# Alcohol as a substitute for acetate in $^{13}$C MRS study of brain metabolism

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## Introduction

Acetate is a known glia-specific substrate and has been used to study cerebral metabolism and neurotransmission in both human subjects and animals [1-3]. In particular, intravenously administered $^{13}$C-labeldd acetate can be used to label cerebral glutamine and glutamate, allowing glutamate-glutamine interactions to be monitored in vivo using $^{13}$C MRS and/or proton-detected $^{13}$C MRS [4-6]. Unlike the well studied procedures for intravenous infusion of glucose, there are only a few reports in the literature on intravenous infusion of sodium acetate into humans [7,8]. Potential risk in intravenous infusion of sodium acetate in patients is unknown in many disorders. In comparison, the effect of alcohol (ethanol) consumption is well understood. Alcohol is predominantly metabolized into acetate in the liver. It is therefore possible to use 13C-labeled alcohol as a substitute for $^{13}$C-labeled acetate for certain studies when the direct effect of alcohol is unimportant or the subject of the study. One main advantage of this strategy is that no intravenous infusion is necessary as alcohol can rapidly diffuse from stomach into the rest of the body. As the first step in this line of research we compare the results of intravenous administration of [$^{13}$C] acetate and [$^{13}$C] ethanol. In the present study, $^{13}$C MRS of the rat brain was performed to detect signals originated from [$^{13}C_6$]-D-glucose, [1-$^{13}$C] acetate and [1-$^{13}$C] ethanol.

## Methods

Male SD rats (178-255g) fasted overnight with free access to drinking water were divided into five groups for the study of neuro-metabolism. Before the *in vivo* $^{13}$C NMRS study, one artery was cannulated for periodically sampling of arterial blood to monitor blood gases (*p*O2, *p*CO2), pH, blood glucose concentration and continuously monitoring the arterial blood pressure level. The ipsilateral vein was also cannulated for the continuous infusion. Glucose infusion used 0.75M [$^{13}C_6$]-D-glucose (10min bolus: 75.5mg/min/Kg BW, 10~270min: 28.5mg/min/Kg BW). Acetate infusion used 0.90M [1-$^{13}$C] acetate (*p*H=7.0, 10min bolus: 18.7mg/min/Kg BW, 10~270min: 7.1mg/min/Kg BW). Alcohol infusion used 33% (*vol/vol*) [1-$^{13}$C] ethanol (10min bolus: 200mg/min/Kg BW, 10~270min: 14.8mg/min/Kg BW). Single infusion (n=15), co-infusion of [$^{13}C_6$]-D-glucose and [1-$^{13}$C] acetate (n=10), and co-infusion of [$^{13}C_6$]-D-glucose and [1-$^{13}$C] ethanol (n=10) were performed. The blood glucose level was maintained within 12~15mM/L. The *in vivo* $^{13}$C NMRS studies were performed on a Bruker AVANCE spectrometer using a home-made $^{13}$C {$^{1}$H} RF coil. Three-section (coronal, horizontal and saggital) MR images (FOV=2.5cm, slice thickness=1mm, TR/TE=200/15ms) were acquired for positioning of the animals. Mixture of 70% $N_2O$, 30% $O_2$, and 1.5% isoflurane was used for anesthesia. 13C spectra were acquired according a previously described method (). Normal physiological conditions were maintained throughout the experiment (pH ~7.4, $PCO_2$ ~35mm Hg and $PO_2$>100mmHg).

## Results and Discussion

All NMRS data were processed using lb=-5, gb=0.1, and zero-order phase correction. Fig. 1 A shows the *in vivo* proton decoupled $^{13}$C NMR time course spectra from the rat brain in the 168-186 ppm region with co-infusion of [1-$^{13}$C] ethanol and [$^{13}C_6$]-D-glucose. Fig 1B shows the corresponding spectra with co-infusion of [1-$^{13}$C] acetate and [$^{13}C_6$]-D-glucose. As described in a separately submitted abstract, the acetate infusion protocol does not produce any visible signal from acetate itself. The co-infusion of differently labeled substrates and the detection of signals in the carboxylic/amide 13C spectral region allow clean separation of contribution to glutamate, glutamine and aspartate from different substrates. As shown in Fig. 2, glutamate C5 originated from [$^{13}C_6$]-D-glucose appears as a doublet with a J coupling constant of 51 Hz while glutamate C5 originated from [1-$^{13}$C] acetate or [1-$^{13}$C] ethanol appears as a singlet. A pseudo quartet was detected in the 178-179 ppm region, allowing easy separation of contributions to glutamine C5 and aspartate C4 from different substrates. Similarly, glutamate C1 originated from [$^{13}C_6$]-D-glucose and singly labeled substrates can also be separated. There is a striking similarity between the steady state spectra acquired using [$^{13}C_6$]-D-glucose + [1-$^{13}$C] acetate and those using [$^{13}C_6$]-D-glucose + [1-$^{13}$C]ethanol (Fig.2). In both spectra, there is a predominant contribution to glial glutamine from the singly labeled substrate (acetate or alcohol). This similarity can be explained by conversion of ethanol in liver into acetate and recirculation of acetate into the brain. In addition, the features of the spectra acquired during co-infusion matches the sum of the corresponding single substrate infusion spectra (data not shown). The present study here demonstrated that the metabolic consequence of ethanol consumption can be detected in the brain by the noninvasive $^{13}$C NMR spectroscopy method. The high similarity of in vivo 13C spectra acquired with ethanol administration to intravenous acetate infusion suggests that oral administration of ethanol may be used for the purpose of using acetate to study brain.

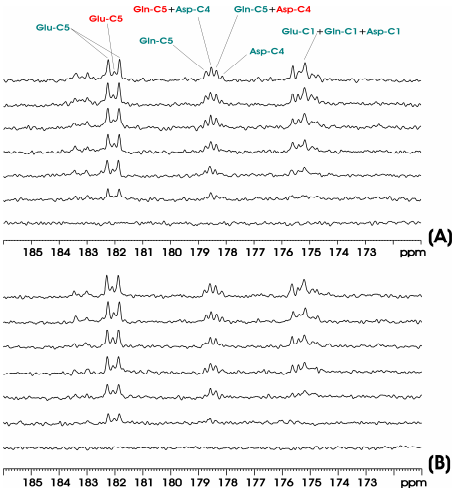


**Fig.1.** (A) Time-course spectra from an individual rat brain after co-infusion of [1-$^{13}$C] ethanol and [$^{13}C_6$]-D-glucose. (B) Spectra after co-infusion of [1-$^{13}$C] acetate and [$^{13}C_6$]-D-glucose. Each individual spectrum was averaged for 20min. Green: signals originated from $^{13}C_6$-D-glucose; Red: signals originated from [1-$^{13}$C] ethanol or [1-$^{13}$C] acetate.

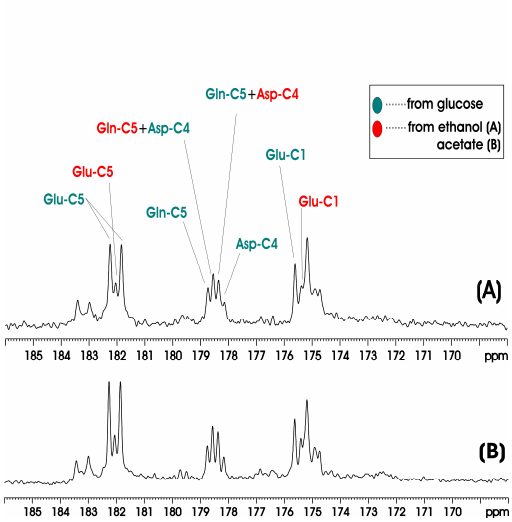


**Fig.2.** (A) Steady state spectrum during co-infusion of [1-$^{13}$C] ethanol and [$^{13}C_6$]-D-glucose from an individual rat brain. (B) Steady state spectrum during co-infusion of [1-$^{13}$C] acetate and [$^{13}C_6$]-D-glucose. Both spectra were accumulated from 120–260 min after the start of infusion. Spectra were processed using the same parameters as in Fig. 1.

## References

[1] Shic F, *et al*. J Magn Reson 2003; 162:259-68. [2] Yang J, *et al*. Neurochem Int 2007; 50:371-8. [3] Lebon V, *et al*. J Neurosci 2002; 22:1523-31. [4] Hassel B, *et al*. J Neurochem 1995; 64:2773-82. [5] Cerdan S, *et al*. J Neurochem 2009; 109: 63-72. [6] Deelchand DK, *et al*. J Neurochem 2009; 109: 46-54. [7] Mason GF, *et al*. Diabetes 2006; 55: 929-34. [8] Befroy DE, *et al*. Diabetes 2007; 56:1376-81.