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Original Contribution

SKELETAL MUSCLE ULTRASOUND: CORRELATION BETWEEN FIBROUS TISSUE AND ECHO INTENSITY

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Abstract—In this study, we examined the correlation between muscle ultrasound and muscle structure. Echo intensity (EI) of 14 muscles of two golden retriever muscular dystrophy dogs was correlated to the percentage interstitial fibrous tissue and fat in muscle biopsy. A significant correlation between interstitial fibrous tissue and EI was found ($r = 0.87$; $p < 0.001$). The separate influence of interstitial fat on muscle EI could not be established as only little fat was present. We conclude that fibrous tissue causes increased muscle EI. The high correlation between interstitial fibrous tissue and EI makes ultrasound a reliable method to determine severity of structural muscle changes. (E-mail: s.pillen@cukz.umcn.nl) © 2009 World Federation for Ultrasound in Medicine & Biology.

Key Words: **Ultrasound, Echo intensity, Neuromuscular disorders, Duchenne muscular dystrophy, Dogs, Muscle.**

INTRODUCTION

Muscle ultrasound is an easy applicable diagnostic tool in the evaluation of neuromuscular disorders (Heckmatt et al. 1988; Pillen et al. 2007; Reimers and Kellner 1996; Walker et al. 2004). The main muscle ultrasound finding in many neuromuscular disorders is increased muscle echo intensity, which is said to be caused by the disruption of normal muscle architecture by infiltration of fat and fibrous tissue (Heckmatt et al. 1982; Reimers and Kellner 1996; Walker et al. 2004). As muscle ultrasound is used for the diagnostic evaluation of neuromuscular disorders, it is important to know the influence of different types of muscle pathology on muscle echo intensity. This knowledge can aid in the interpretation of muscle ultrasound results when used for the detection of neuromuscular disorders or determination of muscle biopsy location. Until now, only two studies have examined the correlation between muscle biopsy findings and muscle echo intensity (Reimers et al. 1993a, 1993b). A significant correlation was found between the amount of interstitial fat and muscle echo intensity. It was not possible to determine the influence of fibrous tissue alone on muscle echo intensity because the percentage fibrous tissue in the muscle specimens of these studies was either low (Reimers et al. 1993b) or strongly correlated to the amount of fat (Reimers et al. 1993a).

In the present study, we aimed to determine the influence of fibrosis on muscle echo intensity. The muscles under investigation had to contain a variable amount of fibrosis and only a small amount of interstitial fat to be able to separately assess the influence of fibrosis. We, therefore, chose to perform this study in golden retriever dogs suffering from muscular dystrophy (GRMD) (Cooper et al. 1988; Valentine et al. 1992). GRMD dogs show prominent necrosis and regeneration in muscle biopsies, with marked fiber size variation and fibrosis and, in dogs over 1 y of age, fibrosis was more prominent than fat infiltration (Valentine et al. 1990). As the muscle specimens were obtained post mortem, it was possible to examine complete muscle cross-sectional areas to avoid sampling error.

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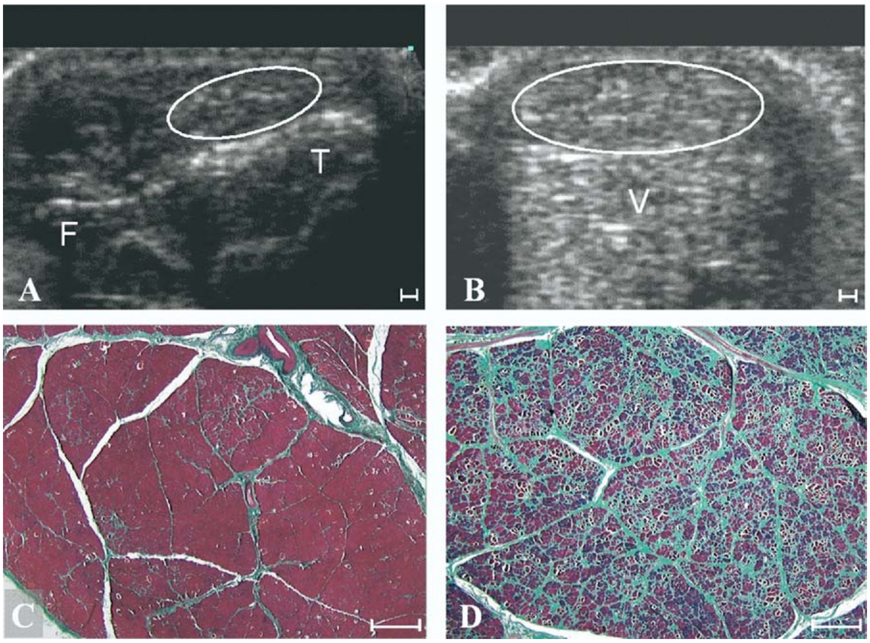

Fig. 1. Ultrasound images and muscle histology of the tibialis anterior muscle (A) and (C) and rectus femoris muscle (B) and (D). The circles in the ultrasound images represent the complete cross-sectional area of the muscle under investigation. Of this region, mean muscle echo intensity was calculated. Muscle histology was examined in the same region (C) and (D). A trichrome staining was applied to the muscle specimen. With this staining perimysial tissue becomes green, whereas muscle tissue is pink. Note the increased amount of fibrous tissue in the rectus femoris muscle which corresponds well with the increased echo intensity (whiter muscle). The bar in the right lower corner of each image represents 1 mm. T = tibia; F = fibula; V = vastus intermedius.

METHODS

Ultrasound measurements of 14 muscles were made in two GRMD dogs aged 27 (dog 1) and 20 months (dog 2) with end-stage cardiomyopathy. The dogs were born from a carrier dam that was inseminated with semen of an affected male GRMD dog. The dam and the semen were obtained from a GRMD colony at the University of Missouri. The study was approved by our institutional animal care and use committee. The following muscles were measured: rectus femoris, adductor magnus, sartorius, tibialis anterior, deltoid (each of the three parts separately), supraspinatus and trapezoid muscle.

Ultrasound measurements

We used a phased array real-time scanner (Sonos 2000 Phased Array Imaging System; Hewlett-Packard Co., Andover, MA, USA) with a 7.5 MHz transducer for dog 1 and a broadband linear 5 to 17 MHz transducer (Philips IU22, The Netherlands) for dog 2.

Despite the differences in system hardware and type of transducer, phantom measurements of both devices had shown that a linear correlation existed for echo intensities between 15 and 70 on a scale from 0 (black) to 255 (white), when measured at a depth between 0.5 and 3.5 cm. Pilot studies had shown that these muscles of GRMD dogs are located up to 2.5 cm deep and muscle echo intensities of GRMD dogs are within this range. Therefore, muscle echo intensities obtained with device 2 were comparable to device 1 after application of a correction equation ($EI_{device\ 1} = 1.38^{*}\ EI_{device\ 2} + 9.14$).

Two ultrasound images were made in the transverse plane at the location of the muscle belly (maximum muscle diameter) and at 1 cm proximal and distal of the muscle belly. Echo intensity was determined quantitatively using computer-assisted grey-scale analysis, as described previously (Scholten et al. 2003). Briefly, the grey-scale analysis was applied after selecting a region-of-interest that comprised the entire muscle without the surrounding fascia (Fig. 1). The mean echo intensity of this region was next calculated with a standard histogram function (Adobe Photoshop; Adobe systems Inc., San Jose, CA, USA) and expressed as a value between 0 (black) and 255 (white) as the ultrasound was created with 8-bit grey-scale. The echo intensity was measured in the six images taken of each muscle group and the mean was taken to reduce measurement variation.

Muscle histology

Before the ultrasound examination, the locations of the ultrasound measurements had been marked with a waterproof stylus. After muscle ultrasound, the dogs were euthanized with a pentobarbital overdose. Cross-sectional muscle specimens were collected at the exact site of the ultrasound examination. Muscle specimens were fixed in 4% formalin and embedded in paraffin. The largest cross-sectional area of each sample was selected and cut in 4 μm thick sections that were stained with Masson trichrome. The percentage perimysial and endomysial tissue (for the larger part containing connective tissue but also blood vessels and nerve bundles) were measured for each muscle using digital image analysis as previously described (van der Steen et al. 1994). Microscopic fields of vision were digitized using a RGB CCD camera, resulting in images measuring 11.7 mm^2 (specimen level pixel size 5.1 $\times$ 5.1um^2). For each section, 4 to 10 digitized images were analyzed and the mean percentage fibrous tissue was calculated. The percentage interstitial fat was determined manually in 20 to 25 systematically randomly sampled fields of vision using a $\times$10 enlargement with a specimen level field size of 3.2 mm^2.

Statistical analysis

Statistical analysis was done using the SPSS package for Windows 12.0.1 (SPSS Inc., Chicago, IL, USA). We performed Pearson's correlations and partial correlations to determine the influence of the amount of fibrous tissue and fat on muscle echo intensity.

RESULTS

Histologic examination of the muscles revealed only small amounts of fat (median 1.5%; range 0% to 11%;). The percentage fibrous tissue varied from 5% to 38% (median 13%). The second dog showed less fibrosis than the first dog, which can be explained by his younger age. Echo intensity ranged from 13 to 55 (median 23). The highest echo intensities were found in the sartorius and rectus femoris muscle, the lowest echo intensities in the deltoid and adductor magnus muscles. Examples of the ultrasound and biopsy measurements are shown in Fig. 1.

Muscle echo intensity was significantly correlated to the amount of fibrous tissue ($r = 0.87$; $p < 0.001$) and this correlation remained after correction for fat content ($r = 0.88$; $p < 0.001$) (Fig. 2). No significant correlation between fat content and echo intensity was found before ($r = -0.05$; $p = 0.82$) or after correction for fibrous tissue ($r = -0.33$; $p = 0.14$) but as only little variation in the amount of fat was present a significant correlation was not expected.

DISCUSSION

Previous studies have already shown a strong correlation between muscle biopsy findings and muscle echo intensity (Heckmatt et al. 1982; Reimers et al. 1993a, 1993b). Especially, the infiltration of fat was held responsible for an increase of reflective interfaces resulting in higher echo intensities (Reimers et al. 1993b). The present study shows that fibrous tissue also leads to increased muscle echo intensity. Both findings combined again indicate that muscle ultrasound is a reliable method to investigate changes in muscle structure.

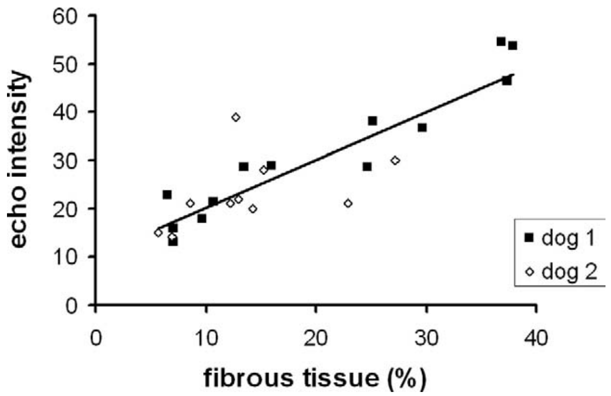

Fig. 2. This figure shows the correlation of muscle echo intensity and percentage of fibrous tissue.

The overall fibrous tissue and fat content was correlated to echo intensity but the exact location and organization of these structures was not taken into account. It is hypothesized that when these structures are smaller than the wavelength, no increase of echo intensity will be found because scattering instead of reflection of the sound beam occurs. Further studies are necessary to determine the influence of the organization and location of fat and fibrosis on the muscle ultrasound appearance.

In this study, a higher correlation between muscle structure and muscle echo intensity was found compared with previous studies (Reimers et al. 1993a, 1993b). This can be explained by the fact that the muscle specimens used in this study comprised the muscles' complete cross-sectional area, whereas previous studies were susceptible to sampling error because of the use of smaller biopsy samples. Other variables such as overlying skin and superficial fascia as well as measurement variations induced by alterations in probe position can also influence muscle echo intensity but their influence appeared to be relatively small in this study because most of the variance in echo intensity was explained by the fibrous tissue content.

As muscle echo intensity can be caused by both fibrosis and fatty infiltration it is impossible to know in an individual patient whether an abnormal muscle ultrasound has resulted from fibrosis, fatty infiltration or both, based on calculation of mean muscle echo intensity alone. To determine the exact kind of muscle pathology, other investigations such as a muscle biopsy are currently necessary.

Future types of quantitative ultrasound image analysis, such as texture analysis, might become helpful for further differentiation, as these methods not only describe the level of echo intensities but also their spatial distribution. Texture analysis has already shown to be capable of differentiating between specific groups of neuromuscular disorders (Pohle et al. 2000) but the diagnostic value of this method in the diagnostic evaluation of neuromuscular disorders is still under investigation.

We conclude that increased echo intensity is both caused by the infiltration of fat and fibrous tissue. As muscle echo intensity is strongly correlated with these structural changes, quantitative muscle ultrasound is a reliable method to detect structural muscle changes and determine the severity of muscle pathology. Muscle echo intensity can be used in the diagnostic evaluation of patients with suspected neuromuscular disorders, guiding muscle biopsy or in follow-up or intervention studies.

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