Chapter 5

T2 relaxation times are increased in skeletal muscle of DMD but not BMD patients

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ABSTRACT

Introduction

Exon-skipping drugs in Duchenne muscular dystrophy (DMD) aim to restore truncated dystrophin expression, which is present in the milder Becker muscular dystrophy (BMD). MRI skeletal muscle T2 relaxation times as a representation of oedema/inflammation could be quantitative outcome parameter for such trials.

Methods

We studied T2 relaxation times, adjusted for muscle fat fraction using Dixon MRI, in lower leg muscles of DMD and BMD patients and healthy controls.

Results

T2 relaxation times correlated significantly with fat fractions in patients only ($p<0.001$). After adjusting for muscle fat, T2 relaxation times were significantly increased in 6 muscles of DMD patients ($p<0.01$), except for the extensor digitorum longus. In BMD, T2 relaxation times were unchanged.

Discussion

T2 relaxation times could be a useful outcome parameter in exon-skipping trials in DMD but are influenced by fat despite fat suppression. This should be accounted for when using quantitative T2 mapping to investigate oedema/inflammation.

INTRODUCTION

Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) are X-linked muscle diseases caused by a mutation in the dystrophin encoding *DMD* gene [10]. As a result there is generally no functional dystrophin in DMD patients and shortened, partially functional dystrophin in BMD patients. Initially muscle fibers undergo processes of degeneration and regeneration accompanied by inflammation in the muscle. As the regenerating capacity of the muscle progressively disappears, muscle fibers are increasingly replaced by adipose and fibrotic tissue [38, 54, 113]. However, the exact pathophysiological process underlying the progressive muscle damage is not understood fully [65, 178]. Clinically, both DMD and BMD patients have progressive muscle weakness, predominantly in proximal limbs in early stages of the disease, with more generalized weakness later on. DMD patients become wheelchair-bound in their early teens and die prematurely due to cardiac or respiratory failure, whereas BMD patients have a more variable and usually less severe disease course [136].
Although there is no curative therapy available for DMD, several clinical trials aim to restore expression of truncated dystrophin using antisense oligonucleotide-mediated exon skipping [77, 78, 117]. This would potentially change the DMD patients into a milder phenotype similar to BMD patients. As a result, there is interest in objective and reliable tools to assess outcome measures for patients of different ages and at different disease stages. Quantitative magnetic resonance imaging (MRI) could be such a tool, as it is non-invasive, has good spatial resolution over a large imaging plane, can be applied repetitively, and has shown promise in monitoring disease progression in DMD [103-106, 108, 113, 114, 179, 180]. Inflammation is present in both DMD and to a lesser extent in BMD from early disease stages when muscle damage is less extensive and is considered potentially reversible in contrast with fatty infiltration [17, 181]. As such, assessment of inflammation could be a valuable outcome parameter, especially as therapies would preferably start in young patients who have minimal irreversible structural changes [108].
In MRI, the proton transverse relaxation time (T2) depends on the presence of oedema/inflammation as well as the level of fatty infiltration in muscle. T2 relaxation times in inflamed muscle are longer than relaxation times of normal muscle tissue and the T2 relaxation times in muscles with fatty infiltration are even longer [90, 103, 182]. Therefore, especially in fat infiltrated muscle, it is essential that the fat is taken into account in the analysis, as otherwise it is unknown to what extent longer T2 relaxation times are a result of increased fat fractions or due to oedema/inflammation [183]. Several studies have quantitatively measured the T2 relaxation times in DMD patients and found it to be increased [90, 109, 114]. However, without fat suppression it is unknown to what extent the observed longer T2 relaxation times result from increased

fat fractions or oedema/inflammation [183]. Two recent studies sought to circumvent this problem by applying fat suppression in quantitative T2 images in a subset of their DMD patients [103, 108]. Unfortunately, fat suppression does not always suppress the entire fat signal present in skeletal muscle, and hence the results could still have been influenced by increased fat fractions [183]. Similar MRI studies assessing oedema/inflammation have not been done in BMD patients. To evaluate if the T2 relaxation time could be a possible outcome parameter in clinical trials, a better understanding of how changes in muscle of DMD patients relate to BMD patients is needed. Therefore, the aim of our study was to compare T2 relaxation times in lower leg muscles of DMD and BMD patients and healthy controls, while taking the increased fat levels into account.

METHODS

Subjects

DMD and BMD patients were recruited from the Dutch dystrophinopathy database. The diagnosis of DMD or BMD had been confirmed by genetic testing for mutations in the *DMD* gene or from a muscle biopsy. Healthy age-matched controls were recruited from local schools and from the Leiden University Medical Center Radiology database for healthy controls. The local medical ethics committee approved the study, and informed consent was obtained from all subjects. For participants under age 18, informed consent was obtained from both the subjects and their parents.

MRI

MR images were acquired on a 3T Philips Achieva Scanner (Philips Healthcare, Best, The Netherlands) from the left lower leg using a 14-cm 2-element receive coil with body coil excitation. The receive coil was positioned directly distal to the patella on the anterior and posterior sides of the leg. The scanning protocol consisted of a T1-weighted turbo spin echo (TSE) sequence [25 slices, 5 mm thickness, 0.5 mm gap, repetition time (TR) 600 ms, initial echo time (TE) 16ms, TSE factor 5], a 3-point gradient echo Dixon sequence (25 slices, 5 mm thickness, 0.5 mm gap, TR 400 ms, TE 4.41 ms, echo spacing 0.71 ms, flip angle 8˚) and a multi-echo sequence with fat suppression for quantitative T2 mapping [5 slices, 5 mm thickness, 22.5 mm gap, TR 2500 ms, 7 echo times (13, 26, 39, 52, 65, 78 and 91 ms), flip angle 90˚, spectral pre-saturation with inversion recovery (SPAIR) fat suppression] using a mono-exponential fit and maximum likelihood estimation [118, 122]. The total scanning protocol, including positioning of the patients, could be completed in 20 minutes.

Data analysis

T2 values were calculated using a maximum likelihood estimation algorithm supplied by the scanner manufacturer. Regions of interest were drawn using Medical Image Processing, Analysis and Visualization software (www.mipav.cit.nih.gov) on the second, third, and fourth slices of the shortest echo image and superimposed on the calculated T2 maps to obtain the mean T2 values of the following muscles: medial and lateral head of gastrocnemius muscle (GCM and GCL), soleus (SOL), posterior tibialis (TP),

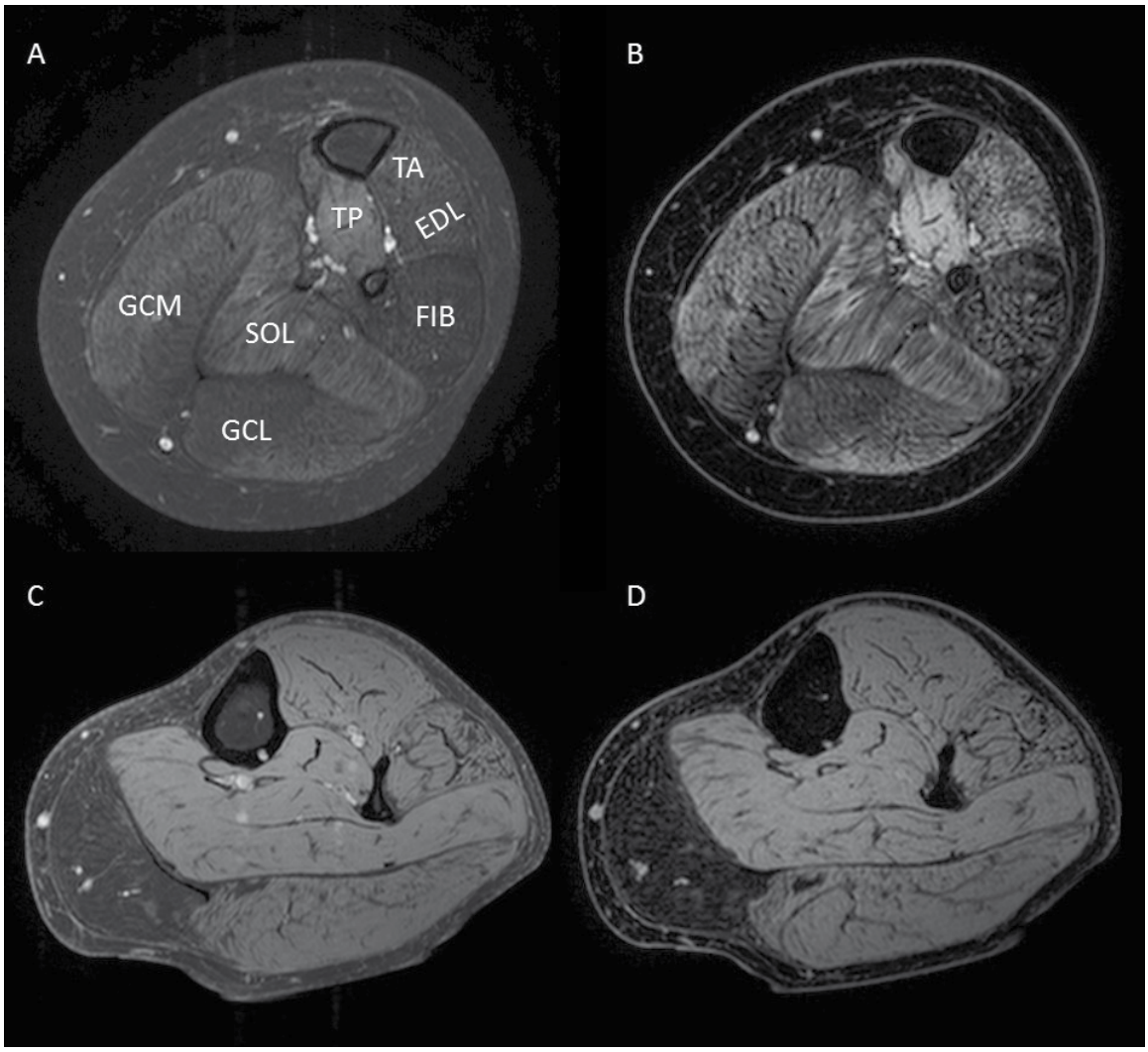

Figure 1. Axial image of lower leg showing the first echo time of the quantitative T2 image of a 12 year-old DMD patient (a), a 63 year-old BMD patient, and (c) a Dixon water image of the DMD patient (b), and the BMD patient (d).
The Dixon image clearly shows diffuse fatty infiltration in virtually all muscles in the DMD patient, whereas in the BMD patient the medial gastrocnemius is most severely affected while the other muscles are still relatively spared.
Muscles shown are medial gastrocnemic muscle (GCM), lateral gastrocnemic muscle (GCL), soleus muscle (SOL) posterior tibialis muscle (TP), anterior tibialis muscle (TA), extensor digitorum longus muscle (EDL) and fibularis muscle (FIB).

fibularis (FIB), anterior tibialis (TA), and extensor digitorum longus (EDL) (Figure 1). Dixon images were co-registered to T1 images, on which regions of interest in every other slice were drawn for the previously mentioned muscles. The middle 11 slices were used to cover the same FOV from which the T2 relaxation times were calculated. Fat and water images were generated using multipeak fitting, and fat fractions were calculated as described previously [143].

Statistical analysis

One-way between-groups analysis of covariance (ANCOVA) was conducted to compare the T2 values in patients and controls. Disease status was used as fixed factor, and fat percentage and age were used as covariates. By including the muscle fat fraction as a covariate the T2 relaxation times adjusted for fat fractions could be calculated and were subsequently incorporated in the analysis. A Bonferroni-Holm model was used to correct for multiple comparisons. A Pearson correlation was performed to test the relationship between fatty infiltration and T2 relaxation time in DMD and BMD patients and in healthy controls. SPSS statistical package Version 20.0 for Windows (SPSS Inc., Chicago, IL) was used for the analysis. The level of significance was set at $p<0.05$.

RESULTS

Patient characteristics

Nineteen DMD patients (age 10.5±2.7, range 6-14 years), 11 healthy boys (age 10.7±2.1, range 8-15 years), 7 BMD patients (age 39.6±11.7, range 26-63 years), and 8 healthy male adults (age 34.4±12.6, range 24-62 years) participated in the study, and all completed the protocol. Of the DMD patients, 10 were fully ambulant, 2 used a wheelchair intermittently, and 7 were completely wheelchair dependent. All but 3 DMD patients used corticosteroids: an 8-year old boy who was still completely mobile and had never used steroids and 2 13-year old boys who were both wheelchair dependent and had been on steroids for approximately 5 years, but had stopped 1 and 2 years, respectively, prior to this study. Of the BMD group, 2 men (aged 26 and 38) experienced no problems or weakness in their daily activities. Three patients (aged 63, 39, and 31) reported muscle weakness when carrying out daily activities such as climbing stairs, but none required a walking aid. Two patients (aged 37 and 43) were completely wheelchair-dependent. All BMD patients experienced occasional muscle cramps.

Fatty infiltration

To adjust for potential effects of fatty infiltration on the T2 values, the fat fractions for the analysed muscles were calculated using Dixon MRI. The mean fat fraction of all analysed muscles was 20.7±7.0% for DMD patients, 5.6±0.9% for paediatric controls, 15.3±11.8% for BMD patients, and 4.4±0.5% for adult controls. Of the analysed muscles, the GCL had the highest mean fat fraction in DMD patients (28.4%), and the TP had the lowest (8.2%). In the BMD patients, the GCM had the highest mean fat fraction (31.8%), and the TA had the lowest (2.2%).

T2 relaxation times

There was a significant correlation between unadjusted T2 values and fatty infiltration in the DMD and BMD patients ($R=0.70$, $p<0.001$), but not for healthy controls ($R=1.13$, $p=0.15$) (Figure 2). Uncorrected average T2 relaxation times in DMD patients were 43±2.4 ms, 42±4.2 ms in BMD patients, 36±1.4 ms in paediatric controls, and 38±1.8 ms in adult controls. After adjusting for the fat percentage the average T2 relaxation times were 42.2±2.2 ms in DMD patients, 39.1±3.3 ms in BMD patients, 37.3±1.4 ms in paediatric controls, and 39.8±2.6 ms in adult controls. The adjusted T2 relaxation times were significantly higher in DMD patients compared to controls for the GCM, GCL, SOL, and TP (all $p<0.001$), TA ($p=0.005$) and FIB ($p=0.002$), but not for the EDL ($p=0.96$) (Figure 3). There were no significant differences between BMD patients and controls after adjusting for the fatty infiltration in any of the investigated muscles (Figure 3).

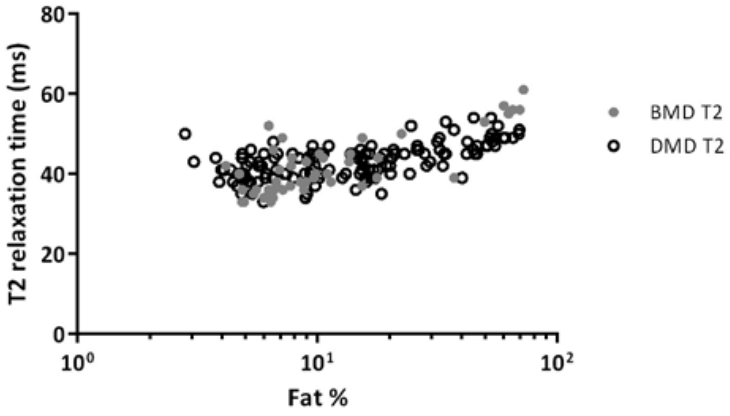

Figure 2. Correlation between fatty infiltration and fat suppressed T2 values in DMD patients (open circles) and BMD patients (grey dots).
Although fat suppression was applied, there remains a significant influence of the fatty infiltration on the T2 values, increasingly towards the higher fat fractions.

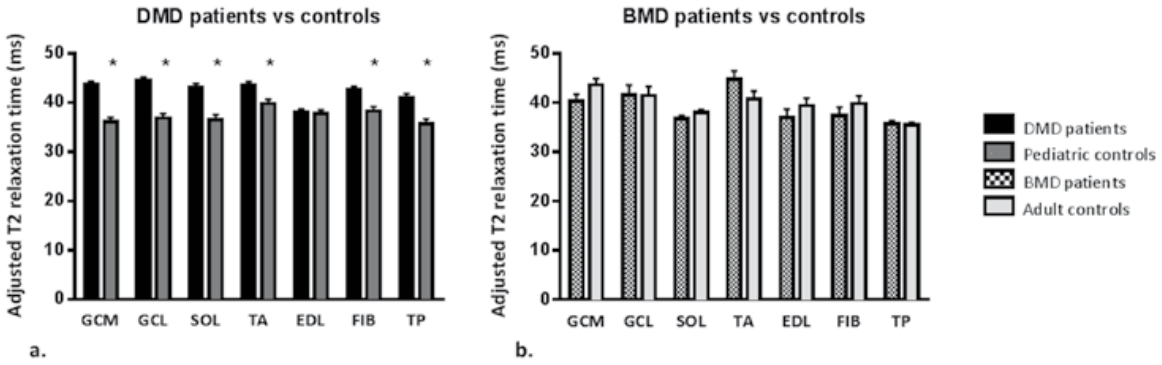

Figure 3. Mean adjusted T2 relaxation times ±SD of DMD patients (black) versus controls (dark grey) (a) and BMD patients (checked) versus controls (light grey) (b) of the investigated muscles [medial and lateral heads of gastrocnemius (GCM, GCL), soleus (SOL), anterior tibialis (TA), extensor digitorum longus (EDL), fibularis (FIB) and posterior tibialis (TP)). Muscles with significantly different T2 values are shown with an asterisk (*).

DISCUSSION

In this study we show that in DMD patients the T2 relaxation times, with adjustment for the increased fat percentage, are significantly increased compared to healthy boys in all analysed muscles except the extensor digitorum longus. In contrast, BMD patients did not show significant increases in adjusted T2 values compared to healthy controls. This suggests that the T2 relaxation time could be a useful outcome parameter in DMD trials that aim to restore truncated dystrophin expression, which is typically expressed in BMD patients.

Increased T2 relaxation times in skeletal muscle can originate from a number of factors, including inflammation, necrosis, or moderate-severe exercise [183]. The T2 relaxation time has also been shown to increase with age [184]. In muscle tissue of DMD and BMD patients mononuclear cell infiltrates predominantly consist of T-lymphocytes and macrophages [54-56, 181, 185]. Several mechanisms have been hypothesized to contribute to the inflammation. It has been suggested that inflammation is a non-specific event, which could be triggered by surrounding necrotic muscle fibers [55, 59]. Activation of an immune response via toll-like receptors triggered by myofiber damage before more structural changes are present is believed to be another explanation [55, 62]. A specific cellular autoimmune response to an unknown antigen has also been suggested [61]. The various components involved in the inflammatory response are thought to contribute directly to some of the muscle damage [56, 62, 63, 185, 186]. In addition to the potential effects of inflammation, a recent sodium MR study demonstrated oedema-like changes in muscle of DMD patients and suggested that increased intracellular sodium might also play a direct role in the pathogenesis of

progressive muscle degeneration[187]. These mechanisms could all contribute to an increase in the T2 relaxation time, as observed in our DMD patients

In contrast to DMD patients, there are no specific anatomical pathology studies describing to what extent inflammatory processes contribute to muscle degeneration in BMD patients. As BMD patients have some, albeit not full-length, dystrophin it could be that the mechanism(s) causing inflammation are less prominent compared to DMD patients. This is supported by the observation that inflammation is less in muscle biopsies of BMD patients than in DMD patients [17, 181]. This could explain why no significant increase in adjusted T2 relaxation time was seen in our BMD patients.

While several studies have quantitatively measured the T2 relaxation times in DMD patients and found it to be increased [90, 109, 114], only two recent studies described increased T2 relaxation times in lower leg muscles of DMD patients using fat suppression[103, 108]. Our results show that even though fat is suppressed using SPAIR, the T2 values and level of fatty infiltration were correlated in many of the investigated muscles. Therefore, an influence of the lipid signal on the T2 values in previous studies cannot be ruled out. Recently, it was shown that suppressing fat with just SPAIR aimed at the aliphatic fat region around 2.6 parts per million is not sufficient to eliminate the entire fat signal[188]. This could be solved by using additional methods to suppress the olefinic fat signal [188, 189]. Alternatively, applying proton MR spectroscopy and separately assessing the T2 relaxation time of water and fat can circumvent this problem. However, this will only yield results over a small volume of interest and is too time consuming to perform for a large number of muscles. Thirdly, a chemical shift method can be employed in which water and fat images are obtained at multiple echo times, but unfortunately this sequence is not currently available on all commercial MR scanners [190]. Finally, water and fat can be separated in post-processing, as proposed by several authors [145, 183, 191-193], or by adjusting for the fat by statistical analysis, as we have done here.

Several previous studies have suggested the use of the fatty infiltration in DMD patients as a measure of disease progression and potential outcome parameter [103, 104, 106, 194]. In the dystrophic process, oedema/inflammation is thought to occur prior to fatty infiltration and could therefore be used as an earlier marker of therapy effectiveness compared to changes in levels of fatty infiltration. In addition, it is questionable if severely fatty infiltrated muscles can still be rescued, and as such fatty infiltration in these patients might be irreversible. Alternatively oedema/inflammation is potentially more likely to be reversible and could therefore be of value as an outcome parameter in assessment of future therapies, especially in early disease stages when effect of the lipid signal on the T2 relaxation times is minimal.

Recently it has been suggested that T2 relaxation times in DMD patients are reduced as a function of age [115, 195, 196]. However there remains some discussion as to these

findings, since some authors report a decrease and others report an increase [103, 108]. The cause for this potential decrease is still uncertain and could be due to technical reasons or pathophysiology, i.e. a decrease in oedema or an increase in fibrosis. So far the available data imply that if the T2 relaxation times decrease, they do not normalize to the levels of healthy controls, and they have only been described for older DMD patients. Therefore, even if such an effect were to be present, the T2 relaxation time remains a potential valuable parameter in the follow-up of younger patients.

This study has some limitations; firstly, the group of BMD patients participating in the study was relatively small. However, as we obtained data from several muscles ranging from patients with very few clinical symptoms to wheelchair-dependent patients we felt that our findings are likely to be representative of the whole spectrum of the disease. Secondly, 3 of our DMD patients did not use corticosteroids. Although a previous study showed no significant effect on the T2 relaxation time in DMD patients before and after steroid treatment, a recent report suggested that corticosteroids do in fact result in a decrease of the T2 relaxation time [114, 197]. However, a reduction of the T2 relaxation time as an effect of steroids would decrease possible differences between patients and controls. As the number of steroid-free patients in our cohort was very small, it is unlikely that the patients not on steroids represented the sole contribution to the significant difference between patients and controls. Thirdly, a TR of 2500ms was used to avoid very long acquisition times, which result in partial saturation effects due to the long T1 of water. Finally, we only assessed the lower leg in our study, as the B1 field in this region is much more homogeneous compared to the upper leg. However, the upper legs are known to be involved earlier in both DMD and BMD patients. For future studies it would be valuable to compare proximal muscles in either the leg or arm of both DMD and BMD patients.

In conclusion, we show that skeletal muscle T2 relaxation times are significantly increased in DMD patients, which is indicative of the presence of inflammation/oedema. We also show no such increase is present in the generally less severely affected BMD patients. Therefore we believe that quantitative T2 mapping, while taking the lipid signal into account can be a useful method for evaluation of oedema/inflammation of DMD patients and could be an outcome parameter in treatment trials aiming to ameliorate DMD to a BMD phenotype.