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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Background: Proton magnetic resonance spectroscopy (^{1}H-MRS) of the human heart is deemed to be a quantitative method to investigate myocardial metabolite content, but thorough validations of in vivo measurements against invasive techniques are lacking.
Purpose: To determine measurement precision and accuracy for quantifications of myocardial total creatine and triglyceride content with localized ^{1}H-MRS.
Study type: Test–retest repeatability and measurement validation study.
Subjects: Sixteen volunteers and 22 patients scheduled for open-heart aortic valve replacement or septal myectomy.
Field Strength/Sequence: Prospectively ECG-triggered respiratory-gated free-breathing single-voxel point-resolved spectroscopy (PRESS) sequence at 3 T.
Assessment: Myocardial total creatine and triglyceride content were quantified relative to the total water content by fitting the ^{1}H-MR spectra. Precision was assessed with measurement repeatability. Accuracy was assessed by validating in vivo ^{1}H-MRS measurements against biochemical assays in myocardial tissue from the same subjects.
Statistical Tests: Intrasession and intersession repeatability was assessed using Bland–Altman analyses. Agreement between ^{1}H-MRS measurements and biochemical assay was tested with regression analyses.

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Results: The intersession repeatability coefficient for myocardial total creatine content was 41.8% with a mean value of 0.083% ± 0.020% of the total water signal, and 36.7% for myocardial triglyceride content with a mean value of 0.35% ± 0.13% of the total water signal. Ex vivo myocardial total creatine concentrations in tissue samples correlated with the in vivo myocardial total creatine content measured with ^{1}H-MRS: $n = 22$, $r = 0.44$; $P < 0.05$. Likewise, ex vivo myocardial triglyceride concentrations correlated with the in vivo myocardial triglyceride content: $n = 20$, $r = 0.50$; $P < 0.05$.
Data Conclusion: We validated the use of localized ^{1}H-MRS of the human heart at 3 T for quantitative assessments of in vivo myocardial tissue metabolite content by estimating the measurement precision and accuracy.
Level of Evidence: 2
Technical Efficacy Stage: 2

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The first proton magnetic resonance spectra (^{1}H-MRS) of the human heart were published more than 25 years ago.[1] With this noninvasive technique, metabolically and (patho)physiologically relevant compounds such as creatine and triglyceride can be detected in in vivo myocardial tissue.[2] The amplitudes of the acquired resonance signals are proportional to the amount of the associated compounds that is present within the tissue. As such, ^{1}H-MRS is deemed to be a quantitative method. Indeed, ^{1}H-MRS of the human heart has been used in various cross-sectional studies, e.g., reporting on myocardial creatine depletion in myocardial infarction[3] and heart failure,[4] and in longitudinal studies of myocardial triglyceride content, e.g., with nutritional interventions in normal volunteers,[5,6] with endurance exercise,[7] and with therapeutic interventions in patients, such as type 2 diabetes[8] and aortic stenosis.[9]

Localized ^{1}H-MRS of the heart is challenging. Surrounding tissue can contaminate the myocardial signal, and air-filled lungs and flowing blood in the ventricles perturb the magnetic field homogeneity that is required for high sensitivity and adequate signal localization. These issues are further exacerbated by cardiac and respiratory motion. Several approaches that alleviate these issues for localized ^{1}H-MRS of the human heart have been proposed.[2] Adequate performance of a measurement technique requires precision and accuracy. *Precision* can be represented by the method's repeatability, i.e., how closely subsequent within-subject measurements agree. For measurements of in vivo myocardial triglyceride content with ^{1}H-MRS, repeatability in terms of the within-subject coefficient of variation has been reported to be as low as 5% at 1.5 T[5] and 3 T.[10] Measurement repeatability for myocardial total creatine content at 3 T has not been reported. There are large discrepancies between the various reports on how the reported repeatability was estimated[4,5] and whether this reflected intrasession or intersession repeatability of the measurements,[11,12] inevitably leading to variations in the reported repeatability that was achieved with various ^{1}H-MRS methods (Table 1). *Accuracy* can be estimated by how well measurements agree with an external validation. Such validations are complicated and would require biochemical assays in tissue samples that are obtained from the same region from which the localized ^{1}H-MRS data were acquired. Such studies have been conducted for human skeletal muscle[23] and liver,[24] but thorough validations for measurements in the human heart are lacking. Myocardial creatine measurements with ^{1}H-MRS have only been validated in dogs.[3] Reported attempts on a small number of human tissue samples ($n \leq 10$) involved high-field NMR[12] or histology[9] and did not include any biochemical assays of triglyceride content (Table 2). Validations of in vivo measurements with ^{1}H-MRS of the human heart against conventional invasive techniques are required to establish and confirm that ^{1}H-MRS is indeed a quantitative method for noninvasive assessments of myocardial tissue metabolite content.

Thus, the purpose of this work was to determine measurement precision and accuracy of myocardial total creatine and triglyceride content with ^{1}H-MRS of the human heart at 3 T.

Materials and Methods

The work in normal volunteers was performed according to recommendations by the local institutional review board (W15_373; Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands). The validation study in patients scheduled for open-heart surgery was approved by the local institutional review board (NL52084.018.15; Academic Medical Center). All participants provided written informed consent.

^{1}H-MRS Protocol

All subjects underwent a cardiac MR protocol with ^{1}H-MRS of the myocardial interventricular septum using a prospectively ECG-triggered respiratory-gated free-breathing single-voxel point-resolved spectroscopy (PRESS) sequence as introduced previously.[10] All examinations were performed with a 3 T MR system (Ingenia, software release 5.1.8; Philips, Best, The Netherlands). Subjects were positioned supine with ECG and respiration sensors connected. After scout imaging, standard retrospectively ECG-triggered cinematographic image series were acquired during breathholds to obtain long-axis views and a contiguous stack of short-axis views covering the left ventricle (LV) from apex to base. Subsequently, an 8–15 × 20–30 × 20–30 mm^3 voxel (triglyceride-methylene at 1.30 ppm on-resonance) was carefully positioned in the interventricular septum (Fig. 1) at the location corresponding to 200 msec after detection of the R-wave in the ECG signal,

TABLE 1. Literature Reports on Proton Magnetic Resonance Spectroscopy (^{1}H-MRS) Measurement Repeatability in the Human Heart

	Year of Publication	Field Strength (T)	Number of Subjects (*n*)	Within-Subject CV (%)	Methodological Repeatability
Total creatine					
Felblinger et al[13]	1999	1.5	9	10[a]	Intersession
Nakae et al[4]	2003	1.5	8	7.4	Not defined
This work	2021	3	16	19.8	Intrasession
This work	2021	3	16	21.3	Intersession
Triglyceride					
Felblinger et al[13]	1999	1.5	9	13[a]	Intersession
Szczepaniak et al[11]	2003	1.5	6	17	Intrasession and intersession combined
Reingold et al[5]	2005	1.5	6	5	Intersession[b]
Kankaanpää et al[14]	2006	1.5	9	17	Intrasession
van der Meer et al[15]	2007	1.5	20	17.9	Intersession
O'Connor et al[16]	2009	1.5	11	9	Not defined
O'Connor et al[12]	2011	1.5	16	6.9	Intrasession and intersession combined
Rial et al[17]	2011	3	15	19	Intrasession[c]
Weiss et al[18]	2012	1.5[d]	12	21	Intersession
Weiss et al[19]	2014	1.5	5	11	Not defined
Ith et al[20]	2014	3	8	6.3[e]	Intersession
de Heer et al[10]	2016	3	8	5	Intrasession
de Heer et al[10]	2016	3	7	6.5	Intersession
Gastl et al[21]	2019	1.5	15	5.2	Intrasession
Gastl et al[22]	2019	1.5	10	9.5	Not defined
This work	2021	3	16	21.3	Intrasession
This work	2021	3	16	18.7	Intersession

[a]Coefficient of variation (CV) reported after exclusion of spectra with "inferior quality."
[b]Sessions were separated by a 90-days interval.
[c]CV reported for same voxel location, but with reference and calibration scans invalidated and re-calibrated between consecutive scans.
[d]Using echo-planar spectroscopic imaging.
[e]CV for triglyceride content quantified with triglyceride/creatine ratios (which was "essentially identical" when referenced to water), total creatine/water ratios not reported.

ensuring that the chemical-shift displaced voxel for total creatine at 3.02 ppm remained within the myocardial tissue as well as within the shim volume (20–30 × 40 × 50 mm^3). Voxel size was adjusted to septal wall thickness. Multiple optimizations to improve suppression trains (MOIST; bandwidth 220 Hz centered on water at 4.7 ppm)[26] water-suppressed spectra (8 × 8 acquisitions; number of points, 1024; bandwidth, 1500 Hz) were acquired at a fixed echo time (TE) of 40 msec and a long repetition time (TR) of >6 seconds to minimize partial saturation effects. Acquisition of the water-suppressed spectra was interleaved with acquisitions of 8 unsuppressed water spectra at a TR of >9 seconds (water at 4.7 ppm on-resonance) to obtain fully relaxed water signals from a voxel identical in size and position to the voxel for the water-suppressed acquisitions. Signals were stored separately and

TABLE 2. Literature Reports on Proton Magnetic Resonance Spectroscopy (^{1}H-MRS) Measurement Validation in the Human Heart

	Year of Publication	^{1}H-MRS Method	Number of Samples (*n*)	Correlation Coefficient (*r*)	Method of Validation
Total creatine					
Bottomley and Weiss[3]	1998	Bottomley et al[25]	12	Not reported	Fluorometric assay of total creatine in ex vivo canine myocardium
This work	2021	de Heer et al[10]	22	0.44	Spectrophotometric assay of total creatine in tissue samples from patients
Triglyceride					
O'Connor et al[12]	2011	O'Connor et al[16]	6	0.91	400 MHz magic angle spinning NMR spectroscopy in endomyocardial biopsies from heart-transplanted patients
O'Connor et al[12]	2011	O'Connor et al[16]	8	0.98	600 MHz high-resolution NMR spectroscopy in endomyocardial biopsies from heart-transplanted patients
Mahmod et al[9]	2013	Rial et al[17]	10	0.66[a]	Histology with Oil Red O-staining in biopsies from patients
This work	2021	de Heer et al[10]	20	0.50	Colorimetric assay of triglyceride in tissue samples from patients

[a]Correlation not shown in the study by Mahmod et al.[9]

processed offline prior to myocardial metabolite content quantification.

Repeatability in Normal Volunteers

Volunteers (male/female, 8/8) underwent the MR exam protocol as described above. To test intrasession repeatability, ^{1}H-MRS acquisition was repeated by copying and executing the same scan directly after the first acquisition. Afterward, the volunteer was removed from the magnet and briefly exited the scanner room, after which the process was repeated immediately in a second MR examination of the same volunteer for an assessment of intersession repeatability. The entire MR protocol was repeated and included subject repositioning, scout imaging, voxel positioning, B_0 shimming, and RF power optimization. The procedure took approximately 1 hour to complete, minimizing any metabolic or physiological variation between scans.

Validation in Patients

We recruited 26 patients with a clinical indication for open-heart surgery at the Departments of Cardiology and Cardiothoracic Surgery at the Amsterdam University Medical Centers, University of Amsterdam, Amsterdam, The Netherlands between April 2016 and March 2020. Patients were eligible if they had severe aortic valve stenosis and were scheduled for surgical aortic valve replacement (AVR), or if they had hypertrophic obstructive cardiomyopathy and were scheduled for septal myectomy. Exclusion criteria were the presence of any non-MR compatible implants and other contraindications for MR examination. All participants underwent the MR protocol as described above. In addition, venous blood samples were collected just prior to MR examination and processed immediately according to the standard blood panel assays. Within 1 week after MR examination, myocardial tissue samples were collected intraoperatively, either via biopsies of the LV endocardial septum during surgical AVR or via septal myectomy through Morrow's procedure.[27] Samples collected via biopsies in patients who underwent AVR weighed 11.9 ± 5.1 mg wet weight. More tissue could be obtained from patients undergoing septal myectomy, yielding larger samples of 32.5 ± 21.1 mg wet weight. Any fibrotic tissue was removed upon collection, and tissue samples were immediately snap-frozen in liquid nitrogen and stored at $-80°C$ until further analyses.

MR Data Analyses

^{1}H-MRS. Spectral fitting for signal quantification was performed in the time domain using the AMARES fitting routine in jMRUI[28] as

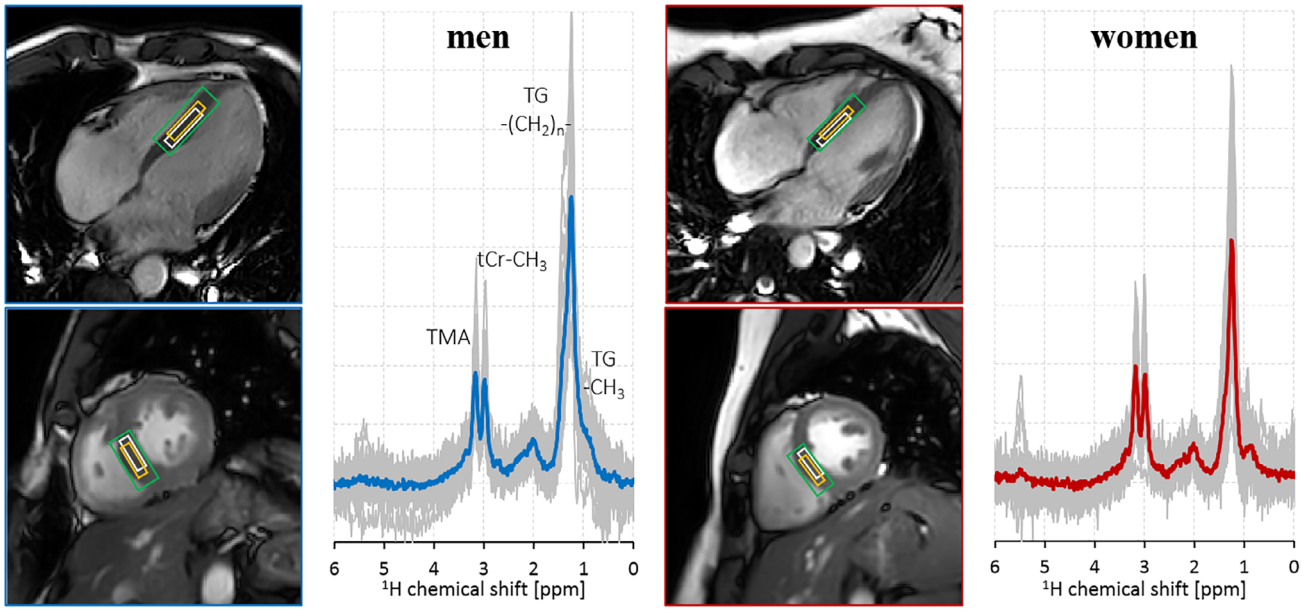

FIGURE 1: Localized ^{1}H-MRS of the human heart. A PRESS voxel (triglyceride-methylene at 1.30 ppm on-resonance for water-suppressed spectra, and water at 4.7 ppm on-resonance for unsuppressed spectra; ocher box) was carefully positioned in the interventricular septum at the location corresponding to 200 msec after detection of the R-wave in the ECG signal, ensuring that the chemical-shift displaced voxel for total creatine at 3.02 ppm (white box) remained within the myocardial tissue as well as within the shim volume (green box). Repeatability assessments were performed in men (n = 8; group mean spectrum in blue) and women (n = 8; red) and yielded three spectra per subject (n = 8 × 3 = 24; gray). Peaks of trimethylamine-containing compounds (TMA; 3.2 ppm), total creatine-methyl (tCr-CH_3; 3.0 ppm), triglyceride-methylene (TG—CH_2—)$_n$ at 1.3 ppm, and triglyceride-methyl (TG-CH_3; 0.9 ppm) are indicated. All spectra are scaled relative to the unsuppressed water signal acquired from the same voxel (ocher box).

described in detail in the Supporting Information. 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 the triglyceride-methylene and triglyceride-methyl signal amplitudes divided by the unsuppressed water signal amplitude.

CINEMATOGRAPHIC MRI. Heart function and morphology were assessed using quantitative analyses of the cinematographic

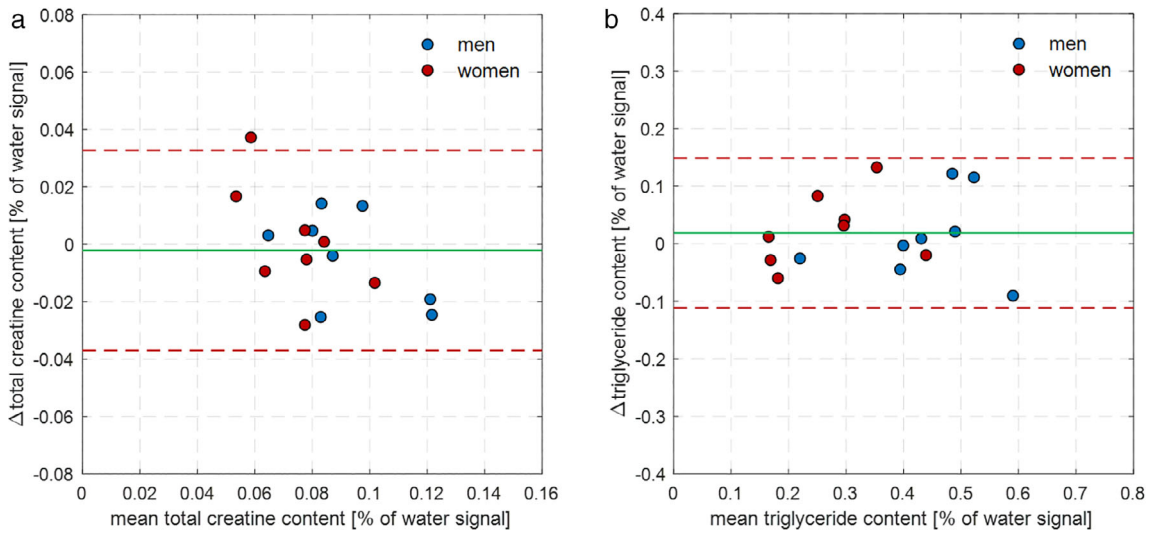

FIGURE 2: Precision. Bland–Altman analyses of in vivo myocardial total creatine content (a) and myocardial triglyceride content (b) measured with localized ^{1}H-MRS in men (blue symbols, n = 8) and women (red symbols, n = 8). The intersession repeatability coefficient for myocardial total creatine content was 41.8% with a mean value of 0.083% ± 0.020% of the total water signal, and 36.7% for myocardial triglyceride content with a mean value of 0.35% ± 0.13% of the total water signal. Dashed red lines represent the 95% confidence interval at 1.96 times the standard deviation of the differences between the repeated measurements around the mean difference between consecutive measurements (green solid line).

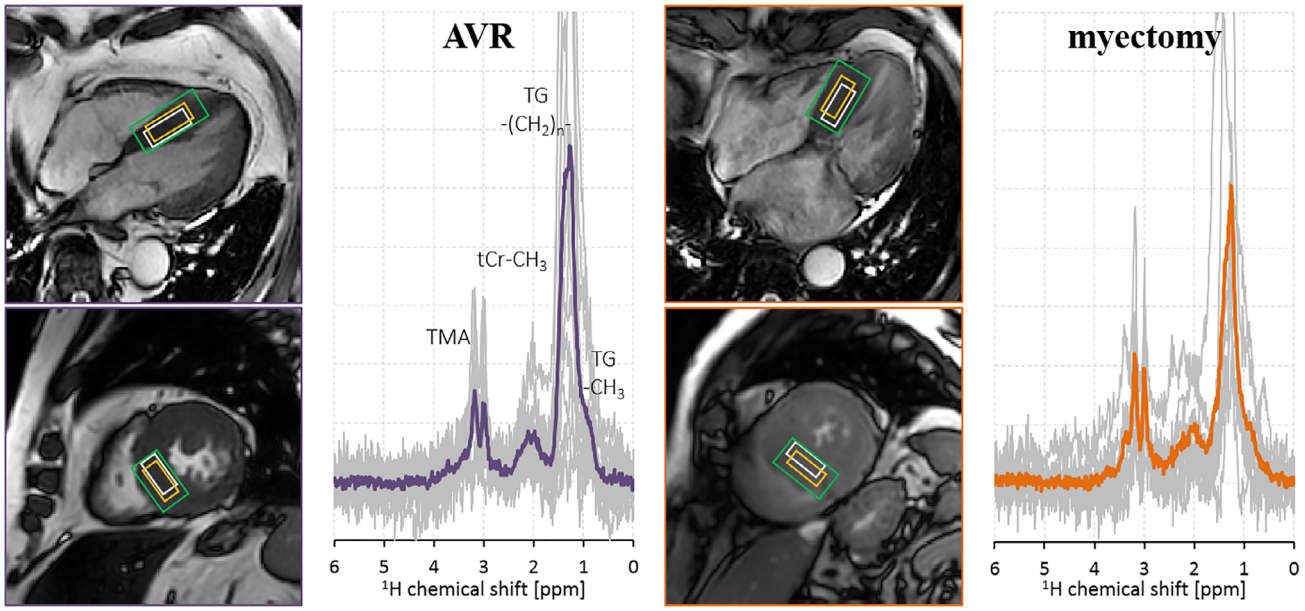

FIGURE 3: Localized ^{1}H-MRS of the heart in patients scheduled for surgical aortic valve replacement (AVR; *n* = 13; group mean spectrum in purple) and in patients scheduled for septal myectomy (*n* = 9; orange). Note the variability for myocardial triglyceride content between subjects, illustrated by differences in triglyceride-methylene signal amplitudes at 1.30 ppm in the individual spectra (gray). Peaks of trimethylamine-containing compounds (TMA; 3.2 ppm), total creatine-methyl (tCr—CH_3; 3.0 ppm), triglyceride-methylene (TG—CH_2—)$_n$ at 1.3 ppm, and triglyceride-methyl (TG—CH_3; 0.9 ppm) are indicated. All spectra are scaled relative to the unsuppressed water signal acquired from the same voxel (ocher), which is identical to the scaling used in Fig. 1.

MRI series acquired in patients. Endocardial and epicardial contours of the LV were manually drawn by an experienced investigator (A.J. B.; 12 years experience) in QMass 8.1 (Medis medical imaging systems BV, Leiden, The Netherlands), and used to quantify end-diastolic (EDV) and end-systolic (ESV) LV volumes, LV stroke volume (SV), LV ejection fraction (EF), LV cardiac output, LV global longitudinal strain (GLS), and LV myocardial mass.

Biochemical Assays

TOTAL CREATINE ASSAY. Tissue samples were weighed and freeze-dried overnight, and then weighed again to estimate tissue water content. The myocardial total creatine concentration (μmol/g wet weight) was measured with an enzymatic spectrophotometric assay[29] as described in detail in the Supporting Information.

TRIGLYCERIDE ASSAY. Total myocardial triglyceride concentration (μmol/g wet weight) was determined biochemically via colorimetric quantification as described previously,[30] with minor modifications (Supporting Information).

Statistical Analyses

Data are presented as mean ± standard deviation (SD). Intrasession and intersession repeatability of localized ^{1}H-MRS measurements was assessed using Bland–Altman analyses of the myocardial total creatine and triglyceride content quantifications.[31] The repeatability coefficient is given by 1.96 times the SD of the differences between the repeated measurements and is expressed as a percentage of the mean value. The 95% confidence interval is defined as the repeatability coefficient offset by the mean difference between consecutive measurements. The within-subject coefficient of variation (CV) was determined as described by Bland and Altman.[31] Regression analyses were performed using MATLAB (The MathWorks, Inc., Natick, MA, USA) assuming a relative equal weighting of error between a linear model fit and in vivo ^{1}H-MRS measurements and biochemical assays. Covariance matrices for the slope and intercept parameters were estimated as described by Landaw and DiStefano,[32] and used to determine 90% confidence bounds on the linear model fits. Differences between groups were tested with two-sided Student's *t*-tests. The level of significance was set at $P < 0.05$.

Results

Precision

The men in our cohort of normal volunteers were slightly older than the women (31.3 ± 3.3 vs. 27.5 ± 1.8 years; $P < 0.05$), and their height (1.87 ± 0.07 vs. 1.70 ± 0.07 m; $P < 0.05$) and body weight (81.1 ± 8.7 vs. 61.1 ± 6.3 kg; $P < 0.05$) were higher, which translated in a somewhat higher body mass index (BMI) for men compared to women (23.3 ± 1.7 vs. 21.0 ± 1.6 kg/m^2; $P < 0.05$). Overall, water linewidth was similar in men and women (13.8 ± 2.8 vs. 13.1 ± 2.4 Hz; $P = 0.351$). In addition, water linewidth correlated with heart rate during MR examination (16 volunteers × 2 sessions; $n = 32$, $r = 0.39$; $P < 0.05$), suggesting that B_0 shimming was better at lower heart rates. Localized ^{1}H-MR spectra were obtained from the interventricular septum, and revealed distinct peaks of the total creatine-methyl resonance (3.02 ppm) and triglyceride-methylene (1.3 ppm) and -methyl (0.9 ppm)

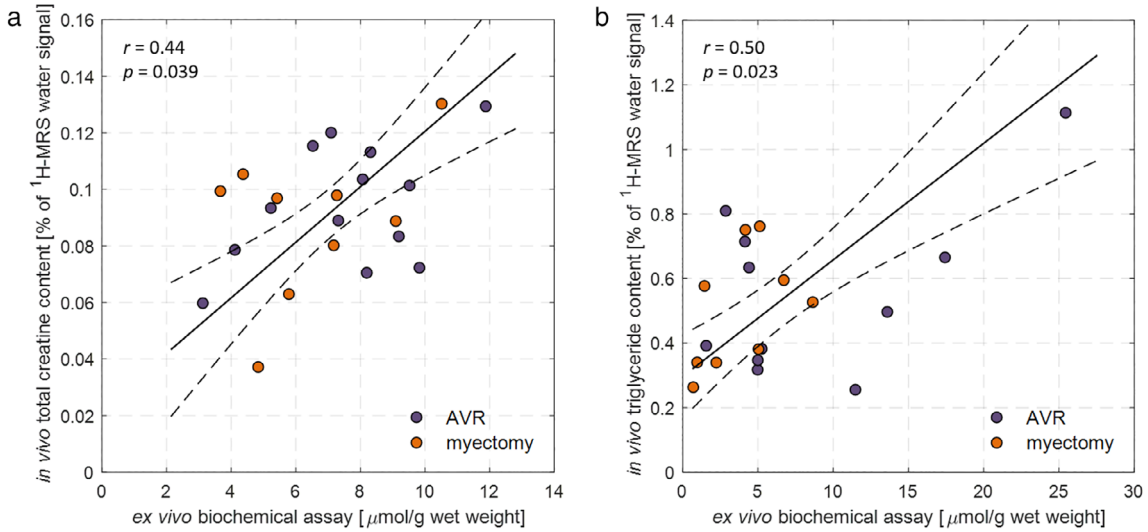

FIGURE 4: Accuracy. Regression analyses of in vivo myocardial total creatine content (a) and triglyceride content (b) measured with localized ^{1}H-MRS against ex vivo myocardial total creatine concentration (n = 22, r = 0.44; P < 0.05) and triglyceride concentration (n = 20, r = 0.50; P < 0.05) determined biochemically in tissue samples obtained from the same subjects. Dashed lines indicate 90% confidence bounds of the linear model fits (solid lines). AVR, aortic valve replacement.

resonances (Fig. 1). Overall, the relative Cramér Rao Lower Bound (CRLB) was 4.8% ± 2.5% for the fit of total creatine, and 3.3% ± 2.6% for triglyceride-methylene. Myocardial total creatine content was higher in men than in women (0.094% ± 0.021% vs. 0.075% ± 0.018% of the total water signal; P < 0.05). The same was found for myocardial triglyceride content (0.44% ± 0.11% vs. 0.26% ± 0.09% of the total water signal; P < 0.05). The intrasession repeatability coefficient for myocardial total creatine content was 38.8% at a mean of 0.084% ± 0.018% of the total water signal with a mean difference between consecutive measurements of 0.005% ± 0.017% of the total water signal. The intra-session within-subject CV of myocardial total creatine content was 19.8% (Table 1). The intrasession repeatability coefficient for myocardial triglyceride content was 41.8% at a mean of 0.35% ± 0.12% of the total water signal with a mean difference between measurements of 0.02% ± 0.08% of the total water signal. The intrasession within-subject CV of myocardial triglyceride content was 21.3% (Table 1). Intersession repeatability was similar to intrasession repeatability for both measurements, with a repeatability coefficient of 41.8% for myocardial total creatine content (Fig. 2(a)) and 36.7% for myocardial triglyceride content (Fig. 2(b)). Within-subject CVs were 21.3% and 18.7%, respectively (Table 1). These data indicate that at 95% confidence of detecting a (patho)physiological change rather than an effect of measurement error, the present approach for ^{1}H-MRS can detect changes of approximately >40% in myocardial total creatine or myocardial triglyceride content between repeated measurements in a single subject.

Accuracy

Out of a total of 26 patients scheduled for open-heart surgery that we recruited for this study, three patients indicated experiencing shortness-of-breath, dizziness, or claustrophobia during their MR examination, upon which the session was aborted before the acquisition of ^{1}H-MRS data. No endomyocardial biopsies were performed in these patients. In one patient, no biopsy could be taken due to insufficient access to the septum during surgery. As such, complete MR datasets with associated tissue samples of 13 patients who underwent AVR and of 9 patients with hypertrophic cardiomyopathy who underwent septal myectomy were available for analyses (Fig. 3). Characteristics, blood panel results and MR results for both groups are reported in the Supporting Information Table.

Water linewidth was similar for AVR and myectomy patients (14.7 ± 2.4 vs. 13.4 ± 3.6 Hz; P = 0.337) and was similar to water linewidths achieved in volunteers (P = 0.289). Myocardial total creatine content measured with ^{1}H-MRS was similar for AVR and myectomy patients (0.095% ± 0.021% vs. 0.089% ± 0.027% of the total water signal; P = 0.575) and was well within the normal range that we established in volunteers. Myocardial triglyceride content was 0.67% ± 0.36% and 0.50% ± 0.18% of the total water signal (P = 0.223) for AVR and myectomy patients, respectively, which was above the normal range, and had a larger variability between subjects than in normal volunteers.

Samples of 22 patients were available for biochemical assays of the myocardial total creatine concentration. Because endomyocardial biopsy did not yield enough tissue from two patients that underwent AVR, myocardial triglyceride concentration was determined in samples of 20 patients. Tissue water content, measured by comparing sample wet weights with sample dry weights, was 81.9% ± 3.7%. Myocardial total creatine concentrations were similar for AVR and myectomy samples: 7.6 ± 2.4 vs. 6.4 ± 2.3 μmol/g wet

weight (P = 0.290). Importantly, these ex vivo myocardial total creatine concentrations determined with biochemical assays in tissue samples correlated with the in vivo myocardial total creatine content measured with ^{1}H-MRS in the same subjects: n = 22, r = 0.44; $P < 0.05$ (Fig. 4(a)). Myocardial triglyceride concentrations were highly variable between subjects and were 8.7 ± 7.4 and 3.9 ± 2.8 μmol/g wet weight ($P < 0.05$) for AVR and myectomy samples, respectively. The ex vivo myocardial triglyceride concentrations determined via biochemical assays in tissue samples correlated with the in vivo myocardial triglyceride content measured with ^{1}H-MRS in the same subjects: n = 20, r = 0.50; $P < 0.05$ (Fig. 4(b)). These results show that the present approach for ^{1}H-MRS can accurately estimate in vivo myocardial total creatine and triglyceride content.

Heart Function and Morphology

Functional and morphological parameters for hearts of patients are reported in the Supporting Information Table. LV SV (74 ± 12 vs. 92 ± 17 mL; $P < 0.05$) and LV EF (57.8% ± 13.1% vs. 67.9% ± 6.4%; $P < 0.05$) were higher in myectomy patients compared to AVR patients. LV GLS in myectomy patients was also larger compared to AVR patients (−17.8% ± 6.8% vs. −24.2% ± 4.4%; $P < 0.05$). In absolute sense, the LV EDV and LV myocardial mass were similar in both groups, translating in a similar LV mass-to-volume ratio (Supporting Information Table). With a difference in BSA ($P < 0.05$), LV EDV indexed to BSA (63.4 ± 10.1 vs. 72.7 ± 8.8 mL/m^2; $P < 0.05$) and LV myocardial mass indexed to BSA (79.3 ± 13.6 vs. 95.5 ± 15.5 g/m^2; $P < 0.05$) were much higher in patients who were scheduled for septal myectomy than in those scheduled for AVR, reflecting hypertrophic cardiomyopathy in the former. Myocardial total creatine and triglyceride content measured with ^{1}H-MRS did not correlate with any of the functional or morphologic parameters, or with any of the circulating metabolite levels measured in blood collected just prior to MR examination. The relatively high Hb1Ac values, a marker for hyperglycemia, did not reach a significant positive correlation with myocardial triglyceride content measured with ^{1}H-MRS (n = 22, r = 0.38; P = 0.077).

Discussion

This work shows that the precision in terms of measurement repeatability of localized ^{1}H-MRS is sufficient to detect (patho)physiological changes of myocardial total creatine and triglyceride content of >40% with 95% confidence in a single subject. Moreover, we established the accuracy of ^{1}H-MRS by demonstrating a good agreement between measurements of myocardial total creatine and triglyceride content quantified by non-invasive ^{1}H-MRS and biochemical assays of metabolite concentrations in myocardial tissue samples obtained intra-operatively from the same patients.

Precision

The repeatability of the ^{1}H-MRS approach used in the present study is comparable to previously reported work (Table 1), with the CV for myocardial triglyceride content on the high end of what has been reported for measurements at 1.5 T[15] and 3 T[17] (Table 1). Importantly, its intrasession and intersession repeatability were very similar for myocardial total creatine content as well as for triglyceride content, suggesting that subject repositioning and initial voxel positioning are not the dominant factors contributing to within-subject variation. Instead, we suggest that despite triggering signal acquisitions to the ECG R-wave, physiological variations in heart rate lead to small displacements of the interventricular septum with respect to the voxel positioned for ^{1}H-MRS acquisition, and consequently lead to myocardial signal fluctuations.[10,33] Besides such variations in signal acquisition, measurement errors can arise in signal quantification through spectral fitting. Using a dedicated fitting model based on biochemical, physical, and empirical prior knowledge, and aided by the high B_0 shim quality[34] that yielded well-resolved resonance peaks, we achieved CRLBs of <10% for essentially all fits. Note that we did not use high relative CRLBs as a criterion to exclude any spectra, in order to avoid bias toward higher tissue metabolite content.[35] Nonetheless, with the CRLB being the lower threshold of the fitting error, such fitting errors likely contributed markedly to within-subject variation. Together, these results emphasize the importance of adequate localization of signal acquisition and B_0 shimming[34] as well as an appropriate spectral fitting approach for precise quantitative ^{1}H-MRS readouts of the human heart.

Accuracy

We established the accuracy of in vivo myocardial total creatine and triglyceride content measurements with an optimized approach for localized ^{1}H-MRS of the human heart at 3 T[10] in a direct comparison with biochemical assays of myocardial tissue samples collected from more than 20 patients. We assumed a homogeneous spatial distribution of myocardial total creatine and triglyceride content in these nonischemic patients, and that the small tissue samples collected from the LV endocardial septum would reflect the content within the relatively large voxel required for localized ^{1}H-MRS of the septum. Indeed, ^{1}H-MRS provides an accurate noninvasive measure of in vivo tissue triglyceride content, which was previously demonstrated for human skeletal muscle[23] and liver.[24] Importantly, we have now extended this approach to the technologically challenging application of ^{1}H-MRS in the human heart, and moreover, we provide an accuracy assessment of in vivo myocardial total creatine content as well. Even with the relatively high CVs that we found in the present study, our results now offer confirmation that ^{1}H-MRS is a reliable tool to noninvasively assess

myocardial tissue metabolite content, one that has been used in many studies for over 25 years for investigations of cardiac physiology and intervention efficacy in health and disease.[2]

Pathophysiology

It has been recognized since the 1930s that myocardial total creatine content can be lower in the failing heart than in the healthy heart.[36] As such, an accurate estimate of the total myocardial creatine content is crucial for a meaningful interpretation of commonly used phosphocreatine-to-ATP ratio measured with phosphorus-31 MRS as a parameter that reflects the myocardial energy status.[37] As expected, in this cohort of patients with severe aortic valve stenosis or hypertrophic cardiomyopathy who had preserved systolic function, we did not observe any correlations of in vivo myocardial creatine content with heart function or with myocardial mass. Indeed, the myocardial total creatine content in patients was similar to volunteers. Myocardial triglyceride content in patients was nearly twice as high as in volunteers. Elevated myocardial triglyceride content has been related to decreased myocardial function in calorie-restricted healthy volunteers[6] and type 2 diabetes.[38] Yet, in vivo myocardial triglyceride content did not correlate with any functional or morphological parameters in the present cohort of patients with preserved systolic function. This suggests that measurements in larger cohorts with a broader range of (diabetic) cardiomyopathies are required to establish such relationships.

Limitations

We did not convert our estimates of in vivo myocardial total creatine and triglyceride content into absolute concentrations. Doing so requires a number of assumptions that we could not verify in vivo, including tissue water content, myocardial T_2 relaxation time constants for creatine-methyl and triglyceride signals, and the chemical composition of myocardial triglycerides (e.g., fatty acid chain lengths and degree of saturation). Assuming myocardial T_2 values of creatine-methyl and water to be similar to those in skeletal muscle at 3 T (135 msec and 30 msec, respectively[39]), 55.5 M concentration of water, a 3-hydrogen nuclei signal for creatine-methyl and a 2-hydrogen signal for water, a tissue water content of 0.82 liter/kg (this work) and an in vivo myocardial total creatine content of 0.09% of the total water signal measured at a TE of 40 msec and a long TR for fully relaxed conditions (this work), we would arrive at an in vivo myocardial total creatine concentration estimate of 9.7 μmol/g wet weight. This value appears to be twofold lower than reported values for in vivo ^{1}H-MRS estimates ranging from 20 μmol/g wet weight[18] up to 29 μmol/g wet weight,[3,4] but is in good agreement with our ex vivo estimates of ~7 μmol/g wet weight determined biochemically in biopsies, and is more consistent with early reports on normal human myocardium (9.3 μmol/g wet weight[36]). Following similar calculations and assuming a T_2 value of 90 msec for triglyceride-methylene,[39] a 68.6-hydrogen nuclei signal for triglyceride-methylene[40] and a 3-hydrogen nuclei signal per triglyceride-methyl group, the in vivo myocardial triglyceride concentration estimate would be 2.9 μmol/g wet weight. This is on the low end of the biochemical estimates in tissue samples (4–9 μmol/g wet weight) and may be attributed to the assumed number of methylene hydrogens per triglyceride that could be an overestimation with unsaturated bonds not being taken into account. Furthermore, tissue samples were collected intra-operatively on a different day after the MR exam. Indeed, as we[6,7] and others[20] have shown previously, the myocardial triglyceride pool is highly dynamic, and diurnal or nutritional differences between MR examination and surgery may contribute to discrepancies between measurements. Notably, fasting has been shown to elevate myocardial triglyceride content.[5] Patients were fasted for >9 hours prior to surgery, which could explain the higher myocardial triglyceride content determined biochemically in tissue samples compared to those found with ^{1}H-MRS. Myocardial total creatine content is considered to be more stable, which is reflected by the good agreement between in vivo ^{1}H-MRS measurements and ex vivo assays of myocardial tissue collected during surgery.

Conclusion

We have validated the use of localized ^{1}H-MRS of the human heart at 3 T for quantitative assessments of in vivo myocardial tissue metabolite content by establishing measurement precision and accuracy. Evidenced by correlations between in vivo myocardial total creatine and triglyceride content measured with ^{1}H-MRS and ex vivo biochemical assays in myocardial tissue samples from the same subjects, we confirmed that ^{1}H-MRS is a quantitative tool for noninvasive assessments of myocardial tissue metabolite content. Such validations provide valuable guidance for quantitative interpretations and comparisons of future and past ^{1}H-MRS studies of the human heart.

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