# [3-$^{13}$C]PYRUVATE: USEFUL ALTERNATIVE TO LABELED GLUCOSE FOR *IN VITRO* METABOLIC STUDIES IN PRIMARY MOUSE HEPATOCYTES

**S. Gottschalk[1], M. Hohnholt[2], D. Leibfritz[2], M. Bilodeau[1], and C. Zwingmann[1]**

[1]Department of Medicine, University of Montréal, Montréal, Québec, Canada, [2]Department of Organic Chemistry, University of Bremen, Bremen, Germany

## Introduction :

The application of stable isotope labeling in cell culture experiments is a widely used and potent model for the study and characterization of metabolic pathways and fluxes. In particular, incorporation of $^{13}$C-labels at certain carbon positions of a given metabolite may not only provide important information about pathways and fluxes but may even reveal unexpected metabolic phenomena without prior knowledge. Depending on the cellular model and the area of interest, one has to take into consideration which labeled substrate will give the most valuable information.

Primary hepatocytes in cell culture – a widely used model to study liver physiology and diseases – are expected to use a variety of substrates for their cellular and energy metabolism. So far, $^{13}$C-NMR studies have been rarely performed in isolated hepatocytes. Labeled glucose is the most widely used substrate in cells isolated from various organs. However, we have shown in preliminary experiments that hepatocytes in primary culture do not readily metabolize glucose taken up from the medium. Since hepatocytes have a very high mitochondrial activity, we have therefore decided to use one- and two-dimensional multinuclear NMR-spectroscopy to characterize the metabolism of [3-$^{13}$C]pyruvate and the metabolic isotopomers derived trough various pathways in these cells.

## Aims :

**A)** To assess whether metabolites of [U-$^{13}$C]glucose can be detected in extracts of primary mouse hepatocytes.

**B)** To evaluate the use of [3-$^{13}$C]pyruvate, as an alternative to labeled glucose, for metabolic studies in primary hepatocyte cultures and identify the different metabolites and isotopomers derived from pyruvate metabolism in these cells.

## Methods :

*Cell culture:* Hepatocytes were isolated from the livers of Balb/c mice. The cells were allowed to attach overnight. Afterwards, the medium was changed to a physiological medium (DMEM) without glucose and pyruvate. [U-$^{13}$C]glucose or [3-$^{13}$C]pyruvate (each 5mM) was added and cells were extracted after 4 h or 6 h.

*Extraction:* At the end of the incubation period with the labeled substrates, cells were immediately frozen in liquid nitrogen and extracted with 5% perchloric acid. After centrifugation, the supernatant was neutralized and lyophilized.

*NMR analysis:* Lyophilized samples were redissolved in 0.5 ml $D_2O$. One- and two-dimensional $^{1}$H- and $^{13}$C-NMR spectra were recorded on a Bruker DRX 600 MHz Spectrometer to quantify and identify metabolic isotopomers derived from [3-$^{13}$C]pyruvate metabolism.

## Results :

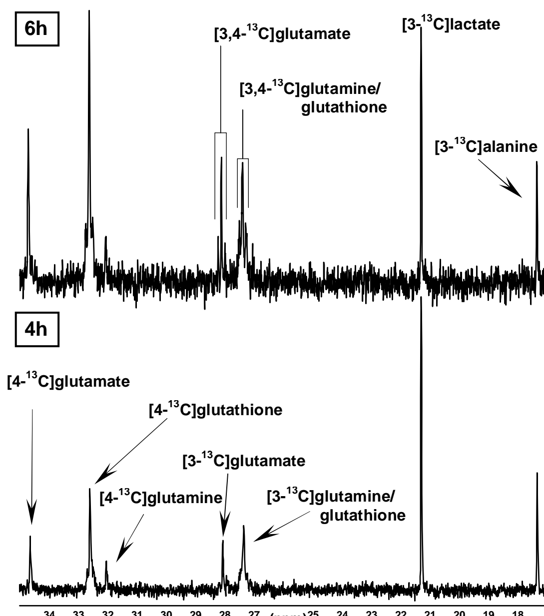
**A)** $^{1}$H- and $^{13}$C-NMR spectra showed that primary mouse hepatocytes in culture readily take up [U-$^{13}$C]glucose. However, no other $^{13}$C-labelled metabolites were detected in the cell extracts or in the incubation media, showing that glucose is not processed trough glycolysis and subsequent metabolic pathways.

**B)** After 4 hours of incubation with [3-$^{13}$C]pyruvate, NMR-spectra showed that pyruvate is readily metabolized to various metabolites of glycolysis and trough the TCA-cycle. The following metabolites and isotopomers were identified in $^{13}$C-NMR spectra, given in the order of increasing chemical shifts (GSx = reduced + oxidized glutathione) :

**[3-$^{13}$C]alanine, [3-$^{13}$C]lactate** – directly derived from pyruvate.

**[3-$^{13}$C]glutamine/GSx-glutamate, [3-$^{13}$C]glutamate, [4-$^{13}$C]glutamine, [4-$^{13}$C]GSx-glutamate, [4-$^{13}$C]glutamate, [2-$^{13}$C]glutamine/GSx-glutamate, [2-$^{13}$C]glutamate** – derived trough the first turn of pyruvate carboxylase (PC) and pyruvate dehydrogenase (PDH) of the TCA-cycle.

After 6 hours of incubation additional labeled isotopomers were identified : **[3,4-$^{13}$C]glutamine/GSx-glutamate, [3,4-$^{13}$C]glutamate, [3-$^{13}$C]aspartate, [3-$^{13}$C]malate**. The doubly labeled isotopomers originate from a second TCA-cycle turn via PC and PDH.




**Sections of $^{13}$C-NMR spectra of cell extracts after 4h and 6h of incubation with [3-$^{13}$C]pyruvate. See text for details. (glutathione = glutamate-residue in reduced+oxidized form)**

[2,3-$^{13}$C]glutamate was not detected, indicating that no 3rd-turn of the TCA-cycle after entry of [3-$^{13}$C]pyruvate occurred during the 6-hr incubation period. It is noteworthy, that the signal intensity in glutamine was very small in comparison to the other labeled carbon-atoms and no doubly-labeled [3,4-$^{13}$C]glutamine was detected. This points towards a low glutamine synthetase activity in these hepatocytes. Enrichments in lactate and alanine are accessible from $^{1}$H-NMR spectra through their well separated $^{13}$C-satellite signals.

## Conclusions :

Labeled glucose cannot be used to investigate metabolic pathways in primary mouse hepatocytes. The block in glucose metabolism takes place at the glucokinase level. Instead, we were able to demonstrate that [3-$^{13}$C]pyruvate is a useful alternative for metabolic studies in primary mouse hepatocyte cultures. Our results clearly show that [3-$^{13}$C]pyruvate was readily taken up and metabolized by the cells. Our results also demonstrate metabolism of [3-$^{13}$C]pyruvate by lactate dehydrogenase, alanine aminotransferase, aspartate aminotransferase, PC, PDH and subsequent metabolic pathways through the TCA-cycle (glutamate dehydrogenase, glutamine synthetase, pathways of glutathione synthesis). Isotopomers from a 2$^{nd}$ turn of the TCA-cycle were detected only after 6 h. Considering that almost no $^{13}$C-NMR studies in isolated hepatocytes have been performed so far, $^{13}$C-labeled pyruvate will provide an important physiological substrate to assess different hepatocellular pathways and the *de novo* synthesis of major metabolites in these cells under normal and pathological conditions.