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Caffeine Supplementation Altered Metabolic Profiles in High-intensity Interval Training

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Objective Caffeine supplementation is a commonly used nutritional practice. Exogenous metabolites from caffeine, such as paraxanthine, theobromine and theophylline, are eventually excreted through urine. Yet, it is less clear whether caffeine would induce endogenous metabolites altered during exercise. Urine metabolomics is non-invasive method, which mainly focus on alterations of endogenous metabolic profiles caused by diseases, drugs, and lifestyle and nutritional interventions as well. Therefore, the purpose of the present study was to examine the effects of supplementation with caffeine in a well-designed high intensity interval training (HITT). We identified significant alterations in urinary metabolite levels and revealed key metabolic pathways involved in caffeine supplementation in HITT.
Methods We performed a randomized, double-blind, placebo- controlled crossover study. Twelve women basketball players (age:19.12 ± 2.64 years, mass: 174.73 ± 5.18 cm, height: 62 ± 5.09 kg, with 8.50±2.11 years training period for basketball) were randomized to placebo (PLA) or caffeine (CAF) with dosage of 3mg on the basis of body weight (kg) 45min before a field HITT test. The test was repeated after three days when players were crossed over to the alternate test. The test began with a 30 min warmup, followed by a high intensity intermittent exercise trail with incremental load for about 25min, and a cool-down. Players are familiar with the test program which included 55 sets of dribble shuttle-run, pass, shoot, and rebound with basketball with a distance of 1540m (55 × 28m), the interval between two sets was gradually reduced. Performance (completed time), heart rates immediate (HR_{0min}) and 1 min (HR_{1min}) after test, blood lactate (BLa), proteinuria and ratings of perceived exertion (RPE) were collected during each protocol. Urine samples were obtained before and 1 h after of the test. ^{1}H-NMR spectra (Bruker AVANCE III HD 600MHz) were obtained and then processed by NMR spectra (MestReNova 9.0). The binning values of NMR spectra are imported into MATLAB, and the peaks are aligned with the icoshift algorithm. Then concentrations of the aligned metabolites were calculated by converting the integral area of proton signals with that of the TSP. Pattern recognition was performed to the processed NMR data, including principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA). Characteristic metabolites were identified that contribute most to the metabolic pattern between groups according to the OPLS-DA models. Finally, we analyzed the metabolic pathway by importing characteristic metabolites with concentrations into the Enrichment Analysis (MetaboAnalysis 3.0) to determine the metabolic pathways with the greatest disturbance related to caffeine during exercise. Moreover, the main effects of exercise, caffeine and the interaction between exercise and caffeine were determined by Repeated measure GLM analysis (Spss 22.0).
Results (1) Compared with PLA, CAF had no significant difference in the completed time (25.9 min vs. 26.8 min). Repeated measured analysis showed that there was significant overall time effect on the routine training monitoring parameters, while no statistically group differences in HR_{0min}, $HR_{1min,}$ BLa (199.02±21.36 vs.189.00±22.38 bpm; 148.02±12.60 vs.148.02±20.34 bpm, and 8.89±2.23 vs. 9.52±2.91 mmol/L, respectively). For the qualitative indexes, the positive rate of urine ketone bodies was increased, while RPE did not changed. (2) We identified 32 metabolites in urine sample. PCA

showed distinct differentiation of metabolic patterns between each two groups in the four groups (PLA_{before}, PLA_{post}, CAF_{before}, CAF_{after}). By using OPLS-DA, we found that the urine metabolic profiles were differences in between caffeine supplementation group and placebo group during the test. OPLS-DA revealed the identified metabolites of exercise and caffeine respectively, among them, lactate, butyric acid, isobutyric acid, 3-hydroxybutyric acid and pyruvic acid could be used as metabolic biomarkers in the HITT response. Supplementation of caffeine increased the production of fat metabolites in urine compared to the PLA. Enrichment analysis showed that the disturbed metabolic pathways shared by PLA and CAF were purine metabolism, glycolysis, insulin signal transduction, galactose metabolism, gluconeogenesis, glucose-alanine cycle, sphingolipid metabolism, alanine metabolism and citric acid cycle. Yet, when compared to the PLA, CAF enhanced fat metabolism and increased pyruvate metabolism, cysteine metabolism and mitochondrial electron transport. These results suggest that caffeine could promote fatty acid metabolism and amino acid metabolism to improve aerobic metabolism and to reduce oxidative stress, and thus promote exercise capacity. (3) Covariance analysis showed that there were significant individual-specific effects of caffeine supplementation.

Conclusions Caffeine supplementation during HITT promoted the fat metabolism, and upregulated the TCA, pyruvate metabolism and mitochondrial electron transfer. It is suggested that caffeine could, to some extent, promote energy supply shift from anaerobic metabolic to an aerobic manner, and the enhancement of fat oxidation would be beneficial to glycogen storage for intensively long-duration exercise. Moreover, there are obvious individual differences in caffeine response on sports.