学位論文要約

論文題目　Exercise training increases expression of mitochondrial translation factors and CISD family
（運動トレーニングはミトコンドリア翻訳因子およびCISDファミリーの発現を増加させる）

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Mitochondrial function in skeletal muscle and white adipose tissue (WAT) is associated with aging, and the progression of type 2 diabetes (T2D) and insulin resistance. Accordingly, mitochondrial dysfunction is observed in the skeletal muscle of patients with T2D and elderly people. In WAT of obese human, mitochondrial gene expressions were also lower than these of lean human. Interestingly, overexpression of peroxisome proliferator-activated receptor gamma coactivator (PGC)-1α, a master regulator of mitochondrial biogenesis, inhibited protein degradation and suppressed induction of ubiquitin ligases in skeletal muscle with disuse atrophy. Muscle-specific PGC-1α overexpression showed the increases in mitochondrial contents and insulin sensitivity. In addition, adipose tissue-specific PGC-1α knockout mice resulted in reduced the expression of mitochondrial genes and, when challenged with a high-fat diet, showed insulin resistance. Therefore, interventions that improve mitochondrial function and enhance mitochondrial biogenesis in skeletal muscle and WAT are clinically important in slowing aging and preventing T2D and insulin resistance.

Exercise increases mitochondrial biogenesis and function in skeletal muscle and WAT. It is classically well-known that exercise is a potent enhancer of mitochondrial biogenesis and functions in rodent skeletal muscle. Recently, chronic exercise also is reported to increase the mitochondrial content and activity in rodent WAT. In addition, clinical studies showed that exercise training enhanced mitochondrial function and biogenesis in skeletal muscle of healthy, T2D, and aged subjects. In parallel with mitochondrial adaptation, exercise improves insulin sensitivity in healthy and insulin-resistant human, and suppresses the progression of T2D. Indeed, aerobic training in T2D patients increased mitochondrial contents and insulin sensitivity, and these improvements were correlated. Additionally, in aging human, exercise training is reported to increase mitochondrial bio-mass in skeletal muscle. On the other hand, the molecular mechanism of exercise-induced mitochondrial biogenesis in skeletal muscle and WAT is not fully understood. As a molecular regulator of exercise-induced mitochondrial adaptations in skeletal muscle and WAT, PGC-1α has been the most studied. Sequentially, several previous studies have reported that exercise upregulates the PGC-1α contents in skeletal muscle and WAT, which is concomitant with mitochondrial biogenesis. In addition, modest overexpression of PGC-1α in skeletal muscle at physiological level showed the increase in mitochondrial proteins and improvement of insulin sensitivity. On the other hand, it is noteworthy that exercise-induced muscular mitochondrial biogenesis was not diminished in muscle-specific PGC-1α knockout mice, suggesting that other molecules might also be involved in exercise-induced mitochondrial biogenesis.

In this study, we focused on mitochondrial translation factors and CDGSH iron sulfur domain-containing protein family (CISDs) as novel candidates which may regulate exercise-induced mitochondrial biogenesis. Several molecular biological studies showed both candidates were reported to regulate mitochondrial biogenesis and activity. In clinical studies, these genes were suggested to play important roles in the maintenance of mitochondrial integrity in human. On the other hand, it is unclear whether exercise training

affect the expression levels of both candidates and whether these candidates mediate exercise-induced mitochondrial adaptation. Therefore, we investigated 1) whether chronic exercise upregulates the protein expression of mitochondrial translation factors and CISDs, 2) whether the exercise-induced expressions occur concomitantly with mitochondrial biogenesis.

Study 1

Background and aims: The process of mitochondrial translation, in which mitochondrial (mt)DNA-encoded genes are translated into proteins, is crucial for mitochondrial function and biogenesis. In each phase, a series of mitochondrial translation factors is required for the synthesis of mtDNA-encoded mitochondrial proteins. Two mitochondrial initiation factors (mtIF2 and mtIF3), 3 mitochondrial elongation factors (mtEFTu, mtEFTs, and mtEFG1), 1 mitochondrial release factor (mtRF1L), and 2 mitochondrial recycling factors (mtRRF1 and mtRRF2) are mitochondrial translation factors that coordinate each translational phase. Exercise increases both nuclear DNA- and mtDNA-encoded mitochondrial proteins, resulting in mitochondrial biogenesis in skeletal muscles. Therefore, mitochondrial translation factors are likely regulated by exercise; however, it is unclear whether exercise affects mitochondrial translation factors in the skeletal muscles. We investigated whether exercise training comprehensively increases this series of mitochondrial translation factors, as well as mtDNA-encoded proteins, in the skeletal muscle.

Materials and methods: To investigate the relationship between mtDNA-encoded proteins and mitochondrial translation factors in metabolically heterogeneous hindlimb muscles, male C57BL/6J mice were sacrificed and then skeletal muscles (gastrocnemius, plantaris, soleus, extensor digitorum longus, and tibias anterior muscle) were rapidly excised. Male C57BL/6J mice were randomly assigned to either the sedentary or exercise group and housed in standard cages with or without a running wheel for 1 and 8 weeks. The morning after the last day of the running period, the mice were sacrificed; the muscle tissues were rapidly excised. The expression levels of mitochondrial translation factors and mitochondrial oxidative phosphorylation (OXPHOS) proteins in the skeletal muscles were then measured by western blotting. Equal protein loading in western blotting was confirmed by Coomassie Brilliant Blue (CBB) staining.

Results: The expression of mitochondrial OXPHOS proteins and mitochondrial translation factors in red oxidative muscles, particularly in the soleus muscle, was higher than that in white glycolytic muscles. We found significant correlations between the expression levels of all mitochondrial translation factors and mtDNA-encoded protein, mitochondrial encoded cytochrome c oxidase I (MTCO1), in metabolically heterogeneous muscles. Exercise training for 1 and 8 weeks upregulated the expression levels of mitochondrial translation factors and mitochondrial OXPHOS proteins in the plantaris muscle. Moreover, these exercise-induced adaptation of mitochondrial translation factors was strongly correlated with MTCO1. However, in the soleus muscle, these comprehensive upregulations were not detected.

Conclusion: These results indicate that exercise-induced mitochondrial biogenesis coincides with the upregulation of mitochondrial translation factors.

Study 2

Background and aims: Mitochondrial function in skeletal muscle and WAT declines with aging and the progression of T2D and insulin resistance. Although exercise increases mitochondrial biogenesis and function in both tissues, the molecular mechanisms are not fully understood. CISDs are a novel family of proteins that regulate mitochondrial activity and biogenesis. However, the relationship between exercise and CISD

expression is unclear. We addressed this in the present study by examining changes in the expression of CISDs and mitochondrial proteins in skeletal muscle and WAT of mice subjected to chronic exercise training.

Materials and methods: Male C57BL/6J mice were randomly assigned to either the sedentary or exercise group and were housed for 4 weeks in a standard cage without or with a running wheel, respectively. To distinguish between chronic and acute effects of exercise, mice also were subjected to single-day exercise intervention. The morning after the last day of exercise, mice were euthanized by cervical dislocation, and the plantaris and soleus muscles and epididymal WAT were removed. CISDs and mitochondrial OXPHOS protein levels in the plantaris and soleus muscles and epididymal WAT were evaluated by western blotting. Equal protein loading in western blotting was confirmed by CBB staining.

Results: Chronic exercise for 4 weeks increased the expression levels of CISD1 and CISD2 as well as mitochondrial OXPHOS protein in plantaris muscle and WAT but not soleus muscle. In addition, this exercise-induced adaptation of CISDs was strongly correlated with mitochondrial OXPHOS protein expression. On the other hand, one day of exercise did not significantly alter CISDs or mitochondrial OXPHOS protein expression in plantaris muscle and epididymal WAT.

Conclusion: Thus, mitochondrial biogenesis induced by chronic exercise coincides with the expression of CISDs in specific tissues, which may be critical for the maintenance of mitochondrial integrity.

In the present studies, we investigated whether chronic exercise upregulates mitochondrial translation factors and CISDs. Two studies showed that both factors are exercise-inducible, and these expression levels are correlated with those of mitochondrial proteins. Therefore, we proposed that both factors may be a critical for exercise-induced mitochondrial adaptations. Main limitation of these studies is that it did not definitively establish a causal relationship between the expression of mitochondrial translation factors or CISDs and that of mitochondrial proteins; as such, it is unclear whether mitochondrial translation factors or CISDs directly mediate exercise-induced mitochondrial biogenesis. Therefore, to elucidate the physiological roles of both factors, additional studies using different physiological models and loss- and gain-of-function approaches are needed.