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Long-term moderate exercise enhances specific proteins that constitute neurotrophin signaling pathway: A TMT-based quantitative proteomic analysis of rat plasma

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ABSTRACT

Physical exercise has been reported to increase neurotrophin in brain tissues as hippocampus as well as increased neurotrophic level peripherally in blood plasma and might have an effect on/or affect molecular processes of energy metabolism (and homeostasis). In this study, using quantitative proteomic analysis, we obtained a plasma protein profile from the rat with long-term moderate exercise. A total of 752 proteins were identified in the plasma. Among them, 54 proteins were significant up-regulated and 47 proteins were down-regulated in the plasma of exercise group compared with the control group. Bioinformatic analyses showed that these altered proteins are widely involved in multiple biological processes, molecular functions and cellular components, which connect with 11 signaling pathways. Interestingly, 5 up-regulated proteins Rap1b, PTPN11, ARHGDIA, Cdc42 and YWHAE, confirmed by Western blots, are involved in the neurotrophin signaling pathway which shows the lowest *P* value among the identified pathways. Further analyses showed that the 5 neurotrophin-signaling-pathway-related proteins participate in two important protein-protein interaction networks associated to cell survival and apoptosis, axonal development, synapse formation and plasticity. This study provides an exercise-induced plasma protein profile, suggesting that long-term exercise enhances the proteins involved in neurotrophin signaling pathway which may contribute to health benefit.

Significance: Physical activity contributes to myriad benefits on body health across the lifespan. The changes in plasma proteins after chronic moderate exercise may be used as biomarkers for health and may also play important roles in increase of cardiovascular fitness, enhancement of immune competence, prevention of obesity, decrease of risk for neurological disorders, cancer, stroke, diabetes and other metabolic disorders. Using a TMT-based proteomic method, this study identified 101 altered proteins in the plasma of rats after long-term moderate treadmill running, which may provide novel biomarkers for further investigation of the underlying mechanism of physical exercise. We confirmed that exercise enhances 5 proteins of the neurotrophin signaling pathway that may contribute to health benefits.

1. Introduction

Insufficient physical activity is known to be a major risk factor for global mortality [1]. Accumulating evidences have shown that physical exercise (PE) contributes to myriad benefits on the body across the lifespan, including increase of cardiovascular fitness, enhancement of immune competence, prevention of obesity, decrease of risk for several disorders as neurological disorders, cancer, stroke, diabetes and other metabolic disorders [2, 3]. PE alters metabolic, physiological and biomechanical processes to adapt to the requirements of motor, nervous, cardio-vascular, endocrine and respiratory systems [4, 5]. Despite the multiple and profound impacts of PE on various organ systems, the

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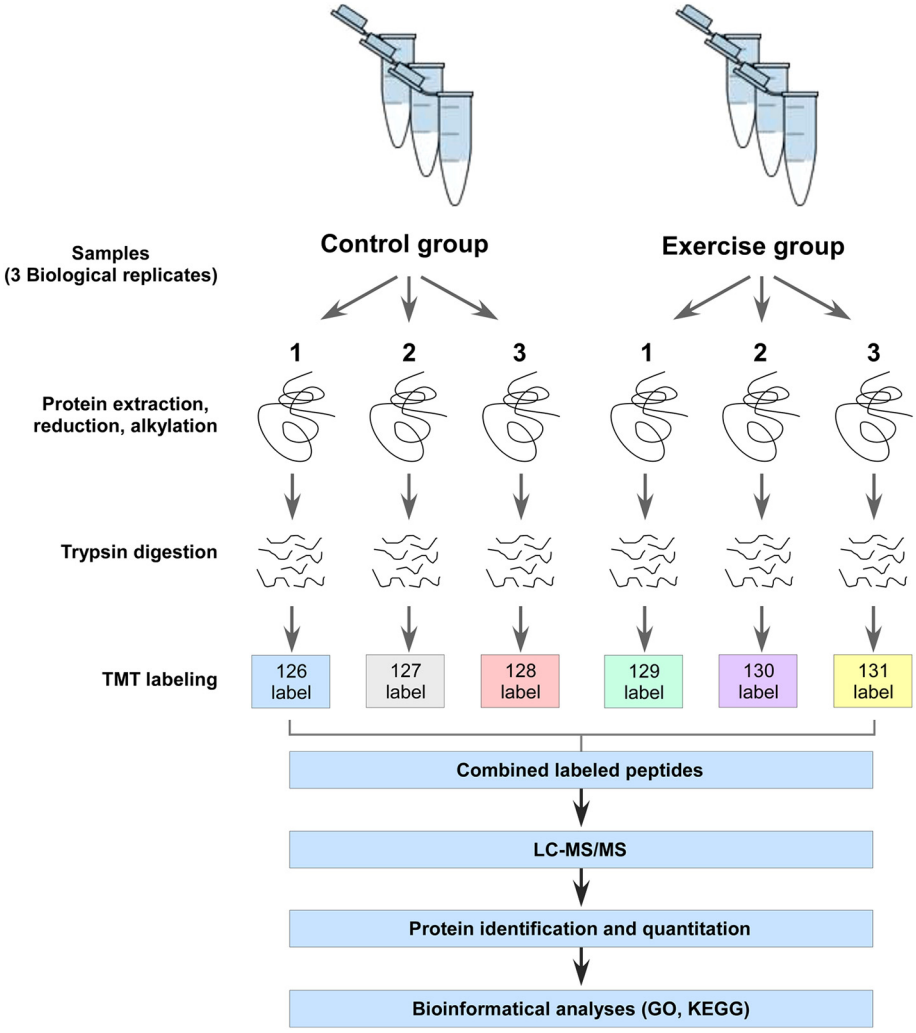

Fig. 1. An experimental workflow for protein profiling of the plasma samples from 3 exercise rats and 3 control rats, independently.

detailed and exact mechanisms of PE's actions on human health remain poorly understood.

The effect of exercise on the nervous system has drawn attention due to the protective benefits of PE on several neurological diseases such as Alzheimer's disease, Parkinson's disease, and ischemic stroke [6–8], as well as several mental disorders including affective disorders as major depression, schizophrenia, anxiety [9–12]. Chronic exercise has been shown to improve cognition, memory and learning in older age [13]. Neuroimaging studies demonstrate that regular exercise reduces age-related brain atrophy, and increase gray matter volume in brain regions including hippocampus, frontal and temporal lobes [14, 15], which are often associated with improved cognitive performance. Neurotrophin signaling pathway is involved in PE-mediated protection of neurological and mental disorders, for example, in rodent models, chronic exercise elevates the levels of several brain neurotrophins including brain-derived neurotrophic factor (BDNF), neurotrophin-3, and nerve growth factor in both central and peripheric systems [16, 17]. These exercise-induced neurotrophins enhance cell proliferation, neurogenesis, axon regeneration and outgrowth, and synaptic plasticity, which may contribute to the benefit of PE to brain health. However, the cellular and molecular mechanisms need to be further elucidated.

The exercise-induced adaptive changes of proteins constitute certain biological process, molecular functions and cellular components that are believed to contribute to the PE's benefits on health [2]. Using proteomic and other post-genomic analyses, changes of protein levels in important pathways under exercise condition have been identified from various tissues including brain, muscle and heart, and so on [18–20]. Two paralleled studies on the plasma protein profile indicate that extreme/prolonged physical exercise affects stress-related proteins and related pathways [21, 22], and another study on metabolic profiling of human plasma proteins provides metabolic signatures of exercise that may contribute to salutary effects [23]. Therefore, comprehensive study of protein changes in plasma under moderate exercise should be help for understanding the benefits of excise on health.

In the present study, we used a TMT-based quantitative proteomic method to detect the change of plasma protein profile in rats with chronic moderate exercise. We showed that 5 upregulated proteins RAS related protein 1b (Rap1b), protein tyrosine phosphatase, non-receptor type 11 (PTPN11), Rho GDP dissociation inhibitor alpha (ARHGDIA), cell division cycle 42 (Cdc42) and tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, epsilon polypeptide (YWHAE), are specific proteins of neurotrophin signaling pathway linking with cell survival and apoptosis, axonal development, synapse formation and plasticity. Thus, this study suggests a plasma proteome profile after long-term moderate exercise and a molecular pathway that may modulate the salutary effects of exercise on health benefit.

2. Materials and methods

2.1. Animals

Twenty male 8-month-old Sprague Dawley rats were obtained from