COMPREHENSIVE ANALYSIS OF BIOLOGICAL PROCESSES OCCURRING IN ATROPHIED MUSCLES

Yuta Kawamura[1], Toshiya Hayashi[2] and Mao Nagasawa[2*]

[1]Graduate School of Agriculture, Meijo University, Japan

[2] Faculty of Agriculture, Meijo University, Japan

*Corresponding author email: mnagasawa@meijo-u.ac.jp

I. INTRODUCTION

Skeletal muscle is the largest tissue in the human body and the essential organ for exercise, standing posture, and elevation of body temperature. On the other hand, it was reduced by diabetes, hypoactivity and aging. Long-term disuse can result in a loss of 0.5~0.6% mass of skeletal muscle per day [1] and the resultant muscle dysfunction compromises quality of life, particularly older adults. Therefore, the elucidation for the mechanism of muscle atrophy is necessary to prevent the muscle disorders. Additionally, such research can contribute not only to understanding skeletal muscular hypertrophy but also to preventing the loss of the skeletal muscle mass of livestock animals which reduced activity due to the small size of the grazing land, such as in Japan.The purpose of the present study was to elucidate for the mechanism of muscle atrophy by metabolome and transcriptome analysis.

II. MATERIALS AND METHODS

In hindlimb suspension (HS) model, the tail of the ICR mice (8 weeks old) was suspended for 14 days and the hind limbs were kept just off floor of the cage with the body of the mouse at a ~30° angle from floor of cage (Figure 1). 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 released their hold hands from the wire netting (Figure 2). The tension (cN) of five trials 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 (Figure 3). In addition, the *soleus* and *gastrocnemius* muscle removed from the hindlimb were weighed wet muscle mass and were analysed by GC/MS for metabolomic analysis. Subsequently, the significantly changed metabolites of *soleus* muscle were imported into MetaboAnalyst 5.0 [2] for pathway enrichment analysis to clarify the metabolic pathway influenced by muscle atrophy. Furthermore, *soleus* was carried out of transcriptome analysis by MinIon and the data were performed enrichment analysis using ExpressAnalyst [3].

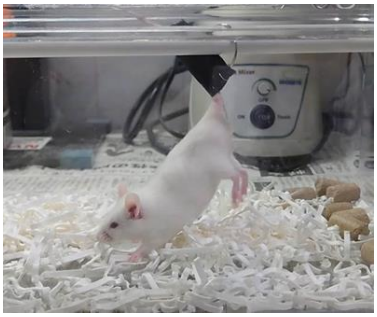

Figure 1. Hindlimb suspension

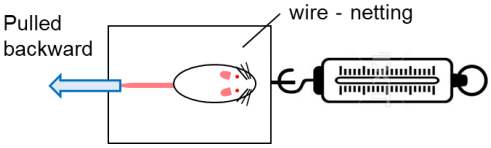

Figure 2. Grip strength test

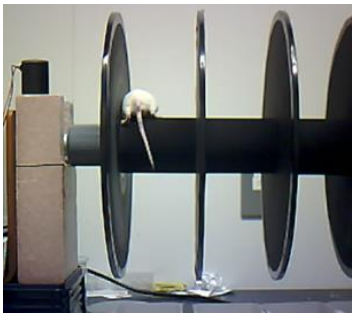

Figure 3. Rotor rod test

III. RESULTS AND DISCUSSION

In the mass of *soleus* and *gastrocnemius* muscles, the significant decreases were observed compared with the control group. Among them there was a greatly decrease in *soleus* muscle. In the grip strength test, the maximum and average grip strength were significantly decreased. Muscle endurance of HS group was significantly decreased compared with that of CON group in the rotor rod test [Figure 4].
The *soleus* muscle with higher decreased ratio was applied to metabolome analysis. In the pathway enrichment analysis, 15 pathways were significantly influenced by muscle atrophy. In particular, we focused on the Galactose metabolism, Citrate cycle (TCA cycle), Pantothenate and CoA biosynthesis. In addition, the *soleus* muscle was applied to transcriptome analysis. In the KEGG pathway enrichment analysis, there were significantly affected the 44 pathways by muscle atrophy. Out of those, we focused on Glycolysis/Gluconeogenesis, Oxidative phosphorylation, Fatty acid degradation and Peroxisome. As a result, it was confirmed that the skeletal muscle atrophy affected the carbohydrate metabolism and energy metabolism. From these results, we hypothesized that gluconeogenesis was accelerated by breaking down skeletal muscles to supply deficient ATP by mitochondrial dysfunction.

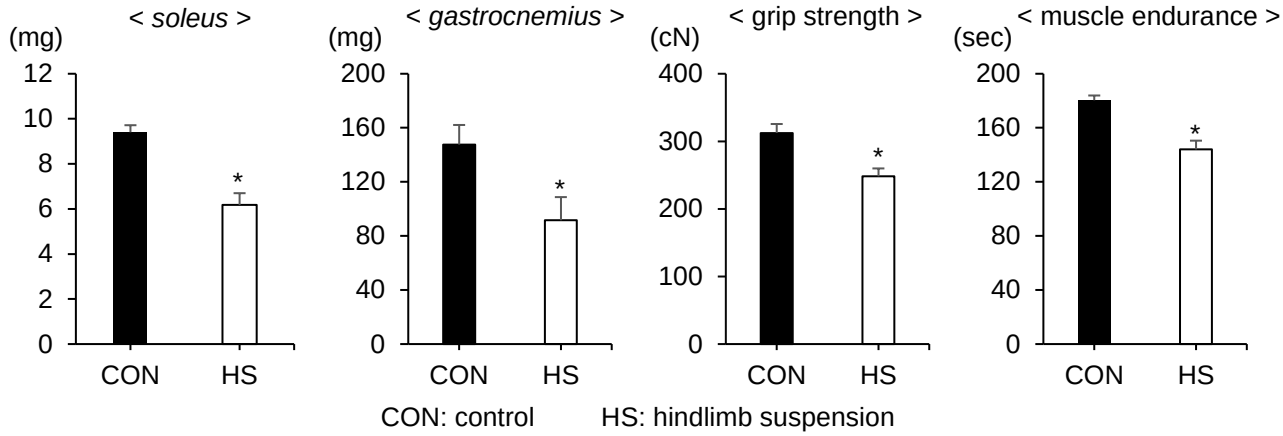

Figure 4. Biological parameters (T-Test, *P<0.05)

IV. CONCLUSION

In the present study, it was confirmed that hindlimb suspension caused the reductions in skeletal muscle mass, instantaneous muscle strength, and muscle endurance. Additionally, the metabolome and transcriptome analysis revealed that the skeletal muscle atrophy influenced the carbohydrate metabolism and energy metabolism by abnormalities in mitochondrial function. Meanwhile, it was suggested that there were differences in the extent to which the atrophy rate of each muscle site affected pathway in the present study. The present results contribute to select the appropriate animal model in the studies of muscle atrophy.

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