Effects of Sodium Pyruvate on Exercise-Induced Lactic Acidosis and Exercise Performance

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Abstract

Background: The beneficial effects of pyruvate on lactic acidosis and energy metabolism have been studied. The ability to attenuate acidosis and improve aerobic system contribution are essential for team sports athletes to perform multiple sprints in a limited time. This study aimed to investigate the impact of pyruvate supplementation on energy metabolism and lactic acidosis during high-intensity interval exercise (HIIE) and to evaluate its role in repeated sprint exercise (RSE) performance.

Methods: Fourteen national-level male soccer athletes from China Football College (age: 20 ± 2 years, body fat: $13.11 \pm 3.50\%$) participated in a randomized, double-blind, crossover study. Each subject underwent two HIIE and RSE tests after one week of supplementation with 0.1g/kg/d of pyruvate or placebo. Venous blood gas (pH, bicarbonate (HCO_3^-), base excess (BE) and oxygen partial pressure (pO_2)) were measured at baseline, pre-HIIE, post-HIIE, pre-RSE and post-RSE. Finger-stick capillary lactate were collected at baseline, immediately after each bout of HIIE, and 3, 5, 7 and 10 min post HIIE. The energy system contributions during HIIE and exercise performance of RSE were analyzed.

Results: Blood pH ($p < 0.01$), HCO_3^- ($p < 0.01$) and BE ($p < 0.01$) were significantly lower than their baseline levels at post-HIIE, pre-RSE and post-RSE in both Group pyruvate (PYR) and Group placebo (PLA), respectively. Blood pH, HCO_3^- and BE were significantly improved in PYR at pre-HIIE ($p < 0.01$), post-HIIE ($p < 0.01$) and pre-RSE ($p < 0.01$) than in PLA. Furthermore, blood BE remained higher in PYR than in PLA until the end of RSE ($p < 0.05$). Blood pO_2 was higher at post-HIIE ($p < 0.05$), pre-RSE ($p < 0.01$) and post-RSE ($p < 0.01$), compared to baseline, in two groups and further higher in PYR than in PLA at post-HIIE ($p < 0.05$) and post-RSE ($p < 0.05$). During HIIE, the contribution of the aerobic system in the fourth bout of HIIE was higher in PYR than in PLA ($p < 0.05$). The contribution of the anaerobic energy was higher than that of the aerobic energy in PLA ($p < 0.01$), but without difference in PYR ($p > 0.05$). In addition, compared with PLA, the relative peak power (RPP) of the first and fifth sprints, the relative average power (RAP) of the fifth sprint and the average of RPP and RAP during RSE were significantly improved in PYR ($p < 0.05$). However, the blood lactate ($p > 0.05$), PD% of each bout ($p > 0.05$) or average PD% of RSE ($p > 0.05$) showed no significant difference between the two groups.

Conclusion: Pyruvate supplementation with 0.1g/kg/d for a 1-week enhances aerobic metabolism and buffers exercise-induced metabolic acidosis but not lactic acidosis during HIIE, improving RSE performance in moderate acidosis. Thus, after 1 week pyruvate supplementation, athletes are expected to balance aerobic and anaerobic energy contributions and attenuate metabolic acidosis during team sports, improving exercise performance.

Introduction

Most team sports involve a number of high-intensity exercises and sprints with incomplete recovery intervals¹. During high-intensity interval exercise (HIIE), the demand for ATP is greatly increased and exceeds the energy generation by aerobic metabolism. Therefore, the dependence on anaerobic

(phosphagen and glycolysis) energy supply is promoted. The amount of hydrogen ion (H^+) created by anaerobic metabolism is uncoupled with that eliminated with aerobic metabolism². Intracellular H^+ is transported to the blood together with lactate. In the blood, H^+ is eliminated by bicarbonate (HCO_3^-) and lactate is transported or accumulated³. Blood pH, HCO_3^- and BE decrease as the H^+ accumulated during HIE exceeds the maximum buffering potential and lactate accumulates simultaneously, resulting in intracellular and extracellular metabolic acidosis². Exercise-induced metabolic acidosis is an important form of metabolic acidosis and is specifically classified into the category of lactic acidosis. Lactic acidosis described as the blood pH level < 7.35 , HCO_3^- level < 20 mmol/L, base excess (BE) level < -2 mmol/L and accompanied by the lactate level > 5 mmol/L⁴. The pH value of skeletal muscle could drop to 6.8 along with extracellular lactic acidosis in an intense soccer match⁵. Although the effect of lactic acidosis on exercise performance is controversial^{6,7}, the large accumulation of H^+ in skeletal muscle may impair muscle contraction and exercise efficiency⁸.

For improving exercise performance, many studies have focused on the effect of alkaline buffers. Typical alkaline buffers mainly reduce extracellular acidosis by acting as physical and chemical buffers with limited metabolism⁹. On the other hand, pyruvate can be transferred into cells as an intracellular buffer to efficiently reduce lactic acidosis^{10,11}. It mainly relies on regulating intracellular energy metabolism to reduce lactic acidosis additionally with the specific low dissociation constant of pyruvate ($pK_a = 2.49$)^{11,12}. Studies have shown that pyruvate is a crucial intermediate of glucose metabolism and a natural inhibitor of pyruvate dehydrogenase kinase (PDK), which improves pyruvate dehydrogenase (PDH) activity and benefits aerobic metabolism^{13,14,15}. Moreover, reports have demonstrated that pyruvate can maintain glycolytic metabolism through its spontaneous reductive reaction by lactate dehydrogenase and improvement of key glycolytic enzymes^{11,14}.

In fact, pyruvate is a superior buffer, as demonstrated in oral rehydration of shock that only can pyruvate reverse lactic acidosis, other than bicarbonate or citrate on an equimolar basis in shock resuscitation of animal models¹⁶. Previous studies found that the blood pH values were raised with reversal of hypoxic lactic acidosis after resuscitation by pyruvate administration in hemorrhagic shock models^{17,18}. It was also discovered that pyruvate could boost blood pH and BE after reperfusion in a rat model of severe intestinal ischemia-reperfusion injury¹⁹. Moreover, perfused with hyperpolarized tracer $[1-^{13}C]$ pyruvate in the heart of rats, the production of $H^{13}CO_3^-$ was increased²⁰. Particularly, pyruvate was reported to raise intracellular pH in failing human myocardium and to minimize hyperchloremic acidosis in a rat model^{10,12}.

Sodium pyruvate is a safe substance ($LD_{50} > 10$ g/kg oral pyruvate in rats)²¹. With oral pyruvate treatment, the subjects reported no gastrointestinal symptoms with single doses less than 10 g²¹. Human studies have reported that single ingestion with 0.1 g/kg pyruvate can increase the blood pH, HCO_3^- and BE of healthy individuals at rest 60 min after intake and its buffering effect can last for 120

min^{22,23}. However, acute supplementation with pyruvate seemed to be ineffective for high-intensity exercise-induced lactic acidosis²³. Whether multiple pyruvate supplementation can further regulate the energy metabolism and attenuate lactic acidosis during exercise remains unknown.

Repeated sprint exercise (RSE) is generally considered to be the determining part of team sports performance despite the fact that sprinting activities account for only 10% of the total distance of team sports²⁴. Team sports performance is affected by RSE fatigue factors, including insufficient creatine phosphate (PCr) resynthesis and intramuscular H⁺ accumulation²⁵. Therefore, reducing lactic acidosis and increasing the potential of the aerobic metabolism to resynthesize PCr are of great benefits in the performance of team sports athletes.

To our knowledge, few studies have documented the effects of multiple sodium pyruvate supplementation this topic. This present study evaluated the role of oral sodium pyruvate on exercise-induced lactic acidosis and team sport performance from the perspective of energy metabolism. We hypothesized that multiple pyruvate ingestion would contribute to energy availability and environmental alkalization during RSE, leading to better performance.

Methods

Subjects

Fifteen national-level male soccer athletes from China Football College volunteered to participate in this study. Exclusion criteria included: 1) hypertension, diabetes, cardiovascular risk factors and any other diagnosed metabolic disorders (e.g., acid-base imbalance); 2) smoking, ingested alcohol, caffeine and any other ergogenic supplements (e.g., sodium bicarbonate and creatine). Inclusion criteria: 1) age 18-24 years; 2) BMI ranging from 18.5 to 23.9 kg/m²; and 3) at least 5 years of regular soccer training experience with maximal oxygen uptake over 50 ml/kg/min. The study protocol was approved by the Internal Review Board of Beijing Sport University (2020057H). All subjects were informed of the study aims and were asked to sign an informed consent form. One participant dropped out after the graded cycling exercise test. Fourteen athletes completed all the sessions and were included in the data analyses (Table 1).

Study design

Randomized, double-blind, crossover trials were used in this research. There were five laboratory visits, each separated by 7 days. The first visit involved personal information collection, body composition measurement, familiarization with the exercise test protocol, and performed a graded cycling exercise test (GXT). In the second visit, participants took sodium pyruvate (PYR) or maltodextrin (Placebo, PLA) (0.1 g/kg/d) for 7 days parallelly and randomly in PYR (n=7) and PLA (n=7), respectively. During the third visit, 45 min after the last supplementation, the subjects took a 15-min resting oxygen uptake test (15-RO₂), performed high-intensity interval exercise (HIIE) test to induce lactic acidosis, and collected a 6-min

oxygen uptake immediately after HIIE (6-VO₂). An RSE test was performed 10 min after HIIE. In the fourth visit, participants who were supplied with pyruvate/maltodextrin in the second visit were switched to maltodextrin/pyruvate, parallelly and randomly in PYR (n=7) and PLA (n=7). After a 1-week, they returned to the laboratory and repeated the tests they had done during the third visit. Each subject performed the tests at the same time of day and in the same laboratory environment.

Participants arrived in a resting and thoroughly hydrated condition following ≥ 2 h postprandial, avoiding any strenuous exercise and consumption with carbonate, alcohol and caffeine 24 h before each exercise test. A 3-day dietary recall was used to measure calorie intake and macronutrient consumption (2 weekdays and 1 weekend). Subjects used a 24-h recall to measure dietary consumption the day before the first trial and were asked to replicate the same diet as accurately as possible in subsequent studies. To ensure compliance, participants were expected to ingest the supplement under supervision. Fig. 1 presents a timeline for this study.

Body composition measurements

A calibrated electronic scale was used to calculate height to the nearest 0.1 cm (GMCS-SGJ3, Jianmin, Beijing, China). Body composition was assessed 2 h after a meal by multifrequency bioelectrical impedance measurement device (Inbody 230, Biospace, Seoul, Korea).

Graded cycling exercise test (GXT)

Each subject underwent GXT on an electromagnetically braked cycle ergometer (EC 3000e, CUSTO Med, Ottobrunn, Germany) to determine peak power ($W_{\max}$) and maximum oxygen uptake (VO_{2max}). Participants adjusted the seat and warmed up for 3 min at 50 W. Then, the pedal frequency was kept between 75-80 rpm and the load was increased at 30 W/min. The subjects' standards for exhaustion included the following: 1) Failed to keep 75 rpm for 5 s; 2) Increased the power load with the oxygen uptake (VO₂) rise ≤ 150 ml/kg/min; 3) Respiratory exchange rate (VCO₂/VO₂) ≥ 1.10 and 4) Heart rate reached 220-age. VO_{2max} is the average oxygen consumption 30 s before reaching exhaustion²⁶. $W_{\max}$ is determined as the power of the last completed stage plus the fraction of time spent in the final uncompleted stage multiplied by 30 W²⁷. During the test, the Borg scale (6-20)²⁸ was used to determine the rate of perceived exertion (RPE) and Polar V800 (Polar Electro Oy, Oulu, Finland) was used to measure heart rate.

Supplementation protocol

Sodium pyruvate in high quality (Lianlu industrial Co., Ltd., Shanghai, China) and maltodextrin were randomly packed in capsules A or B; both were similar in appearance, size and weight. Each participant took 0.1 g/kg/d²³ pyruvate or maltodextrin for 7 days (ingested 2 capsules just after a meal and 1 capsule before sleep on the first 6 days; ingested all the remaining capsules 60 min prior to the exercise test on the 7th day²³). Subjects took the capsule A were switched to the capsule B and vice versa during the test timeline.

Oxygen uptake test

VO₂ was measured with breath-by-breath at GXT, 15-RO₂, HIIE test and 6-VO₂, using a portable gas analyzer (Cortex Metamax 3B, CORTEX Biophysik, Leipzig, Germany). Participants took a break in a sitting position to measure 15-RO₂ and 6-VO₂. The RO₂ was determined by using the average of the last 10 min of data collected²⁹. The gas analyzer was calibrated before each test in accordance with the manufacturer's instructions.

HIIE protocol

HIIE was conducted in an electromagnetically braked cycle (Ergoline Ergoselect 100K, Ergoline, Bitz, Germany). During the test, subjects took a 5-min warm-up at 60 W. HIIE consisted of 4 x 1 min high-intensity cycling at 110% W_{max} and separated by 1 min rest. Cadence was constant (90-100 rpm) during high-intensity bout. Subjects remained seated on the ergometer, taking a 10-min break after finishing the last bout³⁰.

RSE test

RSE consisting of 6 x 6 s of all out maximal cycling was performed on a mechanically braked cycle ergometer (894E, Monark, Vansbro, Sweden). Exercise load equaled weight (kg) × 0.087 kp/kg (weight)³¹. During the test, participants adjusted the seat and began to fully ride following the staff countdown "3, 2, 1, start". Once the cadence reached 110 rpm, the load of inertial ergometers was adjusted to the predetermined load. Subjects kept fully pedaling to complete a 6 s cycling exercise and encouragement was provided to enable subjects to exert maximum ability. Each sprint had a 24 s rest interval²⁵.

Relative peak power (RPP): the highest power output (PP) relative to body mass observed in each 6 s;
Relative average power (RAP): the average power output (AP) relative to body mass maintained in each 6 s;
Power drop (PD%): the percentage of mechanical power decay over the period of the test relative to the peak power, according to the following equation: $PD\% = [(PP - AP)/PP \times 100]$ ²⁵. All the parameters were calculated via Monark Anaerobic Testing software (Version: 3.3.0.0, developed in cooperation with HUR Labs).

Blood collection and analyses

Capillary blood samples with 10 µl of fingerstick were collected (wiped away the first drop of blood) in a Biosen capillary tube (EKF Diagnostics, Barleben, Germany) at baseline, the end of each bout of HIIE, and 3, 5, 7 and 10 min after HIIE. The samples were used to measure blood lactate concentrations with a lactate analyzer (Biosen C-Line, EKF Diagnostics, Barleben, Germany).

Blood samples of 1.0 ml were obtained from the ulnar vein at baseline, pre-HIIE, post-HIIE, pre-RSE and post-RSE. The samples were collected in sodium heparin tubes (YA1430, Solarbio, Beijing, China) and immediately assessed for blood pH, HCO₃⁻, BE and oxygen partial pressure (pO₂) by a blood gas analyzer

(Radiometer ABL80, FLEX CO-OX, Willich, Germany). Additionally, the blood HCO_3^- was calculated from the partial pressure of carbon dioxide and pH values according to the Henderson-Hasselbalch equation and the blood BE was calculated from HCO_3^- and hemoglobin (Hb) determined by gas analyzer according

to the following equation³²:

$$BE = (1.0 - 0.0143 * Hb) * [(HCO_3^-) - 24.8 + (7.7 + 1.43 * Hb) * (pH - 7.4)]$$

Estimation of energy contribution

The aerobic energy contribution was estimated by subtracting the resting oxygen consumption from the oxygen consumption obtained during each 110% W_{max} bout. The consumed oxygen was transferred to energy. One liter O_2 is converted into 20.92 kJ energy equivalent³³. The lactate difference before and after each 110% W_{max} bout and the lactate accumulated during HIIE were assumed to calculate the glycolytic energy contribution of each bout and total HIIE, respectively. The accumulation of 1.0 mmol/L lactate corresponds to 3.0 ml O_2 /kg of body weight³³. The phosphagen energy system contains two sections. First, resting oxygen uptake was subtracted from the oxygen consumption obtained during each 1-min interval. Second, fast component of excess post-exercise oxygen uptake (EPOC fast) accessed during 6- VO_2 was adjusted by a biexponential model. The EPOC fast is assumed to be the product of the amplitude and time constant of the first exponential model (OriginPro 8.0, OriginLab, Microcal, Massachusetts, USA)³⁴.

Statistical analyses

A power analysis (Power $1-\beta = 0.9$ and $\alpha = 0.05$) was performed with a priori in G*Power software version 3.1.9.6 (Universitat Kiel, Germany), and at least 12 participants were needed for the present study. Data analyses were carried out by using SPSS, version 22.0 (SPSS Inc. Chicago, IL, USA). The Shapiro-Wilk test was used to verify the assumptions of normality of the data. A non-parametric Kruskal-Wallis test was used to analyze the power drop (%) of each sprint and Dunn-Bonferroni test as a post-hoc test. Two-way repeated-measures ANOVA was performed to assess the interaction between time (baseline, each bout of HIIE and each sprint of RSE) and the cohort (PYR and PLA). Fisher's least square significant difference (LSD) post-hoc analyses were performed when significant interactions were observed. Independent samples t-tests were used to measure relative energy contribution during HIIE and average RPP, RAP and PD% during RSE. Values were expressed as the mean $\pm$ SD or median (P25, P75). The statistical significance level was set at $p < 0.05$.

Results

All subjects were supplemented with pyruvate or placebo as required and under the supervision of research staff. There were no side effects reported by the participants with the supplementation and there was no significant effect of treatment order in the research. During the study, participants maintained their typical eating habits and did not change their physical activity patterns.

Exercise induced lactic acidosis

Table 2 shows the venous blood pH, HCO_3^- , BE and lactate for each group with no significant difference at baseline. In PYR, blood HCO_3^- ($p < 0.01$) and BE ($p < 0.01$) were significantly increased at pre-HIIE compared with baseline. Blood pH ($p < 0.01$), HCO_3^- ($p < 0.01$) and BE ($p < 0.01$) were significantly decreased, while blood lactate ($p < 0.01$) was significantly increased at post-HIIE, pre-RSE and post-RSE compared with baseline in each group. Compared to PLA, blood pH, HCO_3^- and BE were significantly improved in PYR at pre-HIIE ($p < 0.01$), post-HIIE ($p < 0.05$) and pre-RSE ($p < 0.05$). Moreover, blood BE in PYR was higher than PLA until post-RSE ($p < 0.05$).

Energy contribution

Figure 2A-2C display the energy contribution during HIIE for each group. In both groups, compared to bout 1, aerobic energy system contribution of bouts 2–4 ($p < 0.01$) and the phosphagen energy system contribution of bouts 2–3 ($p < 0.01$) were significantly increased, while the glycolytic energy system contribution of bouts 2–4 ($p < 0.01$) showed a significant decrease. Compared with PLA, the aerobic energy system contribution of the fourth bout ($p < 0.05$) was significantly improved in PYR. However, the contributions of glycolytic and phosphagen energy systems were not significantly changed between PYR and PLA during the four bouts of high-intensity exercise ($p > 0.05$).

Figure 2D shows the percentage of aerobic and anaerobic energy contributions during HIIE. There was no significant difference in the ratio of aerobic ($p > 0.05$) or anaerobic ($p > 0.05$) energy contributions during HIIE between PYR and PLA. But the ratio of the anaerobic energy contribution during HIIE was higher than that of aerobic energy in PLA ($48.07\% \pm 3.22\%$ vs. $51.93\% \pm 3.22\%$, $p < 0.01$), but no difference was observed in PYR ($49.47\% \pm 1.77\%$ vs. $50.53\% \pm 1.77\%$, $p > 0.05$).

Figure 3 displays the venous blood pO_2 response during tests. Compared with baseline, the blood pO_2 increase at post-HIIE ($p < 0.05$), pre-RSE ($p < 0.01$) and post-RSE ($p < 0.01$) in both groups. Compared with PLA, the blood pO_2 significantly improved in PYR at post-HIIE ($p < 0.05$) and post-RSE ($p < 0.05$).

RSE performance

Compared to PLA, pyruvate significantly increased the average RPP ($p < 0.05$) and RAP ($p < 0.05$) but the average PD% was not changed ($p > 0.05$) during RSE (Fig. 4A).

Figure 4B-4D display the RPP, RAP and PD% during RSE for each sprint. In PYR, compared to sprint 1, the RPP of sprints 3–4 and sprint 6 ($p < 0.05$), the RAP of sprint 3 ($p < 0.05$) and sprints 4–6 ($p < 0.01$) were decreased, while the PD% of sprint 4 ($p < 0.05$) and sprints 5–6 ($p < 0.01$) were increased during RSE. In PLA, compared to sprint 1, the RPP of sprint 5 ($p < 0.05$) and the RAP of sprints 4–6 ($p < 0.05$) were decreased, while the PD% of sprints 4–6 ($p < 0.01$) were increased during RSE. Compared to PLA, the RPP

of the first and fifth sprints ($p < 0.05$) and the RAP of the fifth sprint ($p < 0.05$) were significantly improved in PYR. There was no significant difference in the PD% of each sprint during RSE between PYR and PLA.

Discussion

This was the first study to explore the effects of sodium pyruvate supplementation for 1 week on energy metabolism and lactic acidosis during HIIE and the RSE performance of well-trained soccer players. The same doses of pyruvate and maltodextrin were supplemented to eliminate the caloric interference factors. Further, we used two independent exercise programs in the investigation: equal bouts of HIIE for inducing lactic acidosis and estimating energy metabolism and RSE for evaluating exercise performance. The results demonstrated that the strenuous exercise generated severe lactic acidosis, as showed with the robust decrease of blood pH, HCO_3^- and BE with increase of blood lactate in the two groups (Table 2), which was in consistence with previous findings^{5,30}. Present data first showed that the multiple pyruvate supplements significantly reduced the imbalance between aerobic and anaerobic metabolisms and attenuated metabolic acidosis during HIIE. Furthermore, the RPP of the first and fifth sprints, the RAP of the fifth sprint and the average RPP and RAP during RSE were significantly increased in PYR. However, blood lactate, energy contribution of phosphagen and glycolytic energy systems during HIIE as well as PD% of RSE had no significant change by pyruvate intake within this protocol.

One of main findings is that pyruvate supplementation for a 1-week elevated the blood buffering capacity before exercise, which was similar to the single acute supplementation protocol^{22,23}. Another main finding suggested that one week of pyruvate supplementation increased the alkaline reserves and buffered HIIE-induced metabolic acidosis in a certain extent. In contrast, single pyruvate supplementation could not buffer lactic acidosis induced by 6 min high-intensity ($90\%\text{VO}_{2\text{max}}$) exercise²³. These data demonstrated that multiple pyruvate supplementation may play a better role in attenuating exercise-induced metabolic acidosis than single acute supplementation, as shown that the present protocol boosted the aerobic metabolism and the HCO_3^- production during intense exercise. The H^+ was eliminated intracellularly and the efflux into blood was reduced³⁵. In addition, it was also found that pyruvate bridged the gap between the aerobic and anaerobic energy contributions during HIIE (Fig. 3D). As a result, pyruvate eliminated the difference of H^+ generated by anaerobic metabolism and removed by aerobic metabolism, reducing the net accumulation of H^+ and alleviating exercise-induced metabolic acidosis².

Our finding also provided the direct evidence that the venous blood pO_2 gradually increased and blood oxygen saturation (SpO_2) remained 96.5%-98% (data not showed) along with HIIE and RSE in soccer athletes, suggesting that sufficient oxygen was obtained to support aerobic metabolism³⁶. As observed in previous study, the pO_2 of earlobe arterialized blood was significantly increased after progressive exhaustive exercise³⁷. These results strongly supported the concept that severe lactic acidosis could be caused by an imbalance of aerobic and anaerobic metabolism other than lack of oxygen during high-

intensity exercise³⁸. Accordingly, strenuous exercise induces metabolic, instead of hypoxic, lactic acidosis in this study, as demonstrated previously³⁸. Furthermore, current research first demonstrated pyruvate significantly increased the venous blood pO₂ at post-HIIE and post-RSE in PYR relative to PLA (Fig. 3). According to previous works, pyruvate was substantiated to improve the glycolytic energy generation and oxygen-carrying capacity of red blood cells during bypass surgery and storage, *in vitro*^{39, 40}. Pyruvate improved the blood pO₂ during intestinal ischemia reperfusion, reducing ischemia reperfusion injury in rats⁴¹. A small dose of pyruvate in cardioplegia for cardiac bypass surgery showed robust cardioprotection although there is no data that pyruvate effects on the normal human heart during exercise⁴². Moreover, intravenous or oral pyruvate multi-organ protection of metabolism and function, including brain, heart, liver, kidney and intestine with preservation of visceral blood flow was reproducibly demonstrated in shock resuscitation of various animal models⁴³. Therefore, present data strongly suggested that oral pyruvate in this protocol probably facilitated to benefit cardiac function, oxygen-carrying capacity and systemic hemodynamics during the strenuous exercise.

According to the results, the energy supply of aerobic and phosphagen systems increased, while the glycolytic system decreased along with HIIE test in both groups (Fig. 2). These results were compatible with data from other literature on HIIE energy metabolism³⁰. We also discovered that pyruvate enhanced the aerobic energy contribution in the fourth bout of HIIE. Moreover, pyruvate eliminated the gap between aerobic and anaerobic energy contributions during HIIE (Fig. 3D). As shown in previously, pyruvate activated PDH activity and promoted mitochondrial aerobic metabolism and produced more NADH and ATP^{14, 15}. Thus, pyruvate may increase the activity of the key rate-limiting enzymes of aerobic metabolism, such as PDH, balancing the gap between anaerobic and aerobic metabolisms during intense exercise.

Our study showed no significant differences in blood lactate and glycolytic metabolism between two groups during HIIE. These results may be superficially inconsistent with previous findings, as prior research demonstrated that pyruvate could alleviate blood lactate by promoting aerobic metabolism while protecting glycolytic metabolism^{11, 40}. Moreover, single pyruvate supplementation also demonstrated its protection effect on glycolytic metabolism during high-intensity exercise, however, which showed a significant lactate rise post-exercise²³. One possible explanation for the absence of further lactate increase in PYR was that pyruvate promoted aerobic energy metabolism and consumed more lactate in mitochondrial oxidative phosphorylation during the interval period of HIIE, as previously shown^{10, 44}. In addition, pyruvate consumed intracellular H⁺ and decreased the muscle-to-blood H⁺ gradient, reducing lactate output into blood³⁵. Alternatively, the current pyruvate protocol just partially attenuated lactic acidosis induced by intense exercise. No further decrease of blood lactate in PYR relative to PLA may indicate the insufficient pyruvate dose absorbed in this protocol.

Studies have shown that athletes often need hundreds of short-term sprints to ensure the possession and shooting rate during a team sports game⁴⁵. The repeated sprint ability is mainly determined by the

resynthesis of PCr and the removal ability of H^+ during the interval period ^{8,25}. However, as a match progresses, the consumption of PCr and the accumulation of H^+ limited the repeated sprint ability of athletes of high or ordinary levels ^{8,46}. In line with previous studies, the RPP and RAP declined and PD% increased throughout each sprint during RSE in both groups, indicating a decrease in RSE efficiency ⁴⁷. However, compared to PLA, the RPP of the first (+ 11.41%) and fifth (+ 14.92%) sprints, the RAP of the fifth (+ 10.72%) sprint and the average RPP (+ 7.06%) and RAP (+ 5.56%) during RSE were significantly increased in PYR. Therefore, pyruvate may enhance the ability of aerobic metabolism, facilitate to PCr resynthesis and attenuate the severe metabolic acidosis induced by exercise, significantly improving RSE performance. Present results provide additional evidence that the decline of exercise capacity is mostly associated with the severity of metabolic acidosis, rather than the blood lactate level.

Acidosis and dehydration are common events in intense soccer games, which have become critical factors that limit exercise performance ⁵. Considering recent reports with pyruvate-enriched oral rehydration salt (Pyr-ORS) in effective treatments of hypoxic lactic acidosis in shock resuscitation or diabetes ¹⁴, it is necessary to explore the effect of Pyr-ORS in intense team sports, which may enhance the oral pyruvate absorption and provide further benefits in lactic acidosis correction and exercise performance ⁴⁸. Further research should examine the dose-effect relationship between effective blood pyruvate levels and exercise.

Limitations

The participants were college soccer athletes. Due to training commitments, collecting muscle tissue for assessments of PCr, ATP and PDH was challenging. The present study evaluated the acid-base response by using the venous blood; if the arterial blood samples had been used, the effects of pyruvate on skeletal muscle cells during exercise could have been more efficiently evaluated.

Conclusions

Intensive exercise induced severe lactic acidosis caused by the imbalance of anaerobic metabolism in cytosol and aerobic metabolism in mitochondria, rather than systemic hypoxia. Supplementation with 0.1 g/kg/d pyruvate for one week significantly attenuated the severe metabolic acidosis and improve the balance of energy metabolic contributions during HIIE, which are beneficial in the RSE performance. However, the dosage of current pyruvate supply was not sufficient to significantly reduce exercise-induced lactic acidosis to an ideal extent. To increase the pyruvate supplementation dose efficiently absorbed may further improve pyruvate benefits in exercise performance.

Abbreviations

ATP: Adenosine triphosphate; AP: Average power output; BE: Base excess; Bout: High-intensity exercise bout; EPOC fast: Fast component of excess post-exercise oxygen consumption; GXT: Graded cycling exercise test; H^+ : Hydrogen ion; Hb: Hemoglobin; HCO_3^- : Bicarbonate; HIIE: High-intensity interval exercise; HR: Heart rate; NADH, Nicotinamide adenine dinucleotide; PD%: Power drop%; PDH: Pyruvate dehydrogenase; PLA: Group placebo; PDK, Pyruvate dehydrogenase kinase; PLA: Group placebo; pKa: Dissociation constant; pO_2 : Oxygen partial pressure; Pre-HIIE: Pre-high-intensity interval exercise; Pre-RSE: Pre-repeated sprint exercise; Post-HIIE: Post-high-intensity interval exercise; Post-RSE: Post-repeated sprint exercise; PP: Peak power output; PYR: Group pyruvate; RPE: Rate of perceived exertion; RSE: Repeated sprint exercise; RAP: Relative average power; RPP: Relative peak power; VO_{2max} : maximum oxygen uptake; VO_2 : oxygen uptake; W_{max} : Maximal power output; S: Sprint exercise; 15- RO_2 : Resting oxygen uptake test; 6- VO_2 : oxygen uptake test 6-min immediately after HIIE.

Declarations

Ethics approval and consent to participate

The research proposal was approved by the Internal Review Board of Beijing Sport University (BSU IRB). All participants signed written informed consent prior to participating in this study (2020057H).

Consent for Publication

Not applicable, no individual person's data was presented.

Availability of data and material

The datasets generated and/or analyzed as part of the current study are not publicly available due to confidentiality agreements with subjects. However, they can be made available solely for the purpose of review and not for the purpose of publication from the corresponding author upon reasonable request.

Competing interests

No authors declare competing interests.

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Authors Contributions

YY was responsible for the study design, data collection, data interpretation, writing and revision of the manuscript under the supervision of JQ, who finally evaluated the manuscript. MW was in charge of participant recruitment and management. FZ shared the idea with JQ and YP, assisted data analysis and

improved manuscript, DL, LF, and RG assisted in the completion of the manuscript. The authors declare no conflicts of interest with the current publication, and all authors approved the final version of the manuscript.

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Note: The opinions or assertions contained herein are not a reflection of the view of Fresenius Medical Care, Dialysis Centers in Chicago, Illinois.

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Tables

Table 1 Participant characteristics

Age (year)	Height (cm)	Weight (kg)	Body fat (%)	BMI (kg/m ²)	VO _{2max}	W _{max}	Training year (year)
20 ± 2	178.31 ± 6.36	69.58 ± 7.22	13.11 ± 3.50	21.84 ± 1.33	55.93 ± 5.36	298.54 ± 41.7	9 ± 3

Note. Values are mean ± SD. BMI, body mass index; VO_{2max}, maximal oxygen uptake; W_{max}, maximal power output.

Table 2 Venous blood pH, HCO₃⁻, BE and lactate for each group

	Group	Baseline	Pre-HIIE	Post-HIIE	Pre-RSE	Post-RSE
Blood pH	PYR	7.38 ± 0.04	7.40 ± 0.03**	7.20 ± 0.05*△△	7.24 ± 0.06*△△	7.23 ± 0.07△△
	PLA	7.36 ± 0.03	7.37 ± 0.03	7.14 ± 0.08△△	7.17 ± 0.09△△	7.18 ± 0.07△△
Blood HCO ₃ ⁻ (mmol/L)	PYR	24.55 ± 1.15	26.09 ± 0.82**△△	14.56 ± 1.83*△△	15.24 ± 2.10*△△	14.04 ± 2.32△△
	PLA	24.52 ± 0.96	24.86 ± 1.03	12.57 ± 1.86△△	13.02 ± 2.05△△	12.52 ± 1.61△△
Blood BE (mmol/L)	PYR	1.06 ± 1.15	3.39 ± 0.86**△△	-12.58 ± 3.24*△△	-11.68 ± 3.49*△△	-13.64 ± 3.50*△△
	PLA	1.16 ± 1.30	2.00 ± 1.11	-15.61 ± 4.39△△	-14.98 ± 4.37△△	-16.56 ± 3.07△△
Blood lactate (mmol/L)	PYR	2.23 ± 0.61	1.69 ± 0.44	13.58 ± 3.63△△	12.80 ± 2.76△△	14.24 ± 2.81△△
	PLA	2.40 ± 0.68	2.09 ± 0.59	14.23 ± 2.56△△	13.42 ± 2.52△△	14.70 ± 2.26△△

Note. Values are mean ± SD.

Abbreviations: PYR, Group pyruvate; PLA, Group placebo; Pre-HIIE, pre-high-intensity interval exercise; Post-HIIE, post-high-intensity interval exercise; Pre-RSE, pre-repeated sprint exercise; Post-RSE, post-repeated sprint exercise

*: $p < 0.05$, compared to PLA; **: $p < 0.01$, compared to PLA; △: $p < 0.05$, compared to baseline; △△: $p < 0.01$, compared to baseline

Figures

Figure 1

Supplementation and exercise tests timeline

Figure 2

Energy contribution for each group (A-C); the percentage of aerobic and anaerobic energy contributions during HIIE (D). Values are mean $\pm$ SD. PYR, Group pyruvate; PLA, Group placebo; Bout, high-intensity exercise bout; the percentage of aerobic energy contribution = (aerobic energy/total energy) $\times$ 100; the percentage of anaerobic energy contribution = ((phosphagen energy + glycolytic energy)/total energy) $\times$ 100; *: $p < 0.05$, compared to PLA; #: $p < 0.05$ compared to bout 1; ##: $p < 0.01$, compared to bout 1; δδ, $p < 0.01$, difference between aerobic and anaerobic energy contributions

Figure 3

Blood pO₂ response during tests. Values are mean $\pm$ SD. PYR, Group pyruvate; PLA, Group placebo; Pre-HIIE, pre-high-intensity interval exercise; Post-HIIE, post-high-intensity interval exercise; Pre-RSE, pre-repeated sprint exercise; Post-RSE, post-repeated sprint exercise; *: $p < 0.05$, compared to PLA; β : $p < 0.05$ compared to baseline; $\beta\beta$: $p < 0.01$, compared to baseline

Figure 4

Average RPP, RAP and PD% during RSE (A). RPP, RAP and PD% during RSE for each sprint (B-D). Values are mean $\pm$ SD. PYR, Group pyruvate; PLA, Group placebo; S, sprint exercise; RPP, relative peak power; RAP, relative average power; PD%, power drop; *: $p < 0.05$, compared to PLA; Δ : $p < 0.05$, compared to S1; $\Delta\Delta$: $p < 0.01$, compared to S1