Non-invasive assessment of skeletal muscle pathology and treatment for Duchenne muscular dystrophy

K Gutpell1,3,4, L Hoffman1,2,3,4*

Abstract
Introduction
Duchenne muscular dystrophy (DMD) is a severe neuromuscular disorder caused by mutation(s) in the gene encoding dystrophin. While recent advances in palliative care have increased life expectancy to 20–30 years of age, this disease remains invariably fatal. Muscle pathology and therapeutic efficacy is primarily assessed using invasive muscle biopsies or muscle strength measurements, both of which have considerable disadvantages. The use of non-invasive imaging to monitor DMD progression and efficacy of therapeutic intervention strategies may further be used for the development of treatments for this devastating childhood disorder.

This review will highlight the use of three medical imaging technologies routinely used in clinics today that may be valuable in monitoring disease muscle pathology and therapeutic efficacy in animal models of muscular dystrophy and in DMD patients. Specifically, this review will focus on the use of various imaging modalities to assess disease progression in skeletal muscle tissue where myofiber degeneration is the hallmark pathology of DMD. Research involving magnetic resonance imaging (MRI), positron emission tomography (PET) and computed tomography (CT) will be reviewed and their potential use in future studies will be discussed.

Conclusion
While the research discussed in this review highlights the promise for the use of non-invasive imaging to monitor muscle degeneration/regeneration in DMD patients, a few key limitations must first be addressed before these techniques are implemented as a mainstay in DMD patient care. These limitations include long scan and study times that are not ideal for paediatric patients, potential health and safety concerns due to current radiation doses, inconsistent imaging protocols between imaging centres and limited access to imaging centres for some patients.

Introduction
Duchenne muscular dystrophy (DMD) is the most commonly diagnosed fatal childhood disorder, affecting approximately one in 3500 live male births. Although recent advances in palliative care have resulted in prolonged life span, this devastating disease remains invariably fatal by approximately 30 years of age. Considerable promising research is currently being conducted to treat DMD. These areas of research include viral (and other) delivery of mini or microdystrophin variants1-2, stem/progenitor cell transplantation3, exon skipping4 and analog (eg, utrophin) upregulation5. While each of these approaches offer their own potential benefits, they are all hindered by one common limitation. Currently, the typical way to assess the impact of these approaches on muscle health is by invasive and painful muscle biopsies that may themselves contribute to further disease progression. In addition to the invasive nature of muscle biopsies, information gleaned from this tissue is limited to histological (H&E, Masson’s trichrome stains), biochemical and molecular (western blotting, gene expression) analyses, that preclude any sort of longitudinal, functional assessment6. Thus, there is a critical need for ways to non-invasively assess disease progression and subsequent efficacy of therapeutic interventions7. The purpose of this review is to examine the potential use of various non-invasive imaging modalities for their use in monitoring disease progression and/or therapeutic outcome in DMD patients. Specifically, this review will highlight the benefits and limitations of magnetic resonance imaging (MRI), positron emission tomography (PET) and computed tomography (CT) as they may pertain to DMD research. While this review focuses on the use of these imaging modalities as they pertain to skeletal muscle specifically, their use for monitoring disease progression and therapeutic approaches in DMD-related cardiomyopathy is also promising and relevant to this discussion.

Magnetic resonance imaging
Magnetic resonance imaging (MRI) is an ideal imaging modality for visualising the anatomy of soft tissue within the body, thus offering many potential benefits for use in assessing muscle pathology in both animal models of DMD, DMD patients and other patients suffering from a broad spectrum of myopathies8-10. To this end,
MRI has been utilised to distinguish healthy versus dystrophic muscle in an mdx mouse model of DMD\textsuperscript{11}. Specifically, proton transverse relaxation time-weighted images or T2 images, illustrated that degenerating muscle in mdx mouse hind limbs appeared more heterogeneous on an MR image compared to hind limb musculature of a wild-type mouse, which appears largely homogeneous; higher T2 values were attained in control muscles and these regions of interest appeared “lighter” than in the degenerating muscle in mdx mice. Histologically, the “dark” areas on a T2 map were found to correspond to areas of inflammation and macrophage infiltration in dystrophic muscle lesions. Additionally, some areas on a T2 map of dystrophic hind limbs exhibited an intense signal, indicating the presence of fatty infiltrate in the mdx mouse that was not present in healthy wild-type hind limb musculature\textsuperscript{12}. Recent work by Vandenbourne and colleagues, confirms the use of T2 mapping to assess changes in skeletal muscle of the lower extremities in DMD patients aged 5–15\textsuperscript{13}. Specifically, this group confirmed that T2 values are elevated in lower limb muscles of DMD patients versus healthy, age-matched control subjects, including younger subjects between the ages of 5 and 8. Interestingly, these differences in T2 values correlated well with functional tests of muscle contractile strength tests in these individuals. Additionally, 2004 study by Amthor et al., investigated whether the use of a contrast agent could further elucidate the anatomical structures characteristic of progressive muscle dystrophy\textsuperscript{14}, since albumin is known to accumulate in damaged myofibres due to sarcosomal membrane disruption\textsuperscript{15}; the authors used human serum albumin conjugated to a paramagnetic compound, gadolinium-DPTA, to assess its accumulation in dystrophic skeletal muscle. The results of this study indicated that this contrast agent did, in fact, accumulate in the degenerating myofibres of mdx mice but not in wild-type mice. Another recent study from 2010 reported the use of T2 mapping to examine the effect of corticosteroid treatment—the current gold standard therapy to inhibit/reduce inflammation—in DMD patients relative to both untreated and healthy patients\textsuperscript{12}. While patient compliance is always a potential issue given the age of DMD patients, MR imaging may be safely repeated and confers no pain to the individual. Furthermore, MRI does not involve ionising radiation, making it an attractive tool for paediatric patient use. Taken together, these studies provide compelling evidence that MRI may be a valuable tool for providing information on global changes in muscle composition as disease progresses, with or without the use of therapeutic interventions.

### Computed tomography

X-ray computed tomography (CT) technology utilises computed-processed X-rays to produce a topographic image to produce a three-dimensional image of the internal parts of an object. Dense tissues such as bones will attenuate an X-ray more than tissues that are composed largely of water or air. CT resolutions between tissue densities allow researchers to distinguish between tissues that vary by only 1% in their densities, making it an ideal tool to image fat and muscle tissue. Given these characteristics, CT has been in use since the 1980’s, to monitor structural changes in dystrophic skeletal muscle, becoming a gold standard in assessing muscle degeneration\textsuperscript{16–18}. Specifically, a 1990 study, described the use of CT as a tool for diagnosing DMD in patients given the density patterns on CT images of neck, shoulder, back, pelvis and leg muscles in DMD versus healthy patients\textsuperscript{17}. A 1993 study by Liu et al., further used CT to measure the percent cross-sectional area (%CSA) of the hind limb muscles that were composed of muscle or fat. Out of the 71 patients tested, they were able to significantly correlate an increased %CSA of fat with decreased muscle function as DMD progressed. A recent 2013 study, however, highlights specific limitations to using CT to assess muscle involvement in DMD patients, which may explain the lack of routine clinical use. First, there are concerns with x-ray exposure to patients; repeated scans during a longitudinal study may be unsafe for paediatric patients. Additionally, the authors note that most CT methods are unable to distinguish between connective tissue and muscle, which makes it difficult to assess extent of fibrosis, another hallmark of DMD\textsuperscript{19}. Dynamic contrast-enhanced CT (DCE-CT) may address this issue by providing measurements of perfusion in skeletal muscle. Since fibrotic tissue is for the most part, inert tissue, blood flow to those areas is limited and thus may be detected via DCE-CT. The use of a radioopaque contrast, such as iodine, is also ideally suited to monitor changes in muscle ischemia that are inherent to disease progression in DMD. Evidence in support of this comes from a study utilising weakly-affected mdx mice and severely-affected mdx:utrn-/- mice; here, authors correlated inflammation/regeneration indicative of the early stages of DMD with a transient increase in muscle perfusion (blood flow and blood volume). As muscle pathology and fibrosis ensues in both dystrophic mouse models, a progressive decline in perfusion was observed\textsuperscript{20}. This study provides rationale for using DCE-CT to monitor vascular-targeted therapies for treating DMD.

### Positron emission tomography

While MRI and CT are useful for their detailed anatomical data and high resolution, PET imaging may offer more information in regards to muscle function in animal models and in DMD patients. PET involves the use of a radiosensitive compound that is delivered to the desired area of the body. Positron emission tomography (PET) is a functional imaging technique that measures the uptake and metabolism of a radiolabelled compound. PET imaging provides information about the metabolic activity of tissues and can be used to detect early changes in muscle composition, which are not easily visible on CT images. PET imaging has been used to detect changes in muscle composition in DMD patients and can be used to monitor the effects of therapeutic interventions.

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of a radio-labelled substrate that will emit positrons after injection into the body. When a positron collides with a nearby electron, this coincidence event is captured by the PET scanner, allowing for localisation of the collision. Thus, depending on the radiolabelled substrate, positrons may be imaged in various types of cells and tissues. The most widely used substrate in patients today, mainly as a standard diagnostic imaging tool for cancer is fluoride-18 labelled fluorooxyglucose (18F-FDG), a glucose analog. 18F-FDG is taken up by cells that express the GLUT-1 glucose transporter, thus allowing for visualisation of metabolically active cells. While some studies have used PET-FDG for assessing muscular dystrophy-related cardiomyopathy in patients, it has yet to be assessed in significant studies of skeletal muscle metabolism in DMD patients. There is strong evidence, however, that PET-FDG will be a useful tool for monitoring degeneration in DMD patients since it is a direct indicator of skeletal muscle glucose metabolism.[2,21]. Areas of myofibre necrosis and subsequent fibrosis can be detected since fibrotic tissue is not metabolically active and thus should not accumulate 18F-FDG. As evidence of this, our group has also reported the use of 18F-FDG-PET for monitoring skeletal muscle changes in dystrophic mice. In support of their parallel CT study, authors observed an early phase of inflammation and regeneration as evidenced by an increase in 18F-FDG uptake in hind limb musculature. As muscle pathology and formation of fibrotic tissue were progressed, however, degeneration of myofibres was also accompanied by a progressive decrease in FDG uptake compared to wild-type controls.20

While PET imaging may be a promising avenue for longitudinally assessing muscle degeneration and therapeutic interventions, there are a few limitations that must also be considered. A study by Yeung et al. in particular has highlighted some concerns with assessing 18F-FDG uptake in skeletal muscle containing adipose tissue; this 2003 study used retrospective patient data to demonstrate that adipose tissue represents a source of false-positive 18F-FDG uptake. This is a concern for imaging in DMD patients considering the fatty infiltration that eventually dominates the degenerated muscle tissue. Additionally, a 2006 study by Jackson et al., cited some other sources of variability in terms of measuring FDG uptake in skeletal muscle. Specifically, 12% of the 1,164 total patients scanned exhibited excessively elevated 18F-FDG uptake in neck, masseter, chest wall, forearm and lower leg muscles. These findings were due to neck strain from being on a stretcher, gum chewing, laboured breathing, reading in the waiting room and nervous foot tapping, respectively, as indicated by technician notes. Although this study was performed on patients with suspected tumours, the findings of this study shed light on some potential sources of variability in 18F-FDG uptake in skeletal muscle and therefore highlight the need for tight experimental controls and patient reporting.

### Hybrid scanners

Many of the current limitations to the use of MRI, CT and PET imaging of skeletal muscle in DMD may be addressed with the use of hybrid scanners. Hybrid PET/CT and PET/MRI scanners provide the functional metabolic information offered by 18F-FDG-PET while at the same time co-register those areas of uptake with anatomical data provided by CT or MRI.[19,20]. Since it is currently difficult to differentiate between connective and muscle tissue using MRI and CT, PET-FDG may provide further insight to metabolically active areas (muscle) versus metabolically inactive areas (fibrotic tissue). Table 1 outlines the advantages and disadvantages of the various imaging modalities to highlight their utility as standalone tools or as components of hybrid scanners.

### Table 1: A summary of the advantages and disadvantages of MRI, CT and PET for monitoring disease progression and therapeutic efficacy in animal models and patients with DMD

<table>
<thead>
<tr>
<th>Imaging Modality</th>
<th>Type of information</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRI</td>
<td>Anatomical data-adipose versus muscle tissue</td>
<td>No radiation or biological hazards reported</td>
<td>Expensive</td>
</tr>
<tr>
<td></td>
<td></td>
<td>High resolution</td>
<td>Long scan times</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Good resolution of soft tissue</td>
<td>Cannot distinguish between connective tissue and muscle</td>
</tr>
<tr>
<td>CT</td>
<td>Anatomical data-adipose versus muscle tissue</td>
<td>Relatively short scan times</td>
<td>Exposure to radiation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Most cost-effective than MRI</td>
<td></td>
</tr>
<tr>
<td>PET (18F-FDG)</td>
<td>Functional data-metabolically active tissue</td>
<td>Can image biological processes in the body</td>
<td>Exposure to radiation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>May distinguish between fibrotic and non-fibrotic areas</td>
<td>Radiotracer uptake period can lead to a long wait time</td>
</tr>
</tbody>
</table>

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Discussion

The authors have referenced some of their own studies in this review. The protocols of these studies have been approved by the relevant ethics committees related to the institution in which they were performed. Animal care was in accordance with the institutional guidelines.

The potential benefits that non-invasive imaging offers for both monitoring disease status and therapeutic outcome are numerous and may change our understanding of DMD progression and treatment in patients. From the perspective of a patient, non-invasive imaging would eliminate the need for painful muscle biopsies, which are the typical method of assessing muscle damage. Not only is this painful for the patient, but also the biopsy itself may incur further damage to already injured muscle. From a research standpoint, non-invasive imaging allows for more global analysis of what is happening in vivo, which is not offered by ex vivo histological analyses. DMD manifests as a very heterogeneous disease affecting certain muscle groups and even different areas within a muscle group differently. In fact, some muscle groups appear nearly unaffected in DMD. Depending on the tissue region selected for biopsies, this sample may drastically over or underestimate the true extent of damage in the patient. It has also been suggested that changes observed non-invasively may precede changes in physiological outcome, i.e. muscle contractile strength and endurance, which is important when considering the timing of therapeutic intervention. It should be noted, however, that non-invasive imaging is not yet able to definitively diagnose DMD in patients. While new blood test techniques for detecting specific mutations in the dystrophin gene are being developed, it would be advantageous to investigate the use of non-invasive imaging for diagnostic purposes as well.

Prior to the standard use of these imaging modalities for monitoring DMD, there are a few limitations that must be addressed. First and foremost, paediatric patient compliance will continue to be a hurdle for researchers, particularly for certain protocols that currently take up to 2 h to complete. Safety is another primary concern for the use of PET and CT in children since they both involve radiation. Radiation doses must be taken into account considering that children are at a higher risk of lifetime cancer mortalities attributable to radiation than adults. Current research is attempting to implement dose reduction techniques using various software and hardware techniques that may reduce radiation dose by 2–3 fold. Lastly, the non-invasive imaging techniques described in this review focus on their use for measuring muscle mass versus adipose tissue. As the disease manifests, however, fibrosis is perhaps as debilitating as muscle degeneration itself.

Conclusion

Current literature is unclear whether fibrosis in skeletal muscle may be non-invasively measured reliably in DMD patients since it is difficult to distinguish between fibrotic and muscle tissue using non-invasive imaging techniques. Thus, there exists a need to develop protocols to measure fibrosis using these and other imaging techniques. Regardless, the use of non-invasive imaging in standard clinical care is relatively new and is expected to increase over the coming years. Given the limitations of muscle biopsies and muscle strength tests, non-invasive imaging has the potential to shed new light on the efficacy of various therapeutic approaches and may enhance quality of life and increase life expectancy for DMD patients.

Abbreviations list

CT, Computed tomography; DMD, Duchenne muscular dystrophy; FDG, Fluorodeoxyglucose; MRI, Magnetic resonance imaging; PET, Positron emission tomography.

References