INTRODUCTION: Hyperpolarized 13C magnetic resonance spectroscopy and imaging is a rapidly expanding field with many applications, in particular in the field of cancer. Polarization of a number of enzyme substrates enabled the studies of key steps in glycolysis and TCA cycling [1]. [1-13C]-pyruvate has been extensively used in this context. The most commonly used experimental tumor models are (i) mice implanted with tumors and (ii) genetically manipulated mice that develop spontaneous lesions [2]. In this study we compared the lactate dehydrogenase (LDH) activity by measuring the conversion of [1-13C]-pyruvate to [1-13C]-lactate by (i) using the same type of tumor cells (4T1 mammary carcinoma cell line derived from BALB/c mammary tumor) growing at different implantation sites (subcutaneous versus orthotopic) and using mice of different genetic background (immune compromised versus immune competent) thereby assessing effects of the tumor environment on the enzymatic conversion rate, and (ii) by using different tumor cell lines (4T1, C51 colon carcinoma, GL261 glioma) in order to assess differences in the metabolic status amongst the tumor cell lines.

MATERIAL and METHODS: Animals: 9 Balb/c nude (forming 3 groups), 4 Balb/c and 2 C57BL/6 female mice of 6 - 8 weeks of age were used. The mice were anesthetized using sulfurane (2 - 2.5%) and oxygen - air mixture (1/4), with the body temperature maintained at 36.5±0.5°C and respiration monitored. All animal experiments were performed in strict adherence to the Swiss Law for Animal Protection. Tumor: The animals were injected 6 days before the first experiment with 100ul or 50ul of a suspension containing 10^6 4T1 breast cancer cells either into the right thigh flank (in Balb/c) or fourth left mammary fat pad (inguinal group) (in Balb/c or Balb/c nude) respectively. Alternatively, 10^5 C51 colon carcinoma cells were injected in the right thigh flank of Balb/c nude, while the mouse glioma GL261 cell line was intracerebrally injected in a population of 2×10^5. Sample production: Samples of [1-13C] pyruvic acid containing 15 mM triyl radical and 1.5mM Gd(DOTA) were hyperpolarized at 1.4K using a homemade DNP hyperpolarizer [3]. The sample was rapidly dissolved with Tris/NaOH/EDTA buffer solution to a concentration of 90mM and pH 7.5, and a bolus of 0.25ml was injected intravenously. MRS experiment: All in vivo MRS measurements were performed on a Bruker BioSpec 94/30 (Bruker BioSpin MRI, Ettlingen, Germany) horizontal bore MR system using a combination of volume resonant coil, for proton imaging and a custom built 19mm diameter surface coil for 13C measurements. A FLASH sequence was used for anatomical reference images, which were used to accurately position the slices for the spectroscopy experiments and also to calculate the tumor volume. The MRS experiments were carried out using a slice selective spectroscopy sequence with the following parameters: 1 slice with thickness 7.5mm, echo/repetition time TE/TR = 0.5712ms/3000ms, Gaussian shape excitation pulse with 0.1ms duration.

RESULTS: Fig 1 shows a representative spectrum and time profile of pyruvate and lactate intensity for an orthotopic 4T1 tumor in Balb/c mouse. The signal intensity was used to calculate the rate constant, kLDH, for LDH catalysed enzymatic reaction. The LDH forward rate constant (averaged over the tumor volume) for 4T1 tumors implanted either orthotopic in Balb/c or nude Balb/c mice or subcutaneous in nude Balb/c mice was found not to depend on the site of implantation and the mouse strain used. Also we did not observe any change in the conversion rate in relation to the tumor volume (Fig. 2).

DISCUSSION: This study showed that the pyruvate to lactate conversion depends on the tumor type as we found significant differences in the LDH rate constant for three distinct tumor types. On the other hand, the influence of the tumor environment on these metabolic processes appears to be minimal, as we did not detect any difference in the LDH activity when implanting 4T1 tumor cells at different implantation sites or when using different host mouse strains. This indicates that the pyruvate processing through the LDH reaction reflects an intrinsic property of the tumor cell itself, which at least for 4T1 tumors is not altered during tumor growth. Important next steps would be to consider tumor heterogeneity, as the current analysis is based on data averaged over the entire tumor. Furthermore, the differences found among different tumor types have to be validated by comparing the in-vivo MRS findings with immunohistochemical analysis of LDH activity in these in vivo evaluated tumors.