Effects of muscle atrophy induced by hindlimb suspension and chronic dexamethasone administration on metabolic pathways of skeletal muscle

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Objective: Skeletal muscle is the largest tissue in the human body and the essential organ for movement of exercise, standing posture, and elevation of body temperature. It was reduced by diabetes, hypoactivity and aging, and the resultant muscle dysfunction compromises quality of life. Therefore, the elucidation for the mechanism of muscle atrophy and muscle diseases can contribute not only to prevent the muscle disorders but also to understand the skeletal muscular hypertrophy. The purpose of the present study was to elucidate for the mechanism of muscle atrophy by comparison of the skeletal muscle metabolism between hindlimb suspention model and dexamethasone administration model.

Materials and Methods: In hindlimb suspension (HS) model, the tail of the ICR mice (8 weeks old) was suspended for 14 days and the height of suspension was adjusted in the condition that the hindlimbs were kept off the floor of the cage. In dexamethasone ad- ministration (DEX) model, the ICR mice (8 weeks old) were administered dexamethasone (20 mg/10 ml/kg, i.p.) for 14 days. The grip strength test and rotor rod test were performed to evaluate muscle strength. In the grip strength test, mice were placed on the wire netting connected with the spring scale, and the tail of each mouse was pulled backward at a steady speed until the they re- leased their hold hands from the wire netting. The measurements were conducted 5 times, and the tension (cN) at that time was measured as an index of instantaneous muscle strength. In the rotor rod test, mice were placed on the rotating rod with a rotation, and the time until the mice fall off the rod was measured as an index of muscle endurance. In addition, the soleus and gastrocne- mius muscle removed from the hindlimb were weighed wet muscle mass and were analyzed by GC/MS for metabolomic analysis. Subsequently, the significantly changed metabolites of gastrocnemius and soleus muscle were imported into MetaboAnalyst 5.0 (https://www.metaboanalyst.ca/) for pathway enrichment analysis to clarify the metabolic pathway influenced by muscle atrophy.

Results and Discussion: In the mass of soleus and gastrocnemius muscles, the significant decreases in the HS model and DEX model were observed compared with the control group. Whereas there was a greatly decrease in gastrocnemius muscle in the DEX group and in soleus muscle in the HS group. The difference was indicated that it was caused by whether the hindlimb could move freely. In the grip strength test, the maximum and average grip strength were significantly decreased in the HS model. In contrast, only for average grip strength was significantly decreased in the DEX model. In the rotor rod test, muscle endurance was significantly de- creased in both models. The muscle sample with higher decreased ratio in each atrophy model was applied to metabolome analysis. In the pathway enrichment analysis, 15 pathways in HS models and 13 pathways in DEX models were significantly influenced by muscle atrophy. In particular, we focused on the common changed pathway in both models. As a result, the skeletal muscle atrophy affected the carbohydrate metabolism including Galactose metabolism. Furthermore, the energy metabolism including Citrate cycle (TCA cycle), Pantothenate and CoA biosynthesis was altered by muscle atrophy. From these results of metabolomics, we hypothe- sized that gluconeogenesis was accelerated by breaking down skeletal muscles to supply deficient ATP.

Conclusions: In the present study, it was confirmed that hindlimb suspension and dexamethasone administration caused the reduc- tions in skeletal muscle mass, instantaneous muscle strength, and muscle endurance. Additionally, the metabolome analysis re- vealed that the skeletal muscle atrophy influenced the carbohydrate metabolism and energy metabolism. Meanwhile, the different alterations in the reduction rate of each muscle mass and influenced metabolic pathways were observed in the two models applied in the present study. The present results contribute to select the appropriate animal model in the studies of muscle atrophy.

Key words: Muscle atrophy, Metabolome analysis, Hindlimb suspension, Dexamethasone