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Multivoxel proton magnetic resonance spectroscopy in facioscapulohumeral muscular dystrophy

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Abstract

Introduction—Facioscapulohumeral muscular dystrophy (FSHD) is a hereditary disorder that causes progressive muscle wasting. The purpose of this study was to evaluate the use of proton magnetic resonance spectroscopy (¹H MRS) as a biomarker of muscle strength and function in FSHD.

Methods—36 individuals with FSHD and 15 healthy controls underwent multivoxel ¹H MRS of a cross-section of the mid-thigh. Concentrations of creatine, intramyocellular and extramyocellular lipids, and trimethylamine-containing (TMA) compounds in skeletal muscle were calculated. Metabolite concentrations for individuals with FSHD were compared with those of controls. The relationship between metabolite concentrations and muscle strength was also examined.

Results—The TMA/creatine ratio in individuals with FSHD is reduced compared to controls. The TMA/creatine ratio in the hamstrings also showed a moderate linear correlation with muscle strength.

Discussion—¹H MRS offers a potential method of detecting early muscle pathology in FSHD prior to the development of fat infiltration.

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None of the authors has any conflict of interest to disclose.

Keywords

facioscapulohumeral muscular dystrophy; magnetic resonance spectroscopy; skeletal muscle; metabolite quantification; biomarkers; trimethylamine

Introduction

Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominant muscle disease with an estimated prevalence of 3.96 per 100,000.¹ There has been great progress in elucidating the genetic and epigenetic mechanisms of disease in FSHD in recent years.² However, the ability to translate basic scientific discoveries into treatment is limited by the absence of quantitative biomarkers of muscle disease.³ Recent studies have suggested that magnetic resonance spectroscopy (MRS) can differentiate between diseased and healthy muscle in FSHD.^{4, 5} These studies utilized phosphorus-based MRS protocols, which identify metabolites involved in energy consumption in muscle. Proton (¹H) spectroscopy can likewise detect multiple metabolites in muscle, including creatine, trimethylamines (TMA), and extramyocellular and intramyocellular lipids. These metabolites could be particularly relevant to the muscular dystrophies; muscle replacement by fat is a feature common to all muscular dystrophies, and creatine serves as a key substrate for energy metabolism in muscle.⁶ In brain spectroscopy, the TMA peak is comprised of choline-containing compounds, including phospholipids that are implicated in cell membrane metabolism.⁷ Studies in muscle also indicate that the TMA peak is comprised of several molecules involved in muscle metabolism, including carnitine and acetylcarnitine.⁸

To evaluate the use of proton spectroscopy as a biomarker of muscle strength and function, we performed a cross-sectional study of multivoxel ¹H MRS in people with FSHD and healthy volunteers.

Materials and Methods

Study design and setting

The study protocol was approved by the Institutional Review Board. Individuals with genetically-confirmed FSHD and healthy adults (older than 18 years) provided written consent to participate in a single-center, cross-sectional study. Participants with FSHD were asked to provide copies of their genetic testing results. Participants with a clinical diagnosis of FSHD were asked to undergo confirmatory genetic testing for FSHD if it had not been done prior to participation. Healthy volunteers were asked to undergo genetic testing for FSHD to confirm the absence of disease. Each potential participant was screened for contraindications to MRI scanning. Participants with FSHD were asked to return for an optional second scan between 18 and 24 months following the initial scan. All subjects were enrolled and scanned between April 16, 2012 and January 30, 2015.

Clinical data collection

Demographic information (age, gender, height, weight) was collected for each participant. On the day of each scan, participants underwent strength testing of the bilateral hip flexors,

knee flexors, and knee extensors using hand-held dynamometry (Chatillon DPP series mechanical force gauge). A 10-meter walk time was also obtained.

MRI protocol

Each participant was scanned using a 3 Tesla MAGNETOM Trio system (Siemens, Erlangen, Germany). Subjects were scanned in a supine, feet-first position. Two 'body matrix' receiver coil arrays were placed over the upper and lower thighs. T1-weighted localizer sequences were used to identify an axial slice at the mid-thigh in each leg (scan parameters: MPRAGE; repetition time (TR)/echo time (TE) 2300/3.39 ms; field of view (FOV) 40X20 cm; inversion time (TI) 900 ms; slice thickness 1mm; acquisition time 2:31 minutes). Multivoxel MR spectroscopy with water suppression was performed in each slice (scan parameters: TR/TE 1800/135 ms; 16×16 matrix size; slice thickness, 1 cm; 16×16 cm field of view; total data acquisition time, approximately 7:17 minutes). Nominal voxel size was 1.0 cm^3 . The echo signal was digitized with 512 data points and a spectral width of 2,000 Hz. Multivoxel MRS of the unsuppressed water signal was also acquired (scan parameters: TR/TE 1800/30ms; 16×16 matrix size; slice thickness, 1 cm; 16×16 cm field of view; total data acquisition time, approximately 4:35 minutes). Shimming and water suppression were optimized using automated routines provided by the manufacturer. Spectroscopy data were processed using LCModel software using the vendor-provided basis set for muscle spectra.⁹ Metabolite concentrations were estimated relative to the unsuppressed water signal (in Institutional Units (IU)), and ratios with respect to creatine were also calculated.¹⁰

The T1-weighted images of the slice used to place voxels for the MRS protocol were examined to exclude: 1) voxels that were not entirely embedded in muscle, and 2) intramuscular voxels that were infiltrated by fat. Voxels completely enclosed within the vastus lateralis, vastus medialis, rectus femoris, vastus intermedius, adductor magnus, sartorius, gracilis, long and short heads of the biceps femoris, semitendinosus, and semimembranosus muscles were selected for analysis (Supplementary Figure 1). Because of the location and tissue composition of the voxel, coil placement limits, as well as other technical difficulties, spectral quality did not meet processing needs in some collections. Spectra were excluded if they contained excessive noise or did not demonstrate identifiable metabolic peaks.

Statistical methods

The distribution of metabolite concentrations was explored and examined for outliers using histograms and box plots. Excessively right-skewed data were log-transformed, and q-q plots were used to confirm the normal distribution of the resultant data. Multilevel mixed-effects linear regression models were used to assess differences in metabolite concentrations (the response variable) with respect to disease status (the independent variable). Because metabolite concentrations were expected to cluster within individual muscles and within participants, random effects parameters were included in the models to adjust for voxels nested within muscles that were nested within a subject and to generate accurate p-values. Muscle metabolite concentrations in the quadriceps muscle group (vastus lateralis, vastus medialis, vastus intermedius, and rectus femoris) and the hamstrings group (semitendinosus,

semimembranosus, long head of the biceps femoris, and short head of the biceps femoris) were also examined with respect to measured strength in those muscle groups and the 10-meter walk times. Age, gender, and deletion size were explored as potential confounders in these multilevel models.

For the subset of participants who returned for follow-up scans, acquisition and analysis of spectra were performed using the same protocol as the baseline scan. The metabolite concentrations from the baseline and follow-up scans were compared using two methods. First, the mean concentration of each metabolite was calculated for each muscle for which data was available. The difference in the mean concentration between the baseline and follow-up scans was analyzed using histograms, t-tests, and Bland-Altman plots. Second, multilevel linear regression models were also used to study the changes in concentration of each metabolite with respect to time (again grouped by individual subject and muscle). Data analysis was performed using the Stata 12/IC statistical software package (StataCorp LP, College Station, Texas).

Results

Cross-sectional data comparing FSHD and control subjects

A total of 52 individuals participated in the observational study (36 with clinical diagnoses of FSHD and 16 healthy controls). One control was found to have somatic mosaicism for the FSHD mutation and was excluded from the analysis. The demographic features of the affected and unaffected participants, as well as strength measurements in various muscle groups (values from the right and left sides are averaged) are summarized in Table 1. Genetic testing results from participants with FSHD showed a broad range of chromosome 4 deletion sizes (restriction fragment sizes range from 10 to 33 kb).

Proton MRS was completed on both thighs for most participants (3 participants had only one leg studied). Spectra were collected from 256 individual voxels in each leg. Screening for voxels that were completely enclosed with muscle excluded 78% of all voxels, and screening for spectral fit excluded an additional 13% of the acquired voxels. Preliminary examination of the fat and water spectroscopy imaging indicated that there was insufficient field homogeneity in the majority of studies performed in the right lower extremity, and therefore only spectra and strength measurements from the left lower extremity were included in the analysis. 1165 voxels (representing 32 individuals with FSHD and 14 controls) were included in the statistical analysis. No usable voxels were identified in 5 individuals (4 affected and 1 control), and the remaining participants contributed between 7 and 38 voxels to the data set. These voxels represent 11 individual muscles of the thigh (Supplementary Figure 1).

The most commonly identified metabolite peaks included trimethylamines (TMA) at 3.2ppm, creatine (Cr) at 3.0ppm, an extramyocellular lipid (EMCL) peak at 1.5ppm, and an intramyocellular (IMCL) lipids peak at 1.3ppm (Figure 1). Multilevel regression models demonstrated a statistically significant difference in TMA/Cr ratio between affected and unaffected participants ($p=0.001$) (Figure 2). No statistically significant differences were identified in the concentrations of creatine, TMA, IMCL, and EMCL or the ratios of IMCL

and EMCL to creatine (Table 2). Age, gender, and deletion size were evaluated as potential confounders and were not found to modify the associations between disease status and metabolite concentrations; these factors were therefore not included in the final regression models.

Comparison of metabolite concentration and clinical measurements in FSHD

The concentrations of creatine, TMA, IMCL, and EMCL of voxels located in the quadriceps (vastus lateralis, vastus medialis, vastus intermedius, and rectus femoris muscles) and hamstrings (semitendinosus, semimembranosus, long and short heads of the biceps femoris muscles) were averaged for each participant with FSHD. These muscle group concentration means were then compared to muscle strength in these respective muscle groups. Consistent with the cross-sectional analysis, only TMA/Cr showed a moderately strong linear correlation with strength in the hamstrings indicating that muscle strength increases with increasing $\log[\text{TMA/Cr}]$ ($r=0.62$, $p=0.0002$). There was a weaker correlation between TMA/Cr concentration and the 10-meter walk time, but higher $\log[\text{TMA/Cr}]$ values were associated with a decrease in the 10-meter walk time ($r=-0.41$, $p=0.026$) (Figure 3). No statistically significant association between strength or function and $\log[\text{TMA/Cr}]$ was seen in the quadriceps, which had a much narrower range of strength measurements. No significant association with muscle strength was observed in any of the other metabolites studied.

Longitudinal data analysis

Longitudinal spectroscopy data were obtained from 10 participants with FSHD between 18 and 24 months from the baseline scan. There were no statistically significant differences in knee flexion and knee extension strength between the baseline and follow-up scans. Neither the exploratory analysis nor the multilevel linear regression models showed a significant change in concentration from baseline to follow-up for any of the metabolites analyzed.

Discussion

This study demonstrates that multivoxel proton MRS can detect metabolic abnormalities in the muscles of subjects with FSHD, even among voxels that contain no visible fat infiltration on T1-weighted imaging. Comparing individuals with FSHD to unaffected controls, there is a statistically significant reduction in the TMA/Cr ratio. These findings are similar to a prior study which likewise identified a decreased TMA/Cr ratio in individuals with Duchenne muscular dystrophy (DMD) versus controls.⁶ However, the DMD study also found statistically significant differences in the TMA/water and creatine/water ratios, which were not found in the current FSHD study.

Examination of the TMA and Cr data suggest that the difference in TMA/Cr is primarily driven by the difference in TMA concentration, which is reduced in affected subjects compared to controls. Although the TMA concentration did not differ significantly between cases and controls, this may be due to the large variability in concentration found for this particular metabolite. The mechanisms by which TMA and Cr concentrations may be altered in the early stages of FSHD are as yet unknown. However, given our knowledge of the

composition of the TMA peak in brain and muscle, the relative reduction in TMA/Cr in affected muscles may reflect early muscle degeneration resulting in loss of muscle membrane and mitochondrial components. Further studies on the pathophysiologic changes that occur early in skeletal muscle in people with FSHD are needed to elucidate these mechanisms.

This study has some limitations. Although the multivoxel technique was successful in collecting data from multiple voxels in multiple muscle groups in a single acquisition, a large percentage of voxels could not be included in the analysis because they were not completely enclosed in normal-appearing muscle or had insufficient spectral fit. An examination of outliers also demonstrated marked differences in metabolite concentrations in the postero-lateral aspect of the right lower extremity compared to the left. This may be related to isolated scanner-specific field inhomogeneities. To compensate for this potential confounder, only the left lower extremity was analyzed for each participant, and this reduction in data may reduce the power to detect changes in metabolite concentrations.

This study also only analyzed the metabolic profiles of voxels comprised entirely of muscle without visible fat infiltration. As fat infiltration is one of the primarily pathologic features of muscular dystrophy, the metabolic profiling of the fat infiltrated voxels is also of interest as a biomarker, and prior studies have demonstrated changes in the intramuscular lipid concentration in muscular dystrophy using spectroscopy.^{11, 12} However, inspection of the individual spectra raised concerns regarding the inclusion of fat infiltrated voxels in our analysis. Because intramuscular lipids are comprised of multiple molecules, multiple lipid peaks were likewise observed in the resultant spectra, some of which overlapped the range of the creatine and TMA signals. As metabolites were subsequently scaled to creatine, we felt that the presence of these additional large lipid peaks could confound the quantification of those metabolites. By restricting our analysis to voxels without fat infiltration, we were able to investigate potential early differences in metabolic profiling that precede fat infiltration in FSHD.

We also did not collect data on several factors that may influence MRS, including exercise levels and medications. Global function scales that are specific to FSHD are also increasingly being used in clinical studies in FSHD, and may provide greater insight into the associations between imaging and clinical function in this disease population.^{13, 14} Fluid sensitive MRI sequences (such T2-weighted or STIR images) were also not included in the study protocol. T2 or STIR hyperintensity indicates the presence of edema or free water, and is associated with active inflammation seen in affected muscles in people with FSHD. As T2 values can influence spectroscopy measurements, future investigations should consider incorporating these fluid sensitive sequences. The relative utility of MRS as a biomarker could be even further defined by studying these scans in conjunction with other quantitative MRI sequences that have been studied as potential biomarkers in muscular dystrophy, such as Dixon estimation of muscle fat fractions and T2 mapping.^{15–17} This study protocol also did not include repeatability and reliability testing (in part due to time constraints). Although prior studies have demonstrated acceptable repeatability and reliability for multiple spectroscopic techniques,^{18–20} the application of a multivoxel MRS to future multicenter

observational studies and clinical trials will require further internal validation of both repeatability and reliability.

In the 18 to 24 month longitudinal data that was collected, no statistically significant changes in metabolite concentration over time were found. This observation differs from prior MRS data from patients with DMD that showed a time-dependent change in fat and water concentrations.²¹ However, this difference may be related to the fact that only voxels corresponding to normal-appearing muscles on T1-weighted imaging were included in the current analysis. Doing so would necessarily exclude voxels that had been replaced by fat in between the baseline and follow-up scans. It is also possible that changes in spectroscopy are detectable over shorter periods of time in DMD because of the more rapid progression of disease compared to FSHD. Further study is required to fully characterize the longitudinal changes in metabolite profiles in FSHD.

In conclusion, this study demonstrates that the TMA/Cr ratio in skeletal muscle of individuals with FSHD differs significantly from those of unaffected controls. This difference was found in normal-appearing muscle, suggesting that it is an early pathological change. There is also evidence that differences in the TMA/Cr ratio are associated with muscle strength. Further study is needed to determine the ways in which TMA/Cr levels change over time and whether or not these changes are predictive of deterioration of muscle function in muscular dystrophy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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List of abbreviations

FSHD	facioscapulohumeral muscular dystrophy
MRS	magnetic resonance spectroscopy
TMA	trimethylamine
Cr	creatine
DMD	Duchenne muscular dystrophy
¹H	proton

STIR	short-tau inversion recovery
IMCL	intramyocellular lipid
EMCL	extramyocellular lipid
TR	repetition time
TE	echo time
FOV	field of view
TI	inversion time
MPRAGE	Magnetization Prepared RAPid Gradient Echo
IU	institutional units

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Figure 1.

Representative spectrum from a single voxel in a 54 year-old female with FSHD. Peaks from trimethylamines (TMA), creatine, extra- (EMCL) and intra-myocellular lipid (IMCL) are identified. The red line is the spectrum fitted by LCMModel. Inset T1-weighted MRI shows MRS voxel location.

Figure 2.

Histogram representing the distribution of log[TMA/Cr] ratio in all voxels from controls (transparent bars) and subjects with FSHD (blue bars). The log[TMA/Cr] ratio is statistically significantly lower in the FSHD population compared to controls.

Figure 3. Metabolite concentrations with respect to muscle strength and function. Fitted regression lines showing the association between hamstrings strength (a) and 10-meter walk time (b) as a function of the mean log[TMA/Cr] ratio of the hamstring muscles in individuals with FSHD.

Table 1

Demographic information for study participants.

	FSHD	Controls
Number	36	15
Gender	15 male/21 female	6 male/9 female
Age (years)	50 ± 13.6 [20–71]	51.5 ± 14.3 [23–70]
Weight (kg)	77.6 ± 17.9 [49.9–111.1]	77.0 ± 15.1 [59–106.6]
Height (cm)	170.2 ± 7.9 [157.5–188]	167.1 ± 9.9 [155–182.9]
Restriction fragment (kb)	25.3 ± 5.3 [10–35]	77 ± 29.3 [12–130]
10-meter walk (seconds)	6.41 ± 2.9 [3.2–15.3]	4.92 ± 1.0 [3.6–6.69]
Hip flexion strength (lbs)	42.69 ± 10.2 [15–55]	48.17 ± 7.7 [28–62]
Hamstrings strength (lbs)	38.41 ± 12.2 [8–55]	45.6 ± 7.8 [28–53]
Quadriceps strength (lbs)	42.66 ± 8.9 [10–55]	48.84 ± 5.8 [30–55]
Deltoid strength (lbs)	34.33 ± 9.29 [6–55]	42.69 ± 7.7 [27–51]
Biceps brachii strength (lbs)	32.78 ± 10.6 [2–51]	42.71 ± 4.0 [35–51]
Triceps strength (lbs)	28.38 ± 10.6 [3–51]	38.34 ± 7.6 [22–51]
Number of voxels	17920	7424
Voxels enclosed by muscle	3967	2295
Voxels with good spectral fit	1665	847
Left side voxels only	812	353

Concentrations of 7 metabolites in subjects with facioscapulohumeral muscular dystrophy and controls.

Table 2

Metabolite	FSHD	95% Confidence interval	Control	95% Confidence interval	p-value
Creatine	0.00145	0.00115, 0.00184	0.00153	0.00107, 0.00219	0.802
TMA	0.00027	0.00021, 0.00035	0.00042	0.00028, 0.00063	0.065
TMA/Cr	0.17652	0.15569, 0.20014	0.25895	0.21450, 0.31262	0.001
IMCL	0.01076	0.00813, 0.01425	0.01565	0.01027, 0.02385	0.147
IMCL/Cr	7.21004	5.20847, 9.98079	10.05499	6.16943, 16.38772	0.267
EMCL	0.06963	0.04990, 0.09718	0.05519	0.03341, 0.09116	0.449
EMCL/Cr	48.91152	41.12672, 58.16990	39.13538	30.13647, 50.82142	0.163

FSHD – Facioscapulohumeral muscular dystrophy; TMA – trimethylamines; Cr – creatine; IMCL – intramyocellular lipid; EMCL – extramyocellular lipid