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Effect of carbohydrate-protein supplement on anaerobic capacity after physical activity

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ABSTRACT

The study examined how carbohydrate-protein (CHO-PROT) beverages affect anaerobic capacity after exercise. Fifteen male university football players, aged 20–25, participated in a 90-minute cycling session while consuming one of three drinks: CHO-PROT, carbohydrate-only (CHO), or a placebo (PLA). Results analyzed with two-way repeated measures ANOVA and pairwise comparisons (Bonferroni's method, p<.05) showed that the CHO-PROT group experienced less decline in peak power during and after exercise compared to the PLA group (p<.01). Both CHO-PROT and CHO groups showed smaller reductions in mean power at the 90th minute of exercise compared to the PLA group (p<.01), and the CHO-PROT group maintained better mean power 24 hours after exercise (p<.05). Blood glucose levels increased more in the CHO-PROT and CHO groups than in the PLA group at all times (p<.01). The CHO group also showed a smaller drop in blood glucose at the 90th minute of exercise compared to the PLA group (p<.01). No significant differences were found in blood lactate levels between groups. Muscle soreness was lowest in the CHO-PROT group and lower in the CHO group compared to the PLA group at all times (p<.01). In summary, CHO-PROT drinks improved anaerobic performance, raised blood glucose levels, and reduced muscle soreness, with no effect on blood lactate levels.

1. Introduction

The ratio of energy used in a football match is 70% generated by the anaerobic system and the other 30% by the aerobic system (Bahtra et al., 2023). Throughout the entire 90 minutes of the match, the athletes' average moving distance is 10–13 kilometers. It was discovered that players consistently exhibit behaviors such as moving at their highest possible speed, jumping, changing direction, moving while in possession of the ball, evading opponents' attempts to mark them, and intercepting the ball. All of those actions are dependent on the energy generated by the anaerobic system. The anaerobic system produces energy through the metabolism of glycogen in the muscles, or the glycolysis system, which requires the breakdown of branched-chain amino acids in the muscles (Hargreaves and Spriet, 2020). In addition, athletes utilize energy obtained from carbs and free fatty acids in the bloodstream at a comparable proportion while walking or jogging. Protein does not primarily serve as an energy source for the body, unlike carbohydrates and fats. Nevertheless, it is capable of conversion into an intermediary chemical within the Krebs cycle and thereafter be utilized to produce fuel or glucose through the gluconeogenesis pathway (Ling et al., 2023). Protein has unique characteristics as a result of the branched-chain amino acids (BCAAs) including leucine, isoleucine, and valine. These amino acids can be metabolized to provide energy for the muscles, which is approximately 10–15 percent of the body's total energy requirement (Doma et al., 2021).

Nowadays, it is popular to consume carbohydrate and carbohydrate-protein beverages in sports competitions. This practice aims to increase energy, endurance, and performance duration while reducing tiredness during physical activity (Gervasi et al., 2020) and recovering muscles after exercise (Dahl et al., 2020). Supplements containing 6–8% carbohydrates can enhance athletic performance during training or competition by minimizing dehydration, replenishing electrolytes depleted by perspiration, and providing energy to the muscles to reduce glycogen use (Tambalis, 2022). In addition,

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they also prevent a decrease in blood glucose levels (Hulton et al., 2022), extend the duration of physical activity, and quickly restore physical fitness after training or competition (Kaviani et al., 2020). Football athletes are frequently advised to consume carbohydrate-protein beverages for energy storage. These supplements facilitate muscular energy production, promote muscle growth, help in protein synthesis, and assist in muscle recovery post-competition (Dobrowolski et al., 2020). Athletes participating in activities that involve moderate to high energy consumption often rely on protein supplements, commonly consuming around 1.2–2.2 grams per kilogram of body weight per day (Raizel et al., 2019). Research has shown that taking carbohydrate-protein supplements before engaging in resistance exercise or marathon running leads to higher levels of protein in the blood, which can be quickly broken down to provide energy for the muscles. Consuming an adequate amount of BCAAs might effectively inhibit the breakdown of other amino acids in the muscles, hence reducing muscle fiber catabolism (Goldstein et al., 2023). As a result, this can result in a decline in muscular injuries, a quicker recovery of muscles to their usual state, a decrease in the sensation of muscle soreness, and a reduction in the buildup of lactate in the bloodstream. At the same time, consuming a carbohydrate-protein drink before and during physical activity uses energy derived from the metabolism of carbohydrates and protein components. It affects the increase in blood glucose levels and limits the release of insulin during exercise (Malone et al., 2021). Hence, it is possible that the intake of carbohydrate-protein beverages affects the anaerobic capacity, reduces muscle injury, and enhances muscle recovery after physical activity. Therefore, the researcher aimed to examine the impact of carbohydrate-protein supplements on anaerobic capacity after physical activity. The research hypothesis is that consumption of carbohydrate-protein drinks results in improved anaerobic capacity and increased metabolism of nutrients from the beverage received.

2. Methods

This research is an experimental research design that has passed human research ethics certification by Thailand National Sports University at SCI 015/2021 on September 28, 2022.

2.1. Populations and samples

Populations: The population used in this study was 60 football players, aged between 20 and 25 years, of the National Sports University, Chaiyaphum Campus, academic year 2022, who all had the same study schedule, meals, daily tasks and stayed in the athlete dormitory. They were supervised and under the rules and regulations of the university.

Samples: In order to identify the sample group in the formula and develop a sample group that is a good representative of the population, the researcher studied related research documents to acquire information for determining the effect size, power of the test, and statistical significance level (α). Purposive sampling was used to choose the sample group, which consisted of 15 individuals. The researcher conducted the following measures:

1. To determine the maximum oxygen consumption (VO_2max), the researcher gathered a sample group who voluntarily participated in the test.
2. Using criteria from Schantz et al. (2022), the VO_2max test was performed in line with the "Astrand" Per-Olof procedure, and the VO_2max values were ranked from highest to lowest.
3. The first 15 samples with the highest VO_2max were chosen by the researcher to participate as the sample group for this research.

2.2. Sample size calculation

Using Crossover Design's sample size calculation and referencing the study by Sollie et al. (2018), the change in the mean power output (Watts) before and after the experiment was recorded as 1068.00 and 1000.00, respectively. The standard deviations (SD) before and after were 50.00 and 52.00, respectively. Based on this data, the sample size calculation was performed as follows:

$$n = \frac{\left(Z_{\frac{\alpha}{2}} + Z_{\beta}\right)^2 \times \delta}{\triangle^2}$$

where, n is the sample size per group. α is the significance level. $Z\alpha/_2$ is the Z-score for a two-tailed test at α level. β is type II error probability. Z_β is the Z-score for β level. δ is the variance estimate. Δ is effect size or difference in mean.

Using the above equation, this research therefore used a sample of 12 people. However, to account for potential dropouts or withdrawals from the study, the total sample size was increased to 15 participants.

2.3. Inclusion, exclusion, and discontinuation criteria

2.3.1. Inclusion criteria

1. The participants were recruited voluntarily and provided written informed consent prior to their inclusion in the study.
2. All participants maintained a consistent training program, exercising a minimum of 3days/week.
3. Each participant underwent a thorough medical evaluation, confirming the absence of chronic conditions that could interfere with the research outcomes. They were healthy, free from medication use, and without chronic diseases such as cardiovascular conditions, hypertension, diabetes, or long-term musculoskeletal injuries.

2.3.2. Exclusion criteria

1. Participants either chose not to continue volunteering or decided to withdraw from the research study.
2. Participants encountered unforeseen circumstances, such as health issues or injuries sustained during daily or training activities, which prevented them from continuing their participation in the research.

2.3.3. Discontinuation criteria

1. During the experiment, some participants experienced side effects or allergic reactions to the beverage they were given, such as diarrhea. However, this study utilized both the type and quantity of beverages that previous research has shown to be free of adverse effects on the body (Gervasi et al., 2020).
2. Some participants did not adhere to the study protocol as agreed upon in the research agreement established between the researchers and participants.

2.4. Measurement tools/research instruments

1. Weight scale, TANITA brand, model TBF-531 A.
2. Height scale, FBT brand.
3. Digital blood pressure and resting heart rate monitor (Automatic Blood Pressure Monitor), OMRON brand, model SEM-1.
4. Perceived muscle soreness form, with a reliability of 0.78 based on Magoffin et al. (2020)
5. Bicycle ergometers: Monark brand, model 828 E, and Monark brand, model 894 E, equipped with the Wingate Anaerobic Test (WanT) program.
6. Bicycle ergometer, Monark brand, model 828 E.
7. Portable blood glucose analyzer, ACCU-CHEK Performa (version I).
8. Portable blood lactate analyzer, Lactate Scout.

2.5. Research procedures

1. Content organization and validation: The researcher organized the content of sports drinks and exercise patterns and checked content validity. An exercise pattern appropriateness assessment form was created, and its content validity was evaluated by three experts in exercise physiology, physical education major in sports science. The Index of Objective Congruence (IOC) value obtained was 0.92. Exercise patterns were tested with five individuals who were not football players and not part of the sample group (Pilot Study). Based on the test results, revisions were made before collecting data with the actual sample groups.
2. Volunteer recruitment and consent: Volunteers were recruited and selected based on specified criteria. The researcher met with the sample group, explained the research project in detail, and obtained signed consent forms from volunteers who agreed to participate.
3. Preparation of beverages: Three types of drinks were prepared: Carbohydrate-protein (CHO-PROT) drink, carbohydrate (CHO) drink, and placebo (PLA) drink. A total of 135 bottles of these drinks (45 bottles of each type) were prepared. After preparation, the drinks were separated and stored in the refrigerator, with the total number of bottles matching the number of times each volunteer would consume a drink.

a. Drink preparation and distribution: The researcher was responsible for preparing the drinks and explaining the experimental procedures and distribution to the research assistant. The research assistant administered the drinks to the volunteers during the experiment and had no influence on the experimental data. This study utilized a randomized, double-blind, crossover design, ensuring that neither the researcher nor the volunteers knew which beverage was being administered at each session.
b. Experimental procedure: A sample of 15 participants was randomly assigned to receive each type of drink: CHO-PROT, CHO, and PLA. Following Dahl et al. (2020), participants consumed the drink three times: First and second times 30 minutes before exercise and immediately after exercise at the 90th minute, with each serving being 250 ml. The third serving, an hour after exercise, was 500 ml as follows:

- Carbohydrate-protein drink: This drink contained 60 grams of total ingredients: 44 grams of glucose, 4 grams of isoleucine, 8 grams of leucine, 4 grams of valine, 1,000 ml of water, 60 milligrams of sodium, and 5 milliliters of lemon flavoring, with an osmolality of 320 mOsmol/kg.
- Carbohydrate drink: The drink contained 60 grams of glucose, 60 milligrams of sodium, 5 milligrams of lemon flavoring, and had an osmolality of 300 mOsmol/kg.
- PLA drink: This drink consisted of 1,000 milligrams of plain water, 60 milligrams of sodium, 5 grams of artificial sweetener, 5 milligrams of lemon flavoring, and had no effect on energy provision, with an osmolality of 295 mOsmol/kg.

4. Experiment duration and schedule: The sample group of 15 participants engaged in the experiment for 3 weeks, with sessions occurring twice a week: The first day lasting 180 minutes and the following day 45 minutes. Each participant received all three types of drinks across different experimental groups, with 5 people per group (Table 1).

Table 1 shows the distribution of drink intake among the sample groups. Participants were randomly assigned to receive the beverages before and after exercise. The experiment employed a randomized, double-blind, crossover design, ensuring an equal number of participants (5 per group) in each experiment.

2.6. Research methodology

1. On the first day of the experiment, participants underwent measurements including weight, height, heart rate, blood sugar level, blood lactate level, perceived muscle soreness, peak power, and mean power. Their VO_2max was assessed by cycling on a Monark 828 E-bike while using a Portable Cardiopulmonary Gas Exchange System (Cortex; Metamax 3B). The maximum workload was determined using the "Astrand" Per-Olof protocol with reference criteria from Schantz et al. (2022). Based on each participant's maximum cycling ability, 75 percent of their VO_2max was used to set the intensity for the 90-minute cycling experiment.
2. Three days prior to the study, participants were instructed to avoid strenuous exercise and activities that might cause muscle damage. They were also asked to refrain from eating or drinking anything other than plain water for 3 hours before the research.
3. Participants began the research by arriving at the physical fitness test room at 4:00 p.m. They rested for 20 minutes while measurements were taken for weight, heart rate, blood sugar level, and blood lactate level. Subsequently, they consumed 250 ml of a randomly selected beverage and rested for 30 minutes. At 5:00 p.m., participants cycled for 90 minutes at 75 percent of their VO_2max. They consumed 250 ml of the same beverage again, at the 90^{th} minute of exercise and 500 ml one hour after exercise. Peak power and mean power were assessed 24 hours post-exercise.
4. Research results were recorded as follows:

a. Measuring the maximum power output (peak power) and mean power output from the Wingate Anaerobic Test (WanT), recorded at 30 minutes before exercise, 90 minutes after exercise, and 24 hours after exercise.
b. Blood glucose levels were measured using a portable blood glucose analyzer at 30 minutes before exercise, immediately before exercise, during 45 minutes of exercise, at 90^{th} minutes of exercise, 30 minutes after exercise, and 60 minutes after exercise.
c. Blood lactate levels were measured using a portable blood lactate analyzer at immediately before exercise, during 45 minutes of exercise, at 90^{th} minutes of exercise, 30 minutes after exercise, and 60 minutes after exercise.
d. Muscle pain perception was assessed using a perceived muscle soreness evaluation form at 0 minutes before exercise, during 45 minutes of exercise, at 90^{th} minutes of exercise, 30 minutes after exercise, 60 minutes after exercise, and 24 hours after exercise.

5. Participants rested for at least 7 days before repeating the study twice more, following the same procedures outlined in items 2-4 but with different beverages.
6. To minimize the risk of illness, blood samples were collected using a fingertip sampling method by a sports scientist from the participant's middle or ring finger, with a maximum of 6 microliters collected at a time. Blood tests were performed using a portable blood glucose meter and a portable blood lactate meter (Lactate Plus).

2.7. Data processing and analysis

The research's data analysis examines basic statistics, such as mean and SD, differences in peak power, mean power, blood glucose, blood lactate, and perceived muscle soreness among the groups that consumed different beverages at different periods, using two-way repeated measurement and one-way ANOVA to compare the differences between the groups at specific periods. Additionally, comparing differences in pairs using Bonferroni's method. The statistical significance value was set at .05 ($p<.05$).

Table 1: Displays the grouping of beverage intake for the experiment

Sample groups (people)	1st experiment	2nd experiment	3rd experiment
5	CHO-PROT	CHO	PLA
5	PLA	CHO-PROT	CHO
5	CHO	PLA	CHO-PROT

3. Results

Table 2 showes that the general characteristics of football athletes include body weight and height within the standard range, normal body mass index (BMI), normal resting heart rate, and normal blood pressure during both the systolic and diastolic phases. Their maximal oxygen consumption (VO_2 max) was good. According to a questionnaire, the sample group exercises three days per week, and they had experience in football competitions last year. The test compared the differences, including the peak power, anaerobic capacity (mean power), blood glucose levels, blood lactate levels, and perceived muscle soreness. The samples were tested before exercise into three groups: the group that received the carbohydrate-protein beverage, the carbohydrate beverage, and the placebo beverage. The variance differences were analyzed by using one-way ANOVA, and the result was no statistically significant differences between the groups before the experiment.

This experiment stated that the peak power before exercise was no different in the three groups (Fig. 1). However, the comparison of the peak power of the three groups found that the carbohydrate-protein beverage group was significantly higher than in the placebo group, with a statistical significance level of.01 among exercises on the 90th minute. The result on the 90th minute of exercise and 24 hours after exercise had a different statistical significance level of .01 (p<.01).

Table 2: General characteristics of the football athlete sample group (N=15)

General characteristics of the sample group	Mean	SD
Age (year)	20.27	0.80
Body weight (kilogram)	66.45	2.94
Height (meter)	170.00	4.03
Body mass index (kilogram per meter^2)	22.96	1.37
Resting heart rate (beats per minute)	76.67	5.29
Resting systolic blood pressure (mmHg)	118.27	5.08
Resting diastolic blood pressure (mmHg)	77.67	5.81
Maximal oxygen consumption (milliliter per kilogram per minute)	48.94	2.52

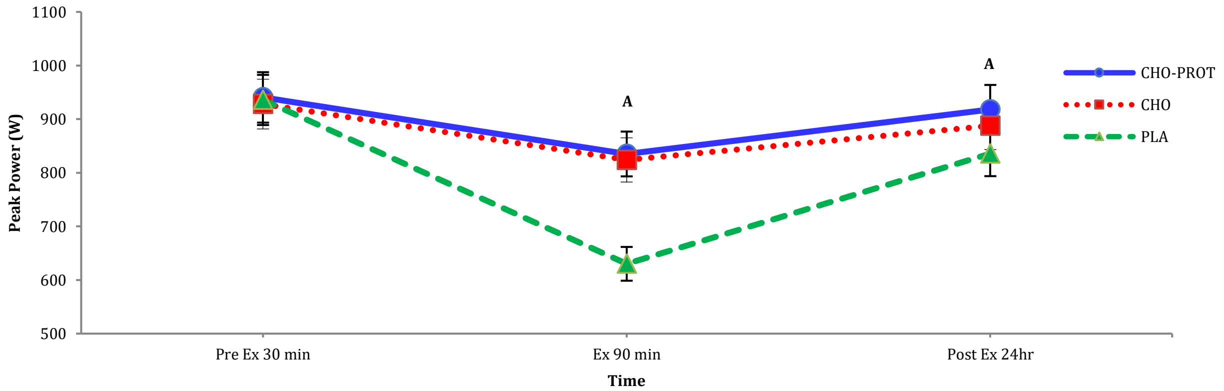

Fig. 1: Differences in peak power (watts) among groups receiving carbohydrate-protein, carbohydrate, and placebo beverages; A = Significant difference between the carbohydrate-protein and placebo groups (p < .01)

This experiment found that anaerobic capacity (mean power) before exercise among the three groups of football athletes was not different (Fig. 2). However, when comparing the differences between the groups, the carbohydrate-protein beverage group and the carbohydrate beverage group showed significant differences from the placebo group at the 90th minute of exercise, with statistical significance at the .01 level (p<.01). Additionally, the difference between the carbohydrate-protein beverage group and the placebo group 24 hours after exercise had statistical significance at the .05 level (p<.05).

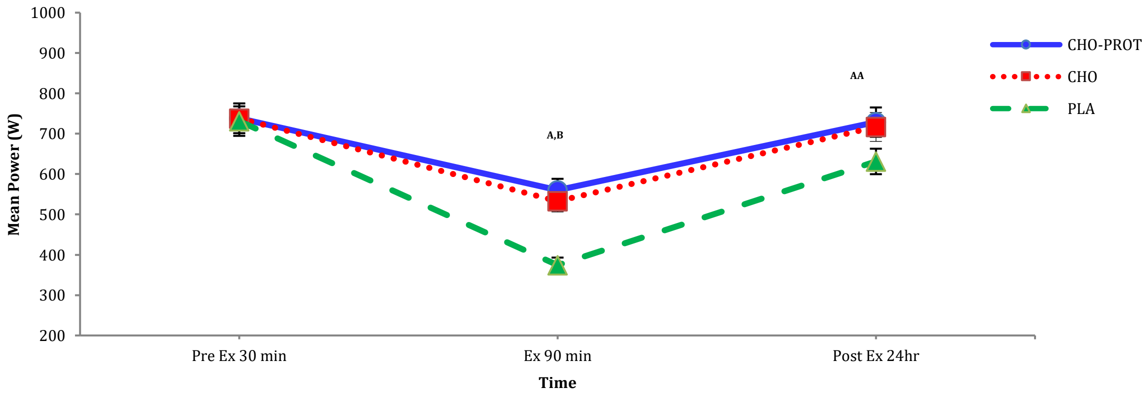

Fig. 2: Differences in mean power (watts) among carbohydrate-protein, carbohydrate, and placebo groups; A = Significant difference between carbohydrate-protein and placebo (p < .01), B = Carbohydrate and placebo (p < .01), BB = Carbohydrate and placebo (p < .05)

This experiment found that the blood glucose levels before exercise among the three groups of football athletes were not different (Fig. 3). However, when comparing the differences between the groups, the group receiving the carbohydrate-protein beverage and the group receiving the carbohydrate beverage had significantly higher increases in blood glucose levels than the placebo group, with statistical significance at the .01 level (p<.01). This difference was tested before exercise while exercising for 45 minutes, and after exercise for 30 and 60 minutes. Additionally, the carbohydrate beverage group experienced a significantly smaller decrease in blood glucose levels compared to the placebo group at 90 minutes into exercise, with statistical significance at the .01 level (p<.01).

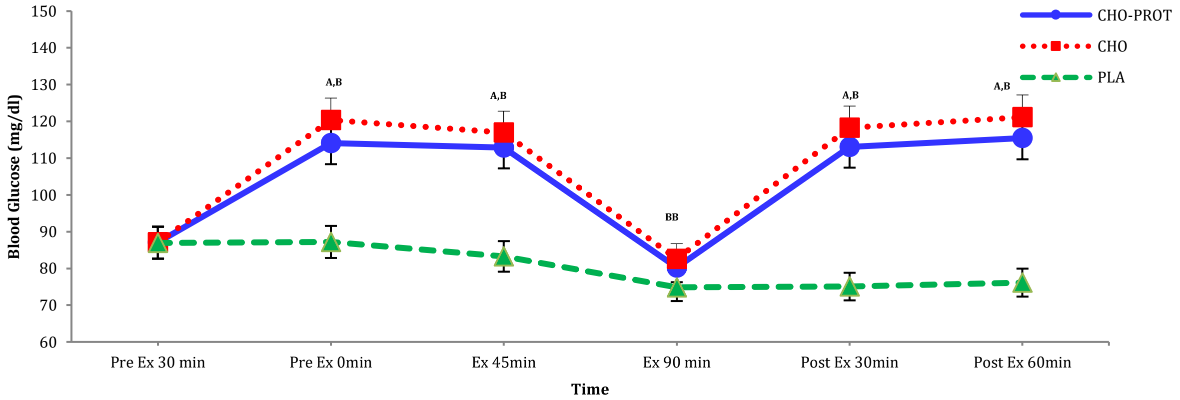

Fig. 3: Differences in blood glucose levels (mg/dL) among carbohydrate-protein, carbohydrate, and placebo groups; A = Significant difference between carbohydrate-protein and placebo (p < .01), B = Carbohydrate and placebo (p < .01), BB = Carbohydrate and placebo (p < .05)

This experiment stated that the blood lactate levels before, during, and after exercise of the three groups including; the carbohydrate-protein beverage group, the carbohydrate beverage group, and the placebo group were not different significantly (Fig. 4). However, it was observed that blood lactate levels reached their peak at the 90th minute of exercise.

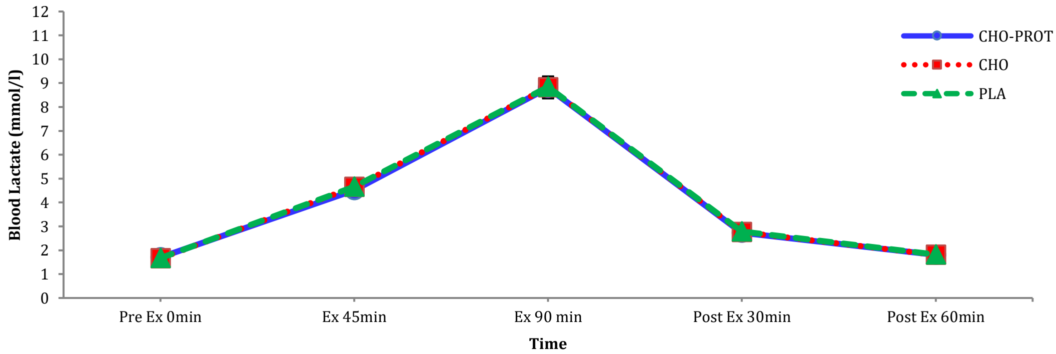

Fig. 4: Differences in blood lactate levels (millimoles per liter) between the carbohydrate-protein beverage, carbohydrate beverage and the placebo beverage

Before exercise, there was no significant difference in perceived muscle soreness among the three groups of football athletes (Fig. 5). However, after exercise, statistically significant differences (p < .01) were observed between the carbohydrate-protein and placebo groups, the carbohydrate and placebo groups, and the carbohydrate-protein and carbohydrate groups. The carbohydrate-protein group experienced the smallest increase in perceived muscle soreness compared to both the carbohydrate and placebo groups at all time points. Additionally, the carbohydrate group showed a smaller increase in perceived muscle soreness than the placebo group at every time point.

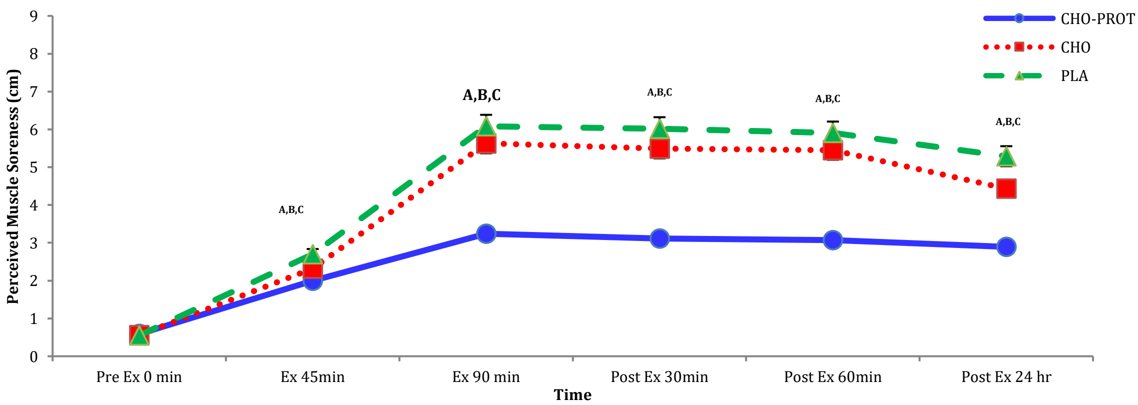

Fig. 5: Differences in perceived muscle soreness (cm) among carbohydrate-protein, carbohydrate, and placebo groups; A = Significant difference between carbohydrate-protein and placebo (p < .01), B = Carbohydrate and placebo (p < .01), C = Carbohydrate-protein and carbohydrate (p < .01)

4. Discussion

4.1. Peak power (watts)

Consuming a carbohydrate-protein drink resulted in a lower decrease in peak power than drinking a carbohydrate drink and placebo water. Exercising for over an hour consumes energy from the aerobic system, which is responsible for metabolizing carbohydrates, fats, and proteins in the oxidative system. As these resources deplete, the body switches to the anaerobic system, which comprises two subsystems: The ATP-CP system (also known as the adenosine triphosphate-phosphocreatine system) and the glycolysis system.

Carbohydrate-protein beverage consumption induces glucose to be broken down through the glycolysis pathway, which signifies that cells absorb glucose as energy sources, reflected by peak power (Russo et al., 2021).

The breakdown of glucose, which involves numerous processes and is catalyzed by enzymes, produces pyruvate as a final product, which is found in oxygen-containing cells. Pyruvate is transported to the mitochondria and metabolized to carbon dioxide and acetyl CoA. In the Krebs cycle, acetyl CoA is combined with oxaloacetate; Oxaloacetate (OAA) and transforms to citrate, which is then converted to isocitrate, succinyl CoA, fumarate, malate, and finally returned to oxaloacetate; OAA. Acetyl groups are broken down in the Krebs cycle and exported as Nicotinamide adenine dinucleotide (NADH) and Flavin adenine dinucleotide (FADH2), with the final product being adenosine triphosphate (ATP) via the electron transport chain (ETC). The Krebs cycle is essential for aerobic fitness because it integrates other nutrients to generate glucose when the body is deficient in glucose and energy and when cells need it for exercise (Manaf et al., 2021). Thus, the anaerobic system breaks down a carbohydrate-protein drink by glucose and BCAAs, storing energy in the muscles such as phosphocreatine (PC). PC is a high-energy substance that the body can temporarily use to create ATP.

Muscle fibers contain phosphocreatine, which provides phosphate groups to convert adenosine diphosphate (ADP) into ATP. The muscles then break down ATP to obtain energy at the end of the exercise (McMahon and Thornbury, 2020). According to the research study by Nielsen et al. (2020), consuming CHO-PROT causes the body to use the energy from the drink and replenish its glycogen stores. This is crucial for a quick energy boost of 3–15 seconds during training and competition, as well as for recovery and peak power performance. The groups that received 50 grams of a CHO-PROT drink and a CHO drink had 200 calories more than the group that consumed PLA water. Hall et al. (2022) studied muscle fibers in athletes who received BCAAs beverages. The study's results showed that the intake of BCAAs enhances muscle breakdown, which is then turned into energy by the enzyme branched-chain aminotransferase (BCAT) and stimulates the synthesis of protein in skeletal muscles, hence increasing energy stores for physical activity. BCAAs contain leucine, which significantly increases ATP synthesis by raising the phosphorylation state of important proteins in the mechanistic target of rapamycin (mTOR) signaling pathway, which controls the beginning of translation in human muscle (Kaspy et al., 2024).

In addition, Mor et al. (2022) documented that the use of a carbohydrate-protein supplement enhances the levels of branched-chain amino acids, leading to improved anaerobic capacity, delayed fatigue, and prolonged athletic performance. This is consistent with the study of Quinones and Lemon (2022), where it was found that during the end of the physical exercise, the body uses blood glucose and glucose from carbohydrates in a received drink, which affects the peak power that was decreased less than the group that consumed a placebo water that does not have replenishment energy during exercise.

4.2. Mean power (watts)

The group that consumed carbohydrate-protein supplements experienced a greater increase in mean power 24 hours post-exercise compared to the group that consumed placebo. During training or football competitions, the athletes engage in high-intensity exercise by repeatedly walking, moving with the ball, jumping, running to block the ball, jogging, and sprinting at full speed. The body mainly uses energy derived by breaking down glycogen in the muscles and free fatty acids in the blood in a similar ratio (Kumar and Pandey, 2023). In intense and short physical exercise, the body burns glycogen in muscles using the glycolysis system, resulting in a decrease in glycogen stored in the muscle (San-Millán et al., 2020). Researchers indicate that consuming carbohydrate-protein beverages (Rankin et al., 2019) and carbohydrate beverages (O'Brien et al., 2021) increases the rate of carbohydrate oxidation, helping to replenish as much glycogen as possible and utilizing it by the end of the competition. Studies have also indicated that taking 1.0-1.85 grams of carbohydrate per kilogram of body weight per hour within 15-60 minutes after a competition leads to the most efficient replenishment of muscle glycogen. This process continues for up to 5 hours following the competition.

Spriet (2022) concluded that when exercising, muscle cells that create force require glucose, which is converted into ATP via the glycolysis pathway. Blood sugar levels start to drop during a 90-minute football game, particularly during the second half when players are performing without oxygen. Therefore, it is essential to break down glucose from stored glycogen in order to keep blood sugar levels from decreasing too low. Another method is for producing it from substances other than carbohydrates, which comes from a metabolism known as gluconeogenesis. Brito-da-Silva et al.

(2024) explained that pyruvate and lactate are substances from the breakdown of proteins, specifically amino acids (glucogenic amino acids), which come from the muscles and enter the bloodstream through the cytoplasm and mitochondria. The enzyme lactate dehydrogenase in the liver converts lactate to pyruvate once it enters the liver, and gluconeogenesis can convert pyruvate back into glucose to boost energy for muscles that function without oxygen, increasing the mean power value.

Millard-Stafford et al. (2021) discovered that the concentration of carbohydrate-protein and carbohydrate supplements is optimal for absorption from the stomach into the small intestine and immediate utilization as energy. This leads to a gradual reduction in the depletion of glycogen in the muscles and liver. This is in marked contrast to the placebo drink, where no energy substitutes resulted in a significant depletion of glycogen. In addition, glycogen controls the release and absorption of calcium from the sarcoplasmic reticulum (SR). Therefore, a decrease in muscle glycogen may result in decreased muscle contraction ability, which may adversely affect the decrease in mean power (Staśkiewicz-Bartecka et al., 2024).

4.3. Blood glucose level (milligrams per deciliter)

The groups that had the carbohydrate beverage had the highest blood glucose levels than the group receiving carbohydrate-protein and placebo drinks in every period. In addition, the group that consumed the carbohydrate-protein drink had higher blood glucose levels than the placebo intake group at every stage. The blood glucose levels of the two sample groups had reached the highest at 0-minutes before exercise; when compared with the 30-minute pre-exercise period, the group that received the carbohydrate-protein drink increased by 23.67%, and the group that got the carbohydrate drink increased by 27.60%.

While the group that had the placebo decreased by 0.31%. The lowest blood glucose levels of all three sample groups are during the 90-minute exercise period. When compared to the 30-minute pre-exercise, the group consuming the carbohydrate-protein drink decreased by 8.47%, the placebo drink intake group decreased by 16.10%, while the group that received the carbohydrate drink increased by 5.48%. Additionally, the blood glucose levels at the 30-minute and 60-minute post-exercise periods gradually increased in all sample groups, but they were not as high as the 30-minute pre-exercise periods.

Football players rely heavily on muscle glycogen as they strive to win the competition by running, evading obstacles, tackling the ball, blocking it, and shooting the opponent's goal. There is a coordinated action of muscles, both slow twitch fiber and fast twitch fiber (Sannicandro and Cofano. 2023). Receiving carbohydrate supplements results in adequate glucose levels in the bloodstream, which then reduces muscle glycogen use (Craven et al., 2021). Exercise and consumption of carbohydrate-protein drinks. Carbohydrate-protein beverages raise blood sugar levels, which are then transferred to intestinal epithelial cells along with sodium (Na+) via a transport protein known as Na+ glucose co-transporter, or Sodium-glucose linked transporter 1 (SGLT1).

Additionally, pancreatic β-cells release more insulin hormone, which can be stimulated by several kinds of stimuli, including glucose, amino acids, and leucine. Glycolysis is the process by which glucose is phosphorylated by glucokinase, a glucose sensor, into glucose-6 phosphate and then undergoes metabolism to create pyruvate once it enters the β-cell through glucose transporter 2 (GlUT2). After entering the mitochondria, it undergoes metabolism to produce ATP, which can be used as energy (Wang et al., 2020). Additionally, the insulin signaling pathway occurs when the insulin hormone binds to the insulin receptor, a receptor on the tissue's cell membrane. This stimulates the tyrosine kinase process and the Mitogen-activated protein (MAP) kinase pathway, which leads to the generating of proteins and glycogen within the cell. As a result, it promotes the movement of the glucose transporter type 4 (GLUT4) receptor to the cell membrane in muscle and fat cells, facilitating glucose uptake into the cell. This process stimulates muscle cells to produce stored glycogen, enhances protein synthesis, and utilizes glucose for energy (Farra, 2023).

It was discovered that the blood glucose level of the carbohydrate-protein drinking group had increased as well. In addition, there is a breakdown of branched-chain amino acids from the consumed drink, which are then used to create glucose by the glucose-alanine cycle, and the leucine in the branched-chain amino acid stimulates increased secretion of the hormone insulin. This hormone works at the genetic level of the insulin hormone, stimulating protein synthesis in the body and inhibiting muscle protein breakdown. Gervasi et al. (2020) found that consuming sufficient branched-chain amino acid and carbohydrate drinks influences the breakdown of nutrients, which the muscles use as fuel. This was demonstrated by a higher increase in peak power and mean power when compared to the placebo-drinking group. For football players, carbohydrate intake is important because of energy expenditure throughout the 90 minutes of competition.

Since carbohydrates and glycogen serve as the primary energy sources, a decrease in blood glucose levels leads to the utilization of glycogen. Drinking carbohydrate beverages aimed to prevent a decrease in blood glucose levels and reserve energy for working muscles (Malone et al., 2021). As well as Craven et al. (2021), studied the effect of carbohydrate intake on the rate of synthesizing muscle glycogen during post-exercise recovery. It was found that receiving carbohydrate drinks stimulates the storage of more glucose in the cells due to an increase in the insulin hormone. Especially during moderate physical exercise. As a result, the muscles store more energy reserves in the form of glycogen (glycogen synthesis), which raises blood glucose levels and provides enough energy for physical exercise.

4.4. Blood lactate level (millimoles per liter)

Blood lactate levels in all sample groups increased to the highest at 90 minutes of physical training and gradually decreased at 30 minutes and an hour after exercise, but they were still higher than before exercise. Researchers reported that during intense exercise lasting more than 1 hour, muscles repeatedly contract and relax, leading to a decrease in accumulated energy and a shortage of oxygen. The researcher found a decrease in the intermediate substances of the Krebs cycle, namely alpha-ketoglutarate, citrate, and succinyl-CoA. This indicates a change in the energy source from amino acids and glucose; the reduction in energy generation in the aerobic system leads to a significant rise in blood lactate levels during exercise, which then gradually decreases during the post-exercise recovery phase.

Lactate is an acid that results from the anaerobic metabolism process, which is produced during continuously intense physical activity. According to reports, blood lactate levels can rise up to 25 mmol per liter following a football game (Martinho et al., 2022). High lactate levels limit muscular function by reducing muscle contractile force. This is caused by a rise in the acidity levels within the cell, resulting in a reduction in the release of calcium from the sarcoplasmic reticulum and affecting the ability of calcium to bind with troponin. Furthermore, it restricts the activity of phosphofructokinase, an enzyme responsible for the conversion of fructose-6-phosphate to fructose-1,6-biphosphate. Consequently, this reduces the effectiveness of ADP and Pi, resulting in a decline in the concentration of ATP (Hargreaves and Spriet, 2020). Consequently, the build-up of lactic acid in muscles increases, resulting in a reduction in muscle contraction strength, fatigue, and the possibility of muscle damage. Intense physical exercise demands high energy consumption; the body metabolizes stored glycogen or utilizes it from the consumed carbohydrate-protein and carbohydrate beverages. The body oxidizes glucose to pyruvate and then converts it to lactic acid faster than the tissue can remove it.

Therefore, the concentration of lactate in the blood begins to increase after exercise. The gluconeogenesis process converts the branched-chain amino acids obtained from carbohydrate-protein drinks back to glucose, thereby creating ATP. The gluconeogenesis process produces 20% lactate, a primary contributor to muscle fatigue (Kafrawi and Hidayati, 2022). Research has shown that during the post-exercise recovery period, the body has a decrease in its level of intensive function. During the post-exercise recovery period, the muscular system, heart circulatory system, and other systems in the body return to a normal state after undergoing intense physical activity. In addition, the anaerobic system does not consume energy during the period of rest, so preventing an increase in the concentration of lactic acid in the blood. Instead, it transports the lactate elsewhere, leading to a reduction in blood lactate levels throughout the recovery period following exercise. The rate of recovery is dependent upon the elimination of waste lactate, hydrogen ions, and carbon dioxide, as well as the restoration of depleted energy reserves utilized during physical activity. An efficient oxygen system facilitates rapid recovery of oxygen debt and restoration of the phosphate system to its normal state (Kaufmann et al., 2022).

According to Lee (2021), the oxygen system is important in helping athletes recover from exercise and improve anaerobic ability after exercise because oxygen helps to move lactic acid out of the blood and muscles. As a result, the circulatory system serves a crucial role in facilitating faster muscle recovery. The blood is responsible for carrying oxygen to the body's various muscles and oxidizing lactic acid to produce carbon dioxide (CO_2) and water (H_2O). When oxygen is available, lactic acid can be converted into energy. The Krebs cycle and electron transport system then convert it to pyruvate, which is further transformed into carbon dioxide and water to produce ATP energy. Using lactic acid as a source of energy during exercise. Due to the fact that slow-twitch muscle fibers are capable of effectively oxidizing lactic acid, the skeletal muscle is an essential organ that contributes to the body's recovery subsequent to exercise.

4.5. Perceived muscle soreness level (centimeters)

The carbohydrate-protein drink intake group had the lowest perceived muscle pain compared to the other groups. Furthermore, the group that consumed carbohydrates had a lower perceived muscle soreness level than the group that received placebo water. Studies have found that post-exercise muscle pain is a result of muscle energy depletion and muscle fiber injury (Calle-Jaramillo et al., 2023). Fluid diffuses into the gaps between muscle cells, resulting in swelling and inflammation and increased perception of A delta fiber and C fiber pain (Holland, 2020).

According to AbuMoh'd et al. (2020), one of the reasons contributing to central fatigue is the fluctuation of 5-hydroxytryptamine (5-HT) levels in the central nervous system (CNS) caused by anaerobic exercise. Since tryptophan transport over the blood-brain barrier is the rate-limiting step in the synthesis of 5-HT, it was discovered that consuming BCAAs decreased the amount of 5-HT that was transferred to the brain. Drinking carbohydrates-protein drinks increased the concentration of BCAAs in plasma, which could reduce the brain's uptake of tryptophan and 5-HT production, delaying tiredness. After 90 minutes of exercise, blood sugar levels in the group that consumed the carbohydrate drink started to drop, and muscles required more energy due to the breakdown of glycogen, which resulted in central nervous system fatigue.

The brain's ratio of free tryptophan to BCAAs was the highest in the group that received the placebo. During exercise, the brain absorbs more tryptophan, and the plasma ratio of free tryptophan (unbound to albumin)/BCAAs rises. Thus, exhaustion during and after intense physical activity may be caused by an increase in the plasma ratio of free tryptophan/BCAAs, which will enhance the transport of tryptophan into the brain as well as the synthesis, concentration, and release of 5-HT from certain neurons. Additionally, receiving BCAAs from a carbohydrate-protein supplement before high-intensity resistance exercise (Osmond et al., 2019) resulted in a lower perception of muscle pain after exercise than the group that received carbohydrate and placebo drinks. BCAT directly breaks down and quickly fuels muscles with branched-chain amino acids, unlike other amino acids that require liver enzymes for breakdown. BCAAs can accumulate in the amount of 0.1 gram per 1 kilogram of muscle, which is the highest concentration compared to other amino acids, and it has been found that when exercising hard and for a long time, it will increase the process of breaking down BCAAs to use as energy (Alhebshi et al., 2021).

Leucine, isoleucine, and valine collaborate to metabolize energy for the muscles or inhibit protein degradation in muscles during low-intensity exercise, such as walking or jogging. During this time, the body primarily metabolizes energy derived from carbohydrates and fat. When the body needs more energy, especially when carbs are running low, it can convert branched-chain amino acids straight into energy for the muscles (Jacob et al., 2019). This decreases the occurrence of muscle fiber tearing and injury, as well as the perception of muscular soreness after exercising (Teixeira et al., 2019). The group that had the carbohydrate drink experienced the conversion of carbohydrates into energy through the processes of glycolysis, the Krebs cycle, and glycogenolysis. The duration of this process exceeds the time it takes for branched-chain amino acids to break down in the muscles, resulting in increased damage to muscle fibers (Margolis et al., 2021). The group that ingested the placebo beverage exhibited the most pronounced perception of muscle soreness. The absence of energy-providing nutrients in the drink leads to an increase in muscle soreness following exercise (Ling et al., 2023).

5. Conclusion

Consuming a carbohydrate-protein supplement that contains branched-chain amino acids may be a potential option for football players. After exercise, it boosts anaerobic capacity by converting nutrients into energy through elevated blood glucose levels. However, it does not affect the blood lactate level and reduces the perceived muscle soreness level following physical activity, making it a favorable choice for post-exercise muscle recovery.

List of abbreviations

ATP	Adenosine triphosphate
BCAAs	Branched-chain amino acids
BCAT	Branched-chain aminotransferase
BMI	Body mass index
CHO	Carbohydrate
CHO-PROT	Carbohydrate-protein
CNS	Central nervous system
CO_2	Carbon dioxide
ETC	Electron transport chain
FADH2	Flavin adenine dinucleotide (reduced form)
GLUT2	Glucose transporter 2
GLUT4	Glucose transporter type 4
H_2O	Water
IOC	Index of objective congruence
MAP	Mitogen-activated protein
mTOR	Mechanistic target of rapamycin
Na+	Sodium ion
NADH	Nicotinamide adenine dinucleotide (reduced form)
OAA	Oxaloacetate
PLA	Placebo
PC	Phosphocreatine
Pi	Inorganic phosphate
SD	Standard deviation
SR	Sarcoplasmic reticulum
SGLT1	Sodium-glucose linked transporter 1
VO_2max	Maximum oxygen consumption

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Compliance with ethical standards

Ethical considerations

This study was conducted in accordance with ethical guidelines and was approved by the Thailand National Sports University Ethics Committee (Approval Code: SCI 015/2021, Date: September 28, 2022). Written informed consent was obtained from all participants before their participation. Participants were informed about the study’s purpose, procedures, and potential risks. All data collected were anonymized to protect participants’ confidentiality. The study adhered to the principles of the Declaration of Helsinki.

Conflict of interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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