Effects of protein addition to carbohydrate–electrolyte solutions on postexercise rehydration

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Abstract

Background/Objective: This study aimed to examine the effects of the addition of whey or casein protein, the two major proteins in milk, to carbohydrate–electrolyte (CE) solutions on postexercise rehydration.

Methods: Ten young men aged 20.7 ± 1.4 years with an average VO2max of 60.7 mL/kg/min ran for 60 minutes at 65% VO2max on three occasions followed by 4 hours recovery. During recovery, the participants consumed either CE solution with 66 g/L carbohydrate (CHO), or CE plus whey protein solution (CW trial, 44 g/L CHO, 22 g/L whey), or CE plus casein protein solution (CC trial, 44 g/L CHO, 22 g/L casein); the solutions were matched for energy and electrolyte content.

Results: The participants lost 2.36 ± 0.32% of their pre-exercise body weight after the exercise. Total urine output after recovery was greater in the CE and CC trials than CW trial (CE vs. CW vs. CC: 1184 ± 378 mL vs. 1005 ± 214 mL vs. 1256 ± 413 mL; p < 0.05). Fluid retention after ingestion of CW solution was greater than CE and CC solutions (CE vs. CW vs. CC: 46.9 ± 16.5% vs. 54.9 ± 9.2% vs. 45.8 ± 17.3%; p < 0.05). Lower urine specific gravity and urine osmolality were observed by the end of recovery in the CE trial compared with CW trial (p < 0.05). No difference was found in the changes in plasma volume in all trials.

Conclusion: These results suggest that during the 4 hours’ recovery after a 60-minute run, the CW solution was more effective for rehydration compared with the CE or CC solution.

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Keywords: Carbohydrate–electrolyte solutions; Casein protein; Rehydration; Whey protein

Introduction

Prolonged endurance exercise is known to induce dehydration because of sweat loss. Besides water, some electrolytes such as sodium are also lost.1 If the recovery time is limited (< 12 hours), effective rehydration strategies are generally recommended for a swift recovery after exercise.2–5 It is recommended that athletes should drink a volume equal to 150% of their body weight (BW) loss during recovery,5,5 and the rehydration solution should contain a certain amount of sodium and potassium because they can replace the major electrolytes loss in sweat.6–8 Commercial sports drinks that contain water, carbohydrates (CHO), and electrolytes are helpful for postexercise rehydration as reported in previous studies.9,10 Recently, it was reported that the low-fat milk is more helpful in fluid retention than common carbohydrate–electrolyte (CE) solutions.11,12 Milk naturally contains as high a content of CHO and electrolytes as CE solutions, the
specific element of milk protein may have some additional effects on fluid retention. However, the potential specific effects of milk protein cannot be illustrated because the low-fat milk and CE solutions in previous studies were not matched for the compositions.

Several studies have demonstrated that the ingestion of solutions containing protein after exercise is also better than that of CE solutions. Seifert et al reported that the addition of protein to a traditional sports drink improves water retention in the body. But the participants drank a volume equal to their BW loss and the drinks were not matched for energy density and electrolyte concentration. In more recent studies, James et al found that the addition of milk protein to a CE solution is more effective in postexercise rehydration than a CE solution alone if the two solutions are matched for energy density and electrolyte concentration.

As is known, two protein groups exist in milk or milk protein. One of the protein groups is whey protein, which accounts for about 20% of the total protein in milk. Another protein group is named casein protein and accounts for the remaining 80% of the total protein in milk. Whey protein is defined as “fast protein” because it can empty from the stomach and be absorbed by the intestine rapidly. By contrast, casein protein is known as “slow protein” because it coagulates when mixed with the gastric acids in stomach, by which the stomach emptying rate will be delayed. Even the contents of total amino acids (AAs) and essential AAs are similar in both whey and casein protein groups, the blood AA concentration is reported to be greater after consumption of whey protein than casein protein. It is interesting to know which protein group plays the major role in fluid retention when milk or milk protein was consumed by participants. Moreover, whether these two types of protein have different effects on postexercise rehydration remains unclear. The purpose of this study was therefore to examine the effects of the addition of whey or casein protein to CE solutions on postexercise rehydration.

Methods

Participants

Ten healthy men [age, 20.7 ± 1.4 years; BW, 65.4 ± 6.3 kg; maximal oxygen uptake (VO2max), 60.7 ± 6.1 mL/kg/min] volunteered to participate in this study. They were all runners in the school team but not experienced athletes. The medical history of all participants was surveyed prior to participation. Written informed consent was obtained from all participants after the details and procedures of the experiment were fully explained. The protocols were approved by the University Clinical Research Ethical Committee of The Chinese University of Hong Kong.

Preliminary test

Participants were asked to complete a preliminary test followed by three main experimental trials. During the preliminary test, participants underwent an incremental sub-maximal running test to determine the relationship between running speed and oxygen uptake (VO2). The test comprised four stages, with each stage lasting for 4 minutes. The participants began running on the treadmill at 7 km/h in the first stage, which gradually increased by 1.5 km/h in each stage. Therefore, the running speed in the fourth stage reached 11.5 km/h. Expired gas was collected and analyzed during the last minute of each stage. The running speed and VO2 value were recorded in each stage throughout the test, so in total there were four coordinate values of these two variables, respectively. Then the relationship between the running speed and VO2 of each volunteer was calculated by linearly regressing the four coordinate values of these two variables. VO2max of each participant was then determined during uphill, incremental treadmill running to volitional exhaustion as described in the existing literature. In the main trial tests, participants were asked to run at a speed that elicited 65% of their VO2max.

Experimental protocol

Three main experimental trials were conducted in a randomized crossover manner. Trials were separated by at least 7 days. All the trials were conducted in an exercise physiology laboratory at similar environmental conditions (temperature: 24°C; relative humidity: 65%). The participants were asked to record their dietary intake and physical activity details 24 hours prior to the first trial. The recorded dietary intake and physical activity patterns were repeated in the other two trials. Participants were also asked to refrain from any strenuous exercise and alcohol consumption for 24 hours prior to each trial.

Experimental trials began early in the morning after overnight fasting (10–12 hours). Upon arrival at the laboratory, participants consumed 500 mL of plain water and rested for 1 hour. This step was performed to ensure that the participants were in a euhydrated state at the beginning of the experiment. The participants were asked to empty their bladders prior to each trial, and urine samples were collected. Nude BW was measured to the nearest 100 g (TB-531A; TANITA Body Fat Monitor, TANITA Health, Tokyo, Japan). After 10 minutes of rest in a sitting position, the baseline capillary blood samples were obtained from the finger.

The participants began to run on a treadmill at 65% of their VO2max for 60 minutes after a 10-minute warm-up. Running speed was determined by the aforementioned preliminary test. Expired gas and ratings of perceived exertion were obtained every 20 minutes during the 60 minutes run. Heart rate (HR) was measured using a HR monitor (Sport Tester PE 4000; Polar Electro, Kempele, Finland). The participants consumed no fluid during the 60-minute run.

Urine and capillary blood samples were immediately collected after the 60-minute run. The participants were then allowed to have a 15-minute shower, after which nude BW was measured again. Postexercise BW was compared with pre-exercise BW to calculate BW loss. A total of 4 hours’ recovery period ensued after data collection. During recovery, one of the three following solutions was consumed: (1) CE,
solution containing CHO and some electrolytes, such as sodium and potassium; (2) CW, solution containing CHO, whey protein (Native Whey Protein Isolate, Protein Factory Inc., Brick, NJ, USA), and the same electrolytes as CE; and (3) CC, solution containing CHO, casein protein (Micellar Casein; Protein Factory Inc.), and the same electrolytes as CE. These three solutions were matched for energy density and electrolyte content (Table 1). The solutions were mixed for 1 hour prior to consumption and stored at room temperature. The total solution volume consumed by the participants was equal to 150% of their BW loss during the 60-minute run. The participants were provided with six aliquots of equal volume, each of which had to be consumed every 30 minutes during recovery. Thus, the participants finished ingesting the solution after 150 minutes during recovery.

Urine and capillary blood samples were collected from the participants at the end of each hour during the 4 hours' recovery period, and the capillary blood samples were collected from participants in a sitting position. Nude BW was then measured. All the urine produced by the participants was collected, and the volume was measured throughout recovery. Participants were asked to rate their subjective feelings, including perceived thirst, abdominal discomfort, and stomach fullness, during running and at the end of each hour during recovery. The answers of the participants were scaled from 0 to 10, in which 0 meant “not so much” and 10 meant “very much”.

Sample analysis

Expired gases were analyzed using a metabolic testing system (MAX-II; Physio-Dyne, New York, NY, USA). Blood glucose concentration was measured with a glucose analyzer (Model 1502; YSI, Yellow Springs, OH, USA). Capillary blood samples in tubes were used to determine the hematocrit levels with a microcentrifuge (Autocrit Ultra 3; Clay Adams, Englewood, CO, USA). Hemoglobin was measured using a clinical chemistry analyzer (Reflotron System; Boehringer Mannheim, Mannheim, Germany). The percentage change in plasma volume (PV) was calculated based on hemoglobin and hematocrit values. Urine osmolality was measured using an osmometer (Vapor Pressure Osmometer 5520; Wescor Inc., Logan, UT, USA), and the urine specific gravity (USG) was measured using a USG analyzer (PEN-Urine S. G.; ATAGO Co. Ltd., Tokyo, Japan). All blood and urine samples were measured immediately after collection.

Table 1

<table>
<thead>
<tr>
<th>Composition of the three solutions. The three solutions were matched for the energy density and electrolyte content.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CE</strong></td>
</tr>
<tr>
<td>Energy density (kcal/L)</td>
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<tr>
<td>Carbohydrate (g/L)</td>
</tr>
<tr>
<td>Protein (g/L)</td>
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<tr>
<td>Na (mM)</td>
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<td>K (mM)</td>
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</tbody>
</table>

CC = CE plus casein protein solution trial; CE = carbohydrate—electrolyte solution trial; CW = CE plus whey protein solution trial; K = potassium; Na = sodium.

Statistical analysis

All data are presented as mean values with standard deviations. SPSS software version 17.0 (SPSS Inc., Chicago, IL, USA) was utilized for data analysis. The participants’ responses to the consumption of the three solutions were compared using two-way (trial x time) analysis of variance (ANOVA) with repeated measures. Significant differences, including the data containing one variable (e.g., % BW loss, % fluid retention, etc.), were determined by repeated measures one-way ANOVA followed by Bonferroni post hoc test. Statistical significance was set at 0.05.

Results

Dehydration and rehydration

During the 60-minute run, the average percentage of VO2max was approximately 65% in each trial with no significant difference (CE vs. CW vs. CC: 65.5% ± 3.8% vs. 65.5% ± 4.7% vs. 65.2% ± 4.5%; p > 0.05). After the 60-minute run, participants lost 2.36% ± 0.32% of their initial BW, and no difference was observed among the trials (CE vs. CW vs. CC: 2.33% ± 0.35% vs. 2.33% ± 0.33% vs. 2.41% ± 0.29%; p > 0.05). No difference was found in the volumes consumed in the three trials during recovery (CE vs. CW vs. CC: 2.24 ± 0.28 L vs. 2.24 ± 0.24 L vs. 2.33 ± 0.27 L; p > 0.05).

Urine output and fluid retention

With regard to cumulative urine volume during recovery (Fig. 1A), the CC trial caused significantly more urine production than the CW trial (p < 0.05) at 2 hours and 3 hours. By the end of the 4-hour recovery period, both the CE and CC trials produced more urine than the CW trial (p < 0.05). With regard to ingested fluid retention (Fig. 1B), the CW trial retained approximately 55% of the ingested solution by the end of the 4-hour recovery period. This value was greater than that of the CE and CC trials (CE vs. CW vs. CC: 46.9% ± 16.5% vs. 54.9 ± 9.2% vs. 45.8% ± 17.3; p < 0.05).

USG and urine osmolality

Prior to exercise, the USG value was similar in all the trials (< 1.020 g/mL; Fig. 2A). Compared with its pre-exercise value, USG increased considerably after 1 hour of recovery in all the trials (p < 0.05). Among the three trials, USG was lower in the CC trial than that in the CW trial (CC vs. CW: 1.005 ± 0.005 g/mL vs. 1.008 ± 0.006 g/mL; p < 0.05) at the 2nd hour of recovery. By the 3rd hour, a lower USG value was observed in the CE trial than that in the CW trial (CE vs. CW: 1.001 ± 0.002 g/mL vs. 1.003 ± 0.002 g/mL; p < 0.05). After 4 hours’ recovery, the CE trial acquired a lower USG value than the CW and CC trials (CE vs. CW vs. CC: 1.002 ± 0.002 g/mL vs. 1.004 ± 0.002 g/mL vs. 1.004 ± 0.002 g/mL; p < 0.05). Fig. 2B shows the changes in...
urine osmolality during recovery. The changes were highly similar to the USG results. In all of the three trials, higher urine osmolality was observed after the 1st hour of recovery than that during pre-exercise \( (p < 0.05) \). By the 3rd hour of recovery, urine osmolality was higher in the CW trial than that in the CE trial \( (p < 0.05) \). After 4 hours of recovery, urine osmolality was lower in the CE trial than those in both the CW and CC trials \( (p < 0.05) \).

**Blood glucose concentration and changes in PV**

Glucose concentration was similar in the three trials before and immediately after the exercise (Fig. 3). In the CE trial, the highest glucose level was observed at the 1st hour of recovery, whereas the lowest level was observed at the end of recovery \( (p < 0.05) \). No difference was observed at any time point in the CW and CC trials. During 1 hour and 2 hours of recovery, the glucose concentration was higher in the CE trial than those in the other two trials \( (p < 0.05) \). However, by the end of the recovery period, the glucose concentration was lower in the CE trial than those in the other two trials (CE vs. CW vs. CC: 3.54 ± 0.46mM vs. 4.04 ± 0.26mM vs. 4.03 ± 0.29mM; \( p < 0.05 \)).

The changes in PV are expressed as a percentage change from the resting levels. PV decreased by approximately 6% after the 60-minute run in each trial (Fig. 4). Individual variability was very large. No difference was found among the trials at all time points \( (p < 0.05) \). The changes in PV were under the baseline throughout the 4-hour recovery period in all the trials.

**Subjective feelings**

No difference in ratings of perceived exertion was observed during the 60-minute run (Table 2). Moreover, no difference was observed in perceived thirst, stomach fullness, and abdominal discomfort in all the trials at any time point during the 4-hour recovery period \( (p > 0.05) \).

**Discussion**

During 4 hours of recovery after 2.36 ± 0.32% BW loss caused by a 60-minute run, the consumption of isocaloric CW solution retained more fluid in the body than that of plain CE or CC solution. Consumption of the CW solution also produced the least urine volume among all the three solutions, with urine loss of approximately 1004 mL in the CW trial compared with approximately 1184 mL and 1255 mL in the CE and CC trials, respectively. This study is the first to
To investigate the rehydrating effect of two different proteins when added to a common CE solution.

The loss of > 2% BW through sweating decreases exercise performance, muscular strength, and even cognitive functions.\(^{23,24}\) Athletes often experience dehydration after exercise because of the large amount of sweat loss and lack of sufficient fluid ingestion during exercise.\(^{1,25}\) Many studies have been conducted to determine how fluid loss can be rapidly and effectively replaced after exercise. Previous findings indicated that the volume and composition of ingested solutions are the two most important factors influencing postexercise rehydration.\(^{5,26}\) Studies have reported that most sports drinks containing CHO and electrolytes are more helpful for rehydration than mere water.\(^{9,10}\) However, recent evidence suggests that rehydration with sports drinks cannot maintain long-term positive fluid balance than drinks containing protein.\(^{13-15}\)

Seifert et al\(^{15}\) reported that solutions containing protein allow for better fluid retention in the body than commercially available CE solutions. James et al\(^{13,14}\) also obtained similar findings in their study in which a certain milk protein was added to a CE solution. Milk protein contains whey (20%) and casein (80%).\(^{18}\) In the present study, the ingestion of a CW solution resulted in greater fluid retention than that of a CC or plain CE solution. This result indicates that whey protein could have a major function in increasing fluid retention in milk protein.

Improved rehydration after the ingestion of the CW solution might be caused by the special properties of whey protein. Whey protein contains many essential AAs,\(^{18}\) and is more effective in increasing blood AAs and protein synthesis than
other proteins, e.g., casein. Moreover, whey protein is digested faster and has a higher absorption rate than casein. Previous studies have illustrated that AAs are helpful for the absorption of sodium and water in animals and humans. There are two major systems responsible for AA transport in mammalian cells, i.e. sodium-dependent AA transport system and sodium-independent AA transport system. Among them, the pathways known as A, ASC in sodium-dependent AA transport system and L in sodium-independent AA transport system are reported to be the key pathways for AA absorption. Furthermore, studies found that many AAs, including glutamine and alanine, can be absorbed by the sodium-dependent AA transport system. Therefore, with the absorption of AAs, more sodium will be absorbed and result in a large osmotic gradient in the circulation. Finally, more water can be absorbed and retained in the circulation. By contrast, AAs can promote plasma albumin synthesis, so more fluid will be absorbed into the circulation to maintain the constant level of albumin.

However, different findings were reported by another study, which found that the percentage of fluid retention is similar between a CE plus whey protein solution and an isocaloric CE solution. The differences in whey protein concentration and rehydration protocol may explain the inconsistent findings. The whey protein concentration in the study of James et al. was lower than that in the present study (15 g/L vs. 22 g/L), and the small amount of whey protein may not significantly increase the fluid retention. To avoid stomach bloating and delayed gastric emptying, participants ingested the solutions in six boluses every 30 minutes for a total of 150 minutes. However, a much shorter rehydration time of four boluses every 15 minutes for 60 minutes was administered in the study of James et al.

Compared with whey protein, casein is digested more slowly in the stomach because casein usually clots in the presence of gastric acid. Thus, casein cannot increase the blood AA level and protein synthesis rate as quickly as whey protein because it cannot be rapidly digested. Although AAs are reportedly helpful for the absorption and retention of fluid, the casein protein utilized in the present study did not present any further benefit for fluid retention. Both the CE solution and CE plus casein solution resulted in the same fraction of fluid retained after recovery. The volumes of urine production during recovery were similar in the CE and CC trials. More fluid was lost through urine in the CC trial (1255 mL) than the CW trial (1004 mL). This result could be ascribed to the fact that casein is digested slowly, as previously mentioned. During the short-term recovery period, casein was probably not completely digested into circulation because of the reduced gastric emptying rate.

As a limitation of this study, the osmotic gradient (plasma osmolality) and synthesis of plasma albumin were not measured. However, we can still reasonably speculate that whey protein in the solutions promoted fluid absorption and retention in the body through the aforementioned mechanisms, thereby resulting in greater fluid retention than plain CE solution or CE plus casein solution. Further studies are necessary to clarify the mechanisms responsible for these findings.

With regard to USG and urine osmolality, all participants had USGs < 1.020 g/mL and urine osmolality < 700 mmol/kg prior to the test. This result indicates that the participants were

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Table 2
Subjective feelings including perceived thirst, abdominal discomfort and stomach fullness during the 4 hours' recovery. Subjective feelings were scaled from 0 to 10, where 0 meant “not so much” and 10 meant “very much”.

<table>
<thead>
<tr>
<th></th>
<th>Recovery time (min)</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>60</td>
</tr>
<tr>
<td><strong>Perceived thirst</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CE</td>
<td>5.4 ± 1.8</td>
<td>1.5 ± 1.2</td>
</tr>
<tr>
<td>CW</td>
<td>5.5 ± 2.3</td>
<td>1.1 ± 1.1</td>
</tr>
<tr>
<td>CC</td>
<td>5.6 ± 1.4</td>
<td>1.6 ± 1.2</td>
</tr>
<tr>
<td><strong>Stomach fullness</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CE</td>
<td>0.4 ± 0.7</td>
<td>0.8 ± 1.2</td>
</tr>
<tr>
<td>CW</td>
<td>0.2 ± 0.4</td>
<td>0.7 ± 1.3</td>
</tr>
<tr>
<td>CC</td>
<td>0.1 ± 0.3</td>
<td>1.0 ± 1.6</td>
</tr>
<tr>
<td><strong>Abdominal discomfort</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CE</td>
<td>0.3 ± 1.0</td>
<td>0.2 ± 0.6</td>
</tr>
<tr>
<td>CW</td>
<td>0.4 ± 1.0</td>
<td>0.3 ± 0.7</td>
</tr>
<tr>
<td>CC</td>
<td>0.4 ± 0.7</td>
<td>0.2 ± 0.6</td>
</tr>
</tbody>
</table>

CC = CE plus casein protein solution trial; CE = carbohydrate—electrolyte solution trial; CW = CE plus whey protein solution trial.
In a normal euhydrated state before the start of the test. In the three trials, USG and urine osmolality significantly decreased at 3 hours and 4 hours of recovery. However, the CW trial demonstrated higher USG and urine osmolality compared with the CE and CC trials. Similar findings were reported by previous studies. High urine production results in low USG and urine osmolality detected because of dilution. Compared with the CW trial, the CE and CC trials outputted more fluid through urine, which possibly resulted in the lower USG and urine osmolality concentration in the present study.

The urine output was approximately 300 mL in all trials during the last hour of recovery, and the volume was higher than the normal urine production in healthy well hydrated individuals. As the participants completed solution ingestion at 150 minutes and urine collection was finished at 240 minutes, large urine production induced by drinking did not end when the experiment was completed at 240 minutes. Moreover, low USG and urine osmolality were obtained at the end of the recovery period. Thus, a longer recovery time is needed in further studies to obtain more information on urine production and fluid retention under this situation.

PV is an indicator of the fluid status of a person. The reduction in PV after exercise is due to the fluid moving from the blood vessels into the surrounding tissues and fluid loss via sweating. A 6% reduction in PV was observed immediately after exercise in the present study. Individual variability was very large during the 4 hours’ recovery period, and PV in the three trials did not return to the pre-exercise level. This finding differed from that of a previous study that employed a similar protocol. In the study conducted by Wong and Chen in 2011, PV was found to be greater than the pre-exercise level after 1 hour of recovery in the CE trial, and continued to increase toward the end of the recovery period. The difference might be caused by the different sodium contents in the solutions, which has an important function in rehydration. The sodium content in the present study was 14mM, whereas that in the study conducted by Wong and Chen was 21mM. Low sodium content resulted in minimal retention of ingested solution in the present study, which was supported by the fact that the final percentage of retention after CE solution consumption was approximately 47% compared with 52% in the study conducted by Wong and Chen.

Given that the CE solution had higher CHO density (66 g/L) than the CW (44 g/L) and CC (44 g/L) solutions, a greater response in blood glucose concentration was observed in 1 hour and 2 hours of recovery in the CE trial than that in both the CW and CC trials. This result was expected because previous studies reported similar findings after a solution with high CHO density was consumed by participants. Although we did not measure the insulin level in this study, a higher insulin level was induced to regulate the high level of blood glucose. Higher insulin levels could have resulted in low blood glucose concentration observed at the end of the 4 hours’ recovery period in the CE trial.

In conclusion, consumption of a CW solution replaced fluid lost after a 60-minute run and produced less urine during a 4-hour recovery period than consumption of CE or CC solutions. A CW solution was more effective for rehydration than CE or CC solution during a short-term recovery period after prolonged exercise.

Conflicts of interest
The authors declare that they have no conflicts of interest.

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Acknowledgments
We would like to thank all the courageous participants for their fearless participation, enthusiasm, and persistence they maintained in this challenging experiment.

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