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Protein Added to a Sports Drink Improves Fluid Retention

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The purpose of this study was to compare fluid retention of carbohydrate plus protein, a carbohydrate-only, and water following 2.5% body weight (BW) loss. Thirteen subjects dehydrated to 2.5% of BW, then ingested a CHO (6%) plus protein drink (1.5%; CP), a 6% CHO drink, or water (WA) at a volume equal to BW loss during a 3-h recovery. Fluid retention was significantly greater for CP (88 ± 4.7%) than CHO (75 ± 14.6%), which was greater than WA (53 ± 16.1%). Serum and urine osmolalities were greater for CP (284.7 ± 5.0; 569.4 ± 291.4 mOsm/kg) than CHO (282.6 ± 5.2; 472.9 ± 291.5 mOsm/kg) which were greater than WA (280.6 ± 5.9, 303.7 ± 251.5 mOsm/kg). Results indicate that fluid retention for CP was 15% greater than CHO and 40% greater than WA. Water ingestion led to a dilution of the serum and resulted in only 53% fluid retention.

Key Words: rehydration, fluid retention, sports drinks, protein

Sports drinks are formulated to provide fluid to minimize dehydration and to supply carbohydrates and electrolytes for fluid absorption and energy, aid in fluid retention, and enhance flavor. The rehydration effectiveness of these beverages can be assessed by the indices of gastric emptying, intestinal absorption, and fluid retention. Retaining fluid is crucial for optimal physiological function, improved performance, and rapid recovery, especially if multiple bouts of exercise are required.

Receptors in the hypothalamus and kidneys respond to changes in blood osmolality, body water (or plasma volume), and blood pressure during disruptions in fluid balance. For example, as blood osmolality increases, signals are ultimately sent to the kidney to retain fluid. On the other hand, when blood osmolality is quickly diluted, fluid is lost to urine formation in order to increase osmolality.

Numerous authors have reported that beverages need to contain sodium to enhance rehydration, usually through a change in blood osmolality (8, 9, 11, 13, 18). Many of these previous investigations have focused on the integral role of water, carbohydrate, and sodium to influence fluid retention during rehydration. Ingesting beverages that contain osmotically active particles will promote water movement across the gut and aid in fluid retention. Sodium would appear to be the ideal candidate and many studies have focused on this aspect. However, simply increasing sodium concentration does not necessarily improve fluid absorption from the gut, fluid balance, or plasma volume (2, 4, 8, 12, 16, 19). Water moves

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passively out of the intestine because of an osmotic gradient created by intestinal solute absorption. Compounds other than sodium, such as carbohydrate and protein, may even be more important than sodium in influencing absorption, and ultimately, fluid retention.

Numerous authors have reported on the benefit of adding protein to a rehydration beverage (3, 6, 7, 14, 17, 22, 23, 24). Amino acids are transported transcellularly by active transport, sodium dependent and independent transporters, and by passive transport (20). It has been well established that proteins enhance sodium and water absorption in the intestine (3, 14, 17, 20, 22, 23). The increased transcellular transport of sodium and protein creates a greater osmotic gradient to attract and retain water. Schedl et al. (17) reported that amino acids also act to open the tight junctions to increase water absorption through paracellular transport.

The purpose of this study was to investigate if adding protein to a carbohydrate-based sports drink would influence fluid retention rate compared to a carbohydrate-only beverage and water following exercise-induced dehydration.

Methods

Following approval from the institutional review board, all subjects provided written informed consent prior to participation. The 13 subjects (5 female, 8 male) ranged in age from 20 to 28 y and were experienced endurance athletes.

Subjects cycled at 25 °C to dehydrate to approximately 2.5% of starting body weight (BW) during three counterbalanced, blinded conditions. Cycling intensity was 80% of maximal heart rate. Maximal heart rate was determined previously by a ramped, cycling protocol. Subjects then ingested one of the test beverages in an amount equal to body weight loss. Recognizing the large variability with ad libitum ingestion during rehydration, we choose to give a rehydration volume based on weight loss. All subjects were given 20 min to complete ingestion. After ingesting the beverage, subjects sat quietly at room temperature and humidity (25 °C and 40%) for the next 3 h during the recovery period.

Subjects ingested one of the three beverages following dehydration. Subjects ingested a carbohydrate/electrolyte/protein drink (CP), a carbohydrate/electrolyte drink (CHO), or flavored water (WA). The CP drink contained 6 g CHO, 1.5 g protein, 53 mg sodium, and 18 mg potassium per 100 mL (Accelerade, Pacific-Health Labs, Matawan, NJ). The CHO drink contained 6 g CHO, 46 mg sodium, and 12.5 mg potassium per 100 mL (Gatorade, Pepsico, Inc.). Beverage osmolality for CP, CHO, and WA were 305 mOsm/kg, 280 mOsm/kg, and 2 mOsm/kg, respectively. Drinks were served at 8 °C.

An indwelling venous catheter was placed in a forearm vein prior to exercise and after subjects voided their bladder; 7 mL blood samples were collected at pre-exercise (0 min) and immediately post-exercise, and 30, 60, 90, 120, and 180 min after beverage ingestion. Following the collection, an aliquot of blood was used for hemoglobin determination (cyanomethemoglobin method, kit #525, Sigma-Aldrich, St. Louis, MO) and hematocrit (following centrifugation). Hemoglobin and hematocrit values were used to determine percent change in plasma volume (1). The remaining aliquot was allowed to clot for 20 min. The clotted blood sample was centrifuged and serum extracted. All blood, with the exception of the hematocrit samples, and urine samples were frozen (–70 °C) and analyzed at the completion of the study. Serum and urine were analyzed for osmolality (Advanced MicroOsmometer, Advanced Instruments, Inc., Norwood, MA). Urine samples and BW were collected after each blood sample. The scale from which BW was collected has a precision of 100 g and was calibrated prior to testing (Health o meter, Jarden Corp., Rye, NY). Free water clearance (FWC) was calculated from the corresponding osmolality levels and urine volume (5). Fluid retention was based on body weight changes and was determined by the following formula: Retention = Ingested fluid volume – urine volume – change in body weight (sweat loss).

A repeated measures ANOVA was used to analyze the data. When a significant interaction was observed, a Tukey's LSD post hoc test was used to differentiate means. Alpha level of significance was 0.05. Data are listed as means ± standard deviation.

Results

All subjects completed the three trials. All subjects completed beverage ingestion within 20 min and none reported adverse side effects from any of the beverages. Exercise duration to lose weight for males ranged from 60 to 75 min and 75 to 105 min for female subjects.

Subjects averaged 1.73 ± 0.68 kg weight loss during WA, 1.73 ± 0.66 kg with CP, and 1.66 ± 0.52 kg with CHO from exercise. When expressed as a percent of starting BW, losses amounted to 2.3%, 2.5%, and 2.4% for WA, CP, and CHO, respectively. No statistical differences were observed between treatments for weight lost during the exercise. Based on BW changes, average ingestion volume was not different between groups and averaged 1726 ± 536 mL for WA, 1726 ± 662 mL for CP, and 1662 ± 519 mL for CHO.

Fluid retention at the end of the 3 h recovery period was significantly ($P = 0.000$) greater for CP (88.0 ± 4.7%) than CHO (74.9 ± 14.6%) and WA (53.2 ± 16.1%). The fluid retention for CHO was also greater than WA. The calculated retention volumes were 1519 mL for CP, 1245 mL for CHO, and 880 mL for WA.

Following beverage ingestion, body weight was restored to baseline for all treatments. Weight change was less for CP than WA at 90, 120, and 180 min. Likewise, BW change at 180 min was less for CP than CHO. No differences were observed between CHO and WA for weight change (Table 1). During recovery, within-treatment analyses indicate that the 60, 90, 120, and 180 min BW changes were significantly different from baseline for WA. For CP, only the 180 min time was lower than baseline while the 120 and 180 min collections were lower than baseline for CHO.

Since retention levels were different between treatments, so too were urine volumes. Total urine volume was significantly less ($P = 0.000$) for CP (206.4 ± 99.3 mL) than CHO (395.3 ± 242.4 mL) and WA (860.6 ± 358.9 mL). Mean urine output for CHO was also significantly less than WA. Urine output data from the individual collection points can be found in Figure 1. Urine output at 60, 90, 120, and 180 min was significantly greater in the WA trial than CP and CHO, while the 90 and 120 min volumes were greater in CHO than CP. Urine volume was significantly increased from baseline to the 60, 90, 120, and 180 min for WA. Urine output increased significantly at 180 min for CP, while outputs at 90, 120, and 180 min were greater than baseline for CHO.

Table 1 Body Weight Changes from Baseline Through Recovery

		Time (min)				
Treatment	Pre	30	60	90	120	180
WA	0	–0.19 (0.40)	–0.52 (0.58)[a]	–0.65 (0.51)[a, b]	–0.86 (0.58)[a, b]	–1.08 (0.80)[a, b]
CP	0	–0.12 (0.23)	–0.27 (0.15)	–0.33 (0.18)	–0.40 (0.23)	–0.51 (0.23)[a]
CHO	0	–0.12 (0.22)	–0.28 (0.22)	–0.45 (0.25)[a]	–0.63 (0.36)[a]	–0.93 (0.55)[a, b]

Values are means ± standard deviation.

Body weight changes in kg.

Pre: pre-exercise body weights used as baseline; 30 to 180 min: post beverage ingestion

WA: Water trial; CP: Carbohydrate-protein beverage; CHO: Carbohydrate only beverage

[a]Significantly different from baseline

[b]Significantly different from CP

Treatment main effect for weight change: WA > CHO > CP

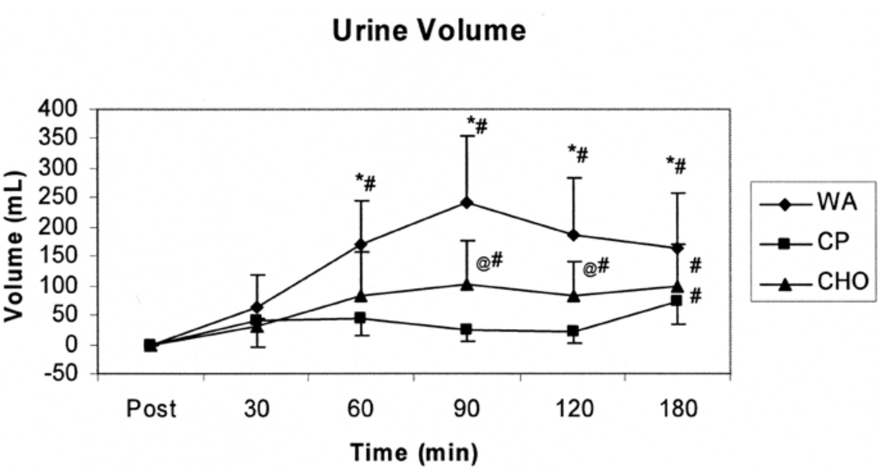

Figure 1 — Urine output during the 3 h recovery period. WA: water; CP: carbohydrate-protein; CHO: carbohydrate-only. Post: Immediately post exercise; 30 to 180 min after beverage ingestion. #: Significantly different from baseline. *: Significantly different from CP and CHO. @: Significantly different from CP. Values are means ± standard deviation.

Figure 2 contains percent change in plasma volume. A significant treatment main effect was observed between CP and WA. The percent change in plasma volume for the CP treatment was 1.9 ± 8.0% vs. –2.3 ± 9.6% for the WA treatment. No treatment differences were noted between CP and CHO (–1.2 ± 8.9%) or between CHO and WA.

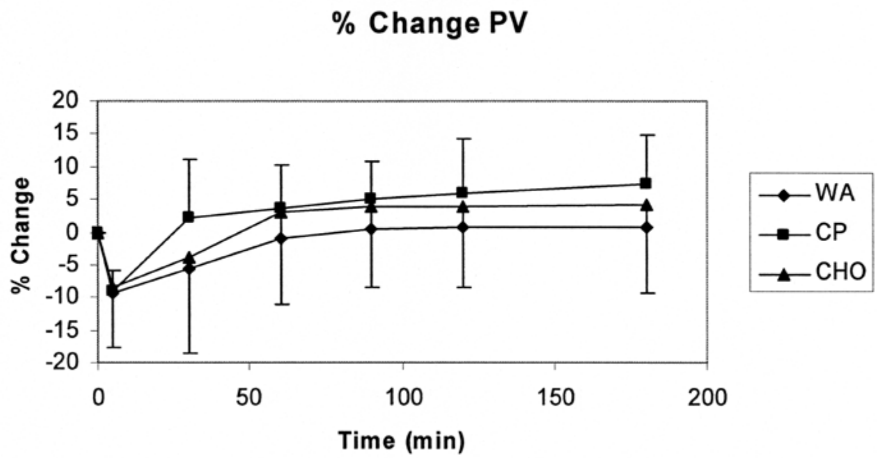

Figure 2 — Percent change in plasma volume. WA: water; CP: carbohydrate-protein; CHO: carbohydrate-only. Values are means ± standard deviation.

Average urine osmolality was greater ($P = 0.001$) for CP (569.4 ± 291.4 mOsm/kg) than CHO (472.9 ± 291.5 mOsm/kg) and WA (303.7 ± 251.5 mOsm/kg). The CHO treatment also resulted in greater urine osmolality than WA. Osmolality data was significantly greater for CP at 60, 90, 120, and 180 min points compared to WA (Figure 3). The 60, 90, and 120 min osmolalities were greater for CHO than WA. Osmolality was also greater for CP at 120 and 180 min compared to CHO. Urine osmolality was significantly elevated above baseline at 30 min post ingestion for all three treatments. It remained elevated only for CP at 90 to 180 min post ingestion.

Average serum osmolality was greater ($P = 0.000$) for CP (284.7 ± 5.0 mOsm/kg) than CHO (282.6 ± 5.2 mOsm/kg) and WA (280.6 ± 5.9 mOsm/kg). Likewise, the CHO trial was greater than WA. Osmolality at 30, 60, and 90 min was significantly greater during CP and CHO than WA (Figure 4). In addition, the 120 and 180 min samples were greater for CP than CHO. Baseline measures for serum osmolality were significantly different from the post exercise and 30 min post ingestion for all three treatments. In addition, the 60 min post ingestion osmolality was significantly less than baseline for WA.

Average free water clearance (FWC) was statistically greater for WA than CP (Table 2). The CHO treatment was not different from CP or WA. The 60, 90, 120, and 180 min data points were significantly greater during the WA trial than

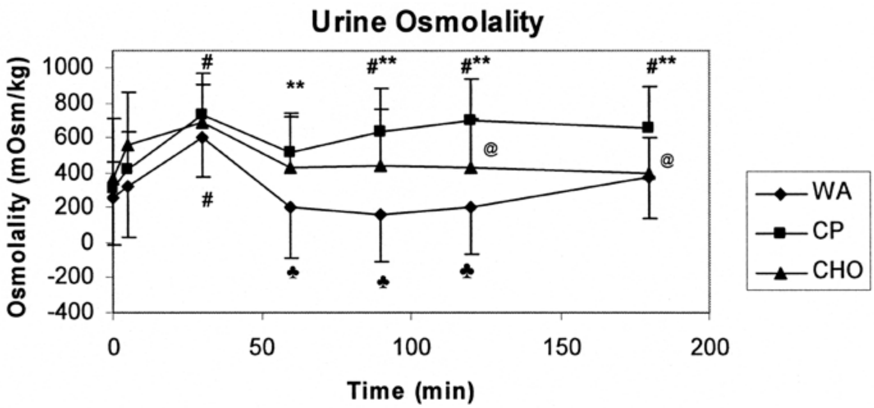

Figure 3 — Urine osmolality. WA: water; CP: carbohydrate-protein; CHO: carbohydrate-only. #: Significantly different from baseline. **: Significantly different from WA; ♣: Significantly different from CHO. Values are means ± standard deviation.

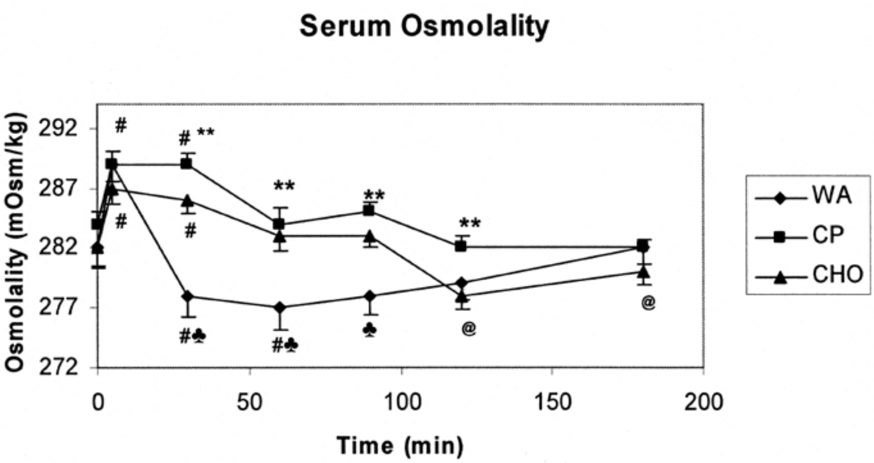

Figure 4 — Serum osmolality. WA: water; CP: carbohydrate-protein; CHO: carbohydrate-only. #: Significantly different from baseline. **: Significantly different from WA. @: Significantly different from CP. ♣: Significantly different from CHO. Values are means ± standard deviation.

CP. While the 60, 90, and 120 min points were greater in the WA trial than CHO. The 90, 120, and 180 min FWC values were greater for CHO than CP. Free water clearance was significantly elevated above baseline for WA at 60, 90, and 120 min post ingestion. However, FWC was not different from baseline for CP and CHO.

Table 2 Free Water Clearance During Recovery

		Time (min)				
Treatment	**Post**	**30**	**60**	**90**	**120**	**180**
WP	0	–1.5 (2.0)	2.8 (4.2)[a, b]	5.3 (5.6)[a, b]	3.3 (5.0)[a, b]	–0.1 (2.3)[c]
CP	0	–1.5 (2.0)	–1.2 (1.8)	–1.3 (1.1)	–1.3 (0.9)	–1.6 (1.4)
CHO	0	–1.2 (1.1)	0.2 (2.5)	0.0 (3.2)[c]	–0.5 (3.6)[c]	–0.3 (1.4)[c]

Values are means ± standard deviation; units in mL/min.

WA: Water trial; CP: Carbohydrate-protein beverage; CHO: Carbohydrate only beverage.

Treatment; Post: Immediately post exercise urine sample; 30 to 180 min: post beverage ingestion.

[a] Significantly different from baseline.

[b] Significantly different from CP and CHO values.

[c] Significantly different from CP value.

Discussion and Conclusion

Results indicate that a small amount of protein, when added to a carbohydrate-based sports drink, does not inhibit rehydration or diminish fluid retention. In fact, based on our results, the CP beverage may be a preferable choice over a carbohydrate-electrolyte sports drink and plain water, when rehydration and fluid retention are a concern. During the CP trial, subjects retained 15% more fluid than CHO and 40% more than WA. Subjects also experienced significantly greater urine loss when rehydrating with WA compared to CP and CHO. As a consequence, only 53% of ingested fluid was retained after the 3 h recovery period during the WA trial.

The most obvious reason for fluid retention differences over WA is related to solute, sodium and protein, content of the CP and CHO beverages. Although adding sodium to a beverage may be important for fluid retention and sensory characteristics (8, 9, 11, 12, 13, 18), the effects seen here do not appear to be a product of the different sodium levels of CP and CHO. Shirreffs et al. (18) reported that a beverage should contain enough cations to maintain an elevated blood osmolality and minimize urine formation. Wapnir et al. (1990) reported that there must be sufficient sodium in the rehydration solution so that the amino acids can be efficiently co-transported. If the sodium content is inadequate, the addition of amino acids may not effectively enhance rehydration. The sodium level of CP (23 mM/L) and CHO (20 mM/L) appear to have been adequate to increase fluid retention, maintain blood osmolality, and minimize urine output when compared to WA under the given conditions.

Urine output and serum and urine osmolalities are interrelated and determine free water clearance. Minimizing free water clearance is one of the key factors in maintaining fluid retention. Free water clearance increases when there is a rapid decrease in blood osmolality (13). The macula densa sense the decrease in osmolality and the nephron responds by retaining solutes and excreting fluid in an attempt to normalize osmolality. Our data supports that of Nose et al. (13) where a rapid decrease in serum osmolality leads to increased FWC and urine production. In contrast to CP and CHO in the present study, serum osmolality for WA decreased from 290 to 278 mOsm/kg water within the first 30 min of recovery and remained below baseline through 120 min. Free water clearance peaked at 90 min post ingestion and was significantly greater than CP and CHO from 60 to 120 min of recovery during the WA trial. Urine output followed the same pattern as FWC during the WA condition. Urine volume only started to decrease when serum osmolality increased and was maintained at levels similar to the baseline sample at 120 min of recovery. The end result was that urine production was 318% and 118% greater during WA than during CP and CHO trials, respectively. Thus, osmotically active particles need to be included in recovery fluids to reduce urine volume and enhance fluid retention (8, 9, 11, 13, 18). In this case, both CP and CHO demonstrated greater capacity to retain significantly more fluid over WA by virtue of the inclusion of carbohydrate, sodium, and, in the case of CP, protein in the fluid.

Ingestion of CP resulted in significantly elevated serum osmolality than CHO and WA during the 3 h recovery period. It is not possible to discern if it was the protein, electrolytes, or a combination thereof that influenced serum osmolality and fluid retention for CP. However, it is unlikely that the small difference in sodium concentration (3 mM/L) between CP and CHO could account for the 15% fluid retention difference. In fact, previous research reported no differences in hydration indices between beverages with sodium levels ranging from 1.3 mM/L to 18.9 mM/L (12). Shirreffs et al. (18) also reported no differences for hydration indices between 23 mM/L and 61 mM/L sodium with high and low fluid volumes. Therefore, the most plausible explanation for increased retention of CP over CHO is not the small sodium difference, but the addition of protein.

The benefit of adding a small amount of protein, or amino acids, to a rehydration beverage, in order to enhance sodium and water absorption from the gut, has been well researched (3, 14, 17, 20, 22, 23). Amino acids are absorbed from the small intestine by multiple transport systems, based on presence of the different types of amino acids. These systems include passive transport (diffusion), sodium independent transporters, and sodium dependent co-transporters. The sodium dependent amino acid co-transporters are separate from the sodium-glucose co-transporter. When both sodium-amino acid and sodium-glucose co-transporters are activated, more sodium can be moved across the intestinal wall. The end result is a greater osmotic gradient by combining protein, glucose, and sodium than with just glucose and sodium. The combination of carbohydrate, amino acids, and sodium provides separate, but additive, mechanisms to enhance water absorption from the intestine beyond that of just the sodium-glucose transport system. However, it appears that the concentration of protein, in a sports drink, is critical in determining hydrating potential of a beverage.

High protein concentrations are known to slow gastric emptying (10). Maughan et al. (10) noted that a 120 g/L protein beverage emptied significantly slower than a 60 g/L protein drink and a 70 g/L CHO drink. It is unclear, however, whether this difference in emptying was due to cholecystokinin release, caloric differences, or beverage osmolality. Regardless, Maughan et al. (10) found there was no difference in gastric emptying rate between the 60 g/L protein and 70 g/L CHO drinks.

In addition, there appears to be a threshold for carbohydrate content and beverage osmolality of 400 mOsm/kg water before gastric emptying is inhibited (15, 21). It is unlikely that the protein in CP diminished gastric emptying because beverage osmolality was 305 mOsm/kg water and both CP and CHO have a carbohydrate content of 6%. Thus, as evidenced by percent change in plasma volume and serum protein data of the present study, the level of protein in the CP beverage of the present study did not appear to inhibit gastric emptying.

Rehydration is often looked at as a singular issue, but rehydration is composed of three interrelated components: gastric emptying, intestinal absorption, and retention. Even though it does not appear that WA affected gastric emptying or intestinal absorption, WA ingestion did lead to reduced retention, compared to CP and CHO, after the 3 h recovery. The percent change in plasma volume returned to baseline levels after 60 min during WA and was maintained for the final 2 h. However, there was still a fluid deficit of about 880 mL at the end of the 3 h recovery period. The implication being that plasma volume alone may not be indicative of the level of rehydration. Since there was no difference in plasma volume restoration, the differences in retention have to be found in the intracellular and interstitial spaces.

In conclusion, contrary to popular misconception, adding small amounts of protein to a carbohydrate-based sports drink did not interfere with fluid retention following dehydration. Carbohydrate plus protein led to improved water retention by 15% over a CHO-only beverage and 40% over plain water. Thus, the CP beverage may be a preferable choice when fluid retention is a concern. We suggest that this result is a consequence of the higher beverage osmolality of CP or possibly water retention properties of the protein. Further research is necessary to determine if other mechanisms might cause this improved retention effect.

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