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EFFICACY OF A MILK PERMEATE-BASED BEVERAGE FOR HYDRATION AND EXERCISE PERFORMANCE

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Kinesiology

by

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ABSTRACT

Fluid homeostasis is challenged during exercise when fluid availability is limited or when fluid loss is not properly replaced. Sports drinks are designed to improve hydration by stimulating fluid ingestion, reabsorption, and retention. Milk has been suggested to be an alternative hydration source to sports drinks due to its higher electrolyte concentrations and similar carbohydrate content. As milk has a high energy density and viscosity that may present gastric discomfort, attempts have been made to develop beverages from milk byproducts, such as milk permeate, that may be more efficacious for consumption during exercise. However, there is limited literature on milk permeate-based beverages for hydration or performance purposes.

This dissertation comprises one published literature review and three empirical studies investigation the efficacy of a novel milk permeate-based beverage as an alternative hydration source and its impact on cycling performance. The first study measured the beverage hydration index (BHI), a measure of the hydrating capacity of a given beverage compared to water, of a novel milk permeate solution (MPS) compared to a traditional carbohydrate-electrolyte solution (CES) and water (WAT) in young, well-hydrated individuals at rest in a thermoneutral environment. Our findings indicated that (1) cumulative urine output was attenuated over the entire four hours following consumption of MPS compared to both CES and WAT, resulting in (2) a higher BHI and (3) attenuated reduction in net fluid balance in MPS compared to the other beverages. Further, there was (4) a similar expansion in plasma volume four hours post- CES and MPS consumption compared to WAT. These findings suggest that a beverage containing milk permeate may serve as an efficacious alternative to more traditional CES as a source for hydration in young adults at rest. The second study examined the efficacy of a MPS as a true sports beverage and how consumption of this beverage may impact hydration and cycling time-

trial performance in young trained cyclists following exercise- and heat-induced dehydration compared to WAT, a traditional CES, or no fluid (NF) consumption. This study concluded that in a hot-dry environment, (1) cycling performance was improved in each of the beverage trials compared to NF, (2) accompanied by similar expansion in plasma volume in these three beverage conditions, but (3) there were no differences in cycling performance among the three beverage conditions. However, (4) in a subset of subjects completing a slightly longer cycling time-trial in thermoneutral conditions, performance was similarly improved in the CES and MPS conditions compared to WAT. These findings collectively indicate that hydration is more important for improving push-to-the-finish cycling performance in the heat, but carbohydrates play an emerging role in a slightly longer cycling time-trial in thermoneutral conditions. The third study investigated how overnight fluid restriction and subsequent rehydration the following morning with WAT, CES, or MPS impacts cycling time-trial performance in recreational athletes and how these beverages differently influence *ad libitum* rehydration following moderate-to-vigorous cycling. This study concluded that 1) time-trial performance was not improved following prescribed consumption of a MPS or a traditional CES compared to WAT, 2) *ad libitum* fluid consumption was not different among beverage conditions during the one-hour recovery cycling period after the time-trial, and 3) changes in plasma volume, an important indicator of hydration, were not different among drink conditions at any time-point, indicating subjects were similarly hydrated among beverage conditions.

Together, these three studies provide insight into the hydration efficacy of a milk permeate solution and its effect on cycling performance, suggesting (1) consumption of a milk permeate solution is more hydrating than a traditional sports drink and water at rest, (2) it similarly improves cycling performance as a traditional sports drink compared to water in thermoneutral

conditions, but not in the heat, and 3) ad libitum consumption of this milk permeate solution following dehydration does not differ from that of a traditional sports drink or water.

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LIST OF ABBREVIATIONS

Ad libitum	AD
Amino Acid	AA
Analysis of Variance	ANOVA
Beverage Hydration Index	BHI
Blood Pressure	BP
Body Mass	BM
Body Mass Index	BMI
Calcium	Ca
Carbohydrate(s)	CHO
Carbohydrate-electrolyte Solution	CES
Chloride	Cl
Core Temperature	T_c
Cumulative Urine Output	CUO
Dehydration	DEH
Estimated Glomerular Filtration Rate	eGFR
Fluid Restriction	FR
Free Water Clearance	CH_2O
Gastric Emptying Rate	GER
Gastrointestinal	GI
Glomerular Filtration Rate	GFR
Glucose	GLU
Heart Rate	HR
Hemoglobin A1C	HbA1c

High Density Lipoprotein	HDL
Insulin	INS
Interclass Correlation Coefficient	ICC
Low Density Lipoprotein	LDL
Magnesium	MG
Mean Arterial Pressure	MAP
Milk Permeate	MP
Milk Permeate Solution	MPS
Oral Rehydration Solution	ORS
Phosphorous	P
Plasma Volume	PV
Potassium	K
Rating of Perceived Exertion	RPE
Rehydration	REH
Relative Humidity	RH
Serum Osmolality	S_{osm}
Sodium	Na
Sodium Chloride	NaCl
Sodium-Glucose Linked Transporter	SGLT
Time Trial	TT
Urine Osmolality	U_{osm}
Urine Specific Gravity	USG
Volume of Oxygen	VO_2

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Chapter 1

INTRODUCTION

Background and Significance

Water is the largest constituent of the human body, accounting for 50-70% of total body weight [1], making it essential for life and the maintenance of proper function in humans. There is an imperative need to tightly regulate and balance total body water. The inability to properly maintain this balance, and thus hydration status, may have severe implications on health and daily function. Fluid loss resulting in ≥2% loss of body mass often leads to reductions in both physical [2-4] and cognitive function [5]. It is essential to evaluate novel strategies for the maintenance of hydration status to prevent reductions in performance and function. Although sports drinks and oral rehydration solutions are often prescribed to improve hydration status and performance, these beverages often contain relatively high sugar concentrations and therefore may not be efficacious for certain populations with impaired glucose handling. Although milk has been suggested as an efficacious alternative to sports drink during and following exercise [6], gastric fullness and discomfort have been reported when consumed in amounts necessary to replace sweat losses [7, 8]. Alternatively, milk permeate, a byproduct created during the ultrafiltration of milk, retains the carbohydrate and electrolyte concentrations of milk, while only containing proteins and fats in trace amounts or not at all, thus lowering the caloric density of the fluid while retaining the hydrating qualities of milk. However, no previous studies have quantified the hydrating qualities of a beverage containing milk permeate. Determination of these characteristics would provide paramount information regarding the efficacy of such a beverage as a potential alternative

hydration source for individuals whom high-sugar beverages may not be desirable or tolerable. Further, a large proportion of milk permeate produced annually during dairy filtration is discarded as waste. Thus, this area represents a largely untapped market that has significant economic and sustainability ramifications

Fluid Balance and Thirst

Fluid balance is determined by the amount of water consumed and metabolically produced by the body versus the amount of water lost. This balance is tightly regulated, resulting in daily fluctuations <1% under normal conditions [9]. A major factor that promotes the maintenance of plasma osmolality and total body water content is the thirst response. Thirst is controlled by both homeostatic and non-homeostatic mechanisms. Non-homeostatic thirst occurs as a result of a variety of behavioral and environmental factors, such as meal consumption, water temperature, water availability, and spontaneous drinking. In contrast, homeostatic mechanisms consist of physiological influences and are triggered by two different stimuli: increased cellular tonicity, sensed by osmoreceptors, and decreased extracellular fluid volume, sensed by baroreceptors [10, 11]. Although thirst alone is typically an adequate stimulus for fluid consumption at rest in thermoneutral conditions, it is often a poor indicator of fluid requirements during and following exercise, especially in the heat, and may not be stimulated until 1-2% of body mass has already been lost [12, 13].

Dehydration and Performance

Physical activity, particularly in the heat, elicits a robust sweating response for the primary purpose of maintaining thermoregulatory balance. Dehydration is defined as the process of losing

body water content in excess of fluid intake and often occurs without proper replacement of lost fluids via sweating during and following exercise in the heat. The current consensus is that dehydration resulting in body mass loss ≥2% impairs both cognitive and physical performance [14]. During endurance exercise at lower intensities for less than 90 minutes in moderate environments (20-21°C), sweating rates are typically low, often result in no more than a 1% loss of body mass, and have mild or insignificant impacts on physical performance [15-17]. However, greater total sweat loss occurring during longer exercise (>90 minutes) [2] or exercise in hotter environment conditions (31-32°C) [18, 19], stimulate greater degrees of dehydration (≥1-2% loss of body mass), and thus are more likely to induce decrements in physical performance [4]. Such relatively large losses of body mass can occur even when fluid is freely available [20], as thirst drives during exercise often lag behind sweat rate [12].

Sports Drinks and Rehydration

Although consumption of water alone improves hydration status and performance, fluids with added components can further stimulate fluid ingestion and promote fluid reabsorption and retention [3]. Beverages designed to promote rehydration are typically defined as either oral rehydration solutions (for consumption at rest) or sports drinks (for consumption during and following exercise). Both contain carbohydrates and electrolytes, though relative concentrations vary among beverages. Sports drink typically contain ~6% carbohydrates [21], which promotes both rapid gastric emptying and intestinal absorption, as well as exogenous carbohydrate supplementation for energy production [22]. However, many of these beverages present a relatively high glycemic index, and therefore may not be suited for consumption in populations with impaired glucose handling. Therefore, it is important to assess other potential beverages with a lower glycemic index.

Milk and Milk Permeate

Annual milk production in the United States has increased over the last decade, with 218 billion pounds being produced in 2019, a 13% increase from 2010. Milk contains naturally high electrolyte concentrations [23, 24] and a carbohydrate concentration similar to most typical sports drinks, and has therefore been proposed as an alternative fluid source for promoting hydration and performance during physical activity [6]. Further, both full-fat and skim milk displayed an elevated beverage hydration index (BHI), a measure of the hydrating capacity of a given beverage relative to water [25]. However, the rehydration and performance-promoting characteristics of milk have only been tested in a small number of studies. Additionally, as milk is a calorically dense and viscous fluid, multiple studies have reported subject sensations of stomach fullness and gastric discomfort following milk consumption.

As much of the large quantity of milk produced annually undergoes ultrafiltration, vast amounts of milk permeate (~523,000 metric tons, or 1.15 billion pounds) are produced each year. Milk permeate has previously been primarily used for animal feed or discarded, with only 20% of produced permeate being used for human consumption. However, milk permeate retains the hydrating and energetic properties of milk and may therefore be efficacious in the production of sports beverages or oral rehydration solutions [26, 27]. Given the increased production of milk and corresponding milk permeate, this represents a largely untapped market that may have important economic and sustainability ramifications for both the dairy and the sports beverage industries. Few studies have examined the efficacy of bringing milk permeate into solution for the production of a palatable and consumable beverage [28, 29]. These studies did not quantify the hydrating characteristics of these beverages, and thus the proposed hydration- and performance-promoting qualities of such a beverage remain theoretical and untested.

Summary

The three studies within this dissertation were designed to investigate the hydration- and performance promoting characteristics of a novel sports drink containing milk permeate. The first study quantified the hydration capacity of this beverage utilizing the beverage hydration index (BHI). The second study how consumption of this milk permeate solution following exercise- and heat-induced dehydration affected indices of hydration and cycling performance compared to consumption of water or a traditional sports drink, or no fluid at all, in highly-trained cyclists. The third study examined how prescribed rehydration with either water, a traditional sports drink, or a milk permeate solution following overnight fluid restriction impacts cycling performance in recreational athletes, and how ad libitum fluid consumption compared among the three beverage conditions following a cycling time trial.

Specific Aims and Hypotheses

Specific Aim 1. The purpose of the study "Hydration Efficacy of a Milk Permeate-Based Oral Hydration Solution" was to utilize the beverage hydration index (BHI) to determine the hydration efficacy of a novel beverage containing milk permeate (MPS) relative to water and a traditional carbohydrate solution (CES) and the extent to which fluid and electrolytes are retained within the vascular space after ingestion of each solution.

Hypothesis 1a: Both CES and MPS would demonstrate a higher BHI than water over a 4-h period after standardized beverage ingestion in euhydrated subjects.

Hypothesis 1b: The BHI of the MPS would be similar to that of the CES.

Hypothesis 1c: Plasma glucose concentrations would be elevated immediately following ingestion of CES and MPS compared to water, though this elevation would be relatively blunted following MPS consumption relative to CES.

Specific Aim 2. The study entitled "Hydration Is More Important Than Exogenous Carbohydrate Intake During Push-to-the-Finish Cycle Exercise in the Heat" consisted of two separate studies to assess the role of dehydration and subsequent rehydration with different beverages in different environmental conditions on cycling time trial performance in highly-trained young cyclists. The aim of *Study 1* was to investigate the rehydration capacity of a milk permeate solution (GoodSport®) following exercise-and heat-induced dehydration compared to no fluid consumption, water, or a traditional CES (Gatorade™).

Hypothesis 2a: Cycling time-trial (TT) performance would be improved following rehydration with all three beverages compared to no fluid replacement.

Hypothesis 2b: Consumption of either Gatorade™ or GoodSport® would improve indices of hydration and TT performance compared to water.

Hypothesis 2c: Consistent with previous results from resting subjects, hydration status would be improved following consumption of GoodSport® compared to water and Gatorade™.

The aim of *Study 2* was to further examine the impact of carbohydrate supplementation on performance during a slightly longer TT in a thermoneutral environment.

Hypothesis 2d: Performance would be improved following consumption of either Gatorade™ or GoodSport® compared to water during a slightly longer TT in thermoneutral conditions.

Specific Aim 3. The purpose of the study "Hydration, Ad Libitum Consumption, and Performance are Similar Among a Milk Permeate Solution, a Traditional Sports Drink, and Water Following Overnight Fluid Restriction in Recreationally Active Adults" was to determine how overnight fluid restriction and next-day prescribed rehydration with either water, a traditional CES (Gatorade™), or a novel milk permeate solution (MPS; GoodSport®) impact cycling time-trial performance in recreational athletes in a warm environment, and to compare the impact of ad libitum consumption of these three beverages on restoration of fluid balance and biomarkers of hydration status post-time-trial.

Hypothesis 3a: Time-trial performance would be similarly improved following consumption of Gatorade™ and GoodSport® compared to water.

Hypothesis 3b: Add libitum fluid consumption post-time-trial would be greater for Gatorade™ and GoodSport® compared to water.

Chapter 2

REVIEW OF LITERATURE

SCIENTIFIC BASIS FOR A MILK PERMEATE-BASED SPORTS DRINK – A CRITICAL REVIEW

INTRODUCTION

Water is the largest constituent of the body, accounting for ~50-70% of total body weight [1]. Water makes up the majority of the composition of cells, tissues, and organs, and serves important roles for physiological function, such as acting as a medium for nutrient transport, hydrolytic reactions of macronutrients, lubrication, and thermoregulation, among other functions. Ordinarily, 2-3 L of water, or 5-10% of total body water content, are turned over daily in sedentary individuals [30]. Fluid balance is determined by the amount of water consumed and metabolically produced by the body versus the amount of water lost from the body. Under normal conditions, daily water loss is ~2500 mL, of which a substantial portion is derived from urinary excretion (1-2 $L \cdot day^{-1}$) [31]. Additional insensible water loss occurs through skin (~450 $mL \cdot day^{-1}$), feces (~200 $mL \cdot day^{-1}$), and respiratory evaporation (~300 $mL \cdot day^{-1}$). In contrast, water gain is achieved through fluid (~1,575 $mL \cdot day^{-1}$ or more) and food (~675 $mL \cdot day^{-1}$) consumption and water produced via metabolic processes (~300 $mL \cdot day^{-1}$) [32].

To maintain euhydration, the current recommendation from the Institute of Medicine for daily water intake is approximately 2.7 $L \cdot day^{-1}$ for women and 3.7 $L \cdot day^{-1}$ for men [9], though these values may increase for individuals with more active lifestyles [33] or who live in hotter climates

where water loss through sweating is greater. It is in these conditions that dehydration, defined as the process of losing body fluid in excess of fluid intake, may occur without proper fluid replacement. As dehydration has clear and profound impacts on multiple facets of human health and performance [34], it is essential to replace these lost fluids to maintain proper water balance and overall physiological function. Although consumption of water alone promotes rehydration, beverages with added components, such as carbohydrates and electrolytes, further stimulate fluid absorption and retention. Beverages designed to promote hydration typically fall in one of two categories: 1) oral rehydration solutions, and 2) sports drinks. Oral rehydration solution have historically been utilized to replace fluid and electrolyte losses associated with diarrheal illnesses [35]. In contrast, sports drinks are designed to replace fluid and electrolytes lost via sweat during exercise and provide exogenous carbohydrates (CHO) for oxidation and energy production by muscles and the central nervous system [22]. Most traditional sports drinks and oral rehydration solutions contain varying concentrations of both CHO and electrolytes, commonly known as carbohydrate-electrolyte solutions (CES) [21]. Beverages with different compositions are likely to have varying effects on promoting hydration. However, until recently, there was no standard method for analyzing the differing hydrating capacities of various beverages.

The beverage hydration index (BHI) was proposed [25] as a measure of the hydrating capacity of a given beverage relative to water. In calculating BHI, the cumulative urine output in the hours following the consumption of 1 L of water is divided by the cumulative urine output at the same time points following consumption of 1 L of the beverage in question. Thus, the BHI of water is set to a value of 1.0. For a given beverage, a BHI lower than 1.0 indicates increased urine excretion, and thereby lowered fluid retention relative to water, while a value greater than 1.0 indicates decreased urine excretion and increased fluid retention relative to water. This strategy has been utilized to characterize the hydration efficacy of several beverages with different compositions compared to water [25, 36-38]. Importantly, BHI is not influenced by differences in

sex or body mass [38], allowing its application to the general population, including older adults [37].

A diverse array of beverages were examined in the original BHI study, including both bovine full-fat and skim milk [25]. Interestingly, both milk beverages had a significantly elevated BHI compared to water, even after adjusting comparisons to account for beverage water content. Only an oral rehydration solution had a similarly elevated BHI after this adjustment among the twelve beverages that were tested (sparkling water, cola, diet cola, orange juice, lager, coffee, tea, and cold tea). These findings indicate that milk may be just as, if not more, hydrating than traditional oral rehydration solutions and superior to sports drinks. The authors attributed the elevated hydrating qualities of milk to its CHO and electrolyte content. However, it should be noted that this study was conducted on well hydrated, resting human subjects. During physical activity, the high energy density and viscosity of milk may present issues regarding gastrointestinal discomfort. To combat this issue, previous attempts have been made to develop beverages from milk permeate, a byproduct created via ultrafiltration of milk [26, 29, 39]. This permeate typically retains the CHO and electrolyte concentration as milk, suggesting that it also retains milk's hydrating qualities. The purpose of this review is to examine the qualities and constituents of milk that contribute to its elevated hydration capabilities, the impact of milk consumption on physical performance and rehydration, and the scientific evidence and recent findings regarding the efficacy of milk permeate-based beverages as a potential alternative to traditional sports drinks.

MILK COMPOSITION AMONG MAMMALS

Historically, milk has been an essential component of the human diet. Human breast milk has been recognized for its ideal composition for promoting newborn nutrition and development [40].

However, as breastfeeding typically terminates within the first two years of life [41], milk must be obtained from other mammalian species to continue consumption over the remainder of the lifespan. Milk is derived from a number of mammalian species, most commonly from cows, water buffalo, goats, sheep, and camels. Humans likely began consuming milk derived from these animals when the animals became domesticated by nomadic herders approximately 8,000-11,000 years ago [42]. All mammalian milk contains the same primary constituents: water, fat, lactose, casein and whey proteins and other bioactive factors (α-lactalbumin, lactoferrin, secretory immunoglobulin IgA, lysozyme, and serum albumin), and minerals, though their respective concentrations differ among species. Bovine milk accounts for nearly 85% of global production, and greater than 80% of production in all regions except for South Asia [43]. Water buffalo, goat, sheep, and camel milk account for about 11%, 3.4%, 1.4%, and 0.2% of global production, respectively [43]. **Table 1** displays the average relative constituents of milk obtained from different mammalian species. Given the nutritional composition of milk, in recent years there has been increased interest in milk as a functional beverage, especially in regards to its potential role as a hydration and recovery beverage following physical activity [6]. In the original BHI study [25], the authors opined that the greater hydration capacity observed in full-fat and low-fat bovine milk was attributable to their physio-chemical compositions that influence fluid absorption and retention.

Table 2-1 : Physio-chemical components of whole milk from different mammalian species.

	Human	Cow	Water Buffalo	Goat	Sheep	Camel
Energy (kcal·100 mL^{-1})	68	69	41	70	105	76
Fat (g·100 mL^{-1})	4.0	3.6	7.4	3.8	7.9	3.8

Protein (g·100 mL^{-1})		1.2	3.2	4.4	3.4	6.2	3.4
Lactose (g·100 mL^{-1})		6.9	4.7	5.0	4.1	4.9	4.5
B Vitamins							
	Thiamin (mg·100 mL^{-1})	0.01	0.04	0.05	0.05	0.07	0.05
	Riboflavin (mg·100 mL^{-1})	0.04	0.18	0.13	0.14	0.36	0.07
	Niacin (mg·100 mL^{-1})	0.21	0.11	0.09	0.28	0.42	0.52
	Pantothenic acid (mg·100 mL^{-1})	0.67	0.36	0.19	0.41	0.31	0.08
	Pyridoxine (mg·100 mL^{-1})	0.09	0.04	0.02	0.05	0.06	0.05
	Folate (mcg·100 mL^{-1})	8.5	4.92	6.15	0.82	6.97	0.41
	Cobalamin (mcg·100 mL^{-1})	0.97	0.44	0.36	0.07	0.71	0.15
Minerals (mg·100 mL^{-1})							
	Sodium	15	58	44	41	44	58
	Potassium	55	152	107	181	136	179
	Chloride	60	100	58	160	136	200
	Calcium	33	122	183	134	193	109
	Magnesium	4	12	18	16	18	14
	Phosphorous	43	119	82	121	158	76

Data, expressed as averages, obtained from Ahmad et al. [44], Konuspayeva et al. [45], Lawrence [46], Park et al. [47], and Shamsia [48].

Absorption of ingested fluids occurs primarily in the intestinal tract, with most fluid absorbed in the proximal portion of the small intestine. However, it has been well established that gastric emptying serves as the rate-limiting step in fluid absorption [49, 50]. Slower rates of gastric emptying (GER) may have profound impacts on the rate at which fluid and nutrients are absorbed in the intestinal tract. In situations where the contents of an ingested beverage are urgently needed, delays in gastric emptying may be detrimental to fluid homeostasis. In contrast, a slower GER may be preferred in certain circumstances, such as when fluid availability is scarce or when an individual is dehydrated, to delay diuresis and prolong fluid retention, though to our knowledge this theoretical assumption has yet to be adequately tested. The energy content of an ingested fluid is considered the main factor influencing GER. Water, which lacks in energy content, is rapidly emptied from the stomach, while beverages with increasing energy content, usually due to increased concentrations of CHO or calories, have a slower rate of gastric emptying. Beverages with CHO contents ~2.5-6% typically empty from the stomach at a similar rate as water [23]. In contrast, impairments in GER relative to water typically occur in beverages with a CHO content ≥6% [51], though interindividual differences may yield impairments in GER following ingestion of beverages containing as little as 4-5% CHO content or not until a solution consists of 10% CHO [52].

CES beverages promote significantly higher fluid retention, lower urine excretion, and greater plasma volume expansion compared to a beverage that contains either carbohydrates or electrolytes alone [53]. In most traditional CES beverages, fluid absorption occurs secondary to sodium (Na^+) uptake [54], as water absorption is linearly related to rate of sodium-chloride (NaCl) absorption [55]. Coupling of sodium with glucose or galactose via the active co-transporter SGLT1 increases water and nutrient absorption. SGLT1 activity relies on the

establishment and maintenance of an osmotic and electrochemical gradient through the energy-dependent sodium-potassium (Na^+/K^+)-ATPase pump [56], where 3 Na^+ ions are pumped out of the cell and 2 K^+ ions enter the cell. For every 2 Na^+ ions that are transported back into the cell via SGLT1, 1 glucose/galactose molecule is transported into the cell. Therefore, sodium and glucose/galactose are absorbed at a fixed ratio of 2:1 [57]. As a result of the increase in solute concentration inside the cell, it is estimated that approximately 260 molecules of water are transported into the cell for each SGLT1 cycle [58]. Beverages with higher concentrations of sodium and glucose/galactose may increase SGLT1 activation and subsequent fluid uptake [59], up to the point where the SGLT1 transporters become fully saturated with glucose molecules. Potassium has been proposed to be equally effective as NaCl in promoting fluid retention [60], though plasma volume expansion, an important marker of hydration status, does not occur to the same extent following potassium consumption compared to sodium consumption [61]. This discrepancy is likely attributable to the high potassium concentration in the intracellular compartment, thus promoting increased water retention within that space as opposed to within the extracellular compartment, where sodium and chloride are the primary ions.

Most mammalian milks contain chloride and potassium in concentrations higher than what is typically included in many commercial sports beverages [62]. Interestingly, milk sodium content is similar to that of many sports drinks, (~20 $mmol \cdot L^{-1}$). Further, milk contains additional minerals, (e.g. calcium, phosphorous, and magnesium), that increase beverage osmolality, which once absorbed create an osmotic impetus that stimulates greater fluid retention within the vascular and intracellular spaces. These additional minerals are often excluded in commercial sports drinks. Beverages with high osmolalities may also slow GER and delay diuresis [63], though this only occurs in beverages with osmolalities >350 $mosm \cdot kg^{-1}$ [51]. In comparison, the osmolality of most mammalian milk, including human breastmilk, is ~300 $mosm \cdot kg^{-1}$ [64], well

below the threshold at which reductions in GER have been observed. These findings collectively suggest that milk may provide optimal amounts of carbohydrates and minerals to initiate gastric emptying at a rate that subsequently promotes efficient and timely fluid absorption in the intestinal tract.

MILK AS A SPORTS DRINK

Physical activity elicits a sweating response for the primary purpose of maintaining thermoregulatory balance, and this response is widely variable among individuals. During endurance exercise at lower intensities for <90 minutes in moderate environments (20-21°C), sweat rates are typically low, often resulting in no more than a 1-2% loss of body mass and mild or insignificant reductions of physical performance [16]. Greater total sweat loss occurring during longer exercise (>90 minutes) [2], more-intense exercise, or exercise in hotter environment conditions (31-32°C) [18], stimulate greater degrees of dehydration (≥2% loss of body mass), and are more likely to induce decrements in physical performance [4]. Although some studies have shown no impairments in performance during dehydration with losses of body mass as high as 3% [65], the current consensus is that dehydration resulting in ≥2% body mass loss significantly and consistently impairs physical performance [2-4], a decrement that is most apparent during physical activity in warm environments. Further, muscle glycogen and glycogen stores in the liver may be substantially reduced during endurance exercise >90 min, significantly reducing CHO availability and subsequently impairing performance.

Consumption of water alone improves hydration status and subsequently improves physical performance compared to no fluid consumption during physical activity [66-68]. However, fluids with added components (e.g., carbohydrates and electrolytes) may further improve hydration

status [3, 18] by promoting drinking, absorption of fluid from the small intestine via sodium-glucose transporter activation [69], fluid retention within the body and specifically the vascular compartment [53, 70] and replacement of fluid and electrolytes lost via sweat. Since the introduction of sports drinks in the 1960's, beverages containing carbohydrates have typically been the option of choice during exercise for their ability to improve performance and sustain hydration during physical activity. [71-73]. A substantial body of research has been established pertaining to recommended ingestion of carbohydrates to provide ideal improvements in performance. The current consensus, established by the American College of Sports Medicine, is that a CHO-consumption of ~30-60 g/h is recommended during endurance exercise [74]. Thus, many leading commercially-available sports beverages contain CHO contents of ~6% [21]. Additionally, although the hallmark of sports drinks is the improvement in performance via increased CHO-derived substrate availability for energy production in active skeletal muscles, these beverages often also contain various electrolytes to facilitate fluid retention, primarily in the extracellular compartment, and thereby improve hydration status. However, the use of CES for the purpose of rehydration during and after exercise does have limitations. The relatively higher glycemic index of CHO-based beverages may present complications for individuals with impaired glucose handling and absorption, such as that which is seen with healthy aging [75]. Similarly, beverages with higher glycemic loads may be contraindicated for clinical populations involving impaired glucose handling and absorption, such as type 2 diabetes [76]. Therefore, it is important to explore potential lower-glycemic beverage alternatives.

Although sports drinks are typically prescribed to restore hydration status and improve physical performance [71-73], more natural alternative fluid sources, such as bovine milk, which has a high electrolyte concentration [23, 24] and similar CHO concentration to traditional sports drinks [6], may serve as an efficacious alternative fluid sources for promoting hydration and

performance. The rehydration characteristics of milk following endurance exercise has been tested in only a small number of studies [7, 77, 78]. For example, the rehydration capacity of milk both with and without added NaCl was compared to a traditional CES and water following exercise-induced hypohydration (-1.8% body mass) [78]. Cumulative urine output was lower in each of the two milk trials compared to the water and CES trials. Further, subjects remained in positive net fluid balance for the entirety of the 4-hour rehydration period in the milk trial, but were in negative net fluid balance 1 hour post-ingestion in the water and CES trials. Importantly, adding NaCl to milk yielded no additional positive impact on fluid retention and balance compared to low-fat milk without added NaCl, indicating that the salt content of milk may already be optimized for promoting rehydration. Desbrow [77] similarly observed greater fluid retention after ingesting cow's milk compared to a traditional sports drink following cycling-induced dehydration (-1.8% loss of body mass). The increase in fluid retention following milk consumption was attributed to the higher energy, protein, and sodium content relative to the sports drink. In contrast, ad libitum ingestion of a milk-based liquid meal supplement (Sustagen Sport) over a two-hour period post-exercise-induced dehydration (-1.9% loss of body mass) resulted in similar fluid retention and fluid balance compared to following Powerade consumption [7]. Sensory data indicated that the milk-based liquid meal was less palatable and induced greater gastrointestinal stress than Powerade [7]. These findings collectively suggest that milk can serve as an efficacious alternative to traditional sports drinks for the purpose of rehydration following endurance exercise-induced dehydration, notwithstanding concerns regarding palatability and gastrointestinal comfort.

Studies examining how milk consumption prior to exercise may influence performance are even scarcer. One study assessed how rehydration with isovolumetric quantities (509.1 ± 36.0 mL) of chocolate milk (CM; 70 g CHO), a fluid replacement beverage (FR; 27 g CHO), or a

carbohydrate recovery drink (CR; 70 g CHO) matched for CHO and energy content may differentially impact cycling time-to-exhaustion [79]. Subjects were permitted to additionally consume water ad libitum during the recovery period, though there were no statistically significant differences in the volume of water consumed among trials (CM: 506.7 ± 212.0 mL; FR: 729.4 ± 400.3 mL; CR: 866.4 ± 631.0 mL). Time-to-exhaustion at 70% VO_{2max} was greater for CM and FR compared to CR (CM: 40.0 ± 14.7 min; FR: 41.3 ± 15 min; CR: 26.8 ± 10.3 min), suggesting that chocolate milk may serve as an effective alternative to a more traditional CES or recovery beverages for delaying fatigue during high-intensity physical activity. Interestingly, on a scale of 1-10, with 1 indicating no symptoms and 10 signifying severe symptoms, subjective scores of gastric distress were greater following CR consumption (2.7 ± 2.1) compared to both CM (2.4 ± 2.4) and FR (1.8 ± 1.4), though there was no statistically significant difference and these scores indicated fairly mild distress among all drink conditions. In contrast, Watson et al. observed no differences in time-to-exhaustion during exercise in the heat after consuming enough of either skimmed milk (39.7 ± 8.1 min) or a CES (39.6 ± 7.3 min) to replace 150% of lost body mass following exercise-induced dehydration (-2% loss of body weight) [80].

To our knowledge, only one study has examined the effect of milk consumption on exercise capacity >1 hour [8]. No significant improvements in exercise capacity were observed in euhydrated subjects while ingesting skimmed milk either with (102.8 min) or without (103.3 min) added glucose compared to water (93.3 min). However, time-to-exhaustion in both milk trials was still similar to that of an additional trial in which a traditional CES (110.6 min) was consumed [8]. Though not significant, 7 of 8 subjects in that study experienced a prolonged time-to-exhaustion in both the milk trials and the CES trial compared to water [8]. The collective findings of these studies suggest that milk and CES promote fluid retention and provides sufficient exogenous CHO to maintain glycogen stores and oxidation rates to a similar degree during

prolonged exercise. Further, considering that adding 10 $g \cdot L^{-1}$ glucose to milk did not prolong time-to-exhaustion compared to milk without added glucose [8] and that the CHO content of milk is similar to that of most sports beverages [21], it is possible that the CHO content of milk, which typically consists of lactose, which can be hydrolyzed to form glucose and galactose, as opposed to the fructose/sucrose/glucose in most sports drinks, is already optimized for performance. Additional research is necessary to assess the influence of milk consumption versus other sports drinks on exercise performance >1 hour, as well as its influence on different measures of exercise capacity other than time-to-exhaustion (i.e. time-trial or sprint-to-the-finish performance).

MILK PERMEATE COMPOSITION

One of the main limitations of milk as a potential hydration source during or following physical activity is its high energy density and viscosity. Previous literature has indicated that milk ingestion presents stomach feelings of fullness [8] or gastric discomfort [7] when consumed in amounts necessary to replace fluid lost via sweat, and may as a result negatively impact performance or recovery. Previous attempts have been made to develop beverages from byproducts created during the ultrafiltration of milk [26, 27, 81] to provide more palatable and digestible alternatives for consumption while still maintaining the hydration-promoting and energetic characteristics of dairy products. For example, large quantities of milk permeate are produced as a byproduct of the ultrafiltration of skim milk in the production of cheeses and other dairy products [82]. During the ultrafiltration process, skim milk is passed over a series of semi-permeable filters designed to promote retention of certain constituents over others. These filters create two streams; 1) permeate, which consists of the constituents that pass through the filter, and 2) retentate, or constituents that were too large to pass through the filter. The size and permeability of these filters can be altered to influence retentate and permeate composition [82].

Thus, milk permeate can take on several different forms, including milk permeate high in lactose or “delactosed” permeate, though the former is much more commonly utilized. Delactosed milk permeate still contains lactose, though in reduced amounts (~50-60% of the original lactose), and additionally contains 3-8% protein, 3-11% ash, 0-1% fat, and 25-30% moisture, and varying concentrations of electrolytes [81, 83]. The high lactose concentration in milk permeate can be drastically reduced by continued filtration, crystallization, or the addition of yeasts or molds high in lactase enzyme that hydrolyze lactose into glucose and galactose [83] thus likely improving beverage palatability. Delactosed permeate is also high in sodium and potassium concentrations, and additionally contains calcium, phosphorous, chloride, and magnesium, such that milk permeate retains the electrolyte profile of milk [81]. Due to the high mineral concentration and the retention of carbohydrates, essential components in the formulation of CES and oral rehydration solutions, it has been suggested that a beverage containing milk permeate may serve as an efficacious source for the purpose of hydration [26, 27].

MILK PERMEATE AS A SPORTS DRINK

In the United States, only 20% of permeate produced from dairy products is used for food, while the rest is used for animal feed or shipped to other countries [83]. Taking this into consideration in conjunction with the increasingly large amount of milk produced and transformed annually in the United States (218 billion pounds 2019, a 13% increase from 2010), and the subsequent vast quantity of milk permeate (~523,000 metric tons, or 1.15 billion pounds in 2019) [84], the use of milk permeate for the production of ingestible goods for human consumption remains a largely untapped market. One potential use of milk permeate is for beverage production, and attempts to do so have previously been conducted [26, 29, 39]. Geilman and colleagues successfully created a milk permeate beverage containing only 20% of the original milk permeate lactose concentration

following hydrolysis, as well as mineral concentrations similar to those in commercially available sports drinks [81]. However, this study only examined potential uses for this previously discarded ultrafiltration byproduct, and specifically the feasibility of creating a milk-permeate beverage with a stable shelf-life, without quantifying the hydration capacity of such a beverage.

El-Khair attempted to develop a milk permeate beverage containing only 5% lactose and trace amounts of protein and ash [26]. The milk-permeate used in production of this beverage underwent a series of treatments, including heating to 80 °C, fermentation to reduce the lactic acid concentration, and clarification to produce a clear liquid milk-permeate. A combination of sugars were added for additional CHO provision (though type and quantity not specified), and orange flavoring added to increase palatability. Two variations of a milk permeate beverage were created: one containing moderate concentrations of electrolytes, specifically sodium and potassium, targeted for recreational activity and thirst quenching, and another containing a higher concentration of electrolytes for endurance performance and replacement of electrolytes lost in sweat. Sensory evaluation indicated these beverages were palatable, though shelf-life was only three weeks before sensory properties deteriorated. Similarly, Hattem and colleagues were able to manufacture a milk permeate-based beverage containing ~4% lactose and trace amounts of ash [39]. Following similar processes used by El-Khair [26] of heat treatment, fermentation, and clarification, fruit homogenates (strawberry and mango) were added to improve the mineral and nutritional profile of the beverage. Addition of fruit homogenates improved palatability compared to a variation of the milk permeate beverage without added fruit homogenates. However, as with Geilman and colleagues, the hydrating capacity of these beverages was not quantified; only the possibility of creating a palatable solution containing milk permeate was assessed.

Currently only one known CES containing milk permeate is commercially available (GoodSport™) and has been tested for its hydrating qualities. This milk permeate solution (MPS) is lactose-free, yet still contains carbohydrates in the forms of glucose and galactose, has no fat or proteins, and consists of a similar mineral profile as milk. Further, this MPS has a similar sodium concentration (21 mmol·L^{-1}), but higher potassium concentration (28 mmol·L^{-1}) and osmolality (578 mOsm·kg^{-1}) and contains more minerals than Gatorade® (20 mmol·L^{-1} sodium, 3.2 mmol·L^{-1} potassium, 322 mosm·kg^{-1} osmolality), a traditional carbohydrate electrolyte solution (CES) and the leading sports drink in the United States. The MPS also has a similar CHO (4% glucose+galactose) and energy (180 kcal·L^{-1}) content as bovine milk, though these constituents are lower than those of Gatorade® (222 kcal·L^{-1}, 6% glucose+sucrose). The similarities in composition of this MPS compared to milk and Gatorade®, a sports beverage specifically formulated to promote fluid absorption and retention for longer periods of time, indicate that it may be an efficacious fluid source for maintenance of hydration status and fluid retention following exercise, though given the relatively high osmolality, it is unknown how such a beverage may affect hydration status during exercise.

Indeed, the hydration efficacy of this beverage has previously been examined in our laboratory [36]. It was determined that MPS consumption (1 L) promoted increased fluid retention for 4 hours post-ingestion compared to both CES and water, and thus had an elevated BHI compared to both Gatorade® and water at all time-points across the 4-h post-consumption period. Both CES and MPS stimulated a similar plasma volume expansion (3-5%) 4 hours post-consumption. Furthermore, the initial rise in serum glucose concentrations following beverage consumption was blunted, and returned to baseline concentrations sooner, in the MPS trial compared to CES. These findings suggest that this enhanced milk permeate product may serve as an efficacious alternative to more traditional CES as a source for hydration in young adults at rest. **Table 2** summarizes these findings in the context of previous studies utilizing BHI.

Table 2-2. Summary of studies utilizing the beverage hydration index (BHI).

Reference	Subjects	Test Beverages	Energy (kcal/L)	Carbs (%)	Sodium (mmol/L)	Potassium (mmol/L)	BHI (2 hours)	Main Findings
Maughan et al. (2016)*	Young healthy men (n=72; 25 ± 4 yrs)	Sparkling water	0	0	1	0	↔	BHI of ORS, full-fat milk, and skimmed milk were similarly higher than water. BHI is a useful measure to identify short-term hydration potential of different beverages when ingested in euhydrated state.
		Cola	420	10.6	2	0	↔	
		Diet cola	4	0	2	0	↔	
		Sports drink	160	3.9	21	4	↔	
		Oral rehydration solution (ORS)	80	1.8	55	20	↑↑	
		Orange juice	470	10.5	1	33	↑	
		Lager	330	2.2	1	6	↔	
		Coffee	4	0.1	1	7	↔	
		Tea	0	0	1	4	↔	
		Cold tea	0	0	1	5	↔	
		Full-fat milk	640	4.7	18	41	↑↑	
		Skimmed milk	350	5.0	19	40	↑↑	
Sollanek et al. (2018)	Young healthy adults n=40 (17M/23F) 20 ± 1 yrs	Amino acid-based ORS (AA-ORS)	21	0	55	10	1.15 ± 0.28	BHI for both AA-ORS and G-ORS were similarly higher than water. BHI may be used and interpreted independently of sex or body mass.
		Glucose-based ORS (G-ORS)	105	2.5	44	20	1.21 ± 0.28	
		Sports Drink	237	6.1	20	3	1.09 ± 0.26	
Clarke et al. (2019)**	Young healthy adults n=12 (7M/5F) 23 ± 3 yrs Older healthy adults n=12 (5M/7F) 68 ± 7 yrs	Gatorade (G-20)	———	6	20	3.2	1.09 ± 0.30	Sodium content progressively increased BHI in young adults independent of glucose or amino acid content. AA-30 displayed highest BHI in older adults. 4-hour BHI may be more appropriate for older adults due to attenuated urine excretion rates.
		Enterade Advanced Oncology (AA-30)		0	30	10	1.11 ± 0.24	
		Pedialyte (G-45)		2.5	45	20	1.08 ± 0.30	
		Enterade Anti-Diarrheal (AA-60)	———	0	60	20	1.24 ± 0.33	

Berry et al. (2020)	Young healthy adults n=12 (6M/6F) 23 ± 2 yrs	Carbohydrate-electrolyte solution (CES) Milk permeate solution (MPS)	220 160	6 4	20 21	3.2 28	1.04 ± 0.09 1.29 ± 0.15	MPS displayed a higher BHI than both water and CES every hour for 4 hours after drinking. Dairy-based beverages may be an efficacious alternative hydration source compared to more traditional carbohydrate-based beverages.

*- specific values not reported in manuscript. **- BHI values represent those reported in young adults.

As stated previously, the objective of sports drinks is not only to provide fluid for the maintenance or restoration of hydration status, but also for exogenous carbohydrate provision for oxidation and energy production. As milk permeate typically contains carbohydrates in the form of lactose, or its corresponding monosaccharides glucose and galactose in de-lactosed milk permeate, it is possible that a beverage containing milk permeate may serve as an efficacious sports drink. Indeed, carbohydrate oxidation rates are similar following consumption of a beverage containing lactose compared to a beverage containing sucrose [85]. Further, when compared to a beverage containing only galactose, endurance cycling performance following consumption of a beverage containing both glucose and galactose is similarly improved compared to a beverage containing both glucose and fructose [86]. These findings indicate that the carbohydrate content of milk permeate may serve as an efficacious source for carbohydrate supplementation during and following exercise, though it is unclear if the carbohydrate content of milk permeate would be sufficient to provide upwards of 90 g/h of carbohydrates per hour during ultra-endurance exercise, as has been previously suggested by the American College of Sports Medicine [74].

Our laboratory more recently assessed the efficacy of a milk permeate solution as a true sports beverage and how consumption of this beverage may impact hydration and cycling performance in young trained cyclists following exercise- and heat-induced dehydration [87]. Ten participants in this study cycled at a moderate intensity in a 40 °C, 20% relative humidity (RH) environment until dehydrated by -2% loss of body mass. Over the following 30 minutes, they then consumed either no fluid or enough water, CES, or MPS to replace 75% of lost body mass. A 30-minute cycling warm-up at a light intensity was followed by a maximum-effort 120-kJ cycling time-trial, similar to a push-to-the-finish effort in the final kilometers of a road-race. Cycling time-trial performance was improved in each of the three drinking conditions compared to no fluid consumption. However, there were no differences in time-trial performance among beverages. Each of the three beverages stimulated a similar plasma volume expansion following ingestion relative to following the dehydration period. Urine excretion was also similar among beverages in the hour following consumption. These findings are contradictory to what we observed in the BHI study examining these same beverages, a difference likely attributable to heat- and exercise-induced blood flow redistribution from renal and splanchnic regions toward the cutaneous and skeletal muscle circulations to meet their thermoregulatory and energetic demands, respectively. These findings collectively suggest that added constituents (i.e. electrolytes and carbohydrates) yield negligible short-term effects on fluid retention and exercise performance in the heat compared to consuming water alone.

To determine if between-beverage differences in time-trial performance, attributable to beverage CHO and electrolyte load, were evident with a longer time-trials in thermoneutral conditions, a subset of subjects repeated the water, CES, and MPS trials and completed a longer 250 kJ time-trial in a thermoneutral environment (35 °C, 20% RH). Interestingly, time-trial performance was improved in all four subjects in the MPS trial compared to water, and in three of four subjects in

the CES trial compared to water. Subjects performed similarly in the CES and MPS trials, indicating that added constituents in beverages are more important for performance during longer cycling time-trials in thermoneutral conditions. In both conditions, subjects completed a post-exercise beverage questionnaire to assess sensory components such as overall beverage likability, taste, aroma, and flavor. Scores were not different between CES and MPS, indicating that MPS was similarly palatable compared to CES. Further, the similarities between CES and MPS in both the hot and thermoneutral conditions indicate that a beverage containing ultra-filtered deproteinized milk permeate may be just as, if not more, hydrating as a traditional sports drink following dehydration and similarly impacts cycling performance. It is important for future studies to explore whether these differences persist in different environments during exercise lasting for longer periods of time (i.e. 60-90 minutes). Future studies should also examine the comparative rehydration capacity of a milk permeate beverage following more severe dehydration (>2%), and whether ad libitum drinking patterns may differ among beverages under these circumstances.

Chapter 3

HYDRATION EFFICACY OF A MILK PERMEATE-BASED ORAL HYDRATION SOLUTION

INTRODUCTION

Maintaining adequate hydration, in both unchallenged and dehydrated conditions, is associated with multiple health benefits. Proper hydration reduces risk for the development of chronic diseases, including cardiovascular, metabolic, and renal diseases, including the development of kidney stones [88]. Additionally, adequate hydration is associated with reductions in cognitive [89-91] and athletic performance [19, 92] impairments. Carbohydrate (CHO)–electrolyte solutions (CES), including sports drinks and oral rehydration solutions have traditionally been the options of choice for promoting euhydration [71-73]. These solutions are designed to maintain or improve hydration status by promoting drinking, absorption of fluid from the small intestine via activation of sodium-glucose transporters [93], and retention of fluid within the body and the vascular compartment [53, 70]. These pro-hydration properties are primarily a function of the carbohydrate and electrolyte composition of the beverage, as well as its total osmolality [23-25, 61, 94].

Maughan et al. [25] proposed the beverage hydration index (BHI) in 2016 as a measure of the hydrating capacity or efficacy of a given beverage relative to water. Since that time, BHI has been used to compare the hydration efficacy of a variety of beverages [25, 37, 38]. This index is able to assess how a beverage impacts post-ingestion body fluid balance in individuals independent of sex or body mass [38]. In calculating BHI, cumulative urine output at various time points

following consumption of 1 L of water is set to a value of 1.0. Following beverage consumption of the same volume as water, beverages that elicit a greater urinary excretion than water over a fixed period have a BHI less than 1.0, while those that elicit greater fluid retention and attenuated urinary excretion have a BHI greater than 1.0. This measurement thereby enables comparisons of the hydration capacity of various beverages both within and across studies. Although the 2-h post-ingestion time point was proposed by Maughan as the standard for comparison [25], additional information can be gleaned from data throughout the entire 4-h testing period, especially for older adults [37, 95] for whom ingested beverages are retained for a longer period of time, or after consumption of beverages that maintain positive fluid balance beyond 2 h.

Dairy-based beverages have been suggested as efficacious alternatives to traditional sports drinks [6]. Maughan et al. determined that the BHI was higher for both whole milk and skim milk compared to water, and similar to that of an oral rehydration solution (ORS) [25]. Those investigators opined that the high BHI of milk was likely due to its high protein (and perhaps fat) content, while the elevated BHI of the ORS was due to its carbohydrate and electrolyte content. To harness the hydrating qualities of dairy, attempts have been made to develop hydration beverages from byproducts produced during ultrafiltration of milk and cheese products [27-29, 96]. For example, large quantities of milk permeate are produced as a byproduct of the ultrafiltration of milk. Milk permeate is a protein-free, fat-free liquid that contains the approximate carbohydrate and mineral content of milk [27]. It is also high in sodium and potassium and has a relatively high osmolality (primarily due to its total mineral content). Therefore, a solution containing milk permeate, developed primarily for use during exercise and other dehydrating conditions, may have hydration characteristics that are, at a minimum, similar to that of a traditional CES beverage. However, it remains to be determined how a milk permeate-

based solution (MPS) impacts hydration status in humans and how this hydration efficacy compares to that of other commercial hydration solutions.

Determining the BHI of various beverages is an important first step in determining hydration efficacy, since the conditions under which this index is calculated are highly standardized and well-described [25], and because it has been measured in individuals varying in size, sex, and age [25, 37, 38]. Therefore, the primary purposes of the present study were: (1) to determine the hydration efficacy of a novel beverage containing milk permeate relative to water and CES, as measured by net fluid balance and BHI; and (2) to determine the extent to which fluid and electrolytes are retained in the vascular space after ingestion of each solution. We hypothesized that both CES and MPS would demonstrate a higher BHI than water over a 4-h period after standardized beverage ingestion in euhydrated subjects and that the BHI of the MPS beverage would be similar to that of the CES beverage.

MATERIALS AND METHODS

Study Population

Twelve young men and women (23 ± 1 years) participated in the study. Subjects were recruited from the community in Centre County, PA using advertisements or from a pool of individuals who had participated in previous studies. All subjects underwent a screening visit consisting of anthropomorphic measurements, resting heart rate and blood pressure, and blood chemistry prior to enrollment. Subjects were excluded if they had reported any prior history of renal, metabolic, prostate, or cardiovascular disease or if they were taking any medications that may impact fluid balance. All procedures were approved in advance by the Pennsylvania State University Institutional Review Board, and all subjects gave written or verbal consent before participation in

accordance with the Declaration of Helsinki. All testing was conducted in Noll Laboratory at the Pennsylvania State University

Study Design

Subject characteristics are displayed in **Table 3-1**. Twelve young men and women participated in the study. Ten subjects completed all three trials, one subject completed the water and MPS trials only, and one subject completed the CES and MPS trials only (due to COVID considerations). Statistically valid mean substitutions were used for the two missing trials during statistical analysis [97]. Subjects completed trials in a randomized order. All trials began between 0600 and 0900.

Table 3-1: Subject Characteristics

	Mean	Range
n (M/F)	12 (6/6)	
Age (yrs)	23	20 - 26
Weight (kg)	69.6	52.9 – 94.6
BMI ($kg \cdot m^{-2}$)	23.7	20.5 – 29.9
Systolic BP (mmHg)	116	100 - 130
Diastolic BP (mmHg)	75	62 - 82
HR ($beats \cdot min^{-1}$)	65	56 - 72
Total cholesterol ($mg \cdot dL^{-1}$)	169	123 - 264
HDL-C ($mg \cdot dL^{-1}$)	56	41 - 74
LDL-C ($mg \cdot dL^{-1}$)	94	56 - 192
HbA1C (%)	5.0	4.8 – 5.5

Values displayed as mean and range and were obtained during the pre-study screening visit. BMI, body mass index; BP, blood pressure; HR, heart rate; HDL, high-density lipoproteins; LDL, low-density lipoproteins; HbA1c, glycated hemoglobin.

The study design followed that of Maughan et al. [25], further described by Clarke et al. in our lab [37]. Prior to each experimental trial, subjects fasted overnight for at least 8 h, refrained from alcohol and caffeine consumption for 12 h, and refrained from vigorous physical activity for 24 h. Additionally, subjects were not permitted to ingest any food for the duration of each trial. This total period of food restriction did not present any adverse consequences to any subjects. Subjects were instructed to maintain normal fluid intake in the 24 h prior to each study. One hour before arriving at the laboratory, subjects consumed 500 mL of spring water (Aquafina, PepsiCo, Harrison, NY, USA). Subjects self-reported that they had consumed 500 mL of spring water. Upon arrival to the laboratory, subjects entered a thermoneutral room (16–20 ◦C, 20–30% relative humidity), where they remained seated in a semi-recumbent position for the duration of the study, except during urine collection. Subjects voided their bladder in a 1-L plastic urine container (designated as the "pre" time point for data presentation) upon arrival to the laboratory. Pre-trial hydration status was assessed using a urine refractometer to determine urine specific gravity, with values between 1.000 and 1.025 confirming euhydration. Following voiding of the bladder, each subject's near-nude body mass was measured. Subjects then sat for 10 min before an intravenous catheter was inserted into an antecubital vein. A 0-mL pre-ingestion venous blood sample ("pre" time point for blood) was then collected.

Following collection of the "pre" time point blood sample, subjects ingested 1-L of a randomly assigned test beverage in 4 equal aliquots over a 30 min period (0.25 L every 7.5 min). Blood samples were collected immediately after completing the final aliquot (0 min) and at 30, 60, 120, 180, and 240 min post-ingestion. Blood samples were collected in 2 serum separator tubes (4 mL each) to measure serum electrolytes and serum osmolality and 1 K2 EDTA tube (2 mL) to measure hematocrit and hemoglobin. Urine samples were collected in 1-L urine containers

following each blood sample, i.e., at 0, 60, 120, 180, and 240 min post-ingestion. At each time point, subjects were instructed to completely empty their bladder to the extent possible. If a subject needed to void their bladder between collection points, urine was collected and added to the urine sample of the following designated time point. A protocol timeline is outlined in **Figure 3-1**.

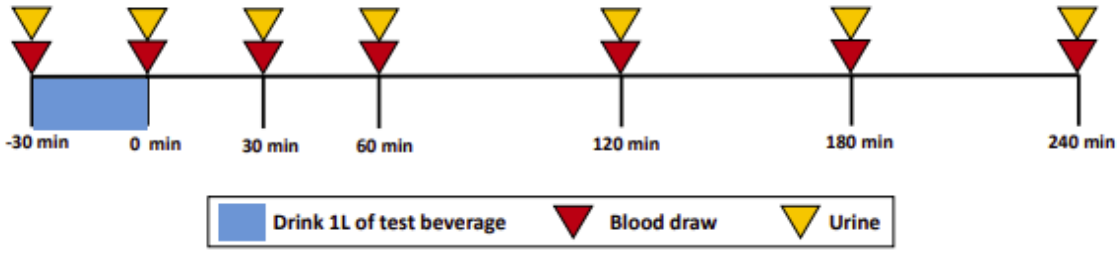

Figure 3-1: Timeline of measurements. Blood samples were collected immediately before (-30 min) and after (0 min) consuming the final beverage aliquot and at 30, 60, 120, 180, and 240 min post-ingestion. Urine samples were collected following each blood sample except at the 30 min time point, i.e., at 0, 60, 120, 180, and 240 min post-ingestion.

Test Beverages

Subjects completed the protocol three times, once for each test beverage. Trials were completed in a randomized order (random number generator) and separated by at least one week. The test beverages were distilled water, CES, and MPS. Beverage composition is displayed in Table 2. Beverage osmolality was tested in triplicate using a freezing-point osmometer (Model 3320, Advanced Instruments, Inc., Norwood, MA, USA); other reported values are label values. All beverage containers were kept sealed and stored at 16-20 ◦C prior to consumption.

Table 3-2: Beverage Composition

Beverage	Energy kcal/L	CHO (%)	Sodium, mmol/L	Potassium, mmol/L	Osmolality, mosm/kg
No Fluid	-	-	-	-	-
Water	0	0	0	0	0
Gatorade	222	6	19	3.6	326 ± 3
GoodSport	180	4	21	28.1	621 ± 5

CES, carbohydrate–electrolyte solution; MPS, milk permeate solution. CHO, carbohydrate content. Values for CHO, kcal, sodium, and potassium are label values. Osmolality was measured in triplicate in our laboratory using a freezing-point osmometer (Model 3320, Advanced Instruments, Inc., Norwood, MA, USA).

Urine and Serum Analysis

Urine mass was assessed using an electronic balance accurate to the nearest 0.1 g, with the mass of the empty 1-L container subtracted from the weighed value. Serum separator tubes were left in an upright position for 30 min to allow serum clotting to occur. Following this 30-min period, blood samples were centrifuged (10 min, 4 ◦C, 4000 rpm).

Urine sodium and potassium concentrations at each time point were measured in triplicate (SmartLyte, Diamond Diagnostics). Urine and serum osmolality were measured at each time point in triplicate using a freezing-point osmometer (Model 3320, Advanced Instruments, Inc.). These analyses were measured at each time point in triplicate using a freezing-point osmometer (Model 3320, Advanced Instruments, Inc.). These analyses were conducted in our laboratory the same day of sample collection. Hematocrit, hemoglobin concentrations, glucose concentrations, serum electrolyte concentrations, and creatinine concentrations were also analyzed for each time point (Quest Diagnostics) within two days of sample collection for each trial.

Data and Statistical Analysis

Baseline blood chemistry and urine analysis were compared among drinks by repeated measures ANOVA to confirm that baseline hydration and renal function status were similar across trials. Main outcomes for this study were cumulative urine output (utilized to calculate BHI and net fluid balance), changes in plasma volume, and plasma glucose responses. BHI was calculated as the cumulative urine output of water divided by the cumulative urine output of the other two beverages at each time point. Plasma volume changes were calculated from hematocrit and hemoglobin concentration using the method of Dill and Costill [98]. Net fluid balance was calculated by subtracting the cumulative urine output at each time from the 1000 g of fluid consumed at the beginning of each trial. Subjects were in positive fluid balance if the obtained value was >0, and in negative fluid balance if this value was <0. Free water clearance (CH2O) was calculated as $CH_2O = \dot{V} - C_{osm}$, where $\dot{V}$ is urine flow in mL/min and C_{osm} is osmolar clearance (mL/min) = $U_{osm} \times \dot{V}/P_{osm}$, with U_{osm} and P_{osm} being urine and plasma osmolality (mOsm/kg), respectively. Urine flow was calculated as urine output (mL) at each time point divided by the amount of time since last urine excretion (minutes). Based on prior publications [25, 37], an $\alpha < 0.05$, and power = 0.8, we determined a priori that a minimum of 10 subjects would be needed to determine statistically significant differences among beverages. Data were analyzed in SAS (Cary, NC, USA) using PROC MIXED two-way ANOVA (beverage x time). With the exception of box plots, all data are presented as mean x SD. Statistical significance was set a priori at $\alpha < 0.05$.

RESULTS

Cumulative Urine Output, Net Fluid Balance, and Beverage Hydration Index

Baseline (immediately before beverage ingestion) serum and urine markers of hydration and renal function status are displayed in Table 3-3. All measurements were similar across trials (all $p \geq 0.05$). As shown in **Figure 3-2A**, cumulative urine output was significantly lower for MPS than for the water ($p = 0.02$) and CES trials beginning at 60 min ($p = 0.01$) and remained lower for the remainder of the trial ($p < 0.01$). Cumulative urine output was lower for the CES trial compared to water at 120, 180, and 240 min ($p \leq 0.03$). The final (4-h) cumulative urine outputs for each trial were: water = 1423 ± 277 mL; CES = 1332 ± 234 mL; and MPS = 1191 ± 194 mL.

Table 3-3: Baseline Characteristics by Trial.

	Water	**CES**	**MPS**
	Mean ± SD	Mean ± SD	Mean ± SD
Fasting glucose ($mg \cdot dL^{-1}$)	87 ± 6	88 ± 5	88 ± 6
Creatinine (mg·dL-1)	0.86 ± 0.17	0.87 ± 0.18	0.89 ± 0.21
Serum Na^+ ($mmol \cdot L^{-1}$)	138 ± 2	137 ± 2	138 ± 2
Urine Na^+ ($mmol \cdot L^{-1}$)	84 ± 48	91 ± 79	75 ± 49
S_{osm} ($mOsm \cdot kg^{-1}$)	291 ± 5	291 ± 5	291 ± 5
U_{osm} ($mOsm \cdot kg^{-}$)	511 ± 296	566 ± 301	574 ± 342
Urine Specific Gravity	1.015 ± 0.008	1.017 ± 0.008	1.017 ± 0.009

Figure 3-2B displays net fluid balance for each beverage. Net fluid balance was higher (more positive) for MPS compared to CES beginning at 60 min ($p = 0.04$) and remained so through the subsequent 3 h ($p < 0.01$) and higher than water from 120 to 240 min ($p < 0.01$). There were no differences in net fluid balance between the water and CES trials at any time point.

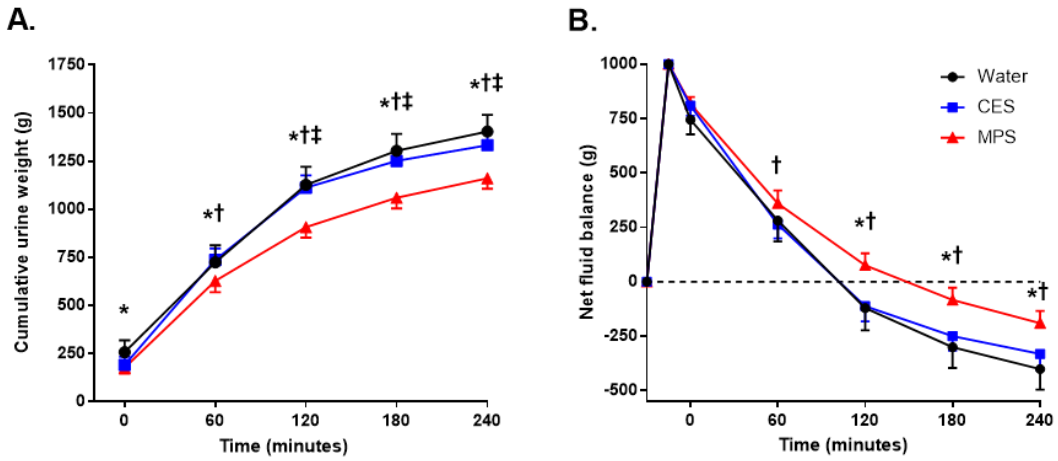

Figure 3-2: Cumulative urine output (A) and net fluid balance (B) 4 h after consumption of water, a carbohydrate–electrolyte solution (CES), and a milk permeate-based solution (MPS). Cumulative urine output was significantly lower in MPS compared to both water and CES at all time points after ingestion and was lower in CES compared to water beginning at 120 min. Net fluid balance was more positive for MPS compared to water and CES from 2 h onward. There were no differences in net fluid balance between the water and CES trials. Values are means ± SD. Differences among beverages were assessed by two-way ANOVA. * $p < 0.05$ MPS compared to water; † $p < 0.05$ MPS compared to CES; ‡ $p < 0.05$ CES compared to water.

Figure 3-3 presents box plots for BHI along with individual subject data. BHI was significantly higher for MPS than water and CES (all $p \leq 0.01$) at all time points. BHI for CES did not differ statistically from water at any time point. Figure 4 displays the differences in BHI for each individual subject between pairs of beverages to better illustrate intra-subject differences (CES = 1.04 ± 0.09; MPS = 1.29 ± 0.15 at 120 min; CES = 1.07 ± 0.06 and MPS = 1.21 ± 0.09 at 240 min).

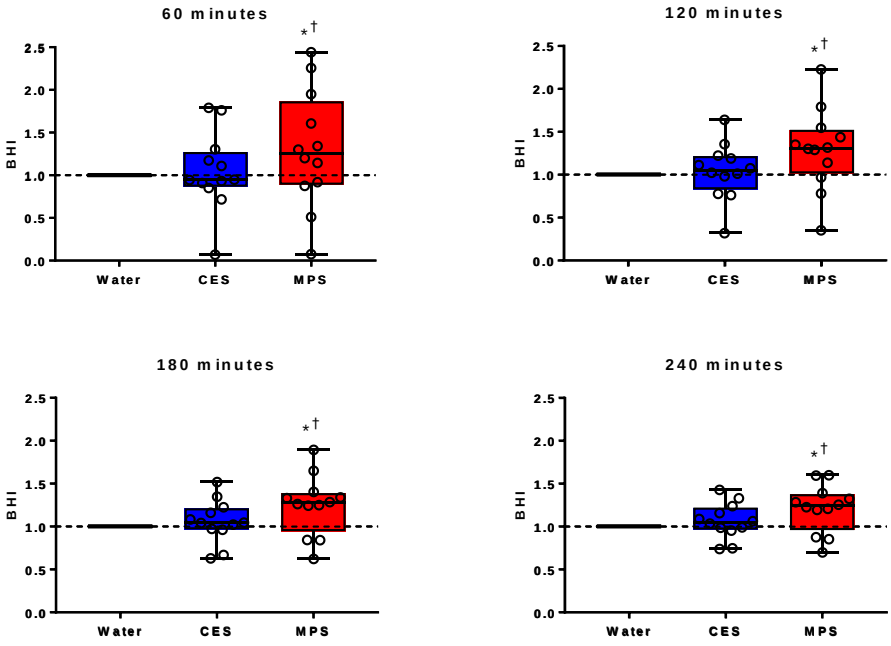

Figure 3-3: Beverage Hydration Index (BHI) for a carbohydrate–electrolyte solution (CES) and a milk permeate-based solution (MPS) relative to water (BHI = 1). The BHI for MPS was significantly higherc ompared to both water and CES at all time points after ingestion. BHI did not differ between water and CES at any time point. Boxes represent first and third quartiles with median values denoted by the horizontal line, while whiskers indicate minimum and maximum observations. Individual subjects' BHIs are displayed as open circles. Differences between beverages were assessed by two-way ANOVA. * $p < 0.05$ MPS compared to water; † $p < 0.05$ MPS compared to CES.

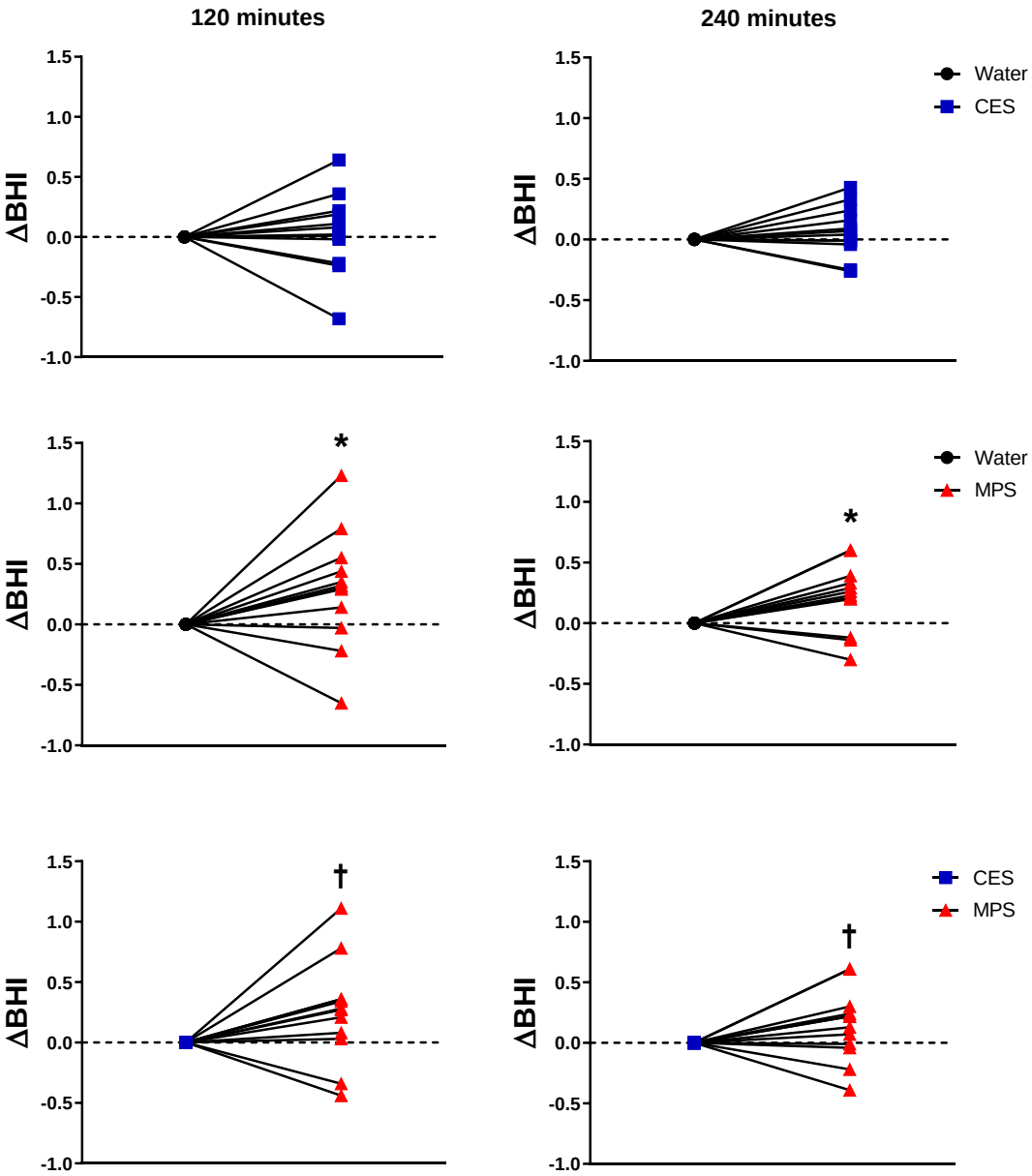

Figure 3-4: Data for individual subjects showing the difference in BHI for paired beverages. Differences between beverages were assessed by two-way ANOVA. * $p < 0.05$ MPS compared to water; † $p < 0.05$ MPS compared to CES.

Serum and Urine Electrolyte Concentrations and Osmolalities

Serum and urine electrolyte concentrations, osmolalities, and free water clearance (CH_2O) across time points are displayed in **Table 3-4**. There were no differences in serum or urine osmolality or

electrolyte concentrations among trials prior to beverage ingestion ($p \geq 0.43$). Immediately post-ingestion, serum osmolality declined in the water trial ($p = 0.02$) but increased in the MPS trial ($p = 0.04$). CES serum osmolality did not significantly change throughout the trial ($p \geq 0.23$). Serum osmolality was greater in the MPS trial at 0, 30, and 60 min compared to water (all $p < 0.001$) and at 60 min compared to CES ($p < 0.01$).

Table 3-4: Serum and urine electrolyte, osmolality, and urine free water clearance (CH_2O) values.

		Pre	0 min	30 min	60 min	120 min	180 min	240 min
Serum								
	Water	138 ± 2	136 ± 3 ‡	135 ± 2 ‡	136 ± 2	137 ± 2	138 ± 2	138 ± 3
Sodium	CES	137 ± 2	136 ± 1 ‡	137 ± 2 *	138 ± 1 *	138 ± 1	138 ± 2	138 ± 1
(mmol/L)	MPS	138 ± 2	135 ± 5 ‡	137 ± 2 *	136 ± 4 †‡	137 ± 1	138 ± 2	138 ± 2
	Water	4.1 ± 0.1	4.3 ± 0.2 ‡	4.2 ± 0.3	4.3 ± 0.3 ‡	4.2 ± 0.4 ‡	4.1 ± 0.4	4.1 ± 0.3
Potassium	CES	4.0 ± 0.2	4.0 ± 0.3 *	3.9 ± 0.2 *	4.1 ± 0.2 *	4.3 ± 0.2 ‡	4.4 ± 0.3 *‡	4.3 ± 0.3 *‡
(mmol/L)	MPS	4.0 ± 0.3	4.3 ± 0.4 †‡	4.4 ± 0.4 *†‡	4.4 ± 0.4 *†‡	4.5 ± 0.3 *†‡	4.4 ± 0.2 *‡	4.4 ± 0.2 *‡
	Water	291 ± 5	288 ± 6 ‡	287 ± 6 ‡	287 ± 9 ‡	291 ± 6	291 ± 5	292 ± 6
Osmolality	CES	291 ± 5	292 ± 4 *	290 ± 5 *	290 ± 4 *	292 ± 3	292 ± 5	293 ± 5
(mosm/kg)	MPS	291 ± 5	293 ± 6 *‡	293 ± 7 *	294 ± 6 *†‡	293 ± 6	294 ± 3 ‡	293 ± 3 ‡
Urine								
	Water	72 ± 49	42 ± 36	----------	28 ± 47	24 ± 19	62 ± 20	95 ± 42
Sodium	CES	87 ± 73	38 ± 30 ‡	----------	29 ± 37 ‡	26 ± 17 ‡	59 ± 30 ‡	103 ± 28
(mmol/L)	MPS	71 ± 50	61 ± 47 ‡	----------	46 ± 32 ‡	78 ± 42 *†‡	100 ± 36 *†‡	115 ± 40 ‡
	Water	28.5 ± 23.2	29.0 ± 18.2	----------	10.2 ± 2.8	11.9 ± 6.8	29.6 ± 22.7	42.6 ± 33.5
Potassium	CES	33.3 ± 20.8	21.4 ± 21.7	----------	3.1 ± 1.5 ‡	10.2 ± 4.9 ‡	40.5 ± 46.7	55.3 ± 32.5 ‡
(mmol/L)	MPS			----------				
		28.5 ± 20.7	32.3 ± 33.5		14.5 ± 13.5 ‡	35.5 ± 27.0 *†‡	51.3 ± 29.8 *‡	63.5 ± 33.3 *‡
	Water	511 ± 282	308 ± 265 ‡	----------	144 ± 217 ‡	113 ± 23 ‡	305 ± 305 ‡	465 ± 465
Osmolality	CES	564 ± 288	352 ± 298 ‡	----------	71 ± 18 ‡	130 ± 45 ‡	309 ± 68 ‡	540 ± 115
(mosm/kg)	MPS	563 ± 345	387 ± 305 ‡	----------	226 ± 147 †‡	370 ± 166 *†‡	537 ± 138 *†	635 ± 134 *
	Water	----------	3 ± 5	----------	6 ± 2	4 ± 1	1 ± 2	-1 ± 1
CH₂O	CES	----------	2 ± 4	----------	7 ± 2	4 ± 2	0 ± 1	-1 ± 0
(mL/min)	MPS	----------	1 ± 5*†	----------	2 ± 3*†	0 ± 3*†	-2 ± 1*	-2 ± 1

Values are means ±SD. Differences among beverages were assessed by two-way ANOVA. * $p < 0.05$ compared to water; † $p < 0.05$ compared to CES; ‡ $p < 0.05$ compared to Pre.

Serum and sodium decreased in all trials immediately post ingestion ($p \leq 0.04$). Serum sodium was elevated in MPS ($p < 0.01$) and CES (p , 0.01) compared to water at 30 min and higher for CES compared to both water ($p = 0.04$) and MPS ($p < 0.01$) at 60 min. There were no differences among beverages at 120 min or beyond ($p \geq 0.22$). Serum potassium was significantly elevated at all time points in the MPS trials (all $p<0.001$), but only at 120 min and beyond in the CES trial ($p < 0.001$). Serum potassium was significantly higher in the MPS trial compared to water at all time points (all $p < 0.01$), and at 0, 30, 60, and 120 min compared to CES (all $p<0.002$).

Urine osmolality was higher in MPS compared to water at 120 min and beyond (all $p < 0.01$) and compared to CES at 60, 120, and 180 min (all $p < 0.02$). There were no differences in urine osmolality between water and CES at any time point. Urine Na+ concentration was significantly higher in MPS compared to water and CES at 120 and 180 min ($p < 0.01$) and urine K+ was higher in MPS compared to compared to water at 120 min and beyond ($p < 0.01$) and vs. CES at 120 min ($p < 0.01$). Free water clearance (CH2O) was lower in MPS compared to water through 180 min (all $p < 0.02$) and compared to CES through 120 min (all $p < 0.04$).

Plasma Volume

There was a significant increase in plasma volume (ΔPV) compared to pre-ingestion beginning at 30 min and continuing for the duration of the study for both MPS (3.5–4.0%; $p < 0.03$) and CES trials (3.8–6.7%; $p < 0.02$), but only at 120 min in the water trial (3.6%; $p = 0.03$). Changes in plasma volume are depicted at 120 and 240 min in **Figure 3-5** as box plots with individual subject data shown. ΔPV was significantly higher for all three beverages compared to pre-ingestion at 120 min (all $p < 0.04$) but only for CES and MPS by 240 min ($p < 0.02$). All subjects experienced a positive change in plasma volume at 120 min in the MPS trial, with relatively small variation in the MPS trial, especially at 120 min.

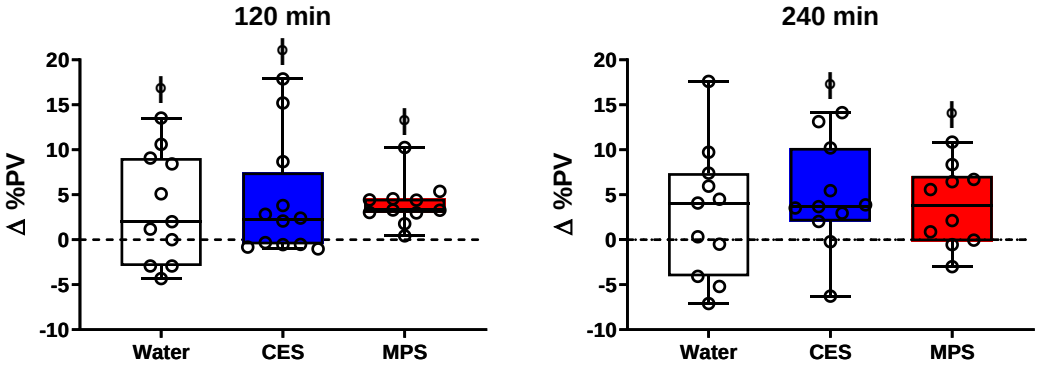

Figure 3-5: Changes in plasma volume at 120 and 240 min post-ingestion. Plasma volume was significantly elevated in all three beverages compared to Pre at 120 min and only in CES and MPS at 240 min. There were no significant differences in changes in plasma volumes between beverages at both time points. Boxes represent first and third quartiles with median values denoted by the horizontal line, while whiskers indicate minimum and maximum observations. Individual subjects' changes in plasma volume are displayed as open circles. Differences between beverages were assessed by two-way ANOVA. ϕ $p < 0.05$ compared to Pre.

Plasma Glucose Responses

There were no differences in fasting plasma glucose concentration across trials prior to beverage ingestion (**Figure 3-6**). Immediately following ingestion (i.e., time 0) of the CES and MPS beverages (but not water; $p = 0.86$), there were significant increases in glucose concentration (both $p < 0.01$). There were no differences in plasma glucose concentration between water and MPS trials after 30 min, but plasma glucose concentration in the CES trial remained elevated compared to both water ($p < 0.01$) and MPS ($p = 0.03$) at 30 min and then lower than both water ($p < 0.01$) and MPS ($p = 0.02$) at 60 min. Plasma glucose concentration for all three beverages returned to pre-ingestion baseline values by 180 min ($p \geq 0.12$).

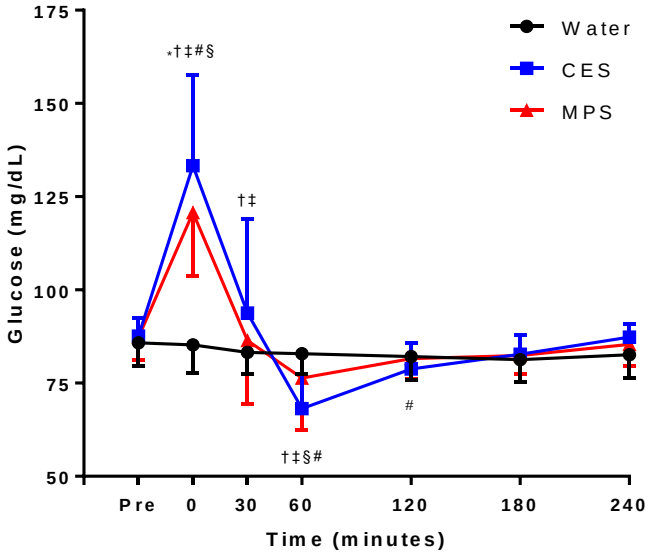

Figure 3-6: Plasma glucose concentration before and after consumption of water, a carbohydrate– electrolyte solution (CES), and a milk permeate-based solution (MPS). Glucose concentrations were significantly higher in CES and MPS compared to water immediately after and 30 min after ingestion of solutions. CES was higher than both MPS and water immediately after ingestion but lower at 60 min post-ingestion. Values are means ± SEM. Differences between beverages were assessed by twoway ANOVA. * $p < 0.05$ MPS compared to water; † $p < 0.05$ MPS compared to CES. ‡ $p < 0.05$ CES compared to water. § $p < 0.05$ MPS compared to pre. # $p < 0.05$ CES compared to pre. & $p < 0.05$ water compared to pre.

DISCUSSION

The present investigation examined the hydration efficacy of a novel milk permeate-based (MPS) solution in comparison to water and a traditional carbohydrate-based electrolyte sports drink (CES). Compared to CES, MPS had a lower carbohydrate content (4% vs. 6%), a similar sodium concentration (20 vs. 21 mmol/L), a higher potassium concentration (28 vs. 3.2 mmol/L), and higher osmolality (621 vs. 326 mOsm/L). The primary finding of the study was that 1 L of the milk permeate solution, consumed in a euhydrated state, was retained in the body longer compared to water and CES. Calculated BHI was significantly higher for MPS compared to both water and CES across the 4 h post-ingestion period, accompanied by a similar vascular fluid compartment expansion. Finally, there were blunted plasma glucose concentration excursions

(i.e., immediate post-drinking increase and subsequent decrease below baseline at 1 h) following MPS ingestion compared to CES.

Maintaining proper hydration status at rest is important for its health benefits and in the prevention of developing chronic disease [88] and deficits in cognitive function [90, 91, 99] and athletic performance [19, 92]. Carbohydrate-based electrolyte solutions have traditionally been recommended for promoting fluid retention and restoring euhydration [71-73], especially during and following physical activity that is accompanied by profuse sweating. The beverage hydration index (BHI), based on cumulative urine output and net fluid balance, is an innovative approach for assessing the hydration efficacy of different beverages [25, 37, 38]. This index is not impacted by differences in sex or body mass [38], allowing its application to the general population, including older adults [37]. In addition, results can be compared across studies that have followed the standardized BHI protocol published by Maughan et al. [25]. For example, following consumption of a traditional carbohydrate- based sports beverage, BHI values in our study were similar to those reported in young men and women by Clarke et al. using a similar CES beverage [37].

Prior investigations have suggested that whole or skim milk is an effective hydration solution at rest and its BHI is comparable to that of an oral rehydration solution beverage [25, 77, 100]. Milk contains electrolytes, proteins, minerals, and other solutes that, when absorbed in the small intestine, promote fluid retention. The milk permeate beverage contains the approximate carbohydrate and mineral content of milk [27], but without fats or proteins. The milk permeate solution tested here comprised approximately 21 mmol/L of sodium, which is similar to that of traditional carbohydrate-based hydration solutions, including the CES beverage utilized in this study (20 mmol/L). However, the potassium concentration of the MPS beverage (28 mmol/L) was considerably higher than the CES beverage (3.2 mmol/L). The MPS beverage also had a

higher osmolality (621 ± 5 mosm/kg) compared to the CES beverage (326 ± 3 mosm/kg). The additional osmolar constituents of MPS consisted of chloride, magnesium, phosphorous, and calcium. The greater osmolality of MPS compared to CES or water likely contributed to the reduced urine production and greater fluid retention.

Indeed, our findings show that the cumulative urine output over the 4 h after ingestion of MPS was significantly lower than either water or CES (**Figure 3-2A**) and was accompanied by a longer time spent in positive fluid balance (**Figure 3-2B**). These results are consistent with prior studies showing that beverages with higher electrolyte concentrations and osmolality promote increased fluid retention in young adults [95]. The increased fluid retention with MPS resulted in an increased BHI across the entire 4 h time course compared to water and CES (**Figure 3-3**). Such an increase in fluid retention could be due to slower gastric emptying following ingestion of a high-osmotic solution, and we cannot rule out that possibility based on the data collected in this study [63].

However, it is unlikely that differences in gastric emptying played a major role in influencing the BHI because other investigators have reported that the primary factor affecting gastric emptying is the energy content of the beverage, even when the osmolalities of the test beverages varied widely. Consumption of 500 mL of isocaloric beverages displayed similar gastric emptying rates, despite large differences in beverage osmolalities [101], and impairments in gastric emptying were not seen at or below beverage glucose concentrations of 6% and osmolalities of 350 mosm/kg [51]. The energy content of the MPS tested here was 27% lower than that of the CES, suggesting that fluid from MPS did not remain in the stomach longer. Additional research is warranted to discern how these differences in BHI are influenced by differences in the rates of gastric emptying, absorption in the proximal small intestine, and renal urine production [102].

Serum osmolality was significantly elevated in the MPS trial compared to both CES and water at 60 min post-ingestion. This was driven, at least in part, by an increased osmolality of the MPS beverage. Intra-individual differences in serum osmolality between trials may explain some of the within-subject variability in urine output between beverages at earlier timepoints (i.e., 120 min), thus influencing BHI, although this variability appears to decrease at later timepoints (i.e., 240 min). Serum potassium concentration in the MPS trial was likewise elevated compared to water and CES in the first hour post-consumption. On the other hand, serum sodium concentration was only significantly elevated in the MPS trial compared to water and CES at 30 min, and was actually lowered compared to the CES trial at 60 min.

One important aspect of efficacious hydration is an expansion of extracellular fluid, specifically in the vascular compartment. There was an expansion of plasma volume (ΔPV) in the MPS trial beginning at 30 min and continuing for the duration of the study, though this mildly increased plasma volume was not different from that of the water trial at any time point. Sustained elevations in PV, i.e., in 120- and 240-min responses are shown in **Figure 3-5**. The %ΔPV was lower for MPS than CES over the initial 10–15 min after drinking but results were highly variable (data not shown), which may reflect a slower initial rate of gastric emptying in response to the higher osmolality of the MPS beverage [51]. Although subjects were in negative net fluid balance by the end of the study, indicating a greater excretion of fluid than what was consumed, there was a sustained plasma volume expansion of approximately 3–5% in the CES and MPS beverages at 240 min. The sustained mild PV expansion over at least 4 h after drinking MPS and CES likely indicates that the increased concentration of serum electrolytes helped retain some of the ingested fluid in the vascular space and possibly promoted some osmotic pull of water from the

intracellular space into the vascular space [103], allowing for hemodilution, an important component of efficacious hydration [104].

The dairy-based MPS beverage in this study was approximately 4% glucose/galactose. In comparison, the carbohydrate–electrolyte solution (CES) tested in this study was 6% sucrose/glucose. It is thus important to elucidate potential differences in the glycemic load stemming from these differences in carbohydrate composition between beverages, which may be a particularly important consideration for populations who are at risk of metabolic dysfunction. The immediate rise in glucose concentration following MPS ingestion was blunted relative to the CES trial (**Figure 3-6**). Additionally, the plasma glucose concentrations returned to baseline sooner after MPS consumption compared to CES consumption, which showed an overshoot below baseline values at 60 min. These plasma glucose profiles may be attributable to the lowered total carbohydrate load and lower glucose concentration in the MPS beverage compared to the CES beverage, supporting its efficacy as a potential lower-glycemic alternative to traditional carbohydrate-based sports beverages.

Important considerations for the benefits of a hydration-promoting beverage include the taste, consistency, and thirst-quenching qualities of the beverage. As such, subjects in the current study completed a sensory evaluation survey for each beverage (data not displayed) regarding qualitative aspects, such as overall likability, taste, sweetness, aroma, and thirst-quenching properties. There were no statistical differences in responses among beverages.

Limitations

In prior BHI studies [25, 37], hydration solutions have been stored at approximately 4–6 °C. All beverages utilized in the current study were stored at 16–20 °C to prevent greater pressor

responses to ingestion of cold beverages compared to room-temperature beverages [105], which may negatively impact effective venous blood sampling within the first 30 min post-ingestion. While the present study used a prescribed drinking protocol, it has previously been reported that stimulation of cold sensitive oropharyngeal receptors results in lowered ad libitum fluid consumption in humans, and that optimal water temperature to encourage ad libitum consumption is approximately 15 °C [106]. However, ad libitum consumption of either 4 or 20 °C water displayed no differential influence on hydration status in mildly dehydrated young adults [107]. The current recommendation of the American College of Sports Medicine is that ingested fluids for the purpose of hydration should be at ambient temperatures between 15 and 22 °C [14]. Therefore, we determined that storing beverages at room temperature was justified. Another limitation of this study, inherent to all BHI studies, is that the location of the fluid remaining in the body is unknown. BHI is determined by differences in the rates of gastric emptying, fluid absorption in the proximal small intestine, and renal urine production. In that regard, future studies measuring the gastric residual contents and duodenal constituent concentrations are warranted.

Although it was outside the scope of the current study to measure circulating concentrations of hormones associated with fluid balance maintenance, it is possible that circulating vasopressin influenced fluid retention in the current study. Following water consumption, there is a rapid decrease in plasma vasopressin concentrations independent of gastrointestinal absorption rate [108-111]. Additionally, these changes appear to occur prior to changes in plasma osmolality [112, 113]. In a prior study examining the potential role of plasma vasopressin in plasma volume changes following consumption of either a glucose polymer–electrolyte solution or water, there were no differences in plasma vasopressin concentrations between the two trials despite a higher plasma volume in the glucose polymer–electrolyte trial compared to the water trial [114].

However, there were no changes in plasma osmolalities in either trial in that study, whereas in the current study there was an increase in plasma osmolality in the MPS trial. Future investigation is warranted to examine the potential role of vasopressin in mediating changes in plasma volume following consumption of beverages with a wide range in osmolalities.

Approximately 36% of the United States population is affected by lactose malabsorption to some degree [115], which may lead to gastrointestinal (GI) discomfort following dairy consumption. This is especially important in individuals who are exercising, as GI discomfort may impair performance. Thus, it is beneficial to explore potential dairy-based alternatives that can provide the same hydrating capacity of milk without the GI discomfort. The current MPS beverage consists of 2% glucose and 2% galactose and is protein- and fat-free. Milk typically contains 4–6% lactose. Although subjects in the current study did not report any GI discomfort at any point following consumption of the MPS beverage, no subjects in the study reported any history of intolerance to dairy-based products.

Subjects in this study started drinking in a euhydrated state and remained at rest for 4 h post-ingestion. When there is any suggestion that individuals may be even mildly dehydrated, clinicians often order prescribed drinking as a first step toward restoring or assuring euhydration. CES solutions are often the drink of choice in such conditions but cross-beverage comparisons have rarely been conducted. This adds situational validity to the BHI approach. However, it is unknown how these findings translate to maintenance of, or return to, euhydration under stressed conditions such as during and after exercise in the heat. Few investigations have examined the efficacy of either skim [116] or low-fat [78] milk for rehydration during or following acute bouts of exercise. Skim milk, both alone and with added sodium, resulted in a lower urine output compared to a traditional sports drink or water in the 4 h following cycling-induced dehydration

[78], though this response was attributed to the high protein concentration of milk. The solution tested in our study contained milk permeate, an ultrafiltrate of milk which is both protein- and fat-free, with the approximate mineral content of milk, and equivalent sodium concentration of common sports drinks. Future investigation is warranted to examine the efficacy of this milk permeate solution as a both a beverage for consumption during and after exercise.

CONCLUSIONS

In summary, a novel dairy-based beverage containing milk permeate promoted better body fluid retention over 4 h post-ingestion. MPS had a lower cumulative urine output, maintained positive fluid balance longer, and thus had a higher beverage hydration index than that of a traditional carbohydrate-based electrolyte beverage or water. The increased fluid retention properties associated with ingestion of the milk permeate solution is likely attributable to its greater total mineral content and higher osmolality. In addition, the initial increase in plasma glucose concentration was lower following consumption of the milk permeate solution compared to the carbohydrate-based electrolyte solution. Together, these findings indicate that a dairy-based beverage containing milk permeate may serve as an efficacious alternative to traditional carbohydrate–electrolyte solutions in healthy young adults at rest. Future research is needed to investigate the efficacy of this milk permeate solution during and following physical activity or environmental stress, or in clinical populations.

AUTHOR CONTRIBUTIONS

Conceptualization, W.L.K. and B.M.; methodology, W.L.K. and B.M.; formal analysis,

C.W.B., S.T.W., B.M., and W.L.K.; investigation and data collection, C.W.B. and S.T.W.; writing—original draft preparation, C.W.B.; and writing—review and editing, C.W.B., S.T.W., B.M., and W.L.K. All authors have read and agreed to the published version of the manuscript.

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CONFLICTS OF INTEREST

Dr. Bob Murray served as a paid consultant to the present project. Dr. Murray serves as a paid consultant for several companies that market and sell beverage products.

Chapter 4

HYDRATION IS MORE IMPORTANT THAN EXOGENOUS CARBOHYDRATE INTAKE DURING PUSH-TO-THE-FINISH CYCLE EXERCISE IN THE HEAT

INTRODUCTION

Physical performance capacity is dependent on a litany of factors that include hydration status, substrate availability for energy production, and the environmental conditions in which activity occurs. Aerobic exercise performance is diminished in the heat compared to cooler conditions and the mechanisms underlying this impaired performance have been reviewed [117]. High-intensity physical activity in the heat elicits a robust sweating response in order to maintain thermal balance which may result in significant loss of body water content if fluid is not replaced. Although this sweating response is widely variable between individuals and depends on a number of factors, including physical fitness, heat acclimation, and exercise intensity [3, 4, 14], it is not uncommon to observe sweat rates exceeding 1 liter per hour [118]. Dehydration resulting in a loss of body mass >2% has been associated with a decline in performance [14, 18, 67, 119-122]. Additionally, during dynamic exercise in the heat, blood flow is redistributed from inactive tissues (i.e., renal and splanchnic tissues) toward active tissues (skin and skeletal muscle). However, dehydration increases competition in blood flow distribution between the skin and skeletal muscle circulatory beds to meet their thermoregulatory and energetic demands, respectively [123]. Concomitant cardiovascular strain (increased heart rate and reduced cardiac filling and stroke volume) attributed to increased skin blood flow limits exercise performance capacity under conditions of heat-induced dehydration [117, 124].

Considering the highly interactive nature of exercise in the heat and dehydration, it is important to assess potential rehydration strategies that preserve and promote fluid homeostasis and how those strategies may differentially affect subsequent performance in both hot and cooler conditions. Water alone is often not sufficient for promoting rehydration, and fluids with added components are necessary to restore lost fluid. In contrast, fluids with added constituents (e.g., carbohydrate and electrolytes) may further improve hydration status[3, 14, 18, 61, 125] and subsequent exercise performance by promoting drinking, absorption of fluid from the small intestine via activation of sodium-glucose transporters [69], encouraging retention of fluid within the body and the vascular compartment [53, 70], and replacement of fluid and electrolytes lost via sweat [14]. The hydrating properties of these beverages are primarily a function of their electrolyte content and composition, caloric content, and total osmolality [23-25, 61, 94]. Although sports drinks are typically prescribed to restore hydration status and improve physical performance [71-73], more natural alternative fluid sources, such as dairy-based beverages, which have a high electrolyte concentration [23, 24] and similar carbohydrate and caloric concentration to traditional sports drinks [6], may serve as efficacious alternative fluid sources for promoting euhydration and rehydration and serve as a reliable source for exogenous carbohydrate supplementation prior to physical activity. Using the Beverage Hydration Index (BHI), Maughan et al. [25] observed a similar hydration capacity of both skim milk and full-fat milk compared to a traditional oral rehydration solution. Our lab more recently showed an elevated BHI after consumption of a novel beverage containing protein- and fat-free milk permeate compared to both water and a traditional sports drink in well-hydrated young subjects at rest [36]. This finding was mainly attributed to the increased osmolality and electrolyte concentration in the milk-permeate beverage. However, it was previously unclear how such a beverage may promote rehydration and subsequently impact

physical performance following exercise- and heat-induced dehydration in young athletes, and how this beverage may compare to other common hydration sources.

The final ~10 minutes of a cycling race are often considered to be the most crucial, involving dramatically increased power outputs [126], and placing at the finish is often decided by a few seconds. Seemingly marginal improvements in late-race performance, therefore, can substantially affect race outcomes. While a plethora of studies have compared the impact of consumption of sports drinks versus water on performance and have been reviewed elsewhere [21, 24, 127, 128], none to our knowledge have examined how rehydration with these distinct solutions may differentially impact performance during the important final stages of a race, i.e, the final push to the finish line after a dehydrating exercise bout. To our knowledge, no previous studies have compared the rehydration capabilities of a beverage containing ultra-filtered deproteinized milk and a traditional sports drink and their subsequent influence on performance. Likewise, whether the ambient conditions in which such an effort is performed influence beverage efficacy is unclear.

The purpose of this study was to determine how hydration status, environmental conditions, and carbohydrate availability affected selected physiological responses and performance during a push-to-the-finish task similar to that occurring during the final kilometers of a cycling race. Specifically, we sought to determine how partial rehydration with two sports drinks -- a new milk permeate-based beverage (GoodSport™) and a traditional sports drink (Gatorade®) – altered push-to-the-finish time trial (TT) performance compared to water or no fluid. TT performance comparisons were made in both hot and cooler environments. We hypothesized that 1) cycling time-trial performance would be improved following rehydration with all three beverages compared to no fluid replacement, 2) consumption of both sports drinks would improve indices of

hydration and TT performance compared to water, and 3) consistent with previous results from resting subjects [36], hydration status would be improved following consumption of GoodSport™ compared to water and Gatorade®.

MATERIALS AND METHODS

Study Population

All data for this study were collected from November, 2020 to May, 2021. Ten young, well-trained (≥ 6 h/wk for ≥ 6 months) cyclists (6M, 4W, 21-38 yrs of age) participated in studies that occurred in a hot-dry environment (35-40 °C, 20% relative humidity (RH)). All studies occurred in an environmental chamber to control these environmental conditions, and were monitored using an ASHRAE box connected to Power Lab and Laboratory Chart software (ADInstruments). A subset of four subjects who participated in that study returned to perform an additional 3 hydration trials in a thermoneutral (21 °C, 20% RH) environment (discussed in subsequent sections). Subject characteristics for hot and thermoneutral studies are detailed in **Tables 4-1A** and **4-1B**, respectively. Additional inclusion criteria consisted of the following: aged 18-45 yrs, body mass index <30 $kg \cdot m^2$, blood pressure <130/80 mmHg, HbA1C < 5.7%, and all premenopausal women either being eumenorrheic or taking oral contraceptives. Subjects were excluded if they reported any prior history of renal, metabolic, prostate, or cardiovascular disease or if they were taking any medications that may impact fluid balance. Fourteen subjects were enrolled in the study. Four subjects dropped out due to time commitment issues. Menstrual cycle phase was not controlled in women participating in the study, as menstrual cycle phase has been previously reported to not influence fluid replacement during or following exercise [129]. All procedures were approved in advance by the Pennsylvania State University Institutional Review

Board, and all subjects gave written or verbal consent before participation in accordance with the Declaration of Helsinki. All testing was conducted in Noll Laboratory at the Pennsylvania State University.

Table 4-1A: Subject characteristics for hot-dry condition.

	Mean	SD	Range
n (M/W)	10 (6/4)		
Age (yrs)	30	6	21 – 38
Weight (kg)	77.1	14.8	53.6 – 97.5
BMI ($kg \cdot m^{-2}$)	24.5	2.9	21.5 – 29.9
Systolic BP (mmHg)	118	12	102 – 142
Diastolic BP (mmHg)	75	8	62 – 88
HR ($beats \cdot min^{-1}$)	58	14	44 – 84
HbA1C (%)	4.9	0.2	4.6 – 5.1

Table 4-1B: Subject characteristics for the thermoneutral condition.

	Mean	SD	Range
n (M/W)	4 (3/1)		
Age (yrs)	31	7	22 – 37
Weight (kg)	83.6	17.6	59.5 – 97.5
BMI ($kg \cdot m^{-2}$)	25.4	1.9	23.8 – 28.2
Systolic BP (mmHg)	124	16	104 – 142
Diastolic BP (mmHg)	80	7	72 – 88
HR ($beats \cdot min^{-1}$)	62	13	52 – 80
HbA1C (%)	4.9	0.2	4.6 – 5.1

Screening and Familiarization

All subjects underwent a screening visit prior to enrollment. Following informed consent, subjects completed a medical health history questionnaire and a fasted blood sample was then obtained for blood biochemistry analysis. Weight and height were measured to the nearest 0.05 kg and 0.01 m, respectively, while subjects wore cycling shorts and socks (women also wore a sports bra) without shoes. Subjects wore the same clothing ensemble for each of the four experimental visits. Heart rate (HR) and blood pressure (sphygmomanometry) were measured after subjects sat quietly in an upright position for at least 5 minutes. An exercise time-trial familiarization session was conducted immediately after screening.

Experimental Protocol

All laboratory visits were completed in a randomized, cross-over, and counter-balanced manner. Subjects were instructed to abstain from vigorous exercise and alcohol for 24 hours, caffeine for 12 hours, and food ingestion for 8 hours before each experiment. Additionally, subjects were asked to maintain their normal dietary and water consumption patterns for the 24 hours prior to each study. A timeline for the study protocol is detailed in **Figure 4-1**. Two hours prior to each experiment, subjects ingested a temperature telemetry pill (BodyCap). One hour prior to arrival, subjects consumed 500 mL of water (Aquafina) and an energy bar (Clif Bar®: 45 g carbohydrates, 5 g fat, 9 g protein, 140 mg sodium).

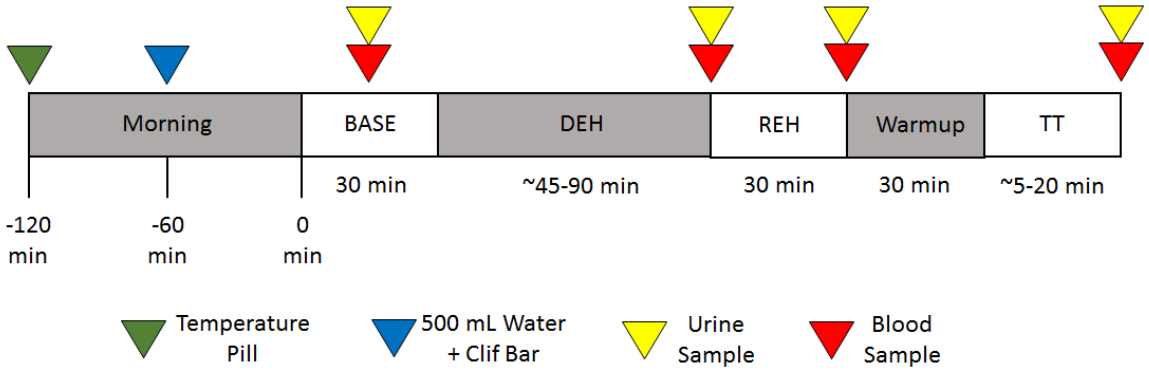

Figure 4-1: Timeline of the study.

All experiments occurred in an environmental chamber. Upon arrival, subjects entered the chamber, which was set at 40 °C and 20% RH. During a 30-min baseline (BASE) equilibration period, a urine sample was obtained to assess urine specific gravity (USG). Body mass was then measured on a scale (Seca Scales) accurate to the nearest 0.05 kg. Subjects then sat quietly in an upright posture for the remainder of the equilibration period. At the end of the equilibration period, subjects were asked to rate their current perception of temperature (0-8; 0 = “unbearably

cold," 4 = "neutral," 8 = "unbearably hot") and thirst (1-9; 1 = "not thirsty at all," 9 = "very, very thirsty").

After completion of the equilibration period, subjects began the dehydration (DEH) period of the study. Subjects mounted their own bicycles attached to a stationary trainer (Wahoo®) and were instructed to pedal at progressively decreasing workloads until they reached a ~2% loss of body mass. The first workload was fixed for a 30-minute period, with subsequent workloads fixed for shorter 15-minute periods. Workload was calculated relative to body mass, with men starting at 2.5 $W \cdot kg^{-1}$ and incrementally decreasing by 0.25 $W \cdot kg^{-1}$ each period, and women starting at 2.3 $W \cdot kg^{-1}$ and incrementally decreasing by 0.3 $W \cdot kg^{-1}$ each period. Body mass was determined at the end of each stage to assess progress toward the goal of a 2% decrease in body mass.

Subjects wore a Polar chest-strap to track heart rate throughout all cycling periods. A Wahoo® phone application connected to the Wahoo® stationary trainer provided instantaneous readings of power, cadence, and speed. Subjects indicated their rating of perceived exertion (RPE) on the Borg 6-20 scale every 15 minutes throughout DEH. Upon reaching the 2% loss of body mass threshold, subjects were again asked to rate their thermal and thirst perceptions. The environmental chamber temperature was then lowered to 35 °C while RH remained at 20% for the remainder of the study.

Following DEH, subjects completed one of four conditions: 1) no fluid (NF), or rehydration REH with 2) water (WAT), 3) a traditional carbohydrate-electrolyte beverage (Gatorade®; GAT), or 4) a novel ultra-filtered deproteinized milk (milk-permeate) beverage (GoodSport™; GS). In each trial, the amount of fluid consumed was 75% of lost body mass. This amount of fluid replacement was chosen for two reasons: 1) to reflect previously reported findings of dehydration in athletes

prior to competition and typically do not voluntarily consume enough fluid to prevent onset of dehydration during competition [130, 131], and 2) to prevent any potential gastric discomfort that may occur during the time trial following prior fluid replacement of 100% lost body mass. Descriptions of beverage composition and total amount of constituent ingestion for each trial are provided in **Table 4-2**. Fluid was consumed in 4 equal aliquots over a 30 min period. During the final aliquot in each of the three drinking conditions, subjects completed a sensory questionnaire for each beverage regarding beverage likability, taste, and thirst quenching properties. At the end of the 30-min REH period, measurements of blood pressure (BP), heart rate (HR), and thirst and thermal perception were obtained prior to collection of a venous blood sample. Body mass was again measured prior to collecting a urine sample.

Table 4-2: Beverage composition and total intake during hot-dry condition.

	Water		Gatorade®		GoodSport™	
	Per Liter	**Total Intake**	**Per Liter**	**Total Intake**	**Per Liter**	**Total Intake**
Energy (kcal)	0	0	222	313 ± 61	180	257 ± 44
Carbohydrates (g)	0	0	61	86 ± 17	64	90 ± 18
Total sugar (g)	0	0	58	82 ± 16	38	54 ± 10*
Sodium (mg)	0	0	444	627 ± 122	480	451 ± 88
Potassium (mg)	0	0	139	196 ± 38	1100	1571 ± 267*
Chloride (mg)	0	0	0	0 ± 0	980	1400 ± 238*
Calcium (mg)	0	0	0	0 ± 0	320	451 ± 88*
Magnesium (mg)	0	0	0	0 ± 0	60	85 ± 17*
Phosphorous (mg)	0	0	83	118 ± 23	350	494 ± 96*
Osmolality (mosm)	0		322 ± 2		578 ± 4	

Values for energy, carbohydrates, total sugar, and electrolytes are label values. Osmolality was measured in quadruplicate using a freezing-point osmometer (Model 3320, Advanced Instruments, Inc., Norwood, MA, USA). Total intake is displayed as mean ± SD and was calculated from the total volume of fluid consumed over the entire study.
*$p < 0.05$ GoodSport vs. Gatorade.

Subjects were then asked to again mount their own bicycles attached to the stationary trainer. HR, core temperature (T_c), and power output (W) were recorded every 5 minutes, and RPE every 15 minutes during a 30-min warm-up period at an intensity of 1.5 $W \cdot kg^{-1}$. In each of the three fluid trials, a 250-mL bolus of the given fluid was provided halfway through the warm-up period and subjects were instructed to finish consuming this bolus within 10 min. The warm-up was immediately followed by a time-trial (TT) in which subjects completed 120 kJ of work as quickly as possible. Subjects were provided verbal feedback every 30 kJ throughout the time-trial. HR, T_c, and W were recorded throughout. Upon completion of the TT, ratings of thirst, thermal

sensation, and perceived exertion were obtained. Subjects then performed a 5-minute cool-down at a self-selected intensity prior to a measure of body mass. To test the repeatability of the time-trial performance, 6 repeat trials were conducted over the course of the study (NF = 2, WAT = 1, GAT = 1, GS = 2).

To further examine the impact of carbohydrate supplementation on performance during a slightly longer TT in a thermoneutral environment, a subset of 4 subjects (3M, 1W) returned to the laboratory for a repeat trials of the WAT, GAT, and GS beverage conditions. The DEH, REH, and warm-up protocols were identical to the original trials; however, the TT was performed at 21 °C, 20% RH. The time-trial performance goal was increased from 120 kJ to 250 kJ. Blood and urine samples were not collected during these thermoneutral trials.

Urine and Serum Sample Analysis

Urine samples were collected following BASE, DEH, REH, and TT in the hot-dry conditions and at BASE in the thermoneutral conditions to assess baseline hydration status. Urine mass was determined using an electronic balance accurate to the nearest 0.1 g with the mass of the empty container subtracted from the total weighed value. USG was measured at each time point using a refractometer. Urine sodium and potassium concentrations and urine osmolality were measured at each time point in triplicate (Smartlyte, Diamond Diagnostics, Massachusetts, United States and freezing-point osmometer (Model 3320, Advanced Instruments, Inc., Massachusetts, United States, respectively).

Blood samples were collected in the hot-dry conditions following BASE, DEH, REH, and TT. These samples were collected in serum separator tubes and left in an upright position for 30 min

to allow serum clotting to occur. Serum separator tubes were then centrifuged (10 min, 4 °C, 4000 rpm) and serum separated. Serum osmolality was measured immediately after centrifugation at each time point in triplicate using a freezing-point osmometer (Model 3320, Advanced Instruments, Inc., Massachusetts, United States). Hemoglobin and concentrations of hematocrit, glucose, insulin, sodium, potassium, and chloride were analyzed for each time point (Quest Diagnostics) within two days of sample collection.

Calculations and Statistical Analysis

Percent change in plasma volume was calculated from hematocrit and hemoglobin concentration using the method of Dill and Costill [98]. Data collected on the Wahoo® application were downloaded and analyzed in Microsoft Excel. A two-way repeated measures ANOVA was performed to examine the effect of beverage and time on urine and serum samples. A one-way ANOVA was used to assess the effect of beverage on time-trial performance. All statistical analyses were conducted using a Tukey's HSD test for post-hoc pairwise comparisons. Hedge's G effect sizes were calculated and reported for select primary outcomes when comparisons were statistically different (small effect = 0.2, medium effect = 0.5, large effect = 0.8). Based on previous data demonstrating an effect size of 4.86 regarding average power output during 5-km time trial performance in either a euhydrated or hypohydrated condition [132], assuming $\alpha < 0.05$, and power = 0.8, it was determined that a sample size of 8 subjects (G*Power) is sufficient to detect meaningful differences in power output during the time trial between different beverage trials. Data were analyzed in SAS (Cary, North Carolina, United States) using PROC MIXED model. With the exception of box plots, all data are presented as mean ± SD. Statistical significance was set a priori at $\alpha < 0.05$.

RESULTS

Hot Dry Condition

Baseline body mass, cardiovascular measures, glucose and insulin, and serum and urine markers of hydration status are shown in **Table 4-3**. There were no significant differences in any baseline measure across trials (all $p > 0.05$).

Table 4-3: Baseline characteristics by trial.

	No Fluid	**Water**	**Gatorade®**	**GoodSport™**
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Weight (kg)	77.3 ± 13.9	77.2 ± 14.0	76.8 ± 14.1	77.5 ± 14.0
Heart rate (bpm)	69 ± 16	69 ± 11	70 ± 14	68 ± 16
Mean arterial pressure (mmHg)	90 ± 8	90 ± 8	90 ± 11	90 ± 10
Glucose ($mg \cdot dL^{-1}$)	96 ± 13	89 ± 9	94 ± 9	93 ± 16
Insulin ($mg \cdot dL^{-1}$)	9.9 ± 11.3	7.5 ± 5.5	10.5 ± 8.5	11.2 ± 10.1
S_{osm} ($mOsm \cdot kg^{-1}$)	298 ± 3	297 ± 3	298 ± 3	298 ± 2
Serum Na^+ ($mmol \cdot L^{-1}$)	139 ± 1	139 ± 1	139 ± 1	139 ± 1
U_{osm} ($mOsm \cdot kg^{-1}$)	298 ± 124	310 ± 226	316 ± 226	320 ± 247
Urine Na^+ ($mmol \cdot L^{-1}$)	67 ± 43	62 ± 56	41 ± 37	38 ± 28
Urine Specific Gravity	1.009 ± 0.004	1.010 ± 0.006	1.010 ± 0.006	1.010 ± 0.006

Values are displayed as mean ± SD. S_{osm}, serum osmolality; U_{osm}, urine osmolality; Na+, sodium; there were no significant differences in any baseline measurements among trials.

Body Mass

Percent change in body mass for the hot-dry experiment is presented in **Figure 2A**. As expected, body mass was similarly reduced from BASE to DEH across all four trials in the hot-dry condition (NF: -2.0 ± 0.1%; WAT: -2.0 ± 0.2%; GAT: -2.0 ± 0.2%; GS: -2.0 ± 0.1%). DEH time was not different among trials (NF: 60 ± 9 min; WAT: 63 ± 11 min; GAT: 60 ± 10 min; GS: 61 ± 9 min; all $p > 0.34$). Body mass remained lower for the remainder of each trial (all $p < 0.0001$) relative to BASE. Body mass was lower in the NF trial compared to the three drinking conditions after REH and TT ($p < 0.0001$).

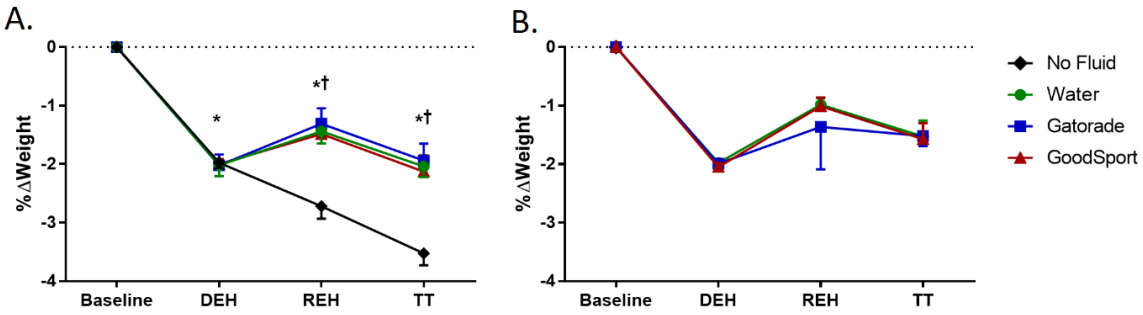

Figure 4-2: Percent change in body mass during (A) hot dry experiments and (B) thermoneutral experiments. Body mass was lower in all four trials at DEH compared to BASE in the hot-dry condition, and this persisted for the remainder of the study. Body mass was lower in NF compared to the other three conditions at REH and TT. *$p < 0.05$ compared to BASE. †$p < 0.05$ compared to no fluid.

Core Temperature (T_c)

T_c was not different among conditions prior to entering the environmental chamber and did not differ among trials at any subsequent time point. T_c was significantly elevated following DEH and TT compared to BASE in all four conditions (all $p < 0.0001$). Following REH, T_c was not different compared to BASE in any of the four trials.

Urine Samples

There were no differences in cumulative urine output (**Table 4-4**) among the four conditions at any time point. There was a main effect of beverage ($p < 0.04$) and time ($p = 0.006$), but no interaction effect (beverage x time: $p = 0.91$) on cumulative urine output. Urine osmolality, sodium, and potassium are provided in **Table 4-5**. For urine osmolality, there was a main effect of time ($p < 0.0001$), but no effect of beverage ($p = 0.67$) or interaction effect (beverage x time: $p = 0.93$). Urine osmolality was significantly elevated compared to BASE in all four trials at both REH and TT (all $p < 0.0001$). There were no significant differences in urine osmolality among the four drink conditions at any time point. There was a main effect of beverage ($p = 0.015$) and time ($p = 0.004$), but no interaction effect ($p = 0.83$) on urine sodium; however, after adjusting for multiple comparisons, there were no differences in urine sodium among time points or beverages. There was a main effect of beverage, time, and beverage x time on urine sodium (all $p < 0.0001$). Urine potassium was higher compared to BASE in all four drink conditions at REH (all $p < 0.02$) and in NF, WAT, and GAT at TT (all $p < 0.0001$). Urine potassium was lower in GS compared to NF and WAT at both REH and TT ($p < 0.02$) and compared to GAT at TT ($p = 0.039$).

Table 4-4: Cumulative urine output and change in plasma volume.

		Base	DEH	REH	TT
Cumulative Urine Output (g)	**No Fluid**	-	150 ± 66	184 ± 65	197 ± 60
	Water	-	124 ± 71	154 ± 87	170 ± 87
	Gatorade®	-	135 ± 91	151 ± 88	167 ± 91
	GoodSport™	-	149 ± 126	202 ± 128	246 ± 129
Δ Plasma Volume (%)	**No Fluid**	0 ± 0	-9.0 ± 3.4*****	-4.8 ± 2.9	-12.8 ± 3.3*****
	Water	0 ± 0	-8.2 ± 4.4*****	1.6 ± 2.2†#	-7.2 ± 3.2*****†

Gatorade®	0 ± 0	-9.1 ± 5.0*****	0.0 ± 4.3#	-9.5 ± 5.5*****
GoodSport™	0 ± 0	-9.3 ± 2.9*****	-3.2 ± 2.7#	-7.6 ± 3.4*****†

Values are displayed as mean ± SD. Differences among beverages were assessed by two-way ANOVA. There were no differences in cumulative urine output at any time-point among trials. Plasma volume was not different among trials through REH. At TT, plasma volume was lower in NF compared to water and GoodSport. *p < 0.05 vs. BASE; †p < 0.05 vs. no fluid; #p < 0.05 vs. DEH.

Table 4-5: Serum and urine electrolyte concentrations and osmolality.

		Beverage	Base	DEH	REH	TT
Serum	**Sodium**	No Fluid	139 ± 1	141 ± 2	141 ± 2*	142 ± 2*
		Water	139 ± 1	141 ± 1*	137 ± 1†	138 ± 2†
		Gatorade®	139 ± 0	141 ± 1*	138 ± 1†	140 ± 1†
		GoodSport™	139 ± 1	141 ± 1*	138 ± 1†	139 ± 2†
	Potassium	No Fluid	4.2 ± 0.1	4.3 ± 0.2	4.3 ± 0.2	4.2 ± 0.2
		Water	4.3 ± 0.2	4.4 ± 0.2	4.5 ± 0.2	4.1 ± 0.2
		Gatorade®	4.2 ± 0.3	4.4 ± 0.3	4.1 ± 0.2‡	4.0 ± 0.2
		GoodSport™	4.1 ± 0.2	4.2 ± 0.2	4.8 ± 0.4*†‡§	4.4 ± 0.2*‡§
	Osmolality	No Fluid	298 ± 3	305 ± 5*	302 ± 4	310 ± 6*
		Water	297 ± 3	305 ± 3*	295 ± 4†	301 ± 5†
		Gatorade®	298 ± 2	306 ± 2*	302 ± 2‡	303 ± 2†
		GoodSport™	298 ± 2	305 ± 2*	305 ± 3*‡	308 ± 5*‡
	Sodium	No Fluid	67 ± 43	36 ± 30	65 ± 40	43 ± 32

		Water	62 ± 56	60 ± 39	85 ± 50	44 ± 36
		Gatorade®	41 ± 37	39 ± 35	63 ± 29	48 ± 30
		GoodSport™	38 ± 28	35 ± 40	53 ± 32	23 ± 9
Urine	**Potassium**	No Fluid	29.4 ± 20.2	26.3 ± 14.3	107.5 ± 35.6*	109.9 ± 31.1*
		Water	36.2 ± 34.3	43.6 ± 33.2	113.4 ± 32.6*	102.6 ± 27.5*
		Gatorade®	26.1 ± 25.4	28.1 ± 22.3	99.8 ± 30.5*	86.6 ± 39.3*
		GoodSport™	33.3 ± 41.1	36.2 ± 32.3	70.0 ± 25.1*†‡	52.4 ± 11.1†‡§
	Osmolality	No Fluid	298 ± 124	231 ± 94	655 ± 169*	768 ± 136*
		Water	310 ± 226	279 ± 147	689 ± 129*	754 ± 138*
		Gatorade®	316 ± 226	254 ± 189	709 ± 85*	793 ± 139*
		GoodSport™	320 ± 247	300 ± 229	681 ± 142*	733 ± 112*

Values are displayed as mean ± SD. Differences among beverages were assessed by two-way ANOVA. *p < 0.05 vs. BASE; †p < 0.05 vs. no fluid; ‡p < 0.05 vs. water; §p < 0.05 vs. Gatorade.

Blood Samples

Percent change in plasma volume (Δ%PV) is also shown in **Table 4-4**. There was a main effect of beverage ($p < 0.001$) and time ($p < 0.001$), as well as an interaction effect of beverage x time ($p = 0.006$). Δ%PV was significantly lower in all four trials after DEH (all $p < 0.0001$). There were no differences in Δ%PV at DEH between drink conditions and Δ%PV was not different after REH compared to BASE in any drink condition. At REH, restoration of PV was significantly greater in WAT compared to NF ($p < 0.002$; $g = 2.38$), but did not differ among WAT, GAT, or GS. There was a significant expansion in PV after fluid consumption in each drink condition, (all $p < 0.004$) but not in the NF condition ($p = 0.209$). At TT, Δ%PV was significantly lower compared to

BASE in all four trials ($p < 0.0002$). The reduction in Δ%PV was less in WAT ($p = 0.013$; $g = 1.65$) and GS ($p = 0.033$; $g = 1.49$) compared to NF.

Figure 4-3A and 4-3B display changes in glucose and insulin concentrations over time in each of the four conditions. For both glucose and insulin, there was a main effect of beverage ($p < 0.001$) and time ($p < 0.001$), as well as an interaction effect of beverage x time ($p < 0.001$). There were no differences in plasma glucose or insulin concentration among conditions at BASE or after DEH. Following REH, there was a significant increase in glucose concentration in the GAT and GS trials compared to BASE (both $p < 0.0001$), as well as compared to NF and WAT (both $p < 0.0001$). The elevation in glucose concentration in GAT was greater than in GS ($p < 0.0001$). Insulin concentration at REH was elevated in GAT compared to BASE ($p < 0.0001$). Insulin concentrations were higher at REH in GAT and GS compared to both NF and WAT ($p < 0.002$), although the increase was blunted in GS compared to in GAT ($p < 0.001$).

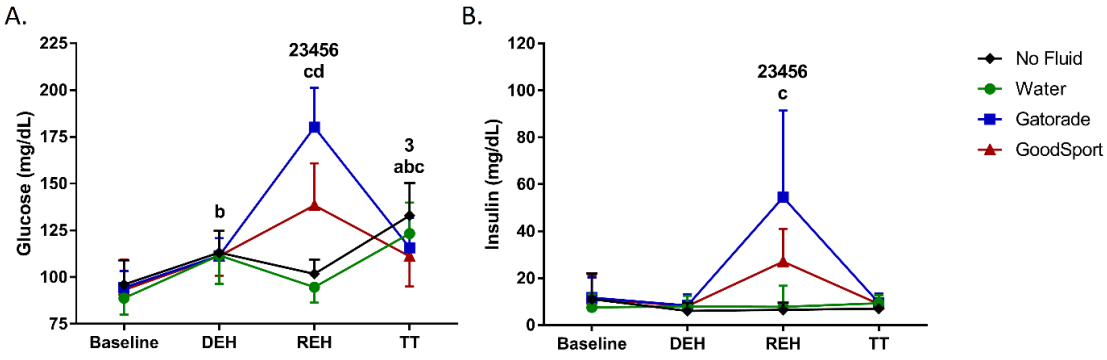

Figure 4-3: (A) Glucose, and (B) insulin concentrations. Glucose and insulin concentrations were significantly higher in Gatorade (GAT) and GoodSport (GS) at REH compared to BASE and compared to no fluid and water. The increase in glucose and insulin concentrations in GAT was higher than in GS. 1, $p < 0.05$ WAT vs. NF; 2, $p < 0.05$ GAT vs. NF; 3, $p < 0.05$ GS vs. NF; 4, $p < 0.05$ GAT vs. WAT; 5, $p < 0.05$ GS vs. WAT; 6, $p < 0.05$ GS vs. GAT; a, $p < 0.05$ NF vs. BASE; b, $p < 0.05$ WAT vs. BASE; c, $p < 0.05$ GAT vs. BASE; d, $p < 0.05$ GS vs. BASE.

Serum osmolality, sodium, and potassium are shown in **Table 4-5**. There were no significant differences in serum osmolality, sodium, or potassium among conditions at BASE and after DEH. There were main effects of beverage (all $p < 0.0001$) and time (all $p < 0.0001$), as well as interaction effects of beverage x time (all $p < 0.0001$), for serum osmolality, sodium, and potassium. Serum osmolality was significantly elevated after DEH compared to BASE in all trials and remained elevated after REH compared to BASE for only GS ($p = 0.002$). Serum osmolality at REH was lower in WAT vs NF ($p < 0.001$) and higher in both GAT and GS compared to WAT (both $p < 0.001$). After the TT, serum osmolality was significantly higher in NF and GS compared to BASE (both $p < 0.0001$). Serum osmolality at TT was significantly lower in WAT compared to both NF and GS (both $p < 0.001$) and lower in GAT compared to NF ($p = 0.008$). Serum sodium was significantly elevated in NF at REH and TT compared to BASE (both $p < 0.03$), but only at DEH in WAT, GAT, and GS (all $p < 0.03$). Serum sodium was lower in WAT, GAT, and GS compared to NF at both REH and TT (all $p < 0.001$). Serum potassium was significantly elevated in GS compared to BASE after REH and TT (both $p < 0.03$). Serum potassium was lower in GAT compared to WAT at REH ($p = 0.03$) and higher in GS compared to the three other conditions after REH (all $p < 0.006$) and compared to WAT and GAT at TT (both $p < 0.04$). Estimated glomerular filtration rate (eGFR) was significantly reduced in all four conditions beginning at DEH and remained lower for the remainder of the study (all $p < 0.0001$). There were no differences in eGFR among beverage conditions.

Perceptual Measures

Rating of Perceived Exertion did not differ between any condition during DEH (NF = 16 ± 3; WAT = 17 ± 2; GAT = 15 ± 3; GS = 16 ± 2) or TT (NF = 19 ± 1; WAT = 19 ± 2; GAT = 19 ± 2; GS = 19 ± 2). Thermal and thirst sensation scores (**Table 4-6**) did not differ between drink conditions at any time point. Thirst sensation was higher for NF compared to the three drinking

conditions at REH (all p <0.0001), and higher compared to both WAT and GAT after the TT (both p <0.01), but not compared to GS (p =0.13). There were also no significant differences among the three drink conditions in beverage sensory questions of overall beverage likability (all p <0.79), taste (p >0.46), thirst quenching (p >0.49), or drinkability (p >0.07). No subjects reported feeling any gastric discomfort following consumption of any of the beverages.

Table 4-6: Thermal and thirst sensation ratings.

		BASE	DEH	REH	TT
Thermal	**No Fluid**	5 ± 0	7 ± 1*	4 ± 0*	6 ± 1*
	Water	5 ± 0	7 ± 1*	4 ± 1*	6 ± 1*
	Gatorade®	5 ± 0	7 ± 1*	5 ± 1*	6 ± 1*
	GoodSport™	5 ± 0	6 ± 1*	4 ± 0*	6 ± 1*
Thirst	**No Fluid**	3 ± 1	7 ± 2*	6 ± 1*	9 ± 0*
	Water	3 ± 1	8 ± 1*	3 ± 1†	7 ± 2*†
	Gatorade®	3 ± 1	8 ± 1*	3 ± 2†	7 ± 2*†
	GoodSport™	3 ± 2	8 ± 1*	3 ± 1†	8 ± 1*

Values are displayed as mean ± SD. Thermal sensation was based on a 0-8 scale, where 0 = "unbearably cold," 4 = "neutral," 8 = "unbearably hot." Thirst sensation was based on a 1-9 scale, where 1 = "not thirsty at all" and 9 = "very, very thirsty." Differences among beverages were assessed by two-way ANOVA. There were no differences in thermal sensation between beverages at any time-point. Thermal sensation was higher (hotter) after DEH and TT compared to BASE, and lower (cooler) after REH compared to BASE in all beverages. Thirst sensation was higher after DEH and TT compared to BASE in all beverages, and higher in no fluid (NF) after REH. Thirst sensation was lower in Water and Gatorade compared to NF after both REH and TT, but only lower in GoodSport after REH. *p < 0.05 vs. BASE. †p < 0.05 vs. no fluid.

Time Trial Performance

Time-trial performance data are displayed in box-plot format in **Figure 4-4**. There was a main effect of beverage on time trial time-to-completion ($p = 0.002$), absolute power output ($p = 0.0002$), and relative power output ($p = 0.0001$). Time-trial time-to-completion (sec) was significantly lower (faster) in WAT (535 ± 214 sec, $p < 0.01$, $g = 0.35$), GAT (539 ± 226 sec, $p < 0.01$, $g = 0.32$), and GS (534 ± 238 sec, $p < 0.01$, $g = 0.34$) compared to NF (631 ± 310 sec). Absolute power (W) was significantly higher in WAT (259 ± 99 W, $p < 0.01$, $g = 0.29$), GAT (260 ± 110 W, $p < 0.01$, $g = 0.29$), and GS (262 ± 105 W, $p < 0.001$, $g = 0.31$) compared to NF (229 ± 96 W). Relative power ($W \cdot kg^{-1}$) was significantly higher in WAT (3.3 ± 0.9 $W \cdot kg^{-1}$, $p < 0.01$, $g = 0.32$), GAT (3.3 ± 1.0 $W \cdot kg^{-1}$, $p < 0.001$, $g = 0.30$), and GS (3.4 ± 1.0 $W \cdot kg^{-1}$, $p < 0.001$, $g = 0.40$) compared to NF (3.0 ± 0.9 $W \cdot kg^{-1}$). There were no significant differences in time-to-completion, absolute power output, and power output relative to body mass for the time-trial among the three beverage conditions (all $p > 0.96$).

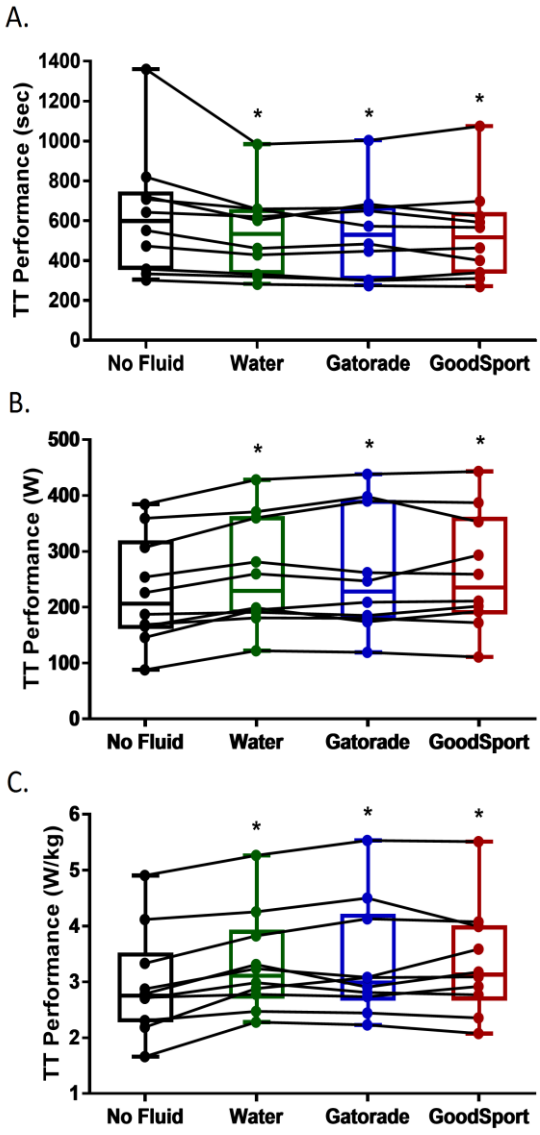

Figure 4-4: 120 kJ time-trial performance in hot dry condition, measured as (**A**) time (s), (**B**) absolute power (W), and (**C**) relative power (W/kg). All three measures of time-trial performance were improved in the three beverage trials compared to no fluid. Boxes represent first and third quartiles with median values denoted by the horizontal line, while whiskers indicate minimum and maximum observations. Dots represent data points for each subject, and lines indicate changes in performance between trials for each subject. *$p < 0.05$ vs. no fluid.

To test the repeatability of the time-trial performance, 6 repeat trials were conducted among four subjects over the course of the study as described previously and an interclass correlation

coefficient (ICC) was calculated. The ICC values for time-trial performance were 0.962 for time, 0.983 for absolute power output, and 0.953 for relative power output, indicating excellent reliability/repeatability for TT performance.

Thermoneutral Time Trials

As only four subjects completed this portion of the study, no statistical comparisons were performed. Percent change in body mass is displayed in **Figure 4-2B**. As in the hot trials, body mass was similarly reduced in each of the WAT (-2.0 ± 0.0%), GAT (-2.0 ± 0.1%), and GS (-2.0 ± 0.1%) trials following DEH and remained lower for the remainder of the study in each trial. DEH time was not different among trials (WAT: 57 ± 13 min; GAT: 54 ± 8 min; GS: 55 ± 9 min; all $p > 0.59$).

Fluid comparison differences for the 250 kJ time-trial performance in thermoneutral conditions are shown for each individual in **Figure 4-5**. Time-trial performance time (WAT: 960 ± 376 sec; GAT: 946 ± 365 sec; GS: 919 ± 353 sec), absolute power (WAT: 283 ± 91 W; GAT: 293 ± 103 W; GS: 300 ± 100 W), and relative power (WAT: 3.43 ± 0.83 $W \cdot kg^{-1}$; GAT: 3.60 ± 0.97 $W \cdot kg^{-1}$; GS: 3.61 ± 0.86 $W \cdot kg^{-1}$) were improved in both the GS and GAT trials compared to WAT. All 4 subjects improved in each measure of time-trial performance in the GS trial compared to WAT, while 3 subjects improved in each measure of time-trial performance in the GAT trial compared to WAT.

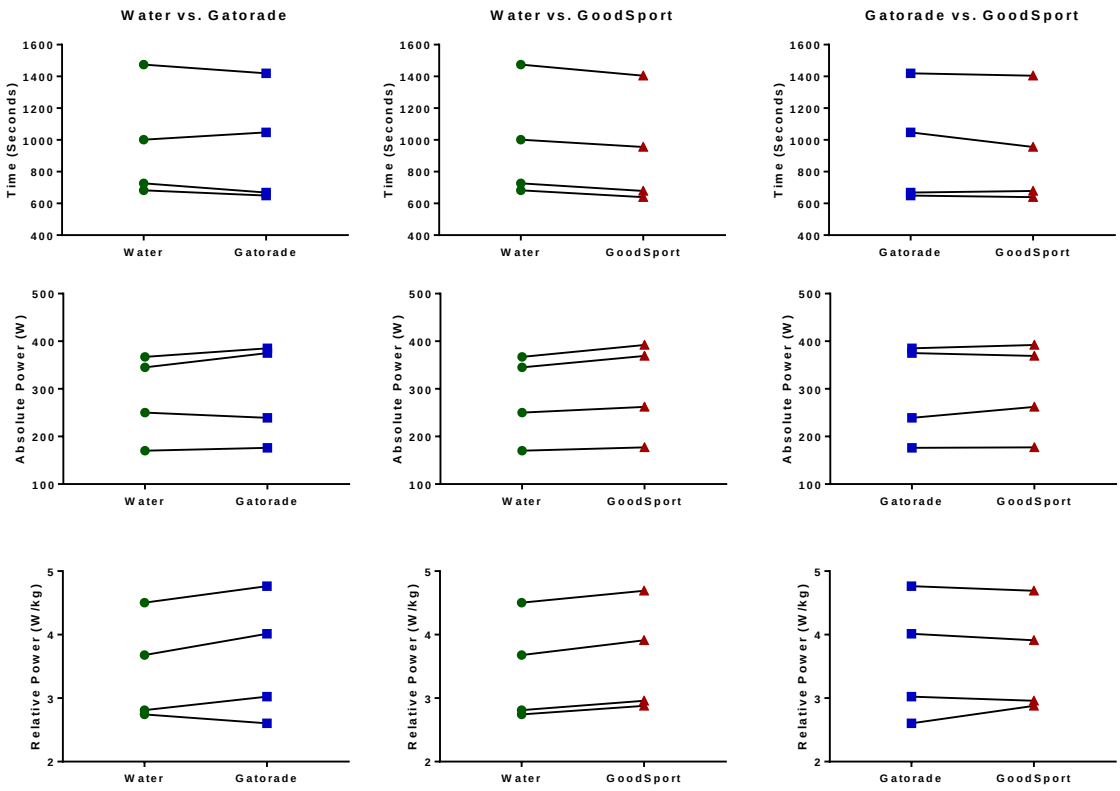

Figure 4-5: Between-beverage comparisons of 250 kJ time-trial performance in thermoneutral conditions, measured as time (s), absolute power (W), and relative power (W/kg). All three measures of time-trial performance were improved on average in both Gatorade (GAT) and GoodSport (GS) compared to water (WAT). All four subjects improved in all measures of time-trial performance in GS compared to WAT, and three of four subjects improved in GAT compared to WAT. Symbols represent data points for each subject, and lines indicate changes in performance between trials for each subject.

DISCUSSION

The present investigation examined how hydration status, environmental conditions, and carbohydrate availability interacted to influence performance during a push-to-the-finish stationary cycling time-trial. The primary finding of this study was that in a hot, dry environment, TT time-to-completion, as well as both absolute and relative power output, were improved

following rehydration compared to no fluid (NF) consumption, but were not different among drinking conditions when consuming water, a traditional carbohydrate electrolyte solution (Gatorade®; GAT), or a novel sports drink containing ultra-filtered deproteinized milk (GoodSport™; GS). However, in a subset of four subjects who completed a slightly longer TT in a thermoneutral environment, performance was improved in all subjects when comparing GS to WAT and in three of four subjects when comparing GAT to WAT. These findings collectively indicate that hydration status has a greater influence on performance during relatively short-duration push-to-the-finish cycling exercise in the heat. In contrast, the importance of carbohydrate supplementation appears to increase with longer TT efforts and/or in thermoneutral environments. To our knowledge, this study is the first to examine how push-to-the-finish cycling performance is affected by combinations of environmental conditions and rehydration with different beverages.

Physical performance is impaired in the heat compared to cooler conditions, attributable to greater cardiovascular strain caused by high skin and core temperatures [133]. Further, exercise in the heat elicits a robust sweating response which, without proper fluid replacement, leads to dehydration. Dehydration ≥2% loss of body mass has been associated with acute deficits in physical performance [133]. Although ingestion of water may partially restore lost fluid, water alone is often not enough for achieving euhydration following exercise- and/or heat-induced dehydration. Ingestion of water alone reduces plasma osmolality, blunting the thirst response, subsequent fluid intake, and fluid retention, and thereby prolonging the time to restore plasma volume compared to beverages with added salt [70]. Fluids with added components, especially salt or carbohydrates, can promote restoration of electrolytes and energy stores. These are often important constituents in the development of beverages for the purpose of promoting rehydration, such as sports drinks.

The components of a given beverage, namely fluid volume, energy content, and osmolality, can impact gastric emptying and absorption in the small intestine. In addition, the mineral constituents of these beverages are also metabolized on different time scales [134]. Thus, beverages with different compositions are likely to have varying effects on promoting hydration. This has led to increased interest in recent years regarding beverages, especially those that occur naturally, that may promote rehydration to a similar or greater extent than common sports drinks. Dairy-based beverages have a high electrolyte concentration [133] and similar carbohydrate and caloric concentration to traditional CES beverages [6] and thus may serve as efficacious alternative fluid sources. Using the Beverage Hydration Index (BHI), Maughan et al. [133] observed a similar hydration capacity of both skim milk and full-fat milk compared to an oral rehydration solution. Our laboratory more recently showed a novel beverage containing protein- and fat-free milk permeate (MPS), GoodSport™, had a higher BHI than both water and a traditional carbohydrate-electrolyte drink (Gatorade®) in well-hydrated young subjects at rest [133]. These findings were attributed to the greater electrolyte and osmolar constituents present in GS compared to the other beverages. However, it is important to note that those subjects were euhydrated at the onset of the study and sat at rest in thermoneutral conditions for the entirety of the study. It was previously unclear how these findings would translate to stressed conditions, such as following exercise in the heat, where dehydration is likely to occur. Based on the findings from the BHI study conducted by our lab, we hypothesized that consumption of the milk permeate beverage would promote rehydration to a greater extent than water or Gatorade following heat- and exercise-induced dehydration. However, in the current study, there were no differences in the amount of plasma volume expansion that occurred in all three drinking trials immediately following fluid consumption. There were also no differences in urine volume at any time point among the three drinking conditions, including after fluid consumption, indicating that

fluid retention was similar across conditions. This suggests that in a hot environment following heat- and exercise-induced dehydration, fluid consumption either with or without added constituents similarly rehydrate young well-trained cyclists.

Previous literature indicates that during shorter bouts of maximal effort cycling in the heat, similar to that which may occur in the final kilometers of a cycling race, maintenance of hydration may be more important than carbohydrate ingestion. Adams et al. [133] reported that during a maximum-effort 5-km cycling TT in the heat (35 °C, 30% RH), fluid consumption following a 2-h dehydration period significantly improved average power output (295 W) compared to a trial in which no fluid replacement occurred (276 W), though fluid replacement occurred during exercise in this study rather than rehydration at rest prior to TT performance. Comparatively, Fan et al. [133] observed no significant differences among trials in 20-km cycling TT performance in the heat (30 °C, 75% RH) following exercise- and heat-induced dehydration following rehydration with water (39 min) or two carbohydrate-based beverages (sports drink: 38 min; oral rehydration solution: 38 min). These findings are similar to those of the current study in that rehydration alone improved maximum-effort push-to-the-finish cycling performance compared to a trial in which no fluid was consumed. In the current study, subjects were instructed to complete a 120-kJ TT, mimicking a push-to-the-finish race condition, in a hot-dry (35 °C, 20% RH) environment. TT performance, measured as time-to-completion, absolute power output, and power output relative to body mass, was improved in each of the three trials in which fluid ingestion occurred compared to the no fluid condition (**Figure 4**), regardless of the type of fluid consumed.

A subset of four subjects repeated the three drinking conditions (WAT, GAT, and GS) to determine whether between-beverage differences in performance, attributable to carbohydrate

load, were evident in a slightly longer TT occurring in thermoneutral conditions compared to the shorter TT in the heat. Interestingly, in a slightly prolonged TT (250 kJ) in thermoneutral conditions (21°C, 20% RH), average time-to-completion in GS (919 ± 353 sec) was 41 sec faster than WAT (960 ± 376 sec) and 27 sec faster than GAT (946 ± 365 sec) (**Figure 5**). All four subjects in this prolonged TT in thermoneutral conditions improved their performance in the GS trial compared to WAT, while 3 of 4 subjects improved in the GAT trial compared to WAT. These findings indicate that different combinations of environmental conditions and time trial length may affect the influence and relative importance of carbohydrate supplementation versus rehydration on cycling TT performance.

In the BHI study conducted by our lab [133], the elevated GS BHI was accompanied by a reduced free water clearance compared to both water and CES in the two hours following fluid consumption, indicating that the kidneys were conserving more fluid following GS consumption relative to the other beverages. However, BHI studies are performed under standardized thermoneutral conditions with subjects at rest, i.e., renal function is unchallenged by the rigors of exercise and heat stress. During exercise in the heat, renal blood flow can be attenuated by as much as 50-60% and redistributed to skeletal muscle and skin for energetic and thermoregulatory purposes, respectively, thus reducing glomerular filtration rate and renal handling of ingested fluids [133]. It was previously unknown how water, GS, or GAT may differentially impact hydration status and subsequent push-to-the-finish exercise performance in either a thermoneutral environment or hot-dry environment, in which renal blood flow, GFR, and subsequent fluid filtration and retention would be challenged. Although kidney function was not directly measured in the present study, we observed similar progressive reductions in estimated glomerular filtration rate, a proxy measurement for kidney function, across all time-points in each drink condition in the hot-dry environment. It is plausible that the reduction in renal blood flow and subsequent

attenuation of eGFR observed in the hot condition utilized in the present study offset the beneficial effects of beverages containing carbohydrates and electrolytes, while such beneficial effects may be preserved in thermoneutral conditions. Thus, it is likely that during a shorter time-trial in hot-dry conditions, maintenance of hydration is more important for push-to-the-finish cycling performance than carbohydrate supplementation, whereas carbohydrate supplementation exerts a greater influence in thermoneutral conditions and/or during push-to-the-finish tasks. Future research should examine how performance is impacted across a wider range of combinations of environments and time-trial lengths.

Limitations

Although all subjects were similarly dehydrated (~2%) following the first bout of cycling and remained similarly dehydrated in the three beverage consumption conditions at each time-point for the remainder of the study, it is possible the subjects were dehydrated to a greater extent than actually observed throughout the study. Body water volume and serum osmolality are tightly regulated such that osmolality is maintained within a range of 275-295 mosm·kg^{-1} under euhydrated conditions. Reductions in body water content without proper replacement can stimulate increases in serum osmolality beyond the upper limit of euhydration of 295 mosm·kg^{-1}. Interestingly, subjects in the current study on average had a baseline plasma osmolality of 297-298 mosm·kg^{-1}, indicating subjects were dehydrated, despite having normal serum electrolyte concentrations and urine specific gravity (1.009-1.010) values on average. In a previous study in our lab examining the BHI of a novel milk-permeate solution similar to the one utilized in the current study, baseline serum osmolality was 291 mosm·kg^{-1}, within the normal range of euhydration [133]. In both studies, subjects were instructed to maintain their normal hydration habits in the 24 hours prior to each trial, and were given a 500 mL bottle of water to consume 1 hour before each trial to ensure euhydration. An overnight fast was also completed prior to each

trial in both studies. However, in the current study, subjects were provided a carbohydrate energy bar to consume along with 500 mL water. This resulted in an increased serum glucose concentration in the current study compared to the previous study, which may in part help explain the further elevation in serum osmolality in the current study compared to the prior BHI study.

Following REH, plasma glucose concentrations were significantly elevated in the GS trial compared to WAT and NF, and in the GAT trial compared to all other conditions. Comparatively, there were no differences in plasma glucose concentrations among the three trials in which a beverage was consumed. Considering the relatively high variance in time trial time-to-completion among subjects, it is possible that the time-course of changes in plasma glucose concentrations may have been different between individual subjects. However, given that the time between collection of the post-REH and post-TT blood samples was <1 hr for all subjects in all trials, it is unlikely that any subject in the hot-dry conditions was sufficiently glucose/glycogen depleted such that the time-course of these glucose responses would differently impact performance among subjects. Further, during short bouts of exercise at a high-intensity (>85% VO2max), oxidation of stored muscle glycogen contributes a large majority (~67%) of the necessary substrate for energy production [135]. Plasma glucose supplies only a small contribution during short bouts of exercise at intensities >85% VO_{2max} when stored muscle glycogen is abundant, but can supply upwards of 40% of energy once stored muscle glycogen is sufficiently depleted [135]. However, it is unlikely that subjects were glycogen depleted prior to the TT. Subjects cycled at a moderate intensity for ~60 min during the dehydration period, and an additional 30 min during the warm-up period at a light intensity. At moderate intensities in endurance-trained subjects, glycogen depletion usually occurs approximately anywhere from 1-3 hours after onset of exercise [135]. It is possible that in the current study, subjects were not glycogen depleted, and plasma glucose derived from ingestion of the two beverages containing carbohydrates (GAT: 82 ± 16 g

sugar consumed; GS: 54 ± 10 g sugar consumed) may have served a minimal role in substrate utilization during the TT. Given that the three drink conditions in the hot dry condition all improved TT performance, but were not different from each other, it is conceivable that hydration status, rather than carbohydrate provision, exerts a greater influence on performance during short bouts of high-intensity exercise in the heat. In contrast, in the thermoneutral condition, it is possible that the slightly extended TT resulted in further muscle glycogen depletion and a shift toward a greater reliance on plasma glucose concentrations for energy production. Thus, carbohydrate supplementation, rather than hydration status, may play a larger influential role on athletic performance during slightly longer push-to-the-finish exercise in thermoneutral conditions.

Subjects remained clothed during each measurement of body mass. While the clothing may have retained some sweat during the cycling periods, limiting the reduction in measured body mass, the dry environmental conditions mitigated retention. Had we assessed nude body mass, subjects would have had to undress for every measurement, extending time spent off the bicycle during cycling sessions and adding to subject burden. Similar methodology has been used in dehydration studies involving cycling [133], and given the cross-over nature of the experimental design, it is unlikely that this limitation had differential effects on specific drink condition trials.

In the current study, rehydration occurred over a 30-min period immediately following an exercise- and heat-induced dehydration of ~2% loss of body mass. However, under normal circumstances, it is likely that thirst sensation would be stimulated before reaching such a degree of dehydration [136]. Indeed, thirst sensation (measured as a 1-9 scale, 1 = “not thirsty at all,” 9 = “very, very thirsty”) was similarly elevated in each of the four trials compared to baseline (NF = 7 ± 2, WAT = 8 ± 1, GAT = 8 ± 1, GS = 8 ± 1), indicating that subjects on average were “very

thirsty" following dehydration. Planned drinking strategies or *ad libitum* fluid consumption are often implemented during road cycling sessions. Kenefick stated that planned drinking is optimal during endurance exercise lasting >90 min, particularly when it is occurring in the heat, while drinking to thirst or *ad libitum* drinking may be more optimal during short duration exercise lasting <60-90 min [137]. Future investigation is warranted regarding how planned and/or *ad libitum* drinking of a beverage containing ultra-filtered deproteinized milk vs. a traditional carbohydrate-based beverage may differentially promote rehydration or maintenance of euhydration during exercise, rather than after exercise has ceased and subjects are already dehydrated, as well as how this may subsequently impact athletic performance.

CONCLUSION

The present study demonstrated that fluid consumption following exercise- and heat-induced dehydration improves high-intensity cycling time-trial performance in young well—trained cyclists. However, neither a beverage containing ultra-filtered deproteinized milk that is high in electrolytes nor a traditional carbohydrate-electrolyte sports drink further improved performance in this short push-to-the-finish type bout compared to water. In a subset of four subjects, cycling performance was improved in a slightly longer time-trial in thermoneutral conditions following consumption of either GS or GAT compared to water. These findings suggest that hydration is more important than carbohydrate provision during shorter push-to-the-finish cycling in the heat. However, carbohydrate provision plays an emerging role in slightly longer push-to-the-finish cycling in thermoneutral conditions. Future investigation is necessary to elucidate whether these differences in performance between beverage and environmental conditions persist with longer periods of exercise (i.e. >1-h in length) and across a wider range of combinations of environmental conditions and push-to-the-finish target length.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

AUTHOR CONTRIBUTIONS

Conceptualization, W.L.K.; methodology, C.W.B., S.T.W., W.L.K.; formal analysis, C.W.B., S.T.W., R.M.C., and W.L.K.; investigation, C.W.B., S.T.W., and R.M.C.; data curation, C.W.B.; writing—original draft preparation, C.W.B.; writing—review and editing, C.W.B., S.T.W., R.M.C., and W.L.K.; supervision, W.L.K.; project administration, W.L.K.; All authors have read and agreed to the published version of the manuscript.

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DATA AVAILABILITY STATEMENT

The data presented in this study are available on request from the corresponding author.

Chapter 5

HYDRATION, AD LIBITUM CONSUMPTION, AND PERFORMANCE ARE SIMILAR AMONG A MILK PERMEATE SOLUTION, A TRADITIONAL SPORTS DRINK, AND WATER FOLLOWING OVERNIGHT FLUID RESTRICTION IN RECREATIONALLY ACTIVE ADULTS

INTRODUCTION

Fluid balance is influenced by a litany of factors, including physical activity, dietary intake, age, and environmental conditions. Body water volume is tightly regulated such that the daily variation in total body water content is less than 1% [9]. Imbalances in fluid volume, particularly in conditions where fluid availability is limited and/or when in a hot environmental conditions, can occur through inadequate fluid intake, excess fluid loss via urination, fecal excretion, or sweat. Under thermoneutral conditions, fluid and food restriction results in body mass losses of 1% after 13 hours, 2% after 24 hours, and 3% after 37 hours [136]. However, when fluid is readily available in these conditions, it is unlikely that individuals would experience such body mass losses without stimulation of thirst sensation and subsequent fluid replacement [136]. Thirst alone is typically an adequate stimulus for fluid replacement under normal resting conditions. Conversely, during and following exercise in the heat, thirst alone is often a poor indicator of water requirements and may not stimulate fluid replacement until 1-2% of body mass has already been lost [12, 13], a phenomenon termed *voluntary dehydration* [138]. This is especially concerning given that physical and cognitive performance typically decline when dehydrated by ≥2% loss of body mass [2-4]. Thus, it is essential to assess strategies that promote fluid replacement and retention during and following exercise.

Ad libitum drinking is defined as consuming fluid whenever and in whatever volume desired [139, 140]. This term is often used synonymously with drinking to thirst, a source of debate, though a recent study reported no differences in performance, fluid consumption, or thirst perception when elite ultraendurance cyclists are instructed to follow either strategy [141]. Similarly, the advantages of ad libitum fluid versus prescribed fluid replacement have been discussed and compared in-depth [137, 142]. To summarize, prescribed fluid replacement to match sweat loss is advantageous for exercises that occur in hotter environments (>30 °C), are longer in duration (i.e. <90 min) or higher in intensity, whereas ad libitum fluid ingestion is sufficient in cooler environments, during and following shorter duration events (<90 min), or at lower intensities, where it is less likely that individuals would approach 2% loss of body mass [137]. Although water consumption alone improves hydration status and physical performance [67], fluid with added components, such as carbohydrates and electrolytes, further stimulate thirst sensation and promote drinking and absorption of fluids. These beverages typically are consumed in the forms of commercially available sports drinks and contain approximately 6% carbohydrates [21]. Sports drinks present a relatively high glycemic index and may be contraindicated for clinical populations or individuals with impaired glucose handling [76], increasing the need to explore lower-glycemic hydration sources.

Bovine milk has been suggested to be an efficacious hydration source attributable to its naturally high concentrations of electrolytes and similar carbohydrate concentration to many commercially available sports drinks [78]. Further, both skim and whole-fat milk display an elevated beverage hydration index (BHI), a standardized measure of the hydrating capacity of a given beverage relative to water [25]. However, milk has a high energy density and viscosity and has been indicated to stimulate gastric discomfort [7, 8] when consumed during exercise in amounts

sufficient for replacement of sweat losses. Alternatively, beverages containing milk permeate, a byproduct created during the ultrafiltration of milk and devoid of protein and fat molecules, thus reducing energy density and viscosity, has been proposed to be an efficacious hydration source [27, 143].

Indeed, our lab reported an elevated BHI in a novel beverage containing milk permeate (GoodSport®) compared to both water and a traditional carbohydrate-electrolyte solution (Gatorade™) [36]. However, more recently, following a 2% exercise- and heat-induced dehydration in highly-trained cyclists, we observed no differences in cycling performance or indices of hydration status after prescribed partial rehydration (75% of lost body mass) with either water, Gatorade, or GoodSport [87]. This difference with our previous BHI study is potentially attributable to exercise- and heat-induced redistribution of blood flow from the renal circulation to the cutaneous and skeletal muscle vascular beds. In contrast, in subset of subjects completing a slightly longer time-trial in thermoneutral conditions, time-trial performance was improved in both Gatorade™ and GoodSport® compared to water. It remains unclear how these findings translate to recreational athletes exercising in a warm environment (i.e. ≤30 °C) and how ad libitum consumption of a milk permeate-based beverage impacts indices of hydration following fluid restriction-induced dehydration. To our knowledge, only one study has compared the impact of ad libitum consumption of a milk-based beverage versus a traditional CES on indices of hydration status. Baguley et al. reported similar fluid retention and fluid balance following consumption of a milk-based beverage (Sustagen Sport™) compared to a commercially available sports beverage (Powerade™) following exercise-induced dehydration (2% loss of body mass) in recreationally active young men [7].

The purposes of this study were 1) to determine how overnight fluid restriction and next-day prescribed rehydration with either water, a traditional CES (Gatorade™), or a novel milk permeate solution (MPS; GoodSport®) impact cycling time-trial performance in recreational athletes in a warm environment, and 2) to compare the impact of ad libitum consumption of these three beverages on restoration of fluid balance and biomarkers of hydration status post-time-trial. We hypothesized that 1) time-trial performance would be similarly improved following consumption of Gatorade™ and GoodSport® compared to water, and 2) ad libitum fluid consumption post-time-trial would be greater for Gatorade™ and GoodSport® compared to water.

MATERIALS AND METHODS

Study Population

Seven young, recreationally active (≥150 min/week for ≥6 months) adults were recruited for the study. Subject characteristics are detailed in **Table 5-1**. Additional inclusion criteria consisted of the following: aged 18-45 years, blood pressure <130/80 mmHg, HbA1C < 5.7%, and all premenopausal women either being eumenorrheic or taking oral contraceptives. Subjects were excluded if they reported any prior history of renal, metabolic, prostate, or cardiovascular disease or if they were taking any medications that may impact fluid balance. Menstrual cycle phase was not controlled in women participating in the study, as menstrual cycle phase has been previously reported to not influence fluid replacement during or following exercise [129]. Based on previous data demonstrating an effect size of 1.03 regarding ad libitum fluid consumption of a milk-based liquid meal supplement and a traditional sports drink during a two hour period following exercise-induced dehydration [7], assuming $\alpha < 0.05$, and power = 0.8, it was determined that a sample size of 6 subjects (G*Power) is sufficient to detect meaningful differences among

different beverage trials. All procedures were approved in advance by the Pennsylvania State University Institutional Review Board, and all subjects gave written or verbal consent before participation in accordance with the Declaration of Helsinki. All testing was conducted in Noll Laboratory at the Pennsylvania State University.

Table 5-1: Subject Characteristics

	Mean	SD	Range
n (M/W)	5 (4/1)		
Age (yrs)	24	2	22 – 26
Weight (kg)	79.3	14.9	66.2 – 97.5
BMI ($kg \cdot m^{-2}$)	25.9	3.5	22.0 – 30.8
Systolic BP (mmHg)	119	7	112 – 126
Diastolic BP (mmHg)	76	5	70 – 82
HR ($beats \cdot min^{-1}$)	58	10	48 – 64
HbA1C (%)	5.0	0.2	4.8 – 5.2
VO_{2max} ($mL \cdot kg^{-1} \cdot min^{-1}$)	43.7	3.7	38.2 – 48.6
VO_{2max} Power (W)	239	49	208 - 326

Screening and Familiarization

All subjects underwent a screening visit prior to enrollment. Following informed consent, subjects completed a medical health history questionnaire and a fasted blood sample was then obtained for blood biochemistry analysis. Weight and height were measured to the nearest 0.05 kg and 0.01 m while subjects wore a robe and undergarments. The robe was then weighed to approximate semi-nude body weight. Heart rate (HR) and blood pressure (sphygmomanometry) were measured after subjects sat quietly in an upright position for at least 5 mins. To determine desired workloads during each experimental trial, a $\dot{V}O_{2max}$ test was conducted on a cycle ergometer. The $\dot{V}O_{2max}$ protocol consisted of maintaining a cadence of 60 revolutions per minute

(RPM) with incrementally increasing resistance, beginning at 75 Watts (W) and increasing by 25 W every 2 minutes until exhaustion, similar to the protocol validated by Muscat and colleagues [144]. Following a 15-minute break after the $\dot{V}O_{2max}$ protocol, an exercise time-trial (TT) familiarization session was conducted. This test consisted of completing a 5-km TT as fast as possible. Subjects controlled their cadence and resistance settings on the cycle ergometer.

Experimental Protocol

All laboratory visits were completed in a randomized, cross-over, and counter-balanced manner. Each experimental study consisted of two laboratory visits; one in the evening on Day 1 (5:00 PM), and one the following morning on Day 2 (8:00 AM). A timeline of the protocol is outlined in **Figure 5-1**.

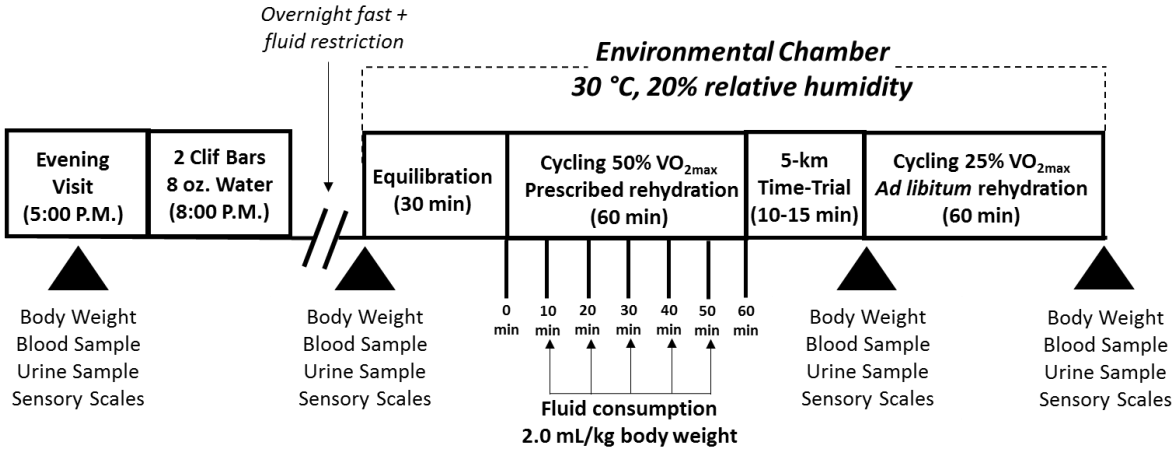

Figure 5-1: Timeline of the study.

Day 1 – Evening Visit

Prior to their Day 1 visit, subjects were instructed to abstain from vigorous exercise and alcohol for 24 h and caffeine for 8 h. Subjects were additionally instructed to consume a meal at 3:00 PM, 2 hours prior to arrival at the lab, with no further food consumption occurring after this time.

Otherwise, subjects were asked to maintain their normal dietary and water consumption patterns for the 24 h prior to each study. Upon arrival to the lab, subjects were asked to provide a urine sample. Subjects then changed into a robe while still wearing undergarments, and a weight was obtained (Seca Scales) to the nearest 0.05 kg. The robe was then weighed to approximate semi-nude body weight. Subjects then sat quietly in an upright posture for 10 minutes prior to measurements of heart rate and blood pressure and collection of a blood sample (EV). Subjects were then provided with 2 energy bars (Clif Bar®: 45 g carbohydrates, 5 g fat, 9 g protein, 140 mg sodium) and an 8 oz. bottle of water (Deer Park®) to be consumed that evening at 8:00 PM. Subjects were instructed to otherwise refrain from any food or fluid consumption until arrival at the lab on Day 2.

Day 2 – Morning Visit

Participants were asked to arrive at the laboratory the morning following the evening visit wearing shorts, socks, and a t-shirt (women also wore a sports bra). Upon arrival, subjects then entered an environmental chamber set to 30°C and 20% relative humidity (RH). Subjects changed into the same robe as Day 1, and body weight was measured. This was followed by collection of a urine sample. Subjects then sat quietly in an upright posture for the remainder 30-minute equilibration period. During this time, a resting heart rate and blood pressure measurement were obtained. An intravenous catheter was then inserted into antecubital blood vessel, and a blood sample was obtained (MORN). At the end of the equilibration period, subjects were asked to rate their current perception of temperature (0–8; 0 = “unbearably cold,” 4 = “neutral,” 8 = “unbearably hot”) and thirst (1–9; 1 = “not thirsty at all,” 9 = “very, very thirsty”). Subjects were then outfitted with a Polar™ heart rate monitor chest strap and watch to measure heart rate.

Following the end of the equilibration period, subjects mounted a cycle ergometer and cycle at a set cadence of 60 RPM for one hour, with resistance fixed at a moderate intensity corresponding to 50% of the maximum power achieved during the screening VO_{2max} test. During this one-hour cycling period, a prescribed amount of fluid was provided to the subjects on five occasions. This amount was equal to 2 mL of fluid per kilogram of body weight, every 10 minutes for the first 50 minutes. This amount of fluid was chosen to match approximate sweat during moderate-intensity physical activity and limit further loss of body mass [14]. The designated fluid for each trial was chosen in a randomized, counter-balanced order using a Latin square. Descriptions of beverage composition are displayed in **Table 5-2**. Heart rate was recorded every 10 minutes and rating of perceived exertion (RPE) every 15 minutes during this one-hour cycling period. At the conclusion of this one-hour cycling period, subjects completed a 5-km cycling time-trial to assess cycling performance. Subjects were allowed to control their cadence and the resistance on the cycling ergometer, and were instructed to finish the time-trial as fast as possible. Average cadence and resistance load were recorded from the cycle ergometer at the end of both the one hour of cycling and the TT. Heart rate, RPE, blood pressure, and perceptions of thermal and thirst sensation were recorded at the conclusion of the TT. Near-nude body mass was measured, and subjects provided a blood and urine sample.

Table 5-2: Beverage Composition.

	Water	Gatorade™	GoodSport®
Energy (kcal)	0	222	180
Carbohydrates (g)	0	61	64
Total sugar (g)	0	58	38
Sodium (mg)	0	444	480
Potassium (mg)	0	139	1100
Chloride (mg)	0	0	980
Calcium (mg)	0	0	320
Magnesium (mg)	0	0	60
Phosphorous (mg)	0	83	350
Osmolality (mosm)	0	292 ± 3	640 ± 1

Values represent amount per liter of fluid, according to product labels. Osmolality is represented as mean ± SD and was measured within the lab in triplicate using a freezing-point osmometer.

To assess beverage palatability, an *ad libitum* rehydration cycling protocol was conducted upon completion of the post-TT measurements. Subjects were instructed to again mount the cycle ergometer and pedal for one hour at a cadence set to 60 RPM, with resistance fixed at a light intensity corresponding to 25% of the maximum power achieved during the screening VO_{2max} test. A large cup containing 700 mL of fluid was provided to subjects and set on a food scale on a table placed next to the cycle ergometer. Subjects were instructed to consume the randomly assigned fluid *ad libitum*. The cup was refilled with an additional 700 mL of fluid each time it was emptied. Heart rate was recorded every 10 minutes and rating of perceived exertion (RPE) every 15 minutes during this cycling period. Average cadence and resistance load were recorded from the cycle ergometer at the end of this cycling period. Heart rate, blood pressure, and perceptions of thermal and thirst sensation were recorded at the conclusion of the TT. Near-nude body mass was measured, and subjects provided a blood and urine sample. At the end of each experimental visit, subjects completed a sensory questionnaire regarding beverage palatability, likability, taste, and thirst quenching properties.

Urine and Serum Sample Analysis

Urine mass was measured with an electronic balance accurate to the nearest 0.1 g, with the mass of the empty container subtracted from the total weighed value. All analyses of urine and blood samples occurred immediately following sample collection. Urine specific gravity (USG) was measured using a refractometer. Urine and serum osmolality were measured in triplicate at each time point using a freezing-point osmometer (Model 3320, Advanced Instruments, Inc., Massachusetts, United States, respectively). Blood samples were collected using serum separator tubes and were centrifuged (10 min, 4□C, 4,000 rpm) after sitting for 30 min to allow for serum clotting to occur. Hemoglobin and concentrations of hematocrit, glucose, sodium, potassium, and chloride were analyzed for each time point (Quest Diagnostics) within 48 hours of sample collection.

Calculations and Statistical Analysis

Percent change in plasma volume at each time point relative to EV was determined using the method of Dill and Costill [98]. A two-way repeated measures ANOVA was performed to examine the effect of beverage and time on urine and serum samples. A one-way ANOVA was used to assess the effect of beverage on time-trial performance. All statistical analyses were conducted using a Tukey's HSD test for post-hoc pairwise comparisons Data were analyzed in SAS (Cary, North Carolina, United States) using PROC MIXED model. With the exception of box plots, all data are presented as mean ± SD. Statistical significance was set a priori at $\alpha < 0.05$.

RESULTS

Body Mass

Percent changes in body mass for each trial are presented in **Figure 5-2**. As expected, body mass was similarly reduced at MORN relative to EV in each of the three conditions following the

overnight fluid restriction and fast (WAT: -2.0 ± 0.8%; GAT: -1.7 ± 0.8%; GS: -1.6 ± 0.7%; all $p<0.0001$ relative to EV). Body mass remained lower relative to EV for the remainder of the trial in all conditions (all $p<0.03$). There were no differences in the percent change in body mass among any condition ($p>0.42$).

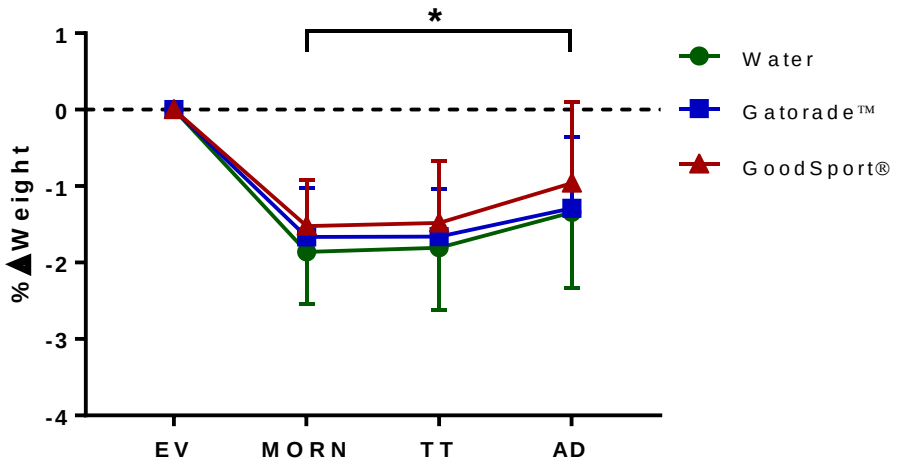

Figure 5-2: Percent change in body mass. Body mass was lower in all three trials at MORN compared to EV, and this persisted for the remainder of the study, with no differences among trials at any time point. *$p < 0.05$ compared to EV.

Urine and Serum

Data collected from blood and urine samples are displayed in **Table 5-3**. Cumulative urine output was significantly higher in the GoodSport® trial following AD compared to the water trial ($p<0.03$). Urine osmolality was elevated relative to EV at all subsequent time points in the water trial ($p<0.002$), but only at MORN and TT in both the the Gatorade™ ($p<0.04$) and GoodSport® ($p<0.03$) trials. Similarly, urine specific gravity was elevated relative to EV at all subsequent time points in the water trial ($p<0.02$), but only at TT and AD the Gatorade™ trial ($p<0.01$), and only at TT in the GoodSport® trial ($p<0.002$).

Table 5-3: Urine output and serum osmolality and electrolyte concentrations.

		Beverage	EV	MORN	TT	AD
Urine	**Cumulative urine output (mL)**	Water	----------	43 ± 51	80 ± 72	113 ± 101
		Gatorade™	----------	29 ± 22	78 ± 54	123 ± 68
		GoodSport®	----------	60 ± 67	109 ± 81	200 ± 114†
	Osmolality (mosm)	Water	647 ± 384	930 ± 114*	977 ± 57*	914 ± 62*
		Gatorade™	685 ± 389	852 ± 141	896 ± 153*	839 ± 178
		GoodSport®	736 ± 243	858 ± 235	959 ± 105*	821 ± 95
	Urine specific gravity	Water	1.019 ± 0.009	1.026 ± 0.003*	1.028 ± 0.004*	1.028 ± 0.004*
		Gatorade™	1.017 ± 0.009	1.021 ± 0.005	1.025 ± 0.004*	1.025 ± 0.005*
		GoodSport®	1.020 ± 0.006	1.024 ± 0.004	1.027 ± 0.003*	1.025 ± 0.002
Serum	**Osmolality (mosm)**	Water	292 ± 4	294 ± 3	296 ± 3	289 ± 4
		Gatorade™	295 ± 7	293 ± 4	295 ± 3	290 ± 4*
		GoodSport®	295 ± 4	295 ± 3	302 ± 7*†‡	299 ± 2†‡
	Sodium	Water	139 ± 1	140 ± 1	139 ± 1	138 ± 1
		Gatorade™	139 ± 2	139 ± 1	139 ± 1	138 ± 1
		GoodSport®	139 ± 1	139 ± 2	139 ± 2	139 ± 1
	Potassium	Water	4.0 ± 0.1	4.1 ± 0.3	4.2 ± 0.2	4.3 ± 0.1
		Gatorade™	4.4 ± 0.6	4.2 ± 0.3	4.3 ± 0.3	4.1 ± 0.3
		GoodSport®	4.0 ± 0.2	4.1 ± 0.3	4.4 ± 0.3	4.6 ± 0.1
	Chloride	Water	103 ± 1	105 ± 1	103 ± 2	102 ± 1
		Gatorade™	104 ± 1	105 ± 3	103 ± 1	104 ± 2
		GoodSport®	104 ± 2	105 ± 1	104 ± 2	105 ± 1

Values are displayed as mean ± SD. Differences among beverages were assessed by two-way ANOVA. *$p < 0.05$ vs. EV; † $p < 0.05$ vs. water; ‡ $p < 0.05$ vs. Gatorade™.

Serum osmolality was elevated relative to EV in the GoodSport® trial only immediately after the TT ($p<0.001$), and lower in the Gatorade™ only after AD ($p<0.03$). Serum osmolality in the GoodSport® trial was elevated relative to both the water and Gatorade™ at both TT and AD ($p<0.004$).There were no differences in serum sodium or chloride concentrations at any time point relative to EV or among the different beverage conditions at any time point. Serum potassium was elevated in the GoodSport® trial compared to EV and the Gatorade™ trial after AD ($p<0.04$). Percent changes in plasma volume are displayed in **Figure 5-**3. There was no difference in percent change in plasma volume relative to EV at either MORN or AD. Plasma volume was significantly reduced relative to EV in all beverage conditions at TT (all $p<0.001$). Percent change in plasma volume was not different among beverage conditions at any time point (all $p>0.73$).

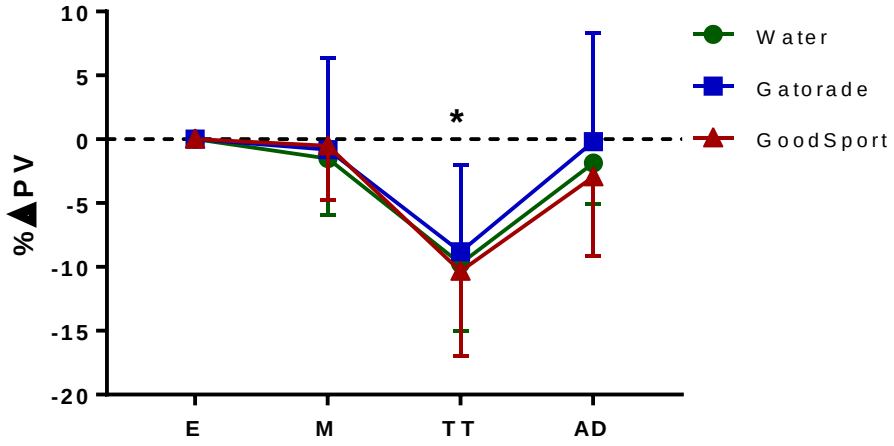

Figure 5-3: Percent change in plasma volume. Plasma volume was lower in all three trials at TT compared to EV, with no differences among trials at any time point. *$p < 0.05$ compared to EV.

Perceptual measures

Rating of perceived exertion did not differ among beverage conditions during either the prescribed drinking cycling period, the time trial, or the *ad libitum* cycling period (WAT: 14 ± 2, 19 ± 2, 11 ± 2; GAT: 14 ± 2, 19 ± 2, 11 ± 2; GS: 15 ± 2, 19 ± 1, 12 ± 3). Thermal sensation did not differ among beverage conditions at any time point (WAT: 5 ± 0; 6 ± 1, 5 ± 0; GAT: 5 ± 1, 6 ± 1, 5 ± 1; GS: 5 ± 0, 6 ± 1, 5 ± 1). Thirst sensation did not differ among beverage conditions at any time point (WAT: 5 ± 1, 7 ± 3, 4 ± 3; GAT: 4 ± 2, 5 ± 2, 3 ± 1; GS: 5 ± 2, 6 ± 3, 4 ± 2). There were also no significant differences among the three drink conditions in beverage sensory questions of overall beverage likability, taste, thirst quenching, or drinkability.

Time Trial Performance and Beverage Consumption

Individual time trial performance data, expressed in both time (sec) and power relative to body mass (W/kg) are displayed in **Figure 5-4**. Neither time-to-completion (WAT: 596 ± 83 sec; GAT: 596 ± 65 sec; GS: 592 ± 56 sec; p>0.69) nor relative power (WAT: 2.2 ± 0.6 W/kg; GAT: 2.2 ± 0.5 W/kg; GS: 2.2 ± 0.6 W/kg; p>0.17) were different among beverage conditions.

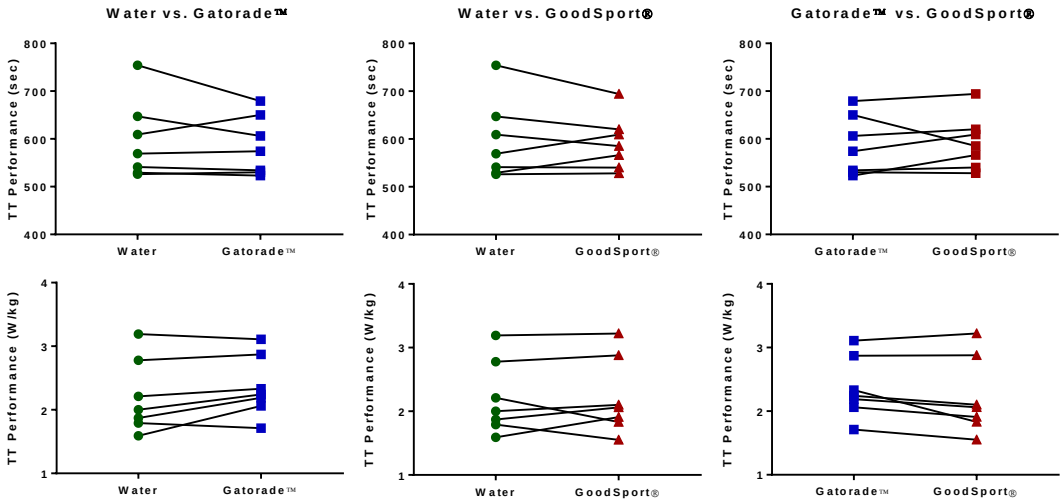

Figure 5-4: Between-beverage comparisons of a 5-km cycling time-trial, measured as both time (s) and relative power (W/kg). There were no significant differences in either measure of performance among beverage conditions.

Beverage consumption data for each subject during both the prescribed drinking and *ad libitum* drinking periods are displayed in **Table 5-4**. Prescribed fluid consumption (2 mL/kg at every 10 minutes for 50 minutes) did not differ among beverage conditions ($p>0.99$). Ad libitum fluid consumption did not differ among beverage conditions ($p>0.61$).

Table 5-4: Individual beverage consumption volumes for both prescribed drinking and ad libitum drinking periods.

	Water			**Gatorade™**			**GoodSport®**		
	Pres.	**Ad Lib.**	**Total**	**Pres.**	**Ad Lib.**	**Total**	**Pres.**	**Ad Lib.**	**Total**
Subject 1	727	972	1699	738	910	1648	713	1393	2106
Subject 2	981	1844	2825	963	1548	2511	966	1446	2412
Subject 3	677	1423	2100	654	1463	2117	690	1679	2369
Subject 4	979	354	1333	981	878	1859	990	567	1557
Subject 5	691	710	1401	700	1169	1869	686	804	1490
Subject 6	656	140	796	667	326	993	666	187	853
Subject 7	528	235	763	540	118	658	538	203	741
Average	748	811	1560	749	916	1665	750	897	1647
SD	170	641	731	164	540	641	166	614	683

Total represents the combined amount of fluid consumed over the course of the study (prescribed drinking and ad libitum drinking). Pres., prescribed drinking; Ad Lib., ad libitum drinking.

DISCUSSION

The present study examined the impact of an overnight fast and fluid restriction and next-day prescribed rehydration with water (WAT), a traditional carbohydrate electrolyte solution (Gatorade™, GAT), or a milk permeate solution (GoodSport®, GS) on 5-km cycling time-trial (TT) performance in a warm-dry environment. We additionally assessed beverage palatability utilizing an ad libitum rehydration period following the cycling TT. The primary finding of this study was prescribed rehydration with either WAT, GAT, or GS following an overnight fast and fluid restriction did not differentially impact 5-km TT performance. Furthermore, there were no

differences in ad libitum fluid consumption among the three beverage conditions during a light-intensity recovery cycling period following the cycling TT. To our knowledge, this study is the first to compare the performance and rehydration characteristics of a novel milk permeate solution compared to a traditional sports drink and water following overnight fluid restriction.

Maintenance of fluid homeostasis is essential for proper function. Fluid homeostasis is tightly regulated such that fluctuations in total body water content typically do not exceed 1% [9]. Under normal conditions, 2-3 L of water (~5-10% of total body water content) is turned over daily in individuals at rest [30], and this daily rate increases with exercise [145]. However, deviations outside of this range can occur when fluid availability is limited or following exercise in the heat where high sweating rates occur. Dehydration ≥2% loss of body mass is associated with reductions in both cognitive and physical function [2-4]. In resting adults, Shirreffs and colleagues reported body mass losses of 1% following 13 hours of fluid restriction and 2% following 24 hours of fluid restriction, though thirst set in long before these losses occurred [136]. Thirst is often a strong enough impetus for drinking to maintain fluid balance under resting conditions. However, during exercise, particularly in the heat, when body water is turned over at a more rapid rate, thirst sensation often does not occur until sweat loss exceeds 2% of body mass [12, 13], a point at which reductions in both physical and cognitive function are often reported. Further, individuals often do not consume enough fluid to replace these excess fluid losses following exercise in the heat [138]. Thus, there is an inherent need to assess hydration strategies aimed at limiting or preventing fluid losses resulting in ≥2% loss of body mass and for promoting proper fluid replacement after excess fluid loss.

Sports drinks are designed to promote fluid intake, rapid reabsorption in the gastrointestinal tract, and stimulate sensations of thirst. Most sports drinks typically contain both carbohydrates (~6%)

and electrolytes to drive these responses [24]. In recent years, milk-based beverages have been discusses as potential alternatives to sports drinks. Utilizing the beverage hydration index (BHI), a standardized measure of the hydrating capacity of a given beverage relative to water, Maughan et al. observed an elevated BHI following consumption of both full-fat and skim milk, exceeding the BHI of a tested sports drink and matched only by that of an oral rehydration solution. Shirreffs et al. observed that milk both with and without sodium promoted greater fluid retention and prolonged positive net fluid balance relative to a traditional carbohydrate-electrolyte solution (CES) and water. Consumption of chocolate milk during exercise similarly prolonged time to exhaustion as a fluid replacement beverage relative to a carbohydrate recovery drink when cycling at 70% VO_{2max} [79] These findings collectively indicate that milk-based beverages are an efficacious alternative hydration source to traditional sports drinks. A major limitation of milk as a sports drinks is its high energy density and viscosity, which has been reported to cause gastric distress and feelings of fullness during exercise [7, 8]. As such, previous attempts have been made to develop beverages from milk byproducts, such as milk permeate [28, 29], created during the ultrafiltration of milk that presents a reduced energy density while retaining the carbohydrate and electrolyte contents of milk.

Our lab observed that a novel solution containing milk permeate displayed an elevated BHI up to four hours post-consumption relative to both water and a traditional CES [36]. In a subsequent study, we observed that consumption of this milk permeate solution improved cycling time-trial performance following exercise- and heat-induced dehydration relative to no fluid consumption, though performance was not different compared to following consumption of water or a CES. However, in a small subset of subjects repeating these three beverage trials in a thermoneutral environment with a slightly longer cycling TT, performance improved in all subjects in the MPS trial and in all but one subject in the CES trial relative to water. This discrepancy between

environmental conditions may be attributable to the exercise- and heat-induced redistribution of blood flow from the renal circulation to the cutaneous and skeletal muscle vascular beds that may counteract the pro-hydration characteristics of fluids with added components. It previously remained unclear how these findings translate to recreational athletes or more moderate environments following a different method of dehydration, such as overnight fluid restriction.

As expected, following a 15-hour overnight fast and fluid restriction, there was a similarly significant reduction in body mass in each beverage condition (WAT: -2.0 ± 0.8%; GAT: -1.7 ± 0.8%; GS: -1.6 % 0.7%) (**Figure 5-2**), comparable to the findings of Shirreffs et al. [136]. Further, following one hour of cycling at a moderate intensity and a 5-km cycling TT, body mass loss was minimally changed, despite consuming the equivalent of 10 mL/kg of body mass over the course of the hour of cycling (WAT: -2.1 ± 0.8%; GAT: -1.8 ± 0.7%; GS: -1.7 ± 0.8%), indicating that fluid consumption matched sweat loss during this period. In contrast, following the *ad libitum* (AD) period, body mass somewhat recovered toward baseline, though was still significantly reduced relative to the evening (EV) body weight (WAT: -1.4 ± 1.2%; GAT: -1.1 ± 1.0%; GS: -0.9 ± 1.2%). Interestingly, there were no differences in percent change in body mass at any time point among the three beverages, indicating that consumption of each beverage elicited similar responses pertaining to overall fluid loss and retention.

Cumulative urine output (CUO), urine osmolality (U_{osm}), and specific gravity (USG) over the entirety of the study were not different following consumption of GS compared to following consumption of GAT or water, though both U_{osm} and USG were elevated in all trials following the TT compared to EV (**Table 5-3**). Similarly, in the morning following the overnight fluid restriction in the current study, percent change in plasma volume an important indicator of fluid balance following both dehydration and rehydration, was relatively miniscule (WAT: -0.62 ±

4.08%; GAT: -1.45 ± 6.23%; GS: +1.03 ± 3.95%) (**Figure 5-3**). However, following the first hour of cycling and the TT, there was a significant similar reduction in plasma volume in each beverage condition relative to baseline (WAT: -9.70 ± 5.75%; GAT: -8.36 ± 7.76%; GS: +10.88 ± 7.80%). In contrast, following AD, plasma volume recovered such that it was not different from EV in any of the three beverage conditions (WAT: -1.60 ± 3.39%; GAT: +0.69 ± 9.45%; GS: -2.40 ± 7.46%).

These results are analogous to findings previously reported by our lab during and following exercise in the heat [87] and in contrast to our findings when resting in thermoneutral conditions [36]. Although the environmental conditions in the current study (30°C, 20% RH) were more moderate compared to our previous study (35-40°C, 20% RH) in young, highly-trained cyclists, the current conditions were still relatively hot. This likely created a drive for redistribution of blood flow from inactive circulations (i.e. renal), to active vascular beds (i.e. muscle and skin) during and following exercise, a competition that is exacerbated in the presence of dehydration, such as that which occurred in the present study [123, 124]. Given the degree of dehydration experienced by the subjects across the course of the study (1-2%) and the lack of complete recovery toward baseline body mass, it is also likely that hormonal and neural mechanisms (renin-angiotensin-aldosterone system) attributed to the small amount of urine production over the course of the study, increase in urine concentration, and recovery of plasma volume following ad libitum fluid consumption for the purpose of preventing further disruptions to fluid homeostasis and reductions in body mass, though measures of this neural activation and circulating hormone concentrations were not measured in this study.

There has been limited research comparing the impact of dairy beverage consumption versus consumption of traditional sports drinks on cycling performance and rehydration following

cycling in the heat. Baguley et al. did not observe any differences in fluid retention following consumption of either a milk-based liquid meal supplement or a traditional sports drink following exercise-induced dehydration (-1.9%) [7]. Inversely, Desbrow et al. observed greater fluid retention following ingestion of bovine milk compared to a traditional CES following cycling-induced 1.8% loss of body mass [77]. The previous study conducted in our lab [87] showed that 120-kJ cycling TT performance was improved with partial prescribed rehydration with either WAT, GAT, or GS versus no fluid consumption following 2% loss of body mass in a hot-dry environment (35°C, 20% RH)). However, there were no differences in TT performance among the three trials in which drinking occurred. In contrast, TT performance was improved in the GAT and GS trials when in a thermoneutral conditions (21°C, 20% RH). It previously remained unclear how these findings would translate to a more moderate, warm environment. Furthermore, to our knowledge, no study had compared the influence of milk permeate consumption versus traditional rehydration beverages on indices of hydration and performance or how ad libitum fluid consumption following performance affects rehydration. In the current study, there were no differences in 5-km TT performance (**Figure 5-4**) when measured as either time (s) or power relative to body mass (W/kg), similar to our findings in the previous study in a slightly hotter condition [87], suggesting that added beverage constituents yield negligible performance benefits effects in the short term compared to water consumption in a warm environment.

Thirst sensation is often does not occur until ~1-2% dehydration when exercising in the heat and may be alleviated prior to full rehydration occurs [12, 13, 138]. Sports drinks are designed to stimulate thirst sensation and promote further ingestion of fluid for maintenance of and recovery to euhydration. *Ad libitum* fluid ingestion is likely sufficient to achieve fluid homeostasis during and following exercise <90 minutes and which occur in moderate environments and when exercise intensity is relatively lower [137]. In the current study, ad libitum consumption of a milk

permeate-based beverage did not differ from that of a traditional sports drink or water in the 1 hour following cycling exercise (**Table 5-4**). This finding is analogous to the findings of the study conducted by Baguley et al. in which *ad libitum* consumption of a milk-based liquid meal supplement (Sustagen Sport™) similarly improved indices of hydration status as a traditional sports drink (Powerade™) over a 2 hour period following cycling exercise resulting in ~1.9% loss of body mass, though less fluid was consumed in the Sustagen Sport™ trial [7]. It remains unclear whether differences would occur in *ad libitum* fluid consumption and markers of hydration status if subjects were given a longer period to rehydrate.

Limitations

Subjects were instructed to maintain their normal hydration habits in the 24 h prior to their EV visit. However, we did not control for euhydration prior to study onset. Plasma osmolality is maintained within a tight range of approximately 275-295 mosm/kg, with osmolalities between 295-300 mosm/kg classified as impending dehydration and >300 mosm/kg classified as clinical dehydration [146]. In the current study, serum osmolality (S_{osm}) was relatively elevated prior to study onset such that on average subjects were fell into the "impending dehydration" category in the Gatorade™ and GoodSport® trials, and near the upper limit of euhydration in the water trial (WAT: 292 ± 4 mosm/kg; GAT: 296 ± 6 mosm/kg; GS: 295 ± 4 mosm/kg). Furthermore, this was accompanied by a relatively higher, yet still within normal range, USG in each condition (WAT: 1.018 ± 0.009; GAT: 1.018 ± 0.009; GS: 1.020 ± 0.006). These findings indicate that subjects on average were predisposed to experiencing dehydration prior to conducting the overnight fast and fluid restriction. It is possible that if hydration were controlled for prior to the evening visit that the degree of dehydration would have been different at the MORN time point prior to onset of exercise and rehydration. A second limitation is in regards to the cycling time trial. During the

screening visit, subjects completed a practice 5-km cycling TT to familiarize themselves with the protocol. This was further done to limit a potential learning effect across experimental visits. TT performance measured as relative power ($W \cdot kg^{-1}$) did not differ among trials based on order (i.e. Trial 1 vs. Trial 2 vs. Trial 3) independent of drink condition. Although TT time-to-completion improved in absolute terms with each successive trial, there were no significant differences between trials (Trial 1: 596 ± 84 sec, 2.2 ± 0.6 $W \cdot kg^{-1}$; Trial 2: 583 ± 55 sec, 2.4 ± 0.5 $W \cdot kg^{-1}$; Trial 3: 577 ± 60 sec, 2.3 ± 0.5 $W \cdot kg^{-1}$. p=0.0295 for Trials 1 vs. Trial 3). Further, given the counterbalanced design of the study, it is unlikely that any potential differences in performance by trial order would have translated to between-beverage differences in time-trial performance.

CONCLUSION

The present study demonstrated that prescribed consumption of a milk permeate-based beverage following overnight fast and fluid restriction does not differentially impact subsequent cycling 5-km time-trial performance or indices of hydration compared to consumption of water or a traditional sports drink. Furthermore, ad libitum consumption of this milk permeate-based beverage during a one-hour recovery period following the cycling time-trial was not volumetrically different than consumption of water or the traditional sports drink and did not result in differences in indices of hydration status or fluid balance. Future investigation is warranted to assess whether ad libitum consumption of a milk permeate-based beverage prior to or during exercise improves indices of hydration status and performance.

Chapter 6

CONCLUSIONS AND FUTURE DIRECTIONS

The studies that comprise this dissertation were designed to examine the hydration- and performance-enhancing characteristics of a novel beverage containing milk permeate. Specifically, this dissertation investigated (1) the hydration efficacy of a milk permeate beverage, utilizing the Beverage Hydration Index (BHI), compared to a traditional carbohydrate-electrolyte solution and water, (2) the influence of rehydration with a milk permeate beverage on cycling time-trial performance in highly-trained cyclists following exercise- and heat-induced dehydration, and (3) the impact of prescribed drinking of a milk permeate beverage on cycling time-trial performance following overnight fluid restriction and subsequent ad libitum rehydration following the cycling time-trial on indices of hydration status. Together, these findings suggest that consumption of a milk permeate beverage at rest and in thermoneutral environments yields beneficial effects on markers of hydration status, but these benefits are negligible compared to ingestion of water or a traditional sports drink when consumed following exercise- and heat-induced dehydration or dehydration following overnight fluid restriction. As such, beverage containing milk permeate may serve as an efficacious alternative to more traditional hydration sources both at rest and during/following exercise. This chapter summarizes the findings of these studies and discusses future directions for this area of research.

Hydration Efficacy of a Milk Permeate-Based Oral Hydration Solution

The primary finding of this study was that cumulative urine output was attenuated over the entire four hours following consumption of MPS compared to both CES and WAT, resulting in a higher BHI and attenuated reduction in net fluid balance in MPS compared to the other beverages.

Additionally, there was an attenuated rise in plasma glucose concentrations following consumption of the MPS compared to CES. Further, there was a similar expansion in plasma volume four hours post- CES and MPS consumption compared to WAT.

Implications

These findings suggest that a beverage containing milk permeate may serve as an efficacious alternative to more traditional sports drink and water as a source for hydration in young adults at rest in thermoneutral environments. Further, a beverage containing milk permeate, which has a lower sugar content, may be more preferable for individuals, such as those with diabetes or renal diseases, who do not desire the relatively higher glycemic index presented in traditional sports drinks while still providing pro-hydration characteristics that exceed those of traditional sports drinks. This study is the first to quantify the hydration capacity of a beverage containing milk permeate and compare it to traditional hydration sources.

Hydration is More Important than Exogenous Carbohydrate Intake during Push-To-The-Finish Cycle Exercise in the Heat

The findings of this two-part study were: (1) cycling time-trial performance is improved after fluid consumption following exercise- and heat-induced dehydration compared to no fluid consumption; (2) cycling time-trial performance in the heat is not differentially improved subsequent to consuming either water, a traditional sports drink, or a beverage containing milk permeate; (3) consumption of these three beverages in the heat do not differentially affect indices of hydration status; and (4) in a subset of subjects completing a slightly longer cycling time-trial

in thermoneutral conditions, performance was similarly improved following consumption of either the traditional sports drink or the milk permeate solution compared to water.

Implications

The results from this study indicate that fluid consumption following exercise- and heat-induced dehydration improves cycling time-trial performance, but fluids with added constituents such as carbohydrates and electrolytes yield negligible benefits for improving push-to-the-finish cycling performance in the heat. However, beverages containing carbohydrates and electrolytes may provide emerging benefits when the time-trial is lengthened and when it occurs in thermoneutral conditions. These data provide insight into the differential impacts of environmental conditions and performance length on the hydration capacity of beverages containing milk permeate compared to traditional forms of rehydration following exercise- and/or heat-induced dehydration.

Hydration, Ad Libitum Consumption, and Performance Are Similar Among a Milk Permeate Solution, a Traditional Sports Drink, and Water Following Overnight Fluid Restriction in Recreationally Active Adults

The principal findings of this study were: (1) prescribed hydration with a milk permeate solution following overnight fluid restriction does not improve cycling performance in recreational athletes in a warm environment compared to consumption of a traditional sports drink or water; (2) ad libitum consumption of these three beverages did not yield volumetric differences during a cycling recovery period immediately following the cycling time-trial; and (3) indices of hydration status, such as change in plasma volume, serum osmolality, and urine output, were not different among beverage conditions at any time point over the course of the study following overnight fluid restriction.

Implications

The results of this study support our previous findings of negligible benefits of consumption of fluids with added constituents on short-term cycling performance following~2% dehydration. Furthermore, the results of the ad libitum consumption period provide important insight into rehydrating characteristics of a milk permeate solution following high-intensity exercise. These findings suggest that the palatability of a milk permeate solution is not different than that of a traditional sports drink or water, and that ingestion of such a beverage does not stimulate further fluid ingestion for achieving rehydration compared to these other beverages, though only over a short 1-hour timeframe.

Future Directions

1) The studies in chapters 4 and 5 of this dissertation, examining the impact of dehydration and subsequent rehydration with a milk permeate solution on cycling performance compared to water or a traditional sports drink, are limited in that subjects were already dehydrated prior to the beginning of each study. In this studies, there was no added benefit to consumption of either a milk permeate solution or a traditional sports drink, which have added constituents such as carbohydrates and electrolytes, compared to water. These findings are in contrast to those of the study discussed in chapter 3 in which consumption of the milk permeate solution results in increased fluid retention and prolonged positive net fluid balance. Subjects in that study were in a euhydrated state prior to study commencement. It is possible that beginning in an already near-dehydrated state prior to initiation of a dehydration protocol in the final two studies comprising this dissertation may have negated the beneficial effects of beverages with added constituents. Future research is warranted to assess whether these beverages with added constituents play emerging roles when subjects are in a euhydrated state prior to exercise rather than

already being in a dehydrated state prior to further dehydration induction.

2) Another important question that needs addressed is whether milk permeate consumption during exercise bouts lasting >90 minutes improves time-trial performance. In the studies comprising chapters 3 and 4 of this dissertation, fluid consumption prior to time-trial performance took place over 30 and 60 minutes, respectively. [2-4]. During endurance exercise at lower intensities for less than 90 minutes or in moderate environments (20-21°C), sweating rates are typically low, often result in no more than a 1-2% loss of body mass, and have mild or insignificant impacts on physical performance [15-17]. However, greater total sweat loss occurring during longer exercise (>90 minutes) [2] or exercise in hotter environment conditions (31-32°C) [18, 19], stimulate greater degrees of dehydration, and thus are more likely to induce decrements in physical performance [4]. Research examining milk permeate consumption during exercise lasting <90 min is thus warranted.

3) Neural and hormonal signaling mechanisms play an influential role on fluid homeostasis. Specifically, the renin-angiotensin-aldosterone system is pivotal in determining fluid balance when homeostasis is challenged, such as during exercise in the heat or when fluid availability is limited. The studies comprising this dissertation did not measure circulating concentrations of key hormones such as arginine-vasopressin that likely modulate several indices of hydration measured in these studies, such as changes in plasma osmolality and concomitant changes in plasma volume. Future research may attempt to elucidate the potentially differing modulating responses of circulating hormones pertaining to fluid balance and retention following consumption of beverages containing milk permeate compared to alternative sources of hydration both at rest and

during exercise, as well is in both euhydrated and dehydrated states.

4) Milk permeate has been proposed to retain the hydrating qualities of milk, attributable to milk permeate's retention of carbohydrates and electrolytes during the ultrafiltration of milk. However, milk is composed of other resources, such as fats and proteins, which have also been proposed to increase the hydration capacity of milk [6, 25], that milk permeate is devoid of. By nature, the beverage hydration index, discussed in-depth throughout this dissertation, allows for the comparison of the hydration capacity of a given beverage compared to other tested beverages both within the same study and across different studies with the same protocol. However, this index only indicates how much fluid is being retained within the body over time following fluid consumption, but not where this fluid is located or being stored (i.e. gastrointestinal tract, renal tubules, vascular space, etc.). To date, no studies have actually compared the hydrating qualities of a beverage containing milk permeate to milk beyond the use of the Beverage Hydration Index. Future research may elucidate whether milk permeate actually does retain the hydration-promoting qualities of milk, and to what degree this retention occurs.

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Appendix

INFORMED CONSENT FORMS

CONSENT FOR RESEARCH
The Pennsylvania State University

Title of Project: Characterization of the effects of amino acid/electrolyte based beverages on hydration status in young and older subjects (IRB #6412)

Principal Investigator: W. Larry Kenney, Ph.D.

Address: 102 Noll Laboratory, University Park, PA 16802

Telephone Number: (814) 863-1672

Subject's Printed Name: _____

We are asking you to be in a research study. This form gives you information about the research.

Whether or not you take part is up to you. You can choose not to take part. You can agree to take partand later change your mind. Your decision will not be held against you.

1. Why is this research study being done?

For some people, it may be difficult to keep enough fluids in the body to maintain hydration. Olderadults tend to avoid drinking during the evenings or during trips to avoid urinating at inconvenienttimes. Certain drinks may be able to keep you hydrated for longer than others when you drink the same amount. This can help keep you hydrated for a longer period of time and by reducing the amount of water that is removed from the body when you go to the bathroom.

In this study, we are using five different drinks:

1. Distilled Water
2. Gatorade
3. Pedialyte
4. Enterade ORS
5. Enterade Lifestyle
6. GoodSport

This study will measure how long the water from these drinks stay in your body. We will look at different hormones and electrolytes in the blood and urine to determine which drink keeps fluid inyour body the longest. We are investigating the impact of aging because it may change the way these drinks are absorbed.

We are asking you to be in this research because you fit our subject characteristic criteria. About 60 people will take part in this research study in our lab.

2. What will happen in this research study?

We may ask you to repeat a trial, procedure, or test. This could happen for many reasons such as equipment failure, power outage, inconclusive test results, etc. You do not have to repeat a trial, procedure, and/or test if you do not wish to do so.

A. Screening Visit:

You drink only water and do not eat for 12 hours before your screening. Report to Noll Lab. We measure your height and weight, blood pressure (BP), heart rate (HR), and measure waist circumference. The research nurse and/or Clinical Research Center (CRC) staff perform the screening. The staff reviews your medical history. The staff draws 30 ml (about 2 Tbsp) of blood from a vein in your arm. We send some of the blood to a lab to see if the proteins, blood cells, electrolytes, etc. are within normal levels. If you are a woman of childbearing age, you submit a urine sample for a pregnancy test. If you take a thyroid drug,you supply the results of a thyroid test taken within the past 6 months. If you do not have thyroid test results, the staff draws 3.5 ml (0.2 Tbsp) of blood from a vein in your arm. We send the blood to a lab that tests it for thyroid levels. The lab destroys the sample after testing. We do not do genetic tests on the blood. We do not test the blood for disease (e.g. HIV).

B. Preparation for all experiments (experiments occur on separate days)

1. Within 24 hours before experiment.
    a. Do not exercise hard. Casual walking is fine.
    b. Please drink at least six 8 oz. glasses of fluid.
    c. Do not take drugs (prescription or over-the-counter), herbals, or supplements that could affect hydration status.
2. For 12 hours before experiment, do not consume caffeine (chocolate, coffee, tea, Pepsi, etc.) and alcohol.
3. For 8 hours before the experiment do not eat.
4. You will collect a morning urine sample in the container we give to you.
5. You will drink 500 ml (2 cups) of distilled water that we give to you 1 hour before the experiment in 15 minutes.

C. Experiment

1. You will bring your morning urine sample to the lab.
2. We measure your heart rate, blood pressure, and oral temperature.
3. You will go to the bathroom to empty your bladder and bowels.
4. You will sit quietly for 10 minutes. Then, the research nurse or the CRC clinicians will put a catheter into the vein of your arm and take a 24 ml (1.6 Tbsp) blood sample. This lets the clinician to take many blood samples without sticking you with a needle each time.
5. We will collect a urine sample in the container we give to you.
6. You will remove your clothes and wear a bathrobe and your underwear to be weighed. We will then subtract the weight of the bathrobe. This is called a near-nude body mass.
7. You will put your clothes back on and sit comfortably in the chair for the experiment.

8. You will drink 1 liter of one of the assigned drink. It will be separated into four equal volumes for easier drinking. You will drink one part of the drink every 7.5 minutes. You will drink the entire drink in 30 minutes.
9. The nurse or CRC clinician draws a blood sample (11 mL, 0.7 Tbsp) at the end of the drinking period. You will go to the bathroom after the blood draw. Urine will be collected into a container we give to you. We will determine the volume of urine by weighing.
10. At 5 minutes after you drink the drink,
    a. We will collect a blood sample (1 mL, 0.67 Tbsp)
11. At 10 minutes after you drink the drink,
    a. We will collect a blood sample (1 mL, 0.67 Tbsp)
12. At 15 minutes after you drink the drink,
    a. We will collect a blood sample (1 mL, 0.67 Tbsp)
13. At 30 minutes after you drink the drink,
    a. We will collect a blood sample (11 mL, 0.7 Tbsp)
14. At 1 hour after you drink the drink,
    a. We will collect a blood sample (11 mL, 0.7 Tbsp)
    b. We will collect a urine sample in the container we give to you
15. At 2 hours after you drink the drink,
    a. We will collect a blood sample (24 mL, 1.6 Tbsp)
    b. We will collect a urine sample in the container we give to you
16. At 3 hours after you drink the drink,
    a. We will collect a blood sample (11 mL, 0.7 Tbsp)
    b. We will collect a urine sample in the container we give to you
17. At 4 hours after you drink the drink,
    a. We will collect a blood sample (11 mL, 0.7 Tbsp)
    b. We will collect a urine sample in the container we give to you
18. At the end of the experiment, we will measure near-nude body mass.

Total blood volume collected per experiment:	106 ml (7.16 Tbsp)
Total blood volume collected for study (including screening)	530 ml (35.8 Tbsp; about 1 pint)

3. What are the risks and possible discomforts from being in this research study?

Blood Pressure: We take blood pressure with the method used in a doctor's office. A cuff inflates onthe upper arm. As the cuff slowly deflates, we listen with a stethoscope at the bend in the elbow.
During the short time we inflate the cuff, your arm may feel numb or tingly. The cuff could causemild bruising. There are no lasting problems from this measurement.

Blood Draws: Blood draws often cause mild pain, bruising, swelling, or bleeding. There is also a slight chance of infection or a small clot. If you are nervous about needles, blood pressure and heart rate may increase for a little while. You may also feel lightheaded, sick to your stomach, or may faint.
Using the same techniques used in hospitals keeps the chance of infection minimal. Do not exercisehard for 24 hours before a blood draw.
Special note about blood draws for the experiments: A trained nurse or CRC clinician inserts a thin tube into a vein in your arm or hand through which she can draw blood. To do this, the clinician inserts a needle with the tube around it into your vein. Then the clinician removes the needle. The tube stays in the vein during the whole experiment. The tube allows the clinician to

take more than one blood sample without sticking you with a needle each time. If the first attempt to insert the tube does not work, the clinician may need to try again. She will try again only if you allow. The clinician uses sterile saline to flush the blood out of the tube between blood draws.
Sometimes the tube can stop letting the clinician draw blood through it. If this occurs, the clinician removes the tube. You may stop the experiment if you wish or you may proceed. If you allow, the clinician may insert a new tube in your vein. Or you may have the clinician stick you with a needle for each of the rest of the blood draws. The tube does not stay in your vein longer than 5 hours. At the end of the experiment, the clinician removes the tube from your vein. The maximum,total volume of blood drawn over whole study is 530 mL. This is about 1 pint. You will take at least 5-6 weeks to complete the study. A typical Red Cross blood donation is 500 ml (1 pint) drawn in less than 15 minutes.

Tape and adhesive disks: You could be sensitive to the adhesive of the tape used in the study. The tape could cause redness, rash, tenderness, and/or itching. We remove the tape carefully. Ointmentis available, if needed.

Screening: You may feel shy about giving health information. The staff collects the information in a private and professional manner. You may feel shy about being measured. You may request someone of the same sex to conduct parts of the screening.

Initial screening form: Only members of our lab group use this form. We use the form to help decide whether you are a good candidate for the study. You may feel shy about answering questions. You may request someone of the same sex to ask you the questions. We collect the information in a private and professional manner. We keep the completed form confidential and secure.

Test Drinks and Placebo: Drinks used are Generally Recognized As Safe (GRAS) by the FDA. The test beverages are: distilled water, a drink with 8 amino acids, 60 mmol/L sodium, 20 mmol/L potassium + citrate, chloride, a drink with 8 amino acids, 30 mmol/L sodium, 10 mmol/L potassium + citrate, chloride, a drink with ultra-filtered milk permeate (containing trace amounts of milk protein), 2% glucose, 2% galactose, 21 mmol/L sodium, 28 mmol/L potassium, Pedialyte, and Gatorade. You drink all test drinks in fluid form. There are no known risks or allergens associated with these drinks. All the drinks have ingredients that are found naturally in the body.

Near-Nude Body Weight: We will measure your body mass using a method called near-nude bodymass. You will be in underwear and a bath robe while on the scale. The known weight of the bathrobe is subtracted to get a near-nude body mass. You may feel uncomfortable during the measurements. All measurements are taken professionally and privately.

There is a risk of loss of confidentiality if your information or your identity is obtained by someone otherthan the investigators, but precautions will be taken to prevent this from happening. The confidentialityof your electronic data created by you or by the researchers will be maintained to the degree permitted by the technology used. Absolute confidentiality cannot be guaranteed.

4. What are the possible benefits from being in this research study?

4a. What are the possible benefits to you?

You receive a screening that informs you about your health such as your current blood pressure.You could gain knowledge about how your body works.

4b. What are the possible benefits to others?
Older adults have a greater risk for dehydration because they do not drink as much and the fluid theydo drink does not stay in the body as long. Sodium can help keep water in the body longer, but adults are often put on low sodium diets. Glucose can also help with staying hydrated, but this can cause upsetstomach and add extra unwanted calories. The results from this study may help keep older adults hydrated. In addition, this project provides valuable experience, education, and partial fulfillment of degree-work for graduate and undergraduate students of The Pennsylvania State University.

5. What other options are available instead of being in this research study?
You may decide not to participate in this research.

6. How long will you take part in this research study?

Description of study/informed consent	1 hour
Screening visit	30 minutes to 1 hour
Experimental visits	5 hours (x6, seven with repetition)

Total: 32 hours (1 screening visit + 6 experimental visits), 37 hours with repetition (1 screening visit + 7 experimental visits)

7. How will your privacy and confidentiality be protected if you decide to take part in this research study?
We make efforts to limit the use and sharing of your personal research information to people who havea need to review this information. We keep the list that matches your name with your code number in a locked file or password protected file on a computer in a room that is locked when unoccupied. Only authorized members of the lab have access to the list. We label your research records with your code number and keep them in a locked file or password protected computer in a room that is locked when unoccupied. Federal law provides additional protections of your medical records and related health information. These are described in an attached document.

In the event of any publication or presentation resulting from the research, we do not share your personally identifiable information.

We do our best to keep your participation in this research study confidential to the extent permitted by law. However, it is possible that other people may find out about your participation in this research study. For example, the following people/groups may check and copy records about this research: The Office for Human Research Protections in the U. S. Department of Health and Human Services, The Institutional Review Board (a committee that reviews and approves research studies), and The Office for Research Protections.

Some of these records could contain information that personally identifies you. We make reasonable efforts to keep the personal information in your research record private. However, we cannot guarantee absolute confidentiality.

8. What happens if you are injured as a result of taking part in this research study?
In the unlikely event you become injured as a result of your participation in this study, medical careis available. It is the policy of this institution to provide neither financial compensation nor free

medical treatment for research-related injury. By signing this document, you are not waiving any rights that you have against The Pennsylvania State University for injury resulting from negligence ofthe University or its investigators.

9. Will you be paid or receive credit to take part in this research study?

Experiments: $50.00 (6 visits)Total =
$300.00
You receive payment for experiments not completed. We pay an amount of money equal to the part you complete. For instance, if you complete half of the experiment, you would get $25.00. ($25.00 is one-half of $50.00). We may ask you to repeat a trial. If you agree to repeat a trial, you receive paymentfor the repeated trial as stated above. We reimburse for gasoline if you live more than 20 miles from Noll Lab.

10. Who is paying for this research study?

The sponsor Dairy Management, Inc. is paying PSU for this research to be done.

11. What are your rights if you take part in this research study?

Taking part in this research study is voluntary.

- You do not have to be in this research.
- If you choose to be in this research, you have the right to stop at any time.
- If you decide not to be in this research or if you decide to stop at a later date, there will be no penalty or loss of benefits to which you are entitled.

The person in charge of the research study can remove you from the research study without your approval. Possible reasons for removal include if the researcher deems that your health or behavior adversely affects the study or increases risks to you beyond those approved by the Institutional Review Board and agreed upon by you in this document. You may decline to answer certain questions. You may decide not to comply with certain procedures. However, your being in the study may be contingent upon answering these questions or complying with the procedures. During the course of the research you will be provided with any new information that may affect your health, welfare or your decision to continue participating in this research.

If you withdraw from the study, the data collected to the point of withdrawal remains part of the study database and may not be removed.

12. If you have questions or concerns about this research study, whom should you call?

Please call the head of the research study

- In charge of experiments:
  - S. Tony Wolf (W: 814-863-8557, M: 559-269-5198)
- The research nurse, Susan Slimak RN (W: 814-863-8556, H: 814-237-4618)

if you:

- Have questions, complaints or concerns about the research.
- Believe you may have been harmed by being in the research study.

You may also contact the Office for Research Protections at (814) 865-1775, ORProtections@psu.eduif you:

- Have questions regarding your rights as a person in a research study.
- Have concerns or general questions about the research.

- You may also call this number if you cannot reach the research team or wish to offer input or to talk to someone else about any concerns related to the research.

INFORMED CONSENT TO TAKE PART IN RESEARCH

Signature of Person Obtaining Informed Consent

Your signature below means that you have explained the research to the subject or subject representative and have answered any questions he/she has about the research.

_____ _____ _____

Signature of person who explained this research Date Printed Name

(Only approved investigators for this research may explain the research and obtain informed consent.)

Signature of Person Giving Informed Consent

Before making the decision about being in this research you should have:

- Discussed this research study with an investigator,
- Read the information in this form, and
- Had the opportunity to ask any questions you may have.

Your signature below means that you have received this information, have asked the questions you currently have about the research and those questions have been answered. You will receive a copy of the signed and dated form to keep for future reference.

Signature of Subject

By signing this consent form, you indicate that you voluntarily choose to be in this research and agree to allow your information to be used and shared as described above.

_____ _____ _____

Signature of Subject Date Printed Name

CONSENT FOR RESEARCH

The Pennsylvania State University

Title of Project: Efficacy of a milk-permeate solution as a sports rehydration beverage in young well-trained cyclists.

Principal Investigator: W. Larry Kenney, Ph.D

Address: 102 Noll Laboratory, University Park, PA 16802

Telephone Number: (814) 863-1672

Subject's Printed Name: _____

We are asking you to be in a research study. This form gives you information about the research.

Whether or not you take part is up to you. You can choose not to take part. You can agree to take part and later change your mind. Your decision will not be held against you and there will be no penalty

KEY INFORMATION

The following is a short summary of this study to help you decide whether or not to be a part of this research. More detailed information is listed later in this form. If you have any questions, be sure to ask the study team.

Why am I being invited to take part in a research study?

We invite you to take part in a research study because we are looking for healthy recreational athletes aged 18-45 years. We think you may be a good fit for this study.

What is the purpose of this research study?

The purpose of this voluntary research study is to see how well different drinks hydrate you after you are dehydrated. We also want to see how these drinks affect your ability to do physical activity.

How long will the research study last?

The study will take about two months to complete. You will be asked to return to the research site for the following visits:

Screening (1 visit) – about 2 hours
Experimental Visits:
Evening Visits (6) – 30 minutes each (3 hours total)Morning Visits (6)
– 4 hours each (24 hours total)
Total – About 29 hours

What will you need to do?
There are 6 trials for this study. The trials in this study are as follows:

1) No drink
2) Distilled water
3) Gatorade
4) Pedialyte
5) BodyArmor
6) GoodSport

Subjects will complete all trials in a random order. For each trial, you will be asked to conduct an overnight fast and restrict fluid ingestion. You will not eat or drink any fluids during this time. The next morning when you return to the lab, you will cycle on a stationary bike at a moderate intensity for 1 hour. During this hour, you will drink 2.5 mL of fluid for each kilogram you weigh at each 20, 40, and 60 minutes. You will drink oneof the five beverages, or drink nothing at all. You will then perform an exercise time-trial on a stationary bike. You will then complete a 1-hour cool-down on the bike at a light intensity. You can drink as much fluidas you want during the cooldown.

What are the main risks of taking part in the study?
For this study, the main risks to know about are: discomfort with exercising in the heat; discomfort with needles during blood draws; allergies to tape or latex; infection from blood draws. The study may also be unsafe for those who with bleeding disorders or nosebleeds. More information about risks can be found in the section labeled "What are the risks and possible discomforts from being in this research study?"

What are the possible benefits to you that may reasonably be expected from being in the research?
We cannot promise any benefits to you from your taking part in this study. You receive a screening that informs you about your health such as your blood pressure and cholesterol levels. You could learn about how your body works. The study may benefit people in the future by helping us learn more about how different drinks affect hydration and performance when exercising in the heat.
What happens if you do not want to be in this research?
Participation in research is completely voluntary. You can decide to participate or not to participate.

DETAILED INFORMATION
The following is more detailed information about this study in addition to the information provided above.

1. **Why is this research study being done?**

This research is being done to find out how a new drink containing milk permeate affects cycling performance in recreational athletes compared to other sports drinks. Staying hydrated is good for health and lowers your risk of disease. Hydration also helps your mental function and can help you do physical activity. Many people use drinks with ingredients that help you go to the bathroom less in order to stay hydrated. This means more fluid stays inside your body. Past studies have shown that milk keeps you just as hydrated as these drinks. A new drink has been made from by-products created during milk filtration. This drink contains milk permeate, which has the same electrolytes asmilk, but no fat or protein. Our lab recently showed that this drink was even more hydrating than a common sports drink in young adults at rest. We do not know if this is true in young adults during exercise.

Approximately 20 people will take part in this research study at Noll Laboratory at PennsylvaniaState University.

2. What will happen in this research study?

_____initial **A. Screening**

You come to the lab for a screening visit to see if you qualify for this study.

1. You drink only water and do not eat for 12 hours before the screening.
2. The research staff measures the following:
    a. Height;
    b. Weight;
    c. Waist circumference;
    d. Blood pressure;
    e. Heart rate;
3. You complete a health history questionnaire.
4. Women of childbearing age have a urine pregnancy test.
5. The research staff will draw 30 mL (2 Tbsp) of blood from a vein in your arm. Some of the blood will be sent to a lab to see if the proteins, blood cells, electrolytes, etc. are within normal levels. We do not look for the presence of disease (e.g. HIV). All the blood tests are common tests to determine your health status.
6. You will complete a test on a stationary bike to see how fit you are.
    a. We will ask that you arrive for the exercise test having refrained from eating, drinking caffeine or alcohol and smoking for 3 hours and vigorous exercise for 24 hours. We will also ask that you wear clothing and shoes that you will be comfortable exercising in.
    b. We will begin by placing a blood pressure cuff around your upper arm to allow us to monitor your blood pressure.
    c. The blood pressure cuff will remain on your arm for the rest of the test, but will only be inflated at the end of each stage of the test.
    d. You will wear a heart rate monitor across your chest. This allows us to observe your heart rate during the test.
    e. We will explain how to rate your effort during the test and we will show you hand signals so that you can communicate with us throughout the test.
    f. Next, you will be asked to wear a snorkel-like mouthpiece and nose clip. This allows us to look at how hard you are working during the test.
    g. You will begin cycling at a rate of 50 rpm. You will cycle at this rate for the whole test.
    h. The resistance will begin at 75 Watts. Resistance will increase by 25 Watts every 2 minutes.
    i. You will pedal at 50 rpm until you have reached your maximal capacity, you request to stop, or if study personnel see something that causes them to stop the test.
    j. Once the test is complete, we will have you cycle for several minutes at a slow speed while we continue to monitor you.
    k. You will then sit for several minutes until your heart rate and blood pressure start to return to pre-exercise levels
7. You will do a practice time trial. You will try to pedal 5 kilometers as fast as possible. This test should take ~10-15 minutes to complete. During this time, you will pedal as hard as you can

until you finish the 5 kilometers. The research team will tell you when you have reached thispoint. This will get you familiar with how the time trial will be during the experimental visits.

8. If you are eligible, we invite you back to the lab for the study visits.

_____initial **B. Day 1 (Evening Visit):**

1. Within 24 hours before experiment.
   a. Do not exercise hard. Casual walking is fine.
   b. Please drink at least six 8 oz. glasses of fluid.
   c. Do not take drugs (prescription or over-the-counter), herbals, or supplements that could affect hydration status.
   d. Do not consume alcohol.
2. For 12 hours before experiment, do not consume caffeine (chocolate, coffee, tea, Pepsi, etc.).3. We will measure your heart rate, blood pressure, and oral temperature.
4. You will provide a urine sample.
5. A nurse will obtain a small blood sample.
6. You will step on a scale to measure your weight.
7. You will be provided a pill that measures your core temperature. You will swallow this pill 2 hours before each morning experiment.

7. You will go home until the following morning. You will be instructed to not eat or drink anything for the rest of the time until the morning experiment.

_____initial **C. Measurements of Body Core Temperature**

Temperature-sensing pill:

1. Intestinal temperature is measured with a temperature-sensing pill.
2. You return the pill wrapping to the research team when you arrive at the lab on Day 2.
3. This temperature is recorded every 5 minutes using a remote detector.
4. This pill should leave your body within about 24-36 hours.

_____initial **D. Day 2 (Morning Visit):**

There are six experimental trials for this study, one for each drink. At any point, the research team may ask you to repeat an experimental trial, if necessary.

Baseline Measurements:

1. You will enter into an environmental chamber. This room is set to 30°C (85°F, 20% relative humidity). The room will remain at these conditions for the whole study. You will be in this room for 30 minutes before beginning any exercise to get used to the temperature. Baseline measurements are obtained during this 30 minute period.
2. We measure your entry vitals (HR, BP, oral temperature).
3. You sit quietly for 10 minutes.
4. The nurse inserts a catheter in an arm-vein and obtains a blood sample (24 ml, 1.6 Tbsp). We measure the amount of plasma in the blood, as well as the number of blood cells and electrolytes in the blood. This will be done for all blood samples obtained during the experimental trials.
5. You go to the bathroom to empty your bladder and give us the urine sample. We measure how much the urine weighs and the particles in the urine. We will do these measurements for all urine samples during the experimental studies.

6. We record your body weight on a scale. The research team leaves the study room while you change into a provided clean robe. You may keep wearing your undergarments under the robe. You then step on a scale that will measure your body weight. We then measure the robe. The weight of the robe is subtracted from your body weight. This gives us an estimate of your actual weight.

Moderate-intensity Cycling and Rehydration Protocol:

1. A blood pressure cuff will be placed around your arm.
2. A heart rate monitor with a strap is placed around your chest at the level of your heart to measure heart rate/rhythm during the test.
3. Resting blood pressure and heart rate are measured.
4. Blood pressure and heart rate will be recorded every 5 minutes during the study.
5. We will measure your core temperature throughout the study.
6. You will exercise on stationary cycle ergometer for 60 minutes. You will pedal at a moderate intensity (50-60% VO_{2max}) ; ~5-6 on a scale of 1-10).
7. You will either drink one of the five beverages or no fluid during this period. If it is a drinking trial, you will drink at 20, 40, and 60 minutes. You will be given a cup with the assigned fluid for that day at each of these time points. Each cup will contain 2.5 mL of fluid for each kilogram you weigh. For example, if you weigh 80 kg, the cup will contain 200 mL of fluid (80 x 2.5 = 200). The order of the beverages is randomized.
    a. No fluid
    b. Distilled water
    c. Gatorade
    d. Pedialyte
    e. BodyArmor
    f. GoodSport
8. After you finish cycling, we measure your vitals (HR, BP, oral temperature).
9. We obtain a blood and urine sample.
10. We record your weight on a scale.

Time Trial:

1. After finishing all measurements following the 1 hour of cycling, you will complete an exercise time trial. This measures your exercise capacity.
2. A blood pressure cuff will be placed around your arm.
3. A heart rate monitor with a strap is placed around your chest at the level of your heart to measure heart rate/rhythm during the test. Resting blood pressure and heart rate are measured.
4. Blood pressure and heart rate will be measured every 2 minutes during the time trial.
5. We will measure your core temperature throughout the study.
6. This stage will occur in the chamber at 30°C (85°F) and 20% relative humidity.
7. You will immediately then pedal 5 kilometers on the cycle ergometer; this will take around 10-15 minutes).
8. You will try to pedal as hard as you can.
9. After the time trial, we collect a blood and urine sample.
10. We record your body weight.

Cooldown Exercise and Ad libitum Fluid Consumption:

1. Immediately after the post-time trial measurements, you will get back on the bike and do a 1 hour cooldown. You will pedal at a light intensity (30-40% VO_{2max}, about a 3-4 on a scale of 1-10).
2. A blood pressure cuff will be placed around your arm.
3. A heart rate monitor with a strap is placed around your chest at the level of your heart to measure heart rate/rhythm during the test.
4. Resting blood pressure and heart rate are measured.
5. Blood pressure and heart rate will be recorded every 5 minutes during the study.
6. We will measure your core temperature throughout the study.
7. You may drink as much of the assigned fluid as you want during this period.
8. Fluid will be in a large cup resting on a scale. You will place the cup back on the scale each time you take a sip so we can measure how much you drank.
9. After you finish this 1 hour of cycling, we record your body weight.
10. We collect a blood and urine sample.
11. We measure HR, BP, and temperature.
12. This concludes the trial. You will be offered a light snack and water.

3. What are the risks and possible discomforts from being in this research study?

- Test Beverages: This study involves the use of natural substances that are known by the FDA as Generally Recognized As Safe (GRAS). The test beverages are: distilled water, GoodSport (ultra-filtered milk permeate), Pedialyte, Gatorade, and BodyArmor. These drinks are consumed in fluid form. There are no known associated risks or allergens with these beverages. All these beverages have ingredients that are found naturally in the body.

- Temperature-sensing pill: Although very rare, there are some risks to taking this pill. The pill could be inhaled into the lungs. The pill could also cause a puncture, blockage or infection of the intestines. This may require endoscopy or surgery to remove it. This pill should not be taken by anybody weighing less than 80 pounds, or by anybody who has or has had any gastrointestinal disease or surgery.

- Exercise: It is possible that you may experience faintness, fatigue, muscle pain, or chest pain during the exercise bouts. These possible side effects are not uncommon in activities that require physical exertion. Your heart rate, blood pressure, and core temperature will be monitored for the duration of the protocol. You may communicate any difficulty you experience. You may end the experiment at any time. All lab personnel are certified and have current CPR/AED and First Aid qualifications. There is an AED in the laboratory. In any emergency, 911 will be called immediately.

- Overnight fluid restriction at home: It is possible that you may experience faintness, fatigue, physical exhaustion, and thirst during the fluid and food restriction protocol. This protocol is designed to only cause mild dehydration. This protocol has been used by our lab in the past without any adverse events. You may communicate any difficulty you experience. You may end the experiment at any time. All lab personnel are certified and have current CPR/AED and First Aid qualifications. There is an AED in the laboratory. In any emergency, 911 will be called immediately.

- Environmental Chambers: The chamber uses a controller to optimize stability and response. Air enters through the ceiling and returns through base molding on three sides. Because air returns behind the walls, wall temperature increases linearly with air temperature. You will complete 6 trials

in a 30°C (85°F), 20% humidity environment. You may feel some discomfort due to increased body temperature and sweat on the skin. This is normal in the heat. These chambers have been used for25 years in our laboratory without any incidence of adverse events.

- Blood Pressure (manual, CardioCap 5): The manual method and CardioCap5 use a cuff that inflates on the upper arm. The cuff slowly deflates while the researchers listen to pulse-sounds at the inside of the elbow with a stethoscope. The CardioCap 5 automatically takes a measurement. The inflated cuff may make the arm feel tingly and numb. The cuff may temporarily bruise the arm. Efficient and competent measurement technique minimizes the duration of cuff inflation. These techniques are unlikely to produce lasting ill effects.

- Blood draw: Blood draws can cause anxiety (with increased heart rate and blood pressure), mild pain, swelling, nausea, lightheadedness, fainting, or bleeding. There is a slight chance of infection. A nurse performs blood draws using standard procedures and techniques that minimize the chance of infection. Participants may recline for the procedure.

- Tape and sticky disks: The tape or sticky disks could cause a rash. During screening, you tell us if you are sensitive to tape. If a disk sticks very strongly, removing the disk could cause an abrasion like a rug-burn on your skin. An abrasion can feel tender or slightly painful, and can increase risk of infection. If you are sensitive to tape, you may have an increased chance for abrasion. An abrasion has occurred only twice during the years that the disks have been used in similar studies in our lab. We may use an adhesive remover like that used in a doctor's office to remove the disks. If you get an abrasion a nurse checks the site. Antibiotic ointment and a sterile bandage are applied. We tell you how to take care of the site. You could have an allergic reaction to the adhesive remover. The reaction could include rash, itching, fever, or breathing problems. Also, it could include changes in pulse, and/or blood pressure, convulsions, shock, and/or fainting. If a bad reaction occurs, we call for medical help right away.

- Screening: You may feel shy about giving health information. The staff collects the information in a private and professional manner. You may feel shy about being measured. You may request someone of the same sex to conduct parts of the screening.

- ECG: The researchers attach three to twelve electrodes to your chest and then attach the electrode wires to an ECG machine. The machine records the electrical activity of the heart. There are no adverse effects from this measure. A subject may be shy about electrodes applied to the chest. The staff carefully remove the tape afterward. They conduct the test professionally and privately.

- Latex: Some gloves and medical materials are made of latex rubber. Some people may be sensitive to latex. We exclude those with a known latex allergy.

There is a risk of loss of confidentiality if your information or your identity is obtained by someone otherthan the investigators, but precautions will be taken to prevent this from happening. The confidentialityof your electronic data created by you or by the researchers will be maintained as required by applicablelaw and to the degree permitted by the technology used. Absolute confidentiality cannot be guaranteed.

4. **What are the possible benefits from being in this research study?**

4a. What are the possible benefits to you?

There is no guarantee that you will benefit from this research. The possible benefits you may experience from this research study include receiving a screening that informs you about your healthsuch as your current blood pressure and blood cholesterol levels. You could gain knowledge about how your body works.

4b. What are the possible benefits to others?

The results of the research may help scientists to better understand how a drink containing milk permeate affects hydration and athletic performance. While a drink containing milk-permeate improves hydration status at rest in young adults, it is not known how these findings translate to stressed conditions, such as exercise in the heat. Currently, popular drinks for improving hydrationcontain carbohydrates and electrolytes that may provide an unwanted amount of sugar. It is important to look at other drinks that can improve hydration during physical activity. The project provides valuable experience and education for graduate and undergraduate students of The Pennsylvania State University.

5. **What other options are available instead of being in this research study?**

You may decide not to participate in this research study.

6. **How long will you take part in this research study?**

If you agree to take part, the study will take about two months to complete. You will be asked toreturn to the research site for the following visits:

Screening (1 visit) – about 2 hoursExperimental
Visits
Evening Visits (6) – 30 minutes each (3 hours total)Morning Visits (6)
– 4 hours each (24 hours total)
Total – About 29 hours

7. **How will your privacy and confidentiality be protected if you decide to take part in this research study?**

7a. What happens to the information collected for the research?

Efforts will be made to limit the use and sharing of your personal research information to people whohave a need to review this information. Reasonable efforts will be made to keep the personal information in your research record private. However, absolute confidentiality cannot be guaranteed.

- We keep the list that matches your name with your code number in a locked file or password protected file on a computer in a room that is locked when unoccupied. Only authorized members of the lab have access to the list.
- We label your research records with your code number and keep them in a locked file or password protected computer in a room that is locked when unoccupied.
- We label your research samples with your code number. We keep the samples in a dedicated ultralow freezer in Noll Lab until analysis.

All research specimens sent to outside labs for analysis (e.g. Quest Labs) are identified only by acode number.

In the event of any publication or presentation resulting from the research, no personally identifiable information will be shared.

We will do our best to keep your participation in this research study confidential to the extent permitted by law. However, the following people/groups may check and copy records about this research.

- The Office for Human Research Protections in the U. S. Department of Health and Human Services
- The Institutional Review Board (a committee that reviews and approves research studies) and Penn State's Office for Research Protections.

7b. What will happen to my research information and/or samples after the study is completed?Your information or samples that are collected as part of this research will not be used or distributed for future research studies, even if all of your identifiers are removed.

8.. What are the costs of taking part in this research study?

8b. What happens if you are injured as a result of taking part in this research study?

In the unlikely event you become injured as a result of your participation in this study, medical care is available. It is the policy of this institution to provide neither financial compensation norfree medical treatment for research-related injury. By signing this document, you are not waiving any rights that you have against The Pennsylvania State University for injury resulting from negligence of the University or its investigators.

9. Will you be paid or receive credit to take part in this research study?

You will receive $75 per **experimental** visit for your participation in this research study. Additionally, you will receive a bonus of $100 if you finish all experimental visits, for a total of $550. You will be compensated the same amount of $75 for any trials that are necessary to be repeated. If you do not complete the study for any reason, you will be paid for the visits you have completed. You will need to provide your social security number and address to receive a check for payment and for tax reporting purposes.

10. What are your rights if you take part in this research study?

Taking part in this research study is voluntary.

- You do not have to be in this research.
- If you choose to be in this research, you have the right to stop at any time.
- If you decide not to be in this research or if you decide to stop at a later date, there will be no penalty or loss of benefits to which you are entitled.

The person in charge of the research study or the sponsor can remove you from the research study without your approval. Possible reasons for removal include if we deem that your health or behavior adversely affects the study or increases risks to you beyond those approved by the Institutional Review Board and agreed upon by you in this document.

During the course of the research you will be provided with any new information that may affect your health, welfare or your decision to continue participating in this research.

11. If you have questions or concerns about this research study, whom should you call?

Please call the following research support staff at the following numbers if you:

- Have questions, complaints or concerns about the research, including questions about compensation.
- Believe you may have been harmed by being in the research study.

- Craig Berry (W: 814-863-8556, C: 937-708-0696)
- Stephen (Tony) Wolf (W: 814-863-8556, C: 559-269-5198)
- Susan Slimak (W: 814-863-8556, C: 814-880-4396)

You may also contact the Office for Research Protections at (814) 865-1775, IRB-ORP@psu.edu if you:

- Have questions regarding your rights as a person in a research study.
- Have concerns, complaints, or general questions about the research.
- You may also call this number if you cannot reach the research team or wish to offer input or to talk to someone else about any concerns related to the research.

You may visit the Office for Research Protections' website at https://www.research.psu.edu/irb/participants for:

- Information about your rights when you are in a research study;
- Information about the Institutional Review Board (IRB), a group of people who review the research to protect your rights; and
- Links to the federal regulations and information about the protection of people who are in research studies. If you do not have access to the internet, copies of these federal regulations are available by calling the ORP at (814) 865-1775.

INFORMED CONSENT TO TAKE PART IN RESEARCH

Signature of Person Obtaining Informed Consent

Your signature below means that you have explained the research to the subject or subject representative, provided the subject or subject representative an opportunity to discuss and consider whether or not to participate in the research, and have answered any questions the subject or subject representative has about the research.

_____	_____	_____
Signature of person who explained this research	Date	Printed Name

(Only approved investigators for this research may explain the research and obtain informed consent.)

Signature of Person Giving Informed Consent

Before making the decision about being in this research you should have:

- Discussed this research study with an investigator,

- Read the information in this form, and

    - Had the opportunity to ask any questions you may have.

Your signature below means that you have received this information, have asked the questions you currentlyhave about the research and those questions have been answered. You will receive a copy of the signed anddated form to keep for future reference.

Signature of Subject

By signing this consent form, you indicate that you voluntarily choose to be in this research and agree toallow your information to be used and shared as described above.

Signature of Subject	Date	Printed Name

VITA

Craig W. Berry

Education

Ph.D.	The Pennsylvania State University, Department of Kinesiology	2022
M.S.	Miami University (Ohio), Department of Kinesiology and Health	2018
B.S.	Miami University (Ohio), Department of Kinesiology and Health	2016

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4. Serviente, C., **C.W. Berry**, W.L. Kenney, L.M. Alexander. Healthy active older adults have enhanced K+ channel-dependent endothelial vasodilatory mechanisms. American *Journal of Physiology: Regulatory, Integrative and Comparative Physiology*. 319(1): R19-R25, 2020.
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6. Wolf, S.T., **C.W. Berry**, G.A. Dillon. A role for endothelin-A receptors in altered blood flow and pressor responses during exercise in hypertensive adults. *The Journal of Physiology*. 598(3): 441-442, 2019.
7. Ballard, K.D., **C.W. Berry**, C.J. Varty, K.B. Arslain, K.L. Timmerman. Acute aerobic or resistance exercise performed the previous day does not attenuate postprandial hyperglycemia-induced endothelial dysfunction in overweight/obese adults. *European Journal of Applied Physiology*. 119(8): 1855-1863, 2019.
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9. Ballard, K.D., R.M. Duguid, **C.W. Berry**, P. Dey, R.S. Bruno, R.M. Ward, K.L. Timmerman. Effects of prior aerobic exercise on sitting-induced vascular dysfunction in healthy men. *European Journal of Applied Physiology*. 117(2):2509-2518, 2017.