Available online at www.sciencedirect.com

ScienceDirect

Neuromuscular Disorders 24 (2014) 574–582

www.elsevier.com/locate/nmd

Assessment of intramuscular lipid and metabolites of the lower leg using magnetic resonance spectroscopy in boys with Duchenne muscular dystrophy

Donovan J. Lott [a,*], Sean C. Forbes [a], Sunita Mathur [b], Sean A. Germain [c], Claudia R. Senesac [a], H. Lee Sweeney [d], Glenn A. Walter [e], Krista Vandenborne [a]

[a] Department of Physical Therapy, University of Florida, Gainesville, FL, USA
[b] Department of Physical Therapy, University of Toronto, Toronto, Ontario, Canada
[c] Department of Anesthesiology and Perioperative Medicine, Oregon Health & Science University, Portland, OR, USA
[d] Department of Physiology, School of Medicine, University of Pennsylvania, Philadelphia, PA, USA
[e] Department of Physiology and Functional Genomics, University of Florida, Gainesville, FL, USA

Received 6 January 2014; received in revised form 24 March 2014; accepted 28 March 2014

Abstract

The purpose of this study was to use proton magnetic resonance spectroscopy to assess intramuscular lipid and metabolites of lower leg muscles in boys with Duchenne muscular dystrophy (DMD) and determine its relationship with strength and functional ability. Spectroscopic measurements were obtained from four muscles of the lower leg in 25 boys with DMD (9.2 ± 3.1 years) and 10 healthy boys (10.2 ± 2.6 years). Lipid fractions and metabolite concentrations were also determined. Muscle strength, a timed functional test, and the Modified Brooke Lower Extremity Functional Scale were also determined. Lipid fractions were higher ($p < 0.01$) for the DMD group than healthy subjects for all muscles, and lipid fraction was found to be greater in the older DMD boys. The peroneal muscle demonstrated a significant difference in lipid fraction in all DMD age groups. Lipid fractions in all muscles correlated with functional measures ($r = 0.52$–0.70, $p < 0.001$), with smaller inverse correlations with the strength measure ($r = -0.36$ to -0.56, $p < 0.05$). These findings provide quantifiable information regarding intramuscular lipid and metabolite levels of different muscles across various age groups in boys with DMD and may be used in determining the effect of interventions in future clinical trials.

Keywords: Duchenne muscular dystrophy; Lipid fraction; Metabolite; Spectroscopy

1. Introduction

Duchenne muscular dystrophy (DMD), the most common form of muscular dystrophy, is an X-linked disease that affects 1 in 3500 [1] to 6291 [2] male births. This degenerative neuromuscular disease impacts muscle tissue resulting in weakness and impaired functional abilities. Clinical manifestations of the disease, such as impaired gait, become apparent while the child is 3–5 years old [3–6]. Historically, children with DMD lose the ability to walk by 12 years of age [7]. With the use of glucocorticoid corticosteroids, most children with DMD can increase this time for ambulation [8–11]. However, even most boys taking steroids become non-ambulatory by the age of 15 years [12]. The vast majority of patients with DMD pass away by their mid to late twenties [13,14], and there is currently no cure for DMD.

* Corresponding author. Address: Department of Physical Therapy, 101 South Newell Drive, PO Box 100154, University of Florida, Gainesville, FL 32610-0154, USA. Tel.: +352 273 9226; fax: +352 273 6109.
E-mail address: djlottpt@phhp.ufl.edu (D.J. Lott).

http://dx.doi.org/10.1016/j.nmd.2014.03.013
0960-8966/

Increased lipid infiltration into skeletal muscles of boys with DMD is associated with the progressive weakness and decreased functional abilities [15–17]. Non-invasive measurements using magnetic resonance imaging (MRI) have provided important information concerning this intramuscular lipid infiltration in boys with DMD [18–25]. Some of the previous MRI approaches have primarily focused on qualitative assessments of dystrophic muscle [18–20,25]. Also, the use of Dixon MRI has been implemented to quantify lipid and water signal in the leg muscles of boys with DMD [13,26]. MRI measurements that are sensitive to changes to muscle composition and fatty tissue replacement appear to be promising biomarkers to monitor disease progression in this patient population.

In addition to MRI, proton magnetic resonance spectroscopy (^{1}H-MRS) can be utilized to provide further information pertinent to muscle tissue in boys with DMD. ^{1}H-MRS is commonly considered the gold standard for measuring lipid and major muscle metabolites. Some investigators have suggested that an alteration in prominent metabolites [the ratio of trimethyl ammonium (TMA) to total creatine (tCr)] may be linked to cell membrane changes that occur from disease progression in DMD [27–29]. Quantifying intramuscular tCr may also be clinically important in boys with DMD as creatine supplementation is commonly used and has been studied as a potential therapy with varying results in DMD [30,31].

Furthermore, ^{1}H-MRS provides a means to determine the location of lipid in skeletal muscle [32–35]. The accumulation of intramuscular lipid impacts the physiological function of muscle differently depending on where the lipid is stored. Intramyocellular lipid (IMCL) is found within skeletal muscle fibers while extramyocellular lipid (EMCL) is located outside of the muscle cell. The amount of IMCL has been shown to affect insulin resistance [36–38]) and is important in people with decreased activity levels (such as boys with DMD). ^{1}H-MRS provides a means to differentiate between IMCL and EMCL and quantify the levels of these fats.

We have previously demonstrated substantial replacement of muscle with non-contractile tissue in the thigh muscles of boys with DMD older than 10 years [39]. In the current study, we chose to examine the lower leg musculature. These muscles may have an advantage over more proximal muscles due to the slower disease progression in the lower leg muscles and therefore may be more applicable for use as an outcome measure over a greater range of ages. Torriani et al. [16] recently measured lipid fraction using ^{1}H-MRS in the soleus (Sol) and tibialis anterior (TA) muscles of a small group of boys with DMD ($n = 9$). They found greater lipid in both of these muscles relative to healthy controls. They also qualitatively noted that the peroneal (Per) muscle had the greatest degree of lipid amongst the lower leg muscles in DMD. However, the extent of lipid infiltration within the Per was not quantified. Additional investigation using ^{1}H-MRS to quantify muscle fatty infiltration in various lower leg muscles of children with DMD in a larger sample size that allows for stratifying subjects by age and disease severity should provide useful information about how this disease impacts the lower leg muscles and its potential impact on strength and walking.

Therefore, the objectives of this study were to: (1) compare intramuscular lipid using ^{1}H-MRS among four key muscles involved in the primary movements of the lower leg in boys with DMD and age-matched healthy controls, (2) examine the intramuscular lipid levels of boys with DMD in different age groups, as well as the contribution of the IMCL and EMCL levels within the lower leg muscles (3) examine metabolite concentrations (TMA and tCr), and (4) determine the association between intramuscular lipid with strength and functional ability in children with DMD and healthy children.

2. Materials and methods

2.1. Subjects

Twenty-five boys with a medical diagnosis of DMD confirmed by genetic testing (mean age 9.2 ± 3.1 years) and 10 healthy control (CON) children (mean age 10.2 ± 2.6 years) participated in the study. Demographic data for all subjects are shown in Table 1. Twenty-one of the 25 DMD subjects were still ambulatory, and all 25 were taking glucocorticoid corticosteroids at the time of testing. To examine the lipid levels at different ages for the second objective of this study, the DMD subjects were further divided into three groups based upon age: 5–7 years ($n = 10$), 8–10 years ($n = 9$), and 11 + years ($n = 6$). All aspects of this research project were approved by the Institutional Review Board of the University of Florida.

2.2. ^{1}H-MRS data acquisition

Spectroscopic measurements were obtained from the Sol, medial gastrocnemius (MG), TA, and Per muscles of the right lower leg. Measurements were performed on either a 1.5T (Signa, GE Medical Systems) or a 3.0T (Achieva, Philips Medical Systems) whole-body scanner.

Table 1
Subject demographics.

	DMD	CON
Number of subjects	25	10
Number ambulatory	21	10
Age (years)	9.2 ± 3.1	10.2 ± 2.6
BSA (m^2)	1.2 ± 0.2	1.1 ± 0.3
Modified Brooke Lower Extremity Score	2 (1–9)**	1

Values are mean ± SD except for Modified Brooke Lower Extremity Score demonstrating median and range. DMD, Duchenne muscular dystrophy group; CON, control group. BSA, body surface area.
** $p < 0.01$ significant difference between groups.