The effects of fasting on myocardial lipid content in fatty acid β-oxidation deficient mice studied with *in vivo* ^{1}H-MRS

A. J. Bakermans[1], S. M. Houten[2], T. R. Geraedts[1], M. van Weeghel[2], K. Nicolay[1], and J. J. Prompers[1]
[1]Biomedical Engineering, Eindhoven University of Technology, Eindhoven, Netherlands, [2]Laboratory Genetic Metabolic Diseases, Academic Medical Centre, Amsterdam, Netherlands

Introduction

A common clinical feature of inherited defects in the mitochondrial fatty acid β-oxidation (FAO) pathway is the presentation of cardiac abnormalities. Chronic energy shortage and accumulation of -possibly toxic- lipid metabolites may be key contributors to the pathogenesis of hypertrophic cardiomyopathy in FAO disorders. Previous studies have shown that increased myocardial lipid content was accompanied by decreased cardiac function in obese [1] and diabetic [2] subjects. A period of fasting will increase the heart's dependency on FAO, as glucose and insulin levels drop and fatty acids are mobilized from adipose tissue. Therefore, it is hypothesized that fasting will increase myocardial lipid content and decrease cardiac function in FAO-deficient subjects.

In this study, the long chain acyl-coenzyme A dehydrogenase knockout (LCAD$^{-/-}$) mouse model [3] was used to investigate the effects of fasting on the FAO-impaired heart. Cinematographic magnetic resonance imaging (cine MRI) was used to investigate cardiac function and morphology. Myocardial lipid content was assessed using localized proton magnetic resonance spectroscopy (^{1}H-MRS).

Methods

Animals: Male LCAD$^{-/-}$ on a pure C57BL/6 background (n=4) and wild type mice (n=4) were kept with ad libitum access to water and a standard rodent diet. At 13 weeks of age, the animals underwent the MR protocol described below to acquire baseline data in the fed state. Two weeks later, the animals were fasted for 24h prior to the fasted state MR experiments.

MR protocol: Mice were anesthetized with 1.6% isoflurane in medical air and positioned supine in a purpose-built cradle. Heart rate and respiration were monitored and used for MR gating/triggering by the SA Monitoring & Gating System (Model 1025, Small Animal Instruments, Inc.). The cradle was positioned into a horizontal bore 9.4T MR system connected to a Bruker Avance III console (Bruker BioSpin) and controlled by the ParaVision 5.0 software package (Bruker BioSpin). A 35mm inner diameter birdcage coil (Bruker BioSpin) was used for RF transmission and signal reception.

Prospective triggered cine MR images of 14-16 frames per cardiac cycle were acquired in 5-7 contiguous 1mm slices in the left ventricular (LV) short-axis orientation together with two- and four-chamber long axis views. Imaging parameters: FOV=30×30mm^2, matrix size=192×192, TE/TR=1.8ms/7ms, flip angle=15º, NA=6.

Localized ^{1}H-MRS was performed in the diastolic interventricular septum using a respiratory gated and cardiac triggered PRESS sequence preceded by a CHESS water suppression module. PRESS parameters: voxel size=1×2×2mm^3, TE/TR=9.1ms/~2s. During respiratory gates, dummy PRESS pulses were transmitted to maintain steady state required for quantification of metabolite concentrations [4]. Water-suppressed spectra (NA = 256) were acquired on-resonance on creatine. Unsuppressed spectra (NA = 32) were acquired on-resonance on water.

Data analysis: Ejection fraction (EF) and LV myocardial mass were determined from the cine MR images using semiautomatic segmentation software (Pie Medical Imaging). MR spectra were analyzed using the time domain fitting software AMARES in jMRUI. Metabolite concentrations were quantified relative to the corresponding unsuppressed water peak amplitude. Results are presented as means ± standard deviation (SD). Statistical significance of fasting and genotype effects was assessed using repeated measures ANOVA with one between-subjects factor (genotype) and one within-subjects factor (fasting). If the interaction term between the factors was found to be significant, the effect of each factor was analyzed separately using two-sided *t*-tests. Level of significance was set at $P<0.05$.

Results

Cine MRI: Ejection fraction was not different between genotypes (Fig. 1, ANOVA: $P=0.707$). LV myocardial mass normalized to body weight (body weight prior to 24h of fasting for normalization in fasted state) was larger in LCAD$^{-/-}$ mice than in controls (fed LCAD$^{-/-}$: 4.57 ± 0.29mg/g vs. fed controls: 4.01 ± 0.06mg/g, unpaired *t*-test: $P=0.010$; fasted LCAD$^{-/-}$: 4.56 ± 0.31mg/g vs. fasted controls: 3.47 ± 0.18mg/g, $P<0.001$). There was a trend for a decrease in EF after fasting (Fig. 1, ANOVA: $P=0.098$). LV myocardial mass normalized to body weight was decreased after fasting in control mice (paired *t*-test: $P=0.013$), but not in LCAD$^{-/-}$ mice ($P=0.871$).

^{1}H-MRS: Creatine concentration (Fig. 2, peak 4) was not significantly different between genotypes (ANOVA: $P=0.148$) and was not affected by fasting ($P=0.459$). The choline/carnitine signal (peak 3) was lower in LCAD$^{-/-}$ compared to controls (ANOVA: $P=0.048$). No significant effect of fasting on myocardial lipid content was observed in wild type animals (paired *t*-test: $P=0.143$). After fasting, myocardial lipid content as derived from peak 9 at 1.3ppm was increased in LCAD$^{-/-}$ mice (paired *t*-test: $P=0.015$) and was larger compared to controls (unpaired *t*-test: $P<0.001$). These effects were also observed for other lipid associated signals (peak 6, peak7).

Discussion

FAO-impaired LCAD$^{-/-}$ mice displayed LV hypertrophy together with increased myocardial lipid content after fasting as revealed by cardiac ^{1}H-MRS. Fasting may have a negative effect on systolic function in LCAD$^{-/-}$ mice, as a trend for a decrease in ejection fraction was observed. The lower signal amplitude found for choline/carnitine in LCAD$^{-/-}$ mice may indicate myocardial carnitine deficiency in these animals.

High temporal resolution cine MRI will be included in future protocols in order to investigate the effect of lipid accumulation on diastolic function in LCAD$^{-/-}$ mice. To be able to identify a possible energy deficiency in the FAO-impaired myocardium, future work will focus on the implementation of *in vivo* cardiac ^{31}P-MRS.

References [1] Szczepaniak, L.S., 2003, *Magn Reson Med*, 49:417-23. [2] Rijzewijk, L.J., 2008, *J Am Coll Cardiol*, 52:1793-9. [3] Kurtz, D.M., 1998, *Proc Natl Acad Sci U S A*, 95:15592-7. [4] Schneider, J.E., 2004, *Magn Reson Med*, 52:1029-35.

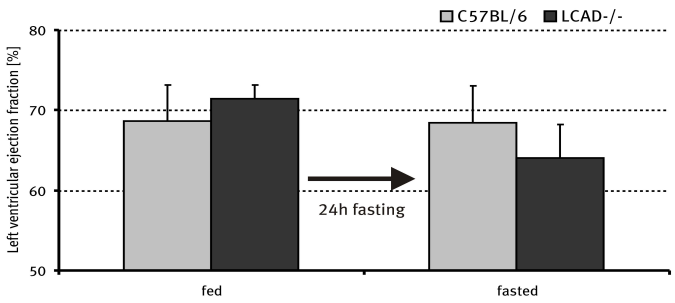

Figure 1 Left ventricular ejection fraction before and after a 24h fasting period as determined from the cine MR images. There was a trend for a decrease in ejection fraction after fasting (ANOVA: $P=0.098$). Error bars indicate SD.

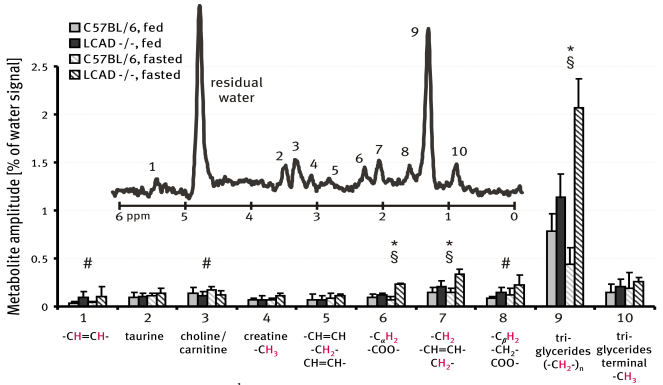

Figure 2 Example of a cardiac ^{1}H-MRS spectrum measured in a C57BL/6 mouse in fed state and average metabolite concentrations in LCAD$^{-/-}$ and control mice before and after fasting quantified from the ten signals annotated in the spectrum.
#, genotype effect (ANOVA: $P<0.05$); §, genotype effect after fasting (unpaired *t*-test: $P<0.01$); *, fasting effect in LCAD$^{-/-}$ mice (paired *t*-test: $P<0.05$).