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Identification of Early Predictive Biomarkers for Exercise-induced Immunodepression by Urinary iTRAQ-proteomic Analysis

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Objective Exercise-induced immunodepression is a common medical problem in competitive sports, leading to upper respiratory tract infection, affecting sports training and sports performance, and increasing athletes' sports disease and injury risk. Finding non-invasive early predictive biomarkers of exercise-induced immunodepression and giving corresponding preventive measures is therefore an important issue in sports training. iTRAQ is an important method that currently looking for and discovering disease-specific protein biomarkers of disease prevention, diagnosis, prognosis, and efficacy monitoring. In this study, early predictive biomarkers of exercise-induced immunodepression will be identified through the iTRAQ proteomics technique, which helps to prevent the occurrence of exercise-induced immunodepression.
Methods Fifteen healthy males were recruited from the student cohort of Guangzhou Sport University. Subjects performed four-week incremental load running exercise. The weekly running load intensity was 60% VO_2max, 70% VO_2max, 80% VO_2max, 90% VO_2max respectively, 5d/w, and 1h/d. The fasting venous blood and urine of the subjects was collected in the morning before the start of the training intervention and at the end of each training week. The white blood cells of the whole blood and the levels of the lymphocyte subtypes $CD4^+$ and $CD8^+$ were tested to monitor the immune function status of the subjects. iTRAQ proteomics technology was used to test and identify differential proteins and their characteristics in urine.
Results During the four weeks of increasing running load, the subject's immune function was progressively reduced. Whole blood white blood cells, and $CD4^+$ and $CD8^+$ lymphocyte fell by more than 10% at the end of the fourth week, showing exercise-induced Immunodepression. Using iTRAQ to test urine proteomes, there were as many as 1854 proteins in the urine during the incremental loading process. The relative molecular weights of most of the proteins were between 10-80 kDa, and the isoelectric point was between 4.5 and 7. During the four weeks of incremental loading running, there were 89, 52, 77, and 148 differential proteins up-regulated, and 66, 27, 68, and 114 differential proteins downregulated respectively in the urine of each week. The differential proteins were mostly found in extracellular and plasma membranes. It is mainly involved in the in vivo biological process, the immune system process, the material transport, and is related to the positive regulatory pathways and immune regulatory pathways for stress response. The up-regulation multiples of four up-regulated proteins such as Semenogelin-1, Prolactin-inducible protein, Platelet-derived growth factor receptor-like protein, and Nucleoside diphosphate kinase increased with increasing exercise intensity. The up-regulated multiples of Glycerol-3-phosphate phosphatase, Secretogranin-1, Prosaposin, and Nephronectin (Fragment) increased with increasing exercise intensity from the second week of exercise. The down-regulation multiples of Ig kappa chain C region, Immunoglobulin lambda variable 3-21 of CUB and EGF-like domain-containing protein 2 and Uromodulin decreased further with the increase of exercise intensity from the second week of exercise, which was consistent with the change of immune function.
Conclusions Urine iTRAQ proteomics technique is an important method to identify early predictive biomarkers of exercise-induced immunodepression, which helps to prevent the occurrence of

exercise-induced immunodepression. In this study, the differential proteins in urine, such as Semenogelin-1, Prolactin-inducible protein, Platelet-derived growth factor receptor-like protein, and Nucleoside diphosphate kinase can be considered as early predictive biomarkers of exercise-induced immunodepression.