1,4-$^{13}C_2$ malate reports on ischemia related reperfusion injury, after administration of hyperpolarized 1,4-$^{13}C_2$ fumarate in mouse skeletal muscle *in vivo*

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Introduction: Hyperpolarized 1-^{13}C pyruvate has been used to visualize that the PDH flux is affected in the stunned myocardium *in vivo* [1] and *in vitro* [2]. Where metabolism of hyperpolarised pyruvate reports on ischemia related injury in the heart we have found that another metabolic marker, hyperpolarized 1,4-$^{13}C_2$ fumarate may be responsive on longer time scales and report on reperfusion injury. Hyperpolarized 1,4-$^{13}C_2$ fumarate was studied in an ischemic model in the resting mouse hind leg skeletal muscle and show the possible complementary value to pyruvate in reperfusion injury after an ischemic insult.

Methods: c57BL/6 mice were anaesthetized with 2% isoflurane in 1:1 mixture of N_20 and O_2. Breathing rate and temperature was monitored and controlled (SA instruments, USA). 1,4-$^{13}C_2$ fumarate or 1-^{13}C pyruvate were polarized using the technology described before [3] to 26-35% in the liquid state. The *in vivo* MR experiments were performed on a 2.35T Bruker Biospec Avance II system. ^{13}C MR spectra were acquired using an 8-mm surface coil placed around the hind leg of the mouse. After the control experiment where hyperpolarized sample was injected i.v. in the tail vain (20 mM, 0.175 µl /6 sec), ischemia was applied for 30 minutes using a setup and model described previously [4]. Within 5 minutes and after 60 minutes of reperfusion DNP sample was injected. ^{13}C-spectra were acquired with a Tr=2s, 10° RF pulse angle and 60 repetitions. Spectra were analyzed using jMRUI 3.0.

Results and discussion: The injection of 1,4-$^{13}C_2$ fumarate leads to a clear $^{13}C_1$ and $^{13}C_4$ malate signal in all MR spectra. 1-^{13}C pyruvate reveals the metabolites lactate, alanine and bicarbonate in healthy muscle. The lactate signal increases right after the 30 minutes ischemic period and is back to pre-ischemic levels after 60 min of reperfusion. The 1,4-$^{13}C_2$ malate signal, however, is still elevated with a factor of 4 after 60 min of reperfusion and with a factor of 2 right after ischemia, compared to the pre-ischemic level. The elevated levels of 1,4-$^{13}C_2$ malate indicates therefore that 1,4-$^{13}C_2$ fumarate can be used as an indicator of mitochondrial dysfunction in ischemia related reperfusion injuries.

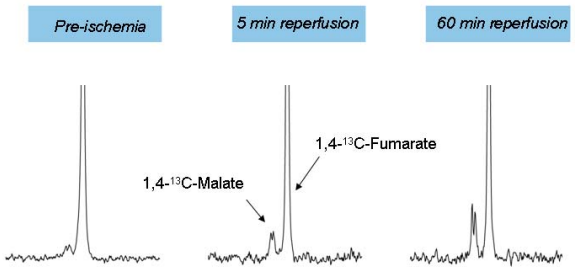

Figure 1: the amplitude of 1,4-$^{13}C_2$ malate before, 5 min after and 30 min after reperfusion after a 30 min ischemic period. 1,4-$^{13}C_2$ malate is significantly elevated 60 min. post ischemia.

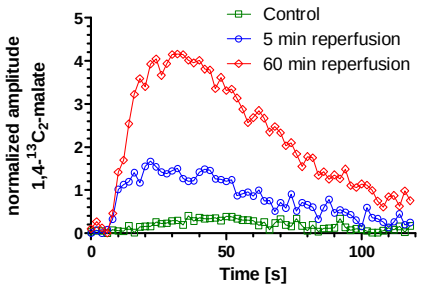

Figure 2: Dynamic profile of the signal from 1,4-$^{13}C_2$ malate in the control muscle, 5 min post ischemia and after 60 minutes of reperfusion.

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References: [1] Golman et al, Magn Reson Med. 2008 May;59(5):1005-13, [2] Merritt et al, Magn Reson Med. 2008,60(5):1029-1036. [3] Ardenkjaer-Larsen et al, PNAS, 2003, [4] In 't Zandt et al, NMR Biomed 1999, 12:327-334