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Skeletal muscle symptoms and quantitative MRI in females with dystrophinopathy

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Abstract

Introduction/Aims: The dystrophinopathies primarily affect males; however, female carriers of pathogenic dystrophin variants can develop skeletal muscle symptoms. This study aimed to evaluate muscle involvement and symptoms in females with dystrophinopathy using quantitative magnetic resonance imaging (MRI), functional assessments, and patient-reported outcomes.

Methods: Controls and females with dystrophinopathy with muscle symptoms of pain, weakness, fatigue, or excessive tightness were enrolled in this cross-sectional study. Participants underwent lower extremity MRI to quantify muscle inflammation, replacement by fat, and disease asymmetry. Cardiac MRI, functional ability, muscle symptoms, and serum creatine kinase levels were also evaluated.

Results: Six pediatric females with dystrophinopathy (mean age: 11.7 years), 11 adult females with dystrophinopathy (mean age: 41.3 years), and seven controls enrolled. The mean fat fraction was increased in females with dystrophinopathy compared to controls in the soleus (0.11 vs. 0.03, $p = .0272$) and vastus lateralis (0.16 vs. 0.03, $p = .004$). Magnetic resonance spectroscopy water T_2, indicative of muscle inflammation, was elevated in the soleus and/or vastus lateralis in 11 of 17 individuals. North Star Ambulatory Assessment score was lower in the dystrophinopathy group compared to controls (29 vs. 34 points, $p = .0428$). From cardiac MRI, left ventricle T_1 relaxation times were elevated in females with dystrophinopathy compared to controls (1311 ± 55 vs. 1263 ± 25 ms, $p < .05$), but ejection fraction and circumferential strain did not differ.

Discussion: Symptomatic females with dystrophinopathy quantitatively demonstrate muscle replacement by fat and inflammation, along with impairments in functional

Abbreviations: BFLH, biceps femoris long head; BMD, Becker muscular dystrophy; CK, creatine kinase; DMD, Duchenne muscular dystrophy; GMAX, gluteus maximus; GMED, gluteus medius; GRA, gracilis; MG, medial gastrocnemius; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; NSAA, North Star Ambulatory Assessment; PedsQL-MFS, Pediatric Quality of Life Multidimensional Fatigue Scale; SOL, soleus; ST, semitendinosus; STEAM, stimulated echo acquisition mode; TA, tibialis anterior; TP, tibialis posterior; VL, vastus lateralis.

ability and cardiac function. Additional research is needed to evaluate how symptoms and muscle involvement change longitudinally.

KEYWORDS
Duchenne muscular dystrophy, fat fraction, inflammation, magnetic resonance imaging, x-inactivation

1 | INTRODUCTION

Carriers of recessive Mendelian disorders often do not develop symptoms of the genetic disorder, but in some cases, a carrier may display a partial or full disease phenotype. The dystrophinopathies are x-linked disorders that primarily affect males; however, it is well-recognized that female carriers of pathogenic dystrophin variants can also develop cardiac and/or skeletal muscle symptoms.[1–5] Skewed x-chromosome inactivation is believed to cause symptoms in the majority of cases, leading to variable levels of mosaic dystrophin expression.[6] However, females may also develop symptoms due to other genetic mechanisms.[7–9] The incidence of female carriers with skeletal muscle symptoms, herein referred to as females with dystrophinopathy, is estimated at 2.5%–22%.[5,10] Muscle symptoms may include myalgias, cramps, fatigue, and symmetric or asymmetric weakness.[3,5,11,12]

Research studies and data from placebo arms of clinical trials have greatly expanded knowledge of the natural history of skeletal muscle involvement in males with dystrophinopathy,[13,14] but neuromuscular experts agree that additional observational and natural history research is needed for females with dystrophinopathy.[15–18] The inclusion of magnetic resonance imaging (MRI) in studies has provided important information about skeletal muscle health in male dystrophinopathy.[19–21] Early foundational MRI work was conducted in a cohort of 12 symptomatic carriers, both with and without weakness (cramps and myalgias only), examining qualitative patterns of lower extremity muscle replacement by fat and T_2-weighted STIR imaging.[22] Few quantitative MR studies have been conducted in female dystrophinopathy, including a longitudinal case study of a single carrier and a study of calf and thigh muscle replacement by fat in symptomatic and asymptomatic carriers.[11,23]

Quantitative MR, in combination with clinical- and patient-reported outcomes, has the potential to further increase understanding of the spectrum of muscle health seen in females with dystrophinopathy. Therefore, the goal of this study was to comprehensively evaluate and quantify skeletal muscle health and symptoms in females with dystrophinopathy across the age-span who report skeletal muscle symptoms. A secondary goal of the study was to determine the frequency of cardiac pathology in female carriers with skeletal muscle symptoms.

2 | METHODS

Females age 5–62 years old with genetically confirmed pathogenic dystrophin variants predictive of Becker muscular dystrophy (BMD) or Duchenne muscular dystrophy (DMD) and skeletal muscle symptoms were invited to participate in this single-site, cross-sectional, observational study. For inclusion, participants had to self-report one or more of the following symptoms: skeletal muscle weakness, muscle pain, muscle fatigue, poor balance, or excessive muscle tightness (intended to capture individuals with ankle contractures). Cardiac symptoms were not an inclusion or exclusion criterion. In addition, controls were recruited from the community with age-matching when possible. Controls could not have cardiac disease or a neuromuscular disorder, but the presence of muscle pain or fatigue was not a specific exclusion criterion. The study was approved by the University of Florida's Institutional Review Board. All adult participants provided written informed consent, while minors provided written assent alongside parent/guardian informed consent.

Medical history, medications, dystrophin variant, family history of muscular dystrophy, and patient-reported outcomes were collected. Adult participants completed the International Physical Activity Questionnaire (IPAQ), while participants <18 years old completed the Physical Activity Questionnaire for Older Children.[24,25] Fatigue was measured using the Fatigue Severity Scale for adults and the Pediatric Quality of Life Multidimensional Fatigue Scale (PedsQL-MFS) for minors.[26,27] Finally, all participants rated muscle pain over the last 7 days on a visual analog scale from 0 to 100, which was converted to a score from 0 to 10. Serum creatine kinase (CK) levels were quantified only in the female carriers, with the laboratory reference ranges used for comparison.

2.1 | Magnetic resonance acquisition and analysis

Participants underwent a contrast-free exam of the skeletal muscles and heart on a Philips Ingenia Elition 3 T MRI scanner (Best, Netherlands) with a 70 cm bore. Details of acquisition parameters and analysis procedures have largely been previously published and are included in Appendix A. Skeletal muscle fatty infiltration was quantified from three-point Dixon MRI acquired bilaterally at the pelvis and thighs and acquired unilaterally at the lower leg. Muscles of interest included the soleus (SOL), tibialis anterior (TA), peroneus group, medial gastrocnemius, tibialis posterior (TP), vastus lateralis (VL), rectus femoris, semitendinosus, biceps femoris long head, gracilis, gluteus minimus, gluteus medius, and gluteus maximus. Single voxel ^{1}H magnetic resonance spectroscopy (MRS) was also unilaterally acquired in the belly of the VL and SOL muscles to determine fat fraction.

Multiple approaches were taken to quantify or visualize muscle inflammation. 2D multi-echo spin-echo images were acquired at the thigh and lower leg to determine muscle MRI T_2 relaxation time. Muscle MRI T_2 is a biomarker of muscle damage, inflammation, and fat, and in muscles without fatty infiltration, elevated muscle MRI T_2 values can be indicative of inflammation.[28] To assess changes in muscle water content, the same single voxel ^{1}H MRS sequence used to calculate fat fraction was used to determine water T_2 relaxation time using a monoexponential decay curve fit. Elevated water T_2 values are reflective of muscle inflammation.[29] Finally, to qualitatively visualize inflammation, a T_2-weighted spin-echo mDixon sequence was acquired.

The cardiac exam was performed using a 32-channel torso coil with ECG monitoring. A left ventricle cine short-axis stack was acquired during free breathing to measure ejection fraction. A single left mid-ventricle slice was acquired with 8 mm grid tags to measure myocardial circumferential strain during free breathing. Finally, a breath-hold, single-slice native T_1 map was generated at the mid and basal levels of the left ventricle using a modified look-locker inversion recovery sequence.[30]

2.2 | Functional assessment

Participants performed the 10-m walk/run, timed four stair climb test, timed supine to stand test, and 6-min walk test as previously described, with the exception of only performing two trials for timed tests.[31,32] The North Star Ambulatory Assessment (NSAA),[33] Mini BESTest (a balanced assessment),[34] and ankle passive range of motion in supine were also performed.

2.3 | Statistical analysis

Descriptive statistics were used to characterize the participant groups (pediatric females with dystrophinopathy, adult females with dystrophinopathy, pediatric controls, and adult controls). Results are reported as sample sizes, means with ranges, or means with standard deviations. Graphs are plotted as box and whisker plots with median, 25th and 75th percentiles defining the box, and error bars indicating the minimum and maximum values. For group comparisons, unpaired *t*-tests were used with Welch's correction if variance differed significantly with alpha set at .05. No statistical comparisons were made between the pediatric control and pediatric female dystrophinopathy groups due to the small sample size of the control group. Statistical analyses and data visualization were performed using GraphPad Prism version 9.5.1 for Windows (GraphPad Software, San Diego, CA, USA; www.graphpad.com).

3 | RESULTS

Seventeen females with dystrophinopathy and seven age-matched controls enrolled between February and September 2022 (Table 1). Fourteen females with dystrophinopathy had out-of-frame deletions or nonsense variants consistent with DMD, two had in-frame deletions in regions associated with DMD in males,[35] and one had an exon 3–7 deletion, which can result in a milder DMD course in males.[36] All participants were ambulant. One pediatric participant took corticosteroids on an intermittent dosing regimen, and three participants took corticosteroids previously. All but two females with dystrophinopathy had a male relative with DMD, and there were four mother/daughter

TABLE 1 Participant characteristics.

	Pediatric females with dystrophinopathy (<18 years old)	Adult females with dystrophinopathy (≥18 years old)	Pediatric controls (<18 years old)	Adult controls (≥18 years old)
Sample size	6	11	2	5
Age (years)	11.7 (8–14)	41.3 (21–54)	13 (12–14)	37.6 (21–52)
Height (cm)	142 (120–158)	162 (153–168)	161 (153–168)	167 (154–175)
Weight (kg)	43.0 (20.6–83.1)	76.0 (52.9–117.5)	50.5 (43.8–57.2)	66.9 (55.4–77.7)
BMI (kg/m^2)	20.5 (14.3–33.3)	28.9 (22.2–44.8)	19.5 (18.7–20.3)	24.1 (19.2–27.2)
10-m walk/run time (s)	5.3 (2.8–13.9)	5.9 (3.1–18.1)	2.5 (2.4–2.7)	3.0 (2.8–3.7)
Time to climb 4 stairs (s)	3.7 (1.4–11.3)	4.3 (1.5–24.2)	1.3 (1.0–1.5)	1.6 (1.3–1.8)
Time to rise from supine (s)	6.3 (1.2–24.8)	4.6 (2.3–9.7)[a]	1.6 (1.5–1.8)	2.3 (1.7–2.9)
6-min walk test (m)	402 (203–482)	436 (141–597)[b]	613 (597–628)	663 (553–750)
MiniBESTest (out of 28)	25.7 (22–27)	24.5 (9–28)	27 (27–27)	27.6 (27–28)
NSAA (out of 34)	29.2 (10–34)	29.9 (9–34)[b]	34 (34–34)	34 (34–34)
Ankle dorsiflexion passive range of motion (right/left)	8° (−5° to 20°)/10° (0°–20°)	9° (5°–15°)/9° (5°–15°)	10° (5°–15°)/15° (10°–20°)	11° (5°–20°)/14° (10°–20°)

Note: Data are presented as mean with range in parentheses.
Abbreviation: NSAA, North Star Ambulatory Assessment.
[a]One participant was unable to perform the supine to stand test.
[b]One participant in this group did not complete the 6-min walk test or NSAA due to fear of excessive pain/fatigue after exertion.

(s) units in the study. Of the females with dystrophinopathy, all identified as White, with six also identifying as Hispanic/Latino. Among controls, one participant identified as Asian, while the remaining identified as White and not Hispanic/Latino.

3.1 | CK, fatigue, pain, and physical activity

Eleven participants self-reported skeletal muscle weakness, seven reported fatigue (often in association with physical activity), five reported muscle pain, and one reported muscle tightness (details in Table S1). CK was elevated in all pediatric females with dystrophinopathy (range = 294–11,357 U/L; lab reference values = 0–180 U/L) and in 8 of 11 adults (range = 56–2278 U/L; lab reference values = <215 U/L; Figure 1A). Muscle pain over the last 7 days averaged 2.1 ± 2.3 points in pediatric females with dystrophinopathy and 4.6 ± 2.6 points in adults with dystrophinopathy (Figure 1B). Adult females with dystrophinopathy had a mean Fatigue Severity Scale score of 5.0 ± 1.0 (Figure 1C), and among the pediatric participants, the mean PedsQL-MFS total scaled score was 51 ± 30 (Figure 1D). From the IPAQ, adults with dystrophinopathy reported 1338 ± 1433 MET/min of walking activity per week, 4299 ± 4793 MET/min of moderate activity, and 1164 ± 2399 MET/min of vigorous activity. For pediatric females with dystrophinopathy, the mean Physical Activity Questionnaire for Older Children score was 1.6 ± 0.47.

3.2 | Muscle replacement by fat

The MRS-derived fat fraction was increased in females with dystrophinopathy compared to controls in the SOL ($p = .0272$) and VL ($p = .0040$; Figure 2A,B). Among pediatric participants, two of six had SOL fat fractions above the highest control value, and three of six had VL fat fractions above the highest control value. Eight of 11 adults had elevated SOL fat fractions and 6 of 11 had elevated VL fat fractions.

Dixon MRI-derived muscle fat fraction was quantified over a larger number of muscles (Figure 2C). There was generally a proximal to distal pattern of involvement in female dystrophinopathy, similar to the pattern seen in male dystrophinopathy,[19,37,38] with a broad range of involvement. Asymmetry of involvement was evaluated in the thigh and gluteal muscles (Figure 3A,B). Six individuals had asymmetric

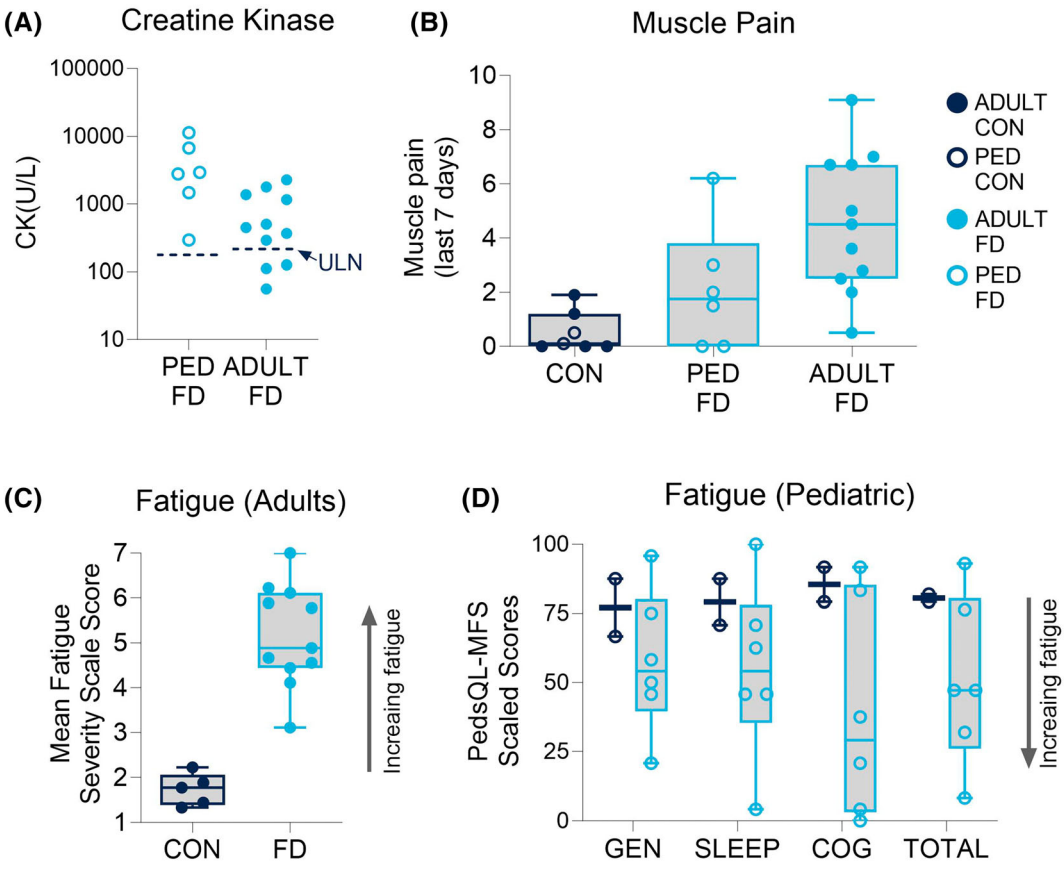

FIGURE 1 Serum creatine kinase and skeletal muscle symptoms. (A) The majority of females with dystrophinopathy had CK levels above the upper limit of normal (ULN), and (B) both pediatric and adult females with dystrophinopathy reported muscle pain over the last 7 days. (C) Self-reported fatigue was increased in both adults and (D) pediatric females with dystrophinopathy. In pediatric females with dystrophinopathy, cognitive fatigue was also commonly reported. COG, cognitive fatigue subscale of the PedsQL-MFS; CON, control; FD, female with dystrophinopathy; GEN, general fatigue subscale of the PedsQL-MFS; PED, pediatric; SLEEP, sleep/rest fatigue subscale of the PedsQL-MFS.

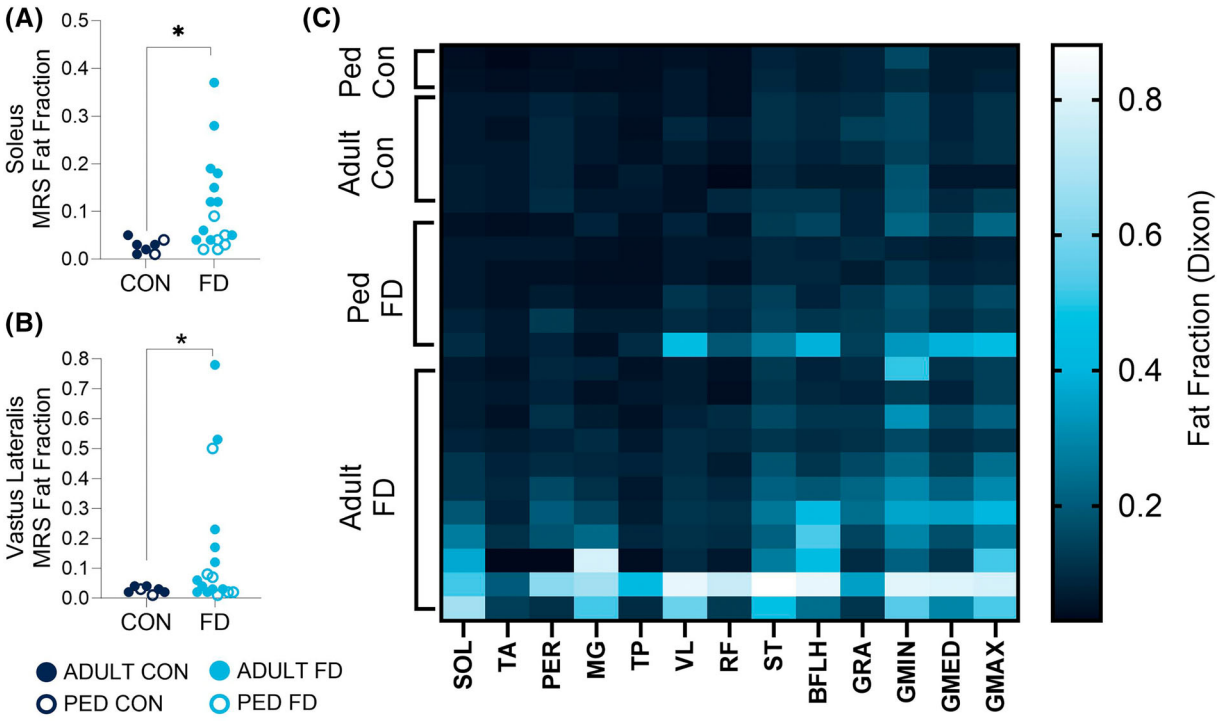

FIGURE 2 Skeletal muscle fat fractions. The MRS-derived fat fraction was significantly higher in the (A) soleus and (B) the vastus lateralis muscles of females with dystrophinopathy compared to controls; however, some females with dystrophinopathy had fat fractions within the range of control participants. (C) The heat map of Dixon MRI derived skeletal muscle fat fractions shows a range of values from control levels of muscle fat to nearly complete muscle replacement by fat. *p < .05. CON, control; FD, female with dystrophinopathy; PEDS, pediatric.

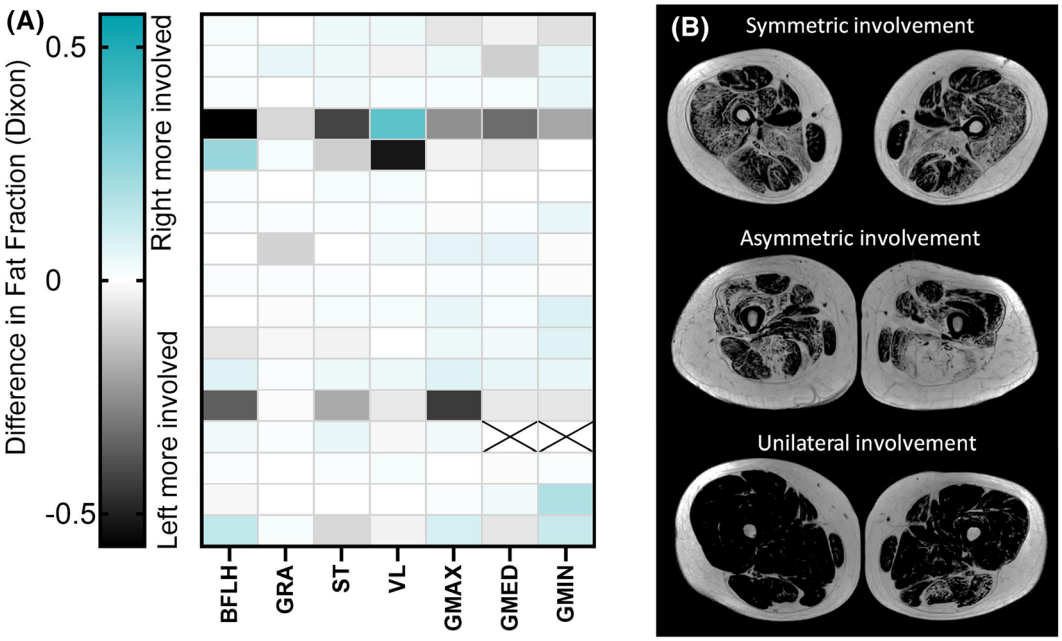

FIGURE 3 Asymmetry of muscle involvement. (A) A heat map of left/right differences in Dixon MRI-derived muscle fat fraction for the thighs and gluteal muscles. Some females with dystrophinopathy had large differences between the left and right sides. (B) Dixon MRI fat maps of the bilateral thighs show that some females with dystrophinopathy show symmetric involvement, some show asymmetric involvement, and one individual had unilateral involvement.

involvement between the left and right sides with FF differences >0.1 in at least one muscle group, and three of these individuals had a fat fraction difference >0.3 in at least one muscle group. In five of the six individuals, there was no clear pattern to the asymmetry, but the remaining individual had elevated muscle fat fraction, along with visually appreciable muscle atrophy, limited to one side of the body.

3.3 | Muscle inflammation

Muscle inflammation was evaluated using multiple methods. Qualitatively, 6 of the 17 females with dystrophinopathy had elevated pixel intensities on T_2-weighted Dixon water maps acquired in the lower leg, indicative of muscle inflammation (Figure 4A). This included three of the pediatric participants, all of whom had low muscle fat fractions in the lower leg muscles. SOL and VL MRS water T_2 relaxation time

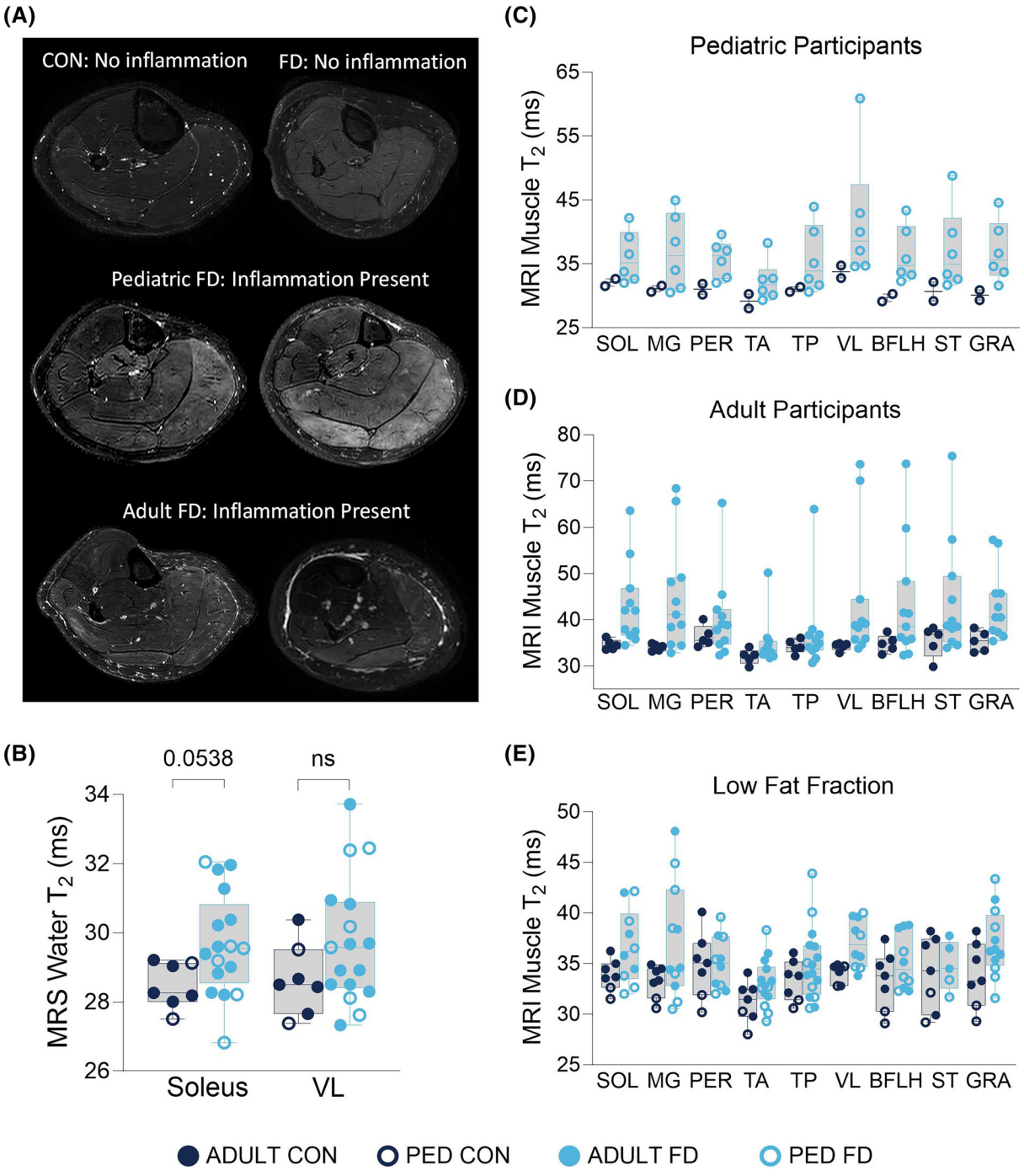

FIGURE 4 Skeletal muscle inflammation. (A) Qualitative T_2-weighted Dixon MRI water maps demonstrate the presence of elevated pixel intensity that likely represents muscle inflammation in both children and adult females with dystrophinopathy. (B) MRS water T_2 values were not significantly different between the controls and females with dystrophinopathy at the group level in the soleus or vastus lateralis, but some females with dystrophinopathy had highly elevated T_2 values. (C) MRI-derived bulk muscle T_2 in pediatric participants and (D) adult participants with a range of muscle fat fractions. (E) MRI-derived bulk muscle T_2 in only participants with control level muscle fat fractions. Values were elevated in many females with dystrophinopathy, suggesting muscle inflammation is present. BFLH, biceps femoris long head; GRA, gracilis; MG, medial gastrocnemius; PER, peroneal group; SOL, soleus; ST, semitendinosus; TA, tibialis anterior; TP, tibialis posterior; VL, vastus lateralis.

was not significantly elevated in the dystrophinopathy group compared to controls; however, water T_2 values were elevated above the highest control value in 10 females with dystrophinopathy in the SOL muscle and 5 females with dystrophinopathy in the VL (Figure 4B). MRI muscle T_2, which is increased in the presence of both muscle inflammation and muscle fat, was elevated above control values in some pediatric and adult females with dystrophinopathy (Figure 4C,D). In females with dystrophinopathy and fat fractions within control ranges, MRI muscle T_2 was elevated in a subset of females with dystrophinopathy, likely indicating muscle inflammation (Figure 4E). There was no clear relationship between pain and fatigue questionnaire results and MR biomarkers of muscle fat fraction or inflammation.

3.4 | Functional ability

At a group level, the females with dystrophinopathy had poorer performance on the 10-m walk/run ($p = .0138$), the timed supine-to-stand test ($p = .0428$), the 6-min walk test ($p < .0001$), and the NSAA ($p = .0428$; Table 1). VL MRS fat fraction correlated with each of the timed tests (Pearson's $r = .77$–$.86$), the 6-min walk test ($r = -.76$), and the NSAA ($r = -.91$; $p < .001$ for all); however, SOL MRS fat fraction was not correlated with any of the functional tests. For the females with dystrophinopathy, there was a ceiling effect for the NSAA, with 11 individuals scoring either 33 or 34 points out of a total of 34. Despite scoring perfectly or nearly perfectly on the NSAA, elevated muscle fat fractions were found (Figure 5A–C).

3.5 | Cardiac MRI

Six of the females with dystrophinopathy reported taking at least one cardiac medication, 13 of 17 were followed by a cardiologist, and 8 of 17 had a prior cardiac MRI. Cardiac MRI was successfully obtained in all participants for this study (Figure 6). Mean left ventricle circumferential strain (control: −20.7 ± 2.1, female dystrophinopathy: −19.34 ± 2.1) and left ventricle ejection fraction (control: 67% ± 4%, female dystrophinopathy: 65% ± 6%) were not significantly different between groups, and ejection fraction was above 50% for all participants. Native T_1 values, however, were significantly elevated in the female dystrophinopathy group compared to the control group at both mid (control: 1263 ± 25 ms, female dystrophinopathy: 1311 ± 55 ms; $p = .0388$) and basal left ventricle levels (control: 1257 ± 24 ms, female dystrophinopathy: 1321 ± 55 ms; $p < .0130$). When evaluating the cardiac MRI variables in only adult participants, differences in strain ($p = .0252$), ejection fraction ($p = .0488$), and basal left ventricle T_1 values ($p = .0176$) reached significance.

4 | DISCUSSION

We have quantified and characterized skeletal muscle replacement by fat and involvement asymmetry in symptomatic female carriers of pathogenic dystrophin variants, finding elevated muscle fat in the majority of participants. Additional key study findings include (1) signs of inflammation in the calf muscles of over half of the participants, (2) a lack of association between reported muscle pain and fatigue

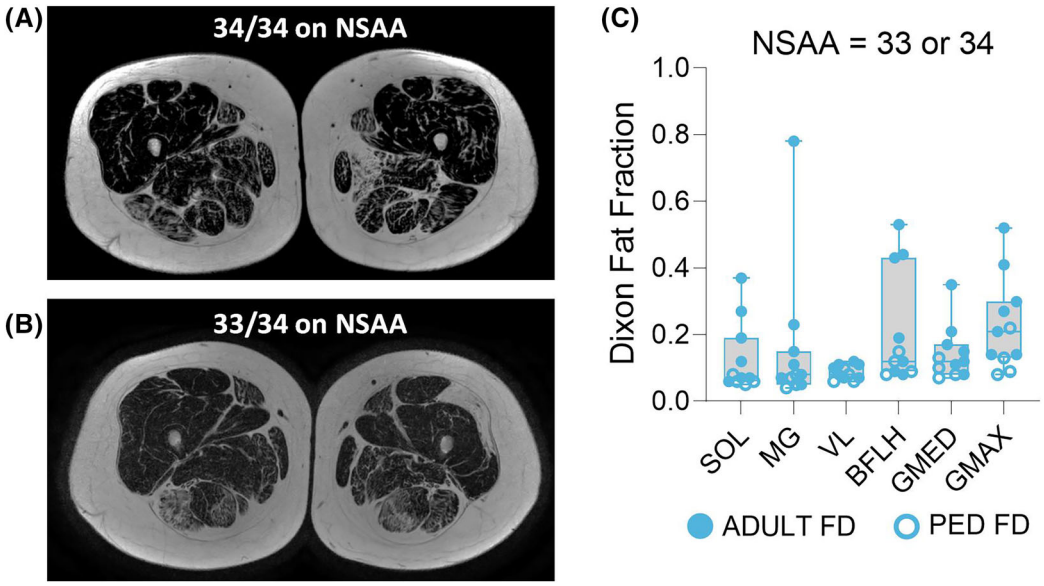

FIGURE 5 Skeletal muscle involvement compared to total NSAA score. (A) Despite perfect or (B) nearly perfect scores on the NSAA, some females with dystrophinopathy had clear muscle replacement by fat in several muscles of the thigh, as seen in these Dixon MRI fat maps. (C) Among all females with dystrophinopathy with NSAA total scores of 33 or 34 points, many had elevated lower leg muscle fat fractions. BFLH, biceps femoris long head; FD, female with dystrophinopathy; GMAX, gluteus maximus; GMED, gluteus medius; MG, medial gastrocnemius; NSAA, North Star Ambulatory Assessment; PED, pediatric; SOL, soleus; VL, vastus lateralis.

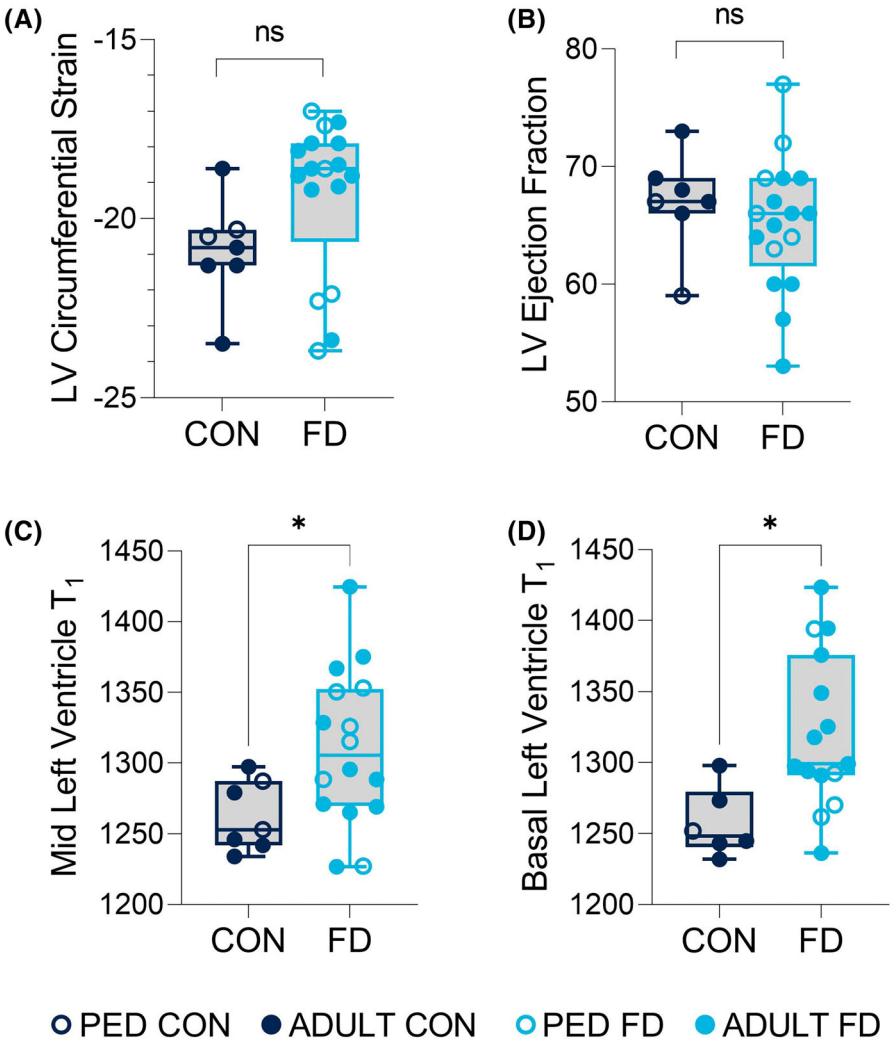

FIGURE 6 Cardiac MRI biomarkers. Neither (A) circumferential strain nor (B) ejection fraction differed between the control and dystrophinopathy groups; however, native T_1 relaxation constants were elevated in the dystrophinopathy group at the (C) mid ($p = 0.0388$) and (D) basal left ventricle levels ($p = 0.013$). CON, control; FD, female with dystrophinopathy; LV, left ventricle; ns, not significant; PEDS, pediatric.

with MRI biomarkers of fat and inflammation, and (3) concomitant cardiac involvement indicated by elevated mid left ventricular native T_1 relaxation values.

Using both MRS and Dixon MRI to quantify lower extremity muscle replacement by fat, 11 of 17 females with dystrophinopathy had fat fractions above control levels, and 6 of those individuals demonstrated >0.10 difference in fat fraction between left and right sides. Findings from a prior qualitative MRI study similarly showed that muscle replacement by fat in carriers typically follows the pattern seen in male dystrophinopathy, albeit with a large number of individuals demonstrating asymmetry of involvement.[22] Unilateral involvement, as seen in one participant in this study, has been reported in the literature in two other women.[39] Another recent study utilized quantitative MRI to evaluate both symptomatic and asymptomatic adult carriers of DMD/BMD variants.[11] The carriers had elevated muscle fat fractions compared to controls; however, it is unclear what differences were present in asymptomatic versus symptomatic individuals.[11]

A novel aspect of this study was the imaging and quantification of biomarkers of muscle inflammation. MRI T_2-weighted STIR image hyperintensity, which can indicate muscle inflammation, was not detected by Tasca et al. in carriers[22]; however, using quantitative methods, we found signs of inflammation in at least one lower extremity muscle in over half of participants, including females without clinical weakness. A longitudinal case report ($n = 1$) from 2012 also found elevated MRS water T_2 in the lower extremity muscles of a female with dystrophinopathy, likely indicative of inflammation.[23] In addition, an inflammatory phenotype mimicking myositis has been published in a case study of a female with dystrophinopathy.[40] The role of inflammation has received little attention in females with dystrophinopathy,[41] but with corticosteroids and corticosteroid alternatives approved and in clinical trials for DMD, additional study is warranted.[17]

Functional test performance was not always sensitive to muscle involvement detected on MRI (Figure 5). For example, some women with biomarkers of muscle inflammation or replacement by fat had ceiling effects on the NSAA. This challenge was also noted in a study of functional ability in females with dystrophinopathy.[3] Because muscle involvement may be milder and/or asymmetric, outcomes validated for DMD may not provide an accurate assessment of function in females. Outcomes such as the new North Star Assessment for Limb-Girdle Type Muscular Dystrophy or the Neuromuscular Gross Motor Outcome could be explored for use in future studies.[42,43]

While most participants demonstrated muscle replacement by fat, muscle inflammation, or decreased functional ability, a few participants presented with only reports of fatigue, exercise intolerance, and/or myalgias. These symptoms have been reported in prior studies of female carriers.[3,5,12] One study reported that ~5% of adult carriers present only with a cramps/myalgia phenotype.[5] This subgroup presents with the clinical challenge of differentiating between signs of mild or late-onset dystrophinopathy, normal aging and sarcopenia, and the potential fatigue associated with caring for a child with dystrophinopathy. Research is needed to improve diagnostics and clinical care of females on the mildest end of the dystrophinopathy spectrum. A similar challenge exists for pediatric female carriers with elevated CK levels but limited symptoms in childhood. Little prognostic information is available for these individuals, and the evolution of symptoms with aging is not well-characterized. However, a recent study followed 12 women who initially presented with symptoms before the age of 18.[44] At follow-up in adulthood, half or more of the participants reported fatigue, myalgias, back pain, and arthralgias with a high percent of perceived constraints due to symptoms.[44] In addition, five individuals developed weakness that limited their functional abilities.[44]

Within the study cohort, there were many individuals with concomitant cardiac involvement. A contemporary cardiac MRI study of adult female carriers of pathogenic dystrophin variants (both asymptomatic and symptomatic) showed that nearly 50% of participants had late gadolinium enhancement, and the carrier group had elevated native T_1 relaxation times, likely indicative of diffuse fibrosis,[45] compared to a control group.[1] Similarly, in our study, 59% of participants had native T_1 relaxation times above that of the highest control; however, none had abnormal ejection fractions, and 29% had left ventricular strain values above −18. The severity of cardiac involvement was not associated with the severity of skeletal muscle involvement.

A study limitation is that participants self-identified as females with dystrophinopathy based on their lived experience of symptoms. Although all had genetic confirmation of a pathogenic dystrophin variant, many only had single gene testing, and only five had prior biopsy confirmation of mosaic dystrophin expression. Thus, other sources of muscle symptoms cannot be completely ruled out. A second limitation was the small cohort size, which limits the ability to generalize study findings. Our control group was particularly small, impairing the ability to perform statistical comparisons for some analyses. Comparisons of patient-reported fatigue and pain outcomes to control values should be interpreted judiciously. Finally, this study was cross-sectional, and no conclusions can be made regarding disease progression or evolution of symptoms over time.

Overall, this study adds quantitative observational data including symptom severity, muscle health, and functional ability for a cohort of females with dystrophinopathy and skeletal muscle symptoms across the age-span. Continued research is needed to establish clinical care guidelines, understand disease progression over time, and move females with dystrophinopathy closer to clinical trial readiness.

AUTHOR CONTRIBUTIONS

Breana M. Jenkins: Formal analysis; data curation; writing – original draft. **Lathan D. Dixon:** Formal analysis; data curation; writing – review and editing. **Kevin J. Kokesh:** Writing – review and editing; resources. **Carla D. Zingariello:** Resources; writing – review and editing. **Krista Vandenborne:** Investigation; funding acquisition; writing – review and editing; software. **Glenn A. Walter:** Investigation; funding acquisition; writing – review and editing; formal analysis; methodology; software. **Alison M. Barnard:** Conceptualization; investigation; funding acquisition; writing – original draft; formal analysis; data curation; methodology.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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APPENDIX A: DETAILED MRI AND MRS METHODOLOGY

Participants underwent a contrast-free exam of the skeletal muscles and heart. The skeletal muscle exam included (1) proton density-weighted Dixon (chemical shift-encoded) imaging, (2) ^{1}H MRS, (3) multi-echo spin-echo imaging, and (4) spin-echo Dixon imaging. All scans were acquired on a Philips Ingenia Elition 3 T MRI scanner with a 70 cm bore. Torso coils were used for all acquisitions except at the lower leg, where a 16-channel knee coil was used.

Skeletal muscle fatty infiltration was quantified from Dixon MRI and ^{1}H MRS scans. For Dixon MRI, a 2D, 3-point Philips mDixon sequence was acquired at the pelvis, thigh, and lower leg (TR = 430 ms, TE = 4.7/5.7/6.7 ms, $0.75 \times 0.75 \times 6$ mm^3, 20° flip angle). Water and fat maps were created using a seven-peak lipid model (Philips v56.1), and regions of interest were drawn just within the border of each muscle using Horos software (Horosproject.org). Muscles of interest included the following: soleus (SOL), tibialis anterior (TA), peroneus group (PER), medial gastrocnemius (MG), tibialis posterior (TP), vastus lateralis (VL), rectus femoris (RF), semitendinosus (ST), biceps femoris long head (BFLH), gracilis (GRA), gluteus minimus (GMIN), gluteus medius (GMED), and gluteus maximus (GMAX). Proton density fat fraction was computed as fat signal/(fat + water signal) within the muscle region of interest, and muscles were analyzed by two separate individuals (AMB, LDD, and/or BMJ). Disease asymmetry was evaluated by comparing muscle fat fractions between the left and right sides of the body. Single voxel ^{1}H MRS was unilaterally acquired in the belly of the vastus lateralis and soleus muscles to determine MRS-derived fat fraction. A stimulated echo acquisition mode (STEAM) sequence was used with TR = 9000 ms, TE = 12/27/54/243 ms, and four phase cycles. The spectra were reconstructed using custom software developed by the ImagingDMD group (IDL version 8.8, L3Harris Geospatial, Denver, CO), and fat fraction was determined at the 27 ms echo time using area integration with T_1 and T_2 corrections.[46]

Multiple approaches were taken to quantify or visualize muscle inflammation. 2D multi-echo spin-echo images were acquired at the thigh and lower leg to determine muscle MRI T_2 relaxation time. Muscle MRI T_2 is a biomarker of muscle damage, inflammation, and fat, and in muscles without fatty infiltration, elevated muscle MRI T_2 values can be indicative of inflammation.[28] This sequence was acquired with TR = 3000 ms, TE = 40, 60, 80, and 100 ms, and slice thickness = 7 mm. Data were fit to a monoexponential decay curve on a pixel-by-pixel basis to create T_2 maps using custom software (IDL) developed by the ImagingDMD group.[47] T_2 values from within each muscle region of interest were averaged across three slices as previously described,[47] and analyses were performed by two separate individuals (AMB, LDD, and/or BMJ). To assess changes in muscle water content, the same single voxel ^{1}H MRS sequence used to calculate fat fraction was used to determine water T_2 relaxation time using a monoexponential decay curve fit. Elevated water T_2 values are reflective of muscle inflammation. Finally, to qualitatively visualize inflammation, a T_2-weighted spin-echo Philips mDixon sequence was acquired with TR = 4018 ms, TE = 100 ms, and slice thickness = 4 mm. Like the proton density-weighted Dixon sequence, these images were reconstructed using vendor algorithms to produce water images. Elevated pixel intensity on the water images was interpreted as a sign of muscle inflammation.

The cardiac exam was performed using a 32-channel torso coil with ECG monitoring for gating purposes. A left ventricle cine short axis stack was acquired during free breathing with a steady state free procession sequence and custom patch to optimize heart and respiratory rate synchronization.[48] Ejection fraction, left ventricular mass, and end-diastolic and systolic volumes were measured using Segment v3.3 software (http://segment.heiberg.se).[49,50] A single left mid-ventricle slice was acquired with 8 mm grid tags (7 mm slice thickness) and retrospective ECG gating to measure myocardial circumferential strain during free breathing, and analysis was conducted using Segment v3.3. Finally, a breath-hold, single-slice native T_1 map was generated at the mid and basal levels of the left ventricle. The scan was acquired with a modified look-locker inversion recovery (MOLLI) sequence[30] with TR = 2.2 ms, TE = 1.03 ms, 20° flip angle, and vendor reconstruction (Philips v56.1). Native T_1 maps were analyzed in Horos (Horosproject.org) by tracing just within the left ventricular myocardium borders.