1. Introduction

Soccer is a high-intensity intermittent exercise which consists of two 45-minute halves separated by a 15-minute half-time period. As one of the most popular sport in the world, many studies focus scientific aspects in soccer. It is well known that frequency of goal scoring is high in the last 15-minute period of the second half, when players suffer from fatigue physically and mentally (Mohr et al., 2003, Armatas et al., 2007; Bradley et al., 2009). Therefore, it is important to maintain performance in this period for soccer players. The causes of fatigue in soccer match are complicated and may involve various mechanisms acting centrally and peripherally (Alghannam et al., 2012). One of the main causes of fatigue in soccer match is the depletion of energy source such as glycogen in muscle and liver (Rico-Sanz et al., 1999, Krustrup et al., 2006a). Many studies show the importance of energy intake in soccer using exercise protocols that replicate soccer match (Currell et al., 2008; Alghannam et al., 2011, Phillips et al., 2011, McGawley., 2012, Kingsley et al., 2014). Goedecke et al. reported that carbohydrate ingestion is useful to maintain performance using Loughborough Intermittent Shuttle Test (LIST), a representative soccer-match simulation protocol (Goedecke et al., 2013). The primary purpose of soccer-match simulations such as LIST is to control movement patterns and standardize physiological demands, resulting that the variability in physiological reactions is limited and the consequence of exercise becomes repeatable (Nicholas et al., 2000). However, simulation protocols do not replicate soccer match completely because they include no physical contacts among players and no gamesmanships. Especially, physical contacts in sports distress players (Johnston et al., 2014). Therefore, player’s fatigue observed in soccer-match simulations might be less than that in actual soccer games. It is more desirable to investigate...
precisely the effect of energy intake on performance in soccer players using actual soccer games rather than simulation protocols.

In this study, we evaluated the practical effect of energy intake on performance in soccer players using designed game-style exercise, which is the combination of actual soccer match play and the Yo-Yo intermittent recovery test (Krstrup et al., 2006b). For energy supply, a mixture of carbohydrate and two glucogenic amino acids, alanine and proline, was used.

2. Methods

2.1. Subjects

Four forwards, eight midfielders and eight defenders (total of 20 players) were recruited and divided into two teams (age: 20.7 ± 0.7 years; height: 171.5 ± 5.4 cm; body mass: 66.8 ± 7.0 kg; percent body fat: 11.4 ± 2.5%). For soccer match play, 2 goalkeepers were prepared. In case of subjects’ dropout, the reserve members participated the soccer match play instead for 11-a-side game. The exclusion criteria included the presence of injuries that make it difficult to perform the study protocol.

The study protocol was approved by the Ethics Review Board of the Japan Institute of Sports Sciences. Written informed consent was obtained from all subjects.

2.2. Test foods

Test foods and control foods were provided as jelly drinks. The test food contained 40.3 g of dextrin, 4.5 g of alanine, and 0.5 g of proline. Dextrin (TK-16/H-PDX), which is a glucose polymer, was purchased from Matsutani Chemical Industry (Hyogo, Japan). Alanine and proline were obtained from Ajinomoto Co., Inc. (Tokyo, Japan). The mass of the test food was 130 g, and the energy content was 180 kcal (high-carbohydrate amino acid-containing food, high-CHO + AA). As a control food, a jelly drink containing 3.6 g of dextrin was prepared. The control food weighs 130 g and contains 19 kcal (low-carbohydrate food, low-CHO). The compositions of the test food and the control food are presented in Table 1. These two foods were packed in a white pouch to ensure that they had no differences in the appearance, taste, and texture. Both foods were grapefruit-flavored and consisted of a yellow, soft gel. After numbering and labeling, the foods were provided to the test facility in a randomized and blind manner.

2.3. Protocol of the experiment

The study design was double-blind crossover. Subjects performed 2 trials separated by a 1-week interval and the order of these trials was randomized. The two trials were conducted during the 2nd and 3rd week of December, respectively. The temperature on the 1st and 2nd trial days was 9.8 ℃ and 5.1 ℃, respectively. The humidity on the 1st and 2nd trial days was 63% and 69%, respectively. At each trial, subjects ingested either the high-CHO+AA or the low-CHO food.

On the day of experiment, the subjects were instructed to eat the same packed lunch box. They finished lunch before 13:00, and after lunch, they were instructed not to eat anything but water. The study started at 16:30. Initially, blood was collected. The subjects rested for 5 min or more after blood collection. Subsequently, they participated in a warm-up period for 20 min, and each subject then ingested test food. After a 15-min rest period, soccer match

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<tr>
<th></th>
<th>high-CHO + AA</th>
<th>low-CHO</th>
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<tr>
<td>Mass (g)</td>
<td>130</td>
<td>130</td>
</tr>
<tr>
<td>Energy (kcal)</td>
<td>180</td>
<td>19</td>
</tr>
<tr>
<td>Dextrin (g)</td>
<td>40.3</td>
<td>3.6</td>
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<tr>
<td>Alanine (g)</td>
<td>4.5</td>
<td>0</td>
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<td>Proline (g)</td>
<td>0.5</td>
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Table 1 Compositions of the test foods. high-CHO + AA indicates high-carbohydrate amino acid-containing food, and low-CHO indicates low-carbohydrate food.
play (exercise loading) for 45 min as the first half of the game was conducted. The half-time duration was 15 min, and one more test food was ingested at the beginning of the half time. Additionally, soccer match play for 30 min as the second half of the game was conducted. The reason why the second half is 15 min shorter than that of standard soccer game is to evaluate performance during the final phase of a game by the Yo-Yo intermittent recovery test (Level 2). After the soccer match play, blood was collected again. Subsequently, exercise performance was measured by the Yo-Yo test. In addition, subjective exercise intensity (ratings of perceived exertion; RPE) surveys were conducted after the soccer match play and the Yo-Yo test, respectively. The running distance during soccer match play was measured using a GPS-type measuring system.

Subjects performed another Yo-Yo test without test food ingestion to assess the basal Yo-Yo test score on a different day 2 months before the experiment. In that case, blood collections, RPE surveys and measurements of running distance during soccer match play were not conducted.

The experimental protocol is shown in Figure 1.

2.4. Blood tests

Blood was collected before the warm-up period and after the soccer match play. 5 mL of blood per session (10 mL in total per trial) was collected through a median basilic or cephalic vein by a nurse. A 5-min or more rest period was followed the blood collection. After hemostasis was confirmed, subjects performed exercise. Hematological parameters included blood glucose, creatine phosphokinase (CPK), creatinine, glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), insulin and lactic acid levels.

2.5. Yo-Yo test

After the soccer match play, the subjects rested for 10 min. The Yo-Yo intermittent recovery test (Level 2) was then performed as described below (Bangsbo et al., 1991; Bangsbo et al., 2008). The Yo-Yo test consisted of repeated two 20-m runs at a progressively increased speed controlled by audio bleeps from a tape recorder. Between each running bout the subjects had a 10-sec rest period. When the subjects failed to reach the finishing line in time twice, the distance covered was recorded and represented the test result.

2.6. RPE

After the soccer match play and the Yo-Yo test, RPE surveys were conducted respectively. RPE was evaluated using 15 grades (6: easy to 20: hard).

2.7. Running distance during soccer match play

The running distance during soccer match play was measured using a GPS-type running distance measuring system (Sports Recorder BT-Q1300S, QSTARZ, Taipei, Taiwan). The systems were attached to the upper arm of the subjects. The running distance was calculated in units of 100 m.

2.8. Test location

The experiments were conducted on the football field (artificial turf) and in the club house of the soccer team.

2.9. Statistical analysis

The results are expressed as the means ± standard error (SE). A one-way analysis of variance (ANOVA) followed by Tukey test was performed to determine the difference between the 3 scores of the Yo-Yo test. The data of blood markers and RPE were analyzed by
a two-way repeated ANOVA. A \( p \)-value of less than 0.05 (two-tailed) was considered to indicate statistical significance.

3. Results

3.1. Review of subjects

Four subjects did not correctly completed the experiments due to injuries and bad conditions. As a result, the data of 16 subjects were analyzed.

3.2. Yo-Yo test score

The Yo-Yo test scores are shown in Figure 2. The basal score without test foods was 710 ± 73 m. The Yo-Yo test score after soccer match play in subjects ingested the high-CHO + AA food was 870 ± 55 m, which significantly increased compared to the basal score \((p < 0.05)\). The Yo-Yo test score with high-CHO + AA food was also significantly higher than that with low-CHO ingestion \((760 ± 59\ m, \text{ difference: }110\ m)\).

3.3 Blood parameters

The results of blood tests are shown in Table 2. Blood glucose level significantly increased after the game. Blood glucose level in the high-CHO+AA group were significantly higher compared to the low-CHO group. The interaction was not observed in blood glucose level. Insulin level significantly decreased after the game. Insulin level in the high-CHO+AA group was significantly higher than that in the low-CHO group. There was no interaction in insulin level. The levels of CPK, creatinine, GOT, GPT, and lactic acid after soccer match play were significantly higher than the pre-game values. Interaction was observed in CPK. Simple main effects of test food on CPK level at pre-game and post-game were not significant \((p = 0.215, \text{ post-game: } p = 0.081)\).

3.4. The running distance during the soccer game

There was no significant difference between the running distances in the high-CHO + AA and low-CHO groups \((7.8 ± 0.1 \text{ km vs. } 7.8 ± 0.2 \text{ km, respectively})\).

3.5. RPE surveys

The RPE scores after the Yo-Yo test were significantly higher than those after the game \((p < 0.001)\, \text{the RPE values before and after the Yo-Yo test were } 16.9 ± 1.8 \text{ and } 18.3 ± 0.6 \text{ in the high-CHO + AA group and } 17.2 ± 1.8 \text{ and } 18.1 ± 0.6 \text{ in the low-CHO group, respectively). The main effect of test food was not observed } (p = 0.576). \text{ There was no interaction } (p = 0.296).
4. Discussion

In this study, we applied the designed game style method to evaluate the practical effect of energy intake on performance in soccer players. A 75-min soccer-style exercise was performed to produce the same load on the subjects as a real soccer game. Given that the running distance may differ among subjects playing a real soccer game, the correctness of this protocol must be validated. The running distances during the game in the high-CHO + AA and low-CHO groups were comparable. With regard to markers for muscle damage (GOT, GPT and CPK) (Lehmann et al., 1991, Saunders et al., 2004), there were no significant differences between the two groups. These results suggest that the intensity of exercise during the game was likely maintained at a constant level, and the Yo-Yo tests were likely conducted under the same conditions. For this reason, we consider the protocol in this study to be appropriate. As is the case with most soccer players, the subjects routinely perform exercise during this study as the exercise of their football club. That may be why plasma CPK levels in both groups before the game were almost elevated above normal. In this context, the present study was conducted under a realistic situation.

In the present study, high-energy intake (360 kcal in total) before and during 75-min soccer game improved the Yo-Yo test score by 14% compared to that in low-energy intake (38 kcal in total) group. Alghannam et al. previously reported that CHO drink (approx. 280 kcal) showed a 49% improvement of the time to exhaustion using a simulation protocol (Alghannam et al., 2011). The performance improvement observed in this study was less despite more energy intake. One possible reason for this is

<table>
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<tr>
<th>Table 2 Blood levels of glucose, CPK, creatinine, GOT, GPT, insulin and lactic acid before and after soccer match play (n=16).</th>
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<tr>
<td>Blood glucose (mg/dL)</td>
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<td>Pre-game</td>
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<td>(NR: 70-109)</td>
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<td>Post-game</td>
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<tr>
<td>CPK (U/L)</td>
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<td>Pre-game</td>
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<tr>
<td>(NR: 62.0 – 287.0)</td>
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<td>Post-game</td>
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<tr>
<td>Creatinine (mg/dL)</td>
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<td>Pre-game</td>
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<tr>
<td>(NR: 0.61 – 1.04)</td>
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<td>Post-game</td>
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<tr>
<td>GOT (U/L)</td>
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<td>Pre-game</td>
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<tr>
<td>(NR: 10.0 – 40.0)</td>
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<td>Post-game</td>
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<tr>
<td>GPT (U/L)</td>
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<td>Pre-game</td>
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<tr>
<td>(NR: 5.0 – 40.0)</td>
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<td>Post-game</td>
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<td>Insulin (pmol/L)</td>
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<td>Pre-game</td>
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<tr>
<td>(NR: 13.2 - 87.6)</td>
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<td>Post-game</td>
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<tr>
<td>Lactic acid (mmol/L)</td>
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<td>Pre-game</td>
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<tr>
<td>(NR: 0.56 - 1.39)</td>
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<td>Post-game</td>
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</table>

The values are represented as the mean ± SE. High-CHO + AA indicates high-carbohydrate amino acid-containing food, and Low-CHO indicates low-carbohydrate food. NR: normal range, CPK: creatine phosphokinase, GOT: glutamic oxaloacetic transaminase, GPT: glutamic pyruvic transaminase
physical contacts. Physical contacts are included in the exercise of the present study but not in the previous study. Johnston et al. investigated the influence of physical contacts on muscle fatigue by small-sided rugby games (Johnston et al., 2014). In that study, physical contacts elevated plasma CPK activity and reduced upper body power following the games. Moreover, running distance during contact games was significantly decreased compared to non-contact games in the same study. These results suggest that physical contacts cause increased fatigue and performance decline in players. Therefore, players in the present study experienced considerable fatigue due to physical contacts during soccer match play, resulting in more energy requirement to keep their performance. That might be the reason why the effect of energy intake on performance in the present study was less than that in the previous study by Alghannam. Furthermore, the difference in improvement by energy intake might resulted from difference in the method of performance test. Alghannam’s study used a running test at constant velocity (at 80% VO\(_2\)max), although the running velocity gradually accelerates and the heart rate increases to 99% HRmax in the Yo-Yo test (Bradley et al., 2009).

It is clear that most soccer players develop fatigue during the final phases of a game (Mohr et al., 2003, Bradley et al., 2009). Mohr et al. reported that the running distance during the final 15-min period of a soccer match was about 150 m shorter than that during other 15-min periods (estimated from the figure in the article) (Mohr et al., 2010). Therefore, to maintain performance until the end of a game is one of the critical challenges for soccer players. The increase of Yo-Yo test score in the present study was 110 m, which is corresponding to more than 70% of the running distance decrease in the final 15-min period in soccer match, suggesting that high-CHO+AA ingestion can mostly prevent the decrease of running distance in the final stage of a soccer game. Most of the sprint distances by players in soccer games are less than 20 m. Therefore, players who intake high-CHO+AA are expected to make five more sprints during the final phases of a game. This may provide significant advantage because repeated-sprint ability is regarded as an important fitness component for superior performance in soccer (Dellal et al., 2011, Girard et al., 2011).

In this study, the mixture of carbohydrate and two glucogenic amino acids, alanine and proline, was used as energy source. Carbohydrate definitely improves the performance in this study because many reports demonstrate that carbohydrate ingestion can reduce fatigue during intermittent exercise (Jeukendrup et al., 1997; Welsh et al., 2002; McGawley., 2012). Huq et al. reported the continuing decrease in serum concentrations of alanine and proline during endurance exercise, suggesting the importance of these amino acids for exercise (Huq et al., 1993). Nogusa et al. demonstrated that a mixture of 1.0 g/kg of maltodextrin and 1.0 g/kg of alanine and proline significantly prolonged the time to exhaustion compared to 2.0 g/kg of maltodextrin alone in mice (Nogusa et al., 2014). Therefore, these amino acids also may contribute to the performance improvement to some extent in this study. However, it is difficult to evaluate the effect of amino acids on soccer performance in the present study due to lack of the suitable control groups, that is, high-CHO without amino acids group. Further studies are needed to reveal the contribution of alanine and proline to the performance improvement observed in this study. Moreover, the studies for the effect of the high-CHO+AA on performance using soccer-match simulation will be necessary to clarify the significance of this study.

5. Conclusion

This study demonstrated the practical effect of energy intake on the performance in soccer game using the combination of actual soccer game and performance test. Energy intake (360 kcal in total) may increase running distance by 110 m in the final phase of the soccer game.

References


tool for evaluation of physical performance in intermittent sports. Sports Medicine, 38: 37-51.


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Conflict of interest
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