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# Hepatic gluconeogenesis influences $^{13}C$ enrichment in lactate in human brain tumors during metabolism of [1,2-$^{13}C$]acetate

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ABSTRACT

$^{13}C$-enriched compounds are readily metabolized in human malignancies. Fragments of the tumor, acquired by biopsy or surgical resection, may be acid-extracted and $^{13}C$ NMR spectroscopy of metabolites such as glutamate, glutamine, 2-hydroxyglutarate, lactate and others provide a rich source of information about tumor metabolism *in situ*. Recently we observed $^{13}C$-$^{13}C$ spin-spin coupling in $^{13}C$ NMR spectra of lactate in brain tumors removed from patients who were infused with [1,2-$^{13}C$]acetate prior to the surgery. We found, in four patients, that infusion of $^{13}C$-enriched acetate was associated with synthesis of $^{13}C$-enriched glucose, detectable in plasma. $^{13}C$ labeled glucose derived from [1,2-$^{13}C$]acetate metabolism in the liver and the brain pyruvate recycling in the tumor together lead to the production of the $^{13}C$ labeled lactate pool in the brain tumor. Their combined contribution to acetate metabolism in the brain tumors was less than 4.0%, significantly lower than the direct oxidation of acetate in the citric acid cycle in tumors.



## 1. Introduction

Metabolic pathways in human tumors *in situ* may be examined by infusion of $^{13}C$-enriched compounds followed by $^{13}C$ NMR spectroscopy of an aqueous extract of the tissue. This approach is readily incorporated into the work flow of the operating room because of the absence of ionizing radiation and simple substrates involved, such as acetate or glucose. Recently we found that glioblastomas and brain metastases have the capacity to oxidize acetate in the citric acid cycle based on an analysis of $^{13}C$-$^{13}C$ spin-spin coupling in glutamate (Mashimo et al., 2014). $^{13}C$-$^{13}C$ coupling due do $J_{1,2}$ and $J_{2,3}$ was also observed in the $^{13}C$ NMR spectra of lactate in the tumors. This finding was unexpected because there is no simple pathway for

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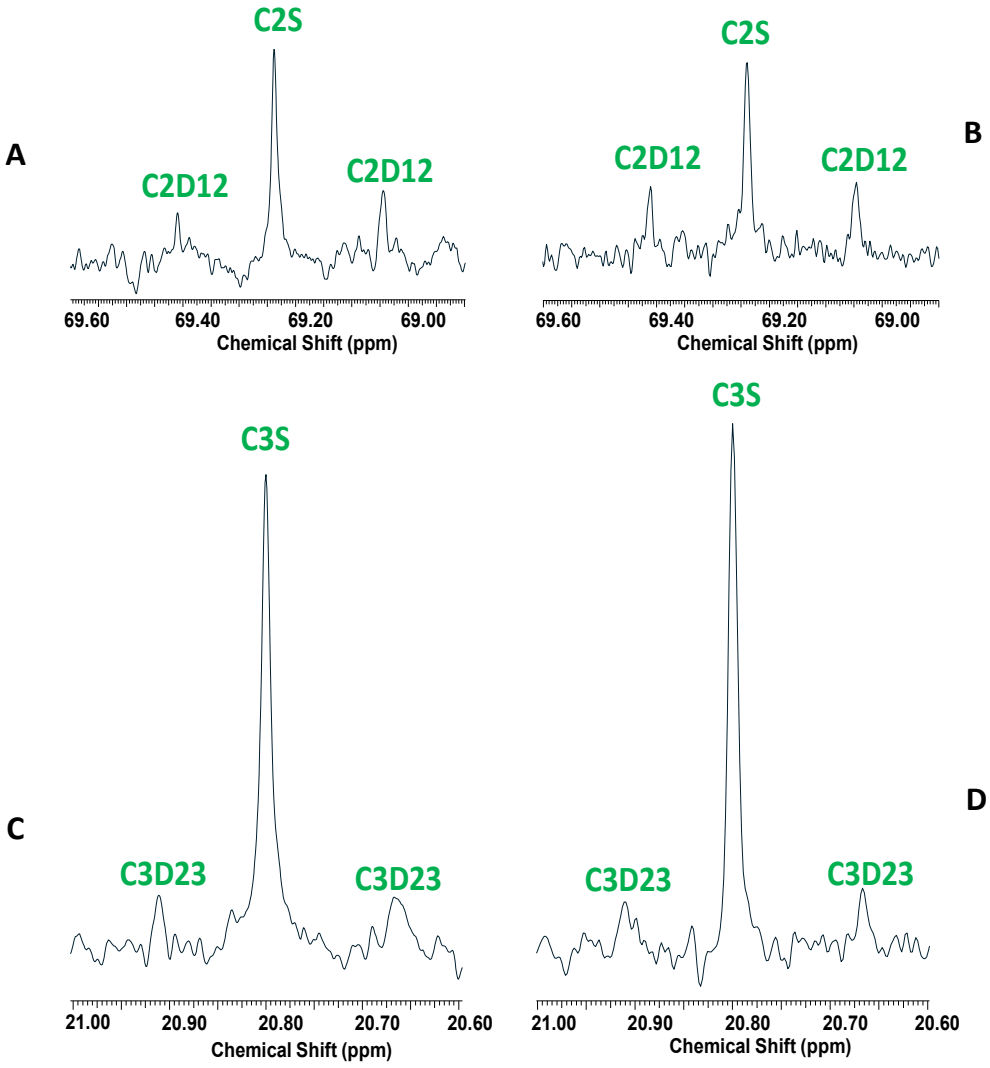


**Fig. 1.** Tumor lactate $^{13}$C-labeling in a patient with GBM (A and C) and a patient with lung metastasis to brain (B, D). $^{13}$C-$^{13}$C doublets C2D12 and C3D23 correspond to [1,2-$^{13}$C] and [2,3-$^{13}$C] isotopomers of $^{13}$C labeled lactate respectively. C2S and C3S are singlet signals from C2 and C3 carbons of lactate respectively.

[1,2-$^{13}$C]acetate to enter the lactate or pyruvate pool (Mashimo et al., 2014; Marin-Valencia et al., 2012a; Cerdan et al., 1990; Haberg et al., 1998a, 1998b; Deelchand et al., 2009). There have been a few reports that discuss the possibility of $^{13}$C-enriched pyruvate or lactate arising from $^{13}$C-enriched acetate through $^{13}$C-labeled acetyl-CoA contributing to $^{13}$C enrichment in oxaloacetate (OAA) followed by decarboxylation and generation of $^{13}$C-enriched pyruvate, known as pyruvate recycling (Cerdan et al., 1990; Haberg et al., 1998a, 1998b; Deelchand et al., 2009; Cruz et al., 1998; Serres et al., 2007). However, systemic acetate may also enter the citric acid cycle of the liver. Although net synthesis of glucose from acetyl groups does not occur in mammalian liver, $^{13}$C from acetate may mix in the oxaloacetate pool and enter gluconeogenesis. Exported $^{13}$C-glucose may be metabolized in the brain tumor. Lactate could also be produced due to the metabolism of acetate elsewhere in the body and made available to the tumor through plasma. Here, we demonstrate that $^{1}$H and $^{13}$C NMR of plasma is a simple and powerful method to study hepatic gluconeogenesis during infusion of [1,2-$^{13}$C]acetate.

## 2. Material and methods

Two patients with glioblastomas and two patients with brain metastases (breast cancer and lung cancer) were enrolled in a protocol approved by the University of Texas Southwestern Institutional Research Board. Subjects were infused with [1,2-$^{13}$C]acetate at 6 mg/kg/min for 5 min, followed by 3 mg/kg/min for 2 h. Methods for sampling tumor tissue and plasma for NMR spectroscopy have been described previously (Mashimo et al., 2014; Marin-Valencia et al., 2012a; Maher et al., 2012). Proton decoupled $^{13}$C spectra of tumor extracts and plasma were acquired at 150 MHz (for $^{13}$C) on an Avance Bruker NMR Spectrometer equipped with 10-mm broadband cryogenically-cooled probe (Bruker Biospin, Billerica, MA). The lactate C3 carbon signal at 20.8 ppm was used as internal chemical shift reference. Examples of $^{13}$C NMR spectra of these brain tumors have been published previously (Mashimo et al., 2014). Signal areas were measured using ACD (Advanced Chemistry Development, Toronto, Canada) (Mashimo et al., 2014; Maher et al., 2012; Marin-Valencia et al., 2012b).