European Journal of Radiology 114 (2019) 160–166

Contents lists available at ScienceDirect

European Journal of Radiology

journal homepage: www.elsevier.com/locate/ejrad

Research article

Feasibility of Dixon magnetic resonance imaging to quantify effects of physical training on muscle composition—A pilot study in young and healthy men

Alexandra Grimm[a,*], Marcel D. Nickel[b], Oliver Chaudry[a], Michael Uder[c], Franz Jakob[d], Wolfgang Kemmler[a], Harald H. Quick[a,e,f], Klaus Engelke[a,g]

[a] Institute of Medical Physics, Friedrich-Alexander University Erlangen-Nuremberg (FAU), Henkestr. 91, 91052 Erlangen, Germany
[b] Siemens Healthcare GmbH, Diagnostic Imaging, Magnetic Resonance, Product Definition & Innovation, Allee am Roethelheimpark 2, Erlangen, Germany
[c] Institute of Radiology, University Hospital Erlangen, Ulmenweg 18, Erlangen, Germany
[d] Orthopedic Center for Musculoskeletal Research, University Wuerzburg, Brettreichstraße 11, 97074 Wuerzburg, Germany
[e] Erwin L. Hahn Institute for Magnetic Resonance Imaging, University Duisburg-Essen, Kokereiallee 7, Essen, Germany
[f] High-Field and Hybrid MR Imaging, University Hospital Essen, Hufelandstraße 55, Essen, Germany
[g] Department of Medicine 3, Friedrich-Alexander University Erlangen-Nuremberg (FAU) and University Hospital Erlangen, Ulmenweg 18, Erlangen, Germany

ARTICLE INFO

Keywords:
Dixon MRI
Fat quantification
Physical training
Muscle
Fat

ABSTRACT

Changes in muscle-fat-composition affect physical performance and muscular function, like strength and power. The purpose of the present study was to investigate whether changes in soft tissue composition of the thigh and changes in muscle size and composition resulting from physical training were detectable with Dixon magnetic resonance imaging (MRI).

A young and healthy subject population (n = 21, 29 ± 5 years) was split into a strength training (G_t, 11 subjects) and a control group (G_c, 10 subjects). The physical training intervention lasted over 13 weeks. Before and after this intervention a muscle performance exam and an MRI exam were conducted on all subjects. To evaluate muscle performance and the training effect, the jump height was measured using a mechanograph. Fascia, pure muscle and subcutaneous fat areas and proton density water fraction (PDWF) and proton density fat fraction (PDFF) of the left thigh were measured with a 6-point Dixon prototype MRI sequence.

Muscle area changed by +7.1 ± 3.3% ($p < 0.05$) and +2.5 ± 5.6% ($p > 0.05$), and PDFF by -16.3 ± 10.4% ($p < 0.05$) and +5.4 ± 6.9% ($p > 0.05$) in G_t and G_c, respectively. Cross-sectional and longitudinal correlation coefficients R between PDFF and muscle performance were moderate (R = -0.43 and R = -0.51, respectively). The correlation was also moderate for muscle performance and a combined muscle fat per area ratio (R = -0.40 and R = -0.55, respectively).

Dixon MRI is capable to measure training-related changes in muscle area and muscular fat. Both parameters correlate to muscle function. Muscle area per se does not always mirror functional parameters. Due to the complex interaction of muscle volume, muscle structure, and inter- and intramuscular coordination during muscle performance, multivariate muscle parameter models should be investigated in the future. Future studies will have to show if structural parameters mirror and explain functional muscle data both in the context of physical training and pathologies like sarcopenia.

1. Introduction

Muscular adipose tissue (AT) is interspersed in skeletal muscle, accumulated beneath the muscle fascia, between muscle groups (perimuscular AT), and within individual muscles (intramuscular tissue) [1]. Due to its proximity to muscle, AT is associated with muscle metabolism. A high content of muscular AT has been reported to reduce insulin sensitivity [2]. Moreover, in muscles with lower AT content, lower levels of proinflammatory cytokines have been observed compared to muscles with higher AT content [3,1,4,2]. High levels of

* Corresponding author at: Henkestr. 91, 91052 Erlangen, Germany.
E-mail addresses: alexandra.grimm@fau.de (A. Grimm), marcel.nickel@siemens-healthineers.com (M.D. Nickel), oliver.chaudry@imp.uni-erlangen.de (O. Chaudry), michael.uder@uk-erlangen.de (M. Uder), f-jakob.klh@uni-wuerzburg.de (F. Jakob), wolfgang.kemmler@ofz.uni-erlangen.de (W. Kemmler), harald.quick@uni-due.de (H.H. Quick), klaus.engelke@imp.uni-erlangen.de (K. Engelke).

https://doi.org/10.1016/j.ejrad.2019.03.019
Received 6 September 2018; Received in revised form 26 February 2019; Accepted 25 March 2019

proinflammatory cytokines have negative effects on muscle regeneration time and consequently on muscular function. This paracrine/endocrine effect on muscle function is amplified by the effect of muscle AT on muscle structure, one of the most important factors contributing to physical performance and muscular function, like strength and power [5–7]. A decrease in muscle AT changes the muscle structure and leads to an increase of elastic energy storage and a decrease of muscle-tendon unit stiffness. These two effects lead to a higher muscle response and activation, both resulting in a decreased energy requirement for muscle contraction [8–10].

It has been shown that sedentary lifestyle, inactivity or limited mobility, for example due to injury cause increased levels of a relative muscular AT content, which results in muscle dysfunction [1,11,5,7] and may lead to further physical inactivity. Manini et al. [11] showed that a four week period of physical inactivity increased muscle AT in healthy young adults by 15–20%. Therefore, exercise and a calorie-restricted diet are often the first interventions prescribed. Compared to diet, exercise interventions have a significantly greater effect to reduce AT and are also accompanied by an improved muscular function. Although the relationship between muscle performance and AT is not entirely clear yet, the relative muscle AT content might be a strong indicator of the actual status of musculoskeletal performance and the success of exercise. Moreover, it might also be a candidate parameter for the risk assessment to develop future mobility impairment as well as the efficacy of a therapeutic intervention, e.g. in myopathies and sarcopenia. Dedicated magnetic resonance imaging (MRI) techniques have been developed to measure AT. Specifically, Dixon MRI [12] has been used to quantify the relative amount of AT in muscular dystrophy [13], myopathies [14], and more recently sarcopenia [15]. Recently, Dixon protocols for quantifying muscular proton density fat fractions (PDFF) of the mid-thigh have been tailored [16]. Further, their excellent accuracy in the muscle [17–20] and their excellent precision [21,22] in the muscle and the liver have been shown.

However, it has not been shown yet, whether 6 pt Dixon MRI is feasible to quantify effects of physical training on soft tissue and in particular on muscle composition of the thigh. The purpose of the present pilot study is therefore to demonstrate that changes in muscle fat resulting from physical training can be quantified with 6-point (6 pt) Dixon MRI [16] and that they relate to changes in muscle performance.

2. Materials and methods

2.1. Subject population

21 young and healthy men (29 ± 5 years, 23–36 years) were recruited for this study. Inclusion criteria were an absence of severe medical conditions, at least three hours of physical training per week for at least six months prior to study enrollment, and experience with strength/ resistance training.

The study was approved by the local ethical review committee. Written informed consent was obtained from each subject.

2.2. Physical training intervention

Participants, who compared to the six-month period before study enrollment, agreed to increase hypertrophic thigh muscle strength training volume and/or frequency by at least 60% and training intensity by at least 20% as were assigned to the training group (G_t, 11 subjects). Participants who kept their training habits as compared to the six-month period before study enrollment were assigned to the control group (G_c, 10 subjects). Thus, group assignment depended on the motivation of the participants to increase their physical training habits.

The training intervention lasted over 13 weeks and focused on hypertrophic strength training of the thigh muscles using 6–14 repetitions with an intensity of 65–80% of the one-repetition-maximum (1 RM). There were no joint training sessions, instead participants received training instructions if needed but most subjects were already familiar with strength training. The focus of the study was not to evaluate a specific training method but to validate 6 pt Dixon MRI to measure effects of validated training methods. In order to keep the training motivation as high as possible, participants could individually choose among different hypertrophic strength exercises, thus the training was not standardized. In addition, participants were encouraged to continue their pre-study physical activities such as endurance, maximal strength, hypertrophic strength, endurance strength, or skill training. The relevant parameters of these physical activities were documented weekly.

2.3. Functional measurement

Jump power was measured using a mechanograph (Leonardo Mechanograph® GRFP, Novotec Medical GmbH, Pforzheim, Germany) in all subjects. The device measures ground reaction forces dynamically. Countermovement jumps [23] were performed and the jump height was measured. Participants were allowed to conduct up to five trials and rest between them. The highest value of all trials was used for the data analysis.

2.4. MR data acquisition and MR examination

2.4.1. MRI sequences

The protocol included a clinically common T_1-weighted (T_1w) Turbo Spin Echo (TSE) sequence (image resolution: 0.5 × 0.5 × 3.0 mm^3, 34 slices, matrix size: 512 × 512, repetition time (TR): 844 ms, echo time (TE): 14 ms, bandwidth: 488 Hz/px, acquisition time: 2:54 min) serving as high spatial resolution anatomical reference.

Fat and water were determined as ‘proton density fat fraction’ (PDFF) and ‘proton density water fraction’ (PDWF), respectively. Both were determined in parameter maps from a prototypical 6 pt GRE (Gradient Echo) VIBE (Volumetric Interpolated Breath-hold Examination) sequence (image resolution: 0.8 × 0.8 × 3.0 mm^3, 36 slices, matrix size: 320 × 320, TR: 14.00 ms, echo times TE^{1-6}: 1.90, 3.73, 5.56, 7.39, 9.22, and 11.05 ms, bandwidth: 710 Hz/px, acquisition time: 1:17 min) using a multi-echo Dixon reconstruction [24].

The 6 pt Dixon prototype sequence used improved water-fat separation to avoid local and global fat/water swaps and a linear principal component analysis (LPCA) denoising algorithm [25]. Moreover, a time-domain calibration of the fat signal dephasing optimized for liver applications [26] with a phase-based fat model was used [24]. A low flip angle of 6° was used to suppress T_1-relaxation effects, which, if uncorrected, lead to an overestimation of fat [20]. Further, T_2*-decay was considered as a degree of freedom in the parameter extraction, and its effect on the extracted water and fat signals eliminated [24].

Accuracy [19,16] and repeatability [22] results of the used Dixon method have been published before.

2.4.2. MR examination

In all subjects an axial scan of the mid-level of the left thigh was obtained using a 3 T MRI system (MAGNETOM Skyra^{fit}, Siemens Healthcare GmbH, Erlangen, Germany). Subjects were positioned in supine position with feet first oriented towards the MRI system. Data acquisition was performed by using a flexible 18-channel body radiofrequency (RF) surface coil for signal reception. For best signal homogeneity and maximum signal to noise, the flexible surface RF coil was wrapped around the mid-level of the thigh. Imaging was repeated after the 13-week intervention period in all subjects. To examine approximately the same anatomical region, the distance of the center of the imaging field of view to the subject’s top edge of the patella was kept constant between both study visits. To prevent temporary training related changes in muscle water content, subjects were ask to avoid intensive physical activity or training 48 h prior to the MRI examination [27].

2.4.3. MR data post-processing

The central five out of 36 acquired slices of the MRI stack were selected for analysis due to the time-consuming semi-automated segmentation process. For the follow-up exam, the corresponding slices were selected manually. Veins were used as landmarks for orientation during slice selection.

For segmentation, first, the outer border of the thigh was segmented automatically in the prototype 6 pt Dixon PDFF maps using in-house software. After exclusion of femoral bone this defined the soft tissue region of interest (ST ROI). Then in the same images the fascia of the thigh muscles was contoured manually using T_1w images to support the identification of the fascia. This defined the fascia (F) ROI. The subcutaneous adipose tissue (SAT) ROI was determined by subtracting F from ST. Therefore, non-fatty components imbedded in SAT, e.g. veins were counted towards SAT. In each of the five slices the areas of the ST, F, and SAT ROIs were measured in cm^2 and average value of the five analyzed slices were determined. These are denoted as ST_A, F_A, and SAT_A, respectively

The fascia masks were used to calculate PDWF and PDFF within the F. By definition, PDFF plus PDWF add up to one. F contains pure muscle and intra and perimuscular adipose tissue. The area of pure muscle tissue was estimated as F_A multiplied by PDWF and will be referred to as "estimated muscle area" (EM_A). Finally, a "fat-muscle-ratio" (FMR), was defined as:

$$FMR = \frac{(PDFF \times F_A) + SAT_A}{ST_A}$$

Which is a measure of the total amount of fat in the thigh.

2.5. Statistical analysis

Longitudinal changes were assessed as percentage differences of follow-up versus baseline measurements. Paired t-tests in both groups were used to determine whether the changes from baseline were significant. Significance of changes between the groups was determined by unpaired t-tests.

Pearson correlation analyses between muscle function (jump height) and MRI data (PDFF, EM_A relative to ST_A (= rEM_A), SAT_A relative to ST_A (= $rSAT_A$), and FMR) were performed for absolute values at baseline and for percentage changes at follow-up. For the correlations both groups were pooled. All statistical analyses were carried out with IBM SPSS l Statistics version 23. A $p < 0.05$ was considered statistically significant. For readability significant always means statistically significant.

3. Results

All subjects were examined successfully. Baseline values are given in Table 1 as group mean value (MV) ± standard deviation (SD) with p-values of the unpaired t-tests between the groups.

Exemplary T_1w images and Dixon PDFF maps are shown in Fig. 1. The T_1w TSE sequence resulted in high-contrast, high-resolution images with a better fascia contrast than PDFF maps obtained from the Dixon sequences. In the PDFF maps, gray values correspond to a percentage fat value. Exemplary fascia segmentation masks superimposed on the PDFF maps of baseline and follow-up exam of another subject are shown in Fig. 2.

Percentage changes after the 13-week study period are shown in Table 2. In G_t significant changes ($p < 0.05$) between baseline and follow-up exams were observed for jump height, F_A, PDWF, PDFF, EM_A, and FMR. PDFF decreased, the other parameters increased. In G_c changes were not significant. Between baseline and follow-up visit, changes were significantly larger in G_t than in G_c for jump height, PDWF, PDFF, EM_A, and FMR.

Correlation coefficients R between muscle function (jump height) and MRI parameters are given in Table 3. Correlations were independently calculated for absolute values determined at baseline and for %changes measured after 13 weeks. At baseline, a moderate cross-sectional correlation was measured between muscle function and PDFF and rEM_A (R = -0.43 and R = 0.64, respectively). For these parameters the longitudinal correlation was low to moderate (R = -0.51 for PDFF and R = 0.14 for rEM_A). Combining fat and muscle area resulted in an increased correlation for FMR (R = -0.40 and R = -0.55 in the cross-sectional and longitudinal analysis, respectively).

Table 1
Baseline data of the training G_t and the control G_c group. Jump height was measured by a mechanograph. and fascia (F_A), estimated muscle (EM_A), subcutaneous fat (SAT_A) and soft tissue (ST_A) areas, and fat-muscle-ratio (FMR) (PDFF * F_A) + SAT_A) / ST_A), proton density water fraction (PDWF), and proton density fat fraction (PDFF) were measured by 6-point (6 pt) Dixon magnetic resonance imaging (MRI). Statistically significant group differences are printed in bold.

	Group G_t (n = 11)	Group G_c (n = 10)	p-value
Age [years]	28 ± 3	31 ± 6	p > 0.1
Weight [kg]	78.8 ± 9.0	75.8 ± 6.0	p > 0.3
Height [cm]	184 ± 7	183 ± 5	p > 0.8
Jump height [cm]	44 ± 6	51 ± 6	p < 0.05
ST_A [cm^2]	217 ± 24	202 ± 28	p > 0.3
F_A [cm^2]	179 ± 19	180 ± 23	p > 0.2
SAT_A [cm^2]	38 ± 12	22 ± 11	p < 0.05
FMR	7.0 ± 2.0	5.1 ± 0.9	p < 0.05
PDWF [%]	92.0 ± 2.6	94.5 ± 1.1	p < 0.05
PDFF [%]	8.0 ± 2.6	5.5 ± 1.1	p < 0.05
EM_A [cm^2]	161 ± 19	169 ± 25	p > 0.2

4. Discussion

This pilot study of a small number of young and healthy subjects confirmed that MRI can be used to quantify longitudinal changes of adipose tissue of the thigh induced by a physical training during a 13-week leg-focused intervention period. The effectiveness of the intervention was validated by a muscle function test. Related changes in muscle structure and muscle fat content were assessed with an advanced 6 pt Dixon prototype MRI technique. A control group was included to ensure that changes were training related.

As the focus of the present study was to evaluate the feasibility of Dixon MRI to measure changes in muscle parameters in training studies, an exercise intervention that from experience was expected to be effective was used. Therefore, only young and healthy subjects without severe medical conditions were included in the study and no randomization was performed. Participants selected one of the two groups by choosing their training intensity, frequency, and volume. All of them had experience with strength training prior to study enrollment. Future studies will target patients with muscle diseases and elderly persons with sarcopenia in which training effects on muscle may be small. Therefore, an intervention period of only 13 weeks was chosen in the present study and all participants had performed regular physical training prior to study enrollment. In both groups, the scope and form of physical training were controlled by a detailed training log.

The study design, the missing randomization and the small numbers of subjects probably explains the observed differences at baseline (Table 1). Moreover, the training group G_t might have had a slightly lower fitness level than the control group G_c. However, the important outcome was that the training increased muscle function and decreased PDFF, while there were no changes in the control group. Thus, the primary aim to show a relation between muscle function and MRI related outcome parameters under exercise was achieved.

Although the group was small, significant training effects were measured in G_t for all parameters except for ST_A and SAT_A (Table 2). Moreover, longitudinal changes of ST_A, F_A and SAT_A, did not differ

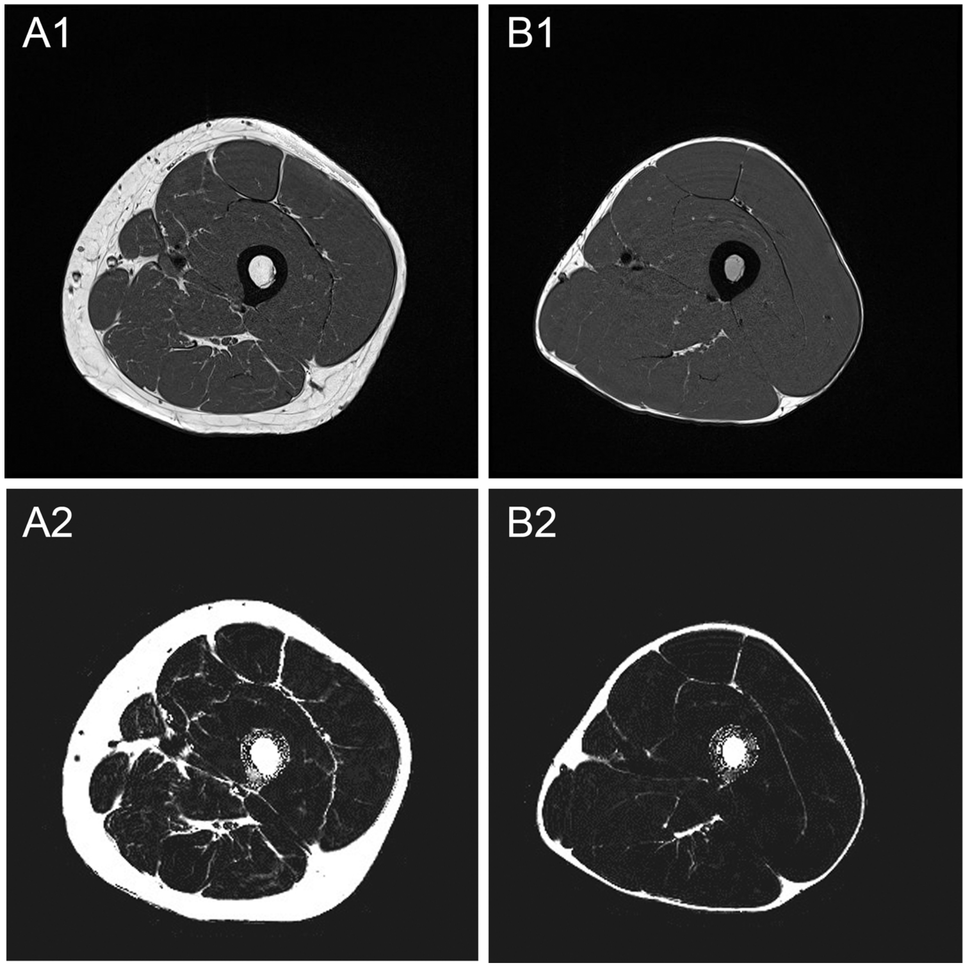

Fig. 1. Exemplary T_1w TSE images (A1 and B1) and Dixon proton density fat fraction (PDFF) maps (A2 and B2) of two subjects (28 years (A) and 29 years (B)) demonstrating the variation of muscle, and of subcutaneous, perimuscular, and intramuscular adipose tissue even in healthy young subjects. Subject A: Jump height: 36 cm, fascia area (FA): 188 cm^2, PDFF: 10.5%, estimated muscle area (EM_A): 168 cm^2, subcutaneous adipose tissue area (SAT_A): 59 cm^2, fat-muscle- ratio (FMR = (PDFF * F_A + SAT_A) / ST_A)): 8.3. Subject B: Jump height: 59 cm, F_A: 177 cm^2, PDFF: 3.6%, EM_A: 171 cm^2, SAT_A: 89 cm^2, FMR: 3.5.

between training and control groups. Therefore, pure muscle tissue area (EM_A) and changes in water and fat composition within the fascia may be better indicators for measuring training effects than the integral fat volume or area, which is dominated by SAT_A. This amplifies the importance of measuring parameters like muscle volume, muscle area or PDFF to evaluate training effects.

Fig. 2 shows that in-plane positions of the leg differed between both exams. This occurred in all subjects and was expected as muscle is a flexible tissue. The soft tissue distribution shown in a single slice depends on the leg rotation during the MRI exam. To minimize this effect, the position of the leg was as straight as possible. The three-dimensional analysis compensates this effect. Precision of Dixon MRI to measure muscle PDFF in the thigh has shown to be high [22] and ST_A was constant in both groups (Table 2). Legs were positioned flat on the scanner table, potentially increasing the degree of muscle deformation. Elevated and cushion-supported knees might have prevented deformation changes between measurements and could potentially increase repeatability in the follow-up exam. However, a pre-investigation showed that such a position was less endurable for most subjects and, caused more movement artifacts. Thus, an elevated knee positioning was not used.

Correlations between muscle function as measured by jumping height and MRI parameters were moderate. As expected for most parameters correlations at baseline were higher than correlations based on %change after the 13-week study (Table 3). Muscle performance is a complex interaction of muscle volume, muscle structure, intramuscular, and intermuscular coordination and paracrine communication of neighboring tissues. Interestingly, changes in PDFF and FMR also showed moderate correlations with change in jump height again emphasizing the importance of adipose tissue within the fascia. The inclusion of PDFF data from single muscles might be useful as intramuscular fat might have a larger effect on physical performance than perimuscular fat. Further, it is noteworthy that the rEM_A was correlated to performance at baseline but its changes after training were not correlated to an improved performance. It seems that loosing fat was more important to improve muscle performance than gaining muscle. This was also supported by the results in Table 2, which showed that in the training group the decrease of PDFF was at least numerically higher than the increase in EM_A. Obviously, these observations, must be confirmed in larger samples.

While changes of physical performance with training have not been correlated with changes in Dixon MRI muscle measurements yet, Manini et al. [11] examined negative effects of short time disuse and reported a 14.5% increase in muscular fat of the thigh and a 20% increase in muscular fat of the calf after 4 weeks of lower-limb suspension as measured in T_1w MRI images in young (19–28 years) subjects. These numbers are in the same range as the 16.3% decrease in PDFF in G_t. A few other studies investigated the correlation between MRI muscle volume and muscle fat measurements with physical performance. Yang et al. [28] showed that intramuscular fat correlated significantly with physical performance as determined by short physical performance battery, chair stand, knee extension, and gait speed (R_F = -0.16 to

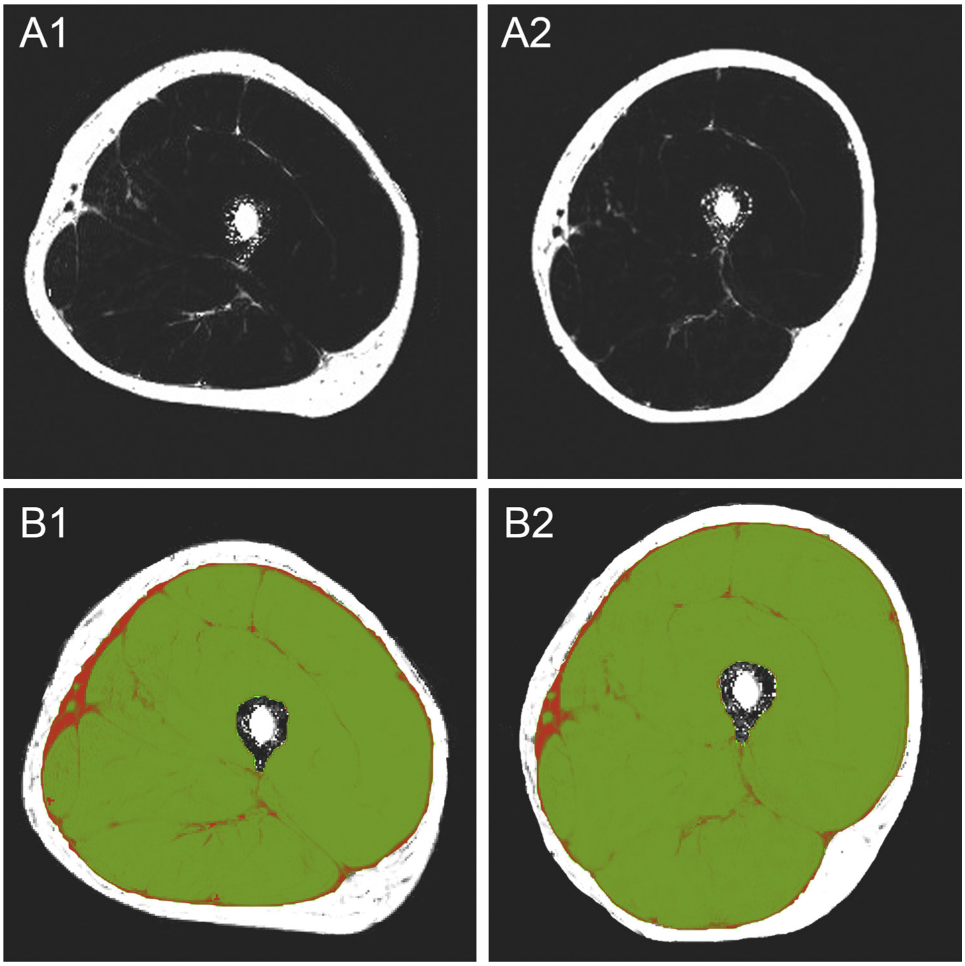

Fig. 2. Exemplary baseline (A1, B1) and follow-up (A2, B2) 6 pt Dixon proton density fat fraction (PDFF) maps of a 27-year-old subject of the training group G_t. B1 and B2: PDFF images with superimposed fascia ROIs. Inside the fascia ROIs, pixels were colored (red: 100% fat, green: 100% water, combinations of red and green: pixels containing fat-water-mixtures). 'Deformations' of the leg between visits are frequently seen on individual slices as shown here, but they are compensated by the three-dimensional analysis. In this subject a decrease of 26.2% in PDFF (from 4.2%–3.1%) and an increase in estimated muscle area of 12.8% (from 151 cm^2 to 170 cm^2) was measured.

Table 2
Percentage change (mean value ± standard deviation (MV ± SD)) after 13 weeks. For G_t, significant changes from baseline are printed in bold (paired t-test). For G_c significant changes from baseline were not observed. The p-value column indicates statistical significance of difference of change between the two groups (unpaired t-tests).

	Percentage change [%] (MV ± SD)		
Parameter	Group G_t (n = 11)	Group G_c (n = 10)	p-value
Muscle function			
Jump height	10.1 ± 4.5	2.7 ± 5.1	0.002
MRI			
ST_A	1.9 ± 4.9	1.8 ± 4.6	0.99
F_A	5.5 ± 4.0	2.9 ± 5.7	0.2
SAT_A	−14.7 ± 6.4	−7.5 ± 17.0	0.3
FMR	−17.7 ± 9.5	3.4 ± 6.1	0.00001
PDWF	1.5 ± 1.5	−0.3 ± 0.4	0.002
PDFF	−16.3 ± 10.4	5.4 ± 6.9	0.00002
EM_A	7.1 ± 3.3	2.5 ± 5.6	0.04

MRI: magnetic resonance imaging, F_A: fascia, EM_A: estimated muscle (F_A • PDWF), ST_A: soft tissue, SAT_A: subcutaneous fat areas, FMR: fat-muscle-ratio ((PDFF * F_A) + SAT_A) / ST_A), PDWF: proton density water fraction, PDFF: proton density fat fraction.

-0.21), but found only a weak correlation of knee extension with muscle volume (R_V = 0.34). Robles et al. [8] found a strong inverse correlation between intramuscular fat infiltration, muscle peak torque, and walking distance (R_F = -0.6 to -0.8) as opposed to fair-to-moderate correlations between muscle volume and the same outcomes (R_V = 0.4 to 0.6). Yoshida et al. [9] found that intramuscular AT and central activation ratio of the knee extensors were inversely related in older adults (R_F = −0.51). Tuttle et al. [7] combined muscle volume and muscle fat in one parameter and showed that fat per muscle volume was inversely related to physical performance (R_{FAR} = -0.30 to -0.48). These results compare well with the correlations at baseline shown in Table 3.

Table 3
Correlation coefficients R of baseline (BL) data and percentage changes (% change) between muscle function (jump height) and magnetic resonance imaging (MRI) data.

Correlation coefficients R		
	BL	%changes
Jump height vs. MRI PDFF	−0.43	−0.51
Jump height vs. MRI rEM_A	0.64	0.14
Jump height vs. MRI $rSAT_A$	−0.63	0.04
Jump height vs. MRI FMR	−0.40	−0.55

PDFF: proton density fat fraction, rEM_A: estimated muscle relative to soft tissue area, $rSAT_A$: subcutaneous adipose tissue relative to soft tissue area, FMR: fat-muscle-ratio ((PDFF * F_A) + SAT_A) / ST_A)).

Limitations of the present study were mostly image-processing-related. As largely manual slice-based segmentation was applied, only five of the acquired 36 slices were used for the analysis. More importantly, only the fascia but not the muscles were segmented, thus intramuscular adipose tissue was not determined separately. From a medical perspective, subcutaneous, perimuscular, and intramuscular AT should be assessed separately [29,1,4,30,31,6,7,2], because intramuscular fat is known to have the strongest effect on and association with decreasing functional performance and muscle function [8,32].

Furthermore, intramyocellular lipids have not been separated from PDFF. Intramyocellular lipids may increase with training as a result of an improved muscle energy storage and have a positive effect on physical muscle performance [33]. Therefore, training might increase the PDFF content to a minor amount. Although intramyocellular lipids cannot be resolved with MRI, a threshold-based image-based analysis could be used to separate intramyocellular lipids from the other intrafascia fat compartment. Moreover, physical training may lead to an increase of muscle water content. Consequently, fat fraction would decrease although absolute fat content would be constant. Reported training related changes in muscle water content were small, causing artificial changes in fat fraction by less than 1% [27]. In the present study, subjects were asked to avoid intensive physical activity 48 h prior to the MRI examination to prevent temporary training-related changes in water content. Furthermore, the physical training was not standardized. The focus of the study was not to evaluate a specific training method but to demonstrate that 6 pt Dixon MRI can be used to measure effects of validated training methods. In order to keep the training motivation as high as possible, participants could individually choose among different hypertrophic strength exercises.

The fat modeling of the Dixon sequences was based on the spectrum of liver but not of muscle fat. Fat quantification was first introduced into clinical routine for liver examinations. In our study, we used an established fat model that originated from a liver application. However, the fat variation throughout the body is not expected to influence fat quantification significantly. In fact, fat models from other body regions and field strengths are often used for the liver [34]. Nevertheless, the adaption of the fat models to the fat spectrum of a particular organ may further improve PDFF accuracy, for example of skeletal muscle.

In conclusion, longitudinal changes in muscle area and PDFF determined by 6 pt Dixon MRI correlated moderately with physical performance in young and healthy volunteers and can serve as an indicator of training effects. Due to the complex interaction of muscle area, muscle structure, and intra- and intermuscular coordination during muscle performance, multivariate muscle parameter models seem promising and should be investigated further to explain functional muscle data and define reliable markers to measure the effect of physical training. This holds also great promise for the diagnosis and functional characterization in pathophysiological conditions such as myopathy and sarcopenia.

Conflict of interest

Co-author Marcel D. Nickel, PhD is an employee of Siemens Healthcare GmbH, Erlangen, Germany. No further conflict of interest exists.

Acknowledgements

This work was supported by the Bayerische Forschungsstiftung (BFS) [grant no. 1044-12]. The works-in-progress 6 pt Dixon prototype sequence was provided by Siemens Healthcare GmbH, Erlangen, Germany.

We thank Stephan Kunzelmann for supporting us with the MR examination, Markus Weineck for image segmentation, and all subjects for participation.

References

[1] O. Addison, R.L. Marcus, P.C. Lastayo, A.S. Ryan, Intermuscular fat: a review of the consequences and causes, Int. J. Endocrinol. 2014 (2014) 309570.
[2] E. Zoico, A. Rossi, V. Di Francesco, A. Sepe, D. Olioso, F. Pizzini, et al., Adipose tissue infiltration in skeletal muscle of healthy elderly men: relationships with body composition, insulin resistance, and inflammation at the systemic and tissue level, J. Gerontol. 65 (2010) 295–299.
[3] L.E. Beasley, A. Koster, A.B. Newman, M.K. Javaid, L. Ferrucci, S.B. Kritchevsky, et al., Inflammation and race and gender differences in computerized tomography-measured adipose depots, Obesity 17 (2009) 1062–1069.
[4] M.J. Delmonico, T.B. Harris, M. Visser, S.W. Park, M.B. Conroy, P. Velasquez-Mieyer, et al., Longitudinal study of muscle strength, quality, and adipose tissue infiltration, Am. J. Clin. Nutr. 90 (2009) 1579–1585.
[5] B.H. Goodpaster, C.L. Carlson, M. Visser, D.E. Kelly, A. Scherzinger, T.B. Harris, et al., Attenuation of skeletal muscle and strength in the elderly: the health ABC study, J. Appl. Physiol. 90 (2001) 2157–2165.
[6] L.A. Schaap, S.M.F. Pluijm, D.J.H. Deeg, M. Visser, Inflammatory markers and loss of muscle mass (sarcopenia) and strength, Am. J. Med. 119 (2006) 526 e9-17.
[7] L.J. Tuttle, D.R. Sinacore, M.J. Mueller, Intermuscular adipose tissue is muscle specific and associated with poor functional performance, J. Aging Res. 2012 (2012) 172957.
[8] P.G. Robles, M.S. Sussman, A. Naraghi, D. Brooks, R.S. Goldstein, L.M. White, S. Mathur, Intramuscular fat infiltration contributes to impaired muscle function in COPD, Med. Sci. Sports Exerc. 47 (2015) 1334–1341.
[9] Y. Yoshida, R.L. Marcus, P.C. Lastayo, Intramuscular adipose tissue and central activation in older adults, Muscle Nerve 46 (2012) 813–816.
[10] A. Faria, R. Gabriel, J. Abrantes, R. Bras, H. Moreira, Triceps-surae musculotendinous stiffness: relative differences between obese and non-obese postmenopausal women, Clin. Biomech. 24 (2009) 866–871.
[11] T.M. Manini, B.C. Clark, M.A. Nalls, B.H. Goodpaster, L.L. Ploutz-Snyder, T.B. Harris, Reduced physical activity increases intermuscular adipose tissue in healthy young adults, Am. J. Clin. Nutr. 85 (2007) 377–385.
[12] T.W. Dixon, Simple proton spectroscopic imaging, Radiology 153 (1984) 189–194.
[13] J. Burakiewicz, C.D.J. Sinclair, D. Fischer, G.A. Walter, H.E. Kan, K.G. Hollingsworth, Quantifying fat replacement of muscle by quantitative MRI in muscular dystrophy, J. Neurol. 264 (2017) 2053–2067.
[14] S. Lovitt, S.L. Moore, F.A. Marden, The use of MRI in the evaluation of myopathy, Clin. Neurophysiol. 117 (2006) 486–495.
[15] A.J. Cruz-Jentoft, J.P. Baeyens, J.M. Bauer, Y. Boirie, T. Cederholm, F. Landi, et al., Sarcopenia: european consensus on definition and diagnosis: report of the European working group on sarcopenia in older people, Age Aging 39 (2010) 412–423.
[16] A. Grimm, H. Meyer, M. Nittka, M.D. Nickel, E. Raithel, O. Chaudry, et al., Evaluation of 2-point, 3-point, and 6-point Dixon magnetic resonance imaging with flexible echo timing for muscle fat quantification, Eur. J. Radiol. 103 (2018) 57–64.
[17] Y.H. Yoo, H.-S. Kim, Y.H. Lee, C.-S. Yoon, M.Y. Paek, H. Yoo, et al., Comparison of multi-echo Dixon methods with volume interpolated breath-hold gradient echo magnetic resonance imaging in fat-sgnal fraction quantification of paravertebral muscle, Korean J. Radiol. 16 (2015) 1086–1095.
[18] M.A. Fischer, C.W.A. Pfirrmann, N. Espinosa, D.A. Raptis, F.M. Buck, Dixon-based MRI for assessment of muscle-fat content in phantoms, healthy volunteers and patients with achillodynia: comparison to visual assessment of calf muscle quality, Eur. Radiol. 24 (2014) 1366–1375.
[19] A. Grimm, H. Meyer, M. Nittka, M.D. Nickel, E. Raithel, O. Chaudry, et al., A comparison between 6-point Dixon MRI and MR spectroscopy to quantify muscle fat in the thigh of subjects with sarcopenia, J. Frailty Aging 8 (2019) 21–26.
[20] J.J. Noble, S.F. Keevil, J. Totman, G.D. Charles-Edwards, In vitro and in vivo comparison of two-, three- and four-point Dixon techniques for clinical intramuscular fat quantification at 3 T, Br. J. Radiol. 87 (2014) 20130761.
[21] K. Sofue, A. Mileto, B.M. Dale, X. Zhong, Bashir, Interexamination repeatability and spatial heterogeneity of liver iron and fat quantification using MRI-based multistep adaptive fitting algorithm, J. Magn. Reson. Imaging 42 (2015) 1281–1290.
[22] A. Grimm, H. Meyer, M. Nittka, M.D. Nickel, E. Raithel, O. Chaudry, et al., Repeatability of Dixon MRI and MRS for quantitative muscle fat assessments in the thigh, J. Cachexia Sarcopenia Muscle 9 (2018) 1093–1100.
[23] S.J. Cormack, R.U. Newton, M.R. McGuigan, T.L.A. Doyle, Reliability of measures obtained during single and repeated countermovement jumps, Int. J. Sports Physiol. Perform. 3 (2008) 131–144.
[24] X. Zhong, M.D. Nickel, S.A.R. Kannengiesser, B.M. Dale, B. Kiefer, M.R. Bashir, Liver fat quantification using a multi-step adaptive fitting approach with multi-echo GRE imaging, Magn. Reson. Med. 72 (2014) 1353–1365.
[25] F. Lugauer, D. Nickel, J. Wetzl, S.A.R. Kannengiesser, A. Maier, J. Hornegger, Robust spectral denoising for water-fat separation in magnetic resonance imaging, in: N. Navab, J. Hornegger, W. Wells, A. Frangi (Eds.), Medical Image Computing and Computer-Assisted Intervention – MICCAI 2015. MICCAI 2015. Lecture Notes in Computer Science, vol 9350, Springer, Cham, 2015.
[26] M.D. Nickel, S.A.R. Kannengiesser, B. Kiefer, Time-domain calibration of fat signal dephasing from multi-echo STEAM spectroscopy for multi-gradient-echo imaging-based fat quantification, Proceedings of the 23rd Annual Meeting of ISMRM, Toronto, (2015) (abstract 3658).
[27] A. Fischmann, S. Kaspar, J. Reinhardt, M. Gloor, C. Stippich, D. Fischer, Exercise might bias skeletal-muscle fat fraction calculation from Dixon images, Neuromuscul. Disord. 22 (Suppl 2) (2012) S107–10.
[28] Y.X. Yang, M.S. Chong, L. Tay, S. Yew, A. Yeo, C.H. Tan, Automated assessment of thigh composition using machine learning for Dixon magnetic resonance images, Magn Reson Mater Phy 29 (2016) 723–731.
[29] J. Machann, O.P. Bachmann, K. Brechtel, D.B. Dahl, B. Wietek, B. Klumpp, et al., Lipid content in the musculature of the lower leg assessed by fat selective MRI: intra- and interindividual differences and correlation with anthropometric and metabolic data, J. Magn. Reson. Imaging 17 (2003) 350–357.
[30] T.N. Hilton, L.J. Tuttle, K.L. Bohnert, M.J. Mueller, D.R. Sinacore, Excessive adipose tissue infiltration in skeletal muscle in individuals with obesity, diabetes mellitus, and peripheral neuropathy: association with performance and function, Phys. Ther. 88 (2008) 1336–1344.
[31] R.A. McGregor, D. Cameron-Smith, S.D. Poppitt, It is not just muscle mass: a review of muscle quality, composition and metabolism during ageing as determinants of

muscle function and mobility in later life, Longev. Healthspan 3 (2014) 9.
[32] R.L. Marcus, O. Addison, L.E. Dibble, K.B. Foreman, G. Morrell, P. Lastayo, Intramuscular adipose tissue, sarcopenia, and mobility function in older individuals, J. Aging Res. 2012 (2012) 629–637.
[33] J.J. Dubé, F. Amati, M. Stefanovic-Racic, F.G.S. Toledo, S.E. Sauers, B.H. Goodpaster, Exercise-induced alterations in intramyocellular lipids and insulin resistance: the athlete's paradox revisited, Am. J. Physiol. Endocrinol. Metab. 294 (2008) E882–8.
[34] J. Ren, I. Dimitrov, A.D. Sherry, C.R. Malloy, Composition of adipose tissue and marrow fat in humans by 1H NMR at 7 Tesla, J. Lipid Res. 49 (2008) 2055–2062.