Trial Protocol

1. Participants

Participants were excluded if they were suffering from hypertension, diabetes, cardiovascular or metabolic diseases; if they had taken cigarettes and/or alcohol; and if they had taken buffering agents within the previous 6 months and throughout this study. The inclusion criteria included the following: (I) participants aged 18–24 years; (II) BMI ranged from 18.5 to 23.9 kg/m^2; and (III) participants had at least 5 years of experience in soccer training with VO_{2max} over 50 mL/kg/min.

2. Study methods

Participants visited the laboratory on 3 separate sessions, including 1 familiarization session and 2 experimental sessions, with each session separated by 14 days. At the first visit, personal information, anthropometric measurements (height and body composition), and graded cycling exercise test (GXT) results were collated. All participants completed the exercise protocol familiarization session. After a 7-day washout period, each participant was then assigned to ingest either 0.1 g/kg/d of PYR or the maltodextrin placebo (PLA) in the following 7 days in a counterbalanced randomized design (i.e., 7 participants were supplemented with PYR and 7 participants were administered the PLA).

During the second and third visits (i.e., on the seventh day of supplementation), 45 minutes following the intake of either PYR or PLA, participants completed a 15-minute resting oxygen uptake test. After a 5-minute warmup, a high-intensity interval exercise (HIIE) cycling test was performed, followed by a 6-minute post-exercise oxygen consumption (EPOC) assessment. Participants undertook the RSE test 4 minutes after the EPOC test.

Oxygen uptake was measured breath-by-breath during GXT, 15 minutes before HIIE, during HIIE, 6 minutes after HIIE, and during RSE, using a portable gas analysis system (Cortex Metamax 3B, CORTEX Biophysik, Leipzig, Germany). Participants completed the oxygen uptake test 15 minutes before and 6 minutes after HIIE in a sitting position. Resting oxygen uptake was taken as the average of the last 10 minutes of data. Before each test, the gas analyzer was calibrated according to the instructions of the manufacturer.

3. Evaluation criteria

3.1 HIIE and RSE tests

HIIE was conducted on a cycle (Ergoline Ergoselect 100K, Ergoline, Bitz, Germany). During the test, participants undertook a 5-minute warm-up at 60 W. Then, the HIIE protocol was performed, consisting of 4 sessions of 1-minute cycling at 110% of their W_{max}, interspersed with 1-minute recovery periods. Cadence was constant (90–100 rpm) during each high-intensity bout.

Following HIIE, participants rested for 10 minutes, and then completed 6 sessions of 6-second maximal cycling, interspersed with 24 seconds passive recovery on a mechanically-braked Monark cycle ergometer (894E, Monark, Vansbro, Sweden). Each sprint exercise was started with a "3, 2, 1, go" countdown. Once cadence reached 110 rpm, the 0.087 kp/kg body mass load was added to the ergometer, and the 6-second sprint was started. All participants were encouraged to exert maximum effort possible during each sprint. The seat height and handlebar position for each participant was adjusted prior to the initial HIIE and RSE tests and remained the same for subsequent tests. The RSE test protocol has been reported to be valid and reliable, and has been previously used to measure exercise performance in soccer players.

All data were calculated via Monark Anaerobic Testing software (version 3.3.0.0, developed in cooperation with HUR Labs). The software automatically recorded the power output per second and calculated the relative peak power output (PPO) and relative mean power output (MPO) for each sprint. The mean PPO and MPO were calculated by taking the average of PPO and MPO for sprints 1–6, respectively.

3.2 Blood collection and analysis

Venous blood samples (1.0 mL) obtained from the ulnar vein at baseline, pre-HIIE, post-HIIE, pre-RSE, and post-RSE were collected in sodium heparin tubes (YA1430, Solarbio, Beijing, China) and immediately assessed for blood pH, HCO_3^-, and BE using a blood gas analyzer (Radiometer ABL80, FLEX CO-OX, Willich, Germany).

Capillary blood samples (10 μL) taken by finger prick were collected with Biosen capillary tubes (EKF Diagnostics, Barleben, Germany) at baseline, immediately after each bout of HIIE, and at 3, 5, 7, and 10 minutes after HIIE. The samples were used to measure blood lactate concentrations with a lactate analyzer (Biosen C-Line, EKF Diagnostics, Barleben, Germany).

3.3 Estimation of energy system contributions

Contributions of the aerobic energy system were estimated by subtracting the resting oxygen uptake from the oxygen consumption obtained during each 110% W_{max} bout. All oxygen used during HIIE was converted to energy assuming one liter of oxygen is equal to 20.92 kJ. The lactate accumulated during each 110% W_{max} bout was used to evaluate the contributions of the glycolytic energy system of each bout of HIIE (1 mmol/L of lactate equals to 3 mL/kg of oxygen). The ATP-PCr resynthesis were calculated by subtracting resting oxygen from the oxygen consumption obtained during each HIIE and RSE recovery periods, and the fast component of post-exercise oxygen consumption ($EPOC_{fast}$) accessed during the 6 minutes after HIIE. The $EPOC_{fast}$ was determined by the product of the amplitude and time constant of the first exponential decay (OriginPro 8.0, OriginLab, Microcal, Massachusetts, USA).

4. Statistical method

Data analyses were performed using SPSS software (version 22.0, SPSS Inc. Chicago, IL, USA) and presented as the mean ± standard deviation (SD). The Shapiro-Wilk test was used to determine the data normality. Two-way repeated-measures ANOVA was performed to assess the interaction between time and the two groups, and post hoc analysis was performed using Fisher's least significant difference test. Effect sizes were calculated as partial eta-squared [η_p^2; small effect (0.01–0.059), medium effect (0.06–0.139), and large effect (≥0.14)]. Independent samples *t*-tests were used to measure average PPO and MPO, and average contributions of the energy system during HIIE and RSE. Effect sizes were expressed as Cohen's d where Cohen's d =0.20–0.49 indicates a small effect, Cohen's d =0.50–0.79 indicates a medium effect, and Cohen's d ≥0.80 indicates a large effect. The significance level was set at $P<0.05$.

Article information: https://dx.doi.org/10.21037/apm-21-3862