Influence of injected pyruvate concentration on metabolism using hyperpolarized ^{13}C

M. Janich[1,2], E. Weidl[3], F. Wiesinger[2], M. I. Menzel[2], J. H. Ardenkjaer-Larsen[4], S. J. Glaser[1], R. F. Schulte[2], and M. Schwaiger[3]
[1]Department of Chemistry, Technische Universität München, Munich, Germany, [2]Imaging Technologies, GE Global Research, Munich, Germany, [3]Institute for Nuclear Medicine, Technische Universität München, Munich, Germany, [4]MST-ASL MR, GE Healthcare, Copenhagen, Denmark

Introduction:
Metabolic imaging with hyperpolarized [1-^{13}C]pyruvate enables the real-time in-vivo observation of metabolism [1]. The typical injected pyruvate dose is a multiple of physiological concentration levels, which may lead to saturation effects in pyruvate metabolism. Under this condition the observed conversion rates between pyruvate and its metabolic products are not only dependent on metabolic activity but also on the injected pyruvate dose. The aim of this study is to investigate the influence of injected pyruvate dose on its cellular uptake and enzymatic conversion in the heart, liver, and kidney of rats. This dose-response study estimates in the healthy rat the lowest dose of pyruvate that gives good metabolite SNR in various organs. Reducing the amount of injected pyruvate is also beneficial for advanced acquisition schemes to avoid a very large difference between signals from pyruvate and its metabolic products. Furthermore, preventing saturation of metabolite concentrations can simplify metabolic modeling.

Methods:
[1-^{13}C]-pyruvic acid doped with Dotarem and trityl radical OX063 was hyperpolarized with dynamic nuclear polarization using a HyperSense DNP (Oxford Instruments, Oxford, UK), and subsequently dissolved in a buffer solution. This solution had physiological pH and temperature, and liquid state polarization levels of 22-34 %. A volume of 5 mL/kg rat mass was injected with 0.17 mL/s into the tail vein of male Wistar rats (~390 g), which were anesthetized with isofluorane at 2 % in oxygen. Animal experiments were approved by the local governmental agency. In this study eight rats were examined, with four rats receiving two injections with a concentration of 40 mM hyperpolarized ^{13}C pyruvate, and four rats receiving two injections of 80 mM. Two rats were examined in one day, one during the morning and one during the afternoon. The time delay between the first and second injection in each rat was 60-80 min.
FID signals were acquired on a GE Signa Excite 3 T scanner (GE Healthcare, Milwaukee, WI, USA) of 5.4 mm thick slices through the heart, liver, and kidneys using a dual-tuned (^{13}C-^{1}H) quadrature coil [2]. Excitation with a 5° flip angle was triggered to breathing, giving a temporal resolution of ~1 s. For data reconstruction the chemical shift (CS) of pyruvate was automatically detected and the CS frequencies of the other metabolites were determined based on their theoretical offset from the pyruvate CS frequency. The signal intensity from each metabolite in the spectrum at each point in time was determined using linear least-squares fitting based on IDEAL CS separation [3]. The resulting signal time curves (Fig. 1) were integrated over 93 s and normalized to the integrated pyruvate signals, in order to get *relative concentrations*, which are independent of polarization level and injected bolus.
For statistical evaluation, a t-test was applied with a significance level of 5 %. It tested the experiments with 40 mM and 80 mM for the null hypothesis of equal relative concentrations of metabolic products against the alternative hypothesis of increased relative concentrations for the 40 mM injections. Another t-test was applied to groups of first and second injections in each animal in order to assess the influence of long anesthetic times. A third t-test compared experiments during morning and afternoon to evaluate the influence of the circadian rhythm on metabolism.

Results:
We measured signal time curves of the metabolites pyruvate, lactate, alanine, and bicarbonate in slices through the heart, liver, and kidneys with a temporal resolution of ~1 s (Fig. 1). The heart shows an increased bicarbonate signal, and decreased alanine and lactate signals compared to liver and kidney.
The 40 mM injections result in a significantly larger relative concentration of metabolic products compared to 80 mM (Fig. 2). This is shown in all slices and for all metabolites, except for bicarbonate in the liver, which can be considered as an error due to low signal-to-noise ratio of the bicarbonate signal in the 40 mM measurements.
There was no significant difference between first and second injections, as well as experiments during morning and afternoon. This is evidence for an unchanged metabolism with the duration of anesthetics and time of the day, but it may also reflect a lack of strong evidence against the null hypothesis due to small sample size.

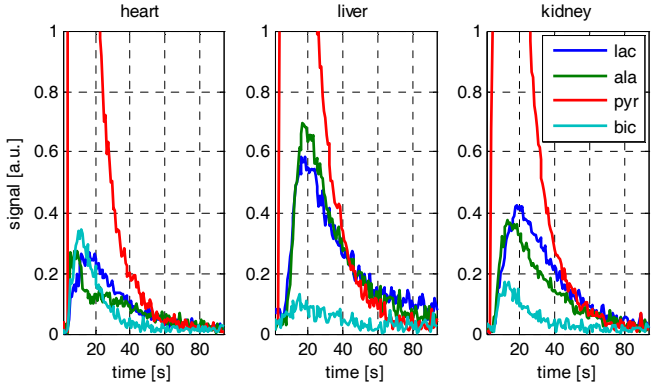

Fig. 1: Time curves of metabolite signals, normalized to integrated pyruvate signal, in slices through the heart, liver, and kidneys with the injection of 5 mL/kg of 80 mM hyperpolarized ^{13}C.

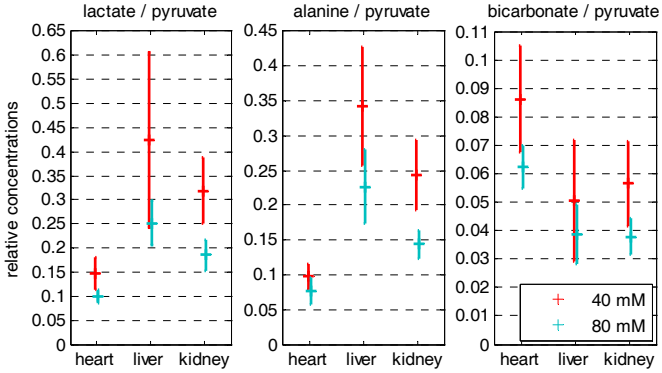

Fig. 2: Relative metabolite concentrations in slices through the heart, liver, and kidneys. The plots show mean ± standard deviation from eight experiments with injected pyruvate concentrations of 40 mM and 80 mM.

Discussion and Conclusions:
In a previous study the influence of injected pyruvate dose on cardiac metabolism was examined with 3 mL/kg injections of 20 mM - 80 mM pyruvate concentrations [4]. We examined this effect in slices through the heart, liver, and kidneys with 5 mL/kg injections of 40 mM and 80 mM concentrations. Saturation of pyruvate conversion to lactate and bicarbonate in the heart was confirmed, and it was also observed in liver and kidney. In this study larger pyruvate masses were injected. With these doses a significant dependency of conversion to alanine on injected pyruvate concentration was recognized in all slices. This indicates that cellular uptake and enzymatic conversion of pyruvate is limited, which causes saturation effects for all metabolic products.
A limitation of this study is the fact that signals from the targeted organs are overlaid by signals from other tissues, which are also present in the slice. While the slice through the heart shows mostly signal from heart muscle and blood, and the slice through the liver covers mainly liver tissue, this limitation is especially present in the slice through the kidneys.
However, the significance of this study is that commonly used dose levels saturate the biological system in many cases and should be considered. The dose of pyruvate should be limited for optimal efficacy to maximize the safety margin.

References: [1] Golman K et al. PNAS 103:11270 (2006); [2] Derby K et al. JMR 86:645 (1990); [3] Reeder SB et al. JMRI 26:1145 (2007); [4] Schroeder MA et al. MRM 61:1007 (2009)
Acknowledgement: This work was partly funded by BMBF MobiTUM grant # 01EZ0827.