

An inducible transgenic mice model of cardiac steatosis; Validation of model and MRS acquisition protocol

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Introduction: Myocardial triglyceride accumulation and its implication on cardiac function have previously been studied in various animal models [1,2]. In the present work, a transgenic mice model of acyl CoA synthase (ACS) overexpression [3,4] is investigated, having the advantages that the expression is cardiomyocyte-specific and that it can be induced in mature animals. *In vivo* triglyceride quantification was performed via MRS and validated against an analytical chemistry technique.

Methods: A transgenic mice model, B6-Tg(Acsl1, rtTA)1Aztc, with cardiomyocyte-specific ACS expression, inducible via doxycycline in the drinking water, was developed in-house. In the study, six transgenic mice (C57Bl/6, male, 16-week-old) (het group), and three age-matched wild-type mice serving as control (wt group), were imaged six days after being put on doxycycline (200 µg/ml). All data acquisition was made on a Bruker Biospec 9.4T/20 USR system. A PRESS sequence (TR>1000ms, TE 11.4ms, BW 4kHz, voxel size 1.0x1.0x1.0mm³, 800 averages) with respiratory gating and ECG triggering to the endsystolic phase was used for the spectroscopic assessment of the triglyceride level. The MRS voxel was placed in the left ventricle wall as illustrated in Fig.1. The water peak and the lipid methylene peak (Fig. 2) were quantified after phase- and baseline correction. The triglyceride level was calculated after compensating for proton density and T1- and T2-decay differences between water and methylene, and for this purpose, the average TR was also calculated in each animal based on the total acquisition time, to reduce the influence of respiratory variations. A series of CINE images was acquired with a retrogated spoiled gradient-echo sequence, IntraGateFLASH (TR/TE/FA 114ms/2.1ms/50°, 12 short-axis slices, slice thickness 1.0 mm, FOV 40x20 mm², 256x128 matrix, 60 averages/cycle, reconstructed to 15 phases per cardiac cycle, T_{acq} 15 minutes) for the evaluation of left ventricle systolic function. In the resulting images, the left ventricle wall was outlined using the software package Segment (Medviso) and the ejection fraction (EF) was calculated. Finally, the animals were sacrificed under terminal Isoflurane anaesthesia by heart excision, and a section of the left ventricle (> 50 mg) was placed in a plastic tube which was submerged in liquid N₂ for subsequent triglyceride analysis (enzymatic colorimetric determination).

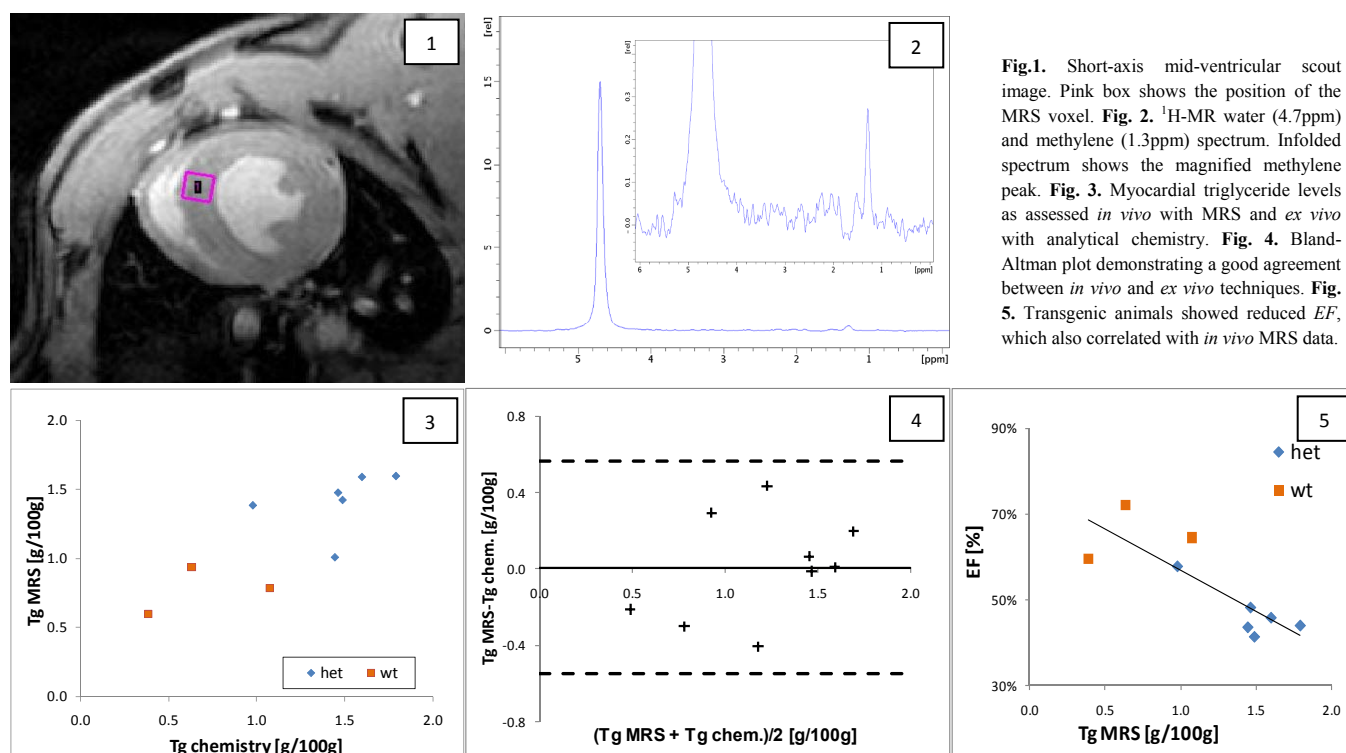


Fig.1. Short-axis mid-ventricular scout image. Pink box shows the position of the MRS voxel. **Fig. 2.** ¹H-MR water (4.7ppm) and methylene (1.3ppm) spectrum. Infolded spectrum shows the magnified methylene peak. **Fig. 3.** Myocardial triglyceride levels as assessed *in vivo* with MRS and *ex vivo* with analytical chemistry. **Fig. 4.** Bland-Altman plot demonstrating a good agreement between *in vivo* and *ex vivo* techniques. **Fig. 5.** Transgenic animals showed reduced EF, which also correlated with *in vivo* MRS data.

Results: The comparison between the triglyceride assessment using MRS and analytical chemistry indicated no presence of bias, and the agreement was good (Fig. 3,4). Increased myocardial triglyceride levels were found in ACS overexpressing mice compared to wild type both when analyzed with analytical chemistry (1.41±0.09g/100g vs. 0.77±0.10g/100g, *p*=0.004), and with MRS (1.46±0.11g/100g vs. 0.70±0.20g/100g, *p*=0.04). The EF was reduced in het animals (47.7±2.2% vs. 60.8±2.1%, *p*=0.02). In addition, there was a high correlation between EF and triglyceride level at day 6 (Fig. 5). A difference in body mass was detected at baseline (het:27.3±0.4g vs. wt:29.2±0.4g, *p*=0.01) which was still present at day 6 (het:27.9±0.5g vs. wt:29.9±0.6g, *p*=0.05).

Discussion/Conclusions: The MRS data were in good agreement with results acquired using the analytical chemistry technique, demonstrating that MRS can be used to assess myocardial triglyceride levels in mice as previously reported by others [1]. The increased triglyceride level in het animals indicates that the investigated model of inducible ACS overexpression produced hearts consistent with the presence of cardiac steatosis. A surprisingly strong functional reduction was found in the het group. This finding needs to be investigated further to rule out factors associated with the transgenic modification, but not related to the accumulation of myocardial triglycerides. An inducible model leading to cardiospecific accumulation of triglycerides, such as the model presented here, can be assumed to be a highly useful tool for investigating the relationship between cardiac steatosis and reduced cardiac function.

References: [1] Hankiewicz *et al.* Circ Cardiovasc Imaging 3(6) 2010 [2] Sharma *et al.* FASEB J 18 2004 [3] Chiu *et al.* J Clin Invest 107, 2001 [4] Lee *et al.* PNAS 101(37), 2004