Non-invasive in vivo measurements of the soleus muscle atrophy in a rat model of muscle disuse using the ultrasound technique

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Ultrasonography is a non-invasive diagnostic imaging technique widely used in clinical setting, including investigations of skeletal muscle. With the recent development of ultrasound equipment for small animals such as rats and mice, it is now possible to extend the use of this technique to the preclinical field. However, there are only a few reports relative to its use for the evaluation of skeletal muscle structural and functional parameters in rodents. The aim of this study was to evaluate the skeletal muscle atrophy of soleus muscle in a model of disuse, the hindlimb unloaded (HU) rat, by using the ultrasound technique (Morey-Holton et al., 2005). Following HU, the postural, slow-twitch soleus muscle shows a progressive atrophy accompanied by slow-to-fast shift of fiber phenotype (Baldwin et al., 1990). Twelve adult Wistar rats (290-330 g) were divided into a control (CTRL) group and hindlimb unloaded (HU) group, and ultrasound image acquisition of soleus muscle was performed at day 0 (D0), and 7 (D7) and 14 (D14) days after suspension. Images were acquired in B-Mode by using an UBM system (Vevo 2100; VisualSonics, Toronto, ON, Canada) operating at a frequency of 40 MHz. The animals were anaesthetized with isofluorane (1.5-2%) and placed in the ventral decubitus position for the measurements of the soleus structural parameters such as pennation angle (PA) and fascicle length (FL). The soleus muscle volume was calculated by using either the conventional truncated cone method (Tomlinsonet al. 2014) or a newly-developed sinusoidal method. The later considers the soleus muscle fibres arranged between two fixed points (tendons) to form a sinusoidal spindle. In this way the soleus volume can be calculated as a solid of rotation of the sinusoidal function around the tendon-to-tendon axis. The physiological cross sectional area (PCSA) was subsequently calculated using the following equation [PCSA = soleus muscle volume (mm^3)/Lf (m)].

In line with the soleus muscle atrophy observed in previous HU studies (Desaphy et al., 2001), the ultrasound analysis demonstrates a significant reduction of soleus volume by 23.0 ± 4.4 % and 32.8 ± 4.9 % at D7 (N=6) and D14 (N=6) with respect to D0 (N=6). After normalization to body weight, the reduction was 19.9 ± 4.9 % and 35.6 ± 4.5 % at D7 and D14 with respect to D0. A satisfactory linear correlation was obtained between the soleus volume calculated from ultrasound analysis and that calculated from weight of soleus removed from both control and HU rats at D14.

The soleus muscle PCSA was significantly reduced by 12.3 ± 6.2 % and 26.8 ± 4.8 % at D7 and D14, while the Lf was significantly reduced by 8.5 ± 3. 8 % and 8.6 ± 2.5 % at D7 and D14. The PA was significantly reduced only at D14, compared to D0.

The Lf and PCSA reductions suggest that soleus atrophy involves a loss of sarcomeres both in series and in parallel. In particular, at D7, the 23.0 % reduction of soleus muscle volume was the result of a 12.3 % reduction of PCSA (sarcomeres in parallel) and a 8.5 % reduction of Lf (sarcomeres in series). At D14 days, the 32.8 % reduction of soleus muscle volume stemmed from a 26.8 % reduction of PCSA and a 8.6 % reduction of Lf.

In conclusion, this study validates ultrasonography as a powerful approach for the evaluation of rat skeletal muscle atrophy in vivo. The non-invasive ultrasound technique allows longitudinal studies in vivo, which may prove very useful for preclinical evaluation of skeletal muscle structural and functional parameters in physiopathological conditions and after therapeutic intervention. Last but not least, such technique would allow to decrease the number of animals necessary to reach statistically significant results.

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