EVALUATION OF THE ERGOGENIC EFFECT OF AN AMINO ACID-BASED DIETARY SUPPLEMENT IN A MURINE MODEL OF PHYSIOLOGICAL EXERCISE

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Introduction: Branched chain amino acids (BCAAs: leucine, isoleucine, valine) account for 35% of essential AAs in skeletal muscle and exert main anabolic actions on protein synthesis. BCAAs are also known to be involved in several metabolic and physiological processes. Accordingly, BCAAs are nowadays highly recommended for athletes and for elderly people, to support muscle health and anabolism. In the present study, we assessed the possibility to maintain the effects of BCAAs exogenous supplementation on muscle function, structure and metabolism, by combining them with DFL24263, a new synthetic amino acid (AA) under current patent confidentiality, involved in the control of BCAA catabolism and protein balance.

Materials and methods: A 4-week treatment with an oral formulation containing BCAAs (2:1:1) and 33% of DFL24263(total dose: 984mg/kg, in drinking water), was performed on male C57BL/6J wild type (wt) mice undergoing a protocol of mild chronic treadmill exercise, resembling an amateur runner training. It consisted in one training session per day, 5days/week; each session lasted 45', and was composed of a 15' warm-up at 15m/min, followed by a phase of gradually increased speed (1m/min each min) until reaching a maximum velocity of 25m/min, maintained until the session ended. Treatment effects were assessed on *in vivo* and *ex vivo* readouts, in comparison to age- and sex-matched wt mice treated with vehicle (drinking water). A group of non-exercised mice was used as internal control, if necessary.

Results: After 4weeks, the formulation significantly increased *in vivo* forelimb force, measured by means of a grip strength meter (0.219± 0.009kg vs 0.187± 0.003kg, $p < 0.005$), and improved by 40% the total distance run assessed by an exhaustion test on treadmill, while there was no change in torque of hind limb plantar flexor muscles, already increased by exercise itself. In parallel, ultrasonography showed a modest reduction of hind limb volume in treated mice vs vehicle group; this was slightly in contrast with the signficant increase in mass of hind limb muscles *ex vivo*. This discrepancy could be due to a decrease of components other than muscle mass (e.g. fat tissue). Histological analysis by hematoxylin & eosin staining of tibialis anterior (TA) muscle, confirmed no structural damage induced by exercise and/or treatment. Immunohistochemistry for succinate dehydrogenase in TA, showed an increased percentage of fast-twitch fibers with respect to total fibers in treated mice. This was accompanied by a significant increase in cross sectional area (CSA) vs vehicle-treated muscles, further confirmed by immunofluorescence for laminin. These data may explain the controversial functional results, since the overall *in vivo* performance may depend on the delicate balance of myofiber composition and size. A significant increase in phosphorylated AMP-activated protein kinase (pAMPK) on total AMPK, was found by western blot in TA muscles of vehicle-treated exercised mice vs non-exercised ones, while no significant differences were found between formulation- and vehicle-treated mice. A significant reduction in interleukin-6gene expression was found by qRT-PCR in treated mice vs vehicle, likely in relation to an ability of the treatment to control a potential pro-inflammatory action of this myokine. However, genes encoding for proteins involved in muscle homeostasis, metabolism and phenotype were not modified. Finally, a trend toward decrease of creatine kinase and lactate dehydrogenase plasma levels was found in treated mice vs vehicle.

Conclusions: Our study disclosed that the administration of a dietary supplement containing BCAAs combined with DFL24263, enhances indices related to physical performance in healthy mice undergoing a physiological, non-harmful training. These results open up new scenarios on extending the application of AAs-based nutraceutical compounds to disease conditions affecting skeletal muscle, such as muscular dystrophies or muscle atrophy.