Investigation of Metabolic Changes in STZ Induced Diabetic Rats with Hyperpolarized [1-13C]Acetate

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Introduction:

In the metabolism of acetate several enzymes are involved, which play an important role for free fatty acid oxidation. Therefore it might serve as a marker for pathological changes of cells' fuel selection in particular in the myocardium. Here, changes in the preferred energy source occur in several diseases and pathologies among others under diabetes mellitus, where the fatty acid consumption is upregulated at the cost of glycolosis [1] (Fig.1). [1-^{13}C]acetate was used for hyperpolarized (HP) experiments previously, its metabolism was investigated in several studies in skeletal [2] as well as in cardiac [3] muscle. The goal of this study was to investigate whether the ratio of acetylcarnitine (ALCAR) to acetate could serve as a marker for myocardial, hepatic and renal metabolic changes under Streptozotocin (STZ) induced diabetes *in vivo*.

Methods:

A sectrospatial pulse was used to seperately excite acetate and ALCAR with different flip angles (4° acetate; 15° ALCAR), in order to allow separation of the peaks and save substrate polarization. Slice selective spectra of three slices, containing the heart, liver and kidneys, respectively, were acquired in each animal with a TR of 3s for each metabolite. Additionally acetate maps (Fig.2; 2nd column) were acquired according to [4]. A group of n=7 eight-week-old female wistar rats with STZ induced diabetes (average weight: 216 ± 9g; blood glucose level ahead the measurement: 15.9 ± 5.9 mmol/l) was compared against a control group, consisting of n=5 healthy rats (average weight: 222 ± 13g; blood glucose: 7.5 ± 0.3 mmol/l). Diabetes was induced by an in vivo injection of freshly prepared STZ (55 mg/kg body weight; Sigma-Aldrich, Broendby ,DK). The animal were anesthetized with 2.5% of serofluran in oxygen as breathing gas. The body temperature was kept constant at 37.0°C and oxygenation of the blood was monitored with a blood oximeter.

The substrate was prepared by mixing 48 wt% of sodium [1-13C]acetate with 30 wt% water 22 wt% of glycerol and adding 15mM Oxo63 radical [5]. 150ml of the mixture was polarized in a 5T SPINLab (GE Healthcare, Broendby, DK) for 180min and solved in a PBS buffered solution. A liquid polarization level of about 20% was reached. All MR measurements were performed on a 3T GE HDx system equipped with a dual-tuned ^{1}H-^{13}C-volume coil.

The spectra acquired during the first 60 s after the start of the injection were summed up, the signal intensities of ALCAR and acetate were flip-angle corrected and used to calculate the ALCAR/acetate ratio.

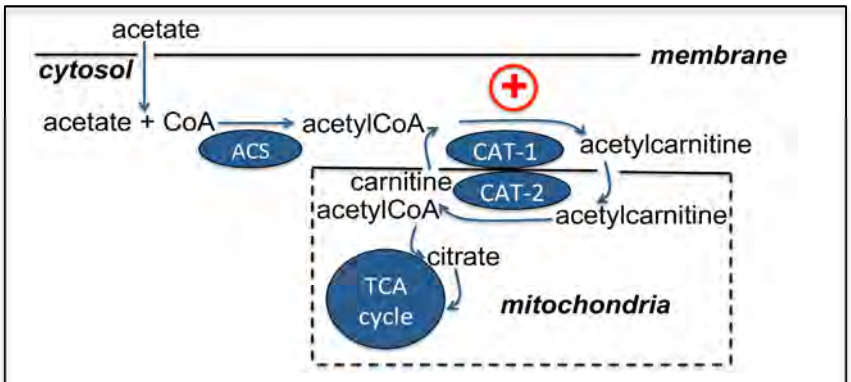

Figure 1: Acetate enters the cytosol where it gets converted to acetylCoA via acetyCoA synthetase (ACS). Then acetylCoA gets converted via carnitineacetyltransferase (CAT)-1 to acetylcarnitine, which can be shuttled into the mitochondria. There it can be metabolised in the TCA cycle, transformed back to acetylCoA via CAT-2. In diabetes CAT-1 activity is increased, which is a crucial step for fuel selection of the myocardium.

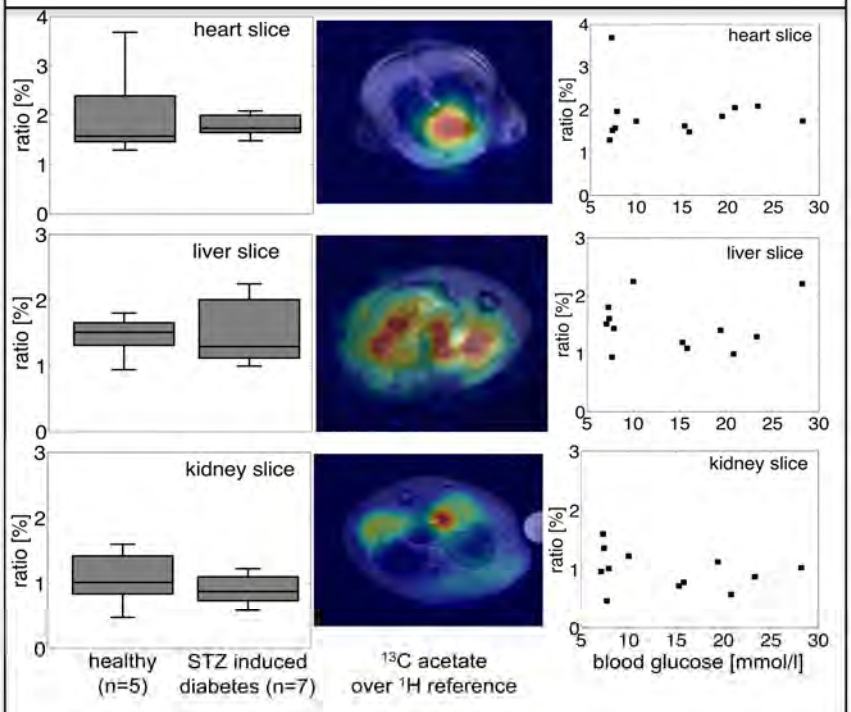

Figure 2: ALCAR/acetate ratio in healthy and diabetic rats. The 1st column shows boxplots containing median, 25 and 75 percentiles as well as the maximal and minimal value for each slice. In the 3rd column the ratio is plotted against the blood glucose concentration.

Results and Discussion:

In Fig.2 the ALCAR to acetate ratio is shown for the three slices which were investigated. There is no significant change between the healthy and the diabetic group (first column) for all three slices, which contradicts the expectations for the heart slice. Furthermore, there is no correlation perceptible between the blood glucose levels and the apparent conversion rates (third column). On the one hand, ALCAR production could be not increased in the myocardium during the time-window of a HP experiment. This could be the case if acetylcoA-synthetase is the rate-limiting step of the conversion described above. On the other hand there might be a higher myocardial ALCAR conversion in the diabetic animals but this product could be shuttled faster towards the TCA cycle. This would lead to production of [5-^{13}C]citrate (179.7ppm), which can not be resolved from the [1-^{13}C]acetate peak (182.5ppm) at 3T due to the high concentration difference and the low chemical shift difference between these two molecules. In the liver slice the variance of the ratio is large, hence it is difficult to draw any conclusion from this data regarding changes in these groups. In the kidney slice there is a slight decrease in the signal-ratio, however, this is below the significance level.

Conclusion

This study demonstrates, that the HP ALCAR/acetate ratio is independent of blood glucose level and prolonged hyperglycemia (2 weeks) following diabetes induction. Investigations at higher field strength might yield more information about the acetate metabolism particularly its contribution to energy production via the TCA-cycle.

[1] Lopaschuk et al., Phys. Rev.2010; [2] Bastiaansen et al., BBA 2013; [3] Jensen et al., JBC 2010; [4] Koellisch, Gringeri et al, MRM 2014; [5] Bowen et al. JMR 2013 **Acknowledgements** : Funding from BMBF grant 13EZ1114 and COST Action TD1103 STMS