IN VIVO ULTRASONOGRAPHIC EVALUATION OF MUSCLE FUNCTION AND STRUCTURE IN ANIMAL MODELS OF MUSCULAR DYSTROPHY AND AGING SARCOPENIA: A NEW APPROACH TO ASSESS DISEASE PROGRESSION AND PHARMACOLOGICAL EFFICACY

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Introduction: Neuromuscular disorders give rise to structural and functional muscle changes important for diagnosis and for assessing disease progression. The absence, in many cases, of specific therapies makes it necessary to improve predictability of pre-clinical studies also regarding methodology advancements. Ultrasonography has been defined as a good initial choice for assessing quantitative changes in human muscle measuring change in muscle size and presence of fat or fibrous tissue infiltrations through echodensity measures. Thanks to development of high frequencies probes, it is possible to apply ultrasound in preclinical settings obtaining more predictive data to be translated in human patients. We proved that ultrasound is an helpful method for pharmacological evaluations in two conditions affecting skeletal muscle. In particular, ultrasound highlighted the impairment of FDL muscle volume in a cis-platin induced model of cachexia and the capability of growth hormone secretagogues to prevent this effect (Conte et al., J Cachexia Sarcopenia Muscle 2017). Also, the selective heart to muscle effect of taurine in late progression of disease in mdx mice, model of Duchenne Muscular Dystrophy (Mele et al., Transl Res 2019). We presently extended our investigations to other clinically relevant assessment, i.e. the early alteration of diaphragm function in mdx mice and muscle alteration in aging sarcopenia.

Methods: Diaphragm function and echodensity were evaluated in 3-and 6-months-old mdx mice. We carried out an ultrasonographic evaluation of volume, pennation angle (PA) and fiber length (Fl) of Sol and FDL muscle in 5(adult) and 24(aging)-months-old male and female rats.

Results: Our data showed a significant decrease in diaphragm contractile amplitude of -39.3% and -36.5% in 3-and 6-month-old mdx mice, respectively. This result correlated with *ex vivo* isometric force reduction of diaphragm strips in dystrophic animals. We observed a no significant increase in mean pixel diaphragm echodensity in 3-months old mdx mice reaching a significant increase of + 27.6% at 6months of age in line with the high values of muscle TGF-beta level.
In male aged rats, we observed a reduction of FDL and Sol muscle volume of -48.5% and -39.8%, respectively. In female rats we observed a reduction of -31.6% and -22.7% in FDL and Sol, respectively. A linear correlation between ultrasonographic volumes and weight-to-density ratio of FDL and Sol muscles excised from the same animals was observed. We measured PA and Fl to calculate Physiological Cross Sectional Area (PCSA) as an index of force developed by a muscle. We observed a significant decrease of PCSA in FDL muscle of male and female rats resulting from a reduction of PA and Fl in male rats and of PA in female rats. In male rats PA and Fl reduction suggests that FDL atrophy involves a loss of parallel and in-series sarcomeres. In FDL of female rats a reduction of in parallel sarcomeres is involved. In Sol muscle, we observed a significant reduction of PCSA only in male rats.

Discussion and conclusion: Our study corroborates the usefulness of ultrasound as a non-invasive tool to monitor mdx mice dystrophic pathology progression since early stages. Pharmacological validation of this preclinical outcome measure is ongoing. We also validated ultrasound as a powerful approach for evaluation of rat skeletal muscle atrophy in different physiopathological conditions (supported by PRIN-MIUR n. 2015MJBEM2_005)