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Exploration of Potential Integrated Biomarkers for Sports Monitoring Based on Metabolic Profiling

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Objective Metabolomic analysis is extensively applied to identify sensitive and specific biomarkers capable of reflecting pathological processes and physical responses or adaptations. Exercise training leads to profound metabolic changes, manifested as detectable alterations of metabolite levels and significant perturbations of metabolic pathways in sera, urine, and rarely, in saliva. Several metabolites have been exploited as biomarkers for generally evaluating physical states in almost all sports. However, alterations of metabolic profile caused by specific sports would be heterogeneous. Thus, developments of new techniques are eagerly required to identify characteristic metabolites as unique biomarkers for specifically accessing training stimulus and sports performances. In the present work, we conducted both metabolic profiling and a binary logistic regression model (BRM) of biological fluids derived from rowing ergometer test with the following aims: 1) to examine changes of metabolite profiles and identify characteristic metabolites in the samples of sera, urine, and saliva; 2) to screen out potential integrated biomarkers for sports-specific monitoring.
Methods A total of 11 rowers (6 male, 5 female; aged 15±1 years; 4±2 years rowing training) underwent an indoor 6000m rowing ergometer test. Samples of sera, urine and saliva were collected before and immediately after the test. 1D ^{1}H NMR spectra were recorded with a Bruker Avance III 650 MHz NMR spectrometer. NMR spectra were processed and aligned, resonances of metabolites were assigned and confirmed, and metabolite levels were calculated based on NMR integrals. Multivariate statistical analysis was carried out using partial least-squares discrimination analysis (PLS-DA) to distinguish metabolic profiles between the groups. The validated PLS-DA model gave the variable importance in the projection (VIP) for a given metabolite. Moreover, inter-group comparisons of metabolite levels were quantitatively conducted using the paired-sample t-test. Then, we identified characteristic metabolites with VIP>1 in PLS-DA and $p<0.05$ in t-test. Furthermore, we screened out potential biomarkers based on the characteristic metabolites identified from the three types of biological fluids using the BRM (stepwise).
Results The rowing training induced profound changes of metabolic profiles in serum and saliva samples rather than in urine samples. Totally, 44 metabolites were assigned in which 19, 20, and 19 metabolites were identified from serum, urine and saliva samples, respectively. Seven metabolites were shared by the three types of samples. Moreover, five characteristic metabolites (pyruvate, lactate, succinate, N-acetyl-L-cysteine, and acetone) were identified from the serum samples. The elevated levels of pyruvate, lactate and succinate suggested that, the rowing training evidently promoted both oxidative phosphorylation and glycolysis pathways. Furthermore, three characteristic metabolites (tyrosine, formate, and methanol) were identified from the saliva samples. Given that tyrosine is the precursor of dopamine, the increased level of salivary tyrosine in all rowers experiencing the test, suggesting that salivary tyrosine could be explored as a potential indicator closely related to nervous fatigue in the test. On the other hand, PLS-DA did not show observable distinction of metabolic profiles between the urine samples before and immediately after the test. Moreover, 20 urinary metabolites

did not display detectable altered levels. We then established the BRM with the identified characteristic metabolites, from which we selected one optimal regression model based on serum pyruvate and salivary tyrosine (adjusted R square was 0.935, $P<0.001$), indicating that the two selected metabolites would efficiently reflect the metabolic alterations in the test.

Conclusions As far as the 6000m rowing ergometer test is concerned, serum samples could be a preferred resource for assessing the changes of energy metabolism in the test, while urine samples might have a relatively lower sensitivity to exercise-induced metabolic responses. Even though metabolite levels in saliva samples are generally lower than those in serum and urine samples, some salivary metabolites potentially have higher sensitivities to exercise-induced metabolic responses. Thus, the integration of multiple biomarkers identified from different type of species could potentially provide more sensitive and specific manners to monitor physical states in sports and exercise. This work may be of benefit to the exploration of integrated biomarkers for sports-specific monitoring.