Addition of Whey Peptides to a Carbohydrate-electrolyte Drink Enhances its Effect on the Early Treatment of Dehydration in Rats

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Previous studies suggested that peptide absorption by peptide transporter 1 (PepT1) contributes to water absorption in the small intestine. Here, we aimed to apply this effect of peptides to a rehydration drink for the effective treatment of dehydration. We first prepared a whey peptide drink (WPD) in which the composition (whey peptides and Na+) was optimized for water absorption in the small intestine. We then evaluated the effectiveness of the WPD in an in vivo dehydration model. The results demonstrated the following: (1) the addition of whey peptides to a carbohydrate-electrolyte drink enhanced the rehydration effect in the rat dehydration model, i.e., it increased the plasma volume more quickly and more abundantly; and (2) the WPD was more effective for rehydration than a general sports drink. The WPD could be considered a new treatment option for the rapid alleviation of dehydration.

Keywords: whey peptide, water absorption, dehydration, rehydration

Introduction

Dehydration inhibits cutaneous blood flow and sweating and thus impairs the regulation of body temperature (Nadel et al., 1980). Dehydration also exerts various negative effects on the human body, such as the deterioration of exercise performance (Sawka, 1992; Yoshida et al., 1997), fatigue (Gonzalez-Alonso et al., 1999), and impaired cognitive function (Wilson and Morley, 2003). It is thus important that dehydration is addressed early by rehydration.

Water as well as minerals, especially Na+, are lost from the human body during sweating. Supplementation of Na+ with water is thus also required. Na+ is absorbed along with glucose by sodium/glucose cotransporter (SGLT1) in the small intestine. Na+ can therefore be absorbed efficiently when it is ingested with glucose. It was reported that water absorption is promoted by SGLT1 along with the absorption of Na+ and glucose (Duquette et al., 2001; Loo et al., 1996, 2002; Sasseville et al., 2011).

The most important requirement for beverages designed to treat dehydration is to rapidly restore the body’s plasma volume. Gastric emptying, water absorption in the small intestine, and the reabsorption of water and Na+ in the kidney are related to this function. So-called sports drinks (SDs) are often used for rehydration. The Institute of Medicine (IOM) in the United States provided general guidance for the composition of SDs designed for individuals performing prolonged physical activity in a hot environment (Institute of Medicine, 1994). They recommended that SDs contain ~20 – 30 mM Na+, ~2 – 5 mM K+, and ~5% – 10% carbohydrates. Many of the commercially available SDs contain ~20 mM Na+ and ~5% – 6% carbohydrates. It was reported that in dehydrated subjects, the recovery effect of SD on plasma volume...
was greater than that of water (Chang et al., 2010; Gonzalez-Alonso et al., 1992; Shirreffs et al., 2007; Wong and Chen, 2011). However, those reports suggested that it takes about an hour for the plasma volume to return to its pre-dehydration level. For the maintenance of exercise performance, beverages that can rehydrate more rapidly are needed.

Some reports have suggested that peptide uptake is linked to water absorption in the small intestine. For example, it was indicated that water is absorbed along with the uptake of the dipeptide derivative glycylsarcosine by peptide transporter 1 (PepT1) in the small intestine (Chen et al., 2010). It was also suggested that PepT1 is expressed in the distal colon and contributes to water absorption (Wuensch et al., 2013). In our previous study, we found that whey peptides provide a greater enhancement of water absorption in the small intestine compared to soy peptides and an amino acid mixture whose composition was equal to that of a whey peptide (Ito et al., in press). However, few studies have focused on the treatment of dehydration by peptide-containing drinks.

The aim of the present study was to apply this effect of whey peptide on water absorption to the method of rehydration. For this purpose, we first investigated the relationship between the concentrations of whey peptides and Na⁺ in test solutions and the water absorption rate in the small intestine, using a rat small intestine perfusion model. Based on the optimal ratio obtained from this model, we prepared a novel whey-peptide drink (WPD). Using the rat dehydration model, we evaluated the effectiveness of the WPD on the alleviation of dehydration. We also compared the effectiveness of the WPD with that of a conventional SD on the alleviation of dehydration.

Materials and Methods

Test solutions To investigate the relationship between concentrations of whey peptides or Na⁺ and the water absorption rate, we prepared test solutions containing 0% – 0.5% whey peptides, 0 – 30 mM Na⁺, 3 mM K⁺, and 0.6% carbohydrate. Trehalose, a disaccharide of glucose, was used as the carbohydrate. We purchased the whey peptide, prepared by hydrolyzing whey protein with proteases, from Arla Food (Viby, Denmark). The whey peptide contained 85.6 g/100 g protein, 0.1 g/100 g carbohydrates, 0.1 g/100 g fat, 4.7 g/100 g ash, 567 mg/100 g Na⁺, and 1150 mg/100 g K⁺. The free amino acids content was 0.18 g/100 g. The average molecular weight of the whey peptide was calculated as 595 Da by size-exclusion chromatography. In experiments to evaluate the dose-dependent effect of whey peptide, we tested concentrations of 0%, 0.1%, 0.3%, and 0.5%. In each case, the concentration of sodium was 10 mM. In experiments to evaluate the dose-dependent effect of Na⁺, we tested concentrations of 0, 10, 20, and 30 mM. In each case, the concentration of whey peptide was 0.3%. Based on the results of these evaluations, we set the compositions of the WPD and a control drink (CD) (Table 1).

Whey peptides (w/v%) 0.5 0 0
Carbohydrate (w/v%) 0.6 0.6 6.2
Sodium (mM) 21 20 21
Potassium (mM) 5 3 5

Table 1. Compositions of the test drinks

The CD was a carbohydrate-electrolyte drink that did not contain whey peptides. For comparison of the WPD with a conventional rehydration drink, we used a commercially available SD (Table 1).

Test animals and rearing conditions We purchased 7-week-old male Sprague-Dawley rats from Japan SLC (Shizuoka, Japan). The rats were maintained at 21 ± 2.0°C with a relative humidity of 55 ± 15% and a 12-h light-dark cycle (light cycle: 07:00 – 19:00). The rats were fed standard chow, Charles River Formula-1 (Oriental Yeast, Tokyo, Japan), and water ad libitum. Each experiment was carried out after a >1-week acclimation period.

This study was approved by the Animal Committee of the Food Science Research Laboratory, Meiji Co., Ltd., and the care of animals was performed in accordance with the guidelines laid down by this committee.

Rat small intestine perfusion We investigated the relationship between the composition of the test drinks and the water absorption rate, and we also compared the water absorption rate of the WPD with that of the SD. The water absorption rate was measured using the rat small intestine perfusion model described by Nishinaka et al. (2004). Briefly, 4–6 rats in each group were anesthetized with urethane (1.5 g/kg, subcutaneously) and then laparatomized. A cannula for infusion was inserted into the proximal duodenum, and a second cannula for collection was inserted into the terminal ileum. The contents of the small intestine were removed by gentle lavage with each test solution, followed by the infusion of a test solution at 0.5 mL/min by a syringe pump (Harvard Apparatus, Holliston, MA, USA). The perfusion was continued for 90 min, and the perfusate was collected during the last 30 min. After the perfusate collection, the rat was sacrificed by exsanguination. The intestines were removed and washed with physiological saline, then lyophilized and weighed.

Phenol red (PR) was added to each test solution at 20 mg/L. PR was used as an internal standard, because it is not absorbed by the small intestine. The test solutions and perfusates were alkalized with sodium hydroxide, and their PR concentrations were determined by spectrometry at 560 nm. The concentrations of peptides/amino acids in the test solutions and perfusates were measured by high-performance liquid chromatography after hydrolysis with hydrochloric acid. The net absorption rates of water and peptide were calculated using the following equations:

Net water absorption rate (mL/min/g)= V·(1 − Ri/Ro)/Wi

where V is the volume of the perfusate, Ri is the initial plasma volume, Ro is the final plasma volume, and Wi is the initial body weight.
Net peptide absorption rate (mg/min/g) = \( V \cdot (P_i - P_o) \cdot R_i / R_o / W_i \) — Eq. 2

where \( V \) is the infusion rate (mL/min), \( W_i \) is the intestine dry weight (g), \( R_i \) is the PR concentration in the pre-infusion fluid (mg/L), \( R_o \) is the PR concentration in the post-infusion fluid (mg/L), \( P_i \) is the peptide concentration in the pre-infusion fluid (mg/mL), and \( P_o \) is the peptide concentration in the post-infusion fluid (mg/mL).

**Evaluation of test solutions using dehydration model rats**

We used the rat dehydration model to determine the effectiveness of each test drink on the alleviation of dehydration. First, 8–10 rats in each group were fasted for 20 h and deprived of water for 16 h from 4 h after the start of the fast. The rats were orally administered 50 mg/kg furosemide at the beginning of the water deprivation. We then administered 20 mL/kg of each test drink to the rats by oral gavage. We collected blood from the jugular vein under isoflurane anesthesia, before fasting, before oral administration, and at 5, 10, 15, 20, and 30 min after oral administration of the test drink. We measured the hematocrit levels and hemoglobin concentrations using an automatic blood analyzer (XT-1800iV; Sysmex, Kobe, Japan), and we calculated the rate of change in plasma volume (Dill and Costill, 1974). To evaluate the effectiveness of whey peptides, we used WPD, CD, and distilled water (W) as test drinks. To compare the effectiveness of WPD with that of conventional rehydration drinks, a commercially available SD was also used as a test drink.

**Statistical analysis**

All values are expressed as means with their standard errors (SEM). In the multigroup analysis, we confirmed that the homogeneity of variance could be assumed using Bartlett’s test, and we carried out Dunnett’s test or Tukey-Kramer’s test. In the comparisons of two groups, we confirmed that the homogeneity of variance could be assumed using an F-test, and we then used a Student’s t-test. Significance was defined as a \( p \)-value <0.05 in each analysis. These tests were performed using Excel Statistics software (SSRI, Tokyo, Japan).

**Results**

**Relationship between whey peptide concentration and water absorption rate**

In our investigation of the relationship between the whey peptide concentration and the water absorption rate, the water absorption rate showed a dose-dependent increase with the whey peptide concentration, and 0.3% and 0.5% whey peptide showed a significantly higher value than 0% whey peptide \( (p < 0.05, p < 0.01, \) respectively; Fig. 1A). The peptide absorption rate also increased dose-dependently with the whey peptide concentration, in which 0.3% and 0.5% whey peptide resulted in a significantly higher value than 0% whey peptide \( (p < 0.01, \) Fig. 1B). Regarding the 0.3% and 0.5% whey peptide data, we plotted the relationship between the peptide absorption and the water absorption rates (Fig. 1C) and observed a significant positive correlation \( (r = 0.95, p < 0.01) \). These results strongly suggested that the addition of whey peptides to the carbohydrate-electrolyte drink enhanced the water absorption in the small intestine.

**Relationship between Na\(^+\) concentration and water absorption rate**

Our evaluation of the relationship between the Na\(^+\) concentration and the water absorption rate revealed that the water absorption rate in the 20 mM Na\(^+\) test solution was the highest in
all groups and was significantly higher than that in the 0 mM Na⁺ solution \((p < 0.05, \text{Fig. 2})\). Although the absorption rate in the 30 mM Na⁺ solution tended to be higher than that in the 0 mM Na⁺ solution, a significant difference was not detected. These results suggested that the effect of whey peptides on the water absorption rate depended on the Na⁺ concentration of the drink.

In light of the results shown in Figures 1 and 2, we chose the composition of 0.5% whey peptides and 20 mM Na⁺ as the WPD, and we performed the subsequent experiment using this WPD.

**Effectiveness of WPD on alleviation of dehydration** We examined the effect of water deprivation and diuretic administration on the body weight and plasma volume (Table 2). The body weight and plasma volume in the rats deprived of both food and water were not significantly different compared with those in the rats deprived of food alone. The administration of the diuretic along with the deprivation of food and water significantly decreased both the body weight and plasma volume \((p < 0.05, \text{respectively})\).

We measured the rates of change in the plasma volume after the oral administration of WPD, CD, and W (Fig. 3A). The WPD showed a significantly higher rate of change in plasma volume compared to the CD at 10, 15, and 20 min after the oral administration \((p < 0.05)\). The WPD showed a significantly higher rate of change in plasma volume compared to W at all time points \((p < 0.05)\). The CD showed a significantly higher rate of change in plasma volume compared to W at 5 min \((p < 0.05)\).

The plasma osmolality after the oral administration of WPD, CD, or W was measured (Fig. 3B). The plasma osmolality decreased at 5 min after the oral administration of each of the three test drinks and then gently increased to baseline by 30 min after the oral administration. The levels of decrease in plasma osmolality after the administration of the WPD or CD were lower than that after the administration of W. The plasma osmolality at 20 min after the administration of WPD or CD was significantly higher than that of W \((p < 0.05)\). These results strongly indicated that the addition of whey peptides to the carbohydrate-electrolyte drink improved the effectiveness of the early treatment of dehydration.

**Comparison of WPD and SD effectiveness** The WPD showed a significantly higher rate of water absorption in the small intestine compared to the SD \((p < 0.05, \text{Fig. 4A})\). The WPD also showed a
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significantly higher rate of change in plasma volume at 5, 10, and 15 min compared to the SD in the rat dehydration model \((p<0.05)\), Fig. 4B). Additionally, the plasma volume was restored to approximately the same level as that at pre-dehydration at 15 min after the WPD administration. These results suggested that the WPD alleviated the dehydration more rapidly than conventional rehydration drinks.

Discussion

The results of the present study demonstrated the following: (1) the addition of whey peptides to a carbohydrate-electrolyte drink enhanced the rehydration effect in the rat dehydration model, namely, it increased the plasma volume more quickly and more abundantly; and (2) the WPD was more effective for rehydration than the general sports drink. This WPD might thus be a new treatment option for the rapid alleviation of dehydration.

Our examination of the whey peptide concentrations showed a dose-dependent promoting effect on water absorption (Fig. 1A) and a strong positive correlation between the peptide absorption rates and the water absorption rates (Fig. 1C). These results suggested that peptide absorption involves water absorption, as was described in a previous study (Chen et al., 2010). It is possible that the mechanism underlying the promotion of water absorption by peptide absorption is similar to the mechanisms underlying the promotions of water absorption by Na⁺ and glucose absorption by SGLT1.

The hypotheses regarding the mechanism of the water absorption-promoting effect of SGLT1 are roughly divided into two types: (1) passive water transport driven by the local osmotic gradient formed by the transport of the substrates (Duquette et al., 2001; Sasseville et al., 2011), and (2) active water transport coupled with the transport of the substrates at the molecular level (Loo et al., 1996, 2002). Peptide transport by PepT1 may also promote water absorption, as described by these mechanisms. Clarifying the mechanism of the promoting effect of whey peptides on water absorption is an attractive and challenging topic to address in the future.

In our previous study, we compared whey peptides with soy peptides (Ito et al., in press). The results demonstrated that soy peptides also enhanced water absorption, but whey peptides enhanced the water absorption to a greater degree. We also assayed the water-absorbing effect of several dipeptides contained in whey peptides, and the results suggested that many dipeptides can promote water absorption, but their effectiveness differed. We infer that the amino acid sequence is one of the main factors influencing the water-absorbing effect of peptides. It is practically impossible to clarify the important factors in the effect of peptides using the method applied in the present study. In the future, we aim to establish a new model that can be performed to assay the water-absorbing effect using only small amounts of samples, and we would like to use this model to clarify the important factor(s) in the promotion of water absorption by peptides.

In this study, we deprived rats of water and administered the diuretic furosemide to establish a dehydration model. Furosemide's diuretic effect involves the inhibition of sodium reabsorption in the kidneys. Furosemide has been used to promote dehydration in both rats (Nakajima et al., 1998) and humans (Ikegawa et al., 2011). In the present study, the overnight deprivation of water was not sufficient to dehydrate the rats, and the administration of the diuretic was thus necessary.

We also observed that in the rat dehydration model, the recovery of plasma volume after WPD administration was faster than that after CD administration (Fig. 3A), suggesting that the addition of whey peptides to the carbohydrate-electrolyte drink enhanced the plasma volume recovery effect as an early treatment of dehydration. It seemed that the promoting effect of whey peptides on water absorption in the small intestine (Fig. 1A) is the most important determinant of this result. In addition, this result suggested that the gastric emptying of the ingested WPD was not...
slow, although the WPD contained peptides and carbohydrates.

Moreover, the plasma volume recovery rate after the administration of the WPD plateaued at a higher level than that of the CD or W. It is possible that the change of plasma osmolality (Fig. 3B) was involved in this result, because plasma osmolality regulates the plasma volume via water diuresis by vasopressin. The Na⁺ and carbohydrates in the WPD or CD may contribute to the suppression of the decrease in plasma osmolality. Additionally, the plasma osmolality after the administration of the WPD showed changes similar to that of the CD, although the plasma volume after the administration of the WPD increased more than that of the CD. It is possible that the decrease in plasma osmolality was suppressed by di/tripeptides or free amino acids generated from the whey peptides.

The WPD alleviated dehydration more rapidly than the SD did (Fig. 4B). We suspect that the difference in the water absorption rate in the small intestine for each test drink (Fig. 4A) was the main determinant of this result. The SD showed a very low rate of water absorption in the small intestine, a result that is similar to that of a previous study (Nishinaka et al. 2004). It has been reported that water absorption was delayed when the carbohydrate concentration in an ingested drink was above 6% (Jeukendrup et al., 2009). It was also reported that a high concentration of carbohydrate delays gastric emptying (Coyle et al., 1978; Foster et al., 1980; Vist and Maughan, 1995). It may thus be possible that the approx. 6% carbohydrates in the SD contributed to the result shown in Figure 4B.

It was reported that the plasma volume after the administration of skim milk showed a change similar to that of a carbohydrate-electrolyte drink with an almost identical composition to that of a sports drink (Watson et al., 2008). Casein and whey protein in skim milk are digested and absorbed as peptides or amino acids. These peptides seem to have a promoting effect on water absorption, similar to that of the whey peptides used in the present study. However, the absorption of casein as amino acids or peptides is slow because casein coagulates in the stomach. This may be the reason why skim milk did not show a stronger effect on the plasma volume than a carbohydrate-electrolyte drink. On the other hand, whey protein was reported to be absorbed more rapidly than casein because whey protein does not coagulate in the stomach (Boirie et al., 1997). It was also suggested that whey peptides are absorbed into plasma as amino acids more rapidly than intact whey protein (Morifuji et al., 2010a). Thus, whey peptides may be most effective for the early treatment of dehydration.

In this study, we found that a whey peptide drink was highly effective for rehydration, which is typically required after exercise. Several studies have also reported the usefulness of whey peptides for ingestion after exercise. For example, whey peptides ingested after exercise were reported to promote protein synthesis (Kanda et al., 2013) and to increase glycogen levels in skeletal muscles (Morifuji et al., 2010b). We thus speculate that a WPD would be particularly useful for post-exercise recovery. In the future, we plan to investigate the usefulness of WPDs for rehydration after exercise in humans.

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