with a 3μM dose having little effect and a 30μM dose completely halting FAS without affecting ATP levels. One notable aspect of this technique is that the difference between vehicle treated livers and livers treated with a fully efficacious dose of Cpd 1 represents a large window (compared to the SEM's) over which to observe the effects of FAS inhibitors. Though *in vivo* studies with orally dosed Cpd 1 were complicated by its poor solubility and absorption properties, the perfused liver technique presented here was able to clearly demonstrate its effect on FAS in the target organ of interest. Hence this technique gives validation for novel compounds as FAS inhibitors, as well as intrinsic compound efficacy independent of pharmacokinetic considerations.

## Conclusion

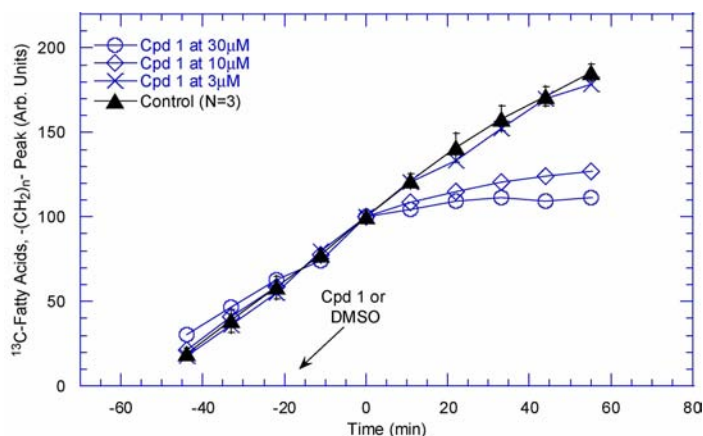
The perfused liver NMR technique has been employed to develop a robust and sensitive functional assay yielding data which is more physiologically relevant than *in vitro* studies, yet avoids the complications of traditional *in vivo* studies. This technique has proven to be a valuable tool for the functional evaluation of FAS inhibitors.

## References

1. Cohen, SM. In Research in Perfused Liver: Clinical and Basic Applications. Ballet, F. and Thurman, RG. Eds. John Libbey: London, 1991, Chapter 4.
2. Cohen, SM, Wermann, JG, and Tota, MR. <sup>13</sup>C NMR Study of the effects of leptin treatment on kinetics of hepatic intermediary metabolism. PNAS, 1998 (95), 7385-90.



**Figure 1.** Essential components of the perfused liver apparatus. Inflow of perfusion buffer is via the portal vein cannula. The expanded view of the bottom of the NMR tube shows an external reference solution which may be used for absolute quantitation.



**Figure 2.** Time course of the integral of the -(<sup>13</sup>CH<sub>2</sub>)<sub>n</sub>- fatty acid peak for livers treated as described in Methods. SEM's are of similar size as the plot markers.