Effects of carbohydrate and protein co-ingestion during short-term moderate-intensity exercise on cognitive function

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Abstract

BACKGROUND: This study aimed to investigate the effects of isocaloric Carbohydrate-electrolyte solution (CES) and Carbohydrates-electrolyte-protein solution (CEPS) consumed during moderate-intensity exercise on cognitive function. METHODS: 23 healthy participants (age: 21.7±1.5 years, body mass index: 21.8±2.0 kg/m^2, and peak oxygen consumption (VO_2peak): 42.4±6.9 ml/kg/min) were recruited in the study. Participants completed two main experiment trials using a cross-over study design. In each trial, they completed 30 minutes of 70% VO_2peak cycling, and one of two solutions (CES or CEPS) was consumed. A battery of cognitive function tests (imPACT Package) were administrated at rest and immediate post-exercise. RESULTS: Blood glucose concentrations decreased in both trials. Heart rate and blood lactate concentrations increased in both trials. However, no significant main effects of the exercise on all cognitive function variables (verbal and visual memory, visual motor speed, reaction time, impulse control, and cognitive efficiency index) were observed (all $P > 0.05$). Also, there was no difference in any of the cognitive function variables between the CES and CEPS trials (all $P > 0.05$). CONCLUSION: In conclusion, the cognitive performance was not affected by the consumption of CHO or CHO-PRO solutions during 30 minutes moderate-intensity cycling.

Key words: Carbohydrate-electrolyte solution, Carbohydrates-electrolyte-protein solution, cycling

1. Introduction

Cognitive function is described as a wide range of brain-mediated functions and processes [1] and is required for the performance of objective tasks that require conscious mental effort [2]. Such tasks require (verbal, spatial, and working) memory, attention, and executive control [3]. Carbohydrate (CHO) and protein (PRO) are two of the most commonly ingested macronutrients and appear to have different beneficial effects on cognitive function in the resting state. One potential mechanism for nutrition-induced changes in cognitive function is the change in blood glucose concentration following acute nutritional intervention, given that blood glucose is the predominant source of energy for brain function [4]. The maintenance of an adequate of blood glucose concentration is required to sustain optimal cognitive function [4] and, as such, it has been reported that the domains of working memory [5], reaction time [6], and executive function [7] are improved after consuming a CHO solution, when comparing with the consumption of a placebo. With regards to PRO, prior work has demonstrated both long- [8,9] and short-term [10,11] beneficial effects of the consumption of a high-PRO meal on cognitive performance such as reaction time and memory, comparing to an isoenergetic high-CHO meal. It has been suggested that this may be caused by the smaller postprandial changes in glucose metabolism and a more constant or higher metabolic activation following PRO consumption [10].

An acute bout of exercise is generally believed to have a small, but positive, effect on cognitive performance across a range of domains, including executive function [12] and reaction time [13], as well as mood [14], an effect often thought to be caused by increased arousal during the recovery period [15]. However, some previous studies have shown that cognitive function is impaired following prolonged high-intensity exercise, which may be as a result of deceased energy availability for neuron activity [16] and hemostatic changes (e.g., lactate accumulation and reduced lower pH) [17]. CHO feeding may attenuate the impairments in cognitive function that occur late in high-intensity exercise. The mechanism by which CHO consumption affects cognitive function could include increasing cerebral glucose uptake and oxygen consumption [18], and altering the balance of neurotransmitters such as serotonin and dopamine in the brain [19, 20]. Three studies [21-23] have evaluated the effect of a CHO solution during prolonged running or team sport exercise on cognitive function, and found an improved choice reaction time, working memory and mood, following CHO, when comparing with a placebo.

Recently, the inclusion of small amounts of protein in a CHO beverage has been suggested to induce certain benefits in physical performance over traditional CHO-only beverages [24-26]. However, only limited research which has investigated the effect of co-ingestion of CHO and PRO on post-exercise cognitive performance and where this has been examined, there are diverse results [27, 28]. In a laboratory-based study, CHO-PRO co-ingestion but not CHO consumption benefited visual motor speed, when comparing with a placebo, whereas no other effects on cognitive performance were observed [27]. Nevertheless, a reduced perception of effort and enhanced affective responses was observed after co-ingestion of CHO and PRO compared with CHO alone during 90 min of strenuous running [28], suggesting that CHO-PRO co-ingestion has the potential to benefit psychological parameters. Therefore, it is reasonable to assume that CHO-PRO co-ingestion during exercise may provide certain additional benefits to cognitive function, when compared with CHO alone.

There is still scarce research investigating the effect of co-ingestion of CHO and PRO on cognitive function following an acute bout of moderate-intensity exercise. This is noteworthy because relative to high-intensity exercise, moderate-intensity exercise is applicable for inactive people and easier to execute repeatedly in daily life [29]. A recent review suggests that moderate-intensity exercise demonstrates a larger effect than light- or vigorous-intensity exercise on neuropsychological tests of cognitive function and academic achievement [30]. However, less is currently known in certain populations such as young adults, due to the lack

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of studies in this population to date[30]. Therefore, the purpose of the present study was to assess the effect of co-ingestion of CHO and PRO solution on cognitive function in college students, following an acute bout of moderate-intensity exercise. It is hypothesized that CHO-PRO co-ingestion would improve the cognitive performance after an acute bout of moderate-intensity exercise as compared with the ingestion of the CHO solution alone.

2. Methods

2.1 Participants

Twenty-three healthy college students (20 males and 3 females, age: 21.7±1.5 years, body mass index: 21.8±2.0 kg/m^2, and peak oxygen consumption (VO_2peak): 42.4±6.9 ml/kg/min) volunteered to take part in this study. All students were recruited from a University population. The number of participant exceeded the minimum sample size required to elucidate differences in the cognitive function tests with a statistical power of 0.8, an estimated effect size of 0.5 SD units, a two-tailed alpha level of 0.05, and an intraclass correlation of 0.5 between repeated measures. All participants completed a health questionnaire to determine whether they had any health conditions that may limit physical activity or their ability to safely participate in the study. Potential participants were also excluded if they were currently using tobacco products, consuming an average of ten or more alcoholic beverages per week, or had undergone surgery, hospitalization, or experienced an emotionally traumatic life event within the past six months. The Education University of Hong Kong Institutional review board approved the study design and procedures. Each participant received an explanation of the purpose and potential risks of the study and was provided with written informed consent.

2.2 Study design

Participants performed two main experimental trials in a randomized, double-blinded, counter-balanced manner. The experimental trials were scheduled at least 7 days apart for each participant. In each main trial, participants consumed one of the two different solutions, namely the carbohydrate-electrolyte solution (CES; 6.3% CHO) and the carbohydrate-electrolyte-protein solution (CEPS; 4.2% CHO + 2.1% PRO). The ratio of CHO:PRO was 2:1 in CEPS trial which was consistent with previous study [27]. The solutions were provided to the participants pre-exercise, at 15 min intervals during the exercise sessions, and post-exercise at a rate of 3ml/kg body mass for each participant at each time point. Therefore, the total CHO intake was around 0.567g/kg in CES trial, whereas the total CHO and PRO intake in CEPS trial were 0.378g/kg and 0.189g/kg separately. Two different solutions were prepared by a third person and were provided to the participants using identical paper cups. The sequences of two trials were arranged using a random number-producing algorithm and were blinded to both the researchers running the trials and participants. The cognitive function tests were administrated at rest and immediate post-exercise (within 2 minutes after the exercise session).

2.3 Preliminary test

Before the main experimental trials, all participants reported to the laboratory for the assessment of VO_2peak and for familiarization with the exercise protocol. VO_2peak testing was conducted on an electronically braked cycle ergometer (LC7, Monark, Sweden). Participants rode at a self-selected cadence above 50 rpm throughout the test. Each participant warmed up for 3 minutes with light resistance before the test began. Workload was initially set at 150 W and then increased by 50 W every 2 minutes during the test until the participants achieved volitional exhaustion. Oxygen uptake and respiratory exchange ratio were assessed at each stage during this test with a metabolic cart system (Cortex Metalyzer II-R, CORTEX, Germany). Heart rate was assessed with a Polar heart rate monitor (PolarTeam System, Polar Electroy, Finland), and rating of perceived exertion (RPE) was measured using the 6-20 Borg scale [30]. The test was considered maximal when at least two of the following criteria were met: heart rate fail to increase with an increase in exercise intensity, a plateau in oxygen uptake with increasing workload, a respiratory exchange ratio of greater than 1.15, and an RPE greater than 17 [31]. On the basis of the preliminary test results, a running speed equivalent to 70% of each participant's VO_2peak was determined. Thereafter, participants completed a familiarization trial to confirm the running speeds equivalent to 70% of the individual VO_2peak. This speed was used in the main experimental trials. All participants were also allowed to repeat the cognitive function tests until they felt comfortable with them, to minimize any potential learning effects.

2.4 Physical activity and nutrition control

Participants were asked to keep their dietary records for 48 hours before their first trial and repeat the same diet for the subsequent trial, as well as to refrain from alcohol consumption, caffeine consumption and unusually vigorous exercise for 24 hours before each trial. To minimize within-participants diurnal variation, the time of the day for each trial was held constant for each participant. In order to maintain euhydration, participants were instructed to consume at least 500 mL of water before the main trial. Constant temperatures (22℃) and relatively humidity (60%) were maintained throughout the experiment by a thermostat.

2.5 Experimental trials

On arrival at the laboratory, participants rested quietly in the seated position for 30 minutes, during which time baseline data were obtained. Participants then performed a standardized 5 minutes warm-up at 50 W on a cycle ergometer. Then, the speed of the cycling was immediately increased to the intensity of 70% of the individual VO_2peak for 30 min. The participants consumed one of the two treatment solutions at a rate of 3 mL/kg body mass for each participant at rest, at 15-min intervals during the exercise sessions, and post-exercise. The two solutions contained equal fluid volumes, were isocaloric and contained thesame electrolyte profile (see Table 1). The drinks were formulated according to one commercial sports drink

(Aquarius, Coca-Cola, Hong Kong). The CES contained 6.3% CHO in the form of sucrose, and the CEPS contained 4.2% CHO plus 2.1% whey PRO (bcshop, Hong Kong).

2.6 Data collection and sample analysis

Data collection procedures are illustrated in Fig 1. Body mass (in underwear only) was measured to the nearest tenth of a kilogram before and after cycling using scales (Body Weight Precisa, DPS-Promatic, Forli, Italy). Heart rate was recorded continuously during the exercise (PolarTeam System, Polar Electroy, Finland). Capillary blood samples were collected to determine the blood lactate and glucose concentrations using YSI 1500 (Yellow Spring Instrument Co. Ltd., USA) and a biochemical analyzer (Roche1 ACCU-CHEK Reflotron plus, USA), respectively. Similar to previous study [31], subjective measures, such as RPE, perceived thirst (PT), and abdominal discomfort (AD) were recorded. The PT and AD varied from 0 to 10, where 0 denoted “not so much” and 10 denoted “very much”.

2.7 Cognitive function tests

The battery of cognitive function tests (imPACT Package, imPACT Application, Inc., Australia) [32] was administrated in a quiet room via a laptop computer and lasted approximately 2 minutes. The battery of tests consisted of th word memory learning, designing memory learning, Xs and Os, symbol match, color match, and three letters, which yield the following composite scores: verbal memory, visual memory, visual motor speed, reaction time, and impulse control, described in detail elsewhere [32]. This testing battery has previously been used to successfully examine both nutritional [27] and exercise-induced [32] effects on cognitive function in an adult population. The cognitive function tests were administered at rest and immediate post-exercise.

2.8 Statistical analysis

This study employed a within-subject repeated-measure design that contrasted the effect of the two solutions on cognitive function. The outcome measures were the cognitive function variables, blood glucose concentration, blood lactate concentration, heart rate and all the subjective measures. Data is presented as mean ± SD unless specifically noted. All data was analyzed using a two-way (trial × time) repeated-measures analysis of variance (ANOVA). When a significant main effect or interaction was identified, data were subsequently analyzed using a Bonferroni *post hoc* test. The significance level was set at $P < 0.05$. Partial eta squared (*η2)* were reported as estimates of effect size. All statistical analyses were conducted using IBM SPSS software (SPSS 21.0, IBM, USA).

3. Results

All participants completed the two trials successfully. There were no differences in all baseline data between two trials (all $P > 0.05$). Also, no order effect was observed regarding all the measurements (all $P > 0.05$).

3.1 Cognitive performance

Table 2 illustrates the results of the cognitive function tests. There was no difference in any of the cognitive function variables between the CES and CEPS trials (all $P > 0.05$). Also, no significant main effects of the exercise on these variables were observed (all $P > 0.05$).

3.2 Blood glucose concentration

Figure 2 shows the changes in blood glucose concentration. Overall, blood glucose concentration was identical between the CES and CEPS trials (main effect of trial, $F_{(2, 23)}=2.1$, $P=0.160$, $\eta^2_p=0.088$). No trial × time interaction effect was observed ($F_{(2, 23)}=0.3$, P=0.733, $\eta 2_p=0.024$). In the CES trial, the blood glucose concentration was lower at 15 minutes than that at rest ($P=0.043$). In the CEPS trial, blood glucose concentrations were significantly lower at 15 minutes ($P=0.026$) and post-exercise ($P=0.014$) than those at rest.

3.3 Other physiological measures

Table 3 summarizes the results of other physiological measures. Significant main effects of exercise on blood lactate [$F_{(2, 23)}$=31.3, P<0.001, η^2_p=0.587] was observed. In both conditions, blood lactate concentration significantly increased during exercise when comparing to rest (P<0.001). However, blood lactate concentration was identical between the CEPS and CES trials (main effect of trial, $F_{(2, 23)}$=0.1, P=0.735, $\eta^2 p$=0.053). Besides, a significant main effects of exercise on heart rate [$F_{(2, 23)}$=421.6, P<0.001, η^2_p=0.950] was found. These results indicated that heart rate gradually rose during the course of exercise among the two trials (both P<0.001). However, there were no differences in heart rate during exercise between conditions (main effect of trial, $F_{(2, 23)}$=2.682, P=0.116, $\eta^2 p$=0.124). For the body weight, a significant interaction effect was observed ($F_{(2, 23)}$=7.115, P=0.014, η^2_p=0.224). However, there were no differences between trials ($F_{(2, 23)}$=0.972, P=0.335, η^2_p=0.042) and time ($F_{(2, 23)}$=3.531, P=0.074, η^2_p=0.138).

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3.4 Subjective measures

Table 4 shows the data for subjective estimates. The main effects of exercise on RPE [F(2, 23)=136.274, P<0.001, η^2_p=0.861], PT [F(2, 23)=30.531, P<0.001, η^2_p=0.581], and AD [F(2, 23)=17.993, P<0.001, η^2_p=0.450] was observed. RPE in both trials (both P<0.001) and PT (P<0.05) in the CES trial increased significantly during the excise. PT in the CEPS trial and AD in the two trials increased during the exercise relative to at rest (P<0.05, respectively). AD was significantly higher at rest in the CEPS trial than in the CES trial (P<0.05). However, there were no differences in RPE, PT, or AD during the exercise between conditions.

4. Discussion

The main finding of the present study was that cognitive performance was similar following moderate-intensity exercise when both a traditional CHO-electrolyte solution and a CHO-

PRO-electrolyte solution were consumed. Furthermore, 30 minutes of cycling at 70% VO_2peak did not affect cognitive performance, nor were the perceptual measures (RPE, perceived thirst and abdominal discomfort) affected by the consumption of either one of the solution. Overall, these findings suggested that ~~cognitive performance was not affected by the addition of PRO to a typical CHO-electrolyte solution post-exercise and thus~~ the consumption of such solutions following moderate-intensity exercise is not recommended to enhance cognition.

Although one previous study suggested that iso-energetic meals consisting of 1:4 ratios of CHO and PRO influenced higher cognitive functions in resting status [10], there has only been very limited previous work examining the effect of the co-ingestion of CHO and PRO on cognitive performance following exercise. The lack of the effect of CHO and PRO co-ingestion comparing with CHO ingestion alone, across the rage of domains of cognition measured, is in line with the findings of Gui et al. [27], who measured cognitive performance following a 21-km running time trial. Interestingly, the study of Gui et al. [27] reported an enhancement in visual motor speed post-exercise following CHO and PRO co-ingestion when comparing with a placebo. It is difficult to compare directly due to the absence of a placebo trial in the present study, yet the findings of the present study are important in demonstrating that following moderate-intensity exercise, there is no extra benefit of CHO and PRO co-ingestion compared to a traditional CHO-electrolyte solution, for cognitive performance.

The lack of the effect of CHO and PRO co-ingestion (compared to a traditional CHO-electrolyte solution) on post-exercise cognitive performance seen in the present study may be explained by the fact that 30 minutes of cycling at 70% VO_2 peak may not have affected any of the measures of cognition. Previous evidence has shown that, overall, exercise has a small but positive effect on cognitive function [33]. However, there are a number of mediators in the exercise-cognition relationship, including the intensity, duration and modality of exercise.

Interestingly, some studies have suggested that following high-intensity exercise cognitive performance may be impaired due to decreased energy availability for neuronal activity [16] and hemostatic changes [17]. Under such conditions, the consumption of CES or CEPS may be proven beneficial to cognitive performance. In contrast to the detrimental effects of high-intensity exercise, acute moderate-intensity of exercise has been shown to improve some aspects of cognitive performance, such as executive function [12], reaction time [13], as well as mood [14]. Therefore, it may be more difficult to identify the potential benefits of nutritional strategies adopted during moderate-intensity exercise to cognitive function, if any. The findings of the present study suggest that the consumption of CEPS may not be necessary for the maintenance or enhancement of cognitive performance following moderate-intensity exercise since the same effect can be fulfilled by CES.

It is possible that there are beneficial effects of CHO and PRO co-ingestion following more strenuous exercise, which in itself may lead to deteriorations in cognitive performance. This notion is supported by the enhanced perceptual and affective responses reported with CHO and PRO co-ingestion following 90 minutes cycling at 70% VO_2 peak [28]. Such perceptual and affective responses have the potential to affect cognitive performance [1]. In the present study, the perceptual measures of RPE, perceived thirst and abdominal discomfort were not different with CHO and PRO co-ingestion comparing with CES ingestion. This lack of difference in the perceptual measures may be a contributory factor to the lack of an effect on cognitive performance. Following more strenuous exercise, there may be differences in such perceptual measures as reported previously [28], and also greater differences in the physiological parameters such as blood glucose concentration. However, the mediating effect of these variables on subsequent cognitive performance warrants further investigation.

There are several limitations in the present study. Firstly, it is more consistent in previous studies that acute exercise may benefit the executive function, but not other domains of cognitive function [30]. Therefore, the future studies should focus more on executive function. Secondly, there was lack of a placebo trial in the present study which made it difficult to judge whether there were certain benefits on cognitive function after CHO or CHO-PRO consumption during exercise especially for moderate-intensity exercise. Nevertheless, the present study is still of value given that young college students may need maintain their cognitive function (or importance for their academic studies), whilst undertaking moderate-intensity exercise. These findings also have potential implication for decision making during such exercise in young college students.

5. Conclusion

In conclusion, there was no difference in the cognitive performance when CHO or CHO-PRO solutions was consumed during 30 minutes moderate-intensity cycling.

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Table 1. Composition of experimental solutions

CEPS: carbohydrate-electrolyte protein solution, CES: carbohydrate-electrolyte solution

Content (per 100 mL)	Supplements	
	CEPS	CES
Energy (kcal)	27	27
Carbohydrate (g)	4.2	6.3
Protein (g)	2.1	0
Sodium (mg)	34	34
Potassium (mg)	8.1	8.1
Calcium (mg)	0.8	0.8
Magnesium (mg)	1.2	1.2
Branched-chain amino acids (mg)	2.5	2.5

Table 2. Cognitive performance in the CEPS and CES conditions

		Rest	Post-exercise
Verbal memory	CEPS	89.5 ± 9.8 [85.3, 93.7]	89.9 ± 7.7 [86.5, 93.2]
	CES	89.1 ± 10.2 [84.6, 93.4]	90.4 ± 8.2 [86.9, 94.0]
Visual memory	CEPS	80.8 ± 12.6 [75.3, 86.2]	82.3 ± 13.3 [76.5, 88.0]
	CES	85.1 ± 10.0 [80.6, 89.3]	80.5 ± 13.6 [74.6, 86.4]
Visual motor speed	CEPS	43.8 ± 6.4 [41.1, 46.6]	45.3 ± 6.5 [42.6, 48.1]
	CES	44.5 ± 6.1 [41.8, 47.1]	45.1 ± 7.2 [41.9, 48.2]
Reaction time	CEPS	0.55 ± 0.10 [0.51, 0.59]	0.54 ± 0.06 [0.52, 0.57]
	CES	0.57 ± 0.08 [0.53, 0.60]	0.54 ± 0.08 [0.51, 0.58]
Impulse control	CEPS	5.17 ± 4.03 [3.43, 6.92]	5.70 ± 3.38 [4.24, 7.16]
	CES	5.39 ± 3.17 [4.02, 6.76]	5.61 ± 5.02 [3.44, 7.78]
Total symptom score	CEPS	2.96 ± 4.51 [1.01, 4.91]	3.52 ± 5.48 [1.15, 5.89]
	CES	2.48 ± 3.31 [1.05, 3.91]	3.00 ± 7.06 [0.06, 6.06]
Cognitive efficiency index	CEPS	0.44 ± 0.13 [0.39, 0.50]	0.41 ± 0.13 [0.36, 0.47]
	CES	0.39 ± 0.17 [0.32, 0.46]	0.41 ± 0.14 [0.36, 0.47]

CEPS: Carbohydrate-electrolyte-protein solution; CES: Carbohydrate-electrolyte solution.

Values are mean ± SD [95% CI].

Table 3. Physiological measures in the CEPS and CES conditions

		Rest	15-min	Post-exercise
Lactate (mmol/L)	CEPS	2.6 ± 1.5	5.6 ± 2.8[a]	5.2 ± 2.6[b]
		[1.9, 3.3]	[4.4, 6.8]	[4.1, 6.3]
	CES	2.3 ± 1.6	5.4 ± 1.8[a]	6.0 ± 3.1[b]
		[1.6, 3.0]	[4.6, 6.1]	[4.7, 7.4]
Heart rate (beats/min)	CEPS	76 ± 12	142 ± 17[a]	147 ± 17[b,c]
		[71, 81]	[135, 149]	[140, 154]
	CES	74 ± 10	137 ± 21[a]	146 ± 17[b,c]
		[69, 78]	[127, 146]	[139, 153]
Body weight (kg)	CEPS	66.2 ± 9.9	-	66.6 ± 10.0
		[61.9, 70.5]		[62.3, 70.9]
	CES	66.7 ± 10.0	-	66.6 ± 10.0
		[62.4, 71.0]		[62.3, 70.9]

CEPS: Carbohydrate-electrolyte-protein solution; CES: Carbohydrate-electrolyte solution

Values are mean ± SD [95% CI]. [a,b] P<0.001 vs. Rest; [c] P<0.001 vs. 15-min.

Table 4. Psychological parameters in the CEPS and CES conditions

		Pre-exercise	15-min	Post-exercise
RPE	CEPS	6.6 ± 1.2	11.8 ± 2.4[a]	13.7 ± 2.9[b,c]
		[6.0, 7.1]	[10.7, 12.8]	[12.4, 14.9]
	CES	6.5 ± 1.2	12.3 ± 2.6[a]	13.7 ± 2.8[b,c]
		[6.0, 7.0]	[11.2, 13.5]	[12.5, 14.9]
PT	CEPS	1.3 ± 1.5	3.0 ± 1.8[a]	3.6 ± 2.2[b]
		[0.6, 1.9]	[2.3, 3.8]	[2.6, 4.5]
	CES	1.0 ± 1.2	2.6 ± 1.8[a]	3.6 ± 2.3[b,c]
		[0.5, 1.5]	[1.9, 3.4]	[2.6, 4.6]
AD	CEPS	1.4 ± 1.7[#]	2.7 ± 2.3[a]	2.7 ± 2.3[b]
		[0.7, 2.2]	[1.7, 3.7]	[1.6, 3.8]
	CES	0.6 ± 1.0	2.1 ± 1.9[a]	2.4 ± 2.3[b]
		[0.1, 1.0]	[1.3, 2.9]	[1.4, 3.4]

RPE: Rating of perceived exertion; PT: Perceived thirst; AD: Abdominal discomfort;

CEPS: Carbohydrate-electrolyte-protein solution; CES: Carbohydrate-electrolyte solution.

Values are mean ± SD [95% CI]. [a,b] $P<0.001$ vs. Rest; [c] $P<0.001$ vs. 15-min; [#] $P<0.05$ vs. CES.

Figure 1 A schematic outline of the present study

Figure 2 Blood glucose concentration in the CEPS (carbohydrate-electrolyte-protein solution) and CES (carbohydrate-electrolyte solution) conditions.

* $P<0.05$, vs. rest.