Chapter 7

7

Summary and general discussion

7.1 Summary

The overall aim of this thesis was to combine various quantitative MR measurements and compare these combined measurements between DMD patients and healthy age-matched controls both on a cross-sectional and longitudinal level, in order to generate a better understanding of the underlying pathophysiology of the disease and ideally to determine the potential of these MRI outcome parameters for monitoring muscle tissue changes in a clinical setting. In order to achieve this aim, we assessed the effect of spatial localization, data quality and confounding effects on the quantification process for various MR outcome parameters. We used a combination of quantitative MRI and spatially resolved ^{31}P MRS to contribute to the understanding of the pathophysiology in DMD.

In **chapter 2** we explored the effect of %fat, SNR and T_2 relaxation time changes on quantitative DTI measurements in skeletal muscle of DMD patients. We showed that sufficient SNR is essential for a reliable estimation of the DTI parameters, and that in vivo measurements of % fat and mean water T_2 are necessary to assess whether detected changes in DTI parameters could be ascribed to pathophysiology or to confounding effects related to low SNR. Overall, we showed that reliable DTI measurements in skeletal muscle can be obtained in DMD patients and healthy controls, if confounding factors are accounted for.

In **chapter 3** we studied fat replacement along the proximodistal muscle axis in DMD patients by using the Dixon technique. We showed that muscle fat fractions were non-uniformly distributed along the proximodistal muscle axis, with higher fat fractions in the muscle end-regions compared to the muscle belly. In addition, we showed the importance of accurate spatial localization along the proximodistal muscle axis when quantifying muscle fat fractions. A slight shift of the slice stack along the proximodistal muscle axis resulted in a difference in mean fat fraction which was on average 1-2%, but with values measured up to 12%. This non-uniformity in fat fraction within an individual muscle has a major influence on quantitative MR measurements that are currently being considered as outcome measures in clinical trials, and highlights the need for accurate repositioning in longitudinal MR studies. In addition, these findings pointed to mechanical disruption of the membrane as one of the key factors in the pathophysiology of DMD.

Chapter 4 presented a combination of quantitative MRI and spatially-resolved (2D-CSI) ^{31}P MRS data in the leg muscles of DMD patients to determine metabolic changes and inflammation in muscles with and without fat infiltration, in order to assess if metabolic changes and inflammation vary at different stages of the disease

process. Both PDE-levels and water T_2 values were significantly increased prior to the occurrence of fat infiltration, and remained elevated in muscles with fat infiltration; whereas Pi/ATP and intracellular tissue pH only changed in muscles that showed fat infiltration. This indicates that we were able to distinguish between early and late pathophysiological changes in DMD patients. More specifically, this suggested that PDE-levels and water T2 values could not only function as early markers for muscle tissue change, but could also reflect potentially reversible pathology in more advanced stages of the disease.

In **chapter 5** we assessed the time course of changes in PDE-levels detected by ^{31}P MRS and the potential value of PDE-levels to monitor muscle tissue changes in DMD patients, using longitudinal and spatially resolved ^{31}P MRS and qMRI data of lower leg muscles that display varying levels of muscle wasting. PDE-levels were significantly higher (two-fold) compared to controls in all analyzed muscles at almost all time points, and did not change over a 2 year time period. Muscle fat fraction significantly increased between the subsequent time points in all analyzed muscles of the DMD patients. In addition, we also assessed the reproducibility of quantifying PDE-levels between two subsequent measurements and the effect of SNR on the accuracy of such quantification. We showed that PDE-levels can reliably and reproducibly be quantified with ^{31}P 2D-CSI in both high and low SNR data sets. The two-fold increase in PDE-levels compared to controls, the stabilization over a two-year time period, its detection prior to structural changes, and its high reproducibility in low and high SNR data confirm the potential of PDE-levels as a marker to monitor muscle tissue changes in DMD patients.

Chapter 6 evaluated the implementation of a fast DREAM-based B_1^+ shimming approach in a whole-body fat/water separation application at 3T. Multi-station DREAM-based B_1^+ shimming showed significantly improved data quality in the stations covering the region from the upper leg to upper body compared to a conventional single shim approach. These improvements were supported by the corresponding B_1^+ maps which showed a more precise flip angle and a more homogeneous B_1^+ field. DREAM-based B_1^+ shimming was shown to be very fast and effective compared to currently available methods, achieving the desired improvements in the transmit RF-field homogeneity, the flip angle accuracy and the image quality while accelerating the calibration process by a factor of ten. This suggests that DREAM-based B_1^+ shimming is a very promising technique for multi-station whole-body imaging applications.

7.2 General discussion

In this thesis, a wide variety of individual MR techniques have been used to assess various pathophysiological changes in healthy and diseased skeletal muscle, on both a cross-sectional and longitudinal level. However, instead of only focussing on individual techniques, this thesis aimed to combine these techniques in a multi-parametric MR approach in order to: 1) assess the effects that methodological aspects can have on quantification, 2) generate a better understanding of the underlying pathophysiology in DMD, and 3) determine the value of these MR outcome parameters to monitor muscle tissue changes. (Figure 7.1) In this chapter, the key findings of the results will be discussed and put into the context of the existing scientific literature. The first part of this chapter will focus on the effect of methodological factors such as data quality, spatial localization and confounding factors on quantification in skeletal muscle applications. The second part of the discussion will focus on how this thesis contributed to new insights in the pathophysiology of DMD, and how these insights can form the foundation for future work.

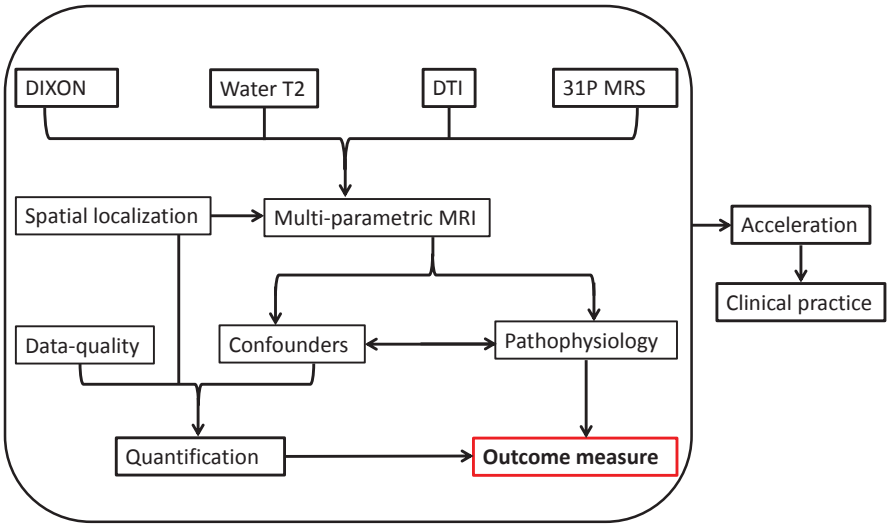

Figure 1. A schematic overview of the inter-connection of all topics in the discussion.

7.2.1 Quantification: Data-quality, Confounders and Spatial localization

In this thesis, ^{31}P 2D CSI and two advanced quantitative imaging techniques have been evaluated with regards to **data quality**, **confounding effects** and **accuracy of spatial localization between measurements,** in order to assess the effect of these factors on **quantification of MR outcome parameters** and **data interpretation**.

7.2.2 Data quality

One of the most common measures used to support a statement about data quality in quantitative MRI is the SNR, where high SNR levels are important to ensure good-quality data. SNR is usually defined as the mean signal divided by the standard deviation of the noise. Previous simulation-based studies from other groups have shown that the reliability of estimating DTI based parameters in skeletal muscle is highly sensitive to changes in SNR, muscle fat fraction and edema/inflammation-like processes. [1, 2] **Chapter 2** of this thesis evaluated the effect of SNR on the DTI parameter estimation in an in-vivo situation, and showed that high-quality data was a prerequisite for a correct DTI parameter estimation in skeletal muscle. To ensure a robust estimation of DTI-based parameters, one could try to fully exclude the effect of data quality on quantification, by acquisition of only high SNR data sets. This can be generalized by reducing spatial resolution or by averaging of more data sets. However, neither "solution" is optimal in a clinical setting, as more averaging is inextricably linked with long measurement times, and a lower resolution results in reduced specificity of the measurements. Moreover, accurate quantification of MR outcome parameters usually involves sufficient levels of SNR rather than maximizing SNR. More specifically, this means that above a certain threshold the robustness of the estimation is relatively stable. The same holds for our data; all five DTI-based parameters (three eigenvalues, MD and FA) can be estimated properly in skeletal muscle with SNR levels above 20, while MD itself can already be estimated properly with SNR levels above 15. Similar findings have been reported in other muscle applications such as STE DTI and BOLD imaging. [3, 4] The influence of data-quality on the quantification process has been evaluated in more detail in **chapter 5** of this thesis by assessing the relation between SNR and PDE-levels and showed that above a certain threshold (SNR PCr peak > 10) PDE-levels could reliably be quantified. Consequently, our recommendation is that the MR protocol needs to be optimized in order to eliminate the effect of SNR on quantification, by reaching sufficient levels to ensure a robust estimation of the desired MR outcome parameter, rather than being aimed at maximizing data quality which may take an impractical amount of scan time. Eventually, it is important that sufficient data quality is reached to quantify and interpret the results, while also taking into account the scan duration. The optimal situation will vary by technique and by application, but scan

durations that exceed 5 minutes are not desirable, especially not in young children and patients who may be more prone to scan discomfort.

7.2.3 Confounding factors

Accurate spatial co-localization in a multi-parametric MR approach, when executed properly, allows among other things the detection of potential confounding factors which can either overshadow pathology or create the impression of pathology. **Chapter 2** of this thesis evaluated the effect of %fat and water T2 on the DTI parameter estimation in an in-vivo situation, and showed that for a robust implementation of DTI measurements, additional factors such as water T_2 changes and %fat need to be measured to prevent unnecessary sources of error when interpreting the results. In addition, our work and experimental data of others showed that, besides the direct effect that %fat and water T_2 changes had on the SNR in a SE-EPI sequence, both measures could also act as independent confounders on the DTI parameter estimation.[1, 2, 5] However, the exact separate effect that these confounders had on the DTI parameter estimation was difficult to assess in our in-vivo approach. This was due to the low inflammation component of the disease and the direct inverse relation of %fat with SNR. Future work should aim at acquisition of high SNR datasets in more pronounced fatty-infiltrated muscles to evaluate the true confounding effect of fat.

The impact of confounding factors has also been shown in other quantitative MR techniques of skeletal muscle, for example Quantitative Magnetization Transfer (QMT) and BOLD imaging.[4] More specifically, both our work and that of others showed that the presence of adipose tissue (muscle fat replacement), low SNR data, and changes in T_2 (fibrosis and inflammatory processes) are common confounding factors in MR techniques used in skeletal muscle applications. However, these common confounding factors are also considered as inevitable structural changes associated with damage, disease and exercise in skeletal muscle. The precise effect that these specific confounders have on the measurement is technique-dependent. Such technique-specific knowledge is essential in order to be able to ascribe detected changes to either pathology or confounding factors, and to ensure accurate interpretation of the results. In the end, the feasibility of multi-parametric MRI in clinical practice can be questioned, but the impact that accounting for confounding factors has on data interpretation is very clear.

7.2.4 Accuracy of spatial localization between measurements

In skeletal muscle applications, repositioning between measurements is usually done by using a combination of bony landmarks, internal muscle references, and

external references such as fish oil capsules placed on the skin. [6, 7] Up to now, there is very little knowledge about the accuracy of spatial localization between measurements based on these guidelines, and more importantly the impact that slight imperfections in spatial localization can have on quantitative measures in skeletal muscle applications.

In **chapter three** of this thesis we showed that slight errors in spatial localization caused artificial differences in mean fat fraction which were on average 1-2% and ranged up to 12% in DMD patients. This effect is caused by the specific non-uniform parabolic distribution of fat replacement along the proximodistal muscle axis. However, this is not specific for DMD or for fat replacement, as in at least two other muscle diseases non-uniform distributions of muscle fat replacement along the muscle axis [8, 9] have been reported. Muscle tears and injuries are highly location specific and exercise effects are muscle- and location-dependent. Therefore, the impact that accuracy of spatial localization can have on quantification is in fact a very common problem.

However, in the end, it is only relevant to know what the impact of these imperfections in spatial localization will be on a group level basis. For example, in the case of a parabolic distribution, if the slice stack is positioned at the lowest point of the parabola, a shift in any direction will result in an artificial increase of the outcome parameter which cannot be levelled out on a group level. However, slice stacks or volumes are generally located at a specific distance from a bony landmark which does not directly match to the lowest point of the parabola in a specific muscle. As a result, parameter estimates can be under- or overestimated, and this difference could possibly be averaged out on a group level. Ultimately, the effect of these imperfections in spatial localization on quantification will be highly dependent on the shape of the distribution of tissue changes (e.g. linear curve, U-shaped curve, focal lesions, exponential curve) along the length of the muscle. Nevertheless, on a group level basis, it will at least affect the discriminative power of the technique to detect change.

The effect of these imperfections on spatial localization becomes even more complicated in longitudinal study set-ups with pediatric study population and after exercise regimes, as muscles will inevitably change between time-points. This complicates repositioning according to bony landmarks and internal references. In addition, the intrinsic parameters involved in the methodology of most imaging and spectroscopy approaches, e.g. slice gaps, slice thickness, field-of-view and restricted voxel size, could result in limited and location-specific information. Both these

methodological aspects magnify the impact that spatial localization imperfections can have on quantitative outcome measures. Therefore it is recommended to establish a protocol which can take these imperfections into account, for instance by using 3D acquisitions with full limb coverage which allows accurate offline matching of datasets in a standardized way.

7.3 Multi-parametric MRI in DMD: Insight in the pathophysiology and the value of MR outcome measures to monitor tissue changes

Quantitative MRI and MRS are important non-invasive methods to follow disease progression in DMD and are considered as promising surrogate outcome measures for clinical trials. [10] So far the main focus of the field has been on individual quantitative imaging techniques with the ability to reflect variations in the extent of fat replacement, inflammation, and metabolic changes; and to correlate these with disease progression, age and function. In addition, it is known and has been investigated extensively with qMRI, that muscles become affected at different time points and at different rates.[11-14] However, both the origin of this variation in disease progression and the pathogenic mechanism behind this degeneration is not fully clear. [15, 16] To date, only little is known about the relationship between the various pathophysiological processes and if/how those pathophysiological processes vary within individual muscles. Such knowledge could contribute to a better understanding of the underlying pathophysiology and to potential strategies aimed at preserving muscle tissue.

7.3.1 Variations within individual muscles

Chapter three of this thesis focused on variations in muscle degeneration within individual muscles and showed that muscle fat replacement was non-uniformly distributed along the proximodistal muscle axis in DMD, with higher fat fractions in the end-regions compared to the muscle belly. The detection of this specific degeneration pattern, combined with the non-uniform mechanical strain distribution along the proximodistal muscle axis in healthy muscle tissue, higher expression levels of dystrophin in the muscle end-regions and the susceptibility of dystrophin-deficient muscle to stress-induced injury, all point towards mechanical disruption of the sarcolemma as one of the key factors for muscle degeneration in DMD.[17-21] However, the specific role of the mechanical component in the degeneration process needs to be investigated in further detail. This knowledge could for example contribute to explaining the specific pattern of muscle involvement in DMD. Closely-related factors such as fiber-type composition and the amount of eccentric muscle contractions have already been associated with this specific pattern of muscle involvement. [22, 23] Future research could also include evaluation

of the stress and strain distributions within individual dystrophic muscle fibers, as well as evaluation of the change in these distributions when muscle degeneration progresses. Ultimately, it would be relevant to evaluate how this specific knowledge could contribute to potential strategies aimed at preserving muscle tissue and how it relates to functional measures.

The difference in parabolic curvature between low, intermediate and highly affected muscles, with a more prominent curvature in the intermediately affected muscles, suggests that at least at the start and at the end of the fat transformation process, the progression of the parabolic distribution cannot be homogeneous over time. It can be questioned how this progression will evolve in the intermediate stages of muscle deterioration. A recent study in the arm muscles of DMD patients suggested that muscle fat transformation evolved over time with similar rates along the proximodistal muscle axis. [24] All these findings together suggest that the muscle end-regions seem primarily affected but do not necessarily evolve more rapidly over time. However, a more detailed and specific analysis will be needed to further evaluate this degeneration process over time.

Furthermore, it would be highly interesting to evaluate whether the other pathophysiological processes, such as muscle inflammation and metabolic alterations, show the same parabolic distribution along the proximodistal muscle axis. Based on the notion that metabolic alterations and inflammation-like processes occur prior to more structural changes, and considering that muscle tissue is known to be progressively replaced with fat in DMD, it is likely that this specific distribution may only be visible in the early disease phases. As such, it could be that in order to be able to visualize this, DMD patients below the age of five have to be recruited. Acquisition of MR data sets in such a young population might not be practically feasible without sedation. In addition, this is one of the reasons, why scanning of such a young study population is not allowed by most medical ethical committees. Alternatively, it is also likely that none of the other pathophysiological changes shows a similar parabolic distribution, as the specific shape of the distribution largely depends on the progressive nature of muscle fat replacement with its well defined begin and end stage.

7.3.2 The inter relation between these pathophysiological processes.

Muscles of DMD patients are characterized by a wide variety of histological changes such as inflammation, changes in resting energy metabolism and fat infiltration. [25] However, so far, the exact time course and relation between these pathophysiological changes is not fully understood. In **chapter 4** of this thesis we showed that that it

is possible to distinguish between early and late pathophysiological processes in individual muscles. PDE-levels and water T_2 relaxation times were elevated prior to the occurrence of structural changes, and remained elevated in later disease stages. In contrast, the other metabolic indices (Pi/PCr and intracellular tissue pH) showed alterations simultaneously with the replacement of muscle tissue by fat or did not change at all (Pi/ATP and PCr/ATP). These findings are partly in line with previous work which reported elevated water T_2 relaxation times in muscles with low fat levels. [26, 27] Knowledge about this time relation is useful to define a time frame in which the trajectory of individual pathophysiological processes can be assessed in more detail. In addition, our work and that of others also showed that analysis of all these individual pathophysiological processes, reflecting multiple aspects of muscle damage, provides a much more comprehensive view of the disease state of a specific muscle. [28]

The trajectories of these metabolic indices and fat fraction have been investigated in more detail over a two-year time period in **chapter 5**. We found that none of the metabolic indices differed between the consecutive time points (Baseline, 12-months and 24-months) while muscle fat fraction did increase with time. These findings suggest that the metabolic alterations are not directly associated with the severity of muscle damage in DMD. However, the absence of this relationship may be confounded by the fact that there are both early and late metabolic alterations, as discussed before in **chapter 4**. This notion is supported by work in the arm muscles of ambulant and non-ambulant DMD patients, and by most previous cross-sectional work in older DMD boys/men not on steroid treatment. [29-34] These findings suggest that detecting a relation between metabolic alterations and disease progression is highly dependent on the disease stage in relation to the outcome parameter. Thereby it is highly likely that the relation with disease progression varies between disease phases. A longer follow-up in younger and older DMD patients should be performed to investigate the trajectories of these early and late pathophysiological processes in more detail. The wide range of muscle involvement in a specific patient together with the large variation in pathophysiological changes along the course of the disease, stress the importance of muscle specific measurements while conducting such investigations. However, it is important to consider that, while muscle-specific analyses are much more sensitive, these type of measurements are not always feasible in practice, due to hardware accessibility and time constraints. From a clinical point of view, it would be interesting to find out if some of the early pathophysiological changes have the ability to predict the course of some of the later pathophysiological changes, and ideally to relate those changes to loss of functional ability. Past studies have shown that muscle fat fraction of a single muscle or a group

of muscles, detected with DIXON imaging, has the ability to predict loss of functional abilities. [35, 36] Therefore, it could be that a combination of pathophysiological changes, as it creates a more complete view of the clinical state of a patient, in one muscle or a group of muscles, is more sensitive to predict loss of certain functional abilities or specific function milestones. Ultimately, the ability to differentiate between slow- and fast-progressing patients based on a combination of MR measurements in one or multiple muscles, would be valuable for disease-monitoring purposes but also for identifying selection criteria for therapeutic interventions. For such analysis, large patient cohorts that cross broad age ranges are required and need to be followed for a much longer time period.

Despite the fact that all findings in this thesis point towards using multi-parametric MRI to advance the understanding of the pathophysiology, it is also important to note that more detailed analysis of individual techniques/processes are still required. For instance, acquisition of high SNR spatially-resolved ^{31}P MRS data sets allows one to distinguish between glycerol 3-phosphocholine (GPC) and glycerol 3-phosphoethanolamine (GPE) in PDE, and as such allows the assessment of the origin of the elevation in PDE-levels, which contributes to a better understanding of the pathophysiology. Alternatively, other so far not yet implemented MR techniques need to be explored as they could be of value as outcome parameters. Such techniques include for example ^{23}Na MRI to assess inflammation, T1 rho mapping aimed at measuring fibrosis, Arterial Spin Labelling (ASL) to assess tissue perfusion and MR Elastography to detect differences in tissue stiffness. Subsequently, more dynamic measurements such as the recovery of PCr and blood flow after sub-maximal exercise or the change of fiber length and pennation angle due to motion, may provide insight into factors that play a role in daily life activities.

7.3.3 Potential MR outcome parameter

With the expansion of therapeutical developments in DMD [37-40], numerous studies have focused on mapping of the natural disease progression of DMD with qMRI. Until now, muscle fat fraction and water T2 measurements seem to be the most promising MR outcome parameters in DMD.[28, 41] However, since therapeutic developments at the moment aim at preserving or improving muscle tissue, an early marker with a high discriminative ability to detect changes in muscle tissue will be essential. Both water T_2 and %fat do not fulfill these criteria. [36, 42-44]

This thesis shows that PDE-levels detected with ^{31}P MRS could reflect muscle tissue changes with a high discriminative ability (there was a two-fold increase in patients compared to controls) in both the early and more advanced disease stages. Despite

the fact that the exact origin of the elevated PDE-levels in DMD is not fully clear, these changes in muscle tissue could have value as outcome parameter. However, several issues need to be investigated in more detail in order to explore this. To start with, it would be crucial to find out if in DMD patients PDE-levels detected with ^{31}P MRS are able to revert back to normal values upon therapy, as previously shown in Golden Retriever Muscular Dystrophy dogs by using adeno-associated virus (AVV) vector therapy. [45] At the moment, the only therapy available for DMD patients are corticosteroids which slows the pace of the disease progression, probably as a result of reducing the inflammatory component. [46] Although it is unclear what the effect of corticosteroids will be on metabolic pathways, it could be interesting to test the short- and long-term effects of corticosteroids on several MR outcome parameters. Subsequently, the full trajectory of PDE-levels along the course of the disease should be assessed, as it is highly valuable to know the natural progression of change, to relate potential therapeutic effects to. Lastly, it is also of interest to know how accurate and reproducible PDE-levels can be detected between measurements within a single imaging center but also across centers. Previous studies assessed the reproducibility of %fat and water T_2 measurements in both HC subjects and DMD patients within and between centers and found CV values ranging between 0.8-8%.[47] The CV values found for quantifying PDE-levels in this work, for high and lower SNR conditions, are similar to these values, and below the general acceptable level of agreement (<10%). This suggests that even in more highly affected patients (simulated by the lower SNR data-sets) one can accurately quantify PDE-levels within a center. In other words, this means that PDE-levels could be used to monitor muscle tissue changes across different stages of the disease. For future clinical studies, it is recommended to establish a standardized protocol which can be used to determine the reproducibility of quantifying PDE-levels with spatially-resolved measurements across centers.

The two-fold elevation of PDE-levels compared to controls, its detection prior to structural changes, and its high reproducibility confirm the value of PDE-levels as an MR outcome parameter to monitor muscle tissue changes. However, it can be questioned how feasible the use of spatially resolved ^{31}P MRS is to detect PDE-levels in clinical trials. To date, most hospitals have only clinical MR systems and surface coils at their disposal, which do not allow such advanced measurements. In practice, this will most likely result in surface coil localized measurements, which generate a weighted value from multiple muscles at once and might not be sensitive enough to detect subtle changes in individual muscles. With respect to therapy developments in DMD, so far it is uncertain if and to what extent there will be changes in the muscle with appropriate therapy. At the same time it is

unclear where these potential therapeutic effects will be manifested. Due to this lack of knowledge, it is too early to choose one single MR measure as an outcome parameter. Therefore it is highly recommended to use a multi-parametric approach in which the assessment of metabolic alterations, PDE-levels specifically, and a variety of other pathophysiological processes are embedded.

7.4 Future perspectives: Acceleration

This thesis provides evidence, from both a pathophysiological and methodological perspective, that it is important to use multi-parametric MRI in skeletal muscle disease. The feasibility of reaching that goal depends on finding an appropriate balance between scan duration and the necessary quality of the data. Acceleration of scan sequences can provide freedom in either increasing variety or quality**. Chapter 6** of this thesis showed that implementation of a fast B_1^+ shimming technique, as applied to a whole-body fat-water separation approach, improved data quality without loss of essential scan time. These mapping approaches do not have any direct diagnostic value but do directly influence data quality. Over the past decade, other approaches aimed at acceleration of diagnostic scans, such as parallel imaging, compressed sensing, interleaving and integrating of sequences, have emerged. Acceleration factors of 2-5 fold have been achieved with these various methods without loss of data quality in skeletal muscle applications. [48-50] These acceleration factors can be highly beneficial for the implementation of multi-parametric MRI, but also in exercise challenges, in which blood flow and energy metabolism very quickly return to resting state settings, and in whole body applications. Although the foundations for speeding up of acquisitions are available, they will need to be further developed to become clinically applicable.

7.5 Conclusions

This thesis showed the impact of data quality and imperfections in spatial localization on the quantification of MR outcome parameters, but more importantly, emphasized the essence of high quality multi-parametric MRI in order to be able to ascribe tissue changes to either pathology or confounding factors in skeletal muscle applications. More specifically, this thesis showed that sufficient levels of SNR resulted in a robust estimation of MR outcome parameters, and that MR protocols need to be optimized accordingly. Subsequently, confounding factors need to be measured and accounted for, in order to have truly quantitative measures that reflect pathology-related changes. In addition, this thesis showed how different MR measures can be depicted in a complementary way to describe pathophysiological processes during the course of the disease. The use of muscle-specific measurements and multi-parametric MRI both contributed to a better understanding of the disease-related changes in DMD. In the

future, multi-parametric MRI and new technical developments should complement each other in an effort to advance the understanding of the pathophysiology and to determine the value of MR outcome measures to assess muscle tissue changes. At the same time, it is important to link this comprehensive MR muscle profile to muscle function, functional abilities and functional milestones.

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