Supplementary material

Subclinical effects of long-chain fatty acid β-oxidation deficiency on the adult heart: a case-control magnetic resonance study

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Methods

MR protocol

Fourteen adult patients (2 with long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency (LCHADD); 4 with carnitine palmitoyltransferase II deficiency (CPT2D); 8 with very long-chain acyl-CoA dehydrogenase deficiency (VLCADD)) and 14 gender-, age-, and BMI-matched control subjects were examined using a comprehensive noninvasive cardiac magnetic resonance (MR) protocol that included functional cine MR imaging, proton MR spectroscopy ($^1$H-MRS), MR tagging, and native T$_1$ and T$_2$ relaxometry. All examinations were conducted on an Ingenia 3 Tesla MR system (Philips, Best, The Netherlands).

First, retrospectively triggered breathhold cine MRI of the heart was performed with a balanced steady-state free-precession (bSSFP) gradient-echo sequence to obtain two- and four-chamber long-axis views of the LV. A stack of 12-15 contiguous short-axis slices covering the left ventricle (LV) was acquired (field-of-view (FOV), 350×350 mm$^2$; slice thickness, 8 mm; temporal resolution, 25 frames/R-R interval) for the quantification of LV and right ventricular (RV) volumes and myocardial mass.

Next, $^1$H-MRS was performed as described previously,$^1$ using a prospective ECG-triggered respiratory-gated free-breathing single-voxel point-resolved spectroscopy (PRESS) sequence. A 10x25x30 mm$^3$ voxel was positioned interventricular septum at 200 ms after R-wave peak detection. A navigator volume for respiratory gating was positioned over the diaphragm at the top of the right liver lobe along the lung-liver interface. Multiple optimizations insensitive suppression train (MOIST) water-suppressed spectra (8x8 acquisitions; number of points, 1024; bandwidth, 1500 Hz) were acquired at an echo time (TE) of 40 ms and a long repetition time (TR) of >6 s to minimize partial saturation effects for signal quantification, interleaved with acquisitions of 8 unsuppressed water spectra at a TR of >9 s (water at 4.7 ppm on-resonance) for metabolite quantification reference.
Then, MR tagging using spatial modulation of magnetization (SPAMM)\textsuperscript{2} was performed to allow for a detailed assessment of LV myocardial contractile function. An orthogonal grid (grid spacing, 7 mm) was applied prior to the prospectively triggered breathhold acquisition of 5 parallel short-axis slices using a multi-shot echoplanar imaging (EPI) sequence (FOV, 350×350 mm\textsuperscript{2}; slice thickness, 8 mm; slice gap, 5 mm; EPI factor, 15×10 segments; temporal resolution, 15 ms/frame).

Subsequently, T\textsubscript{2} and T\textsubscript{1} relaxometry was used for myocardial tissue characterization. A multi-echo gradient spin-echo (GraSE)\textsuperscript{3} sequence (8 echoes; TE, 18 + 7×9 ms) was used to obtain black blood maps of the transversal relaxation time constant T\textsubscript{2} in the four-chamber LV long-axis view. Finally, maps of the native longitudinal relaxation time constant T\textsubscript{1} were acquired of the four-chamber LV long-axis view using a modified Look-Locker inversion recovery (MOLLI) sequence.\textsuperscript{4}

**MR data analyses**

**Cine MRI**

We quantified LV and RV cavity volumes and myocardial mass via segmentation of the LV and RV walls in the cine MR images by manually delineating the ventricular endocardial and epicardial contours using QMass 8.1 (Medis medical imaging systems BV, Leiden, The Netherlands). Papillary muscles were considered as part of the cavities and not included in the myocardial mass.\textsuperscript{5} Measured parameters included the LV and RV end-diastolic volumes (EDV), end-systolic volumes (ESV), stroke volumes (SV), ejection fractions (EF), and LV and RV myocardial masses. Volume and mass parameters were indexed to body surface area (BSA).\textsuperscript{6} The LV mass-to-volume ratio [g/mL] was calculated as a measure of LV hypertrophy and remodeling by dividing the LV myocardial mass with the LV EDV.\textsuperscript{7} Mid-cavity LV wall thickness was measured at end-diastole. Global longitudinal strain (GLS) was estimated with QStrain 2.0 (Medis medical imaging systems BV), and defined as the relative end-systolic shortening of the LV endocardial contour length in the four-chamber long-axis view.

\textsuperscript{1}H-MRS

Individual \textsuperscript{1}H-MRS signals were processed using MATLAB R2016a (The MathWorks, Inc., Natick, MA, USA). Signals were phased and averaged, and eddy-current distortions and DC offset were corrected using the unsuppressed water spectrum as a reference signal. Spectral fitting was performed in the time-domain using AMARES in jMRUI.\textsuperscript{8} The (phospho)creatine-methyl resonance was used as internal chemical shift reference at 3.02 ppm. Peaks of trimethylamine-containing compounds (3.2 ppm) and creatine-methyl (3.0 ppm) were fitted to Lorentzian line shapes. Lipid signals from triglycerides between 0.85 ppm and 2.7 ppm and the triglyceride olefinic protons at 5.3 ppm were fitted to Gaussian line shapes. The unsuppressed water signal was fitted to a Lorentzian line shape and used as a quantification reference. Myocardial lipid content was quantified as the percentage of the sum of the
triglyceride signal amplitudes over the unsuppressed water signal amplitude. The total (i.e., the pool of phosphocreatine + free creatine) creatine content in the myocardium was estimated by expressing the creatine-methyl signal amplitude as a percentage of the unsuppressed water signal amplitude.

**MR tagging**

Myocardial contractile mechanics were assessed by an analysis of tagged short-axis MR images as described previously,\(^9\) yielding quantifications of LV torsion and endocardial circumferential strain. In-plane rotation of the LV wall and endocardial circumferential strain for the 5 short-axis slices were estimated from displacement maps using MATLAB R2016a (The MathWorks, Inc.).\(^9\) Torsion was defined as the epicardial shear angle [rad] between two adjacent slices, calculated as the longitudinal gradient in rotation angle multiplied by the mean epicardial radius. Endocardial circumferential shortening [-] was quantified as the change in mean inner diameter true strain in two adjacent slices.\(^9\) Torsion and shortening time curves were used to derive peak torsion, peak shortening, and the TSR during myocardial contraction.

**Tissue T\(_2\) and T\(_1\) relaxometry**

Quantification of myocardial T\(_2\) and T\(_1\) relaxation time constants was done with QMap 2.2 (Medis medical imaging systems BV). Tissue T\(_2\) and T\(_1\) maps were calculated by pixel-wise fitting of a mono-exponential T\(_2\) decay function and a mono-exponential T\(_1\) recovery function to the multi-echo and inversion recovery data, respectively. Mean T\(_2\) and mean native T\(_1\) values were estimated in a region of interest drawn in the interventricular septum.

**References**

1. de Heer P, Bizino MB, Lamb HJ, Webb AG. Parameter optimization for reproducible cardiac \(^1\)H-MR spectroscopy at 3 Tesla. *J Magn Reson Imaging*. 2016;44:1151-1158.
2. Axel L, Dougherty L. MR imaging of motion with spatial modulation of magnetization. *Radiology* 1989;171:841-845.
3. Sprinkart AM, Luetkens JA, Traber F, et al. Gradient Spin Echo (GraSE) imaging for fast myocardial T\(_2\) mapping. *J Cardiovasc Magn Reson*. 2015;17:12.
4. Kellman P, Hansen MS. T\(_1\)-mapping in the heart: accuracy and precision. *J Cardiovasc Magn Reson*. 2014;16:2.
5. Schulz-Menger J, Bluemke DA, Bremerich J, al. Standardized image interpretation and post processing in cardiovascular magnetic resonance. *J Cardiovasc Magn Reson*. 2013;15:35.
6. Du Bois D, Du Bois EF. A formula to estimate the approximate surface area if height and weight be known. *Arch Intern Med (Chic)*. 1916;XVII:863-871.
7. Dweck MR, Joshi S, Murigu T, et al. Left ventricular remodeling and hypertrophy in patients with aortic stenosis: insights from cardiovascular magnetic resonance. *J Cardiovasc Magn Reson.* 2012;14:50.

8. Vanhamme L, van den Boogaart A, Van Huffel S. Improved method for accurate and efficient quantification of MRS data with use of prior knowledge. *J Magn Reson.* 1997;129:35-43.

9. Lumens J, Delhaas T, Arts T, Cowan BR, Young AA. Impaired subendocardial contractile myofiber function in asymptomatic aged humans, as detected using MRI. *Am J Physiol Heart Circ Physiol.* 2006;291:H1573-H1579.