STUDY TITLE: The Impact of Buoy on Hydration Status of Active Men and Women

IRB: STUDY22090018

NCT05768789

Protocol & Statistical Analysis: 06 JUN 2022

TITLE	The Impact of Buoy on Hydration Status of Active Men and Women
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PROTOCOL VERSION	Version 1.0
PROTOCOL DATE	June 6, 2022

BACKGROUND

Maintaining adequate hydration is essential to optimal health [1,2,3] and athletic performance [4,5]. Dehydration can contribute to a host of issues, including altered cognition and digestion, as well as organ dysfunction in the heart, kidneys, and skin [3]. When individuals exercise (particularly in a warm environment), they can lose excessive amounts of fluids through sweating, along with necessary electrolytes (e.g., sodium, potassium, chloride) [5,6]. With dehydration, athletes may feel sluggish and physical performance can suffer [7,8].

Many attempts have been made to improve the hydration status of active individuals [7,8,9,10]. According to the American College of Sports Medicine's position stand, fluids containing sodium should be slowly ingested leading up to activity to help retain fluids, so that athletes maintain euhydration with normal electrolyte levels [6]. Hydration strategies during physical activity should be aimed at preventing fluid loss greater than 2% of the body weight, which can be optimally accomplished by ingesting a diluted carbohydrate/electrolyte beverage at a rate approximately equivalent to sweat loss. During recovery, enough water and food containing sodium should be ingested to replenish electrolyte losses and reestablish euhydration [6]. This approach seems to work well. However, some debate remains over what the best fluid is to consume, particularly with respect to macronutrient type and the specific electrolyte mix.

Related to the above, it is well-accepted that electrolyte replenishment is of importance both during and following exercise, to aid in rehydration for subsequent exercise bouts [6,11]. Electrolytes (sodium in particular) have been used for decades to aid athlete hydration, and this has led to the development of various sport drinks—which also often include moderate amounts of carbohydrate (e.g., Gatorade® (PepsiCo; Chicago, IL, USA) and Powerade® (The Coca-cola Company; Austin, TX, USA)). However, one problem with carbohydrate ingestion is that some individuals experience gastrointestinal (GI) upset following carbohydrate ingestion before [12] and during an event [13], despite very good physical performance outcomes. Due to this GI

upset, some individuals (in particular, recreationally active individuals who are not competing at high levels) rely solely on water and seek a method to ingest the lost electrolytes. In addition, some individuals prefer to have both plain water and an electrolyte beverage during their training/competition sessions, and in some activities (e.g., running and cycling), carrying multiple bottles of fluid is difficult.

Buoy is an all-natural, organic, FDA compliant dietary electrolyte supplement that can be dissolved in 8-12oz water (or other liquid) and provide electrolytes in servings of 1/3 teaspoon. This study will evaluate Buoy in hydrating active adults.

STUDY DESIGN

The proposed study will be conducted as a single-center prospective, cross-over, placebo-controlled clinical trial at the University of Pittsburgh Medical Center (UPMC). Institutional review board approval will be obtained before eligible patients are recruited and consented. Trial will be registered at www.clinicaltrial.gov before beginning recruitment.

STUDY POPULATION

This study will enroll (30) adult subjects who meet eligibility status. We will attempt to include a racially mixed cohort. Enrolled subjects will include healthy volunteers who are physically active (can walk up a flight of stairs).

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Inclusion Criteria

- Male or female, >18 to 45 years of age
- Freely given written consent
- Non-tobacco users
- Negative pregnancy test in women of childbearing potential
- BMI < 35 kg/m2 (or 35?)

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- GFR > 60 ml/min
- No known underlying medical condition
- Willing to refrain from EtOH for 24h prior to test day
- Willing to refrain from strenuous exercise for 24 h prior to each test day
- Acceptable to have one 8oz cup of coffee/liquid on the morning of the test, but must be consistent each visit
- Without active infection of any kind
- Engaged in exercise three or more hours per week

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Exclusion Criteria

- Abnormal creatinine (Cr > 1.2).
- Proteinuria / hematuria / glucosuria based on urine dipstick.
- Diagnosed medical condition that would impede results (CHF, HTN, CAD, CKD, history of electrolyte abnormality).
- Pregnancy
- Use of diuretics within past 2 weeks
- Obesity (BMI > 35)
- Active infection based on symptoms (bacterial or viral)
- Hemodynamic abnormality at screening visit: Blood pressure less than 100/60 or greater than 140/90.

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STUDY METHODS

After signing an IRB approved Informed Consent, subjects who meet the inclusion/exclusion criterion will be enrolled in the study.

Screening Visit: Prior to initiation of the study, participants will have a screening visit to complete informed consent and health history. The history for females will include their last date for menstruation and/or birth control method to take into consideration the effects of ovulation on water retention. During this visit we will measure heart rate, blood pressure, height, weight.

Females will be administered a urine pregnancy test.

Testing Day Protocol: Participants must refrain from vigorous exercise within 24 hours of study visit. Participants will fast (food) overnight for 10h prior to initiation of test. Upon waking they are asked to empty their bowel & bladder. They can consume one 8oz cup of coffee or other liquid. They will present at 7-8am at which time they will be asked to empty their bladder again. After resting for 5 minutes baseline vitals will be taken, including blood pressure, heart rate, weight, bioimpedance. Urinalysis dipstick for protein/blood/glucose and i-STAT measurement for creatinine and electrolytes on Visit 1 to confirm eligibility. For Visit 2 and Visit 3, the participant will be asked to review medical history and any change in status may warrant an additional baseline creatinine and blood/protein test to confirm eligibility. Additional food or beverage will not be allowed throughout the study period. All studies will be repeated in the same subject using either Buoy (intervention) or water (control) or Nuun (intervention). Urine will be collected at four specific timepoints during the intervention and the volume will be recorded. If participants need to urinate between scheduled collection times, urine will be collected, volume recorded, and combined with the urine collection of the following timepoint. These urine samples will be measured and a fraction of it will be sent to the lab to be tested for the following electrolytes: sodium, potassium, chloride and urine osmolarity. Urine creatinine will also be tested at these timepoints.

TABLE 1: Electrolyte and Volume comparison for Buoy and Nuun				
	Buoy (label recommendations)	Nuun (label recommendations)	Buoy (intervention amount with equivalent amounts of Na+ and water to Nuun)	Nuun (intervention amount with equivalent amounts of Na+ and water to Buoy)
Final Volume	237-355 mL (8-12 oz)	475 mL	4% TBW given in 16 divided doses over 4h For 70kg person: 1.68 L solution containing 9.84 ml of Buoy over 4h (or 105 ml every 15 min)	Given as Nuun 1L bolus and then free water in 14 divided doses for total volume of 4% TBW For 70kg person: 1 L with 2 tabs over 30 min, then 680 ml free water over remaining 3.5 hours (or 48.57 ml every 15 min)
Dose	1 squirt (1/3 tsp or 1.64 ml)	1 tab	9.84 ml	2 tabs
Ca	0.5 mg	13 mg		
Mg	0.5 mg	25 mg (as oxide)		
Cl	80 mg	40 mg		
Na	50 mg	300 mg	600mg	600mg
K	10 mg	150 mg		

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VISIT 1---BUOY INTERVENTION: The goal is to give 600mg of Na+ over 4 hours while measuring urine output over 6 hours. The recommended Buoy dosage is ⅓ tsp in 237 ml of water (50mg Na+) multiple times a day (Table 1). Therefore, to safely achieve a total dose 600mg Na+ (6-fold increase from single dose) we will use 2 tsps (9.84 ml) of Buoy diluted in 4% of total body water (TBW), calculated based on subject's weight. Equation will be [*patient weight (kg) * 0.6 (* 0.8 for women) = total body water * 4.0% = liters of water consumed over 6h*] (Ex:

Assuming a male subject weighed 70 kg: 70 * 0.6 = 42 kg TBW * 4.0% = 1.68 L of water/6h). Not to exceed a threshold of 2.5 L volume total. Therefore, a 70 kg subject would consume 9.84 ml of Buoy in 1.68 L of water over 4h. They will consume 6.25% of the total volume every 15min for 4h (105 ml for a 70kg subject).

Prior to initiation of test blood pressure and heart rate will be measured. Urine will be collected and discarded.

At time 0, the participant will begin to drink fluid (drinking 6.25% of their total amount every 15 min). Urine will be collected at 60, 120, 240, and 360 min. Bioimpedence will be measured after each urine collection. Collect urine for a total of 6 hours.

VISIT 2—WATER CONTROL: Participants will ingest the same quantity of water (4% of TBW) at a rate of 6.25% of the calculated amount of water every 15 min for 4 hours. Not to exceed a threshold of 2.5 L to avoid the risk of water intoxication (see adverse events).

Prior to initiation of test blood pressure and heart rate will be measured. Urine will be collected and discarded.

At time 0, the participant will begin to drink fluid (drinking 6.25% of their total calculated amount every 15 min). Urine will be collected at 60, 120, 240, and 360 min. Bioimpedence will be measured after each urine collection. Collect urine for a total of 6 hours.

VISIT 3—NUUN INTERVENTION: The goal is to give a one-time dose of Nuun (600mg Na+) at the start of the trial, diluted in 1L water to be consumed within 30 min (similar to prior published data, Pence 2020). Then they will drink free water for the remaining 3.5 h to equaling total amount of water consumed during Visit 1 and 2. (Ex: for a 70 kg male subject for a total volume consumption of 4% TBW they will consume 1.68 L over 4 hour. The first 1L consumed with Nuun during the first 30 min, and the remaining 680 ml of electrolyte-free water consumed during the next 3.5h (49 ml free water every 15 min).

Prior to initiation of test blood pressure and heart rate will be measured. Urine will be collected and discarded.

At time 0, the participant will consume the beverage over 30 min. Urine will be collected at 60, 120, 240, and 360. Bioimpedence will be measured after each urine collection. Collect urine for a total of 6 hours. Patients will be compensated $300 after completion of three visits.

Analysis:

Urine collected will be analyzed based on per hour and total excretion. Volume will be measured as well as electrolytes (Na, K, Cl) and osms. Venous whole blood will be collected using phlebotomy and 100ul will be analyzed using an i-STAT. The remaining blood will be centrifuged and plasma will be retained for further analysis. Net fluid balance will be determined by subtracting the cumulative urine output from the total fluid load consumed. The beverage hydration index can be calculated as the cumulative urine output of water divided by the cumulative urine output of the test beverage at the same time point.

SPECIFIC AIMS

Evaluate if giving BUOY will decrease the total volume of urine excretion

Determine if BUOY will lead to bigger increase in Total Body Fluid

Determine if there are quantifiable differences between giving BUOY in divided doses versus a bolus of NUUN.

RESEARCH OUTCOMES/VARIABLES

Net fluid balance, electrolytes in urine, labs

ADVERSE EVENTS

Headache, dizziness, hunger

Water intoxication can occur if consuming 4-5 L of water in a few hours, symptoms include headache, cramping weakness, nausea, vomiting, drowsiness and fatigue. To avoid this, we will limit free water intake to no more than 2.5 L per testing day (PMID 12053855).

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