Supporting Information

Quantification of myocardial creatine and triglyceride content in the human heart: precision and accuracy of *in vivo* proton magnetic resonance spectroscopy

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Methods

^{1}H-MRS analysis

Data were processed in MATLAB R2016a (The MathWorks, Inc., Natick, MA, USA). Individual ^{1}H-MRS signals were phased and averaged. Eddy-current distortions and direct current offset were corrected using the unsuppressed water signals as a reference. Spectral fitting for signal quantification was performed in the time domain using the AMARES fitting routine in jMRUI.[1] The unsuppressed water signal was fitted to a Lorentzian line shape and used as a quantification reference. Water linewidth (full width at half maximum, FWHM) was documented as a surrogate measure of B_0 shim quality. For the water-suppressed spectra, the total creatine-methyl signal was set as the internal chemical shift reference at 3.02 ppm. Any residual water signal was removed using the Hankel-Lanczos singular value decomposition (HLSVD) method available in jMRUI. Peaks of trimethylamine-containing compounds (3.2 ppm) and total creatine-methyl (3.0 ppm) were fitted to Lorentzian line shapes. Peaks from triglyceride-methylene $(\text{-CH}_2\text{-})_n$ at 1.3 ppm and triglyceride-methyl (-CH_3) at 0.9 ppm were modeled with Gaussian line shapes to accommodate small variations in resonance frequencies due to differences in triglyceride composition, fatty acid chain lengths, and specific positions of methylene within the fatty acid chains. Linewidth for the triglyceride-methyl peak was set equal to the linewidth of triglyceride-methylene to prevent a potential overestimation of the triglyceride-methyl signal by erroneously broad linewidths. Linewidths of trimethylamine (LW_{TMA}), total creatine-methyl (LW_{tCr}) and triglyceride-methylene (LW_{TG}) were constrained with respect to the corresponding unsuppressed water linewidth (LW_{water}) according to:

LW_{TMA} (Hz) = 0.93 LW_{water} (Hz) + 2.89 Hz,
LW_{tCr} (Hz) = 0.88 LW_{water} (Hz) + 1.14 Hz, and
LW_{TG} (Hz) = 1.74 LW_{water} (Hz) - 2.54 Hz.

These relations were empirically derived from spectra (n = 20) obtained with identical acquisition parameters in normal volunteers, which featured well-resolved resonance peaks for trimethylamine ($r = 0.73$; $p = 0.0003$), total creatine-methyl ($r = 0.58$; $p = 0.007$), and triglyceride-methylene ($r = 0.72$; $p = 0.0004$). The relative Cramér Rao Lower Bound (CRLB), expressed as a percentage of the associated signal amplitude, was documented as an estimate of the lower threshold of the fitting error.[2] Myocardial total creatine content was quantified as the percentage of the total creatine-methyl signal amplitude of the unsuppressed water signal amplitude. Myocardial triglyceride content was estimated as the percentage of the sum of triglyceride-methylene and triglyceride-methyl signal amplitudes divided by the unsuppressed water signal amplitude.

Biochemical assays

Total creatine assay

Tissue samples were weighed and freeze-dried overnight, and then weighed again to estimate tissue water content. Each sample was homogenized thoroughly using the sharp end of a spatula in a 1.5 mL Eppendorf cup, deproteinized in 4% perchloric acid, followed by 15 s vortexing and centrifugation (2 min; 4°C; 10000*g*). The supernatant was mixed with a neutralizing buffer (triethanolamine/KCl/KOH) at a 2:1 ratio, pH was adjusted to 7.0, and subsequently split in 40-µL aliquots for an assay in triplo. Total creatine concentration was determined by inducing a sequence of enzymatic reactions via (*1*) creatine kinase, (*2*) creatinase, (*3*) urease, and (*4*) glutamate dehydrogenase (GLDH) to ultimately convert nicotinamide-adenine-dinucleotide-phosphate (NADPH) into $NADP^+$, for which the conversion was measured spectrophotometrically.[3] To this end, an aliquot was added to a reaction mixture containing excess amounts: 7.4 mM $MgCl_2$, 134 µM NAPDH, 71.5 U/mL GLDH, 1000 U/mL urease, 13 mM α-ketoglutarate, 2.5 mM ADP, 8.7 U/mL creatinase, and 70 U/mL creatine kinase in a 200 mM phosphate buffer (pH, 7.8). To correct for endogenous urea, a separate series of aliquots underwent reactions (*3*) and (*4*) only. Aliquots were added to the reagent solution in a 1:20 dilution factor and incubated in the dark at 37°C for 30 minutes. The decrease in absorbance at 340 nm was measured spectrophotometrically (Specord 50; Analytik Jena AG, Jena, Germany). Absorbance was corrected for background absorbance of the reagents, samples, and endogenous urea. Myocardial total creatine concentration was calculated using a calibration slope established in 0.325 mM:0.675 mM phosphocreatine:creatine dilutions to correct for any of the forward reactions not running to completion, and reported as the mean of the assay in triplo [µmol/g wet weight].

Triglyceride assay

Total myocardial triglyceride content was determined biochemically via colorimetric quantification as described previously,[4] with minor modifications. Tissue samples were weighed and homogenized in ice-cold Dole's extraction mixture (1 mL; isopropanol:heptane:1 M H_2SO_4, 40:10:1),[5] using a TissueLyser II (Qiagen, Venlo, The Netherlands) at 30 Hz for 4 times 30 s. The lysate was further sonicated until all tissue was dissolved. Then, 1.5 mL Dole's extraction mixture, 1.5 mL heptane, and 0.6 mL 0.4 M 3-(*N*-morpholino)propanesulfonic acid (MOPS) buffer (pH, 6.4) was added to the extract, followed by mixing and centrifugation (4°C; 3000*g*). The upper phase was collected, and the lower phase was extracted again with 1.5 mL heptane. Upper phases were then pooled and evaporated to dryness. The residue was dissolved in 1 mL chloroform with 1% Triton-X100, followed by evaporation to dryness and resuspension in 0.25 mL water. This solution was then used for colorimetric quantification of the myocardial total triglyceride concentration [μmol/g wet weight] after enzymatic hydrolysis with lipases (Triglycerides Liquicolor mono; Human, Wiesbaden, Germany).

References

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Table

Supplementary Table. Characteristics, blood panel results, and MR results for patients that underwent ^{1}H-MRS prior to myocardial tissue collection during surgical aortic valve replacement (AVR) or septal myectomy.

	AVR (n = 13)	Myectomy (n = 9)	p-value
Female [n]	3	4	
Age [y]	68.0 ± 8.5	63.4 ± 7.2	0.198
Weight [kg]	92.0 ± 20.7	71.9 ± 12.6	**0.018**
Height [m]	1.77 ± 0.06	1.74 ± 0.08	0.345
BMI [kg/m^2]	29.3 ± 5.5	23.7 ± 2.9	**0.012**
BSA [m^2]	2.08 ± 0.25	1.86 ± 0.19	**0.033**
Blood panel			
Hematocrit [L/L]	0.43 ± 0.04	0.41 ± 0.04	0.350
Total cholesterol [mM]	4.7 ± 1.0	5.1 ± 1.2	0.449
HDL cholesterol [mM]	1.3 ± 0.5	1.6 ± 0.5	0.118
LDL cholesterol [mM]	2.4 ± 0.9	2.7 ± 1.0	0.575
Triglycerides [mM]	2.2 ± 1.3	1.7 ± 0.8	0.288
Glucose [mM]	6.3 ± 1.1	5.7 ± 1.4	0.322
Insulin [pM]	260 ± 225	209 ± 387	0.702
HbA1c [mmol/mol]	41.1 ± 6.8	38.9 ± 6.5	0.446
^{1}H-MR imaging and spectroscopy			
LV mass [g]	164 ± 31	177 ± 38	0.388
LV EDV [mL]	132 ± 22	136 ± 24	0.707
LV ESV [mL]	58 ± 28	44 ± 13	0.183
LV SV [mL]	74 ± 12	92 ± 17	**0.010**
LV EF [%]	57.8 ± 13.1	67.9 ± 6.4	**0.046**
LV mass-to-volume ratio [g/mL]	1.27 ± 0.29	1.34 ± 0.33	0.589
Heart rate [beats/min]	69 ± 13	59 ± 9	0.066
LV CO [L/min]	5.0 ± 0.9	5.4 ± 1.0	0.407

LV GLS [%]	-17.8 ± 6.8	-24.2 ± 4.4	**0.023**
Water linewidth [Hz]	14.7 ± 2.4	13.4 ± 3.6	0.337
Myocardial total creatine content [% of water signal]	0.095 ± 0.021	0.089 ± 0.027	0.575
Myocardial triglyceride content [% of water signal]	0.67 ± 0.36	0.50 ± 0.18	0.223

Values indicate mean ± standard deviation, with *p*-values reported for two-sided Student's *t*-tests. The level of significance was set at $p < 0.05$, emphasized by values in bold.

BMI, body mass index; BSA, body surface area; CO, cardiac output; EDV, end-diastolic volume; EF, ejection fraction; ESV, end-systolic volume; GLS, global longitudinal strain; Hb, hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LV, left ventricle; SV, stroke volume.