Cardiac lipids show diurnal variations: An in-vivo 1H-MR Spectroscopy study in humans

Michael Ith[1], Christoph Stettler[2], Jian Xu[3], Chris Boesch[4], and Roland Kreis[4]

[1]University Institute of Diagnostic Interventional and Pediatric Radiology, University & Inselspital Bern, Bern, Switzerland, [2]Division of Endocrinology, Diabetes & Clinical Nutrition, University & Inselspital Bern, Bern, Switzerland, [3]Siemens Medical Solutions USA, New York, NY, United States, [4]Depts Clinical Research and Radiology, University Bern, Bern, Switzerland

Introduction: ^{1}H-MR spectroscopy (MRS) is regularly applied to determine lipid content in human non-adipose tissues, mostly skeletal muscle and liver[1,2], thus enabling a variety of metabolic studies of physiologic and pathologic conditions, e.g. insulin resistance. In past years, the concept of myocardial steatosis has received increasing attention and it is hypothesized that excess storage of lipids in cardiomyocytes might represent an early manifestation of type 2 diabetes as well as of associated cardiomyopathy[3]. MRS with double triggering also allows for a non-invasive in-vivo assessment of cardiac lipids (Intra-CardiomyoCellular Lipids, ICCL)[4-6], but its reproducibility is lower and often ill-defined. The goal of this study was to determine reproducibility of navigator-based cardiac MRS and to investigate potential diurnal variations of cardiac lipid content[7] that would profoundly influence the design of physiologic studies.

Methods: Cardiac lipids were determined by single-voxel MRS in nine healthy, young (age: 23.9±2.3 years) and lean (BMI: 22.2±1.2 kg/m^2) male volunteers. Five individual sessions were distributed over two days separated by 1 or 2 weeks. Both days included a measurement in the morning after an overnight fast (>8h) and one in the afternoon (8h after breakfast, 3.5h after lunch). To determine methodological reproducibility, the afternoon measurement was repeated on one of the two days (1h break). Preparation of the volunteers included perpetuation of their normal diet, but restricted physical activity for two preceding days. A diary enabled the repetition of diet and exercise. MRS was performed on a 3T MR system (Siemens Verio) using a PRESS-sequence (TE=35ms; TR≈5s depending on respiratory frequency, ROI=10×20×25 mm^3 placed in the cardiac septum) with water suppression and respiratory (navigator on the diaphragm) and cardiac double-triggering. Spectra were recorded at end-systole in the end-expiratory phase (8×8 scans with water suppression, 16 scans without) after a two step shimming procedure based on 1) a field map obtained with cardiac triggered gradient echo images and 2) cardiac gated FASTESTMAP shimming[8], both recorded in breathhold. Spectra were evaluated with jMRUI (spectra aligned, residual water eliminated, fit in AMARES with prior knowledge constraints) and results of cardiac lipids (ICCL, CH_2-group) and trimethyl-ammonium compounds (TMA) are given relative to the CH_3-signal of creatine (ICCL/Cr; TMA/Cr). Non-water-suppressed spectra were fit scan-by-scan to a single peak to judge stability.

Results: Four out of 45 measurements were excluded from further analysis due to questionable reliability, which was suspected if the 16 scans of unsuppressed water showed strong variations. The average coefficient of variance was CV_{water}=9.1±12.4% (median CV=4.7%). Measurements were excluded if CV_{water}>20%. This yielded a success rate of 91%, resulting in complete datasets from 8 subjects for methodological reproducibility and six complete datasets for day to day physiological reproducibility (morning and afternoon) as well as for morning-evening diurnal (2 per subject) changes. Fig.1 shows a complete set of cardiac spectra from one volunteer to illustrate typical spectral quality. The two spectra from evening 1 are examples for the methodological reproducibility. Paired t-tests showed no significant differences for ICCL/Cr or TMA/Cr. Average CVs were 8.7±3.9% and 7.7±7.8%, respectively. Analysis of intra-individual "long term" reproducibility of cardiac metabolites showed much larger variation for ICCL/Cr, but similar values for TMA/Cr: average CVs of 34±19%/30±26% (morning/evening) for ICCL/Cr and 4.1±3.3%/8.2±4.8% for TMA/Cr. Comparison of morning and evening measurements revealed a strongly significant diurnal variation with a decrease of 37±19% for ICCL/Cr (4.9±1.9 vs. 3.0±1.4; p<0.001), but no change for TMA/Cr (0.92±0.16 vs. 0.92±0.20; p=0.95). This is illustrated in Fig.2a with sum-spectra, and in Fig.2b with individual data for ICCL/Cr.

Discussion & Conclusions: Based on an objective inclusion-criterion of a stable water signal, 91% of all spectra were included for further analyses and showed good spectral quality. An excellent methodological reproducibility for spectra obtained upon immediate repetition in independent examinations (metabolite CV's below 10%) demonstrated the stability of the present implementation of cardiac MRS. This was supported by the fact that TMA/Cr remained stable over at least one week (CV is only slightly higher than for immediate repetition). In contrast, ICCL/Cr showed a higher variation for the "long-term" reproducibility, suggesting physiologic fluctuations that were not prevented by the two day preparation period with reproduced diet and exercise regimens. Furthermore – and to the best of our knowledge seen for the first time in human subjects – this study revealed a significant diurnal difference in ICCL levels between morning (fasted) and afternoon (fed), indicating that the cardiac fuel depot does not remain constant over the course of the day, even though the heart is exercising continuously. Both physiologic findings (day to day variations in spite of equal preparation and diurnal variation) place severe limits on the study designs when investigating physiologic or pathologic cardiac lipid metabolism.

References: **1.** Boesch C JMRI 25:321. **2.** Springer F et al. World J Gastroenterol. 16:1560. **3.** van de Weijer T et al. Cardiovasc Res. 92:10. **4.** Felblinger J et al. MRM 42:903. **5.** van der Meer RW et al. Radiology 245:251. **6.** Szczepaniak LS et al. MRM 49:417 **7.** Tsai JY et al. J Biol Chem 285:2918. **8.** Gruetter R and Tkác I. MRM 43(2):319

Supported by the "Takeda grant for clinical research 2008", and the Swiss National Foundation (31003A_132935)

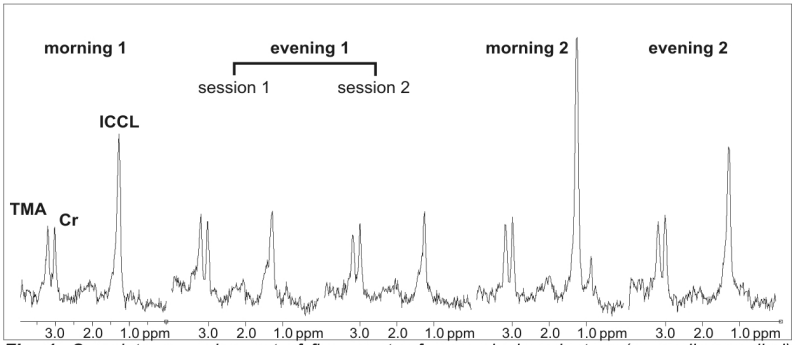

Fig. 1: *Complete exemplary set of five spectra from a single volunteer (no scaling applied) illustrating methodological reproducibility (session 1 vs. session 2), "long-term" reproducibility (morning 1 vs. morning 2; evening 1 vs. evening 2), and diurnal changes (morning 1 vs. evening 1; morning 2 vs. evening 2).*

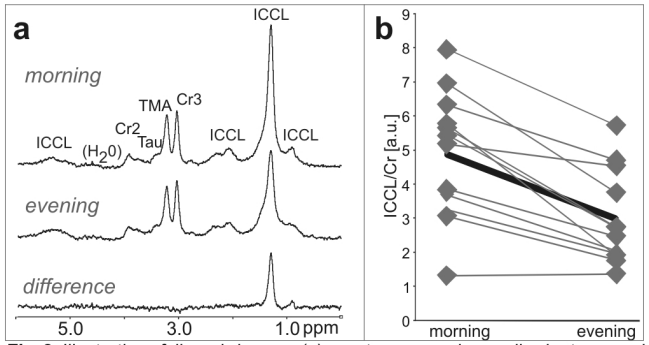

Fig. 2: *Illustration of diurnal changes (a) spectra summed over all volunteers and dates (12 per time point) and their straight difference (no scaling before summation, residual water eliminated by HLSVD). Peak assignments: ICCL at 0.9ppm (-CH3), 1.3ppm (-(CH2)n-), 2.06ppm (CH2-C=C), 2.3ppm (CH2-C=O), and 5.3ppm (CH=CH); Creatine (CH3 at 3ppm and CH2 at 3.9ppm), TMA at 3.2ppm, Taurine at 3.4ppm. (b) individual data points showing an average decrease of 37±19% for ICCL/Cr.*