J Neurol (2012) 259:2385–2392
DOI 10.1007/s00415-012-6512-8

ORIGINAL COMMUNICATION

Permanent muscular sodium overload and persistent muscle edema in Duchenne muscular dystrophy: a possible contributor of progressive muscle degeneration

M.-A. Weber · A. M. Nagel · M. B. Wolf · K. Jurkat-Rott · H.-U. Kauczor · W. Semmler · F. Lehmann-Horn

Received: 8 February 2012 / Revised: 2 April 2012 / Accepted: 3 April 2012 / Published online: 28 April 2012

Abstract To assess the presence and persistence of muscular edema and increased myoplasmic sodium (Na^+) concentration in Duchenne muscular dystrophy (DMD). We examined eight DMD patients (mean age 9.5 ± 5.4 years) and eight volunteers (mean age 9.5 ± 3.2 years) with 3-tesla proton (^1H) and ^{23}Na density-adapted 3D-radial MR sequences. Seven DMD patients were re-examined about 7 months later without change of therapy. The eighth DMD patient was re-examined after 5 and 11 months under medication with eplerenone. We quantified muscle edema on STIR images with background noise as reference and fatty degeneration on T1-weighted images using subcutaneous fat as reference. Na^+ was quantified by a muscular tissue Na^+ concentration (TSC) sequence employing a reference containing 51.3 mM Na^+ with 5 % agarose. With an inversion-recovery (IR) sequence, we determined mainly the myoplasmic Na^+. The normalized muscular ^{23}Na IR signal intensity was higher in DMD than in volunteers ($n = 8$, 0.75 ± 0.07 vs. 0.50 ± 0.05, $p < 0.001$) and persisted at second measurement ($n = 7$, 1st 0.75 ± 0.07, 2nd 0.73 ± 0.06, $p = 0.50$). When compared to volunteers (25.6 ± 2.0 mmol/l), TSC was markedly increased in DMD (38.0 ± 5.9 mmol/l, $p < 0.001$) and remained constant ($n = 7$, 1st 37.9 ± 6.4 mmol/l, 2nd 37.0 ± 4.0 mmol/l, $p = 0.49$). Muscular edema (15.6 ± 3.5 vs. 6.9 ± 0.7, $p < 0.001$) and fat content (0.48 ± 0.08 vs. 0.38 ± 0.01, $p = 0.003$) were elevated in DMD when compared to volunteers. This could also be confirmed during follow-up ($n = 7$, $p = 0.91$, $p = 0.12$). Eplerenone slightly improved muscle strength and reduced muscular sodium and edema. The permanent muscular Na^+ overload in all DMD patients is likely osmotically relevant and responsible for the persisting, mainly intracellular muscle edema that may contribute to the progressive muscle degeneration.

Keywords ^{23}Na magnetic resonance imaging · Duchenne muscular dystrophy · Osmotic muscle edema · Fatty degeneration · Tissue Na^+ concentration

M.-A. Weber (✉) · M. B. Wolf · H.-U. Kauczor
Department of Diagnostic and Interventional Radiology, University Hospital of Heidelberg, Im Neuenheimer Feld 110, 69120 Heidelberg, Germany
e-mail: marcandre.weber@med.uni-heidelberg.de

M.-A. Weber
Department of Radiology, German Cancer Research Center (DKFZ), Heidelberg, Germany

A. M. Nagel · W. Semmler
Department of Medical Physics in Radiology, German Cancer Research Center (DKFZ), Heidelberg, Germany

K. Jurkat-Rott · F. Lehmann-Horn (✉)
Division of Neurophysiology, Ulm University, Albert-Einstein-Allee 11, 89069 Ulm, Germany
e-mail: frank.lehmann-horn@uni-ulm.de

Introduction

Duchenne muscular dystrophy (DMD) manifests as progressive muscle degeneration [1]. Intramuscular fatty degeneration is the principal finding [2], but edema-like muscular changes have been reported in some DMD patients [2, 3]. A recent study described edema-like changes in all muscles that were not completely replaced by fat tissue [3].

These edema-like changes might be mediated by inflammatory processes and thereby mainly extracellular or osmotically driven and mainly intracellular. Recent data support the latter by reporting an increased myoplasmic Na^+ [3]. Also, recent animal studies substantiate the muscular

Na^+ overload in DMD [4]. The absence of dystrophin modifies the expression level, distribution, and gating properties of the skeletal muscle isoform of the voltage-gated Na^+ channel, Na_v 1.4. This leads to an abnormally high Na^+ concentration under the sarcolemma in myotubes of *mdx* mice, which is strongly correlated with increased cell death in *mdx* fibres. The exchange of Na^+ for Ca^+ may account for the subsequent cascade eventually leading to cell death [5]. The specific Na^+ channel blocker tetrodotoxin can reverse both Na^+ overload and cell death [4].

These pilot studies have reported that the elevated muscular Na^+ content may play a role in the pathogenesis of the progressing muscle degeneration in DMD by causing a myoplasmic edema. However, to date, it remains unproven whether these edema-like muscular changes and the Na^+ overload are constantly present in DMD muscles or only a temporary finding.

Thus, we tested the hypothesis that the edema is constantly present in DMD and accompanied by permanently increased myoplasmic Na^+ content. For this purpose, we used, besides standard proton (^{1}H) 3-tesla MRI sequences, dedicated 3-tesla ^{23}Na-MRI sequences that are based on density-adapted 3D radial acquisition techniques with high signal-to-noise ratio (SNR) efficiency [6] and weighting towards intracellular ^{23}Na signal [7] to quantify the muscular Na^+ concentration in vivo.

Methods

Patients and volunteers

The study was approved by the local review board and conducted according to the Declaration of Helsinki in the present form. Written informed consent was obtained from all participants or their parents in case of minority. Eight patients [seven boys, one woman having a X-chromosomal translocation (X:17)] with genetically proven DMD (mean age 9.5 ± 5.4 years) were included prospectively in this study. The control group included eight healthy volunteers without any evidence of muscular or cardiovascular disorders (mean age 9.5 ± 3.2 years). All volunteers had full muscle strength at physical examination and presented regular findings in proton (^{1}H) MRI. Of all study participants, four of the eight DMD patients were treated with glucocorticoids (patients #4, 5, 6, and 7 of Table 1).

Patient examination protocol

We performed ^{1}H and ^{23}Na MRI of both calves in all subjects on a 3-tesla clinical MR system (MAGNETOM Trio, Siemens, Erlangen, Germany) using specific hardware for broadband spectroscopy and a CE (Conformité Européenne) certified double-resonant birdcage coil [32.59 megahertz (MHz)/123.2 MHz, Rapid Biomed Inc., Würzburg, Germany]. All subjects tolerated the entire MRI examination well.

Muscle strength grading

The same physician quantified muscle strength of all patients and volunteers immediately after the MRI examination using the nonlinear grading system defined by the British Medical Research Council (MRC) [8]. He assessed strength of foot dorsi- and plantarflexion, and hip and knee flexion and extension. Additionally, the same physician measured the peak force of the dominant hand using a handgrip dynamometer with an analogue display (SH5001 hydraulic hand dynamometer; Saehan Corporation, Masan, Korea). The maximal voluntary grip strength was assessed at first fist contraction and then again at the fifth contraction in kg.

Follow-up examination

To ensure consistency, we re-assessed seven patients (mean age 7.7 ± 2.0 years) about 7 months (mean 6.9 ± 2.2 months) later. The therapeutic scheme did not change in patients #1, 2, and 3 of Table 1 without glucocorticoid therapy and patients #4, 6, and 7 of Table 1 with glucocorticoid therapy. Patient #4 received constant medication with 15 mg of prednisolone for 2 months prior to visit #1 and for 10 months prior to visit #2. Patient #6 received constant medication with 12 mg of deflazacort for a total of 5 years prior to visit #1 and 5 years and 9 months prior to visit #2. For patient #7, both the 1st and 2nd MRI took place on the 2nd day of the 10-day off-phase with 25 mg of prednisolone. Only in patient #5 of Table 1 was the daily prednisolone dose increased by 5 mg. Patient #5 was started on 15 mg of prednisolone 13 months prior to visit #1 and the 1st MRI took place on the 7th day of the 10-day on-phase. This was changed to 20 mg of prednisolone 1 month prior to visit #2 and the 2nd MRI took place on the 1st day of the 10-day on-phase. Re-assessment comprised ^{1}H and ^{23}Na MRI of both calves, as well as, muscle strength testing.

In the 22-year-old female DMD patient (#8 of Table 1), the only patient of full age in our cohort, we could perform follow-up examinations 5 and 11 months later. She was wheelchair-bound for 8 years and received overnight ventilator treatment for 7 years. Eight years ago she received dorsal spondylodesis due to progressing scoliosis. She consented to an individual experimental therapy involving the aldosterone antagonist and K^+-sparing diuretic agent eplerenone (Inspra, Pfizer, New York, NY) in daily alternating doses of 25 and 50 mg. No additional medication was administered.

Table 1 Overview of study population

Patient #	Age (years)	Diagnosis (deleted exons)	Muscular Na^+ concentration (mmol/l)	Glucocorticoid therapy	Foot dorsi-/plantarflexion (MRC) of right, left leg	Fatty infiltration M. soleus/tib. ant.	Edema M. soleus/tib. ant.
1	5	DMD (45–50)	39.32	No	5/5, 5/5	1/1	4/1
2	6	DMD (10–40)	49.23	No	5/5, 5/5	1/1	3/2
3	7	DMD (12–29)	30.96	No	5/5, 5/5	1/1	2/1
4	7	DMD (51)	32.83	Yes[a]	5/5, 5/5	1/1	3/1
5	9	DMD (13–43)	33.00	Yes[a]	5/5, 5/5	2/1	2/1
6	10	DMD (44–47)	37.94	Yes[a]	4/4, 4/4	2/1	3/1
7	10	DMD (48–52)	42.27	Yes[a]	3/3, 3/3	3/2	3/2
8	22	DMD [1, t(X;17) (p21;q11.2)]	38.49	No	3/4, 3/4	2/3	4/2
9	5	Volunteer	25.26	No	5/5, 5/5	1/1	1/1
10	7	Volunteer	25.72	No	5/5, 5/5	1/1	1/1
11	9	Volunteer	24.20	No	5/5, 5/5	1/1	1/1
12	9	Volunteer	27.91	No	5/5, 5/5	1/1	1/1
13	10	Volunteer	21.80	No	5/5, 5/5	1/1	1/1
14	10	Volunteer	27.67	No	5/5, 5/5	1/1	1/1
15	10	Volunteer	25.70	No	5/5, 5/5	1/1	1/1
16	16	Volunteer	26.80	No	5/5, 5/5	1/1	1/1

The muscular tissue Na^+ concentration was assessed using a spin density ^{23}Na MR image contrast as described in the "Methods" section. Muscle strength of foot dorsi- and plantarflexors was evaluated by clinical examination, quantified with aid of the nonlinear grading system defined by the British Medical Research Council (MRC) [8]. Fatty infiltration and muscle edema-like changes were qualitatively assessed on a four-point visual scale [11]

[a] Glucocorticoid therapy: #4, 1st MRI took place under constant medication with 15 mg of prednisolone for 2 months; #5, 1st MRI took place on 7th day of the 10-day on-phase with 15 mg of prednisolone; #6, 1st MRI took place under constant medication with 12 mg of deflazacort for a total of 5 years; #7, 1st MRI took place on the 2nd day of the 10-day off-phase with 25 mg of prednisolone

MR imaging protocol

The imaging protocol comprised axial T1-weighted turbo spin-echo [repetition time (TR)/echo time (TE) = 700/10 ms, matrix of 275 × 448, section thickness of 3 mm] and axial short-tau inversion recovery (STIR; TR/TE = 6,920/65 ms, matrix of 176 × 320, section thickness of 4 mm) ^1H MR sequences to detect edema-like muscular changes and fatty infiltration. Then, two ^{23}Na pulse sequences based on a density-adapted three-dimensional (3D) radial sequence [6] were performed. Since the muscular 3-tesla ^{23}Na MRI signal is up to 50,000 times smaller than the signal received from ^1H MRI because of the short T2 relaxation time of ^{23}Na causing a low SNR, both ^{23}Na MR sequences had an ultra-short echo time of less than 0.5 ms to minimize T2*-weighting. The reason for this is that the ^{23}Na T2* signal decays bi-exponentially with a fast component ($T2_{fast}$) of 0.5–3 ms [9]. The first sequence was a spin density image contrast [TE/TR = 0.3/100 ms; $\alpha = 90°$; voxel size, 5 × 5 × 5 mm^3, acquisition time (TA) = 8 min 20 s] to quantify the muscular tissue Na^+ concentration (TSC). The second sequence was an inversion recovery (IR) sequence [10] to suppress the ^{23}Na signal emitted by free Na^+ ions (e.g., cerebrospinal fluid, vasogenic edema, or the ^{23}Na signal received from the extracellular space) to achieve a weighting towards intracellular ^{23}Na (TE/TR = 0.3/124 ms; TI = 34 ms; voxel size, 6 × 6 × 6 mm^3, TA = 10 min 20 s) [7].

Analysis of the ^{23}Na and ^1H MRI data

A radiologist with 12 years of experience in musculoskeletal MRI analyzed the ^{23}Na radial MR images by positioning regions of interest (ROIs) on different muscles of the subject's lower leg. For exact positioning, the ^1H MR images were used as reference. When distinct lipomatous degeneration of certain muscles was observed in ^1H MRI, the reader positioned ROIs in an area with more intact muscle. Supplementary ROIs were placed on two reference phantoms, one filled with 51.3 mM Na^+ in saline solution to imitate Na^+ with unrestricted mobility (e.g., within extracellular fluid) and one filled with 51.3 mM Na^+ in 5 % agarose gel to imitate Na^+ with restricted mobility (e.g., within the cytoplasm). Complete signal suppression of the reference tube filled with 51.3 mM Na^+ in saline solution was obtained with the selected inversion

time of the IR ^{23}Na sequence. However, the signal of 51.3 mM Na^+ in the agarose gel reference tube was not eliminated and thus could be used for normalization. For normalization, the ROI values positioned on the soleus muscles on the ^{23}Na MR images were divided by the ROI values positioned on the 51.3 mM Na^+ in agarose gel reference tube. Given the known Na^+ concentration of the reference tube, we calculated the average muscular Na^+ concentration (TSC) by linear extrapolation, and we considered the observed SI to mirror muscular Na^+ concentration. A possible systematic error due to remaining relaxation weighting in the TSC data sets was not considered, because the influence of a systematic offset to the results of interindividual and intraindividual comparisons should be negligible.

Areas of signal intensity (SI) equivalent to the signal received from subcutaneous fat on T1-weighted ^{1}H MR images were interpreted as fatty infiltration caused by chronic myopathy. One reader with 12 years of experience in musculoskeletal MRI scored the lipomatous degeneration with a four-point semi-quantitative visual scale according to Olsen et al. [11]. This scale rates the intramuscular fat using the intensity of subcutaneous fat as reference and has been shown to correlate well with those found by automated computer analysis [11]. Examined calf muscles included the tibialis anterior compartment, the soleus, the medial and lateral gastrocnemius, the peroneal and the deep posterior compartment muscles: grade 1: homogeneous, hypointense signal, contrasting sharply with subcutaneous and intermuscular fat (normal muscle); grade 2: slightly hyperintense, patchy intramuscular signal changes; grade 3: markedly hyperintense, patchy, but widespread intramuscular signal changes; grade 4: homogeneous hyperintense signal in whole muscle, similar to the SI of adjacent subcutaneous or paramuscular fat.

We defined areas of localized hyperintensity on STIR MR images as muscular edema-like changes according to Marden et al. [2]. We evaluated the presence of this criterion with a four-point semi-quantitative visual scale as follows: grade 1: homogeneous, hypointense signal, contrasting sharply with subcutaneous and intermuscular fat (normal muscle, no edema); grade 2: slightly hyperintense, patchy intramuscular signal changes on STIR (<50 % of muscle cross-sectional area); grade 3: markedly hyperintense, patchy, but widespread intramuscular signal changes on STIR (>50 of muscle cross-sectional area); grade 4: homogeneous hyperintense signal in whole muscle on STIR (100 % of muscle cross-sectional area).

Besides the qualitative image analysis, the same reader quantified the lipomatous degeneration of the soleus muscle on T1-weighted images by the ratio of SI of muscle and subcutaneous fat tissue as reference using a ROI analysis ($ROI_{muscle}/ROI_{subcutaneous\ fat}$) according to Jurkat-Rott et al. [12] of the same sections used for ^{23}Na MRI analysis. The reader paid attention to avoid ROI placement close to the rings of the coil, since these regions could be affected by signal inhomogeneities. Moreover, we also semi-quantitatively assessed edema-like changes of the soleus muscle on STIR images according to Jurkat-Rott et al. [12] using a ROI analysis with background noise as reference ($ROI_{muscle}/ROI_{backround\ noise}$). We used the mean of the respective ratios for statistical analysis.

Statistical analysis

Data entry procedures and statistical analysis were performed using SPSS® for Windows®, version 16.0, 2008, SPSS Inc. Chicago, IL. Data were analyzed using two-tailed unpaired Student's *t* test. The data of first and second measurement in $n = 7$ were assessed using two-sided paired Student's *t* test. In all statistical tests, an effect was considered to be statistically significant if the *p* value was 0.05 or less. *P* values were not adjusted for multiple testing and interpretation of *p* values was explorative given the pilot study character. As glucocorticoid medication has been shown to be beneficial in DMD [1], we also performed a subgroup analysis with one group without any glucocorticoid medication ($n = 4$) and one group under glucocorticoid medication ($n = 4$). The effects of glucocorticoid administration were assessed using one-sided Student's *t* test except for fatty infiltration. Follow-up data of these two subgroups were assessed using two-sided paired Student's *t* test. Results were expressed as mean ± standard deviation (SD) for quantitative data and as median and range for the MRC scale results.

Results

^{23}Na magnetic resonance images

In the eight DMD patients, the normalized muscular ^{23}Na SI as assessed by the IR sequence was markedly increased with 0.75 ± 0.07 compared to 0.50 ± 0.05 in the eight volunteers ($p < 0.001$). Also, the tissue sodium concentration was distinctly elevated in the eight DMD patients (TSC 38.0 ± 5.9 mmol/l) when compared to the eight volunteers (TSC 25.6 ± 2.0 mmol/l, $p < 0.001$). The TSC Na^+ content in the subcutaneous fat tissue of all DMD individuals was low with 11.7 ± 0.7 mmol/l and comparable to that of healthy volunteers (11.7 ± 1.0 mmol/l, $p = 0.89$). At second measurement of the seven DMD patients (patients #1–7 of Table 1), the normalized muscular ^{23}Na IR SI persisted (1st MRI examination 0.75 ± 0.07, 2nd MRI examination 0.73 ± 0.06, $p = 0.50$). Also, TSC remained constant at follow-up (1st 37.9 ±6.4 mmol/l,

2nd 37.0 ± 4.0 mmol/l, $p = 0.49$). Figure 1 shows exemplary the MRI findings in a 5-year-old DMD boy (#1 of Table 1) and a 5-year-old healthy volunteer.

^{1}H magnetic resonance images

Edema-like changes were present in all eight DMD patients. The mean muscular SI ratio on STIR images was increased in DMD with 15.6 ± 3.5 versus 6.9 ± 0.7 in volunteers ($p < 0.001$). Also, in DMD, the muscular fat content was increased with an SI ratio on T1-weighted images of 0.48 ± 0.08 when compared to 0.38 ± 0.01 in volunteers ($p = 0.003$). Muscular edema and fatty infiltration could also be confirmed during follow-up. At second measurement of the seven DMD patients (patients #1–7 of Table 1), the normalized muscular STIR signal intensity persisted (1st MRI examination 14.8 ± 2.9, 2nd MRI examination 14.9 ± 1.5, $p = 0.91$; compare Fig. 1b, f). Also, the SI ratio on T1-weighted images remained constant at follow-up (1st 0.48 ± 0.08, 2nd 0.49 ± 0.08, $p = 0.12$).

Qualitatively, the pattern of lipomatous degeneration and edema-like changes was symmetrical in both lower legs. In all eight DMD patients, the triceps surae muscles revealed the highest degree of edema-like changes with a

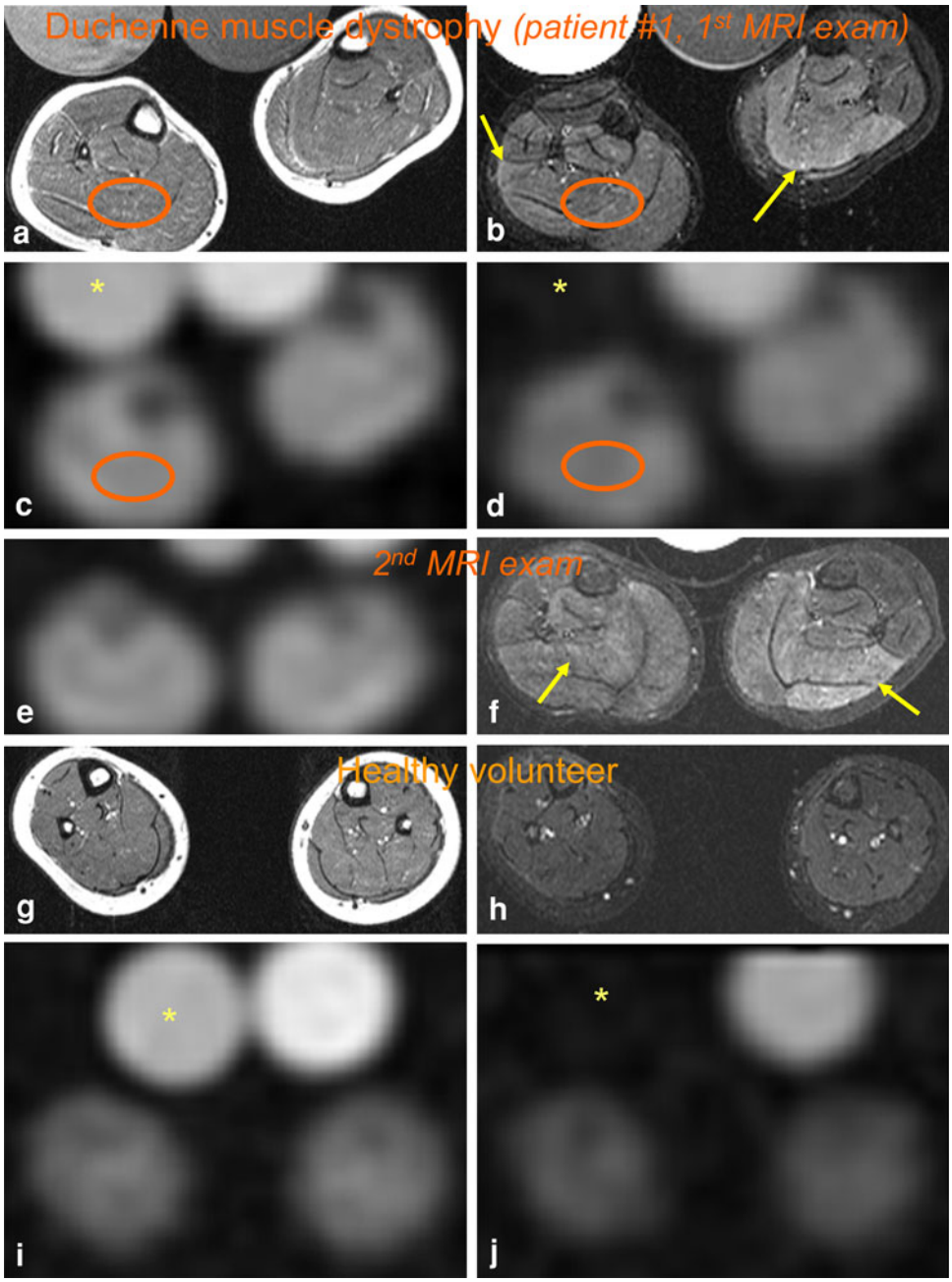

Fig. 1 Edema-like changes and increased muscular sodium are constantly present in DMD. MRI of both calves of a 5-year-old DMD boy (#1 of Table 1; a–f) and a 5-year-old healthy volunteer (#9, g–j); **a**, **g** T1-weighted, **b**, **f**, **h** STIR, **c**, **e**, **i** ^{23}Na TSC images, **d**, **j** ^{23}Na inversion recovery (IR) images. There is no fatty degeneration of the triceps surae muscles both in the DMD boy (**a**) and the age-matched healthy volunteer (**g**). However, muscular edema most pronounced in both soleus muscles (*arrows* in **b**, **f**) are visible, both at first (**b**) and follow-up MR images (**f**) compared to normal findings in the volunteer (**h**). ^{23}Na IR (**d**) and TSC ^{23}Na MRI (**c**) reveal elevated signal in both soleus muscles compared to the volunteer (**i**, **j**) without changes at follow-up (**e**). The tibial bones present with low ^{23}Na signal. With the ^{23}Na IR sequence, the ^{23}Na signal emitted from vasogenic edema and vessels was sufficiently suppressed. Note that the signal of the reference tube containing free 51.3 mM Na^{+} solution (*asterisks* in **c**, **d**, **i**, **j**) is suppressed in the ^{23}Na IR sequence (**d**, **j**), while the contralateral reference tube filled with 51.3 mM Na^{+} in 5 % agarose gel is well visible. Exemplary, the ROI positioning on the right soleus muscles on the ^{23}Na MR and ^{1}H MR images is given in **a**–**d**

median value of 3 (i.e., hyperintense muscular signal changes on STIR >50 of muscle cross-sectional area), whereas the tibialis anterior and peroneal compartments were less affected (Table 2). The median score for edema-like changes of all lower leg muscles of the volunteers was 1 (i.e., normal). In DMD, the mostly patchy intramuscular fatty changes could be observed in the triceps surae and peroneal muscles, whereas the tibialis anterior and the deep posterior compartment were spared (Table 2). The median score for fatty changes of all lower leg muscles of the volunteers was 1 (i.e., normal). The pattern of lipomatous degeneration and edema-like changes remained unchanged at second measurement in those seven DMD patients whose treatment regimen was not changed to eplerenone (patients #1–7 of Table 1).

Muscle strength

DMD patients presented with paresis in the lower legs at physical examination except for a 7-year-old DMD boy (#3 of Table 1). The paresis was symmetric except for the 22-year-old DMD woman (#8 of Table 1). The paresis remained unchanged at second measurement in those seven DMD patients whose treatment regimen was not changed to eplerenone (patients #1–7 of Table 1). Also, the maximum voluntary contraction force (kg) of the dominant hand measured using a handgrip dynamometer (1st measurement, i.e., at first fist contraction) remained constant with a median of 5 kg (range 4–10 kg) at first visit and 6 kg (range 4–9 kg) at second visit. The same applied to the grip strength at the fifth contraction in kg with a median of 6 kg (range 4–8 kg) at first visit and 6 kg (range 4–10 kg) at second visit. The healthy volunteers exhibited normal muscle function of both lower legs (median MRC score 5).

Table 2 Pattern of fatty muscular infiltration and edema-like muscular changes in Duchenne muscle dystrophy at first MRI examination ($n = 8$)

Muscle group	Fatty infiltration Medians (range)	Edema-like changes Medians (range)
Tibialis anterior compartment	1 (1–3)	1.5 (1–2)
Peroneal	1.5 (1–3)	2 (1–2)
Deep posterior compartment	1 (1–1)	2.5 (1–4)
Soleus	1.5 (1–3)	3 (2–4)
Medial gastrocnemius	1.5 (1–3)	3 (1–4)
Lateral gastrocnemius	1.5 (1–3)	3 (1–4)

Fatty infiltration and edema-like muscular changes were qualitatively assessed on a four-point visual scale as described in the "Methods" section [11]. Range, minimum–maximum values. Since the pattern of lipomatous degeneration and edema-like changes was symmetrical, the data are representative of both lower legs. For comparison, the median score for fatty changes and edema-like changes of all lower leg muscles of the volunteers was 1 (i.e., normal)

Effect of glucocorticoid medication

Since glucocorticoid medication is beneficial in DMD, we analyzed two subpopulations (one group without any glucocorticoid medication ($n = 4$; patients #1–3 and #8 of Table 1; mean age 10.0 ± 8.0) and one group with glucocorticoid medication ($n = 4$; patients #4–7 of Table 1; mean age 9.0 ± 1.4). In DMD patients without glucocorticoid medication, the normalized muscular ^{23}Na SI in the IR and TSC sequence were 0.75 ± 0.08 (TSC 39.5 ± 7.5 mmol/l) compared to 0.75 ± 0.07 ($p = 0.47$) (TSC 36.5 ± 4.5 mmol/l; $p = 0.26$) in the treated group at first visit. In the untreated group, muscular edema-like changes were higher, with an SI ratio on STIR images of 17.8 ± 3.5 compared to 13.5 ± 2.0 in the group with glucocorticoid medication ($p = 0.038$). In the untreated group, muscular fat content was 0.43 ± 0.03 on T1-weighted images compared to 0.52 ± 0.08 in the treated group ($p = 0.08$).

At second measurement 7.8 ± 1.4 months later in the four treated DMD patients (#4–7 of Table 1), the normalized muscular ^{23}Na IR SI persisted (0.75 ± 0.07 at first vs. 0.73 ± 0.01 at second measurement, $p = 0.67$). Also, TSC remained constant at follow-up (1st 36.5 ± 4.5 mmol/l, 2nd 36.1 ± 3.1 mmol/l, $p = 0.61$). Likewise, edema-like changes (1st 13.5 ± 2.0, 2nd 14.4 ± 1.8, $p = 0.064$) and fat content remained constant (1st 0.52 ± 0.08, 2nd 0.53 ± 0.08, $p = 0.37$). The same applies to the untreated group of DMD patients. At second measurement 5.7 ± 2.8 months later in the three untreated DMD patients (#1–3 of Table 1), the normalized muscular ^{23}Na IR SI persisted (0.75 ± 0.10 at first vs. 0.72 ± 0.11 at second measurement, $p = 0.69$). Also, TSC remained constant at follow-up (1st 39.8 ± 9.2 mmol/l, 2nd 38.3 ± 5.3 mmol/l, $p = 0.66$). Likewise, edema-like changes (1st 16.7 ± 3.2, 2nd 15.7 ± 0.9, $p = 0.60$) and fat content remained constant (1st 0.42 ± 0.03, 2nd 0.43 ± 0.02, $p = 0.33$).

Effect of therapy with an aldosterone antagonist

Eplerenone therapy slightly reduced TSC (by about 5 %) and edema (by about 11 %, Table 3) in the 22-year-old female DMD patient (#8 of Table 1). Also, the muscle strength improved slightly at physical examination (Table 3). Additionally, the patient reported an improvement in the mobility and flexibility of both hands and an improved ability to speak, but the maximum voluntary grip strength (kg) of the left hand measured using a handgrip dynamometer remained constant with 20 kg at first fist contraction during the first, second, and third visit.

Table 3 Treatment effects of eplerenone in the 22-year-old DMD woman (#8 in Table 1)

Visit (#)	^{23}Na IR signal	Muscular Na^+ concentration (mmol/l)	Edema (STIR ratio)	Fatty infiltration (T1w ratio)	Knee flexion/extension (MRC) of right, left leg	Foot dorsi-/plantarflexion (MRC) of right, left leg
1	0.78	38.49	21.18	0.46	2/2, 3/3	3/4, 3/4
2	0.71	37.26	19.58	0.49	2/3, 3/4	3/4, 4/4
3	0.71	36.61	18.81	0.48	3/3, 4/4	3/4, 4/5

The muscular tissue Na^+ concentration was assessed using a spin density ^{23}Na MR image contrast as described in the "Methods" section. Muscle strength was evaluated by clinical examination, quantified with aid of the nonlinear grading system defined by the British Medical Research Council (MRC) [8]. Eplerenone was administered in daily alternating doses of 25 and 50 mg for 5 weeks before the second visit and 12 weeks before the third visit

Discussion

In our pilot study, we demonstrated that all eight DMD patients exhibited muscular edema-like changes that persisted at follow-up. These edema-like changes were constantly present even in the early stages of the disease that showed no fatty infiltration of the muscles. The presence of edema may also explain the "pseudohypertrophic" aspect of the calves, which are typical of DMD. We concomitantly observed a persisting, elevated myoplasmic Na^+ concentration in DMD in comparison to healthy volunteers by using 3-tesla ^{23}Na MRI. This Na^+ overload may be osmotically relevant and cause the muscle edema.

Our data support the recently reported finding [3] that similarly to patients with hypokalemic periodic paralysis (HypoPP) who exhibit both myoplasmic Na^+ overload and muscular edema [12], the muscular Na^+ overload in DMD may lead to weakness and degeneration. Moreover, our study shows that both muscular edema and Na^+ overload are not a transient but persisting finding in DMD. Both HypoPP and DMD share the pathophysiological similarity of a membrane leakage, an aberrant pore in the voltage sensor in HypoPP [13] and a reduced mechanostability of the sarcolemma in DMD. The latter results from the lack of dystrophin, which may open stretch-activated cation channels as observed in myotubes from dystrophic *mdx* mice [14]. Hence, the intracellular and persisting Na^+ accumulation, as indicated by our ^{23}Na IR MRI results, may cause the edema-like changes in DMD, which could be confirmed consistently in all muscles that were not completely replaced by fat tissue. Thus, both in DMD and HypoPP, the muscle edema might mainly be of osmotic nature and intracellular. This mechanism stands in contrast to the hypothesis that immune-mediated changes in vascular permeability produce a vasogenic edema in DMD [2]. We believe that the significance of the immunologic inflammation in DMD is overestimated, which may explain why immunosuppressive agents like cyclosporin A have no relevant clinical impact on DMD patients [15].

Although the glucocorticoid prednisolone has been shown to have positive effects in DMD on both muscle strength and function [1, 16], there are two drawbacks: (1) the inherent risk of a glucocorticoid-induced myopathy, and (2) since the dose is close to the *Cushing* threshold level, there is the risk of adrenocortical obesity, thus potentially further stressing the already-weak DMD muscles. Recently, it has been postulated that the effect of this glucocorticoid is mainly anti-edematous [3]. Our treated subgroup revealed a less pronounced muscle edema, thus, we substantiate the view that this anti-edematous effect could be one the benefits of glucocorticoids, because it is well known that glucocorticoids decrease the edema surrounding a brain tumor and repolarize neurons in idiopathic facial paresis [17]. Of course, a larger number of patients are required to prove this hypothesis. The decrease of the intracellular edema and cell repolarization could be related. One repolarization mechanism may be the up-regulation of two-pore potassium channels [18]. Our very initial data may provide the rationale for future studies to assess in a randomized and prospective way whether the aldosterone antagonist eplerenone is more efficient and safer than glucocorticoid medication in decreasing intracellular edema in DMD. This particular potassium-sparing diuretic agent has been reported to improve muscular edema and strength in HypoPP [13].

Our ^{23}Na MRI protocol included two dedicated ^{23}Na pulse sequences based on a density-adapted 3D radial sequence: we assessed with a spin density image contrast the total Na^+ concentration that reflects a volume-weighted average of the intracellular and extracellular Na^+ concentrations. Because the extracellular Na^+ concentration in tissue at 140 mmol/l is tenfold higher compared to the intracellular Na^+ concentration at 10–15 mmol/l [19], the analysis of intracellular ^{23}Na is limited when using the aforementioned sequence. Thus, we also performed the ^{23}Na inversion recovery (IR) sequence to suppress the ^{23}Na signal originating from extracellular edema and vessels. However, one of the limitations of this study is that while the IR prepared Na^+ measurement enables weighting of the measurement towards intracellular ^{23}Na, it would still have significant contributions from the extracellular pool. Hence, also the IR prepared ^{23}Na sequence does not provide a sharp separation between intra- and extracellular ^{23}Na. We are hopeful that with the advent of ^{23}Na MRI of

the skeletal muscle at ultra-high field strength, such as possible with a 7-tesla [20], and triple quantum filtered (TQF) twisted projection ^{23}Na MR techniques [21], a better weighting towards intracellular ^{23}Na might be achieved. We also acknowledge the preliminary nature of our findings as a limitation given the low number of subjects. Our local review board, however, consented only to a pilot study. We also believe that therapeutic measures should be assessed in a multi-centric trial.

Our initial data show that ^{23}Na MRI detects a muscular Na^+ overload in DMD, which persisted at follow-up and may be part of a general mechanism in muscular degeneration, because a muscular Na^+ overload is also present in the chronic form of hypokalemic periodic paralysis. The permanent myoplasmic Na^+ overload in DMD may play a role in the development of the muscle edema, which was present and persisted in all studied DMD patients. The edema we examined seemed to be of an osmotic nature and mainly intracellular rather than mediated by inflammation. All in all, we believe that the persistent muscular edema plays an important contributing role in the pathogenesis of DMD. Our very initial findings point out that the aldosterone antagonist eplerenone might represent a treatment alternative to prednisolone with fewer side-effects in order to decelerate muscle dystrophy in DMD patients, if the therapeutic effects of this particular glucocorticoid are proven to be mainly anti-edematous.

Acknowledgments This study was supported by a research grant from the Deutsche Gesellschaft für Muskelkranke e.V. F. Lehmann-Horn is endowed Senior Research Professor of the non-profit Hertie-Foundation. We appreciate the molecular genetics of patient #8 performed by K. Hinderhofer, MD, Institute of Human Genetics, University Hospital, Heidelberg/Germany (Director C.R. Bartram, MD). We also thank A. Behnecke, MD, Institute of Human Genetics, University Hospital, Heidelberg/Germany, H.-M. Meinck, MD, Department of Neurology, University Hospital, Heidelberg/Germany, and M. Sanchez-Albisua, MD, Sozialpädiatrisches Zentrum (SPZ) Tübingen/Germany for referring some patients. We are grateful to the patients and their families for their participation.

Conflicts of interest The authors report no conflicts of interest and no disclosures.

References

1. Bushby K, Finkel R, Birnkrant DJ et al (2010) Diagnosis and management of Duchenne muscular dystrophy, part 1: diagnosis, and pharmacological and psychosocial management. Lancet Neurol 9:77–93
2. Marden FA, Connolly AM, Siegel MJ, Rubin DA (2005) Compositional analysis of muscle in boys with Duchenne muscular dystrophy using MR imaging. Skeletal Radiol 34:140–148
3. Weber MA, Nagel AM, Jurkat-Rott K, Lehmann-Horn F (2011) Sodium (^{23}Na) MRI detects elevated muscular sodium concentration in Duchenne muscular dystrophy. Neurology 77: 2017–2024
4. Hirn C, Shapovalov G, Petermann O, Roulet E, Ruegg UT (2008) Nav1.4 deregulation in dystrophic skeletal muscle leads to Na^+ overload and enhanced cell death. J Gen Physiol 132:199–208
5. Bodensteiner JB, Engel AG (1978) Intracellular calcium accumulation in Duchenne dystrophy and other myopathies: a study of 567,000 muscle fibers in 114 biopsies. Neurology 28:439–446
6. Nagel AM, Laun FB, Weber MA, Matthies C, Semmler W, Schad LR (2009) Sodium MRI using a density-adapted 3D radial acquisition technique. Magn Reson Med 62:1565–1573
7. Nagel AM, Amarteifio E, Lehmann-Horn F et al (2011) 3 tesla sodium inversion recovery magnetic resonance imaging allows for improved visualization of intracellular sodium content changes in muscular channelopathies. Invest Radiol 46:759–766
8. Dyck PJ, Boes CJ, Mulder D, Millikan C, Windebank AJ, Espinosa R (2005) History of standard scoring, notation, and summation of neuromuscular signs. A current survey and recommendation. J Peripher Nerv Syst 10:158–173
9. Weber MA, Nielles-Vallespin S, Essig M, Jurkat-Rott K, Kauczor HU, Lehmann-Horn F (2006) Muscle Na^+ channelopathies: MRI detects intracellular ^{23}Na accumulation during episodic weakness. Neurology 67:1151–1158
10. Stobbe R, Beaulieu C (2005) In vivo sodium magnetic resonance imaging of the human brain using soft inversion recovery fluid attenuation. Magn Reson Med 54:1305–1310
11. Olsen DB, Gideon P, Jeppesen TD, Vissing J (2006) Leg muscle involvement in facioscapulohumeral muscular dystrophy assessed by MRI. J Neurol 253:1437–1441
12. Jurkat-Rott K, Weber MA, Fauler M et al (2009) K^+-dependent paradoxical membrane depolarization and Na^+ overload, major and reversible contributors to weakness by ion channel leaks. Proc Natl Acad Sci USA 106:4036–4041
13. Jurkat-Rott K, Holzherr B, Fauler M, Lehmann-Horn F (2010) Sodium channelopathies of skeletal muscle result from gain or loss of function. Pflugers Arch 460:239–248
14. Lansman JB, Franco-Obregón A (2006) Mechanosensitive ion channels in skeletal muscle: a link in the membrane pathology of muscular dystrophy. Clin Exp Pharmacol Physiol 33:649–656
15. Kirschner J, Schessl J, Schara U et al (2010) Treatment of Duchenne muscular dystrophy with cyclosporin A: a randomised, double-blind, placebo-controlled multicentre trial. Lancet Neurol 9:1053–1059
16. Manzur AY, Kuntzer T, Pike M, Swan A (2008) Glucocorticoid corticosteroids for Duchenne muscular dystrophy. Cochrane Database Syst Rev 1:CD003725
17. Salinas RA, Alvarez G, Daly F, Ferreira J (2010) Corticosteroids for Bell's palsy (idiopathic facial paralysis). Cochrane Database Syst Rev 17:CD001942
18. Yamamoto T, Ohara A, Nishikawa M, Yamamoto G, Saeki Y (2011) Dexamethasone-induced up-regulation of two-pore domain K(+) channel genes, TASK-1 and TWIK-2, in cultured human periodontal ligament fibroblasts. In Vitro Cell Dev Biol Anim 47:273–279
19. Ouwerkerk R, Bleich KB, Gillen JS, Pomper MG, Bottomley PA (2003) Tissue sodium concentration in human brain tumors as measured with ^{23}Na MR imaging. Radiology 227:529–537
20. Chang G, Wang L, Schweitzer ME, Regatte RR (2010) 3D ^{23}Na MRI of human skeletal muscle at 7 tesla: initial experience. Eur Radiol 20:2039–2046
21. Borthakur A, Hancu I, Boada FE, Shen GX, Shapiro EM, Reddy R (1999) In vivo triple quantum filtered twisted projection sodium MRI of human articular cartilage. J Magn Reson 141: 286–290