Quantitative muscle MRI to unravel the physiology of dystrophic and healthy muscle

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Chapter 1

General introduction
Muscular dystrophies are a group of rare inherited muscle disorders that cause wasting and weakening of skeletal muscles. Approximately 20 to 25 per 100,000 persons are affected with a type of muscular dystrophy. Each type of these dystrophies has its own genetic profile, order in which muscles are primarily affected, severity of muscle wasting, rate of progression, and age of onset. Nevertheless, muscles of muscular dystrophy patients show similar histopathological features, like muscle degeneration reflected by changes in muscle fibre size, increased interstitial fibrosis and infiltration of adipose tissue cells, although there are also some distinctive histopathological differences.

Recent molecular studies provided insight in the genetic character of muscular dystrophies and extensive clinical natural history studies have characterized their clinical features at the patient level. This has been a great step forward in the molecular understanding of these diseases, but has not yet led to successful curative treatments. Partly, this is because for most muscular dystrophies the pathways through which the genetic defects lead to the observed pathophysiological processes of muscle degeneration and muscle weakness are poorly understood. The regular way to elucidate these processes are further molecular studies. However, in this thesis, we decided to take a different point of view to get new insights by focussing on the muscle function and pathophysiological processes at the whole muscle level using magnetic resonance (MR) techniques.

MR techniques offer a non-invasive and versatile way to quantitatively assess the anatomy, composition and function of single or multiple muscles and are therefore very well suited to study muscular dystrophies. For example, MR imaging (MRI) techniques can provide relevant outcome measures like muscle volume, fat fraction and T2 relaxation time of water. These outcome measures report on the respective pathological processes of muscle atrophy, fat infiltration, and alteration in tissue water distribution (intra- or extracellular oedema), the latter being an early pathophysiological change that is associated with disease activity. Next to this, MRI can be used to assess other physiological conditions of muscles, such as muscle morphology by water diffusion methods, muscle vascularity by perfusion methods and muscle metabolism by MR spectroscopy (MRS) visualizing for instance phosphorylated compounds involved in the muscle’s energy metabolism.

This thesis focuses on the use of these MRI and MRS techniques to unravel the pathological pathways leading to muscle degeneration in muscular dystrophy. Furthermore, as quantitative outcome measures to test the effectiveness of a therapy are lacking, we also studied the value of MRI as biomarker in future clinical trials. In this thesis, we studied the two most frequent types of adult muscular dystrophy, namely myotonic dystrophy type 1 (DM1) and facioscapulohumeral muscular dystrophy (FSHD). Below, I will first shortly describe DM1 and FSHD which will be followed by the objective and outline of this thesis.
Myotonic dystrophy type 1

DM1 has a prevalence of about 1 per 10,000 and is therefore the most common form of muscular dystrophy in adults.\textsuperscript{3,12} Based on age of onset, the disease is classified in a congenital, an infantile, a juvenile, an adult and a late-onset form. Clinically, DM1 is characterized by distal muscle weakness, facial and neck muscle weakness and myotonia.\textsuperscript{13–15} Furthermore, DM1 is not solely a muscle disorder, it is a complex multisystem disease which also presents with cognitive dysfunction, daytime sleepiness, cataracts, cardiac conduction defects, gastro-intestinal problems and endocrine dysfunction.\textsuperscript{14–16}

The genetic cause of DM1 is an expansion of a CTG trinucleotide repeat in the dystrophia myotonica protein kinase (DMPK) gene. In the general population, CTG repeat lengths are about 5-34 and DM1 symptoms have been reported when the repeat becomes larger than 50.\textsuperscript{17} The size of the expansion is a major determinant of the age of onset of symptoms and the severity of the disease.\textsuperscript{17–19} Furthermore, the expanded CTG repeat in DM1 patients is unstable in the germline and the soma. Germline instability leads to progressive increases in CTG length from generation to generation, leading to the clinical phenomenon of anticipation: progressively earlier onset of symptoms and more severe disease in subsequent generations (e.g. grandfather – mother – son).\textsuperscript{20} Somatic instability means that within an individual, the CTG repeat expansion increases in size during life. This somatic instability is tissue dependent and leads to different repeat expansion sizes between different organ systems. Somatic instability may be an important determinant of disease progression.\textsuperscript{18}

The exact mechanism by which the expanded CTG repeat leads to symptoms of DM1 is unknown, but the most recent theory is as follows (figure 1.1). Transcription of the expanded CTG repeat leads to expanded mRNA, which will form stable hairpin loops that accumulate within the cell, called ribonuclear foci. These ribonuclear foci, by sequestration, dysregulate at least two RNA-binding proteins families: the muscleblind-like (MBNL) proteins and the CUG binding proteins and Elav-like family members (CELF). These RNA-binding proteins are required for normal gene splicing, wherefore deregulation of these proteins is expected to cause missplicing of a large amount of gene products.\textsuperscript{19} Some of these misspliced genes can indeed explain features observed in DM1, like the abnormal splicing of the muscle-specific chloride channel and the reduced conductance of the chloride ions in the sarcolemma that explain myotonia.\textsuperscript{21,22}

MRI findings in DM1

Muscle MRI studies in DM1 are limited and have been mainly focussed on the lower extremity muscles.\textsuperscript{23–27} Those cross-sectional semi-quantitative MRI studies revealed a distinct pattern of fat infiltration, whereby the leg muscles appear to be affected earlier than the thigh muscles. Of the leg muscles, the gastrocnemius medialis and soleus are most severely fat infiltrated, and the thigh muscles are characterized by an semilunar anterolateral perifemoral area of fat infiltration. There is one quantitative muscle MRI study
in DM1, focussed on the tibialis anterior, which showed a strong correlation between ankle dorsiflexion strength and residual muscle tissue, fat-to-water ratio and T2 relaxation time.\textsuperscript{28}

**Figure 1.1: Schematic overview of the genetic background in DM1.** In DM1 patients, the DMPK gene contains an expansion of the CTG repeat (>50 is symptomatic). Consequently, the transcribed mRNA forms hairpin structures that will bind the RNA-binding protein MBNL1 (loss of function) and upregulates the RNA-binding protein CELF1 (gain of function), both of which play a role in the splicing of other genes leading to missplicing of mRNA from many other genes.

**Facioscapulohumeral muscular dystrophy**

FSHD is one of the leading forms of muscular dystrophy with approximately 2000 patients in the Netherlands.\textsuperscript{29} FSHD often presents with asymmetric muscle weakness of the face and shoulder muscles, typically leading to a distinct profile of the shoulders with scapular winging, straight clavicles and rounded shoulders.\textsuperscript{30} As the disease progresses, also the trunk and the lower extremity muscles often become affected.\textsuperscript{31} Patients frequently report long periods of quiescence interrupted by periods of rapid deterioration involving a particular muscle group.\textsuperscript{32} The disease severity is highly variable, even within affected families, with about 20% of the patients eventually becoming wheelchair-bound.\textsuperscript{32}

FSHD is caused by a contraction of the D4Z4 repeat array on the subtelomeric region of chromosome 4q (figure 1.2). In the healthy population, the D4Z4 repeat array consists of 8 to more than 100 D4Z4 units. In FSHD, this D4Z4 repeat array is shorted to 1-10 units.\textsuperscript{33,34} This shortened repeat array is associated with a more open chromatin structure which
facilitates the transcription of the DUX4 gene. This DUX4 gene encodes a double homeobox protein, called the DUX4 protein, that is normally expressed in the luminal cells of the testis but epigenetically silenced in somatic cells. In general, the DUX4 protein is unstable and requires a specific haplotype that provides a polyadenylation signal that stabilizes the distal DUX4 transcript. DUX4 expression leads to a complex, currently largely unexplained, cascade of events that eventually lead to muscle cell death. In general, the disease is more severe in individuals with shorter D4Z4 repeats. However, the length of this repeat only explains about 10% of the variance in disease severity in FSHD, indicating that there are other modifying factors as well.

Figure 1.2: Schematic overview of the genetic background in FSHD. A) In healthy individuals the D4Z4 repeat array on chromosome 4q varies between 8-100 units resulting in a relatively closed chromatin structure (blue wave). B) In FSHD patients, a contraction of the D4Z4 repeat array to 1-10 units results in a relatively more open chromatin structure (blue wave) increasing the chance of expression of DUX4 (red box 1). Only when the last repeat unit is followed by a polyadenylation signal (Poly(A), red box 2) this results in stable expression of DUX4.
MRI findings in FSHD

In FSHD, quantitative MR measures for fat infiltration have been used extensively to uncover the pathophysiological features in FSHD and to study the natural disease progression. These studies reported a distinctive profile of fat infiltration in FSHD, with fat replacement being most severe in the hamstring muscles, adductor group, tibialis anterior and gastrocnemius medialis. Furthermore, muscle strength measures correlate with the residual muscle tissue and fat fraction indicating that fatty infiltration and atrophy are major factors for muscle weakness in FSHD. Moreover, fatty infiltration in FSHD is a dichotomous phenomenon; muscles either showed no signs of fat infiltration, or were heavily fat infiltrated, and only a small group of the muscles were in the intermediately fat infiltration state. The latter group had the highest fat infiltration rate and showed a fat infiltration gradient moving from distal to proximal. Together, this indicates that FSHD is a focal disease that tends to start in the distal part of the muscle and that, as soon as muscle degeneration starts, it progresses rapidly. These MRI findings could be linked to a proposed DUX4 activation model: the DUX4 gene is activated in a few myonuclei yielding DUX4 protein molecules that diffuse into the cytoplasm towards neighbouring nuclei. In those neighbouring nuclei, the DUX4 protein molecules activate target genes, leading to a transcriptional cascade of dysregulation that gradually can move along the whole muscle.

Objective and outline of this thesis

The overall aim of this thesis was to unravel the pathophysiological mechanisms in FSHD and DM1 using dedicated MR techniques. Hereby, we first studied the muscular disease processes in natural history and after intervention in DM1 and FSHD patients (Chapter 3 to 5). Second, in search of triggers for disease initiation we assessed the normal muscle function in healthy volunteers (Chapter 6 and 7).

Thesis Outline

Chapter 2 starts with the basic principles of MR and a short description of the MRI and MRS techniques employed in this thesis. Next to MR, this chapter gives a short description of the optical technique near infrared spectroscopy (NIRS), that we used to study muscle oxygenation in chapter 7.

The semi-quantitative muscle MRI studies in DM1 revealed that multiple muscles in the body are affected in different ways. Therefore, it is important to study the muscle pathology on the broadest feasible set of skeletal muscles. In chapter 3 and 4, we applied quantitative MRI outcome measures for fat infiltration, muscle mass and tissue water distribution (oedema) to study the pathophysiological mechanisms in the lower extremity muscles of DM1 patients. Furthermore, we assessed the value of these MRI outcome measures as biomarkers in future clinical trials. To this end, in chapter 3, we compared fat infiltration,
muscle mass and tissue water distribution in lower extremity muscles of DM1 patients with healthy volunteers, determined the specific pattern of fat infiltration in DM1, and associated fat infiltration and muscle mass with age, CTG repeat length and clinical outcomes. In chapter 4, we investigated the natural disease progression of muscle degeneration by assessing the change in fat infiltration, muscle mass and tissue water distribution in the lower extremity muscles over 10 months and we evaluated the effect of a behavioural intervention on the changes occurring in this 10 month period in these muscles.

In FSHD, the natural history and value of quantitative MRI as an outcome measure for clinical trials has been studied extensively.\textsuperscript{5,6,40–42,46} It indicated that fatty infiltration and atrophy are major determinants of the force that a muscle can generate. In chapter 5, we studied a third factor for muscle weakness in FSHD, namely the specific muscle strength (strength/area of muscle tissue) by combining quantitative assessment of fat infiltration and muscle mass with force measurements and a musculoskeletal model.

Literature indicates that fat infiltration moves from distal to proximal in muscles of FSHD patients.\textsuperscript{5,6,46} The reasons for this distal initiation of fat infiltration are unknown but suggest that normal muscle may already contain some variation in muscle structure or function along its proximodistal axis that makes the distal part of a muscle more vulnerable for initiation of muscle degeneration than the proximal part. Therefore, in chapter 6 and 7 we studied the variations in muscle function along the proximo-distal axis of a single muscle in healthy volunteers. We focussed on the tibialis anterior, as this muscle is easy to access and can easily be activated by ankle dorsiflexion. In chapter 5, we evaluated whether there was a variation in oxidative capacity along the proximodistal axis of the human tibialis anterior in healthy volunteers and in chapter 6 we studied the variation in oxygen supply along the tibialis anterior.
References


Chapter 2

Introduction into magnetic resonance and near infrared spectroscopy
Basic principles of magnetic resonance

MRI is based on the principle of nuclear magnetic resonance and that atomic nuclei possess spin. A spin is a fundamental property of an atomic nucleus, it reflects the nucleus’s intrinsic angular momentum. To imagine this property, spin is often described as spinning of the nuclei around its own axis, but it is important to keep in mind that in reality it is not a rotating particle (figure 2.1A).

When the spin is non-zero, this will create a magnetic moment ($\mu$) that depends on the angular momentum ($L$) and the gyromagnetic ratio ($\gamma$) of the nucleus (equation 2.1). This is for example the case for $^1$H, $^{31}$P, and $^{13}$C, who have a spin of $\frac{1}{2}$.

$$\mu = \gamma L \quad (equation \ 2.1)$$

The orientation of a spin’s magnetic moment is random throughout space, wherefore the magnetic moments cancel each other out and thus no net macroscopic magnetization exists (figure 2.1B). This changes when we put the spins in a magnetic field ($B_0$). The spins will start to precess around $B_0$ with the so-called Larmor frequency ($\omega_0$). The Larmor frequency depends on the gyromagnetic ratio and the $B_0$ field strength (equation 2.2).

$$\omega_0 = \gamma B_0 \quad (equation \ 2.2)$$

However, spins do not only experience $B_0$, they also experience small local magnetic fields of surrounding electrons and molecules. Spatially and temporally, these local magnetic fields fluctuate rapidly due to thermal motion, wherefore spins experience a fluctuating effective field. This causes the spins to deviate very slowly from their angle of precession, changing the orientation of the spin’s magnetic moment. It is slightly more probable that the spin will move towards the orientation with the lower magnetic energy, which is parallel to $B_0$. As a consequence, the distribution of spins will be slightly skewed towards $B_0$ and net magnetization ($M_0$) forms (figure 2.1C). The above description of $M_0$ is based on quantum mechanics, while in the classical description of MR spins are defined as being in only two states, parallel (lower energy state, $n_\alpha$) and anti-parallel (higher energy state, $n_\beta$) to $B_0$. The population ratio between the parallel and anti-parallel spins depends on the temperature and is described by the Boltzmann equation (equation 2.3):

$$\frac{n_\beta}{n_\alpha} = e^{-\Delta E/k_BT} = e^{-h\nu/k_BT} \quad (equation \ 2.3)$$
With \( h \) the Planck constant, \( \nu \) the Larmor frequency, \( k_B \) the Boltzmann constant and \( T \) the temperature. At 3T and body temperature, this population difference is \(~0.001\%\) for \(^1\text{H}\). The resulting magnitude of \( M_0 \) is described as:

\[
M_0 = \frac{\gamma^2 h^2 n B_0}{2\pi 4k_B T} \quad \text{(equation 2.4)}
\]

With \( n \) the spin density of the sample.

---

**Figure 2.1: Basic principle of net magnetization.** A) A single nuclear spin that precesses with the Larmor frequency when placed in the main magnetic field \( B_0 \). B) Distribution of spins without the presence of main magnetic fields, all spins point randomly in space and no net magnetization exits. C) Distribution of spins in the presence of \( B_0 \) which will be slightly skewed toward the direction of \( B_0 \) causing net magnetization (\( M_0 \)). The whole sample of spins will precess around \( B_0 \) with the Larmor frequency. Figure adapted from Hanson et al.\(^1\)

**Excitation**

As long as \( M_0 \) is pointing parallel to \( B_0 \), it cannot induce a current in a radiofrequency coil. Therefore, \( M_0 \) is turned perpendicular to \( B_0 \) into the transversal plane, by transmitting an additional magnetic field \( B_1 \) referred to as the radiofrequency (RF) pulse (figure 2.2). This RF pulse has a relative small amplitude compared to \( B_0 \) but because it oscillates at the Larmor frequency, it still has a large effect on the precessing spins. The strength and duration of the RF pulse determine how far \( M_0 \) is tipped away from \( B_0 \), i.e. determine the rotation angle or flip angle (FA).
Figure 2.2: Excitation. The 90° degree radiofrequency (RF) pulse will flip the net magnetization \( (M_0) \) to the transverse plane. Figure adapted from Hanson et al.\textsuperscript{1}

Relaxation

Because of the RF pulse, the spin distribution is no longer in equilibrium. Therefore, when the RF pulse is turned off, the spins will return back to their original state. First of all, small fluctuating fields of the environment will change the precession axis of the spins causing the net magnetization to return to the thermal equilibrium condition, parallel to B\textsubscript{0}. The recovery of this longitudinal net magnetization \( (M_z) \) is called T\textsubscript{1} relaxation or longitudinal relaxation, and occurs mono-exponentially, whereby the T\textsubscript{1} relaxation time is defined as the time required to recover 63\% of M\textsubscript{z} (figure 2.3A and equation 2.5).

\[
M_z(t) = M_0 \left(1 - e^{-\frac{t}{T_1}}\right) \quad (equation 2.5)
\]

Next to this, there is T\textsubscript{2} relaxation, or transverse relaxation, which describes the decay of the transversal magnetization \( (M_{xy}) \) (figure 2.3B). The order of the spins who had a preference in the z-direction is preserved and tipped as whole in the transversal plane. The spins will be asymmetrically clustered in the transverse plane, called phase coherence. T\textsubscript{2} relaxation describes the loss of this spin coherence by small changes in the local magnetic field that alter the individual precession frequency causing dephasing of the spins and mono-exponential loss of M\textsubscript{xy} (equation 2.6).

\[
M_{xy}(t) = M_{xy}(0)e^{-\frac{t}{T_2}} \quad (equation 2.6)
\]

Furthermore, transversal magnetization is lost due to B\textsubscript{0} inhomogeneities at the macroscopic level. Together with T\textsubscript{2} relaxation, this process is described as T\textsubscript{2*} relaxation (equation 2.7).

\[
\frac{1}{T_2^*} = \frac{1}{T_2} + \gamma \Delta B_0 \quad (equation 2.7)
\]
Magnetic resonance imaging

The T1 and T2 relaxation times differ between tissues. MR imaging makes use of this variation in T1 and T2 relaxation time to generate contrast in the MR images. However, before we can reconstruct an image we need to know which spin signal comes from where in the body. This is commonly achieved by spatial encoding using the scanner’s gradient system. There are many sequences to acquire images, but discussing them all is beyond the scope of this thesis. Therefore, below I will describe this spatial encoding in case of a basic spin echo sequence (figure 2.4).

A spin-echo sequence starts with a 90° excitation pulse. Due to magnetic field inhomogeneities the spins will quickly start to dephase (T2* relaxation). After a certain time, a 180° inversion pulse is given. This inversion pulse will mirror the magnetization vectors with respect to their axis. Since the spins still experience the same field inhomogeneities, they will rephase and an echo is formed. The time between excitation pulse and the echo is defined as the echo time (TE).

The first step in the spatial encoding is the slice selection. This is achieved by turning on the slice encoding gradient during the excitation and inversion pulse. This gradient produces a small magnetic gradient on top of the external magnetic field, wherefore the magnetic field that the spins experience will vary along the slice selection axis. Consequently, the Larmor frequency of the spins will vary along the slice selection axis as well. This means that only the spins with the Larmor frequency in the bandwidth of the RF pulse are excited, enabling the selection of a specific slice.

Second, frequency and phase encoding gradients are applied achieve localization within a slice. For frequency encoding, a gradient is turned on in the frequency encoding direction during the acquisition of the echo. As for the slice encoding, the Larmor frequency will vary
as a function of position, but now in the frequency encoding direction. Consequently, the acquired signal will consist of a range of frequencies that can be related to their position.

In case of phase encoding, a short gradient is applied shortly before the acquisition. During the time that this gradient is turned on, the Larmor frequency will vary as a function of its location along the phase encoding direction. When the gradient is turned off, the spins will return to their original frequency, but will have acquired a spatially varying phase shift along the phase encoding direction. One phase encoding step is not sufficient to obtain the spatial information in the phase encoding direction. Therefore, the echo is acquired multiple times while varying the phase encoding gradient strength. The time between two acquisitions is the repetition time (TR).

The choice of TE and TR (and flip angle) relative to the tissues T1 and T2 relaxation time determine whether an image is T1-weighted, T2-weighted or proton density weighted. Besides the spin-echo sequence, many other pulse sequences exist to generate specific contrast, including a gradient echo, a Dixon sequence, a diffusion weighted sequence and more.

![Spin echo sequence diagram](image)

**Figure 2.4: Spin echo sequence.** The spin echo sequence consists of a 90° and 180° degree pulse. Spatial encoding is achieved by the slice encoding gradient, phase encoding gradient and frequency encoding gradient. The amplitude of the phase encoding gradient is altered for each acquisition. The time between the 90° RF pulse and the echo is defined as the echo time (TE).
MR techniques to assess muscle degeneration
In chapter 3, 4 and 5, we quantitatively assessed the disease processes in muscles of patients with DM1 and FSHD. We specifically looked at fat infiltration, muscle mass or atrophy, and alterations in tissue water distribution. Below, I shortly describe the two quantitative MR techniques used in chapter 3, 4 and 5.

3D Dixon to assess muscular fat infiltration and atrophy
The Dixon sequence is nowadays the most commonly used method to quantitatively assess muscular fat infiltration and atrophy. The primary objective of the Dixon sequence is to separate the water signal from the fat signal based on the chemical shift difference between water and fat. Chemical shift is caused by the shielding of a nucleus from the main magnetic field by its surrounding electrons. This shielding varies between nuclei and depends on the chemical environment. As a result, different nuclei experience a different magnetic field and therefore have a different resonance frequency. This difference in resonance frequency between nuclei, defined as chemical shift, is described as (equation 2.8):

\[ \vartheta = \frac{\omega - \omega_{\text{ref}}}{\omega_{\text{ref}}} \times 10^6 \quad (\text{equation 2.8}) \]

With \( \omega_{\text{ref}} \) the resonance frequency of the reference compound; The chemical shift between water and fat is 3.4 ppm (parts per million; figure 2.5).

Figure 2.5: Chemical shift in a \(^1\)H spectrum of skeletal muscle at 3T. The largest peak reflects the water signal (1) and resonates at 4.7 ppm. In this spectrum, fat is represented by five peaks with different chemical shifts: 2) olefinic fat, 3) methylene (CH\(_2\)) groups bound to carbonyl (COO), 4) methylene groups bound to a double carbon bond (C=C), 5) methylene (CH\(_2\)), 6) methyl (CH\(_3\)). The chemical shift between the methylene peak (5) and water (1) is used in the original 2pt-Dixon approach.
Directly after the 90° RF pulse all protons, fat and water, will precess in phase. However, over time, a phase difference will occur between water and fat because of their difference in resonance frequency. The amount of phase difference is a function of the echo time (TE) (equation 2.9).

$$\Delta \varphi = \Delta \omega TE \quad (equation \ 2.9)$$

Thomas Dixon proposed to acquire two images with two different TE’s (2pt-Dixon), one where fat and water precess in-phase and one where they precess out-phase. As graphically presented in figure 2.6, adding and subtracting these images allows us to separate the signal from water and fat. The resulting fat fraction map can be used to quantitatively estimate the percentage of fat within a muscle. Furthermore, these images are often used to estimate the cross-sectional area or volume of a muscle as a measure for muscle mass and atrophy.

This 2pt-Dixon approach is well-established, but there are several confounding factors that need to be taken into account. First, the fat-water separation is highly dependent on minor $B_0$ inhomogeneities, wherefore at least a 3pt-Dixon is recommended to compensate for this. The most common approach is in-phase, out-phase, in-phase, but several options are possible. Theoretically, the IDEAL method using phase differences of 0°, 120°, and 240° has the best SNR. Second, the three echoes can be used to correct for T2* relaxation. Third, traditional Dixon methods assume that fat protons have a single resonance frequency (methylene peak), but this is oversimplified as ~30% of the fat signal resonances at other frequencies (figure 2.5). The post-processing algorithm can be modified to take these additional peaks into account, and has shown to improve the accuracy of the fat estimation.

**T2\text{water} mapping with a multi-echo spin echo sequence**

Another relevant MR outcome measure is the T2 relaxation time of muscle, which reflects alterations in tissue water distribution within the muscle and is often used as a measure for disease activity. In muscle disorders, T2 relaxation time should be modelled with at least two components, the T2 relaxation time of muscle water ($T2_{\text{water}}$) and fat ($T2_{\text{fat}}$). This because $T2_{\text{fat}}$ (~140 ms) is relative long compared to $T2_{\text{water}}$ (~30 ms), wherefore in fat infiltrated muscle a single T2 value would primarily reflect the fat content, instead of changes in the T2 relaxation time of the muscle tissue itself. Although increased $T2_{\text{water}}$ is assumed to reflect disease activity, the origin of increased $T2_{\text{water}}$ is highly unspecific, it may reflect oedema due to inflammatory processes, sarcoplasmic leakiness, cell necrosis and denervation, or it could reflect myocyte swelling. Furthermore, decreased $T2_{\text{water}}$ is associated with fibrosis.

The $T2_{\text{water}}$ can be quantitative estimated with the multi-echo spin echo sequence based on the Carr-Purcell-Meiboom-Gill sequence. The 90° RF pulse is followed by multiple 180° RF pulses to obtain multiple echoes in a single acquisition. Following equation 2.6, the
In-phase and out-phase images are acquired to reconstruct fat and water images and a fat fraction map. In this fat fraction map of the upper leg of an unaffected volunteer, the subcutaneous fat and bone marrow are bright (high fat percentage) and the muscle is dark (low fat percentage).

signal will decay as a function of TE, therefore the obtained signal intensity over a range of TE’s can be fitted to estimate T2. As previously stated, in muscular disorders at least two components, T2\textsubscript{\text{water}} and T2\textsubscript{\text{fat}}, are assumed, wherefore the signal decay has been fitted with a bi-exponential or even a tri-exponential model (two components for fat). However, the measured signal decay is not a pure T2 decay curve, it is also sensitive to non-ideal slice pulse profiles and B1 inhomogeneities causing stimulated echoes that influence the estimated T2. Recently, a bi-component extended phase graph approach was proposed to deal with these B1 inhomogeneities (equation 2.10 and figure 2.7).

\[
SI(TE) = A \times FF_{EPG} \times EPG(T_{1fat}, T_{2fat}, ES, B1, \alpha_1, ..., \alpha_{ETL}) + A \times (1 - FF_{EPG}) \times EPG(T_{1water}, T_{2water}, ES, B1, \alpha_1, ..., \alpha_{ETL})
\]  

(equation 2.10)

With, A the signal intensity at TE = 0, FF\textsubscript{EPG} the fraction of the signal coming from fat, ES the echo spacing and \(\alpha\) the refocussing flip angles.

Techniques to assess intramuscular variation in muscle function
In chapter 6 and 7, we assessed the variations in muscle function along the proximo-distal axis of the tibialis anterior. We focussed on variations in energy metabolism, or more specifically, oxidative capacity, and the muscle perfusion and oxygenation. Below, I will give a short description on oxidative capacity and the four techniques used in chapter 6 and 7.
The main fuel for muscle contraction is adenosine triphosphate (ATP), which can be produced anaerobically and aerobically. In the anaerobic pathway, glucose is converted to pyruvic acid (glycolysis) and subsequently to lactate, resulting in 2 ATP per glucose molecule. In the presence of oxygen, theoretically up to 36 additional ATP molecules can be produced by converting the pyruvic acid to acetyl coenzyme, which is subsequently oxidized in the TCA cycle, and by subsequent oxidation of NADH via the mitochondrial electron transport chain. The latter mechanism is defined as oxidative phosphorylation. When the demand of ATP increases, for example due to exercise, the ATP production will be upregulated. In some situations, for example during the beginning of exercise, there is a short temporal mismatch between ATP demand and supply. In that case, the system involving the enzyme creatine kinase (CK) acts as a buffer system by catalysing the conversion of phosphocreatine (PCr) to creatine (Cr), thereby producing ATP from ADP and PCr:

$$\text{PCr} + \text{ADP} + H^+ \leftrightarrow \text{Cr} + \text{ATP} \quad \text{CK reaction}$$

The energy stored in ATP is released by decomposing ATP into ADP and inorganic phosphate (Pi) and used to drive other chemical reactions.

$$\text{ATP} \leftrightarrow \text{ADP} + \text{Pi} \quad \text{ATPase reaction}$$

After exercise, CK will catalyse the recovery of the PCr pool, which will now require ATP. As this ATP is produced by oxidative phosphorylation in the mitochondria, we can state that the rate of PCr recovery correlates to the muscle maximal capacity to use oxygen for ATP production. We refer to this capacity as the oxidative capacity. In this thesis, we...
assessed the recovery rate of phosphocreatine to measure the oxidative capacity of the tibialis anterior muscle using phosphorus magnetic resonance spectroscopy ($^{31}$P MRS).

$^{31}$P MR spectroscopy

Magnetic resonance spectroscopy (MRS) is based on the principle of chemical shift. As discussed for the Dixon imaging technique, chemical shift represents the difference in resonance frequency between nuclei as a result of a different chemical environment (equation 2.8). In $^{31}$P MRS we focus on the frequency range where $^{31}$P nuclei resonate. The Fourier transform of the $^{31}$P free induction decay signal obtained of muscle tissue contains multiple resonances of metabolites that posses a $^{31}$P atom (figure 2.8A). The three most common metabolites in such a muscle $^{31}$P spectrum are PCr, Pi ATP and indirectly $\text{H}^+$. Therefore, $^{31}$P MRS enables us to measure the drop in PCr and increase in Pi during exercise, and the subsequent recovery of PCr and Pi to baseline values after exercise (figure 2.8B). Assessment of the recovery rate of PCr ($k_{\text{PCr}}$) with $^{31}$P MRS is therefore a non-invasive method to measure the muscle’s oxidative capacity. Furthermore, it has been shown that the resonance frequency of Pi is pH dependent while PCr is not. Therefore the chemical shift difference between Pi and PCr can be used to determine the $\text{H}^+$ contraction and thus pH non-invasively (figure 2.8A).

**Figure 2.8:** $^{31}$P Magnetic resonance spectroscopy to measure oxidative capacity. A) Example $^{31}$P spectrum of the tibialis anterior muscle with resonances for inorganic phosphate (Pi), phosphocreatine (PCr), and the three resonances of ATP. The chemical shift between Pi and PCr can be used to estimate pH. B) Dynamic changes in the area under the peak of PCr (grey dots) during rest, isometric exercise until exhaustion at 30% of maximum force (grey block) and recovery after exercise. The black line represents the fitted mono-exponential PCr recovery curve ($k_{\text{PCr}} = 0.44 \text{ min}^{-1}$).

Muscle functional MRI

Muscle functional MRI (mf-MRI) uses changes in T2-weighted or T2*-weighted images during and after exercise to study the cellular energy metabolism and hemodynamics as both alter the muscle’s T2 and T2*. 18
One of the main determinants of T2 and T2* is the tissue water distribution, which can be altered by changes in metabolite concentration. For example, during exercise, breakdown of PCr into Pi and creatine, and the glycogenolysis into lactate will lead to an accumulation of metabolites in the cell, but this will quickly drop after exercise. These variations in metabolite concentration cause changes in osmotic pressure that will drive water-shifts between the intracellular compartment and the extracellular compartment. As the intracellular and extracellular compartment have a different T2, these water-shifts will change the muscle’s T2 during and after exercise.19

Next to this, the signal intensity of T2* weighted images will change as a result of the tissue oxygenation level, known as the blood oxygen level dependent (BOLD) effect. The BOLD effect is based on the magnetic property of hemoglobin, the oxygen carrier in the erythrocytes in blood. Hemoglobin occurs in the diamagnetic oxygenated form (O₂Hb) and the paramagnetic deoxygenated form (HHb). The paramagnetic property of HHb affects the local magnetic field B₀, wherefore a change in the ratio between O₂Hb and HHb will alter T2*. For example, T2* will lengthen when HHb decreases or O₂Hb increases. This occurs following an isometric contraction, as the hyperemic response to this exercise will increase the oxygen supply to meet the metabolic demands of recovery. Initially, this oxygen supply will exceed the demand, resulting in an O₂Hb overshoot and thus increased T2* in the exercised muscle.

Although the amount of contribution of the abovementioned processes is debated, together they result in an increase signal intensity in an exercised muscle on T2*-weighted images after isometric exercise. The amplitude and rate of this signal increase varies between subjects and muscles and depends for example on age, fibre type and training.20

**Intravoxel incoherent motion imaging**

IVIM is based on diffusion weighted imaging (DWI), a technique that images the random movement of water molecules, *i.e.* Brownian motion. In biological tissue, diffusion is restricted by cell membranes during the diffusion time. Therefore, we do not measure the pure diffusion coefficient during diffusion weighted MRI experiments, but we speak of the apparent diffusion coefficient (ADC).

Diffusion weighted MR images are created by applying two symmetric diffusion sensitizing gradients around a 180° pulse as described by Stejskal and Tanner.21 The stationary spins will be unaffected by these gradients as the phase accumulated by the first gradient will be reversed by the second gradient. However, spins that are diffusing through the tissue will experience a different gradient before than after the 180° pulse causing them to dephase and signal loss occurs. This signal attenuation is expressed as (equation 2.11):
With $S_0$ the signal intensity without diffusion weighting, $S_b$ the signal intensity with diffusion weighting, and $b$ the diffusion-sensitizing factor. The $b$-value is determined by the gradient strength, duration and time interval between the gradients. The larger the $b$-value, to more diffusion is allowed, and more signal decay is observed (figure 2.9).

\[ S_b = S_0 e^{-b \times ADC} \quad (equation \ 2.11) \]

IVIM is based on the assumption that the diffusion weighted signal decay also contains the fast movement of water molecules in the microcirculation, i.e. the perfusion in the muscle. Therefore, Le Bihan proposed to describe the diffusion weighted signal decay curve with a bi-exponential model, containing the normal ‘slow’ diffusion component (ADC), in the order of $10^{-3}$ mm$^2$/s, and the fast perfusion component, in the order of $10^{-2}$ mm$^2$/s (equation 2.12 and figure 2.9):\(^{22}\)

\[ S_b = S_0 (1 - F_p) e^{-b \times ADC} + S_0 F_p e^{-b \times D^*} \quad (equation \ 2.12) \]

Where $F_p$ is the perfusion fraction and $D^*$ is the perfusion (or pseudodiffusion) coefficient. More specifically, $F_p$ is the fractional volume of capillary blood flowing in each voxel, or in other words the ratio of the NMR-visible water flowing in the capillaries and the total volume of NMR-visible water in a particular voxel.\(^{23}\) $D^*$ depends on the average blood velocity and the mean capillary segment length. The relative perfusion or blood flow can be estimated.
by the multiplication of $F_p$ and $D^*$. Because the perfusion reflects fast movement of water molecules, it is only observed for relative low b-values (<200 s/mm²).

Near infrared spectroscopy

NIRS is a non-invasive optical technology to measure real-time tissue oxygenation. It makes use of the relative transparency of tissue to near infrared light and the absorbing characteristics of hemoglobin. The near infrared photons (wavelength: 700-1000 nm) are transmitted into the tissue via the transmit optode, where they will be scattered in the underlying tissue and absorbed by naturally occurring chromophores, i.e. the part of the molecule responsible for its colour. In biomedical applications, the three chromophores are hemoglobin, myoglobin and cytochrome-c-oxidase. Hemoglobin is the oxygen carrier in the erythrocytes in the blood and myoglobin is the oxygen binding protein in muscle tissue and thus responsible for intracellular oxygen transport. In this thesis, we focus on the chromophores of hemoglobin and myoglobin. The absorption spectra of myoglobin and hemoglobin largely overlap, wherefore it is impossible to distinguish myoglobin and hemoglobin with NIRS. The estimates of the relative contribution of hemoglobin and myoglobin to the NIRS signal is contradicting and varies between ~90% contribution of hemoglobin to only ~10-20% contribution of hemoglobin, and is likely to depend on whether the muscle is in rest or exercise and on pathology.

The oxygenated and deoxygenated forms of hemoglobin ($O_2Hb$, and $HHb$) have different absorption spectra, wherefore it is possible to obtain information about tissue oxygenation by transmitting near infrared light into the tissue and measuring the reflected light in combination with the Lambert-Beer law (figure 2.10). The original Lambert-Beer law is described as (equation 2.13):

$$OD_\lambda = \log \frac{I_0}{I} = \varepsilon_\lambda \times c \times L \quad (equation \ 2.13)$$

Where $OD_\lambda$ is the optical density of the medium, $I$ the intensity of the received light, $I_0$ the intensity of the transmitted light, $\varepsilon$ the extinction coefficient (mM⁻¹cm⁻¹), $c$ the concentration of the chromophore (mM⁻¹), $L$ the distance between the light entry and exit points, and $\lambda$ the wavelength. This original Lambert-Beer law assumes a clear non-scattering medium, but this is not a valid assumption in biological tissue. Therefore, the Lambert-Beer law needs to modified by incorporating a dimensionless pathlength factor $B$, also referred to as the differential pathlength factor (DPF). Furthermore, we need to take into account the independent light loss due to scattering ($OD_{R,\lambda}$), resulting in the modified Lambert-Beer law (equation 2.14):

$$OD_\lambda = \varepsilon_\lambda \times c \times L \times B + OD_{R,\lambda} \quad (equation \ 2.14)$$
As OD\textsubscript{\textit{R,\lambda}} is difficult to determine, absolute concentration cannot be accurately estimated\textsuperscript{25}. Nevertheless, when we assume that OD\textsubscript{\textit{R,\lambda}} is constant, this allows us to determine the concentration change of a chromophore from the change in optical density (equation 2.15).

\[ \Delta c = \frac{\Delta OD\textsubscript{\lambda}}{\varepsilon\textsubscript{\lambda} \times L \times B} \]  \hspace{1cm} \textit{(equation 2.15)}

Equation 2.15 can be solved when there is only one chromophore in the medium. When there are more chromophores in the medium, we need to measure at least as many wavelengths as the number of present chromophores. In this thesis, we used three wavelengths: 765, 857 and 859 nm to extract O2Hb and HHb concentration changes.

Figure 2.10: Schematic representation of the near infrared spectroscopy setup. The near infrared light is transmitted into the tissue with the transmit optode, and the reflected light is detected at the receive optode. The sensitive volume is banana-like shaped, and the distance between the two optodes determines the measurement depth.
References
Chapter 3

Lower extremity muscle pathology in myotonic dystrophy type 1 assessed by quantitative MRI

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Abstract

Objective: To determine the value of quantitative MRI to provide imaging biomarkers for disease in 20 different upper and lower leg muscles of myotonic dystrophy type 1 (DM1) patients.

Methods: We acquired images covering these muscles in 33 genetically and clinically well-characterized DM1 patients and 10 unaffected controls. MR images were recorded with a Dixon method to determine muscle fat fraction, muscle volume and contractile muscle volume, and a multi-spin echo sequence to determine T2 water relaxation time (T2\text{water}), reflecting putative oedema.

Results: Muscles in DM1 patients had higher fat fractions than muscles of controls (15.6% ± 11.1% vs. 3.7% ± 1.5%). Also, patients had smaller muscle volumes (902 ± 232 cm³ vs. 1097 ± 251 cm³), contractile muscle volumes (779 ± 247 cm³ vs. 1054 ± 246 cm³), and increased T2\text{water} (33.4 ± 1.0 ms vs. 31.9 ± 0.6 ms), indicating atrophy and oedema, respectively. Lower leg muscles were affected most frequently, especially the gastrocnemius medialis and soleus. Distribution of fat content per muscle indicated gradual fat infiltration in DM1. Between-patient variation in fat fraction was explained by age (~45%), and another ~14% by estimated progenitor CTG repeat length ($r^2 = 0.485$) and somatic instability ($r^2 = 0.590$). Fat fraction correlated with the six-minute walk test ($r = -0.553$) and muscular impairment rating scale ($r = 0.537$), and revealed subclinical muscle involvement.

Conclusion: This cross-sectional quantitative MRI study of 20 different lower extremity muscles in DM1 patients revealed abnormal values for muscle fat fraction, volume and T2\text{water}, which therefore may serve as objective biomarkers to assess disease state of skeletal muscles in these patients.
Introduction

Myotonic dystrophy type 1 (DM1) is a multisystem disease characterised by progressive muscle weakness, myotonia, and cognitive dysfunction.\(^1,2\) Its genetic cause is an expansion of a CTG trinucleotide repeat in the dystrophia myotonica protein kinase (DMPK) gene, whereby CTG repeat length correlates with disease severity.\(^3,4\)

Although currently DM1 cannot be treated, promising therapeutic approaches are emerging.\(^3,5\) Objective assessment of these approaches in clinical trials requires quantitative biomarkers that can assess disease in individual muscles. Moreover, such biomarkers are important to follow natural progression of DM1 and to understand its pathophysiological mechanisms. As MRI is a non-invasive, quantitative method that can provide relevant biomarkers like muscle volume, fat fraction, and T2 relaxation time of water, it is very suited to study neuromuscular disorders.\(^6-8\) These biomarkers report on the respective pathological processes of muscle atrophy, fatty infiltration, and intra- or extra-cellular oedema, the latter assumed to associate with disease activity.\(^6\) Previously, it has been shown that each muscle appears to be differently affected in DM1.\(^9\)

The aim of our study is therefore to determine the value of quantitative muscle MRI to provide imaging biomarkers for fat infiltration, muscle mass and T2 relaxation of putative oedema in a broad set of upper and lower leg muscles in well-characterized DM1 patients. We first determined if these imaging measures differ between patients and unaffected controls. Second, we examined if DM1 exhibits a specific pattern and distribution of muscular fat infiltration. Third, we associated these MRI measures with age, CTG repeat length and clinical outcomes.

Materials and Methods

Participants

In this cross-sectional study, we included participants with genetically confirmed DM1 and unaffected, apparently healthy controls comparable in age and sex. The DM1 patients participated in the multicentre randomised controlled trial OPTIMISTIC.\(^10\) We invited patients from the study sites in the Netherlands (Radboud university medical center, Nijmegen) and France (Henri Mondor University Hospital, Paris). Participants who had a pacemaker, a prosthetic implant in the lower extremity, claustrophobia or were unable to lie in supine position for 60 minutes were excluded.

Standard Protocol Approvals, Registrations, and Patient Consents

This study was conducted according to the principles of the Declaration of Helsinki (version October 2013) and the Medical Research Involving Human Subjects Act (WMO). The local medical ethical committees approved this study, and prior written informed consent
was obtained from all participants. This trial is registered with ClinicalTrials.gov, number NCT02118779.

Clinical and genetic assessment of DM1 patients
The complete study design for the clinical and genetic assessment is described by van Engelen. Physical capacity was evaluated by performing a six-minute walk test (6MWT), and disease severity using the muscular impairment rating scale (MIRS). Moreover, activity and social participation were assessed with the DM1-Activ-c questionnaire, and fatigue with the subscale fatigue severity of the Checklist Individual Strength (CIS - fatigue). At the time of recruitment for OPTIMISTIC, blood DNA samples were taken and subjected to small-pool PCR and Southern blotting. Since only a small amount (300 pg) of DNA template was used, distinct bands of varying lengths derived from the CTG repeat expansion were observed. Somatic instability usually results in the repeats increasing in length over time, wherefore the lower edge of these bands on the blot was used to estimate the inherited, or progenitor, allele length (CTG$_{ePAL}$) and the densest region of bands to estimate the most frequent, or modal, repeat length at time of sampling (CTG$_{modal}$). Somatic instability was estimated by subtracting CTG$_{modal}$ from CTG$_{ePAL}$. Furthermore, we tested the presence of Acili-sensitive variant repeats in the expanded allele, since they may result in milder symptoms and delayed disease onset. Individual-specific residual variation in somatic instability (CTG$_{ResVI}$) not accounted for by age at sampling, estimated progenitor allele length, or presence of variant repeats was calculated using data from the entire OPTIMISTIC cohort.

MRI acquisition
The upper and lower legs of patients and unaffected controls were examined using MRI, with random selection of the right or left leg. In patients, this MRI took place within one month of recruitment for the OPTIMISTIC trial. The MRI was performed on 3T MRI systems (Siemens, Tim TRIO (Nijmegen) or Magnetom Verio (Paris), Erlangen, Germany), using a spine coil combined with phased arrays placed around the lower extremity. All images were in the transverse plane, positioned at the thickest part of the lower leg and middle of the femur in the upper leg and oriented orthogonal to the tibia or femur bone, respectively (figure 3.1). First, we collected T1 weighted spin echo (SE) images (repetition time (TR) = 670 ms, echo time (TE) = 10 ms, field of view (FOV) = 192x192 mm, voxel size = 1x1x5 mm, number of slices = 27, slice gap = 0 mm). Thereafter, we acquired turbo inversion recovery magnitude (TIRM) images with a similar FOV, resolution and slices (TR = 4,000 ms, TE = 41 ms, inversion time (TI) = 220 ms). Furthermore, we collected data using 3D Dixon sequences (TR = 10 ms, FA = 3°, FOV = 256x192 mm, voxel size = 1x1x5 mm and number slices = 32) either in a 2-point version (TE1/TE2 = 2.45/3.675 ms) or 3-point version (TE1/TE2/TE3 = 2.31/3.68/5.07 ms). Finally, at the Nijmegen site, multi-spin echo (MSE) images were acquired (TR = 3,720 ms, echo train length (ETL) = 17, echo-spacing (ES) = 8 ms, voxel size = 1.5x1.5x10 mm, number of slices = 5, slice gap = 20 mm).
Figure 3.1: Positioning of image slices, muscle delineation, and data processing pipeline. 
A) The positioning of image slices at the lower and upper leg. The large blocks indicate the total area covered by MRI. Muscles were delineated on 17 slices of the Dixon images, depicted in light and dark grey using the out-phase images, water images, and fat fraction map. The dark grey slices correspond in slice position with the $T_2^{\text{water}}$ map. B) Example of muscle delineation of the 12 quantitatively analysed upper leg muscles on the fat fraction map. C) Example of muscle delineation of the 8 quantitatively analysed lower leg muscles on the fat fraction map D) Schematic overview of the data processing pipeline. Muscle abbreviated as: TA/TP = tibialis anterior/posterior; EDL = extensor digitorum longus; P = peroneus; FDL = flexor digitorum longus; GL/GM = gastrocnemius lateralis/medialis; SOL = soleus; RF = rectus femoris; VL/VI/VM = vastus lateralis/intermedius/medialis; S = sartorius; BFS/BFL = biceps femoris short/long head; ST = semitendinosus; SM = semimembranosus; G = gracilis; and AM/AL = adductor magnus/longus.

Data Analysis
Semi-quantitative MRI: An experienced radiologist (MP) scored 7 lower leg and 12 upper leg muscles (figure 3.1B/C) semi-quantitatively for signs of fat infiltration and putative oedema on T1 weighted SE and TIRM images, respectively. For the T1 weighted images, the ordinal Lamminen score (range 1-4) was used, where 1 represents normal muscle signal intensity and 4 reflects a total, homogeneous hyperintense signal change in entire muscle. The TIRM images were scored with the ordinal Malattia score (range 0-2), with 0 meaning no muscle signal abnormalities and 2 indicating a high degree of signal hyperintensity. The Lamminen and Malattia individual muscle scores of each participant were summed over all lower extremity muscles for comparison between DM1 patients and unaffected controls.
**Quantitative MRI:** Data analysis was performed using Matlab version 2014b (Mathworks, Natick, MA, USA). A fat fraction map, with voxel values ranging from 0% to 100%, was calculated from Dixon sequence data by voxel-wise dividing the signal intensity of the fat image by the summed signal intensity of the fat and water image (equation 3.1):

\[
Fat Fraction map = \frac{Fat}{Fat + Water} \times 100\% \quad (equation\ 3.1)
\]

The T2 relaxation time of muscle water \((T2_{water}\) in ms) was determined per voxel by fitting MSE data with a bi-component extended phase graph model as described by Marty *et al* (equation 3.2).

\[
SI(TE) = A \times FF_{EPG} \times EPG(T1_{fat}, T2_{fat}, ES, B1, \alpha_1, \ldots, \alpha_{ETL}) \\
+ A \times (1 - FF_{EPG}) \times EPG(T1_{water}, T2_{water}, ES, B1, \alpha_1, \ldots, \alpha_{ETL}) \quad (equation\ 3.2)
\]

We chose this model, as it takes into account the effect of B1 inhomogeneities on \(T2_{water}\). Increased \(T2_{water}\) is thought to reflect putative oedema and disease activity.

\(T1_{water}\) and \(T1_{fat}\) were assumed to be 1,400 ms and 365 ms, respectively.

For the quantitative evaluation, we analysed the same 19 lower extremity muscles as evaluated semi-quantitatively, plus the flexor digitorum longus, i.e. in total 8 calf and 12 thigh muscles. They were delineated on five distal, middle, and proximal slices of the Dixon images of the upper and lower leg (figure 3.1A-C), using MIPAV (http://mipav.cit.nih.gov), thereby avoiding subcutaneous fat contamination (figure 3.1B-C). Furthermore, delineation was performed on the five slices of the Dixon images corresponding in position to the \(T2_{water}\) map and transposed to this \(T2_{water}\) map (figure 3.1A). Average fat fraction, \(T2_{water}\), and estimated muscle volume were determined for each muscle (figure 3.1D). Average fat fraction was defined as the mean over all voxels, and muscle volume as the number of voxels multiplied by the voxel volume using the 15 delineated slices of the fat fraction map.

In addition, contractile muscle volume - the remaining muscle tissue still able to contract - was calculated as (equation 3.3):

\[
contractile\ muscle\ volume = muscle\ volume \times (1 - fat\ fraction) \quad (equation\ 3.3)
\]

To obtain \(T2_{water}\), the voxels with severe fat infiltration were excluded (\(FF_{EPG} > 50\%\)), since sufficient water signal is required for reliable fitting of \(T2_{water}\).
voxels were more than 10% of the total number of voxels in that muscle, $T_2_{\text{water}}$ was calculated as the average over these voxels in the five $T_2_{\text{water}}$ map slices. Muscles were excluded from the analysis if visual inspection revealed the presence of movement artefacts. For the fat fraction and contractile muscle volume analysis, muscles were also excluded if artefacts in the fat/water reconstruction made the fat fraction estimate unreliable.

Outcomes for the 20 individual muscles were combined, resulting in per participant a value for the entire lower extremity, and a value for the lower and upper leg level (figure 3.1D). For the fat fraction and $T_2_{\text{water}}$, muscles were combined by taking the average over all 20 muscles per participant, and the average over the 8 lower leg and 12 upper leg muscles separately. For the muscle volume and contractile muscle volume, we took the sum of these muscles per participant. The outcome measures for the entire lower extremity were only calculated if upper and lower leg muscle data was available.

To investigate if muscles with signs of fat infiltration differ in $T_2_{\text{water}}$ with muscles without fat infiltration, muscles were divided in two groups based on their fat fraction. For all 20 muscles a cut-off value was defined as the average fat fraction + two standard deviations (SD) of that muscle in unaffected controls (figure 3.1D). It was observed that, in unaffected controls, $T_2_{\text{water}}$ differed between muscles, e.g. average $T_2_{\text{water}}$ was 27.6 ms for the rectus femoris and 33.8 ms in the gastrocnemius medialis (Friedman test: $p < 0.001$, figure S3.5). Therefore, for each muscle, we determined the absolute difference in ms relative to the average $T_2_{\text{water}}$ over the 10 unaffected controls in that particular muscle ($rT_2_{\text{water}}$). Thereafter, we averaged per participant the $rT_2_{\text{water}}$ of the individual non-fat infiltrated muscles and $rT_2_{\text{water}}$ of the fat infiltrated muscles to obtain for both a value for the lower extremity (figure 3.1D).

**Statistical analysis**

Statistical analysis was performed using IBM SPSS Statistics version 22 (SPSS, Chicago, IL, USA) or R statistics using the RStudio package. Differences between unaffected controls and DM1 patients were assessed using a two-tailed Mann-Whitney U test. A two-tailed one sample $t$-test to zero was applied to determine if $rT_2_{\text{water}}$ in DM1 differed from unaffected controls. The difference in $rT_2_{\text{water}}$ between non-fat and fat infiltrated muscles was evaluated using a two-tailed Wilcoxon signed rank test. Fat fraction and $T_2_{\text{water}}$ were correlated with the Lamminen and Malattia scoring using the Spearman correlation, respectively. Multivariate linear regression was applied to predict fat fraction using age, CTG_{ePAL} and CTG_{ResSI}. Fat fraction, age, and CTG_{e99L} were log transformed to increase linearity and improve normality. Model selection was based on the Akaike information criterion (AIC) for each model using a backwards stepwise selection procedure implemented using the step function in R. Whole lower extremity quantitative MRI measures were correlated with the MIRS and 6MWT. In addition, the 6MWT was correlated with quantitative MRI outcome measures of relevant
muscle groups (knee extensors, knee flexors, ankle dorsiflexors, and ankle plantarflexors), using Bonferroni correction. Significance level was set at \( p = 0.05 \). Data are represented as mean ± SD, unless otherwise stated.

Data availability statement
We encourage researchers wishing to access the generated anonymized data to submit a request to the corresponding author. Requests for access will be reviewed by a panel involving LH, AH and BGMvE, and a data access agreement needs to be signed.

Results
Participants
The age and fraction of males/females were not different between the 33 DM1 patients (24 in Nijmegen, 9 in Paris) and 10 unaffected controls (table 3.1). The clinical forms by which the DM1 patients were affected are presented in table 3.1. According to the clinical scores, like the MIRS, DM1-Activ-c, 6MWT and CIS-fatigue, patients varied from mildly to severely affected, and exhibited a wide range of CTG repeat lengths (table 3.1). Semi-quantitative evaluation of the MRI data included 615 muscles in DM1 patients and 190 muscles in unaffected controls, and quantitative evaluation included 633 muscles in DM1 patients and 200 muscles in unaffected controls. Twelve upper leg muscles were not analysed, because one patient could not lie in supine position for long enough to acquire data with all MRI sequences. Further details on the number of muscles and participants analysed per MRI outcome measure are presented in table 3.2a and 3.2b.

MRI outcome measures differ between DM1 patients and unaffected controls
The T1 weighted MR images of lower extremity muscles in DM1 patients displayed hyperintense lesions indicative of fat infiltration (figure 3.2A-D). The semi-quantitative Lamminen score of these lesions was higher for muscles of DM1 patients compared to unaffected controls (table 3.2a; sum Lamminen score (mean ± SD); DM1: 28.3 ± 9.4; controls: 19.2 ± 0.4; \( p < 0.001 \)), with a more substantial relative increase in the lower leg muscles than in the upper leg muscles.

In TIRM images of DM1 patients, we observed hyperintense areas indicative for oedema (figure 3.2E-H). The semi-quantitative Malattia score of these hyperintense lesions was higher compared to controls (table 3.2a; sum Malattia score; DM1: 9.7 ± 7.6; controls: 0.4 ± 0.7; \( p < 0.001 \)). Most TIRM positive lesions were observed in the lower leg. In the 33 scored patients, the number of lower leg muscles with these lesions ranged from 20 tibialis posterior muscles (Malattia score > 0) to 25 tibialis anterior and gastrocnemius medialis muscles. In the upper leg, the vastus lateralis and intermedius were most often TIRM positive (12 and 14 muscles, respectively), and only 4 of the scored gracilis, sartorius and adductor longus muscles were TIRM positive. Of the 422 muscles that showed no fat infiltration (Lamminen score 1), 83 muscles (20%) showed hyperintense areas on the TIRM images, i.e. Malattia
Table 3.1: Participant characteristics, patient clinical performance, and CTG repeat length.

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>DM1 patients (n = 33)</th>
<th>Unaffected controls (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years</td>
<td>45 ± 12</td>
<td>45 ± 14</td>
</tr>
<tr>
<td>Sex male/female – no. (% male)</td>
<td>18/15 (55%)</td>
<td>5/10 (50%)</td>
</tr>
<tr>
<td>MIRS – median (min-max)</td>
<td>3 (1 to 5)</td>
<td></td>
</tr>
<tr>
<td>Clinical classification</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Congenital form – no. (age of onset: first month)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Infantile form – no. (age of onset: 1 month – 10 years)</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Juvenile form – no. (age of onset: 10-20 years)</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Adult form – no. (age of onset: 20-40 years)</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Late-onset form – no. (age of onset: after 40 years)</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Genetics: CTG repeat length</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTG\textsubscript{ePAL}</td>
<td>241 ± 117</td>
<td></td>
</tr>
<tr>
<td>CTG\textsubscript{modal}</td>
<td>446 ± 230</td>
<td></td>
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<tr>
<td>Somatic instability</td>
<td>205 ± 142</td>
<td></td>
</tr>
<tr>
<td>Acil-sensitive variant repeat – no. of patients</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>CTG\textsubscript{ResSI}</td>
<td>-0.2 ± 1.2</td>
<td></td>
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<tr>
<td>Physical activity and capacity</td>
<td></td>
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</tr>
<tr>
<td>DM1-Activ-c score</td>
<td>59 ± 18</td>
<td></td>
</tr>
<tr>
<td>6MWT in metres</td>
<td>417 ± 111</td>
<td></td>
</tr>
<tr>
<td>Fatigue</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CIS-fatigue</td>
<td>44 ± 7</td>
<td></td>
</tr>
</tbody>
</table>

All data are presented as mean ± SD, unless stated otherwise. MIRS: Muscular impairment rating scale. CTG\textsubscript{ePAL}: Estimated inherited progenitor CTG repeat length. CTG\textsubscript{modal}: modal CTG repeat length in blood at time of recruitment. Somatic instability: Increase in CTG repeat length over lifetime, defined as CTG\textsubscript{modal} – CTG\textsubscript{ePAL}. CTG\textsubscript{ResSI}: Individual-specific residual variation in somatic instability. 6MWT: Six-minute walk test. CIS-fatigue: Checklist individual strength, fatigue subscale.

score 1 or 2. Detailed results on the individual Lamminen and Malattia scores are presented in the supplementary (table S3.1 and figure S3.1).

For the quantitative determination of muscle fat fraction, muscle volume, and contractile muscle volume, we analysed the MR images obtained with the Dixon method (figure 3.3, table 3.2b). These analyses revealed an increased fat fraction for DM1 patients compared to unaffected controls: 15.6 ± 11.1% vs. 3.7 ± 1.5%, respectively (p < 0.001; figure 3.3A). Analysis of these images also revealed that muscle volume was reduced in DM1 patients (DM1: 902 ± 232 cm\textsuperscript{3}; controls: 1097 ± 251 cm\textsuperscript{3}; p = 0.028), as well as contractile muscle volume (DM1: 779 ± 247 cm\textsuperscript{3}; controls: 1054 ± 246 cm\textsuperscript{3}; p = 0.008) (figure 3.3B/C).

To determine T2\textsubscript{water} values as a quantitative parameter for putative oedema-associated disease activity, we analysed MR images of the lower extremity obtained with a multi spin-
echo sequence. The $T_2^{\text{water}}$ maps derived from these images typically reveal increased $T_2^{\text{water}}$ in some muscles of DM1 patients, for example in the adductor magnus (figure 3.3D). On average, $T_2^{\text{water}}$ was elevated in muscles of DM1 patients compared to unaffected controls: $33.5 \pm 1.0$ ms vs. $31.9 \pm 0.6$ ms respectively ($p < 0.001$; figure 3.3E, table 3.2b). To account for differences in intrinsic $T_2^{\text{water}}$ between muscles, we also evaluated $rT_2^{\text{water}}$ to quantify oedema-associated disease activity relative to the muscles of unaffected controls. $rT_2^{\text{water}}$ was elevated in non-fat infiltrated muscles ($+1.4 \pm 1.5$ ms, $p < 0.001$) and fat infiltrated muscles ($+2.4 \pm 1.3$ ms, $p < 0.001$), and elevated in fat infiltrated vs. non-fat infiltrated muscles ($p = 0.015$; figure 3.3F). Although these $rT_2^{\text{water}}$ findings are highly significant at group level, some muscles of DM1 patients also showed normal $T_2^{\text{water}}$.

Figure 3.2: Typical example of T1 weighted and TIRM images of lower extremity muscles. A) T1 weighted image of the lower leg of an unaffected control. B) T1 weighted image of the lower leg of a myotonic dystrophy type 1 (DM1) patient showing fat infiltration in the soleus (white arrow). C) T1 weighted image of the upper leg of an unaffected control. D) T1 weighted image of the upper leg of a DM1 patient showing fat infiltration in the vasti muscles and hamstring muscles (white arrows). E) TIRM image of the lower leg of an unaffected control. F) TIRM image of the lower leg of a DM1 patient showing hyperintense lesions reflecting oedema associated with disease activity in the soleus (white arrow). G) TIRM image of the upper leg of an unaffected control. H) TIRM image of the upper leg of a DM1 patient, showing hyperintense lesions in the vastus lateralis and medialis (white arrow), while fat infiltration results in a hypointense signal in the vastus intermedius and medialis (grey arrow).
Table 3.2a: Semi-quantitative values of MRI measures in myotonic dystrophy type 1 (DM1) patients versus unaffected controls.

<table>
<thead>
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<th>Outcome measures</th>
<th>Number of participants</th>
<th>Number of muscles</th>
<th>Outcome values</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>DM1 patients</td>
<td>Unaffected controls</td>
<td>DM1 patients</td>
</tr>
<tr>
<td>Lamminen sum score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower extremity</td>
<td>32</td>
<td>10</td>
<td>608</td>
</tr>
<tr>
<td>Lower leg</td>
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<td>10</td>
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</tr>
<tr>
<td>Upper leg</td>
<td>32</td>
<td>10</td>
<td>384</td>
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<tr>
<td>Malattia sum score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower extremity</td>
<td>32</td>
<td>10</td>
<td>608</td>
</tr>
<tr>
<td>Lower leg</td>
<td>33</td>
<td>10</td>
<td>231</td>
</tr>
<tr>
<td>Upper leg</td>
<td>32</td>
<td>10</td>
<td>384</td>
</tr>
</tbody>
</table>

Outcome measures are presented as mean ± SD at the participant level
Table 3.2b: Quantitative values of MRI measures in myotonic dystrophy type 1 (DM1) patients versus unaffected controls.

<table>
<thead>
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<th>p-value</th>
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<td>DM1 patients</td>
<td>Unaffected controls</td>
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<td>199</td>
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<td>224</td>
<td>80</td>
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<tr>
<td>Upper leg</td>
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<tr>
<td>Upper leg</td>
<td>23</td>
<td>10</td>
<td>268</td>
<td>120</td>
</tr>
</tbody>
</table>

Outcome measures are presented as mean ± SD at the participant level.
Figure 3.3: Quantitative MRI measures in unaffected controls (controls) versus myotonic dystrophy type 1 (DM1) patients. A) Fat fraction. B) Muscle volume. C) Contractile muscle volume. D) Example of a T2\textsubscript{water} map of the upper leg of a DM1 patient showing increased T2\textsubscript{water} in the adductor magnus (white arrow). E) T2\textsubscript{water}. F) Difference in T2\textsubscript{water} compared to unaffected controls (rT2\textsubscript{water}) in non-fat infiltrated (non-fat) and fat infiltrated muscles (fat) of DM1 patients. Data are presented as Tukey boxplots.*** p<0.001, rT2\textsubscript{water} different from unaffected controls.

The quantitative values for fat fraction and T2\textsubscript{water} in DM1 muscles correlate well with the corresponding semi-quantitative scores (Lamminen score vs. fat fraction, $\rho = 0.631$, $p < 0.001$, and Malattia score vs. T2\textsubscript{water}, $\rho = 0.400$, $p < 0.001$). Average values for quantitative MRI measures per individual muscle are presented in the supplementary (figure S3.2 to S3.5).

To assess prevalence of fat infiltration in individual muscles of DM1 patients, we analysed the fat fractions per muscle (figure 3.4A-D). Fat fraction was higher in lower leg muscles compared to upper leg muscles (lower leg: 22.5±18.2%; upper leg: 10.3±7.6%; Wilcoxon signed rank test: $p = 0.001$). Among the lower leg muscles, the gastrocnemius medialis and soleus exhibited the highest fat fractions, while the vastus intermedius had the highest fat fraction in the upper leg (figure 3.4E/F). The tibialis posterior appeared to be spared in the lower leg. The occurrence of varying fat fractions in individual lower extremity muscles...
Figure 3.4: Fat infiltration pattern in the lower extremity muscles of myotonic dystrophy type 1 (DM1) patients. A/B) Typical examples of fat fraction maps in the lower leg muscles of DM1 patients, showing fat infiltration as hyperintense areas. C/D) Typical examples of fat fraction maps in the upper leg muscles of DM1 patients. E) Fat infiltration pattern of the lower leg muscles presented as Tukey boxplots. F) Fat infiltration pattern in the upper leg muscles presented as Tukey boxplots. G) Prevalence of fat infiltration in the lower leg muscles; each dot represents one muscle in one patient. H) Prevalence of fat infiltration in the upper leg muscles. Muscles are abbreviated as: TA/TP = tibialis anterior/posterior; EDL = extensor digitorum longus; P = peroneus; FDL = flexor digitorum longus; GL/GM = gastrocnemius lateralis/medialis; SOL = soleus; RF = rectus femoris; VL/VI/VM = vastus lateralis/intermedius/medialis; S = sartorius; BFS/BFL = biceps femoris short/long head; ST = semitendinosus; SM = semimembranosus; G = gracilis; and AM/AL = adductor magnus/longus.
exhibited a pyramid-like distribution, with fewer highly fat infiltrated muscles than non-fat infiltrated muscles, indicating a gradual process of fat infiltration (figure 3.4G/H).

The effect of age and CTG repeat length on muscle fat infiltration
To assess the effect of age and CTG repeat length on fat infiltration, we applied a multivariate linear regression model, which uncovered age as the primary predictor of a participant’s average lower extremity fat fraction ($r^2 = 0.447, p < 0.001$). Fitting of the data was further improved by incorporating CTG_{ePAL} ($r^2 = 0.485$, model p-value, $p < 0.001$, parameter p-value, age: $p < 0.001$, CTG_{ePAL}: $p = 0.090$), and CTG_{resSt} with interactions between all parameters ($r^2 = 0.590$, $p = 0.001$; see also table S3.2). For the latter model none of the individual parameters reached statistical significance, but stepwise model comparison using the Akaike information criterion confirmed this as the most informative model. The two participants with Acil-sensitive variant repeats and a successful fat fraction calculation had lower mean fat fractions than the other DM1 patients (3.7% vs. 16.0%, Welch’s $t$-test, $p = 0.002$, figure S3.6).

Fat fraction and contractile muscle volume correlate with disease severity and physical performance
We investigated if the quantitative MRI biomarkers explored in this study, correlate with clinical features of the disease. The average lower extremity fat fraction correlated with the MIRS disease severity score ($r = 0.537, p = 0.005$; figure 3.5A). However, the MIRS score correlated neither with contractile muscle volume (figure 3.5B; $r = -0.197, p = 0.335$), muscle volume ($r = -0.119, p = 0.525$) and $T_2$ water ($r = 0.389, p = 0.074$). In the two patients with a MIRS score of 2, in which a manual muscle test does not find distal weakness, 13 of the 16 muscles in the lower legs showed a higher fat content than these muscles in controls. In 6 of these muscles the fat fraction was more than 10%. In the 16 patients with MIRS score 3, in which no proximal weakness was found in a manual muscle test, we detected increased fat content in 54 upper leg muscles of which 37 had a fat fraction of more than 10%.

Fat fraction and contractile muscle volume both correlated with the physical capacity measure 6MWT (fat fraction: $r = -0.553, p = 0.003$; contractile muscle volume: $r = 0.403, p = 0.041$; figure 3.5C/D), but 6MWT did not correlate with muscle volume and ($r = 0.245, p = 0.184$) and $T_2$ water ($r = -0.251, p = 0.261$). For the individual muscle groups after Bonferroni correction, 6MWT correlated with the fat fraction of the ankle dorsiflexors ($r = -0.498, p = 0.007$) and ankle plantarflexors ($r = -0.574, p = 0.001$). The 6MWT did not correlate with any of the other MRI outcome measures of the functional muscle groups (table S3.3).
Figure 3.5: Quantitative MRI measures vs. disease severity and physical capacity. A) Fat fraction vs. muscular impairment rating scale (MIRS). B) Contractile muscle volume vs. MIRS. C) Fat fraction vs. six-minute walk test. D) Contractile muscle volume vs. six-minute walk test.

Discussion

In this international multicentre cross-sectional quantitative MRI study, we observed ~3 times more fat, a ~26% reduction in muscle mass, and increased values for MRI markers of pathological oedema in 20 lower extremity muscles of DM1 patients compared to unaffected controls. In these genetically and clinically well-characterised DM1 patients, quantitative MRI measures correlated with age, CTG repeat length, and clinical outcomes for physical capacity and disease severity.

Using quantitative MRI derived muscle fat fractions, we observed more fat infiltration in muscles of DM1 patients than those in unaffected controls. This fat infiltration was more severe in distal muscles than proximal muscles, corresponding with DM1 being a distal myopathy. The gastrocnemius medialis and soleus were most severely affected, and thereafter the tibialis anterior. In contrast, clinically, ankle dorsiflexion weakness appears to dominate plantarflexion weakness. MRI changes may precede the soleus and gastrocnemius clinical weakness, considering that strength measurements of this strong muscle group are difficult to assess. In agreement with the MR results, force measurements using a dynamometer
showed a similar decline in ankle plantarflexion and ankle dorsiflexion. In the upper leg, the vastus intermedius was the most severely fat infiltrated muscle. This corresponds with the qualitative observation of a preferential semilunar anterolateral perifemoral area of fatty infiltration on T1 weighted images. The typical fat infiltration pattern recorded by our quantitative MRI investigation largely agrees with the semi-quantitative Lamminen score in this and other qualitative MRI studies. However, in the objective assessment of disease severity and progression, and the evaluation of clinical trials, quantitative analysis of MR images covering entire muscles – as presented in this study – is essential. To date, only one quantitative MRI study, limited to the tibialis anterior, has been performed in DM1.

Although the results of the MRI-determined changes in fat fraction identify it as a promising biomarker to assess treatments, its real value must be investigated in longitudinal studies.

An interesting feature of the lower extremity muscles of DM1 patients is their distribution over different levels of fat infiltration. This shows that, in DM1 patients, the higher the fat content in muscles the less prevalent they are, which is reflected in a pyramid-like shape for their distribution over different fat fractions. This is in contrast to lower extremity muscles in facioscapulohumeral dystrophy (FSHD) patients, where a quasi-binary distribution over low and high fat infiltration was observed. This suggest that, in the muscles of DM1 patients, fat infiltration progresses more gradually than in FSHD, in which individually affected muscles exhibit a relatively fast transition from apparently normal to completely fat infiltrated.

By quantitative MRI we determined an average reduction in muscle volume and contractile muscle volume in DM1 patients compared to unaffected controls, in line with an earlier observation for the tibialis anterior. Reduced muscle cross-sectional areas have also been reported for Duchenne muscular dystrophy, Charcot-Marie-Tooth 1A, and inclusion body myositis. The reduced muscle volume in DM1 patients – containing both fat and muscle tissue – indicates that contractile muscle volume is reduced by muscle atrophy and fat infiltration. These findings support the use of muscle volume and contractile muscle volume as MRI biomarkers to monitor changes in muscle mass.

To analyse the presence of putative oedema associated with disease activity, we determined the Malattia score on TIRM images and the T2 relaxation time of muscle water (T2\text{water}). We found an increased sum Malattia score and increased average T2\text{water} in the muscles of DM1 patients. The results indicate that oedema associated processes occur before the presence of fat infiltration, i.e. 20% of the muscle with Lamminen score 1 had a Malattia score 1 or 2, and T2\text{water} was increased in non-fat infiltrated muscles. Increased T2\text{water} in muscles with apparently normal fat fractions was also observed in Pompe disease, Duchenne muscular dystrophy, inclusion body myositis, and Charcot-Marie-Tooth 1A. This suggests that T2\text{water} is an early marker of pathological changes in muscles of DM1 patients, and may be useful for evaluating early treatment effects. Increased T2\text{water} indicates oedema caused by...
inflammation, necrosis, or swelling of myocytes, although, as in other muscular dystrophies, it remains unknown what the precise origins are for the observed increases in all cases. After establishing the fat fractions for the lower extremity muscles of DM1 patients, we compared the amount of fat infiltration in these muscles with determinants of disease severity, i.e. age and CTG repeat length. In our DM1 patients, age explained ~45% of the between-participant variation in fat fraction. This variation is mainly attributed to the progressive nature of DM1, because in healthy volunteers the effect of age is negligible small (1-2% higher fat fraction in elderly compared to young persons). An additional amount of this variation was explained by the estimated progenitor allele length (~48%) and individual-specific residual variation in somatic instability (~59%). This suggest that on-going somatic CTG expansions accrued during a patient’s lifetime directly contributes to disease severity, as was established for age at onset. Previously, a relation between the radiological scoring of T1 weighted images and the average CTG repeat length at time of recruitment was observed. Furthermore, the DM1 patients who carried AciI-sensitive variant repeats – most likely CCG or CGG variants – exhibited slower increase in fat fractions than DM1 patients without detectable variant repeats. This is consistent with the literature on the effect of variant repeats, and is probably mediated via the stabilising effect of variant repeats reducing the rate of somatic expansion. Individual parameters or interactions in the multivariate regression models were not significant and only two patients with AciI-sensitive variant repeats were assessed. Nevertheless, our data highlights the value of genetic profiling for future randomised controlled trials, since age alongside CTG repeat length determine the severity of muscle involvement at baseline, and most likely its progression over time.

Finally, we investigated if the quantitative MRI outcome measures reflect disease severity and physical capacity of the DM1 patients. We observed that the 6MWT results correlate with fat fraction and contractile muscle volume, and that the MIRS disease score correlates with fat fraction. This is in agreement with other DM1 studies that showed correlations between semi-quantitative scored fat infiltration vs. 6MWT, and between fat infiltration and contractile muscle volume of the tibialis anterior vs. ankle dorsiflexion torque. The fat fraction in the ankle dorsiflexors and plantarflexors had the best correlation with the 6MWT, indicating that assessment of the lower leg gives a good representation of the patients physical capacity. This is probably because, in DM1, these muscles are affected earliest. Furthermore, we observed in patients with MIRS score 2, in which no distal weakness is expected, increased fat fractions in lower leg muscles and in patients with MIRS score 3, in which no proximal weakness is expected, an increased fat fraction in upper leg muscles. This demonstrates that quantitative MRI can detect subclinical muscle involvement and thus may serve in the prediction of clinical muscle affliction. This capacity of quantitative MRI has
also been observed for other muscular dystrophies and further emphasises its value as an potential tool in the evaluation of therapies.\textsuperscript{32,40–42}

We observed one outlier, a patient with a low 6MWT (50 m) and a relatively normal fat fraction (12.5\%) and contractile muscle volume (938 cm\textsuperscript{3}). Here, the 6MWT likely reflected this patient’s overall condition or motivation, as opposed to muscle function alone. This further illustrates the more objective value of quantitative MRI to assess muscle involvement in DM1, as has been established for other muscular dystrophies.\textsuperscript{6,43}

In conclusion, quantitative MRI of 20 different lower extremity muscles in 33 DM1 patients demonstrated increased fat infiltration, reduced contractile muscle mass, and the presence of putative oedema, the latter reflected by an elevated T2 relaxation time of muscle water. Fat infiltration was primarily determined by age, followed by inherited CTG repeat length, and on-going CTG repeat expansion accrued over time. Abnormal MRI parameter values correlated with decreased physical capacity and disease severity, but also identified subclinical involvement, and therefore can potentially serve as objective, quantitative biomarkers to assess disease state and to evaluate therapies. For the latter, larger longitudinal natural history studies are required for validation.

**Acknowledgements**

We would like to thank all participants for participating in this study. The OPTIMISTIC consortium is thanked for their critical remarks on the data analysis and interpretation. In addition, we like to thank Barbara Janssen for help with the MRI protocol, Ferroudja Daidj for participants management in Paris, and Sjaak van Asten, Lydia Overtoom en Justine van Eerden for their help with data-acquisition and/or processing.
References
10. van Engelen B. Cognitive behaviour therapy plus aerobic exercise training to increase activity in patients with myotonic dystrophy type 1 (DM1) compared to usual care (OPTIMISTIC): study protocol for randomised controlled trial. Trials. 2015;16:224.

QUANTITATIVE MUSCLE IN MRI IN DM1


Supplementary

Table S3.1: Frequency distribution per score of the semi-quantitative Lamminen score of T1 weighted images and Malattia score of TIRM images.

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<th>DM1 patients (n = 615 muscles)</th>
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</tr>
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The Lamminen score reflects fat infiltration, and the Malattia score reflects oedema-associated disease activity. Data are presented as the total number of muscles (no) with percentage of muscles for a particular score in brackets.
Figure S3.1: Frequency distribution of the semi-quantitative Lamminen score of T1 weighted images and Malattia score of TIRM images per muscle in myotonic dystrophy type 1 patients. A) Lamminen score for the lower leg muscles. B) Lamminen score for the upper leg muscles. C) Malattia score for the lower leg muscles. D) Malattia score for the upper leg muscles.

Figures S3.2 to S3.5: Values for quantitative MRI measures for the 20 individual lower extremity muscles in myotonic dystrophy type 1 (DM1) patients compared to unaffected controls. Since this is an explorative analysis, DM1 patients were compared to unaffected controls using non-parametric Mann-Whitney-U tests. We displayed the statistical result both with and without post-hoc Bonferroni correction for multiple testing.

Abbreviations for the lower extremity muscles are as follows: tibialis anterior (TA); extensor digitorum longus (EDL); peroneus (P); tibialis posterior (TP); flexor digitorum longus (FDL); gastrocnemius lateralis (GL); gastrocnemius medialis (GM); soleus (SOL); rectus femoris (RF); vastus lateralis (VL); vastus intermedius (VI); vastus medialis (VM); sartorius (S); biceps femoris short head (BFS); biceps femoris long head (BFL); semitendinosus (ST); semimembranosus (SM); gracilis (G); adductor magnus (AM); and adductor longus (AL).
Figure S3.2: Fat fraction in the individual lower extremity muscles. A) Lower leg muscles B) Upper leg muscles. *Muscles showing a statistical difference between unaffected controls and DM1 patients when applying Bonferroni correction for multiple testing (significance threshold: p < 0.0025). # Muscles showing a statistical difference between unaffected controls and DM1 patients when not correcting for multiple testing significance threshold: p < 0.05). The p-values for muscles were as follows: TA, p < 0.001; EDL, p < 0.001; P, p < 0.001; TP, p = 0.001; FDL, p < 0.001; GL, p < 0.001; GM, p < 0.001; SOL, p < 0.001; RF, p = 0.661; VL, p = 0.051; VI, p = 0.002; VM, p = 0.023; S, p = 0.003; BFS, p = 0.003; BFL, p = 0.047; ST, p = 0.040; SM, p = 0.069; G, p = 0.003; AM, p = 0.055; AL, p = 0.483.
**Figure S3.3: Muscle volume in the individual lower extremity muscles.** A) Lower leg muscles B) Upper leg muscles. *Muscles showing a statistical difference between unaffected controls and DM1 patients when applying Bonferroni correction for multiple testing (significance threshold: p < 0.0025). # Muscles showing a statistical difference between unaffected controls and DM1 patients when not correcting for multiple testing (significance threshold: p < 0.05). The p-values for muscles were as follows: TA, p = 0.069; EDL, p = 0.045; P, p = 0.052; TP, p = 0.695; FDL, p = 0.096; GL, p = 0.328; GM, p = 0.052; SOL, p = 0.008; RF, p = 0.440; VL, p = 0.036; VI, p = 0.146; VM, p = 0.259; S, p = 0.096; BFS, p = 0.940; BFL, p = 0.154; ST, p = 0.300; SM, p = 0.248; G, p = 0.172; AM, p = 0.248; AL, p = 0.511.

**Figure S3.4: Contractile muscle volume in the individual lower extremity muscles.** A) Lower leg muscles B) Upper leg muscles. *Muscles showing a statistical difference between unaffected controls and DM1 patients when applying Bonferroni correction for multiple testing (significance threshold: p < 0.0025). # Muscles showing a statistical difference between unaffected controls and DM1 patients when not correcting for multiple testing (significance threshold: p < 0.05). The p-values for muscles were as follows: TA, p = 0.021; EDL, p = 0.007; P, p = 0.003; TP, p = 0.935; FDL, p = 0.088; GL, p = 0.660; GM, p < 0.001; SOL, p < 0.001; RF, p = 0.478; VL, p = 0.020; VI, p = 0.051; VM, p = 0.070;
S, p = 0.022; BFS, p = 0.636; BFL, p = 0.074; ST, p = 0.037; SM, p = 0.128; G, p = 0.069; AM, p = 0.208; AL, p = 0.577.

Figure S3.5: T2 relaxation time in fat and non-fat infiltrated muscles in the individual lower extremity muscles. A) Lower leg muscles B) Upper leg muscles. *Muscles showing a statistical difference between unaffected controls and fat or non-fat infiltrated muscles in DM1 patients when applying Bonferroni correction for multiple testing (significance threshold: p < 0.0025). # Muscles showing a statistical difference between unaffected controls and fat or non-fat infiltrated muscles in DM1 patients when not correcting for multiple testing (significance threshold: p < 0.05). For non-fat infiltrated DM1 muscles, the p-values were as follows: TA, p = 0.582; EDL, p = 0.145; P, p = 0.199; TP, p = 0.371; FDL, p = 0.909; GL, p = 0.492; GM, p = 1.000; SOL, p = 0.454; RF, p = 0.007; VL, p = 0.074; VI, p = 0.132; VM, p = 0.002; S, p = 0.024; BFS, p = 0.709; BFL, p = 0.204; ST, p = 0.346; SM, p = 0.371; G, p = 0.447; AM, p = 0.927; AL, p = 0.508. For fat infiltrated DM1 muscles, the p-values were as follows: TA, p < 0.001; EDL, p < 0.001; P, p = 0.001; TP, p = 0.023; FDL, p = 0.003; GL, p = 0.049; GM, p = 0.028; SOL, p = 0.006; RF, p = 0.001; VL, p = 0.027; VI, p = 0.004; VM, p = 0.002; S, p < 0.001; BFS, p = 0.007; BFL, p = 0.001; ST, p = 0.001; SM, p = 0.068; G, p = 0.077; AM, p = 0.003; AL, p = 0.188.
Figure S3.6: Fat fraction in myotonic dystrophy type 1 (DM1) patients with Acil-sensitive variant repeats in the expanded allele vs. DM1 patients where no Acil-sensitive variant repeats were detected. Four patients in our study possessed Acil-sensitive variant repeats, of which whole lower extremity fat fraction could be estimated for two patients. These two patients exhibited lower mean fat fractions in lower extremity muscles than patients without Acil-sensitive variant repeats.
Table S3.2: Multivariate linear regression analysis of the relationship between fat fraction, age, estimated progenitor CTG repeat length ($CTG_{ePAL}$), and individual specific residual variation in somatic instability ($CTG_{ResSI}$).

<table>
<thead>
<tr>
<th>Model</th>
<th>$r^2$</th>
<th>Model $p$-value</th>
<th>Parameter</th>
<th>Coefficient</th>
<th>Standard error</th>
<th>Parameter $p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\log(\text{fat fraction}) = \beta_0 + \beta_1 \log(\text{Age})$</td>
<td>0.447</td>
<td>&lt;0.001</td>
<td>Constant $\beta_0$</td>
<td>-4.9</td>
<td>1.6</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$\log(\text{Age})$ $\beta_1$</td>
<td>1.9</td>
<td>0.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>$\log(\text{fat fraction}) = \beta_0 + \beta_1 \log(\text{Age}) + $</td>
<td>0.484</td>
<td>&lt;0.001</td>
<td>Constant $\beta_0$</td>
<td>-7.7</td>
<td>2.3</td>
<td>0.003</td>
</tr>
<tr>
<td>$\beta_2 \log(CTG_{ePAL})$</td>
<td></td>
<td></td>
<td>$\log(\text{Age})$ $\beta_1$</td>
<td>2.1</td>
<td>0.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$\log(CTG_{ePAL})$ $\beta_2$</td>
<td>0.9</td>
<td>0.5</td>
<td>0.090</td>
</tr>
<tr>
<td>$\log(\text{fat fraction}) = \beta_0 + \beta_1 \log(\text{Age}) \ast $</td>
<td>0.590</td>
<td>0.001</td>
<td>Constant $\beta_0$</td>
<td>8.6</td>
<td>35.5</td>
<td>0.811</td>
</tr>
<tr>
<td>$\beta_2 \log(CTG_{ePAL}) \ast \beta_3 \log(CTG_{ResSI})$</td>
<td></td>
<td></td>
<td>$\log(\text{Age})$ $\beta_1$</td>
<td>-2.5</td>
<td>9.0</td>
<td>0.782</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$\log(CTG_{ePAL})$ $\beta_2$</td>
<td>-6.9</td>
<td>14.7</td>
<td>0.644</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$CTG_{ResSI}$ $\beta_3$</td>
<td>48.4</td>
<td>28.5</td>
<td>0.108</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$\log(\text{Age}) \ast \log(CTG_{ePAL})$ $\beta_4$</td>
<td>2.23</td>
<td>3.7</td>
<td>0.560</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$\log(\text{Age}) \ast CTG_{ResSI}$ $\beta_5$</td>
<td>-12.7</td>
<td>7.2</td>
<td>0.097</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$\log(CTG_{ePAL}) \ast CTG_{ResSI}$ $\beta_6$</td>
<td>-20.0</td>
<td>11.8</td>
<td>0.107</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$\log(\text{Age}) \ast \log(CTG_{ePAL}) \ast CTG_{ResSI}$ $\beta_7$</td>
<td>5.3</td>
<td>3.0</td>
<td>0.095</td>
</tr>
</tbody>
</table>
Table S3.3: Correlation between the six-minute walk test (6MWT) and the fat fraction of four functional muscle groups: quadriceps, hamstrings, anterior compartment of the lower leg and triceps surae.

<table>
<thead>
<tr>
<th></th>
<th>r</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fat fraction</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Knee extensors</td>
<td>-0.409</td>
<td>0.027</td>
</tr>
<tr>
<td>Knee flexors</td>
<td>-0.326</td>
<td>0.085</td>
</tr>
<tr>
<td>Ankle dorsiflexors</td>
<td>-0.498</td>
<td>0.007</td>
</tr>
<tr>
<td>Ankle plantarflexors</td>
<td>-0.574</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Muscle volume</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Knee extensors</td>
<td>0.319</td>
<td>0.075</td>
</tr>
<tr>
<td>Knee flexors</td>
<td>0.189</td>
<td>0.299</td>
</tr>
<tr>
<td>Ankle dorsiflexors</td>
<td>0.225</td>
<td>0.216</td>
</tr>
<tr>
<td>Ankle plantarflexors</td>
<td>0.103</td>
<td>0.575</td>
</tr>
<tr>
<td><strong>Contractile muscle volume</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Knee extensors</td>
<td>0.375</td>
<td>0.045</td>
</tr>
<tr>
<td>Knee flexors</td>
<td>0.267</td>
<td>0.161</td>
</tr>
<tr>
<td>Ankle dorsiflexors</td>
<td>0.407</td>
<td>0.032</td>
</tr>
<tr>
<td>Ankle plantarflexors</td>
<td>0.365</td>
<td>0.056</td>
</tr>
<tr>
<td><strong>T2\text{_water}</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Knee extensors</td>
<td>-0.248</td>
<td>0.254</td>
</tr>
<tr>
<td>Knee flexors</td>
<td>-0.391</td>
<td>0.065</td>
</tr>
<tr>
<td>Ankle dorsiflexors</td>
<td>-0.142</td>
<td>0.527</td>
</tr>
<tr>
<td>Ankle plantarflexors</td>
<td>0.198</td>
<td>0.366</td>
</tr>
</tbody>
</table>

Knee extensors: Three vastii muscles and rectus femoris; Knee flexors: biceps femoris, semitendinosus, and semimembranosus; Ankle dorsiflexors: tibialis anterior and extensor digitorum longus; Ankle plantarflexors: gastrocnemius lateralis and medialis and soleus. After Bonferroni correction, statistical significance is set at \( p = 0.0125 \).
Chapter 4

The effect of increased activity in myotonic dystrophy studied with muscle MRI

Authors
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* Both authors contributed equally

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Radiology, November 2019
Abstract

Objective: To assess the effect of a behavioural intervention on the lower extremity muscles of myotonic dystrophy type 1 (DM1) patients with longitudinal quantitative muscle MRI.

Methods: In this multi-centre randomised trial, we measured 27 severely fatigued DM1 patients at baseline and 10-month follow-up. Patients were randomized to the behavioural intervention (n = 14) or standard care (n = 13). Per patient, we determined for 20 muscles the fat fraction, cross-sectional area (CSA), and $T_2^{\text{water}}$ as markers for fat infiltration, muscle mass and alteration in tissue water distribution (oedema), respectively, using Dixon and multi-echo spin-echo sequences.

Results: CSA in the intervention group (baseline: $132 \pm 32 \text{ cm}^2$) increased with $5.9 \pm 7.8 \text{ cm}^2$ during the 10-month follow-up ($p = 0.029$), and decreased non-significantly in the standard care group (baseline: $141 \pm 35 \text{ cm}^2$, difference: $-3.6 \pm 7.2 \text{ cm}^2$). After 10 months the mean difference between the groups was $9.5 \text{ cm}^2$ ($p = 0.007$). This effect was stronger in muscles without fat infiltration at baseline ($p < 0.001$). The fat fraction increase between intervention group (baseline: $13.5 \pm 11.8\%$, increase: $0.9 \pm 1.0\%$) and standard care group (baseline: $14.3 \pm 7.8\%$, increase: $1.2 \pm 1.2\%$) was not different ($p = 0.558$). $T_2^{\text{water}}$ remained stable in the intervention (baseline: $33.5 \pm 1.2 \text{ ms}$; $p = 0.479$) and standard care group (baseline: $33.5 \pm 0.7 \text{ ms}$, $p = 0.883$).

Interpretation: A behavioural intervention, targeting physical activity, increases lower extremity muscle mass in DM1 patients, preferentially in healthy appearing muscle. This demonstrates the efficacy and safety of such interventions in DM1, and indicates that starting early in the disease course is preferred. Furthermore, this study validates the use of fat fraction and CSA as responsive biomarkers for trials in DM1.
Introduction
Myotonic dystrophy type 1 (DM1) is the most common form of muscular dystrophy in adults. It is characterized by muscle degeneration, including fat infiltration and atrophy, which leads to progressive muscle weakness. Recently, we showed that an activity stimulating behavioural intervention applied to DM1 patients could increase physical activity, measured with accelerometry, and could improve exercise capacity, measured with the six minute walk test (6MWT). This raises the question to what extent this intervention affects the condition of muscles in the lower extremities of these patients.

A suitable technique for non-invasive in-vivo imaging of muscles is MRI, in particular as it enables quantification of fat infiltration and muscle mass in individual muscles. In addition, MRI allows quantification of changes in tissue water distribution using the muscle water T2 relaxation time ($T_2^{water}$). Increased $T_2^{water}$ is thought to reflect intra- or extracellular oedema, an early pathophysiological change.

Therefore, we aimed to study the effect of an activity stimulating behavioural intervention on fat infiltration, muscle mass and tissue water distribution (oedema) in the 20 lower extremity muscles of DM1 patients by performing a longitudinal quantitative MRI study. First, we assessed the effect of the behavioural intervention on these muscles at the subject level. Second, we assessed the individual muscle response in relation to its disease state at baseline.

Materials & Methods
Study design and subjects
Patients were recruited from the OPTIMISTIC trial, involving cohorts in Nijmegen, the Netherlands, and Paris, France. Inclusion and exclusion criteria were similar to the OPTIMISTIC trial. At baseline, patients were severely fatigued (checklist individual strength subscale fatigue score ≥ 35), and could walk independently. Additional exclusion criteria for this MRI study were contra-indications to MRI scanning, including claustrophobia, a pacemaker, a prosthetic implant or being unable to lie supine for 60 minutes. We also recruited ten apparently unaffected individuals, matched for age and sex, to obtain reference values for the MRI outcome measures. This study was conducted according to the principles of the Declaration of Helsinki (version 2013) and the relevant legislation for medical research involving human subjects. It was approved by local medical ethics committees and all participants gave, prior to enrolment, written informed consent.

Randomisation and masking
Patients were randomized either to the behavioural intervention or the standard care group, via the central Tayside Randomisation web-based system (TRuST). All involved in the data-acquisition and data-analysis were masked for treatment allocation.
**Intervention**

The intervention group received a behavioural intervention comprising cognitive behavioural therapy (CBT) optionally combined with graded exercise therapy in addition to standard care. CBT focused on compensating reduced initiative, optimizing physical activity and reducing experienced fatigue. Patients allocated to the control group received standard care. Details have been published elsewhere.\(^4\)\(^,\)\(^6\)

**Clinical and genetic assessments**

At baseline, we collected the demographic parameters age and sex from both patients and unaffected controls, and in patients also the muscular impairment rating scale (MIRS) and genetic variables.\(^8\) The MIRS is an ordinal scale (0-5), with higher scores reflecting a higher disease severity. Genetic variables included the modal CTG repeat length (CTG\(_{\text{modal}}\)) at time of recruitment and the estimated progenitor CTG repeat length (CTG\(_{\text{ePAL}}\)) inherited at birth.\(^9\)

Clinical measures were assessed at baseline and 10-month follow-up. The DM1-Activ-c, which is the primary outcome measure in OPTIMISTIC, measured capacity for activity and social participation (higher scores are beneficial).\(^10\) Exercise capacity was evaluated using the 6MWT and daily physical activity was measured with a tri-axial accelerometer (GENEActiv, Activ Insights Ltd, UK) and calculated over the five most active hours of the day; higher scores indicate higher level of physical activity.\(^4\)

**MRI acquisition**

All participants underwent an MRI scan of the thigh and leg muscles, randomly chosen left or right. In patients, this baseline MRI scan was performed within one month of the first visit for OPTIMISTIC and for the intervention group, before start of treatment. We acquired a follow-up MRI 10 months (± one month) after baseline. To assess repeatability, five patients underwent the follow-up MRI scan twice on the same day.

All MRI images were acquired on Siemens 3T MR systems (Nijmegen: Tim TRIO; Paris: Magnetom Verio; Erlangen, Germany) using a combination of a spine coil with phased-array coils placed around the lower extremity. Matching of imaging slices between the baseline and follow-up scan was achieved by placing a fish oil capsule. This capsule was placed for the thigh at 2/3 the distance between the spina iliaca anterior superior and top of the patella and for the leg at 1/3 the distance between the lower border of the patella and the lateral malleolus (figure 4.1A). All images were in the transverse plane, and the field of view (FOV) was placed such that the middle slice was on the fish oil capsule.

For semi-quantitative assessment of fat infiltration, we obtained a T1 weighted spin-echo sequence (repetition time (TR) = 670 ms, echo time (TE) = 10 ms, FOV = 192x192 mm, voxel size = 1x1x5 mm, number of slices = 27, slice gap = 0 mm). Quantitative assessment of
Fat infiltration and muscle mass was achieved with a 3D Dixon sequence (TR = 10 ms, flip angle = 3°, FOV = 256x192 mm, voxel size = 1x1x5 mm and number slices = 32), either in a 2-point version (TE1/TE2 = 2.45/3.675 ms) or 3-point version (TE1/TE2/TE3 = 2.31/3.68/5.07 ms). Changes in tissue water distribution and presence of oedema were also assessed semi-quantitatively and quantitatively (T2\text{water}). For semi-quantitative assessment, we acquired a turbo inversion recovery magnitude (TIRM) sequence (TR = 4,000 ms, TE = 41 ms, inversion time (TI) = 220 ms), with similar coverage and resolution as the T1 weighted sequence. Quantitative assessment was performed with a multi-echo spin-echo sequence (TR = 3,720 ms, echo train length = 17, echo-spacing = 8 ms, voxel size = 1.5x1.5x10 mm, number of slices = 5, slice gap = 20 mm).

**MRI analysis**

**Semi-quantitative:** An experienced radiologist (MP) scored 7 leg and 12 thigh muscles on the T1 weighted images and TIRM images with the Lamminen score and Malattia score, respectively (figure 4.1B). The Lamminen score ranges from 1-4, with 1 indicating normal muscle signal intensity and 4 indicating total, homogeneous hyperintense signal change in whole muscle. The Malattia score ranges from 0-2, with 0 indicating no muscle signal abnormalities and 2 indicating a high degree of signal abnormalities.

**Quantitative:** Quantitative analysis was performed using Matlab (version 2014b, Mathworks, Natick, MA, USA). We first processed the 3D Dixon reconstructed fat and water images to a fat fraction map by dividing the signal intensity of the fat image by the summed signal intensity of the fat and water image. The resulting fat fraction map has voxel values ranging from 0-100%. Second, we calculated a T2\text{water} map (in ms) by voxel-wise fitting the signal decays in the multi-echo spin-echo images with a bi-exponential extended phase graph model. For more details on the T2\text{water} fitting procedure, see previous work. An increase in T2\text{water} is thought to reflect more oedema.

An experienced observer (LH) delineated regions of interest (ROIs) for 20 muscles, the 19 semi-quantitative assessed muscles plus the flexor digitorum longus, on five distal, middle and proximal slices of the Dixon images using MIPAV (http://mipav.cit.nih.gov) (figure 4.1C). Furthermore, five additional slices, matching the T2\text{water} map slice position (figure 4.1A), were delineated and transposed to the T2\text{water} map. The observer was aware if it was a baseline or follow-up scan, but blinded for group allocation. The ROIs were used to determine for each muscle the average fat fraction and T2\text{water}, and cross-sectional area (CSA). Fat fraction and T2\text{water} were determined over all 15 slices and 5 slices, respectively. CSA was determined at a single slice. For the leg, this was the last slice where the proximal part of the flexor digitorum longus was visible, and for the thigh, the last slice where the proximal part of the biceps femoris short head was visible (figure 4.1C). As a measure for remaining functional muscle tissue, contractile CSA (CSA x (1-fat fraction)) was calculated. All abovementioned outcome
measures were determined per muscle for baseline and follow-up. The 10-month change was calculated as the difference between baseline and follow-up.

Muscles were excluded if visual inspection revealed movement artefacts. For the outcome measures fat fraction and contractile CSA, muscles were also excluded if the fat fraction estimate was unreliable due fat/water reconstruction artefacts.

Combining results of individual muscles to subject level: For each participant, we combined the semi-quantitative and quantitative measures of individual muscles into aggregate values for the integral lower extremity and four functional muscle groups, namely the quadriceps, hamstrings, ankle dorsiflexors and ankle plantarflexors. For fat fraction and T2\text{water} aggregate scores were determined by calculating the average value from the relevant muscles. For the Lamminen score, Malattia score, CSA and contractile CSA, individual muscle values were summed to obtain a combined lower extremity score per subject. The aggregate scores were used to evaluate the intervention effect.

Predictors for the 10-month change in MRI outcome measures in individual muscles
Subsequently, we studied if the 10-month change in MRI outcome measures at the individual muscle level depended on fat infiltration or TIRM signal hyperintensities (oedema) at baseline. We have set the fat fraction's mean + 2SD of the unaffected healthy volunteers as threshold to separate the DM1 patients’ muscles in two groups, muscle with and without signs of fat infiltration at baseline. Furthermore, muscles were separated based on their Malattia score (0, 1, and 2).

Statistics
Baseline comparison of the intervention and standard care group was performed with an independent-samples t-test, Mann-Whitney U, Pearson’s chi-square or Fisher’s Exact test, as appropriate. Baseline MRI outcome measures and accelerometer measured activity were correlated (Pearson). The 10-month change in MRI outcome measures was normally distributed, therefore within-group changes were assessed with a paired-samples t-test. The between-group changes, i.e. intervention vs. standard care, were assessed with a multivariate regression model with group allocation as binary variable. For the whole lower extremity, sex was added as a binary variable to assess gender effects. For the functional muscle groups, Bonferroni correction was applied to correct for multiple testing. To study the effect of disease state at baseline, we used a two-level linear mixed model as we deal with nested data (muscle data - level 1; patient data - level 2). Fat infiltration (yes/no) or Malattia score (0/1/2) were added as factors. Responsiveness was assessed with the standardized response mean (SRM; mean change/SD change), and repeatability with a Bland-Altman analysis. All tests were performed two-sided with significance set at $p < 0.05$. Data are presented as mean (SD) or mean (95% confidence interval (CI)), unless otherwise stated. All statistical
analyses were performed with IBM SPSS Statistics (version 22, SPSS, Chicago, IL, USA). This trial is registered with ClinicalTrials.gov, number NCT02118779.

Figure 4.1: Graphical representation of methodology. A) Field of view for the MR examination of thigh and leg. Muscles were manually delineated on the depicted slices; in light grey, the five proximal, middle and distal slices of the Dixon fat fraction map and, in dark grey, the five slices for the $T_2$ water map. B) Semi-quantitative MRI outcome measures - T1 weighted images were assessed for fat infiltration with the Lammen score. This example reveals fat infiltration in the quadriceps muscles, semitendinosus, semimembranosus and all scored leg muscles, except the tibialis posterior. TIRM images were assessed with the Malattia score for signs of signal hyperintensity (oedema). This example shows signal hyperintensity in the quadriceps muscles, and gastrocnemius lateralis. C) Quantitative MRI outcome measures - Dixon images were used for quantitative assessment of fat infiltration, cross-sectional area (CSA), and contractile CSA. The $T_2$ water map was used to quantify changes in tissue water distribution and oedema. In this example, the adductor magnus and soleus muscle have increased $T_2$ water. Muscles are abbreviated as: TA/TP = tibialis anterior/posterior; EDL = extensor digitorum longus; P = peroneus; FDL = flexor digitorum longus; GL/GM = gastrocnemius lateralis/medialis; SOL = soleus; RF = rectus femoris; VL/VI/VM = vastus lateralis/intermedius/medialis; S = sartorius; BFS/BFL = biceps femoris short/long head; ST = semitendinosus; SM = semimembranosus; G = gracilis; and AM/AL = adductor magnus/longus.

Results
Subjects and their baseline characteristics
In this study we included 33 DM1 patients, of whom 19 patients (11 male) had been randomized to the behavioural intervention and 14 patients (7 male) to the standard care
group. A follow-up MRI was acquired in 27 of these 33 patients, 13 from the standard care group and 14 from the intervention group; the drop-out occurred because of illness unrelated to the trial, because of logistics or personal reasons. In the intervention group, 9 patients received graded exercise therapy in addition to CBT.

The patients were on average 45 (SD: 13) years old and varied from mildly to severely affected (MIRS score range: 2-5). At baseline, there were no significant differences in demographics or clinical variables (table 4.1), although \( \text{CTG}_{\text{modal}} \) was lower in the intervention group compared to the standard care group (\( p = 0.027 \)).

### Table 4.1: Baseline demographics, clinical measures, and genetic parameters of the myotonic dystrophy type 1 patients.

<table>
<thead>
<tr>
<th>Patients characteristics</th>
<th>Standard care group ((n = 13))</th>
<th>Intervention group ((n = 14))</th>
<th>(p)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years (years)</td>
<td>45 (12)</td>
<td>45 (13)</td>
<td>0.956</td>
</tr>
<tr>
<td>Sex male/female – no (% male)</td>
<td>7/6 (54%)</td>
<td>8/6 (57%)</td>
<td>1.000</td>
</tr>
<tr>
<td>MIRS – median (IQR)</td>
<td>3 (1)</td>
<td>3 (0.5)</td>
<td>0.253</td>
</tr>
<tr>
<td>CIS-fatigue</td>
<td>45 (6)</td>
<td>44 (8)</td>
<td>0.626</td>
</tr>
<tr>
<td>Clinical measures</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM1-Activ-c score</td>
<td>62 (19)</td>
<td>57 (15)</td>
<td>0.517</td>
</tr>
<tr>
<td>6MWT in meters (m)</td>
<td>427 (82)</td>
<td>436 (109)</td>
<td>0.805</td>
</tr>
<tr>
<td>Genetic parameters - CTG repeat length</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \text{CTG}_{\text{modal}} )</td>
<td>574 (191)</td>
<td>384 (217)</td>
<td><strong>0.027</strong></td>
</tr>
<tr>
<td>( \text{CTG}_{e\text{PAL}} )</td>
<td>298 (117)</td>
<td>225 (111)</td>
<td>0.114</td>
</tr>
</tbody>
</table>

Results are presented as mean (SD) unless indicated otherwise. The unaffected healthy subjects (5 male) had an average age of 45 (14) years. IQR: Interquartile range. MIRS: muscular impairment rating scale. CIS - fatigue: checklist individual strength, fatigue subscale. \( \text{CTG}_{\text{modal}} \): modal CTG is the repeat length in blood at the time of recruitment. \( \text{CTG}_{e\text{PAL}} \): estimated inherited progenitor CTG repeat length.

### Longitudinal changes in clinical measures

In the intervention group, the capacity for activity and participation, as measured by the DM1-Activ-c score, improved over 10 months with 6 points (95% CI 1 to 12; \( p = 0.021 \)), while no change occurred over this period in the 6MWT (\( p = 0.116 \)) and accelerometer measured activity (\( p = 0.513 \); table 4.2). In contrast, the standard care group demonstrated a significant drop in the 6MWT of -11 m (-12 to 0; \( p = 0.043 \)), and no change in the DM1-Activ-c score (\( p = 0.318 \)) and accelerometer measured activity (\( p = 0.836 \); table 4.2). The changes in the 6WMT and DM1-Activ-c score differed between the groups, in favour of the intervention (6MWT: \( p = 0.018 \); DM1-Activ-c: \( p = 0.012 \)); no sex-effect was observed.
**Table 4.2**: Clinical outcome measures for fat infiltration in myotonic dystrophy type 1 patients at baseline and follow-up and the longitudinal change over 10 months in the standard care group and intervention group.

<table>
<thead>
<tr>
<th></th>
<th>Standard care group (n = 13)</th>
<th>Intervention group (n = 14)</th>
<th>p-value between-group comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline*</td>
<td>Follow-up</td>
<td>10-month change (95% CI; p-value)</td>
</tr>
<tr>
<td>DM1-Activ-c</td>
<td>62 (19)</td>
<td>59 (21)</td>
<td>-3 (-9 to 3; 0.318)</td>
</tr>
<tr>
<td>6MWT (meters)</td>
<td>427 (82)</td>
<td>416 (86)</td>
<td>-11 (-12 to 0; <strong>0.043</strong>)</td>
</tr>
<tr>
<td>Accelerometer measured activity</td>
<td>41.9 (21.6)</td>
<td>42.4 (20.6)</td>
<td>0.5 (-4.7 to 5.7; 0.836)</td>
</tr>
</tbody>
</table>

Results are presented as mean (SD) or mean change (95% confidence interval (CI)). *No significant differences were observed between the standard care and intervention group at baseline.
MRI outcome measures at baseline
On the MR images of the 27 patients who underwent the baseline and follow-up MRI scan, we analyzed 489 muscles, 223 in the standard care group and 266 in the intervention group. In the standard care group, 24 muscles were excluded because in one subject no images from the thigh had been acquired due to inability to lay down sufficiently long to perform all MRI sequences and in one subject from the intervention group the follow-up MRI scan was mis-positioned compared to the baseline MRI scan.

Hyperintense lesions on T1 weighted and TIRM images revealed the presence of fat infiltration and putative oedema in these muscles, respectively (figure 4.1B). Quantitative assessment using Dixon fat fraction maps and multi-spin echo $T_2_{\text{water}}$ maps exhibited increased fat fractions and $T_2_{\text{water}}$, respectively (figure 4.1C). The fat fractions in individual muscles varied between 1.3% to 90.3%, and at the subject level between 2% and 32%. At baseline, the MRI outcome measures did not differ between the groups (table 4.3a-c). The baseline fat fraction and contractile CSA correlated with the accelerometer measured activity for the whole lower extremity (fat fraction: $r = -0.798$, $p < 0.001$; contractile CSA: $r = 0.665$, $p = 0.005$), for the thigh muscles (fat fraction: $r = -0.684$, $p = 0.002$; contractile CSA: $r = 0.574$, $p = 0.013$), and for the leg muscles (fat fraction: $r = -0.644$, $p = 0.004$; contractile CSA: $r = 0.552$, $p = 0.017$).

Longitudinal changes in MRI outcome measures
Semi-quantitative assessment of muscle fat infiltration in the lower extremity muscles with the sum Lamminen score could not detect a longitudinal 10-month change in fat infiltration in both the intervention (mean (95% CI); 1 (-0.5 to 1.5), $p = 0.291$) and standard care group (0 (-0.7 to 1.6), $p = 0.825$) (table 4.3a). In contrast, quantitative assessment with the Dixon fat fraction showed a 10-month increase of 0.9% (0.2 to 1.7; $p = 0.019$) in the intervention group and 1.2% (0.3 to 2.1; $p = 0.016$) in the standard care group (table 4.3a, figure 4.2A). These longitudinal changes did not differ between both groups for the sum Lamminen score (mean score difference in change between groups: 1, $p = 0.517$) and fat fraction (0.3%, $p = 0.558$) (table 4.3a). Additional explorative analysis on the average fat fraction change in the 20 individual muscles suggests a progression in fat infiltration in almost all muscles (figure 4.3A).

In the intervention group, quantitative assessment of lower extremity muscle mass demonstrated an increase in CSA of 5.9 cm$^2$ over 10 months time (0.7 to 11.0; $p = 0.029$), with a similar trend in contractile CSA of 5.0 cm$^2$ (-1.0 to 10.9; $p = 0.093$) (table 4.3b, figure 4.2B/C). The standard care group exhibited a non-significant drop over 10-months in CSA and contractile CSA, -3.6 cm$^2$ (-8.4 to 1.2; $p = 0.125$) and -4.5 cm$^2$ (-10.0 to 1.0; $p = 0.096$), respectively. Comparison of the 10-month change between the groups showed a difference in CSA (9.5 cm$^2$, $p = 0.007$) and contractile CSA (9.5 cm$^2$, $p = 0.017$), in favour of
the intervention. The gain in CSA and contractile CSA is not dominated by a single muscle, but occurs in almost all lower extremity muscles (figure 4.3B/C). The largest intervention effect on CSA and contractile CSA was observed for the quadriceps muscles (table 4.3b).

Both groups showed no change in outcome measures for tissue water distribution and oedema. The change in semi-quantitative sum Malattia score was 1 (-1.2 to 2.7, \( p = 0.402 \)) in the intervention group and 0 (-2.9 to 2.2, \( p = 0.759 \)) in the standard care group. The \( T_2 \) water change was -0.22 ms (-0.47 to 0.03, \( p = 0.082 \)) and 0.04 ms (-0.58 to 0.51, \( p = 0.0883 \)) in the intervention and standard care group, respectively. Also, between-group comparison of the change in 10-months exhibited no intervention effect for the sum Malattia score (+1, \( p = 0.437 \)) and \( T_2 \) water (-0.18 ms, \( p = 0.479 \)) (table 4.3c, figure 4.2D). Visual inspection of the change in \( T_2 \) water in the 20 individual muscles reveals that on average \( T_2 \) water increases in some muscles and decreases in other muscles (figure 4.3D). We observed no sex-effect on the response of the MRI outcome measures.

**Figure 4.2: Longitudinal 10-month changes in MRI outcome measures.** The 10-months longitudinal change in MRI outcome measures for the lower extremity muscles at subject level for the standard care group (SC, blue) and behavioural intervention group (INT, green). A) Fat fraction. B) Cross-sectional area (CSA). C) Contractile CSA. D) \( T_2 \) water. * \( p<0.05 \), ** \( p<0.01 \), reflect a significant within-group change after 10 months. Arrows with \( p \)-value < 0.05 reflect a significant difference in the change over 10 months between the groups. Data are shown per subject and mean ± SD.
Figure 4.3: Longitudinal 10-month changes in MRI outcome measures for the individual lower extremity muscles. The data is presented for the standard care group (white) and behavioural intervention group (grey). A) Fat fraction. B) Cross-sectional area (CSA). C) Contractile CSA. D) T2\textsubscript{water}. Data are shown per muscle as mean ± standard error of the mean (SEM). Muscles are abbreviated as: TA/TP = tibialis anterior/posterior; EDL = extensor digitorum longus; P = peroneus; FDL = flexor digitorum longus; GL/GM = gastrocnemius lateralis/medialis; SOL = soleus; RF = rectus femoris; VL/VI/VM = vastus lateralis/intermedius/medialis; S = sartorius; BFS/BFL = biceps femoris short/long head; ST = semitendinosus; SM = semimembranosus; G = gracilis; and AM/AL = adductor magnus/longus.
Table 4.3a: MRI outcome measures for fat infiltration in myotonic dystrophy type 1 patients at baseline and follow-up and the longitudinal change over 10 months in the standard care group and intervention group.

<table>
<thead>
<tr>
<th>Lamminen sum score</th>
<th>Standard care group (n = 13)</th>
<th>Intervention group (n = 14)</th>
<th>p-value between-group comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (muscles/subjects)</td>
<td>Baseline*</td>
<td>Follow-up</td>
</tr>
<tr>
<td>Lower Extremity</td>
<td>209/11</td>
<td>29 (9)</td>
<td>29 (9)</td>
</tr>
<tr>
<td>Quadriceps</td>
<td>44/11</td>
<td>5 (2)</td>
<td>5 (1)</td>
</tr>
<tr>
<td>Hamstrings</td>
<td>44/11</td>
<td>5 (2)</td>
<td>5 (2)</td>
</tr>
<tr>
<td>Ankle dorsiflexors</td>
<td>26/13</td>
<td>4 (2)</td>
<td>4 (2)</td>
</tr>
<tr>
<td>Ankle plantarflexors</td>
<td>39/13</td>
<td>6 (2)</td>
<td>6 (2)</td>
</tr>
<tr>
<td>Fat fraction (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower Extremity</td>
<td>175/9</td>
<td>14.3 (7.8)</td>
<td>15.5 (7.7)</td>
</tr>
<tr>
<td>Quadriceps</td>
<td>38/10</td>
<td>7.8 (4.5)</td>
<td>9.5 (5.2)</td>
</tr>
<tr>
<td>Hamstrings</td>
<td>38/10</td>
<td>11.7 (7.8)</td>
<td>12.5 (8.1)</td>
</tr>
<tr>
<td>Ankle dorsiflexors</td>
<td>24/12</td>
<td>19.3 (14.8)</td>
<td>20.3 (15.0)</td>
</tr>
<tr>
<td>Ankle plantarflexors</td>
<td>36/12</td>
<td>21.5 (14.3)</td>
<td>22.7 (14.5)</td>
</tr>
</tbody>
</table>

Results are presented as mean (SD) or mean change (95% confidence interval (CI)). *No significant differences were observed between the standard care and intervention group at baseline. Quadriceps: rectus femoris and three vastii muscles. Hamstrings: biceps femoris short and long head, semitendinosus, and semimembranosus. Ankle dorsiflexors: tibialis anterior and extensor digitorum longus. Ankle plantarflexors: soleus and gastrocnemius medialis and lateralis.
Table 4.3b: MRI outcome measures for muscle mass in myotonic dystrophy type 1 patients at baseline and follow-up and the longitudinal change over 10 months in the standard care group and intervention group.

<table>
<thead>
<tr>
<th></th>
<th>Standard care group (n = 13)</th>
<th>Intervention group (n = 14)</th>
<th>p-value between-group comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (muscles/subjects)</td>
<td>Baseline*</td>
<td>Follow-up</td>
</tr>
<tr>
<td><strong>Cross-sectional area (cm²)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower Extremity</td>
<td>197/11</td>
<td>141 (35)</td>
<td>137 (36)</td>
</tr>
<tr>
<td>Quadriceps</td>
<td>42/11</td>
<td>49 (13)</td>
<td>48 (13)</td>
</tr>
<tr>
<td>Hamstrings</td>
<td>35/11</td>
<td>18 (5)</td>
<td>18 (5)</td>
</tr>
<tr>
<td>Ankle dorsiflexors</td>
<td>26/13</td>
<td>6 (2)</td>
<td>6 (2)</td>
</tr>
<tr>
<td>Ankle plantarflexors</td>
<td>39/13</td>
<td>16 (6)</td>
<td>16 (6)</td>
</tr>
<tr>
<td><strong>Contractile cross-sectional area (cm²)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower Extremity</td>
<td>159/9</td>
<td>125 (31)</td>
<td>120 (34)</td>
</tr>
<tr>
<td>Quadriceps</td>
<td>38/10</td>
<td>44 (11)</td>
<td>42 (12)</td>
</tr>
<tr>
<td>Hamstrings</td>
<td>31/10</td>
<td>16 (4)</td>
<td>15 (5)</td>
</tr>
<tr>
<td>Ankle dorsiflexors</td>
<td>24/12</td>
<td>5 (2)</td>
<td>5 (2)</td>
</tr>
<tr>
<td>Ankle plantarflexors</td>
<td>36/12</td>
<td>13 (7)</td>
<td>12 (7)</td>
</tr>
</tbody>
</table>

Results are presented as mean (SD) or mean change (95% confidence interval (CI)). *No significant differences were observed between the standard care and intervention group at baseline. Quadriceps: rectus femoris and three vasti muscles. Hamstrings: biceps femoris short and long head, semitendinosus, and semimembranosus. Ankle dorsiflexors: tibialis anterior and extensor digitorum longus. Ankle plantarflexors: soleus and gastrocnemius medialis and lateralis.
**Table 4.3c:** MRI outcome measures for alterations in tissue water distribution in myotonic dystrophy type 1 patients at baseline and follow-up and the longitudinal change over 10 months in the standard care group and intervention group.

<table>
<thead>
<tr>
<th>N (muscles/subjects)</th>
<th>Standard care group (n = 13)</th>
<th>Intervention group (n = 14)</th>
<th>p-value between-group comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline*</td>
<td>Follow-up</td>
<td>10-month change (95% CI; p-value)</td>
</tr>
<tr>
<td>Malattia sum score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower Extremity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>209/11</td>
<td>11 (8)</td>
<td>11 (8)</td>
<td>0 (-2.9 to 2.2; 0.759)</td>
</tr>
<tr>
<td>Quadriceps</td>
<td>44/11</td>
<td>k1 (2)</td>
<td>2 (3)</td>
</tr>
<tr>
<td>Hamstrings</td>
<td>44/11</td>
<td>1 (1)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Ankle dorsiflexors</td>
<td>26/13</td>
<td>2 (1)</td>
<td>2 (1)</td>
</tr>
<tr>
<td>Ankle plantarflexors</td>
<td>39/13</td>
<td>3 (2)</td>
<td>3 (2)</td>
</tr>
<tr>
<td>T2 relaxation time (ms)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower Extremity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>161/9</td>
<td>33.5 (0.7)</td>
<td>33.5 (0.9)</td>
<td>-0.0 (-0.6 to 0.5; 0.883)</td>
</tr>
<tr>
<td>Quadriceps</td>
<td>36/9</td>
<td>32.5 (1.6)</td>
<td>32.8 (1.9)</td>
</tr>
<tr>
<td>Hamstrings</td>
<td>35/9</td>
<td>33.4 (0.9)</td>
<td>33.3 (0.9)</td>
</tr>
<tr>
<td>Ankle dorsiflexors</td>
<td>19/10</td>
<td>33.6 (1.9)</td>
<td>33.4 (2.4)</td>
</tr>
<tr>
<td>Ankle plantarflexors</td>
<td>21/10</td>
<td>34.9 (1.4)</td>
<td>34.9 (1.5)</td>
</tr>
</tbody>
</table>

Results are presented as mean (SD) or mean change (95% confidence interval (CI)). *No significant differences were observed between the standard care and intervention group at baseline. Quadriceps: rectus femoris and three vasti muscles. Hamstrings: biceps femoris short and long head, semitendinosus, and semimembranosus. Ankle dorsiflexors: tibialis anterior and extensor digitorum longus. Ankle plantarflexors: soleus and gastrocnemius medialis and lateralis.
Predictors

**Fat infiltration:** Assessment of the longitudinal change in the individual lower extremity muscles revealed that, in the intervention group, fat infiltration at baseline was associated with the increase in fat fraction, CSA, and contractile CSA \( (p < 0.001, p = 0.021, p = 0.016, \) respectively; figure 4.4A/B). CSA and contractile CSA increased more in muscles without fat infiltration (fat fractions comparable to healthy volunteers) than in muscles with fat infiltration. Furthermore, in muscles without fat infiltration, fat fraction increased less. In contrast, the change in fat fraction, CSA, contractile CSA, in the standard care group and the \( T_2 \) water in both groups were independent of baseline fat infiltration (figure 4.4A/B).

**TIRM hyperintensity:** Separating muscles based on their Malattia score revealed that the fat fraction increase depended on the severity of the TIRM hyperintensity at baseline (oedema) (intervention: \( p < 0.001 \); standard care: \( p = 0.045 \); figure 4.4C); fat fraction increased more in muscles with a higher Malattia score. Longitudinal changes in CSA, contractile CSA, and \( T_2 \) water were independent of the Malattia scores in both groups (figure 4.4D).

**Responsiveness and repeatability**

The responsiveness was highest for the fat fraction (SRM of 1.00 on subject level). CSA and contractile CSA had a SRM of -0.50 and -0.63, respectively, in the same range as the clinical measure 6MWT (SRM: -0.65) (table 4.4). The repeatability analysis showed a coefficient of repeatability of 0.4% for fat fraction, 8 cm\(^2\) for CSA, 16 cm\(^2\) for contractile CSA, and 1.0 ms for \( T_2 \) water at subject level (table 4.4). At the individual muscle level, fat fraction, CSA, contractile CSA, and \( T_2 \) water had a coefficient of repeatability of 1.9%, 1.4 cm\(^2\), 2.9 cm\(^2\), 2.5 ms, respectively (table 4.4).

**Table 4.4:** Responsiveness and repeatability of the MRI outcome measures at the subject and individual muscle level.

<table>
<thead>
<tr>
<th>MR outcome measure</th>
<th>SRM</th>
<th>Bias</th>
<th>Limits of agreement</th>
<th>Coefficient of repeatability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subject level</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat fraction (%)</td>
<td>1.00</td>
<td>0.19</td>
<td>[-0.2, 0.6]</td>
<td>0.4</td>
</tr>
<tr>
<td>Cross-sectional area (cm(^2))</td>
<td>-0.50</td>
<td>-3</td>
<td>[-11, 4]</td>
<td>8</td>
</tr>
<tr>
<td>Contractile cross-sectional area (cm(^2))</td>
<td>-0.63</td>
<td>-14</td>
<td>[-29, 2]</td>
<td>16</td>
</tr>
<tr>
<td>( T_2 ) water (ms)</td>
<td>-0.06</td>
<td>-0.1</td>
<td>[-1.1, 0.8]</td>
<td>1.0</td>
</tr>
<tr>
<td>Individual muscle level</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat fraction (%)</td>
<td>0.24</td>
<td>[-1.6, 2.1]</td>
<td>1.9</td>
<td></td>
</tr>
<tr>
<td>Cross-sectional area (cm(^2))</td>
<td>-0.2</td>
<td>[-1.5, 1.2]</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>Contractile cross-sectional area (cm(^2))</td>
<td>-1.0</td>
<td>[-3.9, 1.8]</td>
<td>2.9</td>
<td></td>
</tr>
<tr>
<td>( T_2 ) water (ms)</td>
<td>-0.1</td>
<td>[-2.5, 2.3]</td>
<td>2.5</td>
<td></td>
</tr>
</tbody>
</table>

SRM: Standardized response mean.
Figure 4.4: Predictors for the 10-month change in MRI outcome measures in individual muscles. The 10-month longitudinal change in the individual lower extremity muscles for fat fraction and cross-sectional area (CSA) in the standard care (blue) and behavioural intervention group (green) with muscles separated based on fat infiltration at baseline and presence of signal hyperintensity on TIRM images (oedema). A/B) Muscles are separated in muscles without fat infiltration at baseline (non-fat, light colour) and with fat infiltration at baseline (fat, dark colour) for fat fraction (A) and CSA (B). It shows that the intervention had the strongest effect on non-fat infiltrated muscles. A muscle is defined as fat infiltrated when the fat fraction in that muscle was higher than the mean + 2SD of a muscle’s
EFFECT OF A BEHAVIOURAL INTERVENTION IN DM1 STUDIED BY MUSCLE MRI

fat fraction in the unaffected individuals. C/D) Muscles are separated based on TIRM hyperintensity using the baseline Malattia score (0, 1, and 2) for fat fraction (C) and CSA (D). It shows that fat fraction increased most in TIRM hyperintense muscles, which is not influenced by the intervention. Data are shown per muscle and mean ± SD.

Discussion

This prospective multi-centre quantitative muscle MRI study showed that patients who received a behavioural intervention directed at increasing daily activity gained around 4% of lower extremity muscle mass in 10 months. This gain was most prominent in muscles without fat infiltration at baseline. Fat infiltration in the lower extremity muscles progressed with 0.9% in these 10 months compared to 1.2% in the standard care group, with largest increases in fat fraction in muscles with signs of oedema at baseline. The standard care group showed no change in muscle mass over 10 months, and neither group showed longitudinal changes in tissue water distribution (oedema).

This is the first intervention study in DM1 using quantitative muscle MRI to assess a treatment at the muscle level. In another neuromuscular disease, facioscapulohumeral muscular dystrophy (FSHD), a similar behavioural intervention also had a beneficial effect on the muscles. In the latter study, muscle MRI revealed that fat infiltration rate decelerated. In DM1, the intervention did not influence fat infiltration rate. Nevertheless, despite the progression in fat infiltration, contractile CSA did not decrease. On the contrary, contractile CSA tended to increase, meaning that the muscle mass increase due to training was larger than muscle mass decrease due to fat infiltration (figure 4.5). This effect was most prominent in the quadriceps muscles, probably because these had minimal fat infiltration and oedema at baseline and are large muscles. The clinical relevance of this finding is reflected in the positive effect of the intervention on the activity and participation (measured with the Rasch-built DM1-activ-c) and exercise capacity (measured with the 6MWT). Increased physical activity in the intervention group is a likely cause for increased CSA and contractile CSA and is supported by the correlation between baseline contractile CSA and the accelerometer measured activity. Our findings demonstrate the safety of interventions that aim to increase physical activity in DM1. This is in line with other training studies that did not demonstrate any detrimental effects on skeletal muscles. Most importantly, our work suggests that muscles in DM1 patients can be trained as you would expect in healthy subjects. This especially holds for healthy appearing muscle, which underlines that such interventions should preferably be started early in the disease course.

The changes over 10 months in the standard care group represent the natural disease progression in DM1 and reveal a slow but detectable increase in fat fraction. This progression in fat infiltration was not apparent in the semi-quantitative Lamminen score, likely because the Lamminen score is less-sensitive to small changes. In other muscular
dystrophies, natural history studies have been performed using quantitative muscle MRI, but no such studies have previously been conducted in DM1.\textsuperscript{18–21} The progression in fat infiltration (+1.2%/10 months) in DM1 is comparable to limb girdle muscular dystrophy (1-4%/year), oculopharyngeal muscular dystrophy (1.5%/year) and Charcot-Marie-Tooth 1A (0-1%/year).\textsuperscript{22–24} In contrast, progression in fat infiltration is faster in FSHD, Duchenne muscular dystrophy (DMD), and inclusion body myositis with 7%, 3-7% and 3% per year, respectively.\textsuperscript{24–26} The slow fat infiltration rate in DM1 corroborates with the slow decrease in muscle strength.\textsuperscript{27,28}

The progression of fat infiltration was independent of fat infiltration at baseline. These findings contrast with studies in FSHD, in which the highest fat infiltration rates were seen in muscle with intermediate fat infiltration levels.\textsuperscript{25,29} In our DM1 patients, the largest increases in muscle fat fraction at 10 months was observed in muscles with signal hyperintensity at the TIRM images. This supports the hypothesis that TIRM hyperintensity and increased T2\textsubscript{water} lesions (believed to reflect oedema) are an early pathological change occurring prior or parallel to fat infiltration. These findings corroborate those from other muscular dystrophies such as FSHD, Pompe and DMD.\textsuperscript{5,25,26,30} Therefore, T2\textsubscript{water} has been proposed to assess early pathological changes, and was indeed a successful biomarker for response to corticosteroid treatment in DMD.\textsuperscript{5,26} In our study, we did not detect an intervention effect

Figure 4.5: Schematic representation of the effect of the behavioural intervention over 10 months in comparison to the standard care group. Both groups show a significant increase in fat fraction. However, in the intervention group this does not lead to a drop in contractile cross-sectional area (contractile CSA, only muscle tissue), because cross-sectional area (CSA, muscle + fat tissue) significantly increased. * indicates a significant change over 10 months compared to baseline. All other values are non-significant trends.
on the TIRM hyperintensity (Malattia score) and $T_2^{\text{water}}$, further supporting that increasing physical activity does not promote muscle degeneration and thus is safe.

The measures for repeatability and responsiveness indicate that the whole lower extremity fat fraction is the most responsive MR outcome measure in our study. The observed natural change was higher than the coefficient of repeatability, meaning that the average 10-month increase of 1.2% can be detected in an individual patient with a certainty of more than 95%. Together with the beneficial effects of the behavioural intervention on CSA and contractile CSA, this indicates that fat fraction, CSA and contractile CSA may be used as muscle biomarkers in future clinical trials.

Besides the strong points of this study, it being the first longitudinal quantitative muscle MRI study in a well characterised cohort of DM1 patients, there are limitations. First, we included a small subset of all patients participating in OPTIMISTIC. Nevertheless, demographics and clinical measures showed good agreement with the entire OPTIMISTIC cohort. Second, no significant between-group differences were found with accelerometry, in contrast to the main OPTIMISTIC study. This could be explained by the smaller groups in this MRI study, limiting statistical power. Third, the CTG modal was higher in the standard care than the intervention group. This could suggest that DM1 patients in the intervention group were less affected and would therefore be better trainable. However, at baseline, clinical measures and MRI measures did not differ between the groups suggesting that the groups were comparable.

To conclude, DM1 patients can increase their muscle mass by increasing physical activity, which preferably should start when fat infiltration is still limited. Furthermore, our work indicates that fat fraction, CSA and contractile CSA are responsive biomarkers for future clinical trials with timeframes of 10 months.

Acknowledgements
We would like to thank all participants for participating in this study. In addition, we like to thank Barbara Janssen for her help with the MRI protocol, Ferroudja Daidj for participants management in Paris, and Sjaak van Asten, Lydia Overtoom and Justine van Eerden for their help with data-acquisition and/or processing.
References


6. van Engelen B. Cognitive behaviour therapy plus aerobic exercise training to increase activity in patients with myotonic dystrophy type 1 (DM1) compared to usual care (OPTIMISTIC): study protocol for randomised controlled trial. Trials. 2015;16:224.


Chapter 5

Specific muscle strength is reduced in facioscapulohumeral dystrophy: An MRI based musculoskeletal analysis

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Abstract

The aim was to test whether strength per unit of muscle area (specific muscle strength) is affected in facioscapulohumeral dystrophy (FSHD) patients, as compared to healthy controls. Ten patients and ten healthy volunteers underwent an MRI examination and maximum voluntary isometric contraction measurements (MVICs) of the quadriceps muscles. Contractile muscle volume, as obtained from the MR images, was combined with the MVICs to calculate the physiological cross-sectional area (PCSA) and muscle strength using a musculoskeletal model. Subsequently, specific strength was calculated for each subject as muscle strength divided by total PCSA. FSHD patients had a reduced quadriceps muscle strength (median(1st quartile - 3rd quartile): 2011 (905.4 - 2775) N vs. 5510 (4727 - 8321) N, \( p < 0.001 \)) and total PCSA (83.6 (62.3 - 124.8) cm\(^2\) vs. 140.1 (97.1 - 189.9) cm\(^2\), \( p = 0.015 \)) compared to healthy controls. Furthermore, the specific strength of the quadriceps was significantly lower in patients compared to healthy controls (20.9 (14.7 - 24.0) N/cm\(^2\) vs. 41.9 (38.3 - 49.0) N/cm\(^2\), \( p < 0.001 \)). Thus, even when correcting for atrophy and fatty infiltration, patients with FSHD generated less force per unit area of residual muscle tissue than healthy controls. Possible explanations include impaired force propagation due to fatty infiltration, reduced intrinsic force-generating capacity of the muscle fibres, or mitochondrial abnormalities leading to impaired energy metabolism.
Introduction
Facioscapulohumeral muscular dystrophy (FSHD) is a hereditary disease characterized by progressive loss of muscle strength, starting in the face, shoulder and upper arm region.\(^1\) In addition, the trunk and lower extremity muscles are frequently involved.\(^2\) Histopathological changes can be very diverse, ranging from mild myopathic features to overt dystrophic changes with fatty infiltration and fibrosis.\(^3\) Magnetic resonance imaging (MRI) of the lower extremity muscles reveals that muscles are affected by fatty infiltration in a specific pattern.\(^3-5\) For example, in the thigh there is early involvement of the hamstrings, adductors and rectus femoris, whereas the vasti muscles usually become involved later in the disease course.

To aid the development of new treatments for FSHD it is important to understand why muscles of FSHD patients are weak. Muscle strength depends on the physiological cross-sectional area (PCSA), i.e. the cross-sectional area perpendicular to the direction of the muscle fibres.\(^6\) In FSHD, fatty infiltration, fibrosis and muscle fibre atrophy affect the muscle tissue and are assumed to result in a reduced PCSA and contractile muscle volume, i.e. the portion of the muscle able to generate force. Therefore, fatty infiltration, atrophy and fibrosis may all contribute to muscle weakness. Janssen et al. indeed showed that the amount of contractile muscle tissue in the quadriceps muscles of FSHD patients is related to muscle strength.\(^3\) Furthermore, it has been suggested that reduced specific muscle strength, defined as strength per unit area of contractile muscle tissue, is an additional cause of muscle weakness in FSHD.\(^7,8\) However, Bachasson et al. did not include imaging of the quadriceps muscles and some of us investigated specific muscle strength only on a single muscle fibre level.\(^7,8\)

In this study, we investigate whether the specific strength of the quadriceps muscles is affected in FSHD patients as compared to healthy controls. For this aim we evaluated contractile volume, PCSA and strength of quadriceps muscles by combining quantitative MRI, quantitative muscle assessment and musculoskeletal modelling.

Materials and Methods
Participants
FSHD patients and healthy control subjects were retrospectively recruited from two cohorts. The FSHD patients were selected from a larger database of 140 FSHD patients who are participating in a prospective cohort study (FSHD-FOCUS study, Nijmegen, The Netherlands). The control cohort consisted of a group of ten healthy volunteers who participated in the TLEMsafe project dataset (FP7-ICT-2009-4). All subjects performed a maximum voluntary isometric contraction (MVIC) of the knee extensor muscles and underwent an MRI examination of the quadriceps muscles. On the day of MVIC examinations, all patients performed also manual muscle testing, 6-minute walking test, motor function measure, and
spirometry, as part of the FSHD-FOCUS study protocol. No other tests were performed by the healthy controls on the examination days. Gender, age, height, and weight have been recorded for both groups. FSHD disease severity were assessed with a 10-point clinical severity score (Ricci score). For the present study, FSHD patients and health volunteers were included if the MVIC measurement was performed correctly, and the MR images of the lower extremity covered the entire span of the quadriceps muscles and contained no artefacts, like movement or failed reconstruction of the fat and water images obtained via a 2pt-Dixon sequence.

This study was conducted according to the principles of the Declaration of Helsinki (version October 2013) and in accordance with the Medical Research Involving Human Subjects Act (WMO). In both groups, informed consent was obtained from each participant.

**Experimental measurements**

**MR imaging**

All subjects were examined on a 3T MR system (TIM Trio, Siemens, Erlangen, Germany) using a ¹H spine coil combined with phased array coils placed around the leg.

In the FSHD patients MR data of the thigh were recorded with a 2pt-Dixon sequence to quantify the amount of fatty infiltration (repetition time (TR): 10 ms, echo time (TE) in-phase: 2.45 ms, TE out-phase: 3.675 ms, flip angle (FA): 3°, voxel size: 1.36x1.36x5.00 mm³, 1 or 2 stacks with number of slices: 72). A fat fraction map, ranging from zero to one, was calculated for each slice from the reconstructed water and fat images by dividing the signal intensities of every voxel in the fat image by the signal intensities of the same voxel in the fat and water images summed together F/(F+W).

The dataset of healthy volunteers contained T1-weighted MR images of the lower extremity from hip to ankle (TR/TE: 545/9 ms, voxel size: 1.04x1.04x3.00 mm³, number of slices: 400). These T1-weighted data did not allow for quantitative fat measurements. Visual inspection of fatty infiltration on T1-weighted images did not show any pathological fatty infiltration of the quadriceps muscles in any of the healthy subjects. Therefore, for the healthy group, fat fraction values were assumed equal to 8.4%, 7.1%, 6.7% and 7.5% in the rectus femoris, vastus intermedius, vastus lateralis, and vastus medialis respectively, based on our previous measurements in a separate group of ten healthy volunteers (unpublished data, mean (1st quartile - 3rd quartile); age: 41.5 (38-56) years old; BMI: 24.0 (21.6-24.9) kg/m²).

**Strength assessment in the FSHD group**

Maximum voluntary isometric contraction of the knee extensor muscles was assessed in the dominant leg of FSHD patients. Each participant was seated with both hips and knees flexed at 90° on a fixed quadriceps dynamometer. The ankle of the dominant leg was secured to
the dynamometer using Velcro straps and located at a distance of 24.5 cm from the knee joint centre. In this position, the participants were instructed to extend their leg, pushing maximally against the dynamometer. Isometric force was recorded and was fed back visually to the subject on a computer monitor. The test consisted of three consecutive knee extensor MVICs of 4 seconds each, with a resting period of 20 seconds between the tests. Hand support was not allowed throughout the test. Strong verbal and visual encouragement were employed to motivate the participants to deliver their maximal muscle effort. The maximum out of three measured forces was chosen as the final MVIC force. The MVIC force was then multiplied by the knee-to-sensor distance, resulting in the MVIC torque ($T$).

**Strength assessment in the control group**

The MVIC in healthy controls was assessed using a Biodex Dynamometer setup (Biodex Medical Systems, Inc., Shirley, NY, USA). In brief, participants were seated on the chair of the device with both hips and knees flexed by 90°. Belts were placed around the thorax and the thigh and the ankle of the measured leg were tightened. Only the dominant leg was measured, similarly to the FSHD group. Three consecutive MVICs of 6 seconds each were performed, with a resting period of 20 seconds between the tests. Visual feedback and verbal motivation were employed. The highest of the three measured torques was taken as the final outcome.

A post hoc experiment was carried out in a separate group of 9 healthy volunteers to identify possible systematic differences in the MVIC measures between FSHD and control group, owing to different measurement devices. Bland-Altman analysis revealed a measurement bias and an intraclass correlation coefficient of 1.0 Nm and 0.94, respectively. Hence, we did not find any systematic difference in MVIC assessment when using two different measurement devices.

**Data analysis**

**MRI and segmentation and volume/fat fraction**

MR images were analysed using the Medical Image Processing, Analysis, and Visualization software package (MIPAV, Center for Information Technology, National Institutes of Health, obtainable at http://mipav.cit.nih.gov) and MATLAB (version R2014B, The Mathworks, Inc. Natick, Massachusetts, United States). Muscle contours of the four quadriceps muscles were manually delineated every 2.5 cm in the axial direction from the most distal MRI slice to the most proximal MR slice (see figure 5.1).
Caution was taken to avoid the inclusion of subcutaneous fat, fascia, and large blood vessels. Anatomical muscle volume \( V_a \) was determined as the number of voxels within the muscle mask across all slices multiplied by the voxel area and by the distance between slices of 2.5 cm. In FSHD patients, an average fat fraction \( f_{fat} \) was calculated for each of the quadriceps muscles over the voxels in the muscle mask across all slices at which the quadriceps muscles were delineated.

It was assumed that the fatty infiltrate cannot generate force. Thus, for each muscle, the contractile muscle volume \( V_c \) was determined as (equation 5.1):

\[
V_c = V_a \cdot (1 - f_{fat}) \quad (equation \ 5.1)
\]

where \( V_a \) is the anatomical muscle volume.

**Calculation of specific muscle strength**

Maximal force-generating capacity in a skeletal muscle, or muscle strength \( S \) (in Newton (N)), is proportional to the muscle physiological cross-sectional area (PCSA, in cm\(^2\)). \( S \) and PCSA are related by the specific muscle strength \( \sigma \) (in N/cm\(^2\); equation 5.2):

\[
S = \sigma \cdot PCSA \quad (equation \ 5.2)
\]

\( S \) is also related to the experimentally measured torque \( \tau \) (in Nm) by the relation (equation 5.3):

\[
\tau = r_{knee} \cdot S \quad (equation \ 5.3)
\]
To derive $r_{\text{knee}}$ and PCSA of the quadriceps muscles in all participants we used a musculoskeletal model. Briefly, a standard model was obtained from the AnyBody Managed Model Repository (AMMR 1.6.4) in the AnyBody Modeling System (AMS, version 6.0.5, AnyBody Technology A/S, Aalborg, Denmark).\textsuperscript{11} Body height was used to scale the body segments dimensions uniformly. Since the moment arm of a muscle about a joint depends on the angle of that joint, the model was configured in a sitting position similar to the one used in the experiments, with both hips and knees flexed by 90°, see figure 5.2. Existing algorithms available in the AMMR were used to calculate $r_{\text{knee}}$ for each subject, using the tendon excursion method.\textsuperscript{12}

The PCSA of each of the quadriceps muscles was calculated from the contractile volume ($V_c$) divided by the optimal fibre length ($l_o$) and corrected for the pennation angle ($\phi$) using the relationship (equation 5.4)\textsuperscript{13}:

$$\text{PCSA} (cm^2) = \frac{V_c \cos \phi}{l_o} \quad \text{(equation 5.4)}$$

in which $l_o$ and $\phi$ were derived from a dataset of architectural properties of cadaveric lower extremity muscles.\textsuperscript{14} The total PCSA of the quadriceps ($\text{PCSA}_{\text{tot}}$) was calculated as the sum of the individual quadriceps muscles PCSAs. Finally, a specific muscle strength for each participant was calculated from equation 5.2, dividing $S$ by $\text{PCSA}_{\text{tot}}$.

### Statistical analysis

Statistical analysis was performed using Graphpad Prism 5 for Windows (version 5.03, Graphpad Software, San Diego, California, USA). Two-tailed unpaired Student’s t tests were used to compare the characteristics of FSHD patients and healthy volunteers. Not normally distributed characteristics (according to Shapiro-Wilk test) were compared using the Mann-Whitney U test. Significance level was set to 0.05. The same test was used to compare $\tau$, $S$, $V_a$, $V_c$, $f_{\text{fat}}$, PCSA, and $\sigma$. The relation between $S$ and $\text{PCSA}_{\text{tot}}$ in both the FSHD and control group was analysed using Pearson product moment correlation analysis. We used data on specific muscle strength on 20 adult healthy subjects for use in a sample size calculation for our study because we found no sufficient information regarding specific strength variance over the FSHD population.\textsuperscript{15} They reported specific muscle strength to be equal to (mean ± SD) 56 ± 11 N/cm². Setting a power of 0.8 and a Type I error of 0.05, results in a sample size of 10 patients (and 10 controls) to be able to detect a significant difference of 25% in specific strength, which we consider to be clinically relevant. Due to the small sample size, data are presented as median (1$^{\text{st}}$ quartile - 3$^{\text{rd}}$ quartile)
Results

Participants characteristics

Ten FSHD patients and ten healthy controls met our inclusion criteria and were included in the analysis. Both groups were comparable in age, height, weight, and BMI (see table 5.1 for their characteristics). Four out of the ten FSHD patients included in this study performed the muscle strength on the same day as the MRI examination. In the other six patients the examinations took place between thirty days and eleven weeks apart. The examinations in healthy controls were completed no more than two months apart.

Figure 5.2: A view of the musculoskeletal model used to calculate the specific strength (σ). The model was adapted from the ‘Standing Model’ as available in the AnyBody Managed Model Repository (AMMR 1.6.4) in the AnyBody Modeling System (AMS, version 6.0.5, AnyBody Technology A/S, Aalborg, Denmark). The model was scaled based on the height of each subject and measurements of muscle volume. Contractile quadriceps muscle volumes (V_c) were measured by anatomical MRI and corrected using fat fraction maps. Pennation angle (φ) and optimal fibre length (l_0) values were extracted from the literature.\(^8\) The average moment arm of quadriceps muscles (r_{knee}) was calculated at 90 degrees of knee flexion using the tendon excursion method.
Table 5.1 Characteristics of patients with facioscapulohumeral dystrophy (FSHD) and healthy controls.

<table>
<thead>
<tr>
<th></th>
<th>FSHD (n = 10)</th>
<th>Controls (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female (%)</td>
<td>50.0</td>
<td>50.0</td>
</tr>
<tr>
<td>Age (years)</td>
<td>56.0 (47.8, 66.5)</td>
<td>35.5 (24.5, 57.5)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>174.0 (168.8, 177.3)</td>
<td>176.0 (163.8, 183.5)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>72.0 (54.8, 87.5)</td>
<td>76.8 (62.9, 84.9)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.3 (19.9, 27.2)</td>
<td>24.5 (21.7, 26.4)</td>
</tr>
<tr>
<td>Ricci score</td>
<td>7.5 (2.8, 8.0)</td>
<td></td>
</tr>
</tbody>
</table>

Data are shown as median (1st quartile, 3rd quartile).

Quantitative MRI and strength measurements

The MRI data revealed that fat fraction was significantly increased in quadriceps muscles of FSHD patients relative to normal values (p < 0.01; table 5.2). The rectus femoris and vastus medialis of FSHD patients had a reduced anatomical volume compared to the control group (rectus femoris: p = 0.005; vastus medialis: p = 0.029) (table 5.2). Furthermore, the contractile volume of all quadriceps muscles, except the vastus lateralis, was lower in FSHD patients compared to healthy controls (rectus femoris: p = 0.004; vastus intermedius: p = 0.043; vastus medialis: p = 0.019, table 5.2). Maximum voluntary isometric contraction torque was significantly lower in FSHD patients compared to healthy controls (p < 0.001; table 5.2).

Musculoskeletal modelling: PCSA and specific strength calculation

Quadriceps muscle strength in FSHD patients was significantly lower than in healthy controls (FSHD: 2011 (905.4 - 2775) N, healthy controls: 5510 (4727 - 8321) N, p < 0.001; table 5.2). Total PCSA, i.e. cross-sectional area perpendicular to the muscle fibre direction, of the quadriceps in FSHD patients was significantly lower than in healthy controls (FSHD: 83.6 (62.3 - 124.8) cm², healthy controls: 140.1 (97.1 - 189.9) cm², p = 0.015; table 5.2). Total PCSA correlated very strongly with the quadriceps muscle strength (figure 5.3; FSHD: r = 0.95, p < 0.001; healthy controls: r = 0.86, p = 0.001). Specific muscle strength, i.e. strength per unit of PCSA, was significantly lower in FSHD patients compared to healthy controls (FSHD: 20.9 (14.7 - 24.0) N/cm²; healthy controls: 41.9 (38.3 - 49.0) N/cm², p < 0.0001, figure 5.4). Hence, specific strength was reduced by 56% in FSHD patients relative to controls.
Table 5.2: Maximum voluntary isometric contraction (MVIC) torque, anatomical volume, fat fraction, contractile volume, physiological cross-sectional area (PCSA) and calculated specific strength of quadriceps muscles in patients with facioscapulohumeral dystrophy (FSHD) and healthy controls.

<table>
<thead>
<tr>
<th></th>
<th>FSHD (n = 10)</th>
<th>Controls (n = 10)</th>
<th>p-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>MVIC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Measured torque (Nm)</td>
<td>63.8 (28.5, 91.3)</td>
<td>185.4 (146.1, 286.2)</td>
<td>&lt;0.001 ***</td>
</tr>
<tr>
<td>Calculated strength (N)</td>
<td>2011 (905.4, 2775)</td>
<td>5510 (4727, 8321)</td>
<td>&lt;0.001 ***</td>
</tr>
<tr>
<td><strong>Anatomical volume</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (cm$^3$)</td>
<td>1046 (755.3, 1361)</td>
<td>1515 (1049, 2039)</td>
<td>0.029 *</td>
</tr>
<tr>
<td>Rectus femoris (cm$^3$)</td>
<td>107.2 (44.1, 144.7)</td>
<td>203.5 (139.5, 322.4)</td>
<td>0.005 **</td>
</tr>
<tr>
<td>Vastus lateralis (cm$^3$)</td>
<td>408.8 (346.6, 489)</td>
<td>535.4 (329.0, 697.0)</td>
<td>0.393 ns</td>
</tr>
<tr>
<td>Vastus intermedius (cm$^3$)</td>
<td>322.0 (218.1, 368.3)</td>
<td>346.6 (317.9, 510.6)</td>
<td>0.123 ns</td>
</tr>
<tr>
<td>Vastus medialis (cm$^3$)</td>
<td>212.1 (103.2, 326.4)</td>
<td>358.7 (272.4, 497.9)</td>
<td>0.029 *</td>
</tr>
<tr>
<td><strong>Fat fraction</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (%)</td>
<td>12.2 (9.3, 19.2)</td>
<td>7.3 (7.2, 7.3)</td>
<td>0.001 **</td>
</tr>
<tr>
<td>Rectus femoris (%)</td>
<td>17.3 (8.2, 45.0)</td>
<td>8.4$^a$</td>
<td>0.020 *</td>
</tr>
<tr>
<td>Vastus lateralis (%)</td>
<td>10.5 (8.7, 15.5)</td>
<td>7.1$^a$</td>
<td>0.011 *</td>
</tr>
<tr>
<td>Vastus intermedius (%)</td>
<td>10.4 (8.0, 20.9)</td>
<td>6.7$^a$</td>
<td>0.002 *</td>
</tr>
<tr>
<td>Vastus medialis (%)</td>
<td>15.4 (8.8, 35.1)</td>
<td>7.5$^a$</td>
<td>0.011 *</td>
</tr>
<tr>
<td><strong>Contractile volume</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (cm$^3$)</td>
<td>862.4 (639.7, 1264)</td>
<td>1405 (973.4, 1891)</td>
<td>0.019 *</td>
</tr>
<tr>
<td>Rectus femoris (cm$^3$)</td>
<td>88.7 (24.2, 128.9)</td>
<td>186.4 (127.8, 295.3)</td>
<td>0.004 **</td>
</tr>
<tr>
<td>Vastus lateralis (cm$^3$)</td>
<td>353.1 (312.8, 456.2)</td>
<td>497.4 (305.7, 647.5)</td>
<td>0.315 ns</td>
</tr>
<tr>
<td>Vastus intermedius (cm$^3$)</td>
<td>284.4 (176.5, 331.6)</td>
<td>323.4 (296.6, 476.4)</td>
<td>0.043 *</td>
</tr>
<tr>
<td>Vastus medialis (cm$^3$)</td>
<td>165.9 (71.3, 302.2)</td>
<td>331.8 (252.0, 460.5)</td>
<td>0.019 *</td>
</tr>
<tr>
<td><strong>PCSA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (cm$^2$)</td>
<td>83.6 (62.3, 124.8)</td>
<td>140.1 (97.1, 189.9)</td>
<td>0.015 *</td>
</tr>
<tr>
<td>Rectus femoris (cm$^2$)</td>
<td>11.3 (3.1, 16.5)</td>
<td>23.8 (16.3, 37.8)</td>
<td>0.004 **</td>
</tr>
<tr>
<td>Vastus lateralis (cm$^2$)</td>
<td>33.7 (29.9, 43.6)</td>
<td>47.5 (29.2, 61.8)</td>
<td>0.315 ns</td>
</tr>
<tr>
<td>Vastus intermedius (cm$^2$)</td>
<td>28.6 (17.7, 33.3)</td>
<td>32.5 (29.8, 47.8)</td>
<td>0.043 *</td>
</tr>
<tr>
<td>Vastus medialis (cm$^2$)</td>
<td>14.9 (6.4, 27.1)</td>
<td>29.8 (22.6, 41.4)</td>
<td>0.019 *</td>
</tr>
<tr>
<td>Calculated specific strength (N/cm$^2$)</td>
<td>20.9 (14.7, 24.0)</td>
<td>41.9 (38.3, 49.0)</td>
<td>&lt;0.0001 ***</td>
</tr>
</tbody>
</table>

Data are shown as median (1$^{st}$ quartile, 3$^{rd}$ quartile). MVIC: maximum voluntary isometric contraction; PCSA: physiological cross-sectional area. $^a$Fat fraction of individual muscles of healthy controls is based on unpublished experimental data. * p < 0.05, ** p < 0.01, *** p < 0.001.
Discussion
Our study shows that specific muscle strength is reduced more than 50% in our group of FSHD patients relative to healthy controls. In addition, contractile muscle volume and PCSA are significantly reduced. Therefore, it can be concluded that muscle weakness in FSHD is not only caused by a reduced amount of available contractile muscle tissue, but also by reduced function of the residual muscle tissue.
Muscle weakness in FSHD is partly caused by the reduction in the contractile muscle volume and PCSA. The smaller contractile muscle volume is firstly caused by the infiltration of fat. Furthermore, atrophy plays a role, because the anatomical muscle volume of the rectus femoris and vastus medialis is also reduced. The most important finding of our study is that the specific muscle strength is reduced in FSHD patients. This reduced specific muscle strength is not unique to FSHD, as similar results have been reported for Duchenne and Becker muscular dystrophy patients. The exact reason why specific muscle strength is reduced in FSHD is unknown. Potential explanations may be related to changes at the level of muscle fibres, energy metabolism, or in cytoarchitecture, resulting in biomechanical changes that alter force propagation.

First, reduced specific strength of the quadriceps may reflect reduced strength generation of individual muscle fibres. A pilot study by Lassche et al. found a 30% strength reduction in demembranated FSHD type II fibres obtained from quadriceps muscle biopsies from FSHD patients, compared with healthy controls. Second, the integrity of the sarcolemma can be compromised in FSHD muscle fibres, which may hamper lateral transmission of force generated by muscle fibres. Third, mitochondria in FSHD quadriceps muscle tissue might be dysfunctional. Furthermore, in vivo measurement with MR spectroscopy showed that phosphocreatine to ATP ratio is reduced in moderately fat infiltrated FSHD muscles, indicating that energy metabolism is indeed altered. Both the ATP synthesis and the ratio of phosphocreatine to ATP correlate positively with the force produced by the quadriceps muscles. Fourth, fatty infiltration may hamper the transfer of force to the tendon due to fatty interposition, resulting in reduced muscle strength. Advanced imaging techniques, such as ultrasound strain measurement and diffusion-tensor imaging can demonstrate muscle contraction patterns and force propagation and may characterize possible alterations in muscle fibres in FSHD. Besides fatty infiltration, FSHD muscles may suffer from increased interstitial fibrosis which would reduce the fractional muscular area. As our MRI protocol is not designed to detect fibrosis it was not taken into account in our study. As a consequence, the contractile muscle volume might have been overestimated, resulting in underestimation of the specific muscle strength in FSHD in our study. However, to explain the ±50% reduction of specific muscle strength in our group of FSHD patients compared to healthy controls, the remaining contractile muscle volume should consist for 50% of fibrosis. In humans fractional fibrotic areas are rarely quantified. For the heart it has been reported that myocardial fibrosis may be present up to 15% in coronary disease. In an FSHD mouse model extreme high induction of DUX4 resulted in approximately 15% fibrosis in the gastrocnemius. As our patients only showed a mild to moderate fat infiltration it is unlikely that fibrosis has contributed significantly to the lower specific muscle strength observed in our study.

Quadriceps fat fraction in this study varied from normal (<10%) to moderately affected (46%). This was reflected by the clinical severity scores. Three patients had no lower
extremity involvement and one patient was unable to walk unaided. Thus, FSHD patients in our study show a large variability as is also seen in the FSHD population in general. Due to the retrospective nature of this study, the FSHD patients and healthy controls were recruited from two different cohorts in which a different force measurement device and MR protocol was used. Our post-hoc experiment comparing the two measurement devices in a separate group of healthy volunteers showed no systemic difference between the two devices. The MR protocol in healthy volunteers did not allow quantitative assessment of fat infiltration. Therefore, the fat fractions for healthy controls used in this study were obtained from a separate group of healthy volunteers measured with the same 2pt Dixon protocol as the FSHD patients. For this reason, we conclude that the reduction of specific strength is not due to the different measurement set-up and MR protocol used in the FSHD patients and healthy controls. Since FSHD patients may have been fatigued or felt pain more than healthy volunteers, this might have lowered their MVIC measurement. However, Bacchasson et al. found similar central and peripheral quadriceps fatigability in FSHD patients vs. controls, suggesting that fatigue or pain did not cause the changes in specific strength. In our musculoskeletal model the pennation angle and optimal muscle fibre length were assumed to be similar in FSHD patients and healthy controls, although these may have been altered by disease processes in FSHD. However, measuring optimal fibre length and pennation angle in vivo is not feasible in a clinical setting, if not even impossible with the current techniques. Finally, the rectus femoris was included in the total quadriceps muscle PCSA, even though it has a minor contribution to force generation in the sitting position analysed. However, in our FSHD cohort the rectus femoris is the most fat infiltrated quadriceps muscle. Thus, if the rectus femoris contribution is not taken into account the difference in specific strength between the two groups would become even larger.

This study may serve as a reference for future studies employing computer models of the musculoskeletal systems to study FSHD, as it provides values of specific muscle strength of an FSHD group in comparison to a group of healthy controls. Incorporating FSHD-specific model parameters, such as specific muscle strength, is important as the recruitment of muscles is dependent on the capacity of each muscle to generate force. To the present, it remains unclear, for instance, whether FSHD would also alter the recruitment of muscles during daily activities, which warrants further investigation.

**Conclusion**

Specific muscle strength of the quadriceps muscle is reduced in a group of FSHD patients compared to healthy controls, suggesting an intrinsic impairment in the force-generating capacity, energy metabolism and/or force propagation in FSHD muscles. Total contractile PCSA is also reduced in FSHD muscles compared to healthy controls, indicating that fatty infiltration and atrophy are co-factors associated with muscle weakness in FSHD. This finding is important for two reasons. First, future studies that apply musculoskeletal models...
in FSHD patients should consider reduced specific muscle strength. Second, it tells us that interventions targeting muscle weakness in FSHD should focus on treating or preventing not only fatty infiltration and changes in contractile muscle volume, but also aim to restore specific muscle strength.

Acknowledgements
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References


Chapter 6

Oxidative capacity varies along the length of healthy human tibialis anterior

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Abstract
The rate of phosphocreatine (PCr) recovery ($k_{PCr}$) after exercise, characterizing muscle oxidative capacity, is traditionally assessed with unlocalized $^{31}$P magnetic resonance spectroscopy (MRS) using a single surface coil. However, because of intramuscular variation in fibre type and oxygen supply, $k_{PCr}$ may be non-uniform within muscles. We tested this along the length of the tibialis anterior (TA) muscle in 10 male volunteers. For this purpose we employed a 3T MR system with a $^{31}$P/$^1$H volume transmit coil combined with a home-built $^{31}$P phased-array receive probe, consisting of five coil elements covering the TA muscle length. Mono-exponential $k_{PCr}$ was determined for all coil elements after 40s of submaximal isometric dorsiflexion (SUBMAX) and incremental exercise to exhaustion (EXH). In addition, muscle functional MRI ($^1$H mfMRI) was performed using the volume coil after another 40s of SUBMAX. A strong gradient in $k_{PCr}$ was observed along the TA ($p < 0.001$), being two times higher proximally vs. distally during SUBMAX and EXH. Statistical analysis showed that this gradient cannot be explained by pH variations. A similar gradient was seen in the slope of the initial post-exercise $^1$H mfMRI signal change, which was higher proximally than distally in both the TA and the extensor digitorum longus ($p < 0.001$) and it strongly correlated with $k_{PCr}$. The pronounced differences along the TA in functional oxidative capacity identify regional variation in the physiological demand of this muscle during everyday activities and have implications for the bio-energetic assessment of interventions to modify its performance and of neuromuscular disorders involving the TA.
Introduction

Skeletal muscle disposes of efficient cellular buffer systems that enable sudden and large changes in energy expenditure during transitions from rest to exercise and recovery. Creatine kinase (CK) and phosphocreatine (PCr) provide such a buffering function: during conditions when the demand of ATP cannot be met by oxidative metabolism, typically at the onset of exercise, ATP is produced through hydrolysis of PCr by CK. More than thirty years ago it was demonstrated using $^{31}$P magnetic resonance spectroscopy (MRS) that the ATP required for the recovery of PCr is synthesized aerobically.\textsuperscript{1} Thus, the mono-exponential rate constant of PCr recovery ($k_{PCr}$) after in-bore exercise is a non-invasive correlate of the muscle’s oxidative capacity.\textsuperscript{2–4} It was shown to be higher in trained vs. untrained, young vs. older, and in healthy subjects vs. patients with type II diabetes.\textsuperscript{5–9} Traditionally, $^{31}$P MR spectra of muscles are recorded with a single surface coil and a simple pulse-acquire method. The $^{31}$P signals originate from a region adjacent to the coil. The extent of this region is determined by the size and sensitivity profile of the coil, but otherwise the spectra are unlocalized. However, skeletal muscle is not a homogeneous entity. Most importantly, it is composed of metabolically different fibre types: oxidative type I, and glycolytic type II fibres, of which the spatial distribution is not uniform. In many human muscles, type I fibres are predominantly found in the deep parts, while type II fibres are more prominent in superficial areas.\textsuperscript{10,11} In rodent lower hind limb muscles, including the tibialis anterior, fibre distribution also varies along the length of the muscle, with the proportion of oxidative fibres being higher in the proximal part, and glycolytic fibres more prominent in the distal part of the muscle.\textsuperscript{12,13} As experiments using muscle biopsies point towards faster recovery of PCr in the oxidative type I as compared to the glycolytic type II fibres, $k_{PCr}$ might reflect differential muscle fibre distribution.\textsuperscript{14} In addition, intramuscular variations in $k_{PCr}$ may depend on spatial differences in oxygen supply for maximal oxidative phosphorylation. The presence of such variations along the length of muscles will have implications for the bio-energetic assessment, in individual muscles, of interventions (e.g. exercise programs) and of disease progression in neuromuscular disorders.

The general aim of this study was therefore to investigate whether variations in functional oxidative capacity occur along the length of healthy muscles. More specifically we selected the tibialis anterior (TA) for this study as this muscle is easy to approach and performs in a well-defined and assessable way in foot dorsiflexion. To determine if proximal-distal differences in $k_{PCr}$ exist we employed a dedicated phased-array probe with five coil elements to acquire localized $^{31}$P MR spectra with high signal-to-noise and time resolution along the length of the TA, during and after submaximal and exhaustive foot dorsiflexion.

To confirm that the dorsiflexion exercise properly activates the TA and to examine the possible involvement of other muscles to the exercise, specifically that of the extensor digitorum longus (EDL), we applied muscle functional $^1$H MRI ($^1$H mfMRI) with a $^1$H volume
coil, as an independent high resolution MR approach. Moreover, because the dynamics of the exercise induced signal alterations in $^1$H mfMRI are strongly correlated with $K_{PCr}$ it is relevant to examine intramuscular variations of these signal alterations as well.\textsuperscript{15} This is of additional interest as the signal of apparent transversal relaxation (T2*) weighted gradient-echo (GE) images, used in $^1$H mfMRI, is sensitive to changes in deoxyhaemoglobin concentration and blood flow, although other factors may also be involved.\textsuperscript{16,17}

**Materials and Methods**

**Ethical approval:** This study was conducted according to the principles of the Declaration of Helsinki (version October 2013) and in accordance with the Medical Research Involving Human Subjects Act (WMO), except for registration in a database. The study was approved by the local medical ethics committee (registration number: NL47394.091.14) and all subjects gave written informed consent.

**Subjects:** Ten healthy young (between 18 to 35 y) normal weight (BMI between 18 to 25 kg/m$^2$) male volunteers were recruited for this study. Exclusion criteria were: any form of metallic implants, and claustrophobia. Subjects were instructed to abstain from exercise and alcohol during 24 hours prior to the two experimental sessions. During the week prior to the second trial, the participants were instructed to fill in a validated questionnaire about their level of daily physical activity.\textsuperscript{18}

**Ergometer:** A home-built MR compatible ergometer set-up for dorsiflexion of the foot was used. The volunteer’s right foot was tightly strapped to a shoe attached to a pedal. The pedal, in turn, was connected via a rope to a digital force gauge (Sauter FL 500, Balingen Germany), located outside the magnet room. The force applied during the various exercise bouts was then projected onto the front panel of the magnet housing as online feedback to the volunteers (figure 6.1A/B).

**Design and hardware:** Each volunteer underwent two experimental sessions inside the clinical 3T MR system (Siemens Magnetom Trio, Erlangen, Germany). The first session served to determine the maximum voluntary contraction (MVC, maximum of three attempts) for foot dorsiflexion and was determined in each volunteer inside the MR scanner using the ergometer. Thereafter, the volunteers got acquainted with the set-up and in-bore exercise regime as they performed the same rest-exercise-recovery transitions as during the actual experiments of the second session which are described below.

To assess metabolic differences along the length of the TA, a home-built $^{31}$P phased-array probe was used for signal reception, consisting of five individual coil elements (size: 4 x 4.5 cm each, total size: 4x20cm, overlap of elements for decoupling).\textsuperscript{19} The coil array was positioned on the TA of the right leg, with element 1 (E1) at the distal end of the muscle
placed at a ~12 cm from the lateral malleolus, and fixated with Velcro straps (figure 6.1C). Fish oil capsules in the centre of the two outer most elements allowed for verification of coil position in the MR images. The receive coil was combined with a commercially available $^1$H/$^{31}$P birdcage coil (Rapid, Rimpar, Germany), detunable at the $^{31}$P-frequency and 20 cm in length, which was carefully positioned in the head-foot direction to fully cover the $^{31}$P receive coils. This set-up allowed for a homogeneous phosphorous excitation by the volume coil and recording of free induction decays (FIDs) of high signal-to-noise ratio (SNR) from the TA by the individual coil elements of the surface coil. The birdcage coil’s proton part was further used for anatomical imaging, $^1$H mfMRI and $^{31}$P signal enhancement by employing the $^1$H-$^{31}$P Nuclear Overhauser effect (NOE).

**Figure 6.1: Schematic representation of the experimental set-up.** A) The subject’s right foot was placed in a shoe attached to a pedal which was connected with a rope to a force gauge located outside the scanner room. The leg was positioned in a $^1$H/$^{31}$P birdcage coil with the five-element $^{31}$P surface coil for receive strapped on the tibialis anterior. The force applied during the exercise was then projected onto the scanner as feedback to the volunteers. B) Example of the projection onto the front panel of the magnet housing. The subject was asked to keep his force level (green line) within the two red lines (+5% and -5% of the maximum voluntary contraction). C) $^{31}$P phased array surface coil strapped on the lower leg of a healthy volunteer with the five coil elements depicted.
Experiments in resting muscle
After a series of localizers, anatomical imaging was performed using a T1 weighted turbo spin echo (TSE) sequence with five transversal slices centred to the middle of each $^{31}$P coil element. If the fish oil capsules attached to the outermost coil elements did not appear directly above the TA muscle in the TSE images, the $^{31}$P receive coil array was repositioned. Thereafter, 2D $^{31}$P imaging was performed using a GE sequence (centre-frequency on PCr, TR = 1500 ms, TE = 10 ms, NA = 6, FOV = 199x199 mm, matrix size = 16x16) with localization in the third dimension perpendicular to the coil by the position of the individual coil elements. $^{31}$P MRS: The main magnetic field homogeneity was adjusted on a volume covering all of the muscles within the sensitive area of the transmit coil. Two fully relaxed $^{31}$P MR spectra (90° flip angle, 500 μs hard pulse, TR = 15 s, 6 averages) were performed either with or without NOE ($^1$H decoupling with WALTZ 4, $^1$H frequency on the water frequency).  

Experiments in contracting muscle
The post-exercise signal intensity of $^1$H mfMRI takes at least 15 min to reach baseline levels which is considerably longer than the signals of $^{31}$P metabolites. Therefore, $^1$H mfMRI experiments were always performed before the dynamic $^{31}$P experiments. For the same reason, MVC was not repeated for the second experimental session to avoid any interference with $^1$H mfMRI. Furthermore, the submaximal $^{31}$P experiment always preceded the exhaustive one. $^1$H mfMRI: T2* weighted GE-EPI (TE = 29 ms, TR = 1 s) was applied during 1 min of rest, 40 sec of isometric contractions performed at 60% of MVC (figure 6.2), and during 15 min of recovery using 5 transversal slices (3 mm) corresponding to the centre of each $^{31}$P coil element. In separate experiments on three volunteers, $^1$H mfMRI was also performed without the $^{31}$P phased-array probe tightly strapped on the lower leg to examine if the probe can cause blood flow obstruction along the length of the TA. $^{31}$P MRS: To examine post-exercise $k_{pC_r}$ $^{31}$P MR spectra (TR = 2 s, 2 averages per spectrum, 48° Ernst angle excitation, $^1$H-$^{31}$P NOE enhanced) were acquired during submaximal exercise (SUBMAX) of 30 sec rest, 40 sec isometric contraction at 60% MVC and 5 min recovery (figure 6.2). Finally, the volunteers performed exhaustive exercise (EXH) starting at 10% MVC, increasing by 10% every 30 sec (figure 6.2). The volunteers either stopped voluntarily or the test was interrupted when the volunteers could not maintain the requested force. $^{31}$P MRS (same parameters as for the previous experiment) was acquired during and after EXH for a total duration of 10 min 32 sec.
Figure 6.2: Succession of experiments applying isometric muscle contractions performed inside the clinical MR system. First, $^1$H muscle functional magnetic resonance imaging ($^1$H mfMRI) data was obtained for which the volunteers performed 40 sec of isometric dorsiflexions of the foot while $^1$H gradient echo echo-planar imaging ($^1$H-GE-EPI) was applied for 16 min 40 sec. Thereafter, $^{31}$P MRS was performed before, during, and after exercise of the same intensity and duration (SUBMAX). Finally, an isometric incremental exercise was performed until exhaustion recorded with $^{31}$P MRS (EXH).

**Data post-processing**

**Overlay of $^{31}$P imaging and anatomical imaging:** We delineated the TA and EDL to determine the relative contribution of the two dorsiflexors to the total $^{31}$P signal by overlaying the $^{31}$P images and anatomical images in a subset of 8 volunteers using MIPAV (Medical Imaging Processing, Analysis and Visualization (MIPAV), http://mipav.cit.nih.gov).

$^1$H mfMRI: For the post-exercise data, two ROIs surrounding the TA and EDL, and a third ROI surrounding the remaining muscles of the calf and peronei (C) were drawn using Fiji. In these three ROIs the signal intensity during recovery was normalized to baseline and a moving average filter with span of 11 data points was applied. The amount of signal change relative to the minimal value at the start of the recovery, as well as the slope at which the signal increased in the initial phase of the recovery were determined.

$^{31}$P MRS: After phase correction and frequency alignment spectra were fitted using the AMARES algorithm in JMRUI applying appropriate prior knowledge including Lorentzian line shape and multiplets for ATP. Since no Pi splitting was observed during exercise Pi was fitted as a singlet. PCr recovery was fitted to the following mono-exponential function (equation 6.1) using Matlab (The Mathworks, Inc. Natick, Massachusetts, United States):

$$PCr(t) = PCr_0 + \Delta PCr \ast (1 - e^{-k_{PCr}t})$$  \hspace{1cm} (equation 6.1)

where $k_{PCr}$ is the recovery rate constant, $PCr_0$ is PCr at the end of exercise, and $\Delta PCr$ is the recovery value of PCr ($PCr_{recovery}$) minus $PCr_0$. Relative PCr depletion was obtained from the fitted values and defined as $\Delta PCr/(PCr_{recovery})$. pH was determined from the chemical shift difference between Pi and PCr.
**Statistical analysis**

Linear mixed model procedures for repeated measures were used (SPSS version 22.0, IBM, Armonk, NY) to investigate the linear dependence of the various $^{31}$P MRS and $^1$H mfMRI parameters on coil element or slice number, essentially as described by West. For statistical modelling of the linear dependence of $k_{PCr}$ on coil element number, end-exercise pH ($pH_{endex}$) was included as an additional covariate. For the comparison of SUBMAX and EXH, averages of the five coil elements for $k_{PCr}$, PCr depletion, and pH were computed in each volunteer and paired t-tests were performed. Standard linear regression was used to estimate associations between $pH_{endex}$ and $k_{PCr}$, as well as between $k_{PCr}$ and parameters from $^1$H mfMRI. For this linear regression pooled data of all the volunteers per coil element or imaging slice were used. The level of significance $\alpha$ was set to 0.05. All results are means ± SEM, unless stated differently.

**Results**

**Subject characteristics**

The subjects’ anthropometric data along with their level of self-reported physical activity are given in table 1. All but one volunteer were engaged in sitting/sedentary professional activities. A relatively broad range of total physical activity time was reported, in total between 4 and 45 hours per week at various intensities.

**Table 6.1:** Anthropometric data and self-reported physical activity. Walking included work, displacement and leisure time activities, while cycling was commuting and displacement only. Heavy and medium intensity activities include for all but one volunteers exclusively leisure activities. Results are means ± SEM; minimum and maximum value in brackets.

<table>
<thead>
<tr>
<th>Subject characteristics</th>
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<tbody>
<tr>
<td><strong>Anthropometric data</strong></td>
<td></td>
</tr>
<tr>
<td>Age [years]</td>
<td>29 ± 1</td>
</tr>
<tr>
<td>Weight [kg]</td>
<td>79 ± 3</td>
</tr>
<tr>
<td>Height [m]</td>
<td>1.86 ± 0.03</td>
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<tr>
<td>BMI [kg*m$^{-2}$]</td>
<td>23.1 ± 0.8</td>
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<table>
<thead>
<tr>
<th>Physical activity</th>
<th></th>
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<tbody>
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<td>07:40 ± 00:50 [01:40; 10:10]</td>
</tr>
<tr>
<td>Walking [(hh:min)/week]</td>
<td>02:00 ± 00:30 [00:00; 05:10]</td>
</tr>
<tr>
<td>Cycling [(hh:min)/week]</td>
<td>04:20 ± 01:00 [00:40; 09:00]</td>
</tr>
<tr>
<td>Heavy intensity activities [(hh:min)/week]</td>
<td>03:20 ± 02:40 [00:00; 24:00]</td>
</tr>
<tr>
<td>Medium intensity activities [(hh:min)/week]</td>
<td>02:30 ± 02:00 [00:00; 19:00]</td>
</tr>
</tbody>
</table>
**MVC and Isometric exercise**

Average MVC determined during the practice session was 200 ± 7.5 N. Average force during $^1$H mfMRI and $^{31}$P MRS SUBMAX was 59 ± 0.5% and 59 ± 0.4% of MVC ($p = 0.14$) respectively, indicating a good compliance with the requested force output (i.e. 60% MVC). Average time to exhaustion during EXH was 165 ± 7s.

**General characteristics of $^{31}$P MR spectra**

The proportion of $^{31}$P signal stemming from TA was determined using an overlay of $^{31}$P and $^1$H anatomical images and was found to be 64 ± 3%, 75 ± 3%, 84 ± 1%, 82 ± 1%, 79 ± 1% for E1 to E5, respectively. Thus, a dominant proportion of the signal originates from TA (figure 6.3A). The cross-sectional area of the TA on the $^1$H anatomical images was 371 ± 32 mm$^2$, 586 ± 42 mm$^2$, 749 ± 36 mm$^2$, 663 ± 23 mm$^2$, and 437 ± 26 mm$^2$ for image slices 1 to 5, respectively. For the EDL, the cross-sectional area was 294 ± 16 mm$^2$, 305 ± 12 mm$^2$, 279 ± 5 mm$^2$, 283 ± 15 mm$^2$, and 200 ± 13 mm$^2$ for slices 1 to 5, respectively. $^{31}$P MR spectra obtained during exercise and recovery showed resonance for ATP, PCr, and Pi with good SNR from all coil elements (see examples in figure 6.3B/C). The variation in the signal integral of PCr during the experiments was stable enough for proper analysis (figure 6.3D). Together this demonstrates good data quality given the small volume of muscle tissue that the signals are arising from. The signal enhancement of PCr due to $^1$H-$^{31}$P NOE was modest, but significant and very similar between the different coil elements (15% to 16% signal enhancement on average in a subset of 4 volunteers at rest).

**$^{31}$P MRS after exercise**

The post-exercise recovery rate of PCr varied between the different coil elements as indicated in figure 6.4, which shows the average PCr intensity of the 10 volunteers during exercise and recovery after SUBMAX exercise. Average $k_{PCr}$ varied significantly along the length of TA, being lower in distal compared to proximal regions after both, SUBMAX and EXH (for both: $p < 0.001$, +0.27 min$^{-1}$ with increasing coil number, figure 6.5A/B). The statistical model used accounted for differences in pH at the end of exercise ($pH_{endex}$) between coils and subjects, indicating that the pronounced difference in $k_{PCr}$ between coil elements cannot primarily be explained by alterations in pH.

On average, $k_{PCr}$ was significantly lower after EXH than in SUBMAX ($p = 0.024$, figure 6.5C). A comparison of $k_{PCr}$ with $pH_{endex}$ for EXH and SUBMAX and all coil elements showed that a drastically lower $pH_{endex}$ was associated with an overall slightly lower $k_{PCr}$ in EXH as compared to SUBMAX, but the difference in $k_{PCr}$ between coil elements was unchanged (figure 6.5D). This further supports that pH is not the main reason for the gradient in $k_{PCr}$ along TA. Pooling all subjects and coils, $k_{PCr}$ significantly correlated with $pH_{endex}$ in SUBMAX ($r^2 = 0.17$, $p = 0.003$) and in EXH ($r^2 = 0.14$, $p = 0.008$). $k_{PCr}$ was also fitted with a bi-exponential model and in 80% and 66% of the cases for SUBMAX and EXH respectively, the fit converged to the same
mono-exponential solution. Importantly, there was no difference between coil elements as a mono-exponential solution was found in 14 (coil elements 1 and 3) or 15 (coil elements 2, 4, 5) out of 20 PCr recovery experiments per coil element.

Figure 6.3: Results of the $^{31}$P experiment in one volunteer from coil elements E1 (distal, left), E3 (middle) and E5 (proximal, right). A) Overlay of $^{31}$P maps on $^1$H images, indicating that the majority of the $^{31}$P signal originated from the tibialis anterior. B) $^{31}$P MR spectra at the end of submaximal exercise. C) $^{31}$P MR spectra at the end of recovery. D) The corresponding signal dynamics for phosphocreatine.
Figure 6.4: Average normalized PCr signal intensity (n = 10) during recovery of submaximal (A, C) and exhaustive exercise (B, D). PCr is normalized to pre-exercise levels. A and B show all five coil elements for submaximal and exhaustive exercise, respectively (E1-distal to E5-proximal; no error bars). C and D show results for the two outermost coil elements E1 and E5 including error bars (SEM), for submaximal and exhaustive exercise, respectively. This demonstrated similar depletion of PCr, but a pronounced difference in the recovery rate.

31P MRS during exercise
Along with differences in oxidative capacity within TA measured by post-exercise $k_{PCR}$, parts of the muscle with a lower oxidative capacity might also show an earlier pH drop after the initial pH increase and a more pronounced utilization of PCr during exercise. While the relative maximum depletion of PCr was not correlated with any of the coil elements ($p = 0.22$) during SUBMAX, it varied slightly (estimated fixed effect: +0.02 with increasing coil number), but significantly ($p < 0.001$) with the coil elements during EXH (figure 6.6). pH$_{endex}$ and the lowest pH value during the experiment (pH$_{min}$; reached shortly after the end of the exercise due to protons released by the reversed creatine kinase reaction)$^9$ showed no gradients along the length of the muscle, neither during SUBMAX, nor EXH (figure 6.6C/D). Time evolution of the PCr signal and the Pi-to-PCr ratio (not shown) during EXH was similar between coil elements, while pH started to drop earlier during exercise in E3 compared to the outer elements E5 and E1 (figure 6.7). In EXH, the depletion of PCr was more pronounced ($0.53 \pm 0.02$ vs. $0.40 \pm 0.02$; $p=0.001$), as was the decrease of pH at end exercise ($pH_{endex} = 6.75 \pm 0.03$ vs. $7.07 \pm 0.01$; $p < 0.001$) compared to SUBMAX. The minimal pH was lower in EXH than SUBMAX ($pH_{min} = 6.59 \pm 0.03$ vs. $6.93 \pm 0.03$; $p < 0.001$).
**1H mfMRI response to exercise**

The pronounced increase in the relative 1H mfMRI signal intensity in the early recovery phase after isometric exercise (+15.3 % in TA or +17.9 % in EDL on average in the most proximal image slice 5) indicate that both TA and EDL were recruited for the isometric dorsiflexion exercise, while the rest of the lower leg muscles were not, as reflected by the almost complete absence of post-exercise response for the remaining muscles of the lower leg (figure 6.8). The slope of this signal increase varied with the slice number (TA and EDL: \( p < 0.001 \)), increasing from distal to proximal (figure 6.9A/B). The slope of the 1H mfMRI signal increase appeared to be lower for each slice when the 31P phased-array probe is positioned on the TA (figure 6.9C/D). However, a similar proximal-distal gradient of this slope along the

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Figure 6.5: The post-exercise PCr recovery rate constant (\( k_{PCr} \)) of the 10 volunteers for elements E1-distal to E5-proximal. A) After submaximal exercise (SUBMAX). B) After exhaustive exercise (EXH). A pronounced gradient in \( k_{PCr} \), increasing from distal to proximal regions of the tibialis anterior was found during both, SUBMAX and EXH (\( p < 0.001 \) for both). C) Correlation of \( k_{PCr} \) of SUBMAX with \( k_{PCr} \) of EXH for means of each coil element. D) Plot of average pHendex vs. \( k_{PCr} \) over all subjects of each coil element, indicating that pH cannot be the principal reason for the pronounced variation in \( k_{PCr} \) between elements. Data are presented as mean ± standard deviation.
TA was still present without this probe attached to the lower leg. Figure 6.10A displays the association of the slope of TA and EDL, which were significantly correlated for the average over the volunteers per slice ($r^2 = 0.99; p < 0.001$).

**Correlation of $k_{PCr}$ ($^{31}$P) with $^1$H mfMRI**

The PCr recovery rate was positively associated with TA’s slope of the $^1$H mfMRI signal (figure 6.10B). Highly significant correlations were observed for the average over the volunteers per slice or per coil element ($r^2 = 1.0, p < 0.001$).

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**Figure 6.6**: Relative PCr-depletion (top), and pH at the end of exercise and minimum (pH$_{endex}$, pH$_{min}$). A) and C), submaximal exercise (SUBMAX). B) and D), Incremental exercise to exhaustion (EXH). During EXH, PCr-depletion varied slightly, but significantly (p<0.001) with coil-number, i.e. displayed a gradient along the length of tibialis anterior. Data are presented as mean ± standard deviation.
Figure 6.7: Average normalized PCr signal intensity (n = 10) (A, B) and pH (C, D) during the incremental exercise to exhaustion. A and C show results for the five coil elements (E1-distal, to E5-proximal; without error bars). B and D only show results for three coil elements (E1, E3, and E5) including error bars (SEM). pH values deviating more than ± 2 times the standard deviation from the mean were removed. The average time to exhaustion in the 10 volunteers was 165 ± 7s.
Figure 6.8: Average $^1$H mfMRI signal ($n = 10$) in the recovery phase after 40 s isometric exercise (60% MVC). Data represents five slices (S1, distal to S5, proximal) corresponding to the center of each of the five $^{31}$P-coil elements (error bars omitted for clarity). For visual interpretation the lines were normalized to a minimum value at initial recovery. Both, tibialis anterior (A) and extensor digitorum longus (B) were recruited while the rest of the lower leg muscles (C) were not. The first 600 of 900 sec of recovery are shown. Of note: The high signal intensity in the first few seconds is a movement artefact.
Figure 6.9: Post-exercise dynamics of the $^1$H mfMRI signal. A) and B) Slope during initial recovery from minimum to peak signal intensity in 10 volunteers after 40 sec isometric exercise (60% MVC) for the five imaging slices (S1-distal to S5-proximal) in tibialis anterior (A) and extensor digitorum longus (B). C) Slope of the $^1$H mfMRI response in a subset of 3 volunteers without the placement of the $^{31}$P phased array probe on the tibialis anterior D) Slope of the $^1$H mfMRI response with placement of the $^{31}$P phased array probe on the tibialis anterior in the same three volunteers. Data are presented as mean ± standard deviation.
Discussion

In this study we observed remarkable differences in oxidative capacity along the length of the tibialis anterior in healthy male volunteers by using phased-array localised 31P MR spectroscopy. While the extent of PCr depletion and acidification during exercise was similar, the rate of PCr recovery after exercise was approximately twice as high in proximal as compared to distal muscle parts, indicating a heterogeneous oxidative capacity within this muscle. It is important to note that the magnitude of this difference in $k_{\text{PCr}}$ within the same muscle is similar to the difference in $k_{\text{PCr}}$ between untrained and endurance trained, or sprint and endurance trained TA muscles as assessed by 31P MRS using traditional surface coils.\textsuperscript{6,26} Thus proper placement of the coils may be critical in these assessments. We observed a similar proximal to distal gradient in the slope of the signal increase recorded by $^1$H mfMRI after exercise. These post-exercise dynamics of the $^1$H mfMRI signal strongly correlated with $k_{\text{PCr}}$ derived from 31P MRS. It is noteworthy that these correlations stem from two consecutive exercise experiments, indicating a similar metabolic demand of both exercise bouts, which is also suggested by the very similar overall force during both experiments.

$^{31}$P MRS studies repeatedly showed that end-exercise low levels of pH and/or pronounced PCr depletion are associated with reduced PCr recovery rates.\textsuperscript{27–29} In the present study, we indeed observed this correlation between $pH_{\text{endex}}$ and $k_{\text{PCr}}$ in an evaluation of the results from all subjects and coil elements. Furthermore, $k_{\text{PCr}}$ was suppressed after exhaustive exercise with lower $pH_{\text{endex}}$ and $pH_{\text{min}}$. This demonstrates that although the PCr signals arose from small muscle volumes, the signal-to-noise ratio was sufficient to measure the expected drop in $k_{\text{PCr}}$ and pH associated with intense exercise. The variation in mean $k_{\text{PCr}}$ along the

Figure 6.10: Correlation between post-exercise $^1$H mfMRI dynamics and recovery of phosphocreatine ($k_{\text{PCr}}$)
A) Significant correlation between the tibialis anterior (TA) and extensor digitorum longus (EDL) for slope of the $^1$H mfMRI signal intensity. B) Significant correlation of $k_{\text{PCr}}$ with the slope of the $^1$H mfMRI signal during the initial recovery after 40 sec isometric exercise (60% MVC).
coil elements, however, cannot be explained by pH, as the linear mixed model included pH_{endex} as a covariate. This conclusion is supported by the observation that EXH exercise is associated with substantially lower pH_{endex} compared to SUBMAX, while the difference in mean k_{PCr} between coil elements remains the same.

Recovery of PCr requires an adequate supply of oxygen to the muscle cell.\textsuperscript{1,30} Blood flow to the TA could be reduced due to compression of the anterior tibial artery by the surface coil, and thus, potentially decrease k_{PCr}. The additional \textsuperscript{1}H mfMRI experiments performed in a subset of three volunteers showed indeed that strapping the surface coil to the lower leg can result in a slower response compared to the same measurement without the \textsuperscript{31}P coil array. However, the gradient in the post-exercise kinetics of the \textsuperscript{1}H mfMRI signal was not affected by the placement of the array.

The observed gradient in the rate of PCr recovery of the TA muscle thus has a biological origin which could be a proximo-distal variation in fibre type composition and/or a variation in capillary density and local perfusion. The typical fibre length in TA is less than 7 cm, while this whole muscle is on average 26 cm long in male subjects.\textsuperscript{31} Even though TA muscle is only slightly pennated, the muscle fibres found distally are not the same as those proximally.\textsuperscript{13} In rats and rabbits, the proportion of the area occupied by type I fibres gradually decreases from the proximal to the distal parts of TA.\textsuperscript{13} Given the intrinsically higher capacity for PCr recovery in oxidative type I fibres, a varying muscle fibre distribution along the TA muscle could explain the presently observed gradient in PCr recovery.\textsuperscript{14} There is evidence of non-uniform muscle fibre distribution in cross sections of various human limb muscles, including the TA and the vastus lateralis, but we are not aware of studies on muscle fibre distribution along the length of human TA muscle.\textsuperscript{11,32,33}

Besides \textsuperscript{31}P MRS, muscle functional \textsuperscript{1}H MR imaging was performed before, during and after exercise of the same intensity and duration as for the submaximal exercise in the \textsuperscript{31}P experiments and with imaging slices placed at the middle of each \textsuperscript{31}P coil element. The slope of the signal increase in the TA after exercise increased from the distal to the proximal part of the muscle. Qualitatively, the signal response in \textsuperscript{1}H mfMRI after contraction is very similar to that of the brain: after a stimulus, an initial signal increase, followed by a plateau phase, and subsequent decrease of the signal is observed.\textsuperscript{34} In the brain the response is in the order of 1 to 2\%, lasting only a few seconds and is due to the so-called blood oxygenation level dependent (BOLD) effect, caused by local susceptibility differences due to changing concentrations of blood deoxy- and oxyhaemoglobin. The post-exercise effect in muscles on T2/T2* relaxation is higher, up to 30\%, depending on exercise intensity, and is lasting several minutes.\textsuperscript{35} In skeletal muscle, the exact causes of these signal alterations after exercise are not entirely known, besides BOLD also changes in pH, and osmotically driven fluid shifts may affect T2/T2* relaxation.\textsuperscript{16,17}
If the observed gradient in the $^1$H mfMRI signal slope is mainly due to the BOLD effect, this could indicate a lower capillary density and local perfusion in the proximal to distal direction and as a consequence a slower recovery of PCr in the distal parts. In rats, higher capillary density in proximal vs. distal regions of TA muscle was reported, whereas we are unaware of studies investigating capillary density along human TA muscle. However, studies using other methods such as positron emission tomography and near-infrared spectroscopy have demonstrated heterogeneity in perfusion and oxidative capacity of human skeletal muscle. Therefore, in future work it would be valuable to investigate perfusion along the length of the TA also, for example by MR techniques such as arterial spin labelling or intravoxel incoherent motion imaging.

Next to the BOLD effect it has been shown that tissue pH directly affects the muscle’s intracellular transverse relaxation time. Furthermore, rapid osmotically driven alterations of intra- and extracellular water pool sizes due to changes of intracellular metabolite concentrations (Pi, Cr, PCr, lactate, acetylcarnitine etc.), may dynamically change the overall/apparent T2 and T2* of skeletal muscle. This requires intra- and extracellular (=interstitial and intravascular) water T2/T2* to be substantially different from each other. Intracellular and interstitial water T2 are indicated to be similar (around 30 ms), while that of vasculature is in the order of 140 to 180 ms. In the early phase of recovery, oxidatively produced ATP is utilized to re-establish levels of PCr according to the net equation: Pi + Cr → PCr + H+. The protons produced by the CK-reaction contribute to an initial lowering of intracellular pH, resulting in a (slightly) prolonged cellular T2. Parts of the protons are, however, buffered, or together with lactate efficiently pumped out of the myocyte. Therefore, intracellular metabolite concentration falls off quickly after the end of exercise, essentially as a function of the PCr recovery rate. Thus, the rapidly decreasing muscle metabolite concentration in the early phase of the recovery could lead to a fluid shift from intracellular to interstitial and via capillary exchange to vascular compartments, thereby resulting in a higher overall water T2 and T2*. Thus, increasing overall water T2/T2* as a consequence of changing PCr concentration could be an alternative explanation for the strong correlation of $k_{PCr}$ with the slope of the post-exercise increase of the $^1$H mfMRI signal.

It is beyond the scope of this study to disentangle the complex nature of the $^1$H mfMRI response to exercise. However, our finding that $k_{PCr}$ is correlated with the $^1$H mfMRI response further supports the notion that metabolic events and oxidative capacity of the muscle are directly associated with the kinetics of the $^1$H mfMRI response or the extent and kinetics of muscle T2 after exercise. It was previously shown that the inverse of $k_{PCr}$ correlates with the time to peak of the post-exercise $^1$H mfMRI signal of a single slice. Here these findings are extended, demonstrating that this correlation holds true also for different regions of the same muscle.
In the present study, a pronounced post-exercise $^1$H mfMRI signal increase was also observed for the EDL, while the remaining lower leg muscles do not show such an increase, which is in accordance with the biomechanics of dorsiflexion. Similar to TA, EDL displayed a proximal to distal gradient in the slope of the signal increase. Again, this functional gradient could reflect intramuscular variation in oxidative capacity or oxygenation. Most importantly, the fact that this gradient is also apparent in the EDL indicates that the gradient in $k_{\text{PCr}}$ in our $^{31}$P experiments of the TA muscle cannot be explained by a varying signal contamination by the EDL muscle along the different $^{31}$P coil elements. This is confirmed by the overlay of $^{31}$P and $^1$H anatomical images demonstrating that the vast majority of the $^{31}$P signal stems from the TA.

If oxidative capacity is higher in proximal parts of the TA muscle we anticipated that the higher potential for aerobic energy provision should also be reflected in a slower utilization of PCr and a later drop of pH during exercise. An incremental test to exhaustion is expected to be most sensitive to pick up differences between coil elements during exercise. However, we observed little differences in PCr utilization, Pi to PCr ratio and pH in this phase despite the pronounced gradient in the post-exercise PCr recovery rate. We tested only for linear gradients along the muscle, and can therefore not exclude certain parameters to be different from one coil element to another. These differences must be, however, much smaller than those observed in the recovery phase. A limitation of this study is the use of continuous isometric exercise which is characterized by lower energy expenditure compared to concentric exercise and higher blood flow occlusion compared to dynamic muscle contractions. Thus, low oxidative energy provision in the entire TA during the muscle contractions could explain why the gradient in oxidative capacity is not or only slightly reflected in PCr utilization and pH. It may therefore well be that those parameters would vary to a greater extent if dynamic exercise was used. Alternatively, it cannot be ruled out that force production and therefore energy requirement is not uniform along the TA muscle. If the demand of ATP is higher in the proximal vs. distal regions of TA, the rate of PCr utilization may be similar despite a pronounced difference in oxidative capacity.

In our study we used five small separate coil elements for localization. Alternative technical approaches were recently presented for the localized assessment of $k_{\text{PCr}}$. For instance, using a phosphorous volume coil together with a fast $^{31}$P imaging method accelerated by compressed sensing at 7T. While this elegant method allowed determining PCr recovery rates in various muscles of a cross-section of the lower leg, the simultaneous assessment of pH was not possible. Fast imaging of PCr and ATP in different muscles during exercise and recovery was demonstrated at 7T using selective excitation and chemical shift displacement. With single voxel localization a more pronounced PCr depletion in the medial gastrocnemius during plantar flexions was observed as compared to non-localized experiments. Others reported different PCr recovery kinetics in different muscle groups.
in separate measurements, as well as within a single experiment. We are not aware of other studies investigating PCr recovery rates spatially resolved along the length of the same muscle.

In conclusion, pronounced differences in the functional oxidative capacity along the human tibialis anterior muscle were found by $^{31}$P MRS investigations with a specifically designed multi-array coil, supported by $^1$H mfMRI examinations. These findings identify regional variation in the physiology of this muscle during everyday activities. An important practical implication of the proximal-distal gradient in $k_{PCR}$ is that reproducible positioning of a surface coil on the TA in conventional $^{31}$P MRS studies or voxel selection in localized $^{31}$P MRS are crucial, especially in longitudinal studies, such as to assess the effect of exercise training on $k_{PCR}$. Moreover, for interventions it may be essential to assess multiple locations in muscles for a proper evaluation of their effects. The present and other studies also demonstrated that $^1$H mfMRI and $k_{PCR}$ assessed from $^{31}$P MRS can provide similar spatial information on in vivo oxidative capacity of skeletal muscles. Since $^1$H mfMRI can be done on any clinical MR system, and because of its much higher sensitivity, resulting in higher spatial and temporal resolution, this method may become a promising clinical tool to examine regionalization of metabolic capacity of skeletal muscle. As a possible outlook, the current localized $^{31}$P MRS and $^1$H imaging approach could also be used to investigate mechanisms of disease progression in neuromuscular diseases such as Duchenne muscular dystrophy and facioscapulohumeral muscular dystrophy, for both of which non-uniform muscle fat infiltration along the proximo-distal axis of lower limb muscles were recently observed.

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References


24. West BT. Analyzing Longitudinal Data With the Linear Mixed Models Procedure in SPSS. Eval Heal Prof. 2009;32:207–228.
Chapter 7

Post-exercise intramuscular O2 supply is tightly coupled to the higher proximal to distal ATP synthesis rate in human tibialis anterior

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Abstract

Phosphorus magnetic resonance spectroscopy ($^{31}$P MRS) measurements after exercise of the human tibialis anterior (TA) revealed that the phosphocreatine (PCr) recovery rate constant ($k_{\text{PCr}}$), reflecting the muscle’s oxidative capacity, decreases along the proximo-distal axis of the TA. Since O$_2$ is essential for PCr recovery, we tested if surrogate markers of O$_2$ supply show a similar variation within the TA. We performed $^{31}$P MRS measurements combined with near infrared spectroscopy (NIRS) and intravoxel incoherent motion imaging (IVIM) at multiple locations along the TA. The supply of O$_2$ is proportional to the NIRS assessed change in oxyhaemoglobin ($\Delta$O$_2$Hb) plus the $^{31}$P MRS assessed ATP synthesis rate. Furthermore, IVIM measures muscle perfusion, a main determined of O$_2$ supply.

Fifteen young male volunteers performed continuous isometric ankle dorsiflexion at 30% of their maximum force until exhaustion. Following this exercise, the recovery rate of O$_2$Hb ($k_{\text{O2Hb}}$) showed a linear relation with measurement location ($p < 0.001$), and the $k_{\text{PCr}}$, ATP synthesis rate and muscle perfusion showed a non-linear relation with measurement location ($p < 0.001$, $p = 0.050$ and $p = 0.003$, respectively), all being higher proximally. The ATP synthesis rate correlated with the $k_{\text{O2Hb}}$ and muscle perfusion. Furthermore, $k_{\text{PCr}}$ was studied in five volunteers following dynamic isometric exercise until exhaustion. Again, $k_{\text{PCr}}$ was larger proximally compared to distally ($p = 0.034$).

In conclusion, we confirmed our hypothesis that O$_2$ supply spatially varies within the human TA, and showed that this is tightly coupled to the variation in $k_{\text{PCr}}$. The observed association between O$_2$ supply and $k_{\text{PCr}}$ cannot be explained by ischemia during exercise, but may be due to an intrinsic variation in the mitochondrial oxidative capacity or architectural, vascular and mechanistic differences along the TA.
**Introduction**

Muscle contraction requires energy in the form of ATP, of which the production is dominated by oxidative phosphorylation in the mitochondria. This aerobic process does not always meet the demand of ATP, for example during the onset of exercise. In those situations, the phosphocreatine (PCr) energy buffer system comes in to play as it hydrolyzes PCr by creatine kinase to generate ATP. After exercise, the PCr pool will recover to its resting value, requiring ATP. As this ATP is generated by oxidative metabolism, the recovery rate of PCr constant ($k_{\text{PCr}}$) is taken as a correlate of the muscle’s oxidative capacity.\(^1\)\(^-\)\(^4\)

The recovery rate constant $k_{\text{PCr}}$ can be measured with dynamic phosphorus magnetic resonance spectroscopy ($^{31}$P MRS). Several studies showed that $k_{\text{PCr}}$ varied between muscles of trained vs. untrained subjects, young vs. elderly and healthy subjects vs. subjects with mitochondrial myopathies or type II diabetes.\(^5\)\(^-\)\(^8\) Furthermore, $k_{\text{PCr}}$ is higher in muscles that predominantly contain oxidative type II fibres than in muscles in which glycolytic type I fibres are dominant, corresponding with a difference in mitochondrial capacity between both types.\(^9\),\(^10\) Recently, we revealed that following continuous isometric exercise, $k_{\text{PCr}}$ does not only vary between subjects and muscles but also within a single muscle; $k_{\text{PCr}}$ was significantly larger in the proximal part of the tibialis anterior (TA) compared to the distal part.\(^11\)

The ATP needed for PCr recovery is produced aerobically and for this production vascular $O_2$ supply is expected to meet cellular $O_2$ demand.\(^1\),\(^12\) It is known that in certain diseases, affecting muscular vascularity, reduced $O_2$ supply is limiting PCr recovery and it also has been demonstrated that for normal muscles blood perfusion is correlated with $k_{\text{PCr}}$.\(^13\) Thus, if $k_{\text{PCr}}$ varies spatially along the TA, we hypothesize that $O_2$ supply does so as well.

The aim of this study was to test if this hypothesis is correct. As it is difficult to non-invasively assess $O_2$ supply directly, we addressed this question by combining $^{31}$P MRS with two complementary techniques, namely near infrared spectroscopy (NIRS) and intravoxel incoherent motion imaging (IVIM) as surrogate measures for $O_2$ supply. NIRS measures changes in oxyhaemoglobin ($O_2$Hb) which reflects the temporary imbalance between $O_2$ supply and $O_2$ use ($\Delta O_2$Hb = $O_2$ supply – $O_2$ utilization). Since $k_{\text{PCr}}$ reflects the initial post-exercise $O_2$ utilization we can estimate the ATP synthesis rate as $\Delta \text{PCr} \times k_{\text{PCr}}$, in which $\Delta \text{PCr}$ is the end-exercise decline in PCr.\(^1\) This is independent from basal ATP synthesis. If we assume a similar ATP yield per amount of $O_2$ consumed along the muscle, it follows that $O_2$ supply is proportional to $\Delta O_2$Hb + $\Delta \text{PCr} \times k_{\text{PCr}}$.\(^1\),\(^14\),\(^15\) IVIM measures muscle perfusion, which is main determinant of $O_2$ supply. To exclude that the observed $k_{\text{PCr}}$ gradient is due to variable ischemia caused by continuous isometric exercise, we also performed $^{31}$P MRS measurements following dynamic isometric exercise. The relaxation phase during dynamic isometric exercise permits muscle perfusion and is therefore expected to cause less or no ischemia.
Materials and Methods

Subjects
We recruited 20 healthy male subjects with ages between 18-35 years and BMI of 18-25 kg/m². Exclusion criteria were contra-indications for MRI scanning or a history of muscular disease. This study was conducted according to the principles of the Declaration of Helsinki (version October 2013) and the Medical Research Involving Human Subjects Act (WMO). It was approved by the local medical ethical committees, and prior written informed consent was obtained from all subjects.

Study design
Of the 20 participants, fifteen participants performed continuous isometric ankle dorsiflexion and five participants dynamic isometric ankle dorsiflexion. Daily life activity was determined for the fifteen subjects performing the continuous exercise using a validated questionnaire. For the continuous exercise, the subjects underwent two experimental sessions, the first session for the NIRS and second session for the MR measurements (IVIM and 31P MRS). For the dynamic exercise, we conducted only 31P MRS. The subjects right foot was placed in a custom-built MR compatible ergometer connected to a digital force gauge (Sauter FL 500, Balingen, Germany; figure 7.1A/B). At the start of each session, the volunteers’ maximum voluntary contraction (MVC) for ankle dorsiflexion was determined, defined as the maximum of three attempts. This was followed by 15 minutes rest to reach resting muscle perfusion and PCr levels. The continuous isometric ankle dorsiflexion exercise was performed at 30% MVC until exhaustion, and the dynamic isometric ankle dorsiflexion (frequency: 0.5 Hz) started at 10% MVC and incrementally increased with 10% MVC every 30 seconds until exhaustion.

Data acquisition
NIRS
Concentration changes in oxyhaemoglobin (O₂Hb) were measured simultaneously at seven positions along the TA using an eight-channel continuous-wave NIRS system (OxyMon MK III, Artinis Medical System, Elst, The Netherlands). We used three wavelengths (765, 857 and 859 nm) and a 50 Hz sampling frequency. The dependent differential path length factor (DPF), that accounts for the increased distance travelled by the light due to scattering, was set at 4.0. The total muscle coverage was 15 cm, with the seven positions centred 2.5 cm apart (Figure 7.1C). The transmit-receive inter-optode distance was 3 cm, resulting in a measurement depth of ~1.5 cm. Position P1 was at the distal side, and the middle measurement location was centred at the TA belly, defined as 1/3 the distance between the fibula head and lateral malleolus. Data was acquired during five minutes rest, the exercise period, and fifteen minutes recovery.
Figure 7.1: Schematic overview of the experimental set-up. A) Experimental set-up for the NIRS measurement. The subjects foot was placed in a shoe attached to a pedal connected to a force gauge. B) Experimental set-up for the MRI measurements. The subjects foot was placed in a shoe attached to a pedal and connected with a rope to a force gauge outside the scanner room. The subject received visual feedback by a beamer placed in the controller room. C) Overview of the placement of the four transmit and four receive optodes (all 3 cm apart) for the NIRS measurement, allowing oxyhaemoglobin assessment at seven positions along the muscle. D) Overview of the $^{31}$P phased array surface coil with the five coil elements depicted.

**MR measurements**

The MR measurements were performed on a 3T MR system (Magnetom, Prisma Fit, Siemens, Erlangen, Germany). In case of the continuous exercise, the subject performed the exercise twice, first to assess muscle perfusion with IVIM and second to measure the PCr recovery with $^{31}$P MRS. The muscle perfusion measurement was performed first as it takes considerable longer for muscle perfusion to reach resting values post-exercise than for $^{31}$P metabolites. There was at least 45 minutes time between the two exercise bouts.

**IVIM:** MR images were acquired with a 15-channel Tx-Rx knee coil. We recorded images of nine transversal slices covering a distance of 18.4 cm along the proximo-distal axis. The middle slice was centred on the TA belly, with slice 1 distal and slice 9 proximal. First, an anatomical T1 weighted images were acquired with a turbo spin-echo sequence (repetition time (TR): 685 ms, echo time (TE): 12 ms, flip angle (FA): 140°, field of view (FOV): 176 x 176 mm², voxel size: 0.92 x 0.92 x 10 mm³, slice gap 11.3 mm, number of slices: 9, number
of averages (NA) = 2, turbo spin-echo factor: 3). Second, diffusion weighted images were obtained with a spin-echo sequence using echo-planar imaging (EPI) read-out and SPAIR fat suppression (TR: 2000 ms, TE: 40 ms, FOV: 176 x 176, voxel size 2.75 x 2.75 x 10 mm³, number of slices: 9, slice gap 11.3 mm, receiver bandwidth 2440Hz/pixel, acquisition time: 1 min 18 s). A total of 13 b-values were obtained (0,5,10,15,20,40,60,80,100,150,200,400,600 s/mm²) in three orthogonal directions. We acquired four repetitions of the diffusion weighted scan before the start of the exercise, and twelve repetitions after the exercise.

\[ ^{31}P\text{ MRS:} \] \(^{31}P\) MR spectra were obtained with a custom-built \(^{31}P\) phased array probe for signal reception (figure 7.1D), consisting of five individual coil elements (size: 4 x 4.5 cm, total size: 4 x 20 cm, overlap of elements for decoupling).\(^{11,18}\) We combined this receive coil with a commercially available \(^1H/^{31}P\) birdcage coil (Rapid, Rimpar, Germany) for homogeneous phosphorous excitation. This set-up enabled us to receive free induction decays (FIDs) with a high signal-to-noise ratio (SNR) at five positions along the TA. The surface phased array probe was positioned with element 3 (E3) centred at the TA belly; element 1 (E1) was at the distal side. We first obtained anatomical T1 weighted images in resting muscle, using the same sequence as prior to the IVIM measurements, to verify correct placement of the \(^{31}P\) phased array probe. If the two fish oil capsules, that were fixed on the centre of the two outer most elements of the \(^{31}P\) probe, did not appear directly above the TA, the \(^{31}P\) probe was repositioned. Second, we performed 2D \(^{31}P\) imaging with a gradient echo sequence (centre frequency on PCr, TR: 1500 ms, TE: 10 ms, NA: 6, FOV: 176 x 176 mm², matrix size: 16x16), with localization in the third dimension perpendicular to the coil by position of the individual coil elements. Thereafter, \(^{31}P\) MR spectra (TR: 2.06, 2 averages per spectrum, 48° Ernst angle excitation, \(^1H^{31}P\) NOE enhanced) were obtained. The exercise protocol consisted of 1 minute rest, the continuous or dynamic isometric exercise until exhaustion, and the recovery period. During this time, we recorded \(^{31}P\) MR spectra continuously for 20 min and 36 sec.

**Data processing**
Data analysis was performed using Matlab version 2014b (Mathworks, Natick, MA, USA).

**NIRS**
The \(O_2Hb\) signal was filtered with a moving average filter of 10 seconds to remove high-frequency noise. Next, we selected the recovery part of the \(O_2Hb\) signal from end-exercise until the maximum value was reached, and baseline corrected these by subtracting the end-exercise value. This signal was fitted with a mono-exponential model (equation 7.1):

\[
O_2Hb(t) = O_2Hb_0 + \Delta O_2Hb \times (1 - e^{-k_{O2Hb}x^t}) \quad (equation \ 7.1)
\]
Where $k_{O2Hb}$ is the recovery rate constant, $O_2Hb_0$ is $O_2Hb$ at the end of exercise and $\Delta O_2Hb$ is the recovery value of $O_2Hb$ minus $O_2Hb_0$.

**IVIM**

The TA was delineated on eight slices of the $b_0$ image using MIPAV (http://mipav.cit.nih.gov) for all 16 diffusion weighted acquisitions to determine the average signal intensity for the TA per slice, b-value and diffusion weighted acquisition. The ninth, most proximal slice, was excluded, because the TA was in most cases too small to be accurately delineated. For each slice and diffusion weighted acquisition, the diffusion signal decay was fitted with a bi-exponential model (equation 7.2), in two steps.

$$S_b = S_0' \left( (1 - F_p) e^{-b \times D} + F_p e^{-b \times D'} \right)$$ (equation 7.2)

First, the diffusion coefficient ($D$) was computed by a linear least-squared fit to the log-transformed signal for b-values $\geq 200$ s/mm$^2$ according to equation 7.3:

$$\log(S_b) = -D \times b + \log(S_0')$$ (equation 7.3)

Second, the perfusion coefficient ($D^*$), perfusion fraction ($F_p$) and $S_0'$ were fitted with a non-linear least-squared fit to Eq2 with b-values from 5 to 600 s/mm$^2$ and $D$ fixed. The fitted parameters $D$, $D^*$, $F_p'$ and the blood flow related parameter $F_p \times D^*$ during rest and recovery were defined as the average over the first four and last eleven diffusion weighted acquisitions, respectively. The first acquisition after exercise was excluded, as it was often corrupted by motion artefacts.

**$^{31}$P MRS**

First, we assessed the amount of $^{31}$P signal coming from the TA and the extensor digitorum (ED). The TA and ED were delineated on the five slices of the T1 weighted anatomical images and overlaid on the $^{31}$P images. Thereafter, the sum intensity for both regions of interest was determined to calculate the relative contribution of the two dorsiflexors to the total $^{31}$P signal.

Second, the $^{31}$P spectra were fitted, after phase correction and frequency alignment, using the AMARES algorithm in JMRUI with Lorentzian lineshapes, multiplets for ATP, and a singlet or doublet for Pi, as appropriate. Thereafter, the PCr recovery was fitted with a mono-exponential model (equation 7.4).

$$PCr(t) = PCr_0 + \Delta PCr(1 - e^{-k_{PCr\times t}})$$ (equation 7.4)
Where $k_{\text{PCR}}$ is the recovery rate constant, $\text{PCR}_0$ is PCR at the end-exercise and $\Delta\text{PCR}$ is the recovery value of PCR ($\text{PCR}_\text{recovery}$) minus $\text{PCR}_0$. PCR depletion was calculated from the fitted values as $\Delta\text{PCR}/\text{PCR}_\text{recovery}$ and end-exercise pH (pH$_{\text{endex}}$) from the chemical shift difference between Pi and PCR.\textsuperscript{21} In case two Pi peaks were fitted at end-exercise, the average pH$_{\text{endex}}$ was defined as the average of the two pH pools. The ATP synthesis rate was estimated as $k_{\text{PCR}} \times \Delta\text{PCR}$, whereby the $\Delta\text{PCR}$ was expressed in mM using an assumed ATP concentration of 8.2 mM and applying T1 relaxation correction.

**Statistical analysis**

We assessed the proximo-distal variation in $k_{\text{O2Hb}}$, $D$, $F_p \times D^*$, $k_{\text{PCR}}$, and $k_{\text{PCR}} \times \Delta\text{PCR}$ along in the TA in two ways using IBM SPSS Statistics (version 25, Chicago, IL, USA). First, the most distal position was compared with the most proximal position using a paired samples two-sided sample t-test. Second, the dependence of $k_{\text{O2Hb}}$, $D$, $F_p \times D^*$, $k_{\text{PCR}}$, and $k_{\text{PCR}} \times \Delta\text{PCR}$ to the location along the TA was tested with mixed modes, linear and non-linear. For the linear model, we included location as a covariate (i.e. $y=\beta_1 \times \text{location} + \beta_0$), and for the non-linear model we included location, and the interaction term location $\times$ location (i.e. $y= \beta_1 \times \text{location} + \beta_2 \times \text{location}^2 + \beta_0$). In case of the evaluation of $k_{\text{PCR}}$, the pH$_{\text{endex}}$ ($\beta_3$) was included as an additional covariate. The most appropriate model, linear or non-linear, was chosen based on the Schwarz’s Bayesian Criterion (BIC). Moreover, a Pearson’s correlation was used to estimate the association between the ATP synthesis rate $k_{\text{PCR}} \times \Delta\text{PCR}$ and $k_{\text{O2Hb}}$ and $F_p \times D^*$. For this correlation, we pooled data from all volunteers per coil element, optode position or image slice.

**Results**

**Subjects**

The fifteen healthy volunteers for the continuous isometric exercise were on average 26 ± 3 years old and had a BMI of 22.8 ± 1.5 kg/m$^2$ (table 7.1). Their self reported activity ranged from 4 hours to 69 hours per week at various intensities (table 7.1). The five additional volunteers for the dynamic isometric exercise were 31 ± 4 years old and had a BMI of 21.3 ± 1.6 kg/m$^2$. Two subjects were excluded from the IVIM analyses, because of acquisition problems. Furthermore, $^{31}$P MRS data was not acquired for three subjects in the continuous isometric exercise group due to scanner software problems. In addition, we excluded two subjects from the $^{31}$P MRS analysis in that group, as in one subject no drop in PCR was observed in two probe elements, possible due to movement, and in the other subject acquisition time was not long enough to recover PCR in one of the coil elements.

**MVC and force during exercise**

During the continuous isometric exercise, the subjects had an average MVC of 200 ± 42 N for session 1 and 203 ± 24 N for session 2. The average measured force during the continuous isometric exercise was 29% ± 1%, 29% ± 1%, and 29% ± 1% of MVC and average time to
exhaustion was 296 ± 133 s, 250 ± 103 s, and 145 ± 46 s during the NIRS, IVIM and 31P MRS measurement, respectively. The average time to exhaustion during dynamic isometric exercise was 187 ± 4 s.

**Table 7.1:** Subject characteristics - demographics and self-reported physical activity for the fifteen volunteers doing continuous isometric exercise

<table>
<thead>
<tr>
<th>Demographics</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>26 ± 3</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>76.1 ± 8.8</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.82 ± 0.08</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.8 ± 1.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Self reported physical activity</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sitting (h:min per day)</td>
<td>7:18 ± 2:22 (3:51 to 11:09)</td>
</tr>
<tr>
<td>Walking (h:min per day)</td>
<td>4:58 ± 4:15 (0:30 to 16:40)</td>
</tr>
<tr>
<td>Cycling (h:min per day)</td>
<td>2:46 ± 2:36 (0:00 to 7:00)</td>
</tr>
<tr>
<td>Heavy intensity activities (h:min per day)</td>
<td>2:58 ± 2:35 (0:00 to 10:00)</td>
</tr>
<tr>
<td>Medium intensity activities (h:min per day)</td>
<td>5:48 ± 9:21 (0:00 to 36:00)</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD for demographics and mean ± SD (min to max) for self reported physical activity.

**NIRS**

The O₂Hb signal of the NIRS measurement, that evaluates the imbalance between O₂ supply and O₂ utilization, was stable during rest, reduced during the continuous isometric exercise, and recovered to baseline after exercise (figure 7.2A). The rate of O₂Hb recovery, \(k_{O_2Hb}\), was 5.4 ± 3.8 min⁻¹ at the distal optode and 7.8 ± 4.4 min⁻¹ at the proximal optode (\(p = 0.011\); figure 7.2B/C).

**IVIM**

The diffusion weighted images after continuous isometric exercise revealed an increased signal intensity in the TA and ED compared to the signal before exercise indicating that both muscles were activated during the exercise (figure 7.3A). For the low b-values (0-100 s/mm²), there is more signal decay during recovery compared to pre-exercise, as depicted for the TA in figure 7.3B. In line with this, in the TA, \(F_pD^*\) and \(F_pxD^*\) increased significantly from rest to recovery with 63% ± 37%, 58% ± 37%, and 164% ± 108% (figure 7.3D, table 7.2). Also, the diffusion coefficient D of the TA significantly increased after exercise, with 4.0% ± 1.4% (\(p < 0.001\)), respectively. The \(F_pxD^*\) differed between the distal and proximal slice from 0.8 ± 0.3 \(\times\) 10⁻³ mm²/s distally to 1.1 ± 0.5 \(\times\) 10⁻³ mm²/s proximally (\(p = 0.034\) (figure 7.4A). The D was lowest at the muscle belly with 1.62 \(\times\) 10⁻³ ± 0.04 mm²/s and there was no difference between the distal and proximal slice (\(p = 0.062\); figure 7.4B).
Figure 7.2: Recovery of the tissue oxyhaemoglobin (O$_2$Hb) concentration assessed with near infrared spectroscopy (NIRS). A) Typical example of one volunteer for the change in tissue O$_2$Hb concentration during rest, exercise and recovery. Data is shown from the last 100 sec of rest to 1200 sec. The grey block is depicted in more detail in B. B) Recovery of O$_2$Hb during the first 170 sec after exercise for optode position P1 (dist), P3, P5, and P7 (prox) depicted as dashed lines and the corresponding mono-exponential fit depicted as solid lines. C) Average recovery rate of O$_2$Hb (k$_{O_2Hb}$) over all volunteers per optode position and the individual volunteer data.

Table 7.2: IVIM outcomes in the whole tibialis anterior during rest, recovery and the change from rest to recovery.

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>Recovery</th>
<th>Absolute change</th>
<th>Percentage change</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>F$_p$ (%)</td>
<td>2.6 ± 0.5</td>
<td>4.1 ± 0.6</td>
<td>1.4 ± 0.8</td>
<td>63.1 ± 37.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>D* (x10$^{-3}$ mm$^2$/s)</td>
<td>13.9 ± 2.2</td>
<td>20.3 ± 1.7</td>
<td>6.5 ± 3.4</td>
<td>58.0 ± 37.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>F$_p$ x D* (x10$^{-3}$ mm$^2$/s)</td>
<td>0.36 ± 0.10</td>
<td>0.83 ± 0.18</td>
<td>0.47 ± 0.2</td>
<td>163.6 ± 108.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>D (x10$^{-3}$ mm$^2$/s)</td>
<td>1.57 ± 0.02</td>
<td>1.63 ± 0.04</td>
<td>0.06 ± 0.02</td>
<td>4.0 ± 1.4</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD and reflect the average over all 8 slices.
Figure 7.3: Diffusion weighted images (DWI) and IVIM fit before (acquisition -4 to -1) and after exercise (acquisition 1 to 11). A) DWI image at rest (top) and recovery (bottom, acquisition 6) for $b_0$ with the tibialis anterior (TA) and extensor digitorum (ED) delineated, showing an increased signal intensity in the TA and ED after exercise. B) Example fit of the IVIM model on the experimental data of the TA during rest (blue) and recovery (orange) for $b_0$ to $b_{600}$ (top) and $b_0$ to $b_{100}$ (bottom). C) Map of the blood flow related parameter $F_p \times D^*$ during rest (top) and recovery (bottom, acquisition 6) indicating an increased blood flow in the TA and ED after exercise. D) Blood flow related parameter $F_p \times D^*$ over time for the eight analysed slices.
OXYGEN SUPPLY ALONG THE TIBIALIS ANTERIOR MUSCLE

**Figure 7.4**: Blood-flow related outcome measure $F_p \times D^*$ and diffusion coefficient (D) of the IVIM model in the tibialis anterior during recovery for the eight slices. A) $F_p \times D^*$. B) Diffusion coefficient.

**31P MRS**

**Rest**: To validate that the $^{31}$P MR signals comes from the TA, we analysed $^{31}$P MR images overlaid on the anatomical $^1$H images. This revealed that the $^{31}$P signal stems for 58% ± 3% (mean ± SEM), 75% ± 3%, 79% ± 3%, 78% ± 2%, and 66% ± 3% from the TA for element E1 to E5, respectively, confirming that the dominant proportion of the $^{31}$P signal indeed originates from the TA.

**Continuous isometric exercise**: For all five elements, the $^{31}$P spectra showed the expected drop in PCr and increase in Pi during exercise and recovery of both metabolites after exercise, while the three resonances of ATP remained stable (figure 7.5 for E1/E3/E5). In this example, PCr recovered faster in the proximal element (E5) compared to the distal element (E1). Combining all subjects and coil elements shows that PCr depletion was 48% ± 4%, $p_{endex}$ was 6.78 ± 0.09, and $k_{PCr}$ was 0.73 ± 0.21 min$^{-1}$.

The initial assessment of intramuscular difference along the TA revealed that PCr depletion at end of exercise was largest in the middle element E3 (54% ± 5%), but not different between the distal and proximal element ($p = 0.269$) (figure 7.6A). The corresponding pH at end of exercise ($pH_{endex}$) was higher distally than proximally (6.87 ± 0.09 vs. 6.76 ± 0.12, $p = 0.002$) (figure 7.6B). To determine this $pH_{endex}$, Pi was fitted with two peaks in 47% of the spectra (22% distally vs. 56% proximally). After exercise, PCr recovered slower for the distal element compared to the proximal element (0.44 ± 0.26 min$^{-1}$ vs. 1.50 ± 0.57; $p < 0.001$) (figure 7.6C). Multiplying $k_{PCr}$ with PCr depletion shows that also the ATP synthesis rate ($k_{PCr} \times \Delta PCr$) was lower distally compared to proximally (9 ± 2.4 mM/min vs. 17.6 ± 6.7 mM/min; $p < 0.001$) (figure 7.6D).
Figure 7.5: Typical example of $^{31}$P spectra during rest, end-exercise and end-recovery and the PCr signal intensity over time. A) Example $^{31}$P spectra showing inorganic phosphate (Pi), phosphocreatine (PCr) and the three resonances of ATP and their change from rest to end-exercise to end-recovery. B) Signal intensity of PCr over time for E1 (dist), E3 and E5 (prox), showing a faster PCr recovery ($k_{PCr}$) proximally.

**Dynamic isometric exercise:** As continuous isometric exercise may restrict muscle perfusion during exercise resulting in ischemia, of which the extent may vary along the TA, possibly followed by a variable post-exercise hyperaemic response we also performed $^{31}$P MRS during dynamic isometric exercise. On average over the whole TA, PCr depletion was 44% ± 3%, $pH_{endex}$ was 6.69 ± 0.20, and $k_{PCr}$ was 0.95 ± 0.59 min$^{-1}$.

Intramuscular assessment of the five separate coil elements showed that PCr depletion was with 49% ± 4% largest in element 2 (E2), and that distally PCr depletes more compared to proximally (0.44 ± 0.03 vs. 0.38 ± 0.03, $p = 0.029$) (figure 7.6A). No significant difference in $pH_{endex}$ was observed between the distal and proximal element (6.64 ± 0.21 vs 6.81 ± 0.20, $p = 0.101$) (figure 7.6B). This $pH_{endex}$ was determined as the weighted average of two Pi peaks in 60% of the $^{31}$P spectra (80% distally vs. 40% proximally), in the other 53% of the spectra a signal Pi peak was observed. In line with the recovery after continuous exercise, both $k_{PCr}$ and the ATP synthesis rate were lower distally compared to proximally for the dynamic exercise (0.70 ± 0.65 min$^{-1}$ vs. 1.66 ± 1.00 min$^{-1}$; $p = 0.013$ and 6.6 ± 6.1 mM/min vs. 16.7 ± 10.7 mM/min; $p = 0.010$, respectively) (figure 7.6D/E).
Figure 7.6: $^{31}$P MRS results for the continuous isometric exercise and the dynamic isometric exercise. 
A) PCR depletion for both exercise regimes. B) pH at end-exercise ($\text{pH}_{\text{end-ex}}$) for both exercise regimes. C) Phosphocreatine recovery rate constant ($k_{\text{PCr}}$) during continuous isometric exercise. D) Estimated ATP synthesis rate ($k_{\text{PCr}} \times \Delta\text{PCr}$) during continuous isometric exercise. E) $k_{\text{PCr}}$ during the dynamic isometric exercise. F) Estimated ATP synthesis rate ($k_{\text{PCr}} \times \Delta\text{PCr}$) during the dynamic isometric exercise.

Statistical outcomes of the mixed model and the correlation analysis
The outcome of the statistical analysis with the mixed model for the NIRS, IVIM and $^{31}$P MRS measurements during and following continuous isometric exercise is depicted in table 7.3. For the NIRS, the relation between $k_{\text{O2Hb}}$ and the seven locations along the TA was best fitted with the linear mixed model, whereby optode location had a significant effect on $k_{\text{O2Hb}}$ ($p < 0.001$).

For IVIM, the $F_p \times D^*$ and D were best fitted with the non-linear model with the p-values for location being $p = 0.090$ and $p < 0.001$ and location$^2$ being $p = 0.003$ and $p < 0.001$, respectively.

For the $^{31}$P MRS, the $k_{\text{PCr}}$ and the ATP synthesis rate $k_{\text{PCr}} \times \Delta\text{PCr}$ showed a significant non-linear relationship with coil element number for the continuous isometric exercise (location:
\( p = 0.071 \) and \( p = 0.455 \), location: \( p = 0.001 \) and \( p = 0.032 \), respectively), which for \( k_{\text{PCr}} \) cannot primarily be explained by the covariate \( \text{pH}_{\text{endex}} \) (\( p = 0.231 \)). Furthermore, \( \text{pH}_{\text{endex}} \) and \( \text{PCr} \) depletion showed a significant non-linear association with coil element number with \( p \)-values for location being \( p = 0.003 \) and \( p < 0.001 \) and for location\(^2\) being \( p = 0.010 \) and \( p < 0.001 \), respectively.

For the dynamic isometric exercise, the mixed model did not converge due to the lack of power with five subjects. Nevertheless, it did show a clear similar trend for the \( k_{\text{PCr}} \), \( \text{PCr} \) depletion and \( k_{\text{PCr}} \times \Delta \text{PCr} \) as compared to continuous isometric exercise.

**Correlation analysis**

The correlation analysis revealed that \( k_{\text{PCr}} \) correlated strongly with \( k_{\text{O2Hb}} \) \((r^2 = 0.983, p = 0.003)\) and \( F_p \times D^* \) \((r^2 = 0.954, p = 0.012)\). In addition, the ATP synthesis rate \( k_{\text{PCr}} \times \Delta \text{PCr} \) also strongly correlated with \( k_{\text{O2Hb}} \) \((r^2 = 0.994, p = 0.001)\) and \( F_p \times D^* \) \((r^2 = 0.960, p = 0.009)\).

**Table 7.3:** Estimates of the (non-)linear mixed model and the corresponding \( p \)-values.

<table>
<thead>
<tr>
<th></th>
<th>Estimates of the (non-)linear mixed model</th>
<th>( p )-values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \beta_0 ) (Intercept)</td>
<td>( \beta_1 ) (location)</td>
</tr>
<tr>
<td>NIRS</td>
<td>( k_{\text{O2Hb}} )</td>
<td>4.75</td>
</tr>
<tr>
<td>IVIM</td>
<td>( D )</td>
<td>1.67</td>
</tr>
<tr>
<td></td>
<td>( F_p )</td>
<td>4.15</td>
</tr>
<tr>
<td></td>
<td>( D^* )</td>
<td>17.64</td>
</tr>
<tr>
<td></td>
<td>( F_p \times D^* )</td>
<td>0.80</td>
</tr>
<tr>
<td>( ^{31} \text{P MRS} )</td>
<td>( k_{\text{PCr}} )</td>
<td>-4.30</td>
</tr>
<tr>
<td></td>
<td>( k_{\text{PCr}} \times \Delta \text{PCr} )</td>
<td>4.64</td>
</tr>
<tr>
<td></td>
<td>( \text{pH}_{\text{endex}} )</td>
<td>6.97</td>
</tr>
<tr>
<td></td>
<td>( \text{PCr depletion} )</td>
<td>0.24</td>
</tr>
</tbody>
</table>

According to the Schwarz’s Bayesian Criterion, \( k_{\text{O2Hb}} \) and \( D^* \) were best fitted with a linear model and \( D \), \( F_p \times D^* \), \( k_{\text{PCr}} \), \( \text{pH}_{\text{endex}} \), \( \text{PCr} \) depletion, and \( k_{\text{PCr}} \times \Delta \text{PCr} \) were best fitted with a non-linear model. In case of \( k_{\text{PCr}} \), \( \text{pH}_{\text{endex}} \) was also added as a covariate.

**Discussion**

In this study we combined NIRS, IVIM and \( ^{31} \text{P MRS} \) before, during and after isometric exercise of the TA and found that post-exercise \( O_2 \text{Hb} \) recovery rate, perfusion, \( \text{PCr} \) recovery rate constant and ATP synthesis rate (\( k_{\text{PCr}} \times \Delta \text{PCr} \)) varied along the length of the TA. They all showed a proximo-distal gradient, with the largest values proximally. If we assume that \( O_2 \) supply \( \sim \Delta O_2 \text{Hb} + k_{\text{PCr}} \times \Delta \text{PCr} \), this suggests that \( O_2 \) supply also exhibits a proximo-distal gradient with
the highest $O_2$ supply proximally (figure 7.7). This finding is supported by the proximo-distal gradient in the IVIM assessed muscle perfusion, also being highest proximally. In addition, we found that $k_{PCr}$ also varied along the TA following dynamic isometric exercise, indicating that prolonged ischemia during exercise cannot explain the observed gradient in $k_{PCr}$ and $O_2$ supply following exercise.

**Figure 7.7:** Schematic overview suggesting a proximo-distal gradient in $O_2$ supply. Oxyhaemoglobin recovery rate $\propto O_2$ supply – $O_2$ utilization. At start of recovery, $O_2$ utilization $\propto k_{PCr} \times \Delta PCr$, assuming similar ATP-yield per amount $O_2$ consumed along the muscle and all ATP used for phosphocreatine recovery. Therefore, $O_2$ supply $\propto$ Oxyhaemoglobin recovery rate + $k_{PCr} \times \Delta PCr$.

It is important to note the magnitude of the difference in $k_{O2Hb}$, $F_p \times D^*$, and $k_{PCr}$ that we observed along the TA is comparable to differences in these variables observed as effect of muscle disease or training measured at a single muscle location. For instance, variations in $k_{O2Hb}$ are in the order of the difference between healthy subjects and patients with chronic heart failure and peripheral vascular disease, in $F_p \times D^*$ the variations are as between the soleus and the gastrocnemius muscle, and for $k_{PCr}$, the difference are as between untrained and endurance trained TA muscles.\textsuperscript{5,22–24} This highlights the critical selection of a suitable measurement location and assuring reproducible repositioning in follow-up studies for $O_2$ supply and energy metabolic measurements, as a single location within the muscle might not represent the muscle as a whole.

The significant variation in $k_{O2Hb}$ along the length of TA is in line with our previous finding with muscle functional MRI (mf-MRI), that also revealed a proximo-distal gradient in the slope of the mf-MRI signal increase response after exercise.\textsuperscript{11} However, the $O_2$Hb signal recovered approximately 2 to 3 times faster than the mf-MRI signal suggesting that the mf-MRI response reflects not only changes in oxygenation, but most likely also changes in T2 relaxation time due to other factors, like intra- and extracellular water shifts. Furthermore, our work corroborates with NIRS findings in the vastus lateralis and gastrocnemius. Those
muscles showed a higher exercise induced deoxygenation distally vs. proximally followed by a larger and faster reoxygenation during recovery distally vs. proximally.25–29

The NIRS findings in muscle oxygenation are supported by the proximo-distal gradient in IVIM perfusion parameters $F_p$, $D^*$, and $F_p D^*$. On average over all slices, $F_p$ increased with ~63% and $F_p D^*$ increased with 164% in the TA after exercise, which is in the same order of magnitude as previous muscle IVIM studies.30–34 These previous studies reported significant differences in perfusion between muscles, but did not assess intramuscular variation in perfusion. The presence of intramuscular variation in muscle perfusion is supported by other perfusion measurement techniques. For example, perfusion $H_2O$ PET studies revealed that muscle blood flow was heterogeneous in the four quadriceps muscles during rest and after exercise, and detailed analysis of the vastus lateralis after cycling showed a proximo-distal gradient with the highest muscle blood flow proximally.35,36 Also, NIRS studies showed a larger decrease in blood volume during repeated standing plantar flexion in the distal part of the gastrocnemius, and a subsequent greater increase during recovery afterwards.25

The present study confirms our previous finding that $k_{PCr}$ varies along the length of the TA.11 We have now observed this proximo-distal gradient in $k_{PCr}$ with several exercise regimes including continuous isometric exercises for 40 sec and exercise until exhaustion at several intensities and dynamic isometric exercise indicating that the proximo-distal gradient in $k_{PCr}$ does not depend on the type of exercise. In the present study, exercise was performed until exhaustion, leading to acidosis near end-exercise. Cytosolic acidosis slows down the recovery of PCr, and could therefore be a confounder in our analysis.1,37,38 However, during our continuous isometric exercise, the most severe acidosis was in the proximal part of the TA, the place where the $k_{PCr}$ was highest. During the dynamic exercise, the lowest pH was however found in the distal part of the muscle. Nevertheless, according to the pH$_{endex}$ and $k_{PCr}$ relation reported in literature, the pH difference of 0.2 between is not sufficient to explain a factor 2 difference in $k_{PCr}$ between the distal and proximal part of the muscle.37,39 Together with the mixed model analysis that takes pH$_{endex}$ into account as a covariate, and our previous work, this indicates that proximo-distal variation in pH cannot explain the proximo-distal gradient in $k_{PCr}$.11

The strong relation between $k_{PCr}$, ATP synthesis rate, $O_2$Hb recovery rate and muscle perfusion found in this study suggests that there is tight coupling between $O_2$ flow and use that applies across the spatial gradient of oxidative function. This relation is line with earlier work showing a correlation between $k_{PCr}$ and ASL determined post-exercise perfusion.13,40 The underlying reason for this observed proximo-distal variation in $O_2$ supply and $k_{PCr}$ and ATP synthesis rate, is unknown, but we will discuss two possible explanations. The first is a proximo-distal variation in fibre type distribution and capillary density. Slow oxidative type I fibres have a greater capillary density than the fast glycolytic type II fibres.
The fibre type distribution within the human TA is unknown, but studies in rats and rabbits showed that the area occupied by type I fibres gradually increases from distal to proximal. In line with this, in the TA of the rat, a higher capillary density was found proximally compared to distally. This explanation is supported by studies in the human vastus lateralis. Histology showed a lower percentage oxidative type I fibres in the distal part of the vastus lateralis compared to the proximal part in line with a larger blood flow proximally vs. distally. Furthermore, the superficial part of the vastus lateralis contains less oxidative fibres than the deep part, and visual inspection of the blood flow on PET images showed a lower blood flow in these superficial muscle parts.

Alternatively, variation in architecture along the TA could play a role. The tibialis anterior is a bipennate muscle with a central aponeurosis separating the muscle into a deep and superficial compartment. Using 3D MR diffusion tensor imaging, it was discovered that in-vivo the axial orientation of this aponeurosis changes considerable from the proximal part to the distal part of the TA. This causes the fascicles pennation angle to vary along the TA, which is supported by a 3D ultrasound study showing a pennation angle of 15° proximally vs. 7° distally. Larger pennation angles are associated with larger intramuscular pressures and could therefore lead to a larger inhibition of muscle perfusion during the exercise. Though the dynamic isometric exercise ruled out the role of ischemia in the variation in $k_{PCr}$, the non-ischemic inhibition of muscle perfusion during exercise could still be larger in the proximal part of the TA and as such result in a stronger reactive hyperaemic response after exercise proximally. This hypothesis is supported by a study in the gastrocnemius medialis where during exercise the change in pennation angle and fascicle length measured with ultrasound correlated with NIRS assessed decrease in blood volume and muscle deoxygenation. Intramuscular pressure is also related to fascicle length and the change in pennation angle and fascicle length during contraction, but to our knowledge their variation along the TA has not been studied. A way to look at intramuscular pressure variations caused by heterogeneity in the TA's architecture is cine MR 3D strain measurements, as strain is inversely related to pressure. For example, passive ankle dorsiflexion leads to higher 3D strain distally vs. proximally. Also, visual inspection of 3D strain rate maps following isotonic ankle and plantar- and dorsiflexion revealed an inhomogeneous strain pattern between distal and proximal portions of the TA, but this was not further specified. This suggests that intramuscular pressure varies within the TA during exercise, and, at least during passive exercise, this corroborates with the larger $O_2$ supply after exercise proximally.

In this study, we have assessed young male subject because their subcutaneous fat layer is very thin which maximises SNR of the $^{31}\text{P}$ spectra and NIRS measurements. Studying this observed proximo-distal gradient change with age, differences between men and women, or the effect of training could give additional information on the underlying mechanism and muscle function. In addition, for future work, it would be of interest to study the
intramuscular variation in $k_{\text{PCr}}$ in other muscles, for example the gastrocnemius and vastus lateralis muscle, as several studies showed intramuscular variation in oxygenation and muscle blood flow in those muscles as well. Mapping these intramuscular differences in multiple muscles could help our understanding of the pathophysiological mechanisms in muscle disorders. For example, in muscular dystrophy patients, it has recently been observed that the disease spreads non-uniformly along the proximo-distal axis, while the underlying physiological reason for this is unknown.$^{49,50}$

In conclusion, we provide compelling evidence that $O_2$ supply varies along the human TA muscle. This variation in $O_2$ supply is strongly associated with the variation in $k_{\text{PCr}}$ and ATP synthesis rate observed along the TA, but cannot be explained by ischemia during the exercise. The association between $O_2$ supply and $k_{\text{PCr}}$ and ATP synthesis rate could be driven by an intrinsic variation in the mitochondrial oxidative capacity in particular because of variation in the ratio of oxidative and glycolytic muscle fibres along the TA. Or, it could be driven by architectural and mechanistic differences along the TA leading to a variation in intramuscular pressure along the TA during exercise.
References


Chapter 8

Summary, general discussion and conclusion
Summary
Muscular dystrophies are a generic name for a group of inherited muscle diseases that cause degeneration of skeletal muscles. Patients with a muscular dystrophy experience progressive muscle weakness over time, and may eventually become wheelchair dependent. In this thesis, I focussed on two forms of muscular dystrophy, myotonic dystrophy type 1 (DM1) and facioscapulohumeral muscular dystrophy (FSHD). For both diseases, progress towards new treatments is ongoing, but has not yet been able to provide a curative therapy. Successful treatment development requires 1) understanding of the pathophysiological mechanisms of a muscular dystrophy, 2) understanding of factors that are involved in disease onset and progression, and 3) objective quantitative biomarkers to successfully evaluate the effect of the novel proposed therapy at the level of muscles. This thesis describes the results of studies that aimed to unravel the pathophysiological mechanisms in DM1 and FSHD using dedicated MR techniques and evaluated if these techniques could be used as quantitative biomarkers in DM1. First, chapter 3 to 5 focussed on muscular disease processes in natural history and intervention in DM1 and FHSD. Second, in chapter 6 and 7, we assessed intramuscular variation in normal muscle function in healthy volunteers in search of triggers for disease initiation.

Chapter 1 presents an introduction to this thesis and describes the clinical presentation, genetics, and histopathological findings in DM1 and FSHD. Chapter 2 gives an overview on the basic principles of MR and the MRI/MRS techniques used throughout this thesis. In short, the Dixon sequence provided the muscle’s fat fraction and volume (or cross-sectional area) as respective objective biomarkers for fat infiltration and muscle mass. Furthermore, the muscle’s T2 relaxation time was determined, using a multi-echo spin-echo MR acquisition sequence, as a biomarker for early pathological changes in tissue water distribution (intra- or extracellular oedema). Moreover, in healthy volunteers, we applied phosphorus MR spectroscopy ($^{31}$P MRS), intravoxel incoherent motion imaging (IVIM), and the optical technique near infrared spectroscopy (NIRS) to assess the muscle’s energy metabolism, perfusion and oxygenation, respectively.

In chapter 3 and 4, I present quantitative muscle MRI data obtained in an international multi-centre randomized controlled trial in DM1, named OPTIMISTIC. This trial studied the effect of a behavioural intervention directed to increase patient’s daily life activity over a 10 month period. We determined the proposed MRI biomarkers for fat infiltration, muscle mass and tissue water distribution (oedema) in the 20 lower extremity muscles of 33 DM1 patients at baseline and their change over 10 months.

Chapter 3 describes the cross-sectional muscle MRI baseline data of OPTIMISTIC and shows that lower extremity muscles of DM1 patients have about ~3 times more fat, a ~26% reduction in muscle mass, and increased values of $T_2^{\text{water}}$ (reflecting the presence...
of putative oedema) compared to unaffected controls. The severity of fat infiltration was primarily determined by age, followed by two genetic markers, namely the inherited CTG repeat length, and the on-going CTG repeat expansion accrued over life time. Furthermore, the fat fraction and muscle mass correlated with decreased physical capacity and disease severity. It is of particular interest that fat fraction is already abnormal in subclinical disease progression.

In chapter 4, we studied the effect of the behavioural intervention on the lower extremity muscles in DM1 patients compared to standard care, and determined the natural disease progression. First, DM1 patients receiving this intervention gained around 4% of lower extremity muscle mass in 10 months, while no change in muscle mass was observed in patients receiving standard care. This gain in muscle mass was most prominent in muscles without fat infiltration. Second, fat infiltration in the lower extremity muscles progressed with 0.9% over 10 months in patients receiving the intervention compared to 1.2% in the muscles of patients who received standard care. This progression was fastest in muscle with signs of oedema at baseline. Neither group showed changes in tissue water distribution as assessed with $T_2^{\text{water}}$ (oedema) over 10 months. Our findings demonstrate the effectiveness and safety of behavioural interventions directed to increase daily activity in DM1, it underlines that these interventions should preferably be started early in disease course, and it helps to understand the mechanism of action of increasing physical activity on muscles in DM1 patients.

Together, the findings of chapter 3 and 4 underline that MRI biomarkers for fat infiltration and muscle mass are promising outcome measure for future clinical trials. They can discriminate healthy from diseased muscles, they correlate with functional outcome measures, and they are able to detect the natural disease progression as well as a therapy effect in a reasonable timeframe of 10 months.

Chapter 5 is focused on the specific muscle strength in patients with FSHD, which is defined as the strength per unit area of contractile muscle tissue. We found a reduced specific muscle strength (~50%) in the quadriceps muscles of FSHD patients compared to healthy controls. Muscle weakness is thus not only caused by muscle tissue loss, but also by a reduced function of the residual muscle tissue. The reason for this diminished muscle function needs to be elucidated. Proposed mechanisms are intrinsic impairment in the force generating capacity and/or force propagation of the muscle (fibres), or impaired energy metabolism in muscles of FSHD patients.

Previous studies indicated that FSHD is a focal disease that appears to start in the distal part of the muscle. This suggest that normal muscle may already vary in structure or function along its proximodistal axis such that the distal part of a muscle is more vulnerable for initiation of muscle degeneration than the proximal part. As a first step, in chapter 6,
we used $^{31}$P MRS to study the intramuscular variation in energy metabolism in-vivo in the human tibialis anterior (TA) by measuring the muscle’s maximum capacity to produce ATP by oxidative phosphorylation. This capacity is referred to as the oxidative capacity and was indirectly measured by determining the recovery rate of phosphocreatine (PCr). We developed a unique set-up including a custom-built $^{31}$P phased array coil consisting of five separate coil elements for spatial localization at five positions along the proximo-distal axis of the TA. With this set-up, we showed that PCr recovery rate after isometric ankle dorsiflexion varied along the length of the TA; the rate was approximately twice as large proximally compared to distally. These findings were supported with muscle functional MRI (mf-MRI) examinations, in which we observed a similar proximo-distal gradient in the slope of the signal increase in the TA after exercise. Therefore, it can be concluded that oxidative capacity varies along the TA and is lowest distally.

In chapter 7, we combined our developed $^{31}$P MRS set-up with NIRS and IVIM to study intramuscular variations in oxygen supply, as this is a potential limiting factor in PCr recovery. As depicted in equation 8.1, oxygen supply is proportional to the NIRS assessed change in oxyhaemoglobin ($\Delta O_2$Hb) plus the $^{31}$P MRS assessed ATP synthesis rate ($k_{PCr} \times \Delta PCr$).

$$O_2 \text{ supply} \propto \Delta O_2 Hb + k_{PCr} \times \Delta PCr \quad (\text{equation 8.1})$$

Furthermore, IVIM measures muscle perfusion, a main determined of oxygen supply. We confirmed that oxygen supply also varies along the TA, and showed that it is strongly associated with the variation in $k_{PCr}$ along the TA. This association could be driven by an intrinsic variation in the mitochondrial oxidative capacity due to variation in the ratio of oxidative and glycotic muscle fibres along the TA. Or, this could be a causal relation due to architectural and mechanistic differences along the TA leading to a variation intramuscular pressure along the TA during exercise.

**General discussion and future perspective**

Throughout this thesis, I applied dedicated muscle MR techniques in cross-sectional studies to assess the (patho)physiological processes in healthy and diseased skeletal muscles and in a longitudinal study to assess the value of MR as a biomarker for clinical trials in DM1. The first part of this discussion focuses on what this thesis learned us on the muscle pathophysiology underlying DM1 and FSHD. In the second part, I will discuss our findings in healthy muscle. The third part will be about the value of quantitative muscle MRI outcome measures during future clinical trials in neuromuscular disorders.
1. The MR view on muscle pathophysiology underlying DM1 and FSHD

In this section, I will discuss the new insights gained on disease onset and progression in DM1 and FSHD and how the results of our studies may impact disease monitoring and patient counselling.

In DM1, we first studied fat infiltration at the subject level by combining per subject the fat fraction of the 20 individual muscles to an aggregate fat fraction over the whole lower extremity. This lower extremity fat fraction varied widely in our DM1 cohort and, as expected for a progressive disease, was for a large part explained by age (~45%). However, this leaves about 55% of the variation unexplained, indicating the role of other modifying factors. Identifying those factors will improve the prediction of a patient’s disease progression, which is relevant for patient management/counselling and clinical trials.

In our search for these modifying factors, we first found that the estimated progenitor CTG repeat length and somatic instability explained an additional 14% of the inter-subject variation in fat infiltration. Second, AciI sensitive variant repeats are likely playing a role, because they are thought to have a stabilizing effect and reduce the rate of somatic instability. Indeed, in our DM1 cohort, patients with these variant repeats had fat fractions in the range of unaffected controls. Furthermore, literature reports a later age of onset and milder clinical symptoms in DM1 patients with AciI sensitive variant repeats compared to those without these repeats.

Third, our behavioural intervention study suggests that patient’s life-style and daily life activity could modify muscle degeneration, since the DM1 patients who were stimulated to become more active exhibited a beneficial change in their skeletal muscles. This is supported by a recent study in DM1 mice model, where chronic exercise improved muscle strength and reduced the number of myofibres with a small CSA. Interestingly, this chronic exercise even influenced the main disease mechanism in DM1; the muscles of the exercising mice had a reduced number of myonuclei containing (CUG)\textsubscript{n} foci with sequestered MBNL1. This suggests less dysregulation of RNA-binding proteins like MBNL1 in those mice and a reduction in the subsequent missplicing of many other gene products. Fourth, literature indicates sex as an important modifying factor as males present with more severe muscular disability and muscle weakness.

Our second step was to study the distinctive fat infiltration profile in muscles of DM1 patients. The lower leg muscles had higher fat fractions compared to the upper leg muscles, with the gastrocnemius medialis and soleus exhibiting the highest fat fractions and the tibialis posterior often presenting without fat infiltration. This shows that disease onset and/or progression rate in DM1 varies between individual muscles. FSHD and other muscular dystrophies also exhibit a distinctive fat infiltration pattern, being specific for each type of muscular dystrophy. These patterns might hide clues on the underlying mechanisms that initiate or accelerate muscle degeneration or that protect a muscle from degeneration. This
is expected to be a complex interplay between the disease specific molecular and cellular pathological processes and the muscle activation and tissue deformation during daily life activities. In future studies, muscle MR could shed some light on this complex interplay as besides muscle structure in rest, it can also dynamically study molecular processes, perfusion, muscle activation and deformation during and following exercise, all in a non-invasive quantitative manner. For example, energy metabolism can be assessed with $^{31}$P MRS, muscle perfusion with IVIM or arterial spin labelling (ASL), and muscle activation patterns with T2 or T2* weighted imaging. Furthermore, recently a 4D phase contrast MRI sequence was developed that allowed accurate estimation of the tissue deformation with 3D velocity and strain (displacement) maps. Until now, these techniques have been applied to assess simple isolated movements, like ankle dorsiflexion or plantarflexion. For future work, we need to extend this to more common daily life movements, like walking, cycling or stair climbing. Such studies require the development of more complex ergometer devices. Furthermore, when applying those techniques in muscular dystrophy patients, one should take into account the confounding effect of fat infiltration. For example, the signal to noise ratio of the $^{31}$P spectra and the IVIM signal will be significantly reduced, and alteration in tissue water distribution ($T_2^{\text{water}}$) due to oedema or fibrosis will affect the IVIM and BOLD technique.

Our next step was to study the longitudinal change in fat infiltration in the muscles of DM1 patients. We related this to the presence of oedema and fat infiltration at baseline. On average, fat fraction in the lower extremity muscles of DM1 patients increased with 1.2%. In muscles exhibiting signal hyperintensity at TIRM images (oedema) fat fraction increased more, up to an average 1.9% over 10 months in muscles categorized as severe TIRM hyperintensity (Malattia score 2) compared to 0.9% over 10 months in non TIRM hyperintense muscles. This is much slower than the fat infiltration rate of 7%/year seen in FSHD patients, which even reached 17%/year in intermediately fat infiltrated muscles. These MRI findings are in line with the clinical presentation of DM1 and FSHD. In DM1, muscle strength decreases slowly, while FSHD patients frequently report long periods of quiescence interrupted by periods of rapid deterioration. Together, this highly suggest that muscle degeneration in DM1 is a more gradual process, in contrast to a proposed on-off mechanism in FSHD.

Adding the finding that TIRM hyperintense muscles show the largest fat infiltration rate to the finding that muscles without fat infiltration often exhibit elevated $T_2^{\text{water}}$, suggests that $T_2^{\text{water}}$ increases prior or in parallel with fat infiltration. In other words, muscles showing elevated $T_2^{\text{water}}$ (or TIRM positive lesions) are likely to become fat infiltrated in the following years. These findings corroborate those from other muscular dystrophies such as FSHD, Pompe and Duchenne muscular dystrophy. Therefore, screening for TIRM positive lesions could predict the deterioration in the patient’s muscle function. However, this thesis
also shows that this prediction is not straightforward. Not every TIRM hyperintense muscle exhibited progression in fat infiltration in the 10 months time. Furthermore, clinically, fat infiltration may be masked by the compensating ability of other muscles. Therefore, to fully grasp the course and relation of fat infiltration and oedema, more frequent and longer follow-up studies that combine quantitative muscle MRI measures for fat infiltration, muscle volume and oedema with quantitative muscle strength measurement are necessary.

As shown above, muscle MRI by itself can help to elucidate the pathological processes in muscular dystrophies. In combination with other techniques, its value will become even more evident. By integrating the quantitative MRI assessment of fat infiltration and muscle volume with a mathematical musculoskeletal model, we discovered that the specific muscle strength in FSHD was reduced. In other words, the muscle weakness in FSHD is not solely explained by the amount fat infiltration and atrophy, but also by a reduced function of the residual muscle tissue. This could be related to the altered deformation patterns observed in fat infiltrated muscles of FSHD patients. Other proposed mechanisms are a reduced force generating capacity of the individual muscle fibres and an altered energy metabolism. This work highlights that interventions targeting muscle weakness should not only focus on preventing muscle degeneration, but also aim to restore specific muscle strength. This emphasizes the added value of multimodality assessment for entangling the disease mechanisms in muscular dystrophy.

2. Intramuscular variation in normal muscle physiology

This section focuses on our work in healthy volunteers, which was inspired by the finding that in FSHD, fat infiltration appeared to start at the distal part of the muscle.

To identify potential triggers for this localized disease onset, we studied the variation in muscle function along the length of the TA muscle in healthy volunteers. We found that the proximal part of the TA, following continuous isometric ankle dorsiflexion, exhibited a faster recovery of PCr and O$_2$Hb ($k_{\text{PCr}}$ and $k_{\text{O2Hb}}$, respectively) and higher muscle perfusion ($F_p xD^*$) than the distal part of the muscle. This indicates that oxidative capacity and oxygen supply are not homogeneous within the TA. It is important to note the magnitude of the difference in $k_{\text{PCr}}$, $k_{\text{O2Hb}}$, $F_p xD^*$ along the TA. For $k_{\text{PCr}}$, it was in the order of the difference between untrained and endurance trained TA muscles, for $k_{\text{O2Hb}}$ in the order of the difference between healthy subjects and patients with chronic heart failure and peripheral vascular disease and for $F_p xD^*$ the difference between the soleus and the gastrocnemius muscle. This is a clear warning that all comparative and longitudinal studies that do not take intramuscular heterogeneity into account may come to wrong conclusions.

The distal disease onset in muscles of FSHD patients could be related to the lower oxidative capacity and/or lower oxygen supply in the distal part of the TA. To test this hypothesis,
the work needs to be extended to other muscles and men and women of varying ages. Furthermore, intramuscular variation in oxidative capacity and oxygen supply should be studied following common daily life activities, like walking, cycling and stair climbing in contrast to isolated isometric ankle dorsiflexion. Moreover, the study protocol can be applied in FSHD patients to detect differences with healthy volunteers.

The underlying mechanism for the proximo-distal gradient in oxidative capacity and oxygen supply along the TA following isometric ankle dorsiflexion is still under investigation. In this thesis, I discussed an intrinsic proximo-distal gradient in oxidative capacity vs. a functional gradient caused by the TA architecture. The intrinsic oxidative capacity is reflected by the mitochondrial content. This content depends on the ratio between the two main muscle fibre types, namely glycolytic type II fibres (low mitochondrial content) and oxidative type I fibres (high mitochondrial content). In rats and rabbits, the proportion of the area occupied by type I fibres indeed gradually decreases from the proximal part to the distal part of the TA. This would also explain the proximo-distal gradient in oxygen supply, since oxidative fibres often have a higher capillary density. Unfortunately, in humans, we are not aware of studies on muscle fibre distribution along the TA. The muscle mitochondrial density and fibre composition can be studied in-vivo and non-invasively with MR spectroscopy. For example, $^3\text{P}$ MR spectra may contain a second Pi peak, reflecting an alkaline Pi pool at 0.38 ppm, downfield from the cytosolic Pi signal. The ratio between the amplitude of the alkaline Pi signal and the cytosolic Pi signal provides a measure for the mitochondrial density. Furthermore, $^1\text{H}$ MRS can be used to assess the carnosine concentration, whereby glycolytic muscle fibres have higher carnosine concentrations compared to oxidative fibres.

The second proposed explanation is related to the TA’s architecture. The TA is a bipennate muscle with a central aponeurosis separating the muscle in a deep and superficial compartment. The axial orientation of this aponeurosis changes considerable from the proximal part to the distal part of the TA leading to a larger pennation angle proximally vs. distally. Larger pennation angles are associated with larger intramuscular pressures and possibly more inhibition of muscle perfusion during exercise. Assuming that the oxidative capacity is limited by the oxygen supply, a proximo-distal gradient in oxygen supply along the TA will lead to a similar gradient in oxidative capacity. This assumption can be tested by determining $k_{\text{PCr}}$ several times, while varying the fraction of inspired oxygen. The intramuscular pressure is not only related to the pennation angle in rest, but also to the fascicle length in rest and the change in pennation angle an fascicle length during contraction. Recent developed MRI and ultrasound technique offer the possibility to real-time image these changes in the TA’s architecture during contraction in 3D. The resulting 2D or 3D strain maps give an estimate of the variation in intramuscular pressures along the muscle during contraction as strain is inversely related to pressure. The work describing
the accelerated 4D phase contrast MRI sequence indeed reported an inhomogeneous strain pattern in the TA, but this was unfortunately not further specified.9

Though the underlying mechanism for the proximodistal gradient in oxidative capacity and oxygen supply are not fully explained, our work still has two major practical implications, both for research and clinical practice. First, it underlines the importance of localization when assessing muscle energy metabolism and perfusion and indicates that it is essential to decide if localized assessment at a single location or multiple locations or an average whole muscle assessment suits best for the specified question. Second, in case of longitudinal studies, it implies that reproducible positioning of the measurement device is crucial.

3. Quantitative MRI outcomes as biomarkers in DM1. What is there value?
In this third section, I will discuss the value of quantitative muscle MRI outcome measures (fat fraction, CSA, and T2\textsubscript{water}) as biomarkers for the assessment natural disease progression and therapy evaluation in DM1.

To be a suitable biomarker, an outcome measure must be responsive and sensitive (repeatable) enough to detect natural disease progression and treatment effects, and also able to detect early changes. The most responsive MR outcome measure was fat fraction. In case of 80% power and 50% reduction in disease progression, the needed sample size for fat fraction is only 64 subjects per treatment group. In contrast, the commonly used clinical measure six-minute walk test, requires twice as many subjects (153 subjects) in this scenario. This agrees with studies on Charcot-Marie-Tooth and inclusion body myositis, where fat fraction was also more responsive than clinical outcome measures.33 Natural disease progression and treatment effects could be detected by fat fraction and CSA. Fat fraction can detect the average 1.2% fat fraction increase observed in our DM1 cohort over 10 months in an individual patient with certainty of more than 95%. CSA was sensitive enough to detect the increased CSA in our behavioural intervention study. T2\textsubscript{water} was unresponsive over 10 months; no longitudinal change was observed in T2\textsubscript{water} for the behavioural intervention and standard care group. Early subclinical muscle changes could especially be observed with fat fraction and T2\textsubscript{water}. Fat fraction was increased before clinical muscle weakness was observed, whereby T2\textsubscript{water} is often elevated even before signs of fat infiltration. From these results we conclude that the fat fraction and CSA of muscles are suitable biomarkers for trials in DM1 as they can detect earlier and/or smaller treatment effects than common clinical outcome measures. This reduces the number of patients and time needed for a clinical trial, which is especially important in a rare disease, like DM1 and other muscular dystrophies.

As mentioned above, T2\textsubscript{water} was unresponsive in our longitudinal study. Here, I will shortly discuss why this might be, and why longitudinal changes in T2\textsubscript{water} are still relevant but
should be interpreted with care. It is important to realize that disease progression in DM1 is not reflected by a continuous increase in $T_2^{\text{water}}$. The $T_2^{\text{water}}$ increases in the early stage of the disease, but might normalize or decrease as soon as a muscle becomes fat infiltrated. In this thesis, I assessed the average $T_2^{\text{water}}$ over all 20 lower extremity muscles. Consequently, if in one muscle $T_2^{\text{water}}$ would increase (appearance of oedema) and in another muscle would decrease (oedema $\rightarrow$ fat infiltration), the average $T_2^{\text{water}}$ over all muscles remains stable and individual muscle changes are missed. This could be solved by looking at the average $T_2^{\text{water}}$ changes in individual muscle. However, in DM1 (and FSHD), increased $T_2^{\text{water}}$ presents as lesions in the muscle instead of in a homogeneous increased $T_2^{\text{water}}$. If one lesion in a muscle appears, while another lesion in the same muscle disappears, then an average $T_2^{\text{water}}$ over a single muscle might still miss localized changes within the muscle. Though $T_2^{\text{water}}$ was unresponsive in our study, in the past, it has been a successful biomarker for response to corticosteroid treatment in Duchenne muscular dystrophy.\textsuperscript{15,17} The difference with our study is that in Duchenne muscular dystrophy $T_2^{\text{water}}$ increase is much more homogeneous and with corticosteroid treatment you expect a drop in $T_2^{\text{water}}$ in all muscles. Therefore, it can be concluded that $T_2^{\text{water}}$ can still be promising for therapy evaluation in DM1 in case of treatments that are anticipated to give overall drop in $T_2^{\text{water}}$. Furthermore, the analysis should preferably be on $T_2^{\text{water}}$ in combination with fat fraction on a lesion level. Ideally, $^1$H MRS is used, to avoid any remaining effect of fat infiltration on the calculated $T_2^{\text{water}}$.

Although we and others have shown the added value of quantitative MR biomarkers to describe muscle disease, the clinical implementation of these biomarkers is hampered by the time-consuming task of manual muscle segmentation. Therefore, in many published studies on muscular dystrophy patients, only one or two slices of the MR images are analysed. However, this approach is prone to errors. Fat infiltration is clearly heterogeneous within the muscle and even functional measures in healthy muscle vary along the length of a muscle.\textsuperscript{14,34} Single slice assessment will therefore not be a good representation of the entire muscle and could miss the presence of pathology or disease progression. Accurate monitoring of disease progression and therapy evaluation therefore requires whole muscle assessment. Thus, (semi-)automatic muscle segmentation tools are urgently needed. Promising tools for this (semi-)automatic segmentation of muscles in healthy subjects already exist and some are getting optimized for applications to muscular dystrophy.\textsuperscript{35–37} Ideally, segmentation approaches should be fully automatic. However, fat infiltration and atrophy significantly alter the muscle’s anatomy and contrasts on the MR images, and boundaries between muscles may completely disappear. Therefore, fully automatic segmentation of muscle MR images in neuromuscular disorders seems to remain difficult, although the application of artificial intelligence (deep learning) software, trained on sufficient amounts of relevant images may solve this problem.\textsuperscript{38,39}
Conclusions

• Quantitative muscle MR is a valuable tool to study muscular conditions and changes in health and disease.
• Different MR techniques have complementary properties in studying (patho)physiological processes in skeletal muscle.
• This thesis led to a better understanding of the pathological changes and interventional effects in muscles of DM1 and FSHD patients, and muscle function and metabolism in healthy volunteers.
• Fat fraction and CSA measured by MR are highly recommended as muscle outcome measures for future clinical trials in DM1 as they are responsive and/or can detect early disease involvement.
• Both our physiological and morphological assessments demonstrate that it is absolutely required to take intramuscular heterogeneity into account to avoid errors and to obtain a complete picture of the muscle under investigation.
• To fully exploit the opportunities of quantitative MRI in clinical trials, (semi-)automatic segmentation tools are required to enable whole muscle analysis.
• Macroscopic MR observations of muscles in patients with neuromuscular disease are an important connection between clinical observations of these patients and the physiological and molecular observations of diseased muscles.
• To take the next step in unravelling the disease mechanisms in muscular dystrophies and to support developing curative treatments, we need to further extend our MRI toolbox with other techniques, including musculoskeletal modelling and with other modalities like molecular studies of muscle tissue biopsies.
References
Chapter 9

Nederlandse samenvatting
**Samenvatting**

Spierdystrofie is een verzamelnaam voor een groep erfelijke spierziekten die allen gekenmerkt worden door de degeneratie van skeletspieren. Deze spierdegeneratie houdt het volgende in: spiercellen worden vervangen door vetcellen, spiercellen worden kleiner (atrofie), en de hoeveelheid bindweefsel in de spier neemt toe (fibrose). Mensen met een spierdystrofie tonen progressieve spierzwakte, waardoor ze in de loop van de tijd vaak de mogelijkheid tot zelfstandig lopen verliezen en rolstoelafhankelijk worden. In dit proefschrift heb ik mij gericht op twee vormen van spierdystrofie, namelijk myotone dystrofie type 1 (DM1) en fascioscapulohumerale spierdystrofie (FSHD). Er worden momentele grote stappen gezet in de ontwikkeling van therapieën voor zowel DM1 en FSHD, echter heeft dit nog niet geleid tot een verkend geneesmiddel. Voor de ontwikkeling van een verkend geneesmiddel is het belangrijk om de ziekteprocessen en mechanismen (de pathofysiologie) in de spieren van mensen met spierdystrofie goed in beeld te brengen, zodat we deze pathofysiologie beter kunnen begrijpen. Daarnaast hebben we objectieve kwantitatieve uitkomstmaten nodig om het effect van een nieuw ontwikkelde therapie op spierniveau te kunnen bestuderen.

Dit proefschrift beschrijft de resultaten van onderzoeken die de pathofysiologische mechanismen in DM1 en FSHD proberen te ontrafelen door gebruik te maken van magnetische resonantie (MR) technieken. **Hoofdstuk 3 tot en met 5** richten zich op het natuurlijk beloop van de ziekteprocessen in DM1 en FSHD en hoe dit verloop verandert bij een interventie. In **hoofdstuk 6 en 7** heb ik gekeken naar de variatie van de normale spierfunctie in een enkele spier, de scheenbeenspier (tibialis anterior) bij gezonde mensen zonder spierdystrofie. Hoofdstuk 6 en 7 hadden als doel om mogelijke factoren te vinden die betrokken zijn bij de start van het vervettingsproces in de spier.

**Hoofdstuk 1 en 2** geven een introductie in dit proefschrift. **Hoofdstuk 1** beschrijft de klinische kenmerken en symptomen, de genetica, en huidige bevinden op MR gebied bij mensen met DM1 en FSHD. **Hoofdstuk 2** geeft een overzicht over de basis principes van MR en de gebruikte MR technieken. Voor dit proefschrift heb ik gebruik gemaakt van MR beeldvorming (‘imaging’; MRI) en MR spectroscopie (MRS), zoals hierna samengevat beschreven. Bij de mensen met spierdystrofie (hoofdstuk 3 tot en met 5) heb ik een Dixon sequentie en multi-echo spin-echo sequentie gebruikt. Met de Dixon sequentie heb ik per spier de vetfractie en spiervolume (of spieroppervlak) bepaald als respectievelijke uitkomstmaat voor spiervervetting en spiermassa. Daarnaast heb ik van alle spieren de T2 relaxatietijd van het water in de spier (T2 water) bepaald middels de multi-echo spin-echo sequentie. De T2 relaxatietijd weerspiegelt de verandering in de waterdistributie van het weefsel veroorzaakt door bijvoorbeeld intra- of extracellulair oedeem, één van de vroege pathologische veranderingen in het spierweefsel.
Voor hoofdstuk 6 en 7, bij mensen zonder spierdystrofie, heb ik het energiemetabolisme, de perfusie en oxygenatie van de tibialis anterior spier onderzocht met behulp van de MR technieken fosfor MRS ($^{31}$P MRS), spierfunctionele MRI (mf MRI), ‘intravoxel incoherent motion imaging’ (IVIM) en de optische techniek ‘near infrared spectroscopy’ (NIRS).

In hoofdstuk 3 en 4 presenteer ik de resultaten van onze kwantitatieve MRI spierstudie bij mensen met DM1. Deze resultaten zijn verkregen binnen een internationale multicenter studie, genaamd OPTIMISTIC. De OPTIMISTIC studie betrof een gerandomiseerd studie met een controlegroep (randomized controlled trial) waarbij het effect van een gedragsinterventie bij mensen met DM1 is onderzocht. Deze gedragsinterventie had als doel de dagelijkse activiteit van mensen met DM1 te verhogen. In een subgroep bestaande uit 33 mensen met DM1 hebben we de hiervoor voorgestelde MRI uitkomstmaten voor spiervervetting (vetfractie), spiermassa (volume/oppervlak) en waterdistributie/oedeem binnen de spier ($T_2^{water}$) gemeten aan het begin van de studie (‘baseline’) en 10 maanden later.

Hoofdstuk 3 beschrijft de resultaten van de baseline MRI scan. De deelnemende mensen met DM1 hadden ongeveer 3x zoveel vet, een 26% afname in spiermassa en een verhoogde $T_2^{water}$ (geeft aanwezigheid van oedeem weer) vergeleken met mensen zonder spierdystrofie. De mate van spiervervetting bij mensen met DM1 werd primair bepaald door leeftijd gevolgd door de mate van afwijking in het DNA, namelijk de verlengde CTG repeat. Meer specifiek, de mate van vettigheid werd bepaald door de lengte van de CTG repeat bij de geboorte en de hoeveelheid repeats waarmee deze CTG repeat is toegenomen tijdens het leven. Verder hebben we aangetoond dat de vetfractie en spiermassa bepaald met MRI correleren met klinische maten voor fysieke activiteit en ziekte-ernst bij mensen met DM1. Interessant hierbij is dat de vetfracties reeds verhoogd zijn voordat er klinisch spierzwakte wordt waargenomen.

In hoofdstuk 4 heb ik gekeken naar de verandering die de spieren van mensen met DM1 lieten zien over 10 maanden. Hierbij heb ik in eerste instantie gekeken naar het effect van de gedragsinterventie op de spieren. Hiervoor heb ik de MRI uitkomstmaten van mensen met DM1 die gerandomiseerd waren tot de interventie vergeleken met de mensen met DM1 die gerandomiseerd waren naar de standaardbehandeling. Daarnaast heb ik de MRI uitkomstmaten van de mensen met DM1 die de standaardbehandeling kregen gebruikt om het natuurlijk ziekteverloop van mensen met DM1 te bepalen. Ten eerste, mensen met DM1 uit de interventiegroep lieten ongeveer 4% toename in spiermassa zien na 10 maanden behandeling, in tegenstelling tot standaardbehandeling groep, waarbij de spiermassa niet veranderde. Deze spiermassa toename bij de interventiegroep was het meest prominent in spieren die op de baseline MRI scan nog geen tekenen van spiervervetting lieten zien. Ten tweede, vettigheid in de beenspieren nam
met 0.9% toe in de interventie groep en met 1.2% in de standaardbehandeling groep. Deze toename in vetfractie was het snelst in de spieren die op de baseline MRI scan tekenen van oedeem vertoonden. Ten derde, beide groepen toonden na 10 maanden geen verandering in de waterdistributie van het spierweefsel (T2\textsubscript{water}, oedeem). Op basis van bovenstaande resultaten kunnen we concluderen dat de gedragsinterventie, die stimuleert tot meer activiteit in het dagelijkse leven, zowel veilig als effectief is voor mensen met DM1. De gedragsinterventie leidt namelijk niet tot versnelde spierdegeneratie en wel tot een toename in spiermassa. Aangezien vooral de spieren die nog geen tekenen van vettveting tonen baat lijken te hebben bij de interventie is het aan te raden deze gedragstherapie vroeg in het ziekteverloop in te zetten.

Hoofdstuk 3 en 4 combinerend kunnen we ook concluderen dat de MRI uitkomstmaten, vetfractie en spiervolume/spieroppervlak, veelbelovende uitkomstmaten zijn voor toekomstige klinische trials bij mensen met DM1. Deze uitkomstmaten zijn in staat om gezonde en zieke spieren van elkaar te onderscheiden, ze correleren met functionele uitkomstmaten en zijn sensitief genoeg om het natuurlijk ziekteverloop en een therapie-effect te detecteren in een acceptabele tijdsduur van 10 maanden.

Hoofdstuk 5 gaat over mensen met FSHD waarbij ik de spierkracht van deze mensen heb onderzocht. Bij deze studie heb ik MRI gecombineerd met spierkrachtmetingen en een mathematisch spiermodel om de specifieke spierkracht uit te rekenen. De specifieke spierkracht is gedefinieerd als de kracht per eenheid oppervlak contractiel spierweefsel. De specifieke spierkracht was ongeveer 50% lager in de quadriceps spieren van mensen met FSHD vergeleken met gezonde controles. Dit betekent dat spierzwakte bij mensen met FSHD dus niet alleen wordt veroorzaakt door spierweefseilverlies, maar ook door een afgenomen functie van het overgebleven spierweefsel. Wat de oorzaak is voor deze verminderde spierfunctie moet verder onderzocht worden. Mogelijk is de intrinsieke kracht die een spier/spiervezel kan genereren of overbrengen verminderd of speelt een verstoord energiemetabolisme in spieren van mensen met FSHD een rol.

Eerder onderzoek bij mensen met FSHD suggereert dat FSHD een focale ziekte is; de spiervervetting lijkt te beginnen in het distale gedeelte van de spier. Dit impliceert dat de structuur of functie van gezonde spier zodanig varieert langs zijn proximo-distale as, dat het distale gedeelte van de spier het meest gevoelig is voor ziekte-initiatie. Daarom heb ik, als een eerste stap, in hoofdstuk 6 en 7 in-vivo enkele functionele eigenschappen over de lengte van tibialis anterior spier (i.e. de scheenbeenspier) onderzocht bij vrijwilligers zonder spierdystrofie.

In hoofdstuk 6 heb ik mij gericht op de intramusculaire variatie in energiemetabolisme. Met \textsuperscript{31}P MRS heb ik de verandering in fosforcreatine (PCr) gemeten gedurende vrijwillige spierinspanning van de tibialis anterior middels isometrische enkeldorsiflexie en tijdens
herstel van deze spierinspanning. Hierbij zien we een afname van de PCr concentratie tijdens de inspanning en herstel van de PCr concentratie na de inspanning. Vervolgens heb ik de snelheid van het herstel van deze PCr concentratie ($k_{pcr}$) bepaald. De $k_{pcr}$ is een maat voor de maximale capaciteit van de spier om ATP, de energiedrager in het menselijk lichaam, te produceren via oxidatieve fosforylering. Dit noemen we ook wel de oxidatieve capaciteit van de spier.

Om deze metingen mogelijk te maken hebben we een opstelling ontwikkeld met een zelfgebouwde $^{31}$P ontvangstspoel, bestaande uit vijf aparte spoelelementen (phased array spoel). Deze spoel maakt het mogelijk om op vijf posities langs de proximo-distale as van de tibialis anterior veranderingen te meten in PCr met een hoge tijdsresolutie. Met deze opstelling hebben we aangetoond dat de herstelsnelheid van PCr na isometrische enkeldorsiflexie varieert langs de proximo-distale as van de tibialis anterior. De herstelsnelheid van PCr ($k_{pcr}$) was ongeveer twee keer hoger proximaal dan distaal. Deze resultaten worden ondersteund door spierfunctionele MRI (mf-MRI) metingen, waarbij we ook proximo-distale verschillen vonden in de snelheid van signaaltoename in de tibialis anterior na enkeldorsiflexie. Hieruit kunnen we concluderen dat de tibialis anterior een proximo-distale variatie laat zien in de oxidatieve capaciteit, waarbij deze capaciteit distaal het laagst is.

In hoofdstuk 7 heb ik mij gericht op intramusculaire variatie in spierperfusie en oxygenatie door onze ontwikkelde $^{31}$P MRS opstelling te combineren met NIRS en IVIM. De reden hiervoor is dat zuurstoftoevoer essentieel is voor het herstel van de PCr concentratie. De zuurstoftoevoer is proportioneel aan de verandering in oxyhemoglobine ($\Delta O_2 Hb$), de drager van zuurstof in bloed, en de ATP synthese snelheid ($k_{pcr} \times \Delta PCr$), zie vergelijking 9.1.

$$O_2\,toevoer \propto \Delta O_2\,Hb + k_{pcr} \times \Delta PCr \quad (vergelijking\,9.1)$$

De verandering in oxyhemoglobine heb ik gemeten met NIRS en de ATP synthese snelheid met $^{31}$P MRS. Daarnaast heb ik de spierdoorbloeding onderzocht met IVIM, aangezien spierdoorbloeding één van de bepalende factoren voor zuurstoftoevoer is. Onze resultaten tonen aan dat ook de zuurstoftoevoer en spierdoorbloeding variëren langs de proximo-distale as van de tibialis anterior. Net als de oxidatieve capaciteit, zijn de zuurstoftoevoer en spierdoorbloeding het laagst in het distale gedeelte van de spier. De gevonden intramusculaire variatie in zuurstoftoevoer is sterk geassocieerd met de intramusculaire variatie in PCr herstelsnelheid en ATP synthese snelheid. Een mogelijke verklaring voor deze associatie is een intrinsieke variatie in mitochondriële oxidatieve capaciteit, bijvoorbeeld door variatie in de ratio oxidatieve vs. glycolytische spiervezels langs de tibialis anterior. Of, mogelijk is er een causale relatie tussen zuurstoftoevoer en herstel van PCr concentratie die gedreven wordt door verschillen in architectuur en/of mechanistische verschillen langs de proximo-distale as van tibialis anterior.
In conclusie, met dit proefschrift heb ik laten zien dat kwantitatieve MR van waarde is bij het bestuderen van spieraandoeningen. De verschillende MR technieken zijn complementair aan elkaar bij het begrijpen van de (patho)fysiologische processen in de spier. De macroscopische MRI observaties in de spieren van mensen met spieraandoeningen vormen een belangrijke verbinding tussen de klinische observaties bij deze mensen en de fysiologische en moleculaire observaties op spierniveau. Dit proefschrift heeft in de eerste plaats geleid tot meer inzicht in de pathologische veranderingen en een interventie-effect in spieren van mensen met DM1 en/of FSHD, alsmede spierfunctie en spiermetabolisme in het algemeen. Ten tweede heeft dit proefschrift aangetoond dat vetfractie en spieroppervlak/volume zeer geschikt zijn als spieruitkomstmaat in toekomstige klinische studies bij mensen met DM1. Ten derde, dit proefschrift laat grote intramusculaire verschillen zien in spierfunctie en spiermetabolisme, wat aantoont dat in toekomstige studies het essentieel is dat deze intramusculaire heterogeniteit meegenomen wordt om fouten te voorkomen en een compleet beeld te krijgen van de spier. Om de volgende stap te nemen in het ontrafelten van de ziektemechanismes in spierdystrofieën en het ondersteunen van de ontwikkeling van geneesmiddelen, moeten we onze MRI toolbox verder uitbreiden met andere technieken, onder andere mathematische spiermodellen en andere modaliteiten, zoals spierweefselbiopten.
Appendices
List of abbreviations

$^1$H MRS  proton magnetic resonance spectroscopy
$^{31}$P MRS  phosphorous magnetic resonance spectroscopy
6MWT  six-minute walk test
ADC  apparent diffusion coefficient
ASL  arterial spin labeling
ATP  adenosine triphosphate
BOLD  blood oxygen level dependent
CBT  cognitive behavioural therapy
CELF  CUG binding proteins and Elav-like family members
CIS  checklist individual strength
CK  creatine kinase
Cr  creatine
CSA  cross-sectional area
$\text{CTG}_{\text{ePAL}}$  CTG repeat length inherited at birth
$\text{CTG}_{\text{modal}}$  modal CTG repeat length
DM1  myotonic dystrophy type 1
DMD  Duchenne muscular dystrophy
DMPK  dystrophia myotonica protein kinase
DWI  diffusion weighted imaging
FA  flip angle
FID  free induction decay
FOV  field of view
FSHD  facioscapulohumeral muscular dystrophy
GE  gradient echo
HHb  deoxyhaemoglobin
IVIM  intravoxel incoherent motion imaging
$k_{\text{PCR}}$  recovery rate of phosphocreatine
MBNL  muscleblind-like
mf MRI  muscle functional MRI
MIRS  muscular impairment rating scale
MR  magnetic resonance
MRI  magnetic resonance imaging
MRS  magnetic resonance spectroscopy
MSE  multi-echo spin-echo
MVC/MVIC  maximal voluntary (isometric) contraction
NIRS  near infrared spectroscopy
NOE  nuclear overhauser effect
$O_2$Hb  oxyhaemoglobin
PCr  phosphocreatine
PCSA  physiological cross-sectional area
Pi  inorganic phosphate
ppm  parts per million
RF  radiofrequency
ROI  region of interest
SE  spin echo
SNR  signal to noise ratio
SRM  standardized response mean
T2_{water}  T2 relaxation time of muscle water
T2_{fat}  T2 relaxation time of muscle fat
TE  echo time
TI  inversion time
TIRM  turbo inversion recovery magnitude imaging
TR  repetition time
TSE  turbo spin echo

Abbreviations of studied muscles
AL  adductor longus
AM  adductor magnus
BFL  biceps femoris long head
BFS  biceps femoris short head
EDL/ED  extensor digitorum (longus)
FDL  flexor digitorum longus
G  gracilis
GL  gastrocnemius lateralis
GM  gastrocnemius medialis
P  peroneus
RF  rectus femoris
S  sartorius
SM  semimembranosus
SOL  soleus
ST  semitendinosus
TA  tibialis anterior
TP  tibialis posterior
VI  vastus intermedius
VL  vastus lateralis
VM  vastus medialis
List of publications
L. Heskamp, K. Okkersen, M. van Nimwegen, M.J. Ploegmakers, G. Bassez, J.F Deux, B.G.M. van Engelen, A Heerschap, for the OPTIMISTIC consortium. Quantitative MRI detects increased muscle mass upon a behavioural change in myotonic dystrophy. Submitted


List of conference presentations

ISMRM 2019, Montreal, Canada

L. Heskamp, K. Okkersen, M. van Nimwegen, M.J. Ploegmakers, G. Bassez, J.F Deux, B.G.M. van Engelen, A. Heerschap, for the OPTIMISTIC consortium. Quantitative MRI detects increased muscle mass upon a behavioural change in myotonic dystrophy. Oral presentation

L. Heskamp, M. van Uden, T. Scheenen, A. Heerschap. Phosphocreatine recovery in the tibialis anterior after dynamic exercise shows a proximo-distal gradient, which is not explained by carnosine and acetylcarnitine differences. Oral presentation

ISMRM Benelux 2019, Leiden, The Netherlands

L. Heskamp, K. Okkersen, M. van Nimwegen, M.J. Ploegmakers, G. Bassez, J.F Deux, B.G.M. van Engelen, A. Heerschap, for the OPTIMISTIC consortium. Quantitative MRI detects increased muscle mass upon a behavioural change in myotonic dystrophy. Power pitch + Poster presentation

ISMRM MRS workshop 2018, Utrecht, The Netherlands

L. Heskamp, F. Lebbink, M. van Uden, M. Maas, J. Claassen, A. Boss, A. Heerschap. Both oxygen supply and phosphocreatine recovery rate show proximo-distal gradients along the human tibialis anterior after exercise. Oral presentation

ISMRM 2018, Paris, France

L. Heskamp, F. Lebbink, M. van Uden, M. Maas, J. Claassen, A. Boss, A. Heerschap. Both oxygen supply and phosphocreatine recovery rate show proximo-distal gradients along the human tibialis anterior after exercise. Oral presentation

L. Heskamp, M. van Nimwegen, G. Bassez, C. Jimenez Moreno, M. Ploegmakers, J. Deux, G. Gorman, D. Monckton, B. van Engelen, A. Heerschap. Does muscular fat infiltration and volume determined with MRI correlate with functionality and the DMPK CTG repeat length in myotonic dystrophy type 1? Poster presentation

ISMRM Benelux 2018, Antwerp, Belgium

L. Heskamp, M. van Nimwegen, G. Bassez, C. Jimenez Moreno, M. Ploegmakers, J. Deux, G. Gorman, D. Monckton, B. van Engelen, A. Heerschap. Does muscular fat infiltration and volume determined with MRI correlate with functionality and the DMPK CTG repeat length in myotonic dystrophy type 1? Poster presentation
Imaging in Neuromuscular Disease 2017, Berlin, Germany

**L. Heskamp**, A. Boss, V. Breukels, L. Bains, M. van Uden, A. Heerschap. Muscle functional oxidative capacity varies along the length of healthy human tibialis anterior. *Oral presentation*

**L. Heskamp**, M. van Nimwegen, G. Bassez, C. Jimenez Moreno, M. Ploegmakers, J. Deux, G. Gorman, H. Lochmuller, M. Catt, B. van Engelen, A. Heerschap. Quantitative MRI in Myotonic Dystrophy Type 1: Natural progression and correlation with functionality. *Poster presentation*

**L. Heskamp**, M. Marra, K. Mul, B. van Engelen, A. Heerschap N. Verdonschot. Specific strength is reduced in facioscapulohumeral dystrophy muscles. An MRI based musculoskeletal analysis. *Poster presentation*

World Muscle Society 2017, Saint-Malo, France

**L. Heskamp**, M. Marra, K. Mul, B. van Engelen, A. Heerschap N. Verdonschot. Specific strength is reduced in facioscapulohumeral dystrophy muscles. An MRI based musculoskeletal analysis. *Poster presentation*


ISMRM 2017, Honolulu, USA

**L. Heskamp**, M. van Nimwegen, G. Bassez, M. Ploegmakers, J. Deux, B. van Engelen, A. Heerschap. Disease progression in skeletal muscles of Myotonic Dystrophy Type 1 evaluated using quantitative MRI. *Poster presentation*

ISMRM Benelux 2017, Tilburg, The Netherlands

**L. Heskamp**, M. van Nimwegen, G. Bassez, M. Ploegmakers, J. Deux, B. van Engelen, A. Heerschap. Disease progression in skeletal muscles of Myotonic Dystrophy Type 1 evaluated using quantitative MRI. *Poster presentation*

ISMRM 2016, Singapore, Singapore

**L. Heskamp**, M. van Nimwegen, B. Janssen, B. van Engelen, A. Heerschap. Quantitative MRI evaluation of fatty infiltration and edema-like processes in skeletal muscles of Myotonic Dystrophy Type 1. *Poster presentation*
ISMRM Benelux 2016, Eindhoven, The Netherlands

**L. Heskamp, M. van Nimwegen, B. Janssen, B. van Engelen, A. Heerschap.** Quantitative MRI evaluation of fatty infiltration and edema-like processes in skeletal muscles of Myotonic Dystrophy Type 1. *Oral presentation*

ISMRM 2015, Toronto, Canada

**L. Heskamp, B. Janssen, A. Heerschap.** Muscular fat fraction determination by quantitative T2-MRI, reproducibility in facioscapulohumeral muscular dystrophy and healthy volunteers. *Poster presentation*

**L. Heskamp, B. Janssen, A. Heerschap.** Quantification of the inflammatory process in muscles of patients with facioscapulohumeral muscular dystrophy. *Poster presentation*

ISMRM Benelux 2015, Gent, Belgium

**L. Heskamp, B. Janssen, A. Heerschap.** Quantification of the inflammatory process in muscles of patients with facioscapulohumeral muscular dystrophy. *Oral presentation*
## PhD portfolio

**Name PhD candidate:** L. Heskamp  
**Department:** Radiology and Nuclear Medicine  
**Graduate School:** Radboud Institute for Molecular Life Science  
**PhD period:** 01-09-2014 – 31-5-2019  
**Promotor(s):** Prof. A. Heerschap, Prof. B. van Engelen

### TRAINING ACTIVITIES

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**D) Other**  
Radiotherapy Research meeting | 2014-2019 | 4.0  
BioMR research meetings and journal clubs | 2014-2019 | 8.0  
Organisation committee ISMRM Benelux 2018 | 2017-2018 | 2.0

### TEACHING ACTIVITIES

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<td>Supervision five 10-week Master Internships Technical Medicine</td>
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**TOTAL** | 64.5

^Indicates oral or poster presentation with a * and # after the name, respectively
Data management
This thesis is based on the results of human studies, which were conducted in accordance with the principles of the Declaration of Helsinki. The medical and ethical review board Committee on Research Involving Human Subjects Region Arnhem Nijmegen, Nijmegen, the Netherlands has given approval to conduct these studies.

The paper trial master files and case report forms for chapter 3, 4, 6 and 7 are stored in the department archive (Radboudumc, Long-term storage M330.02.219).

The digital trial master files are stored at the Radiology department network drive (\umcfs097\radngdata\Research\Trialbureau\_Archief\Trialbureau_digitaal).

All paper data were entered into the computer by use of Castor EDC. Data management and monitoring were also performed within Castor EDC. An audit trail was incorporated to provide evidence of the activities that has altered the original data. The privacy of the participants in this study is warranted by use of encrypted and unique individual subject codes. The codes were stored separately from the study data. Data where converged from Castor EDC to SPSS (SPSS Inc., Chicago, Illinois, USA).

The raw data (MR images, MR spectra, and NIRS and force data) are stored on the scientific data storage archive of the department of Radiology and Nuclear Medicine. The data are directly exported from the modalities to the data archive (\rdscience\mrs_data5\LinHes and \rdscience\mrs_data5\AndBos). The processed, analysed data and final outcome values are stored on a different part of the scientific data storage archive, a part only accessible by the researcher and authenticated by password (pathname is: \rdscience\u3\lindaheskamp and \rdscience\u3\andreasbos). The final outcomes values are stored in SPSS format: OPTIMISTIC\Database_Final (chapter 3 and 4), ExTibant (chapter 6) and ExTibant2.0\OutcomeSPSS (chapter 7).

The data will be saved for 15 years after termination of each study. Using these participants data in future research is only possible if explicit permission by the participant is given in the informed consent or after a renewed permission by the participant. The datasets analyzed during these studies are available from the corresponding author on reasonable request.
**Dankwoord**

Met veel plezier heb ik de afgelopen 5 jaar aan dit proefschrift gewerkt en eindelijk is het zover, het is af! Dit had ik natuurlijk niet alleen gekund. Daarom wil ik met deze laatste pagina’s iedereen bedanken die heeft bijgedragen.

Mijn grootste dank gaat uit naar de deelnemers van mijn studies, zonder jullie had dit proefschrift niet bestaan. In het bijzonder wil ik hierbij de deelnemers met spierdystrofie en hun familie benoemen. Jullie kwamen van overal uit Nederland, soms op meer dan 2 uur reizen van Nijmegen, voor een MRI scan van ‘slechts’ een uurtje. Ik heb dan ook grote bewondering voor jullie doorzettingsvermogen en wil om bij te dragen aan de wetenschap. Jullie verhalen over een leven met spierdystrofie waren indrukwekkend en vormden voor mij altijd een extra bron van motivatie.

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Promoveren is natuurlijk niet alleen werken, ontspanning is ook essentieel. Hiervoor zijn vrienden en familie onmisbaar.

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Curriculum Vitae

Linda Heskamp was born on the 1st December 1988 in Enschede, The Netherlands. In 2007, she finished her VWO (pre-university education) at the Bonhoeffer College in Enschede, after which she started studying Technical Medicine at the University of Twente. She obtained her Bachelor degree in 2010 and in the same year she continued with the Master program of Technical Medicine. During the second year of her Master program she completed four 10-week internships at the University Medical Center Utrecht, Medisch Spectrum Twente and the Radboud university medical center. She graduated in 2014 with her Master thesis at the neonatology intensive care unit of the Radboud university medical center, where she worked on predicting fluid responsiveness in neonates.

In September 2014, she started with her PhD at the Radiology department of the Radboud university medical center, Nijmegen. Her project was a collaboration between the Radiology department and Neurology department, under supervision of prof. Arend Heerschap and prof. Baziel van Engelen. She applied MR techniques to study the pathophysiology in muscles of patients with muscular dystrophy and physiology of healthy muscle. This research resulted in the thesis you are currently reading. During her PhD, she also performed contract research for aTyr Pharma. She successfully applied for the Rubicon grant of NWO to work at the Newcastle University on the development of a novel MRI technique to study motor unit activity in muscles of patients with motor neuron disease. In April 2019, she started this post-doc at the in-vivo MR centre at the Newcastle University, Newcastle upon Tyne under supervision of prof. Andrew Blamire.
Quantitative muscle MRI to unravel the physiology of dystrophic and healthy muscle

Linda Heskamp