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Determining Hydration Using Serum Plasma Osmolality Following Consumption of Carbohydrate + Protein, and Carbohydrate Only Sports Drinks During 60km Cycling Time Trials

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**Determining Hydration Using Serum Plasma Osmolality Following Consumption of Carbohydrate +
Protein, and Carbohydrate Only Sports Drinks During 60km Cycling Time Trials**

by

Thomas J. Ebnet

A Thesis

Submitted to the Graduate Faculty of

St. Cloud State University

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Master of Science in

Exercise Science

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Thesis Committee:

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Abstract

The purpose of this study was to determine whether there was less dehydration when drinking a carbohydrate+protein (CHO+P) sports drink compared to a carbohydrate (CHO) only sports drink before, during, and after a 60km cycling time trial. It was hypothesized that the CHO+P sports drink would result in a decrease in plasma osmolality (improved hydration), thus allowing a cyclist to shorten their trial time. Testing was conducted on 14 trained cyclists on 4 non-consecutive days. Individual subjects repeated the same time trial with both types of sports drinks. A VO_{2peak} test was performed on the first day of testing. On the 2nd day, a 40km familiarization time trial was completed. On the 3rd and 4th days, subjects engaged in the 60km time trial on a Velotron Racermate stationary cycle. Subjects ingested 170mL of either supplement (CHO+P: Accelerade, Pacific Health Laboratories Inc., or CHO: Gatorade, PepsiCo Inc.) every 5km throughout the time trial. Blood samples were collected through an indwelling venous catheter at the start, at 20km, at 40km, at 60km, and 24 hours following the completion of the time trial. A minimum of 7 days separated the two 60km time trials. Results indicated no difference in plasma osmolality at the 20km and 40km mark; but, there was a difference at the 60km mark with CHO beverage being slightly better than CHO+P. This suggests that a CHO drink without added protein may benefit cyclists more when completing a time trial that is at least 60km in length.

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Chapter I: Introduction

Sports drinks are composed of water, carbohydrates (CHO) and electrolytes (largely sodium with a little potassium) to enhance fluid retention (6, 10, 24, 29, 39, 42). The efficacy of rehydration using a sports drink is determined by several physiological components: Palatability, gastric emptying, intestinal absorption, fluid retention, and volume (6, 9, 10, 23, 24, 29, 30, 39). In several studies a CHO + protein (CHO+P) sports drink has been shown to be better in performance compared to a CHO-only sports drink (16, 18, 36, 37, 41). The reason for the observed performance improvement following the addition of protein to a sports drink is as yet unclear. There have been numerous studies reporting the addition of protein in a meal (drink) decreases rate of gastric emptying (2, 4, 25). A slower rate of gastric emptying would decrease fluid delivery to the small intestine (SI) and thus its rate of absorption. This would elicit to a lesser extent fluid excretion at the kidney and improve rehydration. Yet Maughn et al. (26) concluded protein is emptied from the stomach at similar rates as CHO. Also, the addition of protein is theorized to aid in greater fluid delivery and retention in the gut as the addition of the protein solute may increase osmolality thereby pulling more water into the small intestine. Athletes should ultimately consider palatability, %CHO, as well as total caloric content as these factors influence gastric emptying and fluid retention as well (27).

Performance in the form of time is what matters most to competitive cyclists and other endurance type athletes. Managing to perform well with one's best (shortest) time occurs when an athlete is properly and adequately hydrated (euhydrated) before and during an exercise bout/trial/race that is at least 90 minutes or more in duration. In addition to hydration, athletes fare better when they ingest adequate substrates or macronutrients that can assist in fueling the exercise (7, 14, 43, 48, 49). Traditionally, the makeup of ready-to-drink sports formulas have consisted of 6%

carbohydrate and 20 mEq/L sodium. Recently, various sports beverages have attempted to find the right balance of the aforementioned combinations to hydrate and fuel athletes quickly, efficiently, and adequately for optimal performance. One such theory is that the addition of protein will aid in macronutrient value and hydration, thus leading to a shorter performance time for competitive cyclists.

Gastric emptying is regulated by the central nervous system (CNS), the enteric nervous system (ENS), and by hormones secreted by the gastrointestinal (GI) tract (13). The enteric system is inhibited by the sympathetic nervous system and stimulated by the parasympathetic nervous system (13). Activation of the sympathetic branch leads to inhibition of smooth muscle contraction and decreased blood flow in the gut, which decreases the movement of nutrients through the GI tract. Parasympathetic activation leads to stimulation within the GI tract by releasing acetylcholine (ACh) to cause contraction of smooth muscle and enhance movement of nutrients within the tract. The ENS is considered part of the peripheral nervous system and contains many neurons that control GI functions. The ENS is composed of the myenteric (Auerbach's) plexus, and the submucosal (Meissner's) plexus (13). The function of the myenteric plexus is to control movement of food through the GI tract through tonicity, and rate and rhythm of smooth muscle contractions. The function of the submucosal plexus is to regulate GI secretion and perfusion. The submucosal plexus is then largely responsible for GI hormone regulation, which can either stimulate or inhibit GI motility. The submucosal plexus secretes several hormones that respond to or affect the presence of protein within the GI tract. Gastrin is released in the presence of protein and leads to an increase in the production of gastric acid and mucosal motility. Cholecystokinin is generally released in the presence of fat and possibly protein. Gastric inhibitory peptide (GIP) is also released in response to all macronutrients and leads to a slower rate of gastric motility to promote digestion. Processes called

enterogastric inhibitory reflexes control the rate of gastric emptying and are affected by duodenal distension, the state of the duodenal mucosa, the acidity and osmolality of chyme, and by the presence of protein or fat (13). Nutrient breakdown, volume of intake, caloric content (density), pH, and osmolality all affect the rate of gastric emptying (10, 23, 24). Although protein and CHO have the same caloric content per volume, it is thought that the presence of protein slows the rate of gastric emptying in an attempt to properly catabolize the protein (19). The volume of ingested food shares a direct relationship to that of gastric emptying as a greater volume induces myenteric reflexes for greater motility (13). Physical activity will decrease the rate of gastric emptying as blood is shunted away from the digestive system to other areas of the body (skeletal muscle) that are more metabolically active. This happens through afferent input from the body's nervous system. Increased osmolality (such as from the addition of protein) will also decrease the rate of gastric emptying as does a gastric pH that is not optimal.

Protein has superior intestinal absorption rates compared to sodium and CHO only (32, 39, 46, 47). Although studies suggest that the time required to break down proteins into an absorbable form leads to a slower rate of gastric emptying, water retention may be enhanced to a greater extent with protein (i.e. slower rate of gastric emptying may or may not occur with protein; however, greater level of fluid absorption/retention outweighs the possible slower rate of gastric emptying when considering the addition of protein to a sports drink). It is precisely this notion that needs further research. Gastric emptying only tells part of the story.

Purpose

The purpose of this study was to investigate the effects of protein added to a CHO and electrolyte sports drink, and specifically how plasma osmolality (hydration status) can be affected by added protein. The theory is that although the addition of protein may decrease the rate of gastric

emptying, the increase in solute (osmolality) will pull more water into the small intestine for absorption and may outweigh the slower rate of gastric emptying to improve hydration status and ultimately race or cycling trial time. This study aims to determine whether adding protein to a carbohydrate sports drink will result in less dehydration during a 60km cycling time trial as well as 24 hours after completion of the time trial and improve performance during the time trial.

Chapter II: Review of Literature

Cycling, like all endurance events ultimately comes down to time. Faster times can only be achieved when an athlete is well hydrated, and also requires the delivery of macronutrients for energy to do external mechanical work (6). Hydration is important to maintain cellular osmolality for normal cell function, as well as to produce sweat to maintain a core temperature that allows further normal bodily function (13).

Recently, multiple published studies have hypothesized the importance of adding protein to conventional carbohydrate-only (CHO) sports drinks (16, 34, 36, 37, 38). Two studies indicated longer time to exhaustion (26% & 29% longer, respectively) when cyclists consumed a CHO and protein (CHO+P) drink versus a CHO-only beverage (16, 36). Similar findings (13% longer) have also been reported for CHO+P gels versus CHO-only gels (37). In addition, some recent studies have reported that CHO+P ingestion can reduce markers of muscle damage following exercise (22, 34, 36, 37). These findings purport that CHO+P beverages are effective in prolonging endurance, decreasing muscle damage, and aiding recovery. These results may have a significant impact on dietary recommendations to endurance athletes considering the use of CHO+P beverages at certain times during training, actual events/races, and recovery to improve athletic performance.

One study reported no improvements in prolonged time-trial performance with CHO+P consumption (44). These researchers suggested that the type of exercise protocol used in previous studies (time-to-exhaustion) may be responsible for the quantifiable differences, and that CHO+P beverages may result in no advantages during timed events that cyclists perform in during competition. However, this study (44) was statistically underpowered using a small sample size (N=14), and an exercise protocol that did not address performance in the final stages of exercise where fatigue would have played a greater role concerning performance. Also, one study with the

same researcher who originally reported greater time to fatigue (36), later reported no difference in time to exhaustion following the addition of protein to a conventional CHO-only sports drink a few years later (34). This begs the question whether adding protein to carbohydrate sports drinks improves performance and reduces dehydration.

Chapter III: Methods

Instrumentation

A Monark model 842e cycle ergometer was calibrated and used for initial determination of maximal aerobic capacity. A Velotron Racermate stationary trainer cycle was calibrated and used for each 60 km time trial. For determination of oxygen consumption, a MedGraphics CPXD open circuit spirometry system was calibrated prior to each test with certified gases and utilized a 30 sec averaging period during gas collection. An indwelling Teflon butterfly catheter and complimentary 5ml vacutainers were used to draw venous blood samples during time trials. For plasma osmolality measures, a National instruments osmometer employing freezing point depression methodology was calibrated and used for each sample.

Subjects

Fourteen endurance-trained male subjects (mostly competitive cyclists, with the addition of several recreational cyclists to achieve a desirable number of subjects for statistical power) with high peak aerobic capacities (mean $VO_{2peak}=60$ mL/kg/min or 4.5 L/min) and high peak power outputs of at least 400 Watts (W) from Central Minnesota and the Twin Cities metropolitan area were recruited to complete the study. Subjects were recruited through area cycling teams, the SCSU cycling team, MCF.net, cycling newsletters, and through bike shops. To be eligible for the study, subjects had to be 18-45 years of age and have engaged in cycling at least 3 days each week for the last 2 months. Subjects were also classified as “low risk” as indicated by the American College of Sports Medicine’s *risk stratification*. No subjects with milk product allergies were recruited and subjects listed all medications that they were currently taking.

All experimental procedures, as well as risks and benefits were explained in full detail to each subject prior to their agreement to participating in this study. All participants also signed an

informed consent witnessed by a member of the research team. All procedures were approved by the Institutional Review Board at St. Cloud State University.

Involvement in this study required all subjects to visit the Human Performance Lab at St. Cloud State University on 6 non-consecutive days.

Procedures

The study consisted of 4 phases: 1) Pre-testing for qualification through minimum aerobic endurance or power assessment; 2) Upon qualification, a 40 km familiarization trial to accustom the subject to the bike and to the protocol (discussed below); and 3) Two 60 km time trials wherein the two treatments (sports drink with and without protein) were administered. Treatment A (sports drink with protein added) and treatment B (sports drink with CHO and electrolytes only) were randomly assigned for all subjects. The treatment A drink was composed of 6% CHO, whereas the treatment B drink was composed of 6% CHO + 1.5% protein. Within each 170 mL treatment, subjects were ingesting 10.2g of CHO or approximately 41 Calories, and 2.55g of whey protein or approximately 10 additional Calories if ingesting treatment B. Before pre-testing, subjects were given informed consent forms to read and sign that provide a thorough description of the study, the risks and benefits of the study, and the maintenance of subject confidentiality. Subjects were also allowed to withdraw from the study at any time under their discretion.

Pre-testing: To assess aerobic endurance, subjects performed a progressive (graded) exercise test to assess their peak oxygen uptake capacity (VO_{2peak}). Subjects rode a Monark cycle ergometer to achieve a peak oxygen uptake. Subjects were properly hydrated, warm, and had not engaged in vigorous exercise the day-of to ensure achieving the greatest effort. Subjects began the pre-test at 1.0 kg resistance at a self-selected pace [most subjects cycled with a cadence between 70-95 revolutions per minute (rpm) assessed through a pulse meter to determine rpm. Subjects completed

a warm-up at 1.0 kg resistance for 5 minutes. For each 3-minute stage after warm-up, ergometer resistance was increased by .5 kg per stage. The pre-test continued until the subject could no longer maintain the self-selected pace (+/-2-3 rpm). This was assumed to be their peak VO_2 and peak power output. Following the end-point of the test, the subject remained on the ergometer for an active recovery of approximately 10 min. using a self-selected resistance at a pace similar to that used for the warm-up. Subjects had to meet one of the qualifying markers to be eligible for the study ($VO_{2peak} \geq 60$ ml/kg/min, 4.5L/min or 400W power output).

Upon qualification, subjects performed a 40 km familiarization trial to ensure comfort and knowledge of the Velotron ergometer and associated procedures. During this trial, 170 ml doses of self-selected sports beverage (not treatment beverages) were given at regular 5 km intervals. Subjects used their own pedals, were given no verbal feedback/encouragement and were only able to view distance completed and power output in W.

During treatments A and B, a non-affiliated research assistant opened treatment beverages and placed 170 ml aliquots in water bottles so that the study was conducted in a double-blind procedure. Treatment beverage order was randomized.

Subjects were instructed to refrain from heavy exercise for 48 hours prior to trials/experimental testing, and were not to exercise between trials and post-trial testing (24 hours after trials). Subjects were instructed to drink 20-32 oz of water the evening before each trial to ensure euhydration. Subjects were given a frozen Healthy Choice meal and a CLIF bar along with 8 oz of water and were instructed to eat and drink this as a meal 2-3 hours before each experimental trial. Subjects were also instructed of the incentive program, which paid a bonus for faster trial times.

Subjects were given 8 oz of water prior to each experimental trial. Subjects voided their bladder immediately prior to each trial. An indwelling venous catheter was inserted to collect blood

at intervals of 0 km, 20 km, 40 km, and 60 km. A post-trial sample was also collected 24 hours later. During all trials, subjects wore a Polar HR monitor. Subjects were able to listen to music, provided the music was the same for both experimental trials. Subjects then engaged in a 60 km time trial, and were given treatment "A" or "B" every 5km, respectively.

Following completion of each 60km trial, subjects were instructed to consume nothing but water for 2 hours post-trial, to follow their regular eating habits between 2 hours post-trial and the 24 hours post-trial testing, to consume 20-32 oz of water the evening of each trial, and to arrive well-rested, 3-4 hours post-meal for the 24 hours post-trial blood sampling.

A minimum of 6 days but not longer than 9 days occurred between each experimental trial. Measurements of the plasma samples were taken to determine osmolality values. Paired t-tests were used to determine differences between treatment conditions at each time interval and a P-value of $P < .05$ was considered significant.

Chapter IV: Results and Discussion

Results and Discussion

Fourteen well trained men participated in this study and repeated the same time trial race having the same measurements taken each time. Trial A and trial B were randomized for each subject, and a non-affiliated graduate student poured the 170 mL aliquots to ensure a double-blind study procedure.

The results of this study are both copacetic and contradictory with previous studies that examined the differences between sports drinks with and without protein. In other words, researchers have found and continue to find that this type of study lacks repeatability. Some have found differences, while others have reported no differences in terms of a sports drink's influence on hydration and performance. The current study found only one significant difference within four time periods (one in five if you include the starting period). An issue with this type of study and relying on measurements of performance to assess benefits is that it is very dependent upon individual subject effort. In spite of maximal efforts to control confounding variables, a subject might choose to give more of an effort between one bout to the next (consciously or subconsciously). If one considers the effects of central and peripheral fatigue, it would appear that central commands or descending drive for an individual can be quite variable. If a subject gives a larger effort during one trial, then it would follow that working skeletal muscle and overall metabolism will increase core temperature leading to greater sweat production. This in turn would dehydrate the subject more quickly. So, whether the assessment is time or hydration (via plasma osmolality), individual effort could have a large impact on plasma osmolality and is therefore quite difficult to fully control.

Figure 1 depicts mean (\pm SD) plasma osmolality immediately before beginning the time trial, at the 20 km mark, at the 40 km mark, at the 60 km mark, and 24 hours post time trial. There was no

difference observed between the two treatment beverages except at the 60 km mark. A paired t-test showed that this was the only time point at which plasma osmolality was significantly different, $t_{(14)} = 2.30$, $P < .038$. The average plasma osmolality after consuming CHO (Gatorade) at 60 km was 289.2 ± 4.4 mOsm; the average plasma osmolality after consuming CHO+P (Accelerade) at 60 km was 293.7 ± 6.4 mOsm. This may suggest that an observed difference in plasma osmolality may only occur during events lasting greater than 2 hours. One possible explanation for this observation at the 60 km mark is that there may be more protein turnover with increasing plasma osmolality at this duration or if the body has been shunting blood away from the splanchnic system for 2 hours or more. The addition of protein may be so diminutive that the effect is largely negligible on supplementing the working skeletal muscles in terms of calories given the intensity of the cycling and the time frame; however, as glycogen stores are used up around 2 hours, protein may become more important in providing the body with supplemental calories. Also, the utility of the addition of protein may only be in attenuating post-exercise muscle damaged as assessed by creatine-kinase and/or lactate dehydrogenase markers (22, 36, 37).

I have found that the results of this study offer a challenge in determining the correct type of sports drink to recommend for endurance athletes. Further research in this area is needed, specifically when considering exercise modality and experimental design. The addition of protein provided subjects with approximately 60 supplemental calories per hour. Protein contribution to skeletal muscle metabolism is largely ignored or assumed constant when considering a single bout of exercise. Even for endurance events, it is considered constant at approximately 15% regardless of the intensity and modality. Ultimately, protein does not provide an immediate noticeable difference in a cycling time trial lasting approximately 2 hours for most subjects. There is, however, a trend that both sports drinks follow over time leading to a difference at 60 km as seen in Figure 1. The average

difference between the treatments at 60 km is approximately 4.5 mOsm. And if we consider a normal plasma mOsm range of 280-300 mOsm, there is likely not enough of a difference to claim that the addition of protein to a sports drink influences plasma osmolality or hydration during a 2 hour cycling time trial.

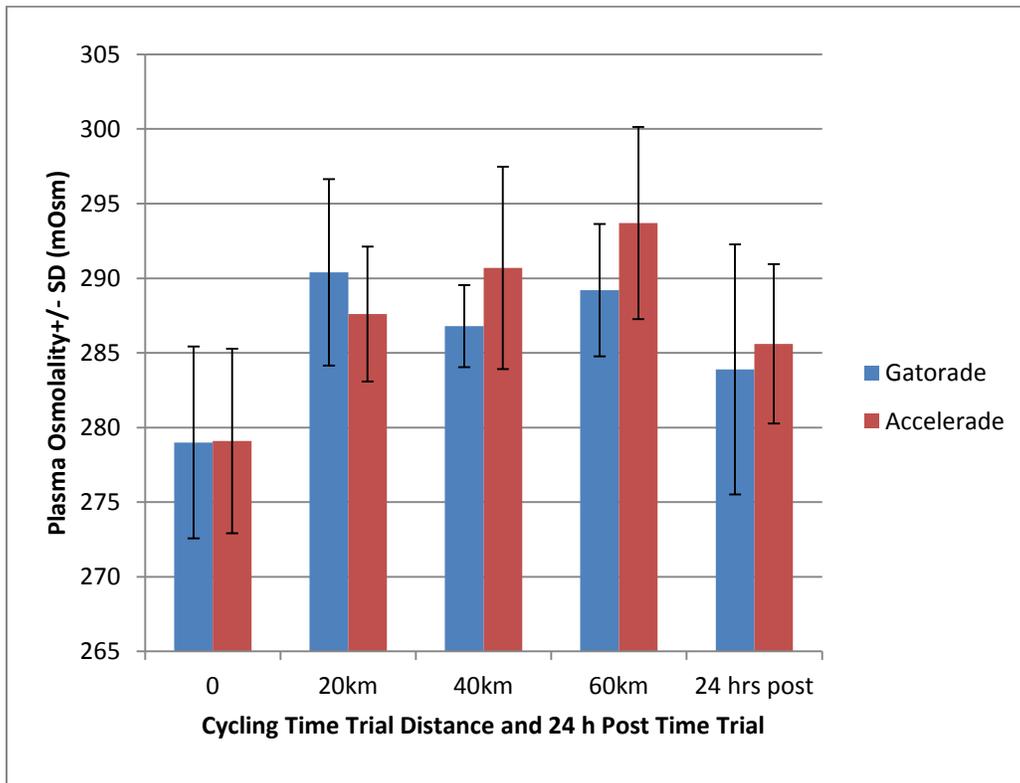


Figure 1. Mean plasma osmolality (\pm SD) values for cycling time trial and 24 h post

Conclusions

Based on results of this study the following conclusions can be made:

1. There is no difference in plasma osmolality (hydration status) at 20 km and 40 km, of a 60 km cycling time trial when comparing sports drinks with carbohydrate-only (Gatorade) to a sports drink with carbohydrate+protein (Accelerade).

2. There is a difference in plasma osmolality (hydration status) at 60 km (the final stage) of a cycling time trial when comparing sports drinks with carbohydrate-only (Gatorade) being lower (better hydration status) than a sports drink with carbohydrate+protein (Accelerade).

Recommendations

Based on the results of this study, the following recommendations can be made:

1. A 6% CHO sports drink with electrolytes may be beneficial to an endurance athlete regarding their hydration status provided their event is cycling and concludes near a 60 km distance.
2. A 6% CHO sports drink with electrolytes may be beneficial to an athlete racing in a cycling event that takes approximately the same time to complete as the time it took these subjects in this study to near the 60 km mark.

Limitations

It was not possible to obtain all of the original study data, thereby limiting results for discussion and conclusions. Even so, changes in plasma osmolality throughout and between the two time trials provide some informative data. Also, the time between the actual experimentation and measurement of plasma samples was less than optimal, samples were frozen and secure. When measured, they were all measured on the same day and all values fell within normal physiologic ranges.

The dependent variable was very likely influenced by many pre-experiment confounding variables that the researchers were unable to control such as: normal water and food intake between subjects as well as activity levels. Considering the actual time trials, effort was very subjective and can vary from subject-to-subject and from day-to-day for one subject. This variability

must then be considered with descending drive or central command and a subject's overall effort. If a greater effort was given, then it likely follows that one increased metabolic heat production and produced greater volumes of sweat thereby increasing plasma osmolality.

A suggestion for future studies would be to assign treatment beverage aliquots in proportion to a subject's body mass, and even a step further to lean body mass. Also, a subject pool with similar training regimens and cycling capabilities would minimize additional potential confounds.

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