IN VIVO $^{1}H/^{13}C$ MR ANALYSIS REVEALS VISCERAL OBESITY, HEPATIC STEATOSIS, AND DISORDERS IN BODY FAT COMPOSITION UPON LONG-TERM MEDIUM CHAIN TRIGLYCERIDE DIET IN MICE WITH A DEFECT IN FATTY ACID OXIDATION

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Introduction: The introduction of newborn screening programs for fatty acid oxidation (FAO) disorders in many countries worldwide led to an enhanced identification of FAO patients, otherwise asymptomatic until severe catabolic situations occur. One of the involved pathologies is the very-long chain acyl-CoA dehydrogenase deficiency (VLCADD) which is considered to be the most common inherited disorder of mitochondrial β-oxidation of long chain fatty acids. Therapeutical approaches to prevent metabolic derangement during situations of increased energy demand include avoidance of fasting and a fat restricted and fat-modified diet, in which long-chain triglycerides (LCT) are replaced by medium-chain triglycerides (MCT)[1]. In fact, medium-chain fatty acids are able to bypass the first step of β-oxidation catalyzed by VLCAD and are supposed to supply tissues and organs with the required energy. However, recently also distinct but still reversible impairments of lipid homeostasis and clearance were observed during short-term MCT diet in a murine model of VLCADD[2]. In order to mimick more closely the clinical treatment of VLCADD, the present study investigated the effects of a long-term MCT therapy on body fat distribution and composition using ^{1}H MRI and $^{1}H/^{13}C$ MRS, respectively.

Methods: After weaning, both wildtype (WT) and VLCAD-deficient ($VLCAD^{-/-}$) mice were fed for one year with (i) a normal LCT diet or (ii) a MCT diet (containing the essential long-chain fatty acids). $^{1}H/^{13}C$ MR was performed at a vertical Bruker DRX Wide Bore NMR spectrometer at 400.1 MHz for ^{1}H and 100.6 MHz for ^{13}C using a Bruker Microimaging unit (Micro 2.5) equipped with an actively shielded 40-mm gradient set (1 T/m maximum gradient strength, 110 µs rise time at 100% gradient switching). For ^{1}H MRI and MRS a 30-mm saw resonator was used, and proton-decoupled ^{13}C MR spectra were acquired with a 12×8 mm transmit/receive ^{13}C surface coil. Abdominal fat was associated to visceral and subcutaneous regions by analysis of anatomical images with and without fat suppression. Non-volume selective proton-decoupled ^{13}C MR spectra were recorded over the entire abdominal region for determination of fat composition. For analysis of liver fat, localized respiratory-triggered ^{1}H MR spectra were acquired from a $3\times3\times3$ mm^3 voxel placed in the mid of the right liver lobe using a PRESS sequence with outer volume suppression.

Results: Quantification of abdominal fat showed that $VLCAD^{-/-}$ mice fed with a long-term MCT diet exhibited an almost twofold higher overall fat content per measured body volume compared to all other mouse groups (n=5-7, $P<0.05$). The classification of abdominal fat in visceral and deep/superficial subcutaneous fat revealed that this was predominantly caused by an increase in visceral fat and only to a minor extent by alterations in subcutaneous fat. In the same experimental setting natural abundance ^{13}C NMR spectra were acquired for analysis of abdominal fat composition. Figure 1A shows sections of characteristic ^{13}C NMR spectra for $VLCAD^{-/-}$ mice after one year of LCT and MCT diet, respectively. The most striking difference is the dramatic drop in signal intensity for polyunsaturated carbons [Δ_p]. Quantitative analysis of the spectra revealed a polyunsaturated fatty acid (PUFA) content of only 13±5% upon MCT diet as compared to 49±6% under control conditions. Concomitantly, we found a massive increase in monounsaturated fatty acids (MUFA) and a moderated up-regulation of saturated fatty acid (SAFA) levels (Fig. 1A). However, these effects were not specific for $VLCAD^{-/-}$ mice, but were similarly found in WT mice. To determine also intrahepatic lipid accumulation, localized ^{1}H MR spectra were acquired from the mid of the right liver lobe. Under control conditions (LCT diet) WT and $VLCAD^{-/-}$ mice did not show substantial differences in intrahepatic lipid levels. However, as can be seen from representative spectra shown in Fig. 1B, ^{1}H MRS showed pronounced alterations in both liver fat content and composition in $VLCAD^{-/-}$ mice upon MCT diet. The ratio of water/fat signals indicates a massive increase in liver fat while the loss of the signal for the double allylic protons (Δ-1_p) reflects the dramatic decrease in PUFA content which was also observed on the entire abdominal level. Quantification of the spectra revealed an almost doubled liver fat content in $VLCAD^{-/-}$ mice on MCT as compared to LCT diet (Fig. 1B). Histology and enzymatic analysis comfirmed the presence of hepatic steatosis associated with markers of oxidative stress in $VLCAD^{-/-}$ mice.

Conclusions: In a murine model of VLCADD, long-term MCT diet results in massive visceral fat infiltration, impaired body fat composition with decreased levels of physiological important PUFA, and hepatic steatosis as assessed by $^{1}H/^{13}C$ MR analysis. Although MCT diet has been reported to prevent development of cardiomyopathy and the myopathic phenotype [1], according to our data its use and dose has to be well considered.

References: [1] Roe et al. J Clin Invest. 2002, 110:259-69. [2] Tucci et al. Mol Genet Metab. 2010, 101:40-7.

Figure 1: Sections and quantifications of (A) ^{13}C MR spectra of the abdomen and (B) localized ^{1}H MR spectra of the liver from $VLCAD^{-/-}$ mice after one year of LCT and MCT diet, respectively. Abbreviations: Carbox., carboxylic carbons; Δ and Δ_p, mono- and polyunsaturated carbons; α/ω, protons bound to α and ω carbons of the fatty acid chain, $[\alpha+\gamma]_g$, protons bound to α+γ carbons of the glycerol backbone.