Effects of Increased Water Intake on Uropathogenic Bacterial Activity of Underhydrated Menstruating Premenopausal Females

by

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ABSTRACT

Urinary tract infections (UTIs) disrupt military women's service obligations and health. Females are more susceptible to UTIs due to their unique anatomical features and hormone fluctuations affecting vaginal flora. During phase 1 of the menstrual cycle (onset of bleeding, menstrual cycle days 1-5), estrogen levels significantly decrease and inhibit the growth of lactobacilli, good bacteria that are essential in warding off harmful bacteria and infections, particularly pathogens of UTIs. To reduce UTI onset, it is recommended to frequently urinate with sufficient urine void volume to facilitate washing out harmful bacteria from the bladder and urethra. While menstruating, increased fluid consumption to support urination frequency and void volume may be critical, as the urethra and urinary tract are more predisposed to pathogenic bacteria found. Yet, there is a lack of research investigating the impact of hydration on urinary tract health during menstruation. The study sought to examine the effects of increased water fluid intake on the uropathogenic bacterial activity of underhydrated menstruating premenopausal females. Thirteen females underwent a 2x2 randomized crossover trial to evaluate the effectiveness of a) additional 1.89 L of water fluid intake and b) maintain habitual fluid intake on two subsequent phase 1 menses. At each phase 1 menses, fluid intake was gathered on days 2 and 5 to determine the fluid amount consumed. First-morning urinations on days 3 and 6 assessed urogenital bacterial activity. Combining data collection days 2 and 5 per intervention (INT) and control (CON), the mean±SD for total fluid intake was INT 2.99±1.05 and CON 1.85±0.89, resulting in a 62% increase, $p < 0.001$, $\eta^2 = 0.459$. For days 2 and 5, a 48% and 80% increase in total fluid in from CON to INT was found, $p_s < 0.01$. However, only four cultures detected uropathogenic bacteria from four participants, with no patterns between conditions or days,

making it difficult to determine the effectiveness of the intervention. Though the intervention results were undetermined, military women's hydration, menstruation, and urinary tract health remain prominent health concerns. Efforts to assess their fluid consumption and urination behaviors during menstruation and UTI risks are warranted.

DEDICATION

I dedicate this dissertation to my spouse, son, family, and friends who have supported me unwaveringly. The pleasure of taking you along my Ph.D. journey inspired me to achieve my academic, professional, and personal goals these last four years. Thank you for your invaluable encouragement and reminding me to celebrate the small successes.

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CHAPTER 1

INTRODUCTION

Uncomplicated urinary tract infections (UTIs) are common but painful bacterial infections of the urinary system, often affecting the urethra and bladder in healthy females with a normal structure and functioning urinary tract.[1] Symptoms may include an urgent need to urinate, frequent small urine passes, blood in urine, and a burning sensation when urinating.[1,2] The average UTI symptoms last 6.1 days, restricting 2.4 days of physical activity, and contributing to 1.2 days of absences and 0.4 days in bed.[3] To prevent UTIs' onset and severity, consuming a sufficient fluid amount is recommended to assist adequate urine volumes and the number of voids.[1,2,4] Withholding fluids and bladder retention increases urine concentration and decreases void frequency.[1,2] Low urine volume and minimal voiding are ill-effective in flushing out bacteria in the urethra and bladder, increasing women's risk of developing UTIs'.[1,2] Consuming 2.2 L of fluid and urinating five times per day is positively associated with an optimal hydration status (euhydrated, a state of total body water balance, urine specific gravity <1.013 in 24-hour urine collections) and a healthy urinary tract (urine absent of nitrites, leukocyte esterase, and uropathogenic bacterial activity), indicators of UTI presence.[1,2,5,6] A clinical study found reduced recurrence of UTIs when premenopausal females (with recurrent UTIs) drank an additional 1.5 L of water per day.[7] Thus, women consuming at least 2.2 L of fluids and urinating five times per day could achieve optimal hydration status, reduce AVP secretion, and protect from urogenital bacterial growth in the urinary tract.

The timing of increasing fluid consumption and urination could be critical in decreasing UTI infections, particularly during the early follicular phase of the menstrual

cycle.[8,9] The menstrual cycle fluctuates in estrogen and progesterone hormone levels, ranging in amounts dependent on the menstrual cycle phases (early follicular, phase 1; late follicular, phase 2; ovulatory, phase 3; and mid-luteal, phase 4) (see Figures 1 and 2).[9]

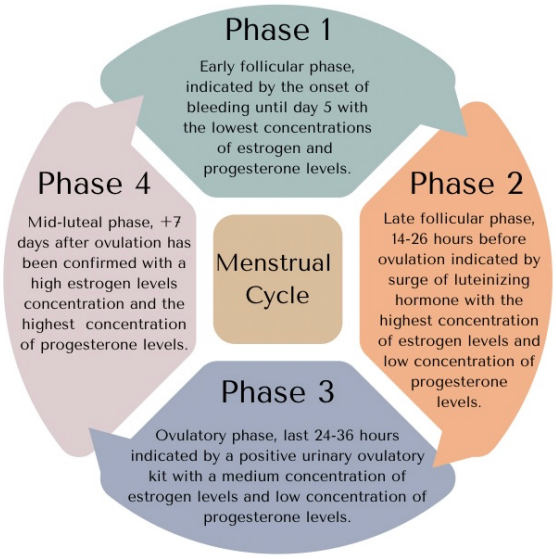


Figure 1. Menstrual cycle phases 1-4 indicated by hormonal fluctuations in estrogen and progesterone.

Estrogen is essential in promoting lactobacilli (good bacteria), which helps maintain an optimal vaginal flora.[1,10] During phase 1 (the onset of bleeding until day 5),[9] estrogen drops to its lowest levels and inhibits the growth of lactobacilli, which is crucial in warding off infections, particularly pathogens of UTIs.[8] Most infections are caused by rectal flora ascending to the bladder after colonizing in the periurethral and urethral environment.[8] The urethra and urinary tract could be most susceptible in hosting an environment favorable for uropathogenic growth when vaginal flora is unstable at phase 1 and lead to an increased risk of UTI onset in phase 2, as rises in estrogen promote continued adhesion. Furthermore, estrogen rises are positively associated with an increased release of arginine vasopressin (AVP; a hormone regulating fluid balance),[2] initiating fluid volume retention and reducing flow in the urinary tract.

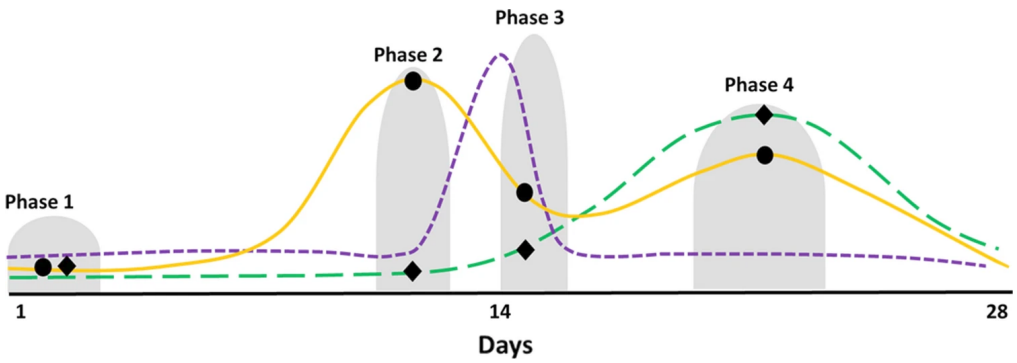


Figure 2. Hormonal changes spanning an average 28-day menstrual cycle, noting each phase's start and end as described in Figure 1. The solid gold line represents estrogen, the short dash purple line represents luteinizing hormone, and the long dash green line represents progesterone. The black dots represent the mean concentration of estrogen during each phase, and the black diamonds represent the mean concentration of progesterone in each phase. Source: Adapted from Elliott-Sale et al.[9]

In the US, approximately 216,000 women serve active-duty in the Department of Defense's military services (Air Force, Army, Navy, Marine Corps) and Department of Homeland Security (Coast Guard).[11] The percentage of women entering the military services has vastly increased from 1.6 percent in 1973 to 16.3 percent in 2018.[11] As more women join the military services, gynecological care is essential to address their unique needs, such as UTIs.[12,13] Over a 13-year surveillance, urinary tract infections affected non-deployed active-duty military females at an overall rate of 130.9 per 1,000 person-years (p-yrs),[14] and in deployed settings, 101.2 per 1,000 p-yrs.[15] Additionally, White Hispanic and non-Hispanic military females had slightly elevated UTI rates, at 72.4 and 72.1 per 1,000 p-yrs, respectively.[14] Compared to non-military women, between 25-40% of women 20-40 years old have had a UTI and account for over 6 million clinic visits, with the estimated number of UTIs being 0.5 p-yr among young adult females.[16]

Thus, military females may not obtain maximal performance and attentional focus necessary to carry out their service obligations if experiencing a UTI. For example, 48% of deployed active-duty military women (n= 841) reported that vaginitis and UTI symptoms

compromised their duties, and 27.4% lost duty time (actively working) due to symptoms.[17] The first-time UTI incident rate among non-deployed active-duty females was 70.4 per 1,000 p-yrs, of which 41.3% (n= 81,948) had a recurrence.[14] Similarly, while deployed, the first-time UTI incident rate was 86.7 per 1,000 p-yrs, with 13.5 percent (n= 978) having a recurrence.[15] UTIs were 26-55% more likely to occur among non-deployed females than deployed females, during the 2008-2013 surveillance period.[15] UTIs' lifetime incidence is prominent among military females (77%).[10] UTI recurrences increases the risk of infertility, sexual transmitted infections (STIs), and HPV, leading to cancer if left untreated[3]; this is because inflammation caused by UTIs increases the risk of persistent infection and weakens the urinary tract, opening an opportunity for HPV if continuing sexual activity, which could further develop into cervical cancer.

Over the last several decades, minimal studies have examined military women's hydration, and those that have found women to not consume enough fluids to maintain a balanced hydration level.[10,18] Military women's occupational demands, long on-duty shifts, the inconvenience of removing protective attire, and access to facilities have led to their limited fluid intake and lack of urinating[4,19-28]; a heightened concern in deployed settings or during training exercises where medical care may not be available or equipped for a suspected UTI.[22-28] The gravity of prolonged dehydration practices negatively impacting military women's urinary tract health and service obligations spurred the development of mobile urinary devices to facilitate urination (extended funnels and battery-powered pads with suction).[19,20] However, innovative studies addressing their hydration and urogenital needs are warranted.

Military females' fluid intake and urination void volume and frequency during phase 1 may influence uropathogenic bacteria found in the urethra and urinary tract. This, in turn, may prevent UTI onset in phase 2 while considering women's fluctuating estrogen levels. Examining the fluid intake and urination behaviors while menstruating (i) voids a gap in the hydration science concerning female's urogenital health and (ii) provides evidence of whether increasing fluids positively impacts uropathogenic bacterial found, potentially fostering improved urogenital health and service obligation maintenance among military women.

Research Questions and Hypotheses

The long-term goal of this research is to develop an effective intervention to promote increased fluid intake, especially during phase 1 of the menstrual cycle, and to reduce military women's UTIs' incidence and prevalence. The overall objective was to determine whether increasing water intake positively impacted uropathogenic bacterial activity found in the urinary tract during phase 1 of the menstrual cycle. To attain the overall objective, the following two questions and specific aims were pursued:

Question 1: What are the fluid intake behaviors and lifetime UTI history of premenopausal Reserve Officers Training Corps (ROTC) cadets?

Specific Aim: To determine the fluid intake behaviors and lifetime UTI history of ROTC cadets. Cadets completed the Beverage Intake Questionnaire (BEVQ) to determine the monthly average of total fluid, water, sweetened-sugar beverages, and alcohol amount consumed. The self-reported lifetime UTI history was collected.

Ha1: It was expected over 50% of ROTC cadets would consume daily monthly average of less than 1.5 L/day and are considered low-volume drinkers potentially risking

urinary tract health.[10,18] It was conservatively estimated that 30% would have a lifetime UTI history.[1,10]

Primary Outcomes: Mean daily total fluid intake and lifetime UTI history

Question 2: Will increased water intake reduce found uropathogenic bacterial activity of underhydrated menstruating premenopausal females?

Specific Aim: To determine whether increased water intake reduced found uropathogenic bacterial activity of underhydrated menstruating premenopausal females. Those identified as underhydrated (consuming a daily monthly total fluid intake average of less than 1.5 L/day and urine specific gravity [USG] ≥1.020, prioritizing those with a UTI history for enrollment) underwent a 2x2 randomized crossover trial to evaluate the effectiveness of a) additional 64 oz (1.89 L, to meet 2.2 L/day) of water fluid intake and b) maintain habitual fluid intake on two subsequent phase 1 menses. At each phase 1 menses, fluid intake and urination diaries were gathered on days 2 and 5 to determine the fluid amount consumed and urination frequency. Additionally, urine samples were collected i) between 4:00-8:00 PM on days 2 and 5 urine to assess hydration status and ii) at the first-morning urinations on days 3 and 6 to assess hydration status and urogenital bacterial activity.

Ha2: It was expected the additional 64 oz of water would increase urination frequency and void volume, leading to and found, reduced uropathogenic bacterial activity compared to their habitual fluid intakes of underhydrated menstruating premenopausal females.

Primary Outcome: Uropathogenic bacterial activity

Secondary Outcomes: Total fluid intake, urination frequency, and USG

Significance of Study

UTIs negatively impact military female's duties with adverse health consequences yet are understudied and ill-addressed within the armed forces.[12,29] The occupational and situational factors of military females may heighten UTI susceptibility compared to non-military females.[22-28] Assessing fluid intake and urogenital health can promote educational-behavioral interventions, potentially leading to restored urogenital health and sustain occupational performances. Preventive measures inclusive of the menstrual cycle phases will most effectively reduce UTIs' onset and long-term health consequences while contributing to service obligations in deployed and non-deployed settings. The results from this study could initiate a notable change of health services, such as offering educational webinars, UTI screenings, self-hydration and UTI assessment training, and fluids supporting urogenital health that will promote females' continuation within the armed services.

Definition of Terms

The following terminology is operationally defined for clarity pertinent to this study regarding hydration[30,31] and promotes a consensus on nomenclature suggested by Elliott-Sale et al. (2021)[9] related to menstrual cycle phases corresponding to hormonal profiles in the sports and exercise science field.

1. Euhydration: A state of total body water balance. The body's systems function most optimally and intracellularly and extracellularly fluid volumes are sustained.[30,31]
2. Underhydration: A low water intake in the absence of a total body water deficit, thirst, or elevated plasma osmolality, though elevated arginine vasopressin and urine biomarkers are seen.[30,31]

3. Hypohydration: A state of total body water deficit due to acute or chronic dehydration.[30,31]
4. Dehydration: The process of fluid loss when fluid output is higher than fluid intake, in which maintaining bodily system functions begin to fail. The most considerable fluid loss during exercise is via sweating. *Acute* dehydration occurs under certain circumstances of extreme heat exposure or extended physical activity. *Chronic* dehydration is recurrent, regardless of daily fluid intake.[31]
5. Overhydration: A higher water intake in the absence of a total body water excess, thirst, or depleted plasma osmolality, though diluted urine biomarkers are seen.[30,31]
6. Hyperhydration: A state of total body water excess, in which the intracellular and extracellular fluid volumes are expanded.[30,31]
7. Exercise-Associated-Hypernatremia: Denoted as EAH, happens when serum sodium levels are less than 135 $mmol/L^{-1}$ during or within 24 hours of physical activity. Thus, too much water is within the body, swelling the organs and tissues and minimal-to-no excretion of fluid excess. EAH is potentially fatal and deemed a medical emergency when serum sodium level drops below 130 $mmol/L^{-1}$.[31]
8. Hydration status: The method(s) to determine the state of fluid balance in the body.[30,31]
9. Uncomplicated urinary tract infection: bacterial infection in a healthy, non-pregnant, premenopausal female with structurally and functionally normal urinary tract.[1]

10. Menstruation/menses: the process of discharging blood and other substances from the uterus lining at intervals approximately one lunar month apart.[9]
11. Naturally menstruating females who undergo menstruation with a cycle length of ≥21 days and ≤35 days without confirmed ovulation.[9]
12. Phase 1: early follicular phase, indicated by the onset of bleeding until day 5 with the lowest concentrations of estrogen and progesterone levels.[9]
13. Phase 2: late follicular phase, 14-26 hours before ovulation indicated by a surge of luteinizing hormone with the highest concentration of estrogen levels and low (<6.36 nmo•L^{-1}) concentration of progesterone levels.[9]
14. Phase 3: ovulatory phase, last 24-36 hours indicated by a positive urinary ovulatory kit with a medium concentration of estrogen levels and low (<6.4 nmo•L^{-1}) concentration of progesterone levels.[9]
15. Phase 4: mid-luteal phase, +7 days after ovulation has been confirmed with a high estrogen levels concentration and the highest (>16 nmo•L^{-1}) concentration of progesterone levels.[9]
16. Oligomenorrhea: a menstrual cycle length >35 days.[9]
17. Secondary amenorrhea: ≥3 consecutive missed menstruations in non-pregnant females with a menses history.[9]
18. Menstrual irregularities: the participation exclusion from the current study self-reporting or diagnosed with oligomenorrhea or secondary amenorrhea.[9]

Scope, Delimitations and Limitations

The study aimed to 1) examine the fluid beverage behaviors and lifetime UTI history of ROTC cadets and 2) determine if there was a significant difference in uropathogenic

bacterial activity after increased water intake of underhydrated premenopausal females. The research focused on increased water intake as a primary prevention to UTIs by increasing urine frequency and void volume among healthy females, though possibly at risk of a UTI. Increased water intake, in turn, facilitate washing harmful bacteria from the urinary tract, reducing bacterial activity (see Figure 3). Participants included full-time ROTC cadets and non-affiliated ROTC females in the metropolitan Phoenix area. The study began in the Athl*ea*t Field Lab in Fall 2021 and was funded by the Arizona State University Sun Devil Athletics Department Research Grant and College of Health Solutions and IRB approved by Arizona State University.

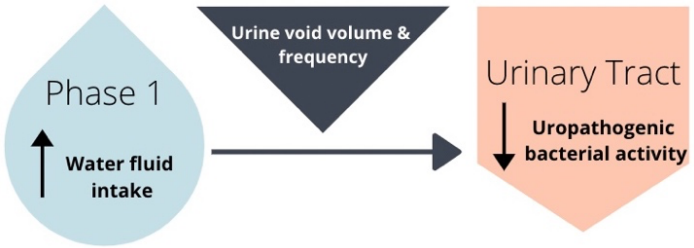


Figure 3. Conceptual framework of fluid intake facilitating urine void and frequency to rid the urinary tract of harmful bacteria found during phase 1 of the menstrual cycle.

Given the limited active-duty military female population in the metropolitan Phoenix area, monetary constraints, and restrained study resources, the first research question focused on ROTC cadets that risked contracting a UTI identified as being underhydrated, with or without a UTI history for the intervention if later qualified for question two. A small population of active-military women (approximately 216,000 members) and military obligations deter participation in health-related clinical research.[11,12] Likewise, service members are generally a hard-to-access population because the military is a transitory organization with unpredictable routines.[12] Thus, research requiring heavy involvement would not appeal to active-duty military women nor be conducive to their variable schedules

and training exercises.[12] Therefore, a proxy population, women within an ROTC program (full-time ROTC cadets and non-affiliated ROTC females in the metropolitan Phoenix area) were first sought, sharing similar demands to active-duty. The study expanded to other uniformed services (military veterans, firefighters, police officers, paramedics, nurses, lifeguards, and park rangers) as a contingency plan for the second study question and intervention component if a low number of ROTC cadets qualified for the intervention, as these organizations too, are similar in occupational demands.

Two of the three University ROTC programs agreed to participate in the study, further downsizing the target sample. Secondly, a change-in-command occurred when the study was initially proposed (January 2021) and collection began (September 2021), shifting leadership priorities. Likewise, a leading female officer was amiss from the two participating ROTC programs, which could have played an integrative role in relaying study information and supportive personnel. Finally, recruitment may have been low due to participation burden of previous and current studies being conducted; two studies were simultaneously collected during Spring 2021 among ROTC programs, with one continuing in Fall 2021 when the present study began. The low participation, and therefore limited qualified cadets for the intervention portion of the study opened the population to women in service (military veterans, firefighters, police officers, paramedics, nurses, lifeguards, and park rangers). Though recruitment was still low, as the study was designed initially within a university setting and those women representing the stated services were unable to commit given shift schedule, emergency calls, low census due to COVID-19, and not living near the University or location where they worked that was close to the University. Recruitment extended to a general premenopausal female population due to logistical concerns of the

local uniformed servicewomen's being unable to commitment to the study involvement. Generalizability on an occupational level is weak, yet physiological measures remain comparable to premenopausal military women consuming low volumes of fluid.

Additionally, this study examined the immediate (24-hours) and short-term (96-hours) effects of increased water intake on uropathogenic bacterial activity of regular menstruating women; future research should explore underhydrated active-duty military females with a UTI history or recurrent UTI diagnosis for an extended prospective increased water intake intervention in (i) deployed and non-deployed settings, (ii) those suppressing menstruation, (iii) variations in hormonal birth control on all menstrual cycle phases, and (iv) with a menstrual cycle irregularity (Oligomenorrhea) or disorder (Premenstrual Dysphoric Disorder). No participants were diagnosed with diabetes or other comorbidities influencing hydration status.

Participants tracked their menstrual cycles in a calendar along with the investigator to help women gauge their expected menstrual start date. Data collection started on women's day 2 of bleeding, when the intervention began. The onset of bleeding (day 1) is unpredictable, making it challenging to begin data collection that's standardized. Day 2 had no additional biological advantage, though had an advantage that's more suitable to conduct less biased results. Participants collected urine samples and completed a form to self-report fluid intake and urination behaviors, though are prone to under-or over-reporting. The urine samples and analysis helped detect discrepancies in self-reported hydration information and actual hydration status given that individuals tend to over- and under-report hydration. All participants were educated on properly reporting fluid intake and urination diaries

and collecting urine samples. To ease participation burden, I did not use a 24-hour urine collection to assess hydration. However, a spot urine sample between 4:00-8:00 PM is comparable against a 24-hour collection.[2] For all urine samples, the clinical practice standard of a "mid-stream clean catch" approach was instructed. Disregarding the "mid-stream clean catch" approach would compromise the integrity of the primary outcome, uropathogenic bacterial activity, by tainting samples with particles not found in the urethra and bladder. Several participants did not follow instructions to handle and store urine samples, thus, recommendations to improve sample quality collection are given in the Discussion section.

To improve adherence to the increased water intake and even consumption throughout the 24-hour day, participants received a 32 oz refillable water bottle, noting the time at which specified water amounts should be consumed. Consuming the total 64 oz at one time point or in short period of time is not recommended, risking hyponatremia or water intoxication. The study examined fluid beverage intake, though 20% of one's daily diet comes from water content in foods.[5,6] The addition of water content from foods was not included to ease the participation burden and lower the costs of added analyses. Furthermore, prospective behavioral interventions may be more successful in motivating and maintaining increased water intake than high water-content foods. Food may introduce unique barriers in taste preferences, costs, and availability; larger-funded studies should examine water content in foods. Any adherence inconsistency to study protocols were reported. No UTI symptoms or incidents occurred during the study timeframe.

CHAPTER 2

REVIEW OF LITERATURE

In 2014, the Armed Forces Health Surveillance Center released the urinary tract infections (UTIs) active component between 2000-2013 and deployed active component between 2008-2013.[14,15] The UTI frequency and recurrences reported by deployed and non-deployed active-duty military females, though not novel, highlighted the need for sex-specific preventative urogenital care. The report suggested increasing urine void volume and frequency to rid the body of uropathogenic bacteria from the urinary tract. However, two recent systematic narratives found minimal investigated studies[2,32]; these differ from systematic reviews because there are not enough studies to support the data analyses required for a review. Furthermore, to the best of my knowledge, there is no research examining the intricacies of military women's hydration status, menstrual cycle phases, and urinary tract health, information that is critical when developing preventative strategies to lessen UTIs. Women play an integrative role in the military services, and as their occupational opportunities expand, so should hydration and urogenital health sciences to best support their needs. The following section provides an overview of (i) UTIs, (ii) hydration and UTIs, (iii) menstrual cycle and UTIs, and (iv) hydration interventions to reduce UTIs and their relation to military women.

Urinary Tract Infections

Definition. Urinary tract infections are bacterial infections associated with the urinary system, affecting the lower (urethra, bladder, and prostate for males; cystitis) or upper (kidneys and ureters; pyelonephritis) tracts.[1] Usual cystitis symptoms are urination

urgency, frequency, painful urination, blood in urine, foul-smelling urine, and suprapubic discomfort; pyelonephritis (characterized by flank pain, nausea and vomiting, fever >38°C), and costovertebral angle pain, with or without cystitis symptoms.[1] Infections can be classified as uncomplicated (healthy non-pregnant female with anatomically and structural sound urinary tract) or complicated (factors increasing bacteria colonization or treatment efficiency); acute (sudden inflammation of the bladder) or chronic (failure to respond to treatment or keep reoccurring).[1] Factors potentially complicating UTIs in women may include being elderly, pregnant, functionally or abnormal urinary tract structure, recent antimicrobial use, diabetes mellitus, immunosuppression, symptoms lasting >7 days, urinary tract surgery and hospital-acquired infections, such as an indwelling urinary catheter (catheter-associated UTI; CAUTI). However, UTIs can be asymptomatic.[1] An acute uncomplicated UTI is the most common,[1] predominantly affecting non-pregnant, premenopausal, and healthy adult females with high recurrence rates (approximately 44-70% of women will have a recurrence within a year after the initial onset),[7] which is the remaining document's focus.

Etiological spectrum. Approximately 80% of uncomplicated upper and lower UTI cases are caused by Escherichia coli (E. coli).[1] Staphylococci saprophyticus (S. saprophyticus) accounts for 5-15% of cases, more common in upper UTI cases.[1] Other causative agents of UTIs include Proteus species, Klebsiella species, Enterococcus species, Pseudomonas species, Enterobacter, Candida species, and Adenovirus type 11.[1]

Diagnosis. Healthcare providers conduct urinalysis and collect urine cultures to diagnose UTIs among non-pregnant premenopausal females suspected of an acute uncomplicated UTI.[1] Urinalysis via the dipstick method looks for elevated white blood cells (pyuria), blood

(hematuria), and nitrites in urine. Pyuria is present in most cases of women exhibiting acute symptomatic UTI. Leukocyte esterase has a 73–84% specificity (ability to designate an individual who does not have an illness as negative) and an 80–92% sensitivity (ability to designate an individual with an illness as positive) for UTI.[1] Positive nitrites are converted from nitrate via certain gram-negative bacteria. Though precise (96–99%), lacks sensitivity due to the conversion.[1] To determine pyuria, urine cultures exhibiting >10 WBC/hpf on a spun specimen via microscopy are most reliable to diagnose an acute uncomplicated UTI.[1] It is highly sensitive (95%) though it has less specificity towards a UTI. Urine microscopy helps identify a contaminated sample, as >15–20 squamous epithelial cells/hpf on microscopy suggest contamination. A $>10^5$ cfu/mL on urine culture is considered the diagnostic for UTI or of at least 10^3 cfu/mL uropathogens with ≥1 UTI symptom.[1,7] However, women with a suspected UTI providing a clean catch, midstream urine sample that is $<10^5$ cfu/mL may require repeating a sample.[1] Likewise, the presence of blood in midstream urine during women's phase 1 menses may be misleading. The combination of a woman's history, physical exam, reported symptoms, urinalysis, and urine culture is considered when making an acute uncomplicated UTI diagnosis.[1]

Treatment. Antibiotics are the first-line treatment for an acute uncomplicated UTI, as they are highly concentrated in the urine and effective in ridding the urinary tract of bacteria.[1] Typical antibiotic regimens are Fosfomycin (3 grams, single dose), Nitrofurantoin (100 mg, 5 days), and Trimethoprim-sulfamethoxazole DS (1 pill, 3 days).[1] Providers will prescribe a stronger antibiotic, Ciprofloxacin (250 mg, 3 days), if bacteria are resistant.[1] Asymptomatic bacteriuria does not require treatment; symptoms and clinical signs should

initiate treatment options to reduce antibiotic use and minimize resistance to them or risk complications in the future.[6]

Prevention. The American College of Obstetricians and Gynecologists,[33] American Urological Association,[1] Centers of Disease Control and Prevention,[34] National Institute of Diabetes and Digestive and Kidney Diseases,[35] US Department of Health and Human Services,[36] and Veteran Affairs Women's Health Guide[4] anecdotally recognize increased fluid consumption as a non-invasive, easy-to-adopt strategy to prevent UTIs listed under "prevention" on their websites. Increased fluid consumption assists in creating adequate urine volumes while increasing the needed number of voids to help rid the body of bacteria that cause UTIs.[2] Other nutritional means via the urinary system have gained interest, including cranberry supplementation. It is believed these products contain proanthocyanidins, a class of polyphenols found in plants, such as berries, that help prevent bacterial adhesion to the urinary tract lining.[1, 37-43] Though promising, research trials which study the use of cranberry supplementation to prevent UTIs lack robust findings and include ill-defined dosages, frequency of consumption, and used various forms of cranberry products including juice, tablets, and cocktails. Further, study populations are not all exclusive to premenopausal women.[37-42] Investigation of other oral non-antibiotic preventative options, such as D-mannose,[37,44] lactobacillus,[45-47] or a combination of cranberry, lactobacillus, and Vitamin C[47] is currently being evaluated. Like the cranberry trials, these studies either lack (i) methodological rigor, (ii) definitions of dosage and frequency, (iii) disregard hydration needs, (iv) and exclusivity to premenopausal women or focus on the gastrointestinal tract.[37,44-48] In addition to increased fluid intake and urination

frequency, other behavioral UTI preventatives include proper genital washing and care and avoid using spermicides, douches, and scented products.[1,4,33-36]

Incidence, morbidity, and risk factors in military women. The Medical Surveillance Monthly Report of February 2014 found 30.4% (n= 198,603) of non-deployed active-duty military females reported a UTI, with 1.6% (n= 3,273) of occurrences requiring hospitalization.[14] The number of UTI recurrences was 41.3% (n= 81,948) with 2.3% (n= 1,914) leading to hospital care.[14] The UTI recurrences ranged from 2-5 UTIs (n= 75,344), 6-10 UTIs (n= 5,888) and >10 UTIs (n= 718) over the surveillance period.[14] UTIs accounted for a yearly average of 2,240 hospital bed days and 4,981 days of lost work time[14]; females accounting for most of these estimates, though sex was not adjusted.

When examining personal characteristics, almost 90% of UTI first-time occurrences were from females ≤34 years of age (n= 180,012) and enlisted personnel (n= 178,320).[14] White Hispanic and non-Hispanic females had slightly elevated UTI rates, at 72.4 and 72.1 per 1,000 p-yrs, respectively.[14] The UTI first-time rates were highest among those serving in the Army (84.8 per 1,000 p-yrs) and Marine Corps (79.6 per 1,000 p-yrs) and recurrence rates for Coast Guard (45.7 per 1,000 p-yrs) females.[14] Those holding armor/motor transport occupations had the highest UTI first-time incident rates (83.5 per 1,000 p-yrs), while communication/intelligence showed the highest recurrence rates (42.2 per 1,000 p-yrs).[14] Lastly, single females had the highest UTI first-time (n= 110,174) and recurrences (n= 46,632) than those married or in other relationship statuses.[14] Compared to non-deployed active-duty males, the UTI first-time and recurrence rates are significantly lower and disproportionately affect females[14]; is this research, circumcision status was not provided.

Over 7,200 deployed active-duty military females reported a UTI incident within the Southwest Asia/Middle East deployment settings.[15] More than half (53.6%) of the females diagnosed with a UTI while deployed also had at least one incident prior in a non-deployed setting.[15] Rates were highest among females under <20 years of age (128.8 per 1,000 p-yrs) and among Blacks and non-Hispanic Whites, 93.8 and 85.4 per 1,000 p-yrs, respectively.[15] Females serving in the Army had the highest UTI incidents (101.0 per 1,000 p-yrs) and recurrences (15 per 1,000 p-yrs).[15] Like non-deployed active-duty females, armor/motor transport occupations had the highest UTI first-time incident rates (108.2 per 1,000 p-yrs), while communication/intelligence showed the highest recurrence rates (15.2 per 1,000 p-yrs).[15] Alternatively, single females had the lowest UTI first-time (75.6 per 1,000 p-yrs) and recurrence (13.1 per 1,000 p-yrs) rates than those married or in other relationship statuses when depoyed.[15] Compared to deployed active-duty males, the UTI first-time (3.3 per 1,000 p-yrs) and recurrence (3.6 per 1,000 p-yrs) rates were significantly lower.[15] The occurrences and rates excluded females deployed at sea (Navy ships) and used confirmed UTI cases within the Southwest Asia/Middle East Theater Medical Data Store (TMDS), underestimating accurate UTI deployment rates.

The risk factors for increasing uropathogenic bacterial activity within the urinary tract and subsequent UTI development for military women include voluntary urinary retention and poor feminine hygiene, a concern in deployed and off-site training environments.[19-28] The combination of poor sanitation conditions, lack of restrooms, minimal privacy, and inconveniences of removing attire led to reduced urine output.[10,19,21,25-28] Between 70-76% of women believed field conditions do not offer sanitary toilet facilities for personal hygiene[10,49]; the sanitation of facilities in non-field conditions were not reported for

comparison. Intentionally restricting fluid intake to avoid the urge to urinate postpones voiding,[26,27,50] compounding UTI risks. Other non-military-specific risk factors positively associated with UTIs in service women were antibiotic use, spermicides, contraceptive diaphragms, douches, hygiene sprays, sexual activity, and multiple sex partners.[1,10]

Hydration and Urinary Tract Infections

Water is regarded as the most fundamental nutrient required to operate bodily systems and safeguard human survival.[2] Approximately 40-62% of an adult's total body mass is water.[51-53] People can survive without water for only a few days.[2,51] The water volume within an individual's body is subject to fluid-food intake and metabolic water and output in the forms of urine, sweat, feces, and evaporation of the respiratory tract and skin (see Figure 4).[53,54] Among women, between 2.8-3.3 L of water per day are turned over, mostly ingested via fluid intake, and excreted in the form of urine.[55] With a high daily water turnover and military women's service requirements,[6,18,555] they must consume adequate fluid amounts to maintain euhydration and, perhaps, protect from UTIs.[2] Increasing fluid intake results in lower urine concentration and increased urine flow, encouraging frequent flushing of the urinary tract, which may help UTI prevention among military women.[2]

Maintaining a water balance state is narrow (1% overhydration and 2% underhydration) as it varies in degree and severity and continuously fluctuates due to the body's minimal storage capacity.[2,31] The water balance state is also tightly regulated by the release of arginine vasopressin (AVP), an antidiuretic hormone acting on the kidneys.[2] Increases in AVP secretion are witnessed when AVP V2 receptors bind to the collecting ducts, initiating renal water saving due to low fluid intake.[2] Copeptin is a surrogate marker of AVP and is positively associated with poor kidney function.[2] Acute and chronic renal water

saving due to the higher AVP release and copeptin circulation could compromise the mechanisms of flushing out uropathogenic bacteria from the urinary tract, risking a UTI. A urine osmolality approaching 500 mOsm/kg or below (or a urine specific gravity <1.013) and urinating 5-7 times per day (2-3 L per day)[2] suggests a sufficient amount of fluid intake (2.2 L of fluid beverages per day)[5,6] to achieve optimal hydration, reduce AVP secretion, and protect from uropathogenic bacterial growth in the urinary tract.[2,7]

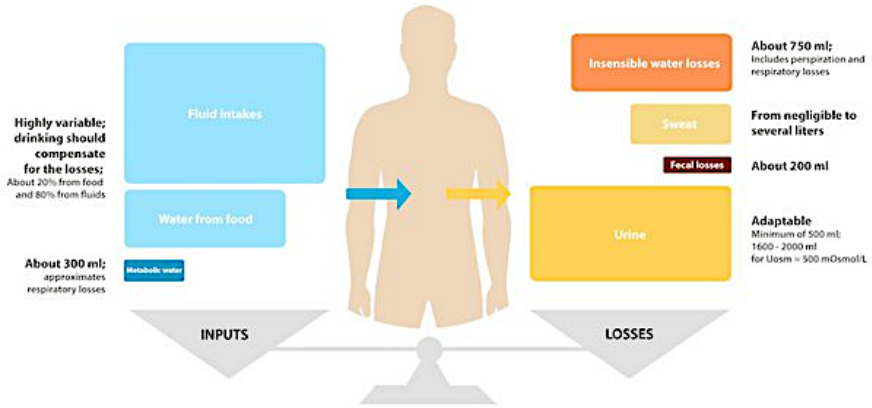


Figure 4. Typical water daily inputs and outputs. Source: Adapted from Hydration for Health (2018).

Hydration and UTI non-experimental studies. Several cross-sectional and observational studies have supported fluid intake's role in women with UTIs and the importance of managing reinfections. Adatto et al. (1979) examined the urine voiding and sexual habits of 84 female university students with a history of recurrent UTI compared to a control group.[56] Sixty-one percent of females with recurrent UTI had a history of voluntary urinary retention of 1-6+ hours compared to 11% of the controls, with fluid intake and sexual practices being similar.[56] Patients underwent a behavioral intervention focusing on regularly urinating when feeling the urge and complete bladder emptying and found it to prevent reinfections.[56] Similarly, Ervin et al. (1980) compared 23 women with dysuria symptoms and more than >105 colonies of a single pathogenic organism per milliliter of a

clean-catch, midstream urine specimen with 64 women with no UTI history at medical centers.[57] Patients with dysuria drank significantly fewer fluids and urinated less frequently than the control group.[57] However, they found no difference in voluntary urinary retention. The differing findings of Adatto et al. (1979) and Ervin et al. (1980) may be in part of the women's age, socioeconomic status, and background differences.[56,57]

Working extended hours could heighten UTIs.[58] Female custodial employees were found to have high UTI rates within an industrial park, consuming low fluid amounts and minimal urination frequency during the workday than custodial males and non-custodial employees.[58] To promote positive hydration behaviors and reduce the incidence of UTIs, Su et al. (2006) implemented a two-year health education intervention.[59] The intervention included providing information about UTIs and UTI prevention during a new employee orientation and seasonal on-the-job training.[59] Additionally, the intervention included promotional campaigns and one-on-one consultations with employees who were found to have previously reported having UTIs.[59] Over 1,600 female employees (n= 1,414 custodial; n= 252 non-custodial) receiving annual health examinations at the park clinic participated.[59] After the intervention, a similar prevalence (both 0.8%) of symptomatic UTIs was found in female custodial and non-custodial employees.[59] Those who participated in the previous study (n= 366) yielded a significant decrease in the prevalence of UTIs (from 9.8% to 1.6%) and increased water intake and urine voiding three times or more during a shift ($p< 0.001$).[59]

Like custodial workers, female healthcare workers may also limit fluid intake and urination frequency during long shift hours.[60] Vyas et al. (2015) conducted a cross-sectional study involving 177 unmarried nursing students who completed a questionnaire regarding UTI symptoms in the previous three months.[60] Almost 20% (n= 35) reported a UTI. Most

consumed 1–2 L of water daily.[60] Those consuming a water intake <1 L were more likely to have a UTI (Odd Ratios, OR = 12.73, 95% CI 3.43-47.2).[60] Over 47% (n= 84) reported urinating only 1–3 times per day, and this population had the highest UTI prevalence.[60] Voluntary urinary retention and UTIs episode was positively associated (p= 0.03).[60] Both Su et al. (2006) and Vyas et al. (2015) target populations have comparable workday hours to military women; additionally, these long working hours and job demands negatively impact fluid intake and urination behaviors.[59,60]

A few researched reported contrary evidence of water intake as a preventive measure for developing UTIS. Remis et al. (1987) did not find volumes of fluid consumed and urination frequency to protect college women from developing UTIs.[61] The cross-sectional study found over 95% ingested three or more glasses (or equivalent) of fluids per day and 85% five or more glasses per day.[61] Women with a UTI urinated a mean of 5.5 times per 24-hour day compared with 5.5 times among upper respiratory infection control and 5.8 times among gynecology control groups.[61] It appears most were euhydrated, accounting for a lack of associations found. However, the authors did not operationally define fluid amounts or hydration states nor included 24-hour urine samples to validate the women's self-reported fluid intake and urination behaviors.[61]

While not strictly among women, Robinson and Rosher (2002) implemented a non-randomized intervention to improve hydration and prevent conditions associated with dehydration, including UTIs among elderly residents in nursing homes.[62] A variety of hot and cold beverages offered on a decorated beverage cart was routinely wheeled to residents throughout the day.[62] Data collection occurred weekly for nine weeks, at baseline (2 weeks), intervention (5 weeks), and post-intervention (2 weeks after cessation).[62] Weekly bioelectric

impedance analysis (BIA) measurements were obtained of each resident's intracellular body water (ICW), extracellular body water (ECW), and total body water (TBW). Conditions, including UTIs, and the amount of fluid intake from the beverage cart, also was recorded. They found a significant increase in fluid in each body compartment and, although not statistically significant, reduced UTIs.[62]

Military women's hydration and UTIs non-experimental studies. Limited studies have investigated the relationship between hydration and UTIs among the military,[10,19,50] let alone their hydration needs.[18,63] Lowe et al. (2003) were among the first to examine situational and behavioral risk factors of contracting genitourinary infections, including UTIs, of deployed military women.[10] They found 18.4% (n= 155) of military women reported a UTI while deployed.[10] Of those reporting a UTI, 91.6% voluntarily retained urine, and 63.4% limited fluid to avoid using restrooms.[10] Albright et al. (2005) found an association between dysuria and self-imposed fluid restriction and postponing urination in field settings.[50] In this study, 112 military women with acute dysuria (not confirmed UTI) and 126 who presented with no symptoms completed a survey regarding drinking and urination behaviors during different duty conditions.[50] Under normal duty hours, women presenting with dysuria drank significantly less than those with no symptoms (21% and 14%, respectively; p= 0.004).[50] When conducting field duty, consuming appropriate fluid amounts was exacerbated, with the dysuria group drinking considerably less than the control group (79% and 19%, respectively; p= 0.002).[50] Women with dysuria postponed urination more than those without symptoms during normal (75% and 53%, respectively; p= 0.006) and field (79% and 65%, respectively; p= 0.008) duty.[50]

Regardless of deployment status and duty conditions, military women struggle to exercise proper fluid intake and urination behaviors, compromising their urinary tract health. Recognizing these ill-practices, Trego et al. (2018) pilot-tested the Women's Health Promotion Program (WHPP) for Austere Environments to 49 military units presented to 443 military women.[64] The primary goal of the WHPP was to educate military women to recognize and prepare for environments that require altering feminine hygiene behaviors, including adequate fluid intake and urination frequency (though with no specific metrics), to maintain genitourinary health.[64] The program included a 30-minute presentation and provided urinary healthcare packages of at-home urine strip tests, mobile urination devices, cleansing materials, and over-the-counter vaginal medications.[64] One year after implementing the WHPP, the participating military women's UTIs rates significantly decreased (1 year before WHPP= 1.3%; 1 year after WHPP= 0.3%, $p< 0.001$).[64] Though the collection of fluid intake, urination frequency, and hydration biomarker data are missing, the WHPP demonstrated promising results of military women integrating positive drinking and urination behaviors to minimize UTI risk.

Measures and instruments of hydration. Multiple instruments are available to measure widely used hydration biomarkers, including blood serum and urine osmolality, urine color, and urine specific gravity.[31,65,66] Osmolality (serum and urine) is the most valid and reliable technique. However, it is invasive (serum) and requires expensive equipment (urine),[31,66] making them difficult to use in the applied settings of a military base or field operation. Furthermore, osmolality is limited in only detecting severe clinical dehydration, and shifts in urine concentration are subtle.[31,66] Alternatively, urine specific gravity (USG) is a field-accessible method and valid measure of urine concentration compared to the more

sophisticated urine osmolality (*U*Osm) laboratory method.[65] Examining urine color via a urine color chart is a noninvasive, inexpensive, and good means for hydration awareness and increased reliability when combined with USG or *U*Osm,[31] yet it can be impractical to assess during phase 1 of a woman's menstrual cycle. Less precise methods include total fluid intake and urine void frequency and volume within a 24-hour day[66]; however these methods provide quantifiable results for military women without the necessary equipment and are associated with USG[67-69] and *U*Osm.[67,69] Other techniques such as thirst and body mass alone are not valid hydration measures and should be paired with more advanced measures.[31] Similarly, thirst is best measured when at rest, and body mass requires multiple first-morning baseline measures,[31] making this less feasible due to military women's time constraints. Urine concertation via USG (with modified collection protocols; mid-stream clean catch approach) and urine void frequency and volume output serve as acceptable hydration measures, especially within a military setting and among military women, factoring in their menstrual cycle changes.

Urine specific gravity includes instruments of floating, handheld, optical, or digital refractometry options.[31] Refractometry measures the refractory index in a liquid, corresponding to moisture content.[31] Urine reagent strips are unreliable and should not measure USG, even when solely designed to.[31,70,71] Urine specific gravity is time- and temperature-sensitive, needing to be measured within 24 hours of urination or seven days stored with a refrigerator at a temperature of 20°C, regardless of instrument type.[72] The preferred urine samples to test are the first morning and afternoon (4:00-8:00 PM) voids, as these represent the most-to-least concentrated samples to detect abnormalities in the urine and best to assess hydration within 24-hours, respectively.[8,9,73,74] A USG value of <1.013 is

the recommended cut-off values for optimal euhydration[2], as this is equivalent to a 24-hour *U*Osm >500 mOsm/kg,[75] though a USG value of <1.020 is considered euhydrated. Perrier et al. (2017) conducted receiver operating characteristic curve (ROC) analyses on N= 817 urine samples of healthy adults and found that a USG <1.013 detects *U*Osm<500 mOsm/kg with very high accuracy (area under the curve [AUC] 0.984).[75] A *U*Osm<500 mOsm/kg is demonstrated with reduced UTI incidents in premenopausal women,[7] and thus, reasonable that a USG <1.013[2] is equivalent and more practical to use when researching with military women.

Menstrual Cycle and Urinary Tract Infections

Menstrual cycle overview during reproductive years. Premenopausal women (time between puberty and perimenopause) typically experience a 21- to 35-day menstrual cycle bleeding for two to seven days, denoted by fluctuations in circulating concentration levels of the ovarian sex hormones, estrogen and progesterone.[9] Estrogen is responsible for developing and regulating the female reproductive system and secondary sex characteristics, including estrone, estradiol, and estriol, the three major endogenous estrogens.[76,77] In premenopausal women, estradiol is the most abundant estrogen found in urine, blood, and saliva samples.[9,76,77] Progesterone is an endogenous steroid and the most significant progestogen in the body, helping to regulate the menstrual cycle, prepare the body for pregnancy, and support embryo development.[76.77] For each natural occurring cycle (no hormonal contraception, such as combined estradiol-progesterone and progesterone-only), estrogen fluctuates fivefold and progesterone more than 50-fold and is influenced by external factors (hormonal contraceptives, circadian patterns, physical activity level, and diet quality), medical conditions (functional hypothalamic amenorrhea [FHA], polycystic ovary

syndrome [PCOS]), and pregnancy.[9] The concentration levels of estrogen and progesterone determine the four menstrual cycle phases: early follicular, phase 1; late follicular, phase 2; ovulatory, phase 3; and mid-luteal, phase 4 (see Figure 5).[9,76,77] Phase 1 (menstrual cycle days 1-5) has the lowest estrogen levels, while phase 2 (14-26 hours before ovulation) has the highest.[9] Progesterone also is lowest during phase 1 and spikes at phase 4 (+7 days after confirmed ovulation).[9] Within a calendar year, premenopausal women will undergo nine to 12 menstrual cycles,[9] experiencing a wide range of estrogen and progesterone concentration levels per cycle. A combined estrogen-progestin contraceptive (pill, patch, and ring) inhibits ovulation, which thins the uterine lining and thickens vaginal mucus, though it does not alter the cyclical menstrual changes, with estrogen low and allowing for phase 1 bleeding.[78] The progestin-only contraceptive (pill, injection, implant, intrauterine device [IUD]) interferes with ovulation and may change the menstrual cycle, with sometimes unpredictable bleeding or none depending on the contraceptive type used.[78]

Recommendation	Rationale (intended to)
Phase 1: indicated by the onset of bleeding until day 5. Estrogen and progesterone levels are low.	Capture the lowest concentrations of estrogen and progesterone
Phase 2: occurs in the 14–26 h prior to ovulation and the LH surge. Estrogen higher than during phase 1, 3 and 4 and progesterone higher than during phase 1, but lower than 6.36 nmol·L^{-1}.	Capture the highest estrogen concentration, while progesterone remains low.
Phase 3: indicated by a positive urinary ovulation kit and lasts 24–36 h. Estrogen higher than phase 1 but lower than phase 2 and 4 and progesterone higher than phase 1 but lower than 6.4 nmol·L^{-1}.	Capture a medium estrogen concentration, while progesterone remains low.
Phase 4: + 7 days after ovulation has been confirmed. Estrogen higher than phase 1 and 3 but lower than phase 2 and progesterone > 16 nmol·L^{-1}.	Capture the highest concentration of progesterone and a high concentration of estrogen.

Figure 5. Definitions based on hormonal profiles (see Figure 2). Source: Adapted and modified from Elliott-Sale et al.[9]

Sex hormones and vaginal flora. The fluctuations in estrogen and progesterone concentration levels for both natural and combined contraceptive users also affect the vaginal flora.[10,77,79] The vaginal environment typically contains acidophilic lactobacilli, coagulase-negative staph, corynebacterium, and streptococci and forms a defense-like barrier to prevent colonization.[10,77-80] Acidophilic lactobacilli (lactobacilli) are considered an essential bacterium to maintain a normal vaginal pH between 3.8 and 4.4 by producing lactate and hydrogen peroxide to subdue normal flora's overgrowth.[10,77,80] Similarly, vaginal epithelial cells secrete a layer known as the basal lamina, which holds mast cells, lymphocytes, and immune globulins (more commonly known as the thick vicious vaginal mucus) that protect against infections by limiting pathogen's travel from the lower (vaginal tract and ectocervix) to the upper (endocervix, uterus, fallopian tubes, and ovaries) genital tract (see Figure 6).[77,79,80] The vaginal mucus contains cervical IgA, which is critical in boosting genital immunity.[79,80] Higher concentration levels of estrogen promote the establishment of lactobacilli, and higher concentration levels of progesterone initiate the discharge of vaginal mucus.[10,77,79-81] Changes in estrogen, vaginal pH, and cervical IgA affect normal flora's colonization.

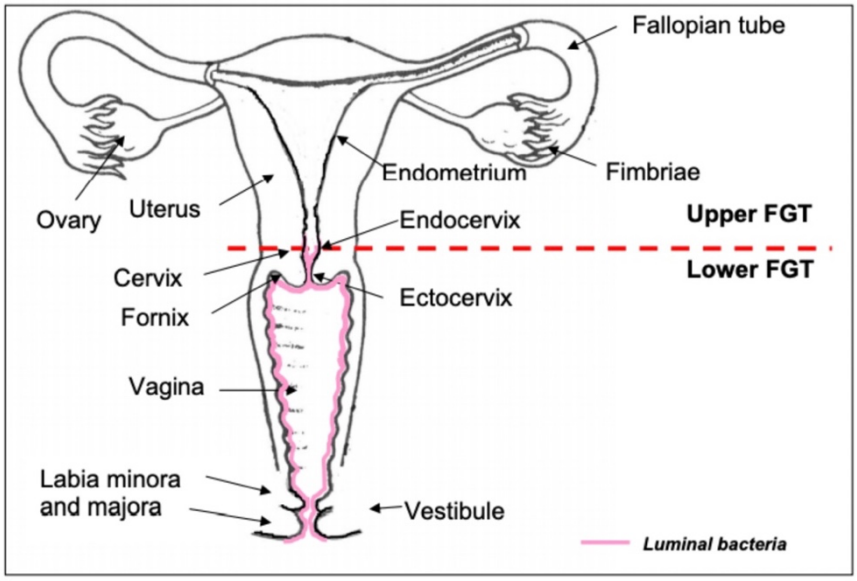


Figure 6. Female genital tract (FGT). Source: Adapted from Nikolaitchouk (2009).

During phase 1 of the menstrual cycle, estrogen and progesterone concentration levels drop to their lowest.[9,76,79-81] The low estrogen levels inhibit lactobacilli's growth, increasing opportunities for "bad" bacteria, such as E. coli, to colonize and disrupt a stable vaginal pH.[3,9,10,19,76,77] Likewise, the lower progesterone concentration levels cease the discharge of vaginal mucus, and therefore, the release of cervical IgA also disturbing the vaginal flora.[76,77] Though the secretion of vaginal mucus ceases, the upper genital tract has some protection from infections developing via the expulsion of the uterine lining, blood, and other particles; it is not known whether hormonal birth control protects from UTI from the increases vaginal secretions during other phases on the menstrual cycle. The lower genital tract and outer vaginal environment (labia minora, labia majora, and vestibule) could be most suspectable in hosting harmful bacteria as the low estrogen levels have restricted its primary defense mechanism, the presence of lactobacilli.[3,9,10,19,76,77] The colonization could expand and seek the nearest opening for an environment that stimulates continued growth and protection,[10] typically is the urinary opening, leading to the urethra, and thus, the urinary

tract. Therefore estrogen, more so the progesterone, may play a role in the colonization and growth of uropathogenic bacteria around and in the urinary tract during phase 1 of the menstrual cycle.[81]

Estrogen fluctuations and uropathogenic bacterium. The temporal association between phase 1 of the menstrual cycle and UTI onset in premenopausal women was first supported by Hooten et al. (1996).[82] In a cross-sectional study, Hooten et al. (1996) examined 577 women (mean age and range, 24 [18-43]) with acute cystitis (E. coli in n= 486 cases [84%]; S. saprophyticus in n= 60 cases [l0%]) and found 41% presented to the clinic with a UTI 8-15 days after the onset of phase 1 of their menstrual cycle ($p< 0.001$).[82] For the primary uropathogens found, the association remained for a UTI caused by E. coli (41% presented 8-15 days after onset of phase 1) and S. saprophyticus (47% presented 8-15 days after onset of phase 1), $p< 0.001$.[82] Furthermore, the study's inclusion criteria for a UTI were if women had dysuria, frequency, urgency and/or suprapubic pain, and 10^2 cfu/mL of midstream urine.[82] Seventy-three percent were found to have $\geq 10^5$ cfu/mL and were similar in presenting (41%) with a UTI 8-15 days after the onset of phase 1 of their menstrual cycle to those with 10^2 cfu/mL ($p< 0.001$).[82] The vaginal colonization of E. coli could surge after phase 1 of the menstrual cycle.[82-84] Several studies have supported that the dramatic rise of estrogen's peak in phase 2 (just before ovulation) positively affected E. coli's adherence and incubation to vaginal and uroepithelia cells, provoking a UTI onset; the occurrence was not witnessed for progesterone.[85,86] Military women's fluid and urination behaviors could be critical during phase 1 to limit uropathogenic bacteria's growth and prevent further adherence and incubation as estrogen begins to rise in the following phase; situational and behavioral factors previously mentioned could magnify growth and UTI risk.[1,10,19-28]

Estrogen fluctuations and AVP. It is critical to note that fluctuations in estrogen are directly related to body fluid balance, such as the synthesis and release of AVP.[87-90] The higher estrogen levels in phases 2 and 4 of the menstrual cycle are associated with increased release of AVP[88] and copeptin circulation,[91] leading to water retention and higher *U*Osm and USG values. The interactions between hydration status and menstrual cycle phases are unknown. Just as the raised estrogen levels after phase 1 spurred the uropathogenic bacterial activity,[82-86] it is plausible the increased estrogen effects on AVP release may amplify uropathogenic bacterial activity by reducing fluid flow in the urinary tract, chancing a UTI. Furthermore, this intensification during phase 2 could be more so for those on a combined contraceptive and, as it is theorized, the additional variable estradiol doses relate to AVP synthesis.[92,93] Therefore, it could be more imperative to increase fluid intake during phase 1 of the menstrual cycle to combat the estrogen fluctuations negatively impacting military women's hydration and urinary tract health.

Measure and instruments of estrogen. As stated earlier, estradiol is the most abundant of estrogens in premenopausal women, and thus, is preferred when determining estrogen levels for this age group.[9,76,77] Estradiol is measured via blood, saliva, and urine.[9,76,77,94-97] The blood analyses are invasive compared to the saliva and urine collection methods.[94] Concerning costs and off-field accessible tools, urine analyses are higher in price due to the multiple measures and are more cumbersome than the saliva approach.[94] Saliva analytes are a convenient and inexpensive method to measure estradiol levels and demonstrates improved compliance with research participants[94]. Salivary estradiol measures free unbound estradiol,[95] expresses dynamic parallels to blood samples,[96] showing comparable validity and reliability.[97] Samples are collected into a vial via the passive drool

method and stored at or below -20°C immediately (or at least within 24-hours) to reduce estradiol composition changes.[94] Estradiol levels are lowest during phase 1 of the menstrual cycle; though, normative values are difficult to establish due to (i) limited collections in phase 1 and (ii) poorly defining menstrual cycle phases.[9,94] However, a source with the salivary estradiol levels of premenopausal females for the "follicular phase" stated to range between 0.9-2.5 pg/mL[98], though could be lower than the provided range.

Hydration Interventions to Reduce Urinary Tract Infections

Hydration is strongly advocated to reduce UTI onset and symptom severity, though it is understudied, loosely defined, and research studies examining its impact to reduce UTIs lack scientific rigor.[2,32,99] Several non-randomized studies have demonstrated a positive relationship between low fluid intake or urine voids and UTI risk[56-60,64]; however, few found no relationship.[61,62] Establishing an optimal fluid volume intake and urine void volume and frequency to reduce UTIs is challenging given the lack of empirical evidence.[2,32,99]

Recognizing the low-body of hydration and urinary tract health literature, three teams of hydration science experts published systematic reviews and narratives in January-July 2020.[2,32,99] Fasugba et al. (2020)[32], Perrier et al. (2020)[2], and Scott et al. (2020)[99] reviewed published literature on how increased fluid intake 1) prevents UTI or 2) directly impacts urine void on urinary tract health. Fasugba et al. (2020)[32] and Perrier et al. (2020)[2] identified two scholarly articles that met their inclusion criteria, both reporting Hooten et al. (2018)[7]. Scott et al. (2020)[99] found eight articles, including Hooten et al.'s (2020),[7] though analyzed studies that examined specifically cranberry supplements, fruit juices, and probiotics on urinary tract health, which their nutrient properties do not generalize to water and other less acidic fluid consumption. Additionally, none of the studies included in Fasugba et al.

(2020),[32] Perrier et al. (2020),[2] and Scott et al.'s (2020)[99] reviews used uropathogenic bacterial growth count as an outcome marker, only UTI symptoms and/or diagnosed occurrences. For this dissertation's focus, the narrative reviews conducted by Fasugba et al. (2020)[32] and Perrier et al. (2020)[7] rather than Scott et al.'s (2020)[99] meta-analysis will be discussed.

A total of three robust interventions were found examining the effect of increased fluid intake on urinary tract health or UTIs.[2,32] While Perrier et al. (2020)[2] reported that in 1995, a small crossover trial found self-assessing urine concentration and encouraged low urine osmolality and UTIs among premenopausal women, the intervention was deemed methodologically unsound (high attrition, lacked proper control group, no assessment of fluid intake).[100] The following paragraphs highlight the three studies' results examining the effectiveness of increased fluid intake on UTI onset in elderly nursing home residents and free-living healthy premenopausal women.

Elderly nursing home residents. Mentes and Cup (2003) examined the effectiveness of an 8-week hydration intervention to reduce hydration-related events (HLEs), such as acute confusion, UTIs, and respiratory infections among nursing home residents.[101] An event was considered an HLE if a USG of ≥1.02 preceded the infection or acute confusion episode and decreased fluid intake found via records.[101] Employing a quasi-experimental design, four long-term care facilities in Iowa were randomly assigned to either 1) calculate a specific hydration goal per participant or 2) maintain current hydration practices, totaling 49 participants (n= 25 intervention; n= 24 control); gender was stratified.[101] Researchers gathered case-line and weekly urine samples and 24-hour fluid diaries to determine if fluid intake was achieved. Strategies to ensure participants consumed fluids included standardized 6 ounces (180 ml) of fluids with all medication administrations,

fluid rounds morning and evening, and at social gatherings twice a week in the late afternoon.[101] Infections and acute confusion episodes were documented, and USG and urine color analyzed.

Neither infection and acute confusion incidents nor time of occurrences differed between groups over the 8-week intervention, $p > 0.05$.[101] Mean USG and urine color were lower in the intervention group than control, and mean fluid intake compliance greater. Mentes and Cup (2003) reported participants in the intervention group to be frailer, more cognitively impaired, and at higher risk for acute confusion than the control group participants.[101] Although no statistically significant differences between the groups yielded, it was clinically relevant that the frailer, more at-risk participants in the treatment group had a lower incidence of HLEs.[101]

Similarly, Lean and colleagues (2019) conducted a quality improvement project, investigating the introduction of seven daily structured drink rounds to reduce UTI onset and hospitalization among elderly nursing home residents.[102] The project's goals were to increase hydration and UTI awareness among staff and encourage residents to consume fluids regularly throughout the day, including a range of hot and cold beverages.[102] The quasi-experimental intervention was implemented between July-September 2016, and researchers observed intervention sustainability from October 2016-March 2018.[102] The study recorded the number of UTIs requiring antibiotics and hospitalizations and compliance of staff delivering drink rounds to nursing home residents.[102] Post-intervention, UTIs requiring antibiotics or hospitalizations reduced to 58% and 36%, respectively.[102] At baseline, the average days between a UTI increased from nine to 121 days by the end of the 18-month intervention.[102] Staff compliance of administering seven daily rounds of beverages

increased from 97% during the intervention to 99% in the sustainability phase.[102] Though the residents did not collect fluid intake, the multiple structured rounds to increase fluid consumption appeared to decrease the severity of the nursing home residents' UTIs.

Healthy premenopausal women. The extent of UTI reductions via hydration was also found by Hooten et al. (2018), who conducted the first randomized control trial among premenopausal women suffering from uncomplicated recurrent UTIs[7]. In this intervention, 140 who reported low fluid intake and low urine volumes were randomized to a) increase water intake by 1.5 L or b) maintain habitual fluid intake for 12 months.[7] The women were asked to keep three-day fluid diaries and collect a 24-hour urine samples at study baseline, six months, and 12 months.[7] The number UTI occurrences were reported, along with the antimicrobial regimens prescribed. The study found that increasing water intake to 2.8 L/day and urine volume to 2.2 L/day yielded a 48% decrease in UTI episodes in the intervention group (3.4 to 1.7 mean episodes) compared to a 9.6% decrease for the control group (3.3 to 3.2 mean episodes).[7] Equally, increased water intake reduced antibiotic use for UTI prevention and treatment; the mean number of antibiotics uses for the intervention (1.9 [95% CI: 1.7-2.2]) and control (3.6 [95% CI: 3.3-4.0]) groups.[7] Hooten et al. (2018) proposed increasing fluid intake supports urine void frequency and volume to facilitate flushing bacteria from the urinary tract to reduce bacterial activity.[7]

Overall, these studies provide preliminary evidence of the effectiveness of increased fluid intake interventions to decrease UTIs. However, these studies are not generalizable to premenopausal women not with recurrent-UTI nor account for the menstrual cycle phase's hormonal fluctuations. Adequately powered and robust studies that evaluate the

effectiveness of increased water intake on urinary tract health considering one's menstrual cycle phases unique to military women are needed.

CHAPTER 3

METHODOLOGY

Study Participants and Recruitment

For my first research question (fluid intake behaviors and lifetime UTI history), the target participants were cadets from the participating university ROTC programs. To partake in the intervention portion of the study, the target participants expanded to healthy premenopausal females, regardless of ROTC or military service status, which will be the remainder of the Methodology description. To assess whether potential participants met the inclusion criteria, women completed a self-reported pre-screening questionnaire (2 minutes) via Qualtrics (Qualtrics, Provo, UT, USA) or paper. This pre-screening questionnaire asked women to report any pre-existing conditions or use of medications influencing body fluid balance. The inclusion criteria were as follows: (i) female, (ii) age 18-34 years (premenopausal), (iii) naturally menstruating (21-35-day menstrual cycle, without ovulation confirmation), (iv) no changes in hormonal contraceptives within the last three months; a list was provided on the types and brands allowed, certain intrauterine devices, such as the ParaGard were acceptable, since this is a non-hormonal contraceptive, (v) no current UTI symptoms, and (vi) be willing to engage in two consecutive phase 1 menstrual cycle data collections. The exclusion criteria included the following: (i) pregnant, (ii) lactating, (iii) menstrual irregularities, (iv) progesterone-only contraceptive use, (v) antibiotic use, (vi) hormone replacement therapy, (vii) symptomatic vulvovaginitis (vaginal inflammation, visible redness, itchiness, or discomfort and not requiring a pap smear), and (viii) have recurrent UTI.

Recruitment occurred between September-October 2021 and data collection between September-December 2021. When recruiting ROTC cadets, an in-person brief was arranged by ROTC leadership where prospective cadets received study information and a pre-screening questionnaire if requested. Before taking the pre-screening questionnaire, participants completed informed consent to use de-identified data in future publications. Once expanding to uniformed servicewomen and eventually a healthy premenopausal female population, recruitment occurred via (i) flyer circulated on social media platforms, word of mouth, and posted on physical bulletin boards and (ii) authorized department and university personnel sending a mass email with study information and link to the pre-screening questionnaire. Additionally, the contact information of the lead investigator was included, allowing prospective participants to have an opportunity to request an informal meeting, ask questions regarding the study, and decide whether to volunteer to sign a paper or electronic informed consent form. The Arizona State University Institutional Review Board approved the study before recruitment and data collection began (STUDY00014289). The lead investigator registered the study as a clinical trial at Clinicaltrials.gov at least 14 days before enrolling the first participant (NCT05015400). All participants received a $50.00 gift card for their contribution and were given individual health information profiles to provide to their primary physician after signing a Research Results Acknowledgement Statement if the study identified a high amount of uropathogenic bacterial activity.

Study Design

This 2x2 randomized crossover study entailed two consecutive phase 1 menstrual cycle data collections, comparing intervention (A) and control conditions (B). At each phase 1 data collection, participants (i) recorded fluid intake and urination diaries and (ii) collected

a 4:00-8:00 PM urine sample on days 2 and 5 along with a first-morning urine sample on days 3 and 6 of their phase 1 menses. Salivary estradiol samples were collected on days 3 and 6 in the morning. A UTI symptoms severity and impairment questionnaire (UTI Symptoms Assessment Questionnaire [UTISA]) was administered via Qualtrics on day 16 (see Figure 7).

Fluid intervention (A). The purpose of the intervention was to investigate whether increased water intake reduced uropathogenic bacterial activity of underhydrated menstruating premenopausal females. The increased fluid intervention began on day 2 after the onset of bleeding (day 1) of their phase 1 menses through day 5. For the increased fluid intake intervention, participants i) consumed an additional 64 oz (1.89 L) of plain water only and ii) aimed to at least urinate 5 times per day. The increased daily water recommendation was based on Hooten et al.'s study[7] to strive to meet the US National Academies of Sciences, Engineering, and Medicine (2005)[5] daily fluid beverage intake recommendations of ≥2.2 L for females ≥19 years old (total fluid intake from beverages and food 2.7 L) and urination frequency from Perrier et al. (2020).[2] Given that the participants resided in a dry desert climate and to coincide with the provided water bottle for ease, the additional fluid amount was slightly increased to 64 oz. The aim was for participants to consume an absolute beverage fluid intake of at least 2.2 L, of which 1.89 L of being only plain water; it was expected participants would adjust their habitual beverage choices during this condition. All participants in the fluid increase intervention received a 32 oz refillable water bottle (to be refilled at 1:00 PM), noting the time at which specified water amounts should have been consumed to ensure an even spread of fluid consumption within a 24-hour day and adherence to the protocol. Plain water was only allowed, no additives were permitted to be added. Filtered water was supplied to the participants if they requested it.

Control condition (B). To compare the effects of the increased fluid condition, participants were asked to maintain their habitual fluid intake volume and beverages choices on day 2 after the onset of bleeding (day 1) of their phase 1 menses through day 5. The 32 oz refillable water bottle was not used, and participants were continuously reminded not to consume the additional 64 oz of water via text reminders; all participants received the bottle to keep after completing the study.

Follow-up UTI symptom severity and impairment. On day 16 of the menstrual cycle for months for both conditions, each participant completed the UTISA to determine if a UTI symptom(s) occurred, and if so, its severity and impairment rating. The questionnaire was conducted via Qualtrics.

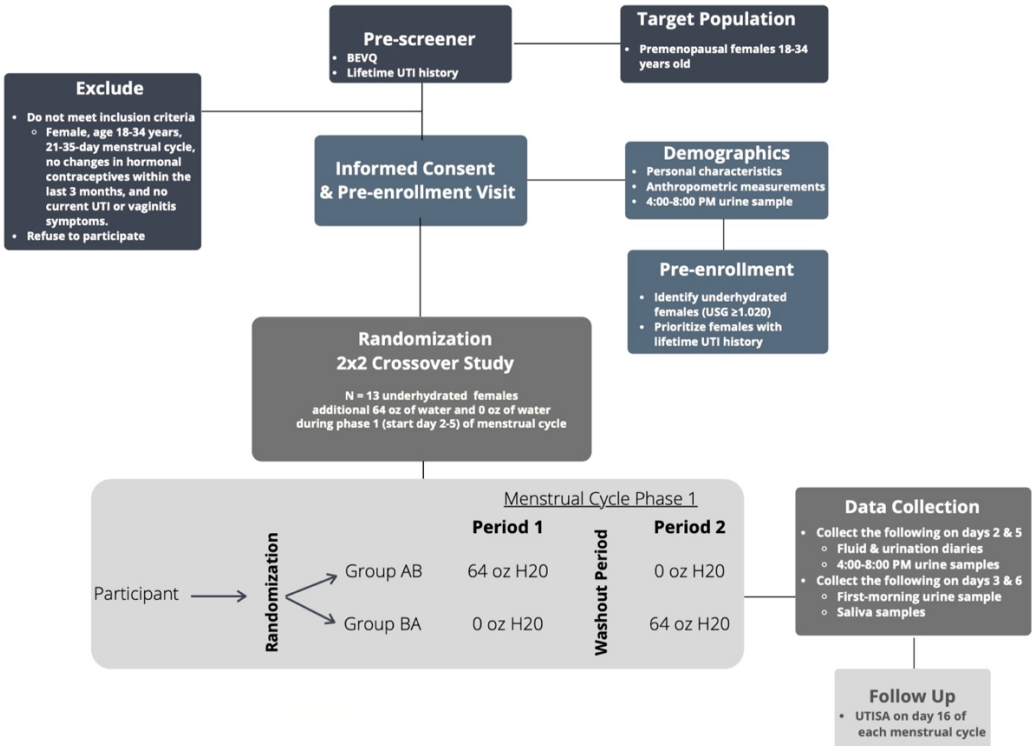


Figure 7. Study flow and design. Randomization order sequence (A= Intervention; B= Control).

Study Protocol

After pre-screening potential participants for eligibility (including Beverage Intake Questionnaire [BEVQ] and UTI history) and after they completed informed consent, the women attended a screening pre-enrollment visit (10 minutes) to complete a personal characteristics questionnaire, anthropometric measurements, and provided an afternoon (4:00-8:00 PM) urine sample at the laboratory facility.

The pre-enrollment aimed to confirm hydration status and occurred during phases 2-4 of their menstrual cycle. Participants classified as underhydrated continued with the intervention based on fluid intake and USG, in which underhydration was defined as having a low fluid intake (based on the BEVQ daily monthly average fluid intake in the past month <1.5 L) and a high urine concentration (based on a urine specific gravity (USG) ≥1.020). Normally a USG <1.013 is preferred though rarely is found in first-morning urinations. Therefore, a less stringent USG requirement of ≥1.020 was used that has also shown to be highly correlated with a *U*Osm<500 mOsm/kg.[75] Enrollment prioritized identified underhydrated premenopausal females with a UTI history, followed by those with no UTI history but at an increased risk based on their hydration status. All participants underwent each condition, randomized via dice roll (evens= AB sequence; odds= BA sequence).

After establishing a participant's randomization sequence, the women received instructions to increase (64 oz water) or maintain habitual fluid intake during phase 1 (days 2-5) of their subsequent two menstrual cycles. Fluid and urination diaries and urine sample collections occurred on days 2-3 and 5-6 at each phase 1 menstrual cycle. The distribution and collection of diaries and urine samples per participants' menses phases occurred at the laboratory facility or picked up at their residence during the early morning hours (5:30-9:30

AM) to coincide with in-person saliva sample testing at the exchange of materials (see Figure 8). If materials were picked up and saliva sample testing occurred offsite the laboratory facility, all samples were placed inside a biohazard cooler filled with ice until stored within the facility. Instructions on how to complete diaries and samples were given during distributions, along with to-go instructional information sheets attached to diaries and collection containers.

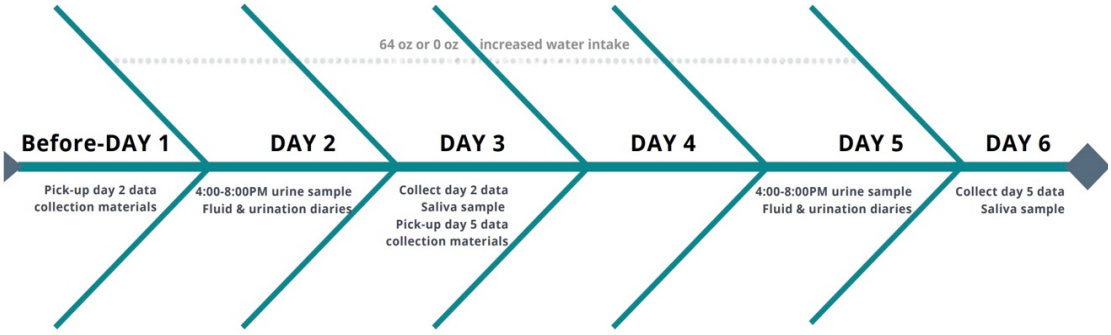


Figure 8. Study materials distribution and data collection process. Material distributions and exchanges at the laboratory facility or convenient pick-up at residences occurred during early morning hours (5:30-9:30 AM) to coincide with in-person saliva sample testing.

The health and safety of all participating females were of the utmost importance. The lead investigator minimized all potential risks and adhered to the university's IRB regulations. If a UTI incident or related UTI risk occurred, the lead investigator would have removed the participant from the study, provided necessary care, and documented the event to the Arizona State University IRB. However, during the study, no UTI incidents or risks occurred. Furthermore, the lead investigator and participants adhered to the current COVID-19 safety and social distancing recommendations when distributing and handling data collection materials.

Instruments

Personal characteristics. The personal characteristics included (i) demographics (age, race, ethnicity, marital status), (ii)occupation, (iii) family planning, sex, and hygienic

practices (hormonal contraceptive type, sexual activity, and the number of partners, and use of menstrual and washing products), and (iv) lifestyle behaviors (alcohol and tobacco use, weekly average physical activity level, and perceived stress within the last month). On average, this assessment took five minutes to complete.

Perceived Stress Scale Four (PSS-4). The PSS-4 is a validated self-administered questionnaire to determine perceived stress levels within the last month.[103] It has four questions, ranging from 1 (Never) to 5 (Very Often). Compared to the PSS-14, internal reliability is lost ($r = 0.60$ vs. $r = 0.85$), yet the brevity of the PSS-4 is useful in time-sensitive settings (average one minute to complete) and when a general sense of perceived-stress level is wanted.[103] Stress can impact hormonal fluctuations, and thus, it was used to determine if the sample perceived similar levels and was embedded within the personal characteristics questionnaire.

Anthropometric measurements. Participant's physical measurements included (i) height (cm) and weight (kg) were derived from mobile devices at the screening visit (SECA 213 portable stadiometer, Hamburg, Germany and Omron Mobile BIA weight scale, Kyoto, Japan). On average, these measurements took three minutes to complete.

Beverage Intake Questionnaire (BEVQ). The BEVQ (2 minutes) is a self-administered frequency questionnaire to determine the mean daily beverage intake of 19 beverage types over a month.[104] For example, questions regard the frequency of and amount at each time consumed for water, tea, soft drinks, alcohol. The BEVQ is validated against urine samples and dietary intake records and demonstrates excellent test-retest reliability ($p < 0.001$).[104] USG was negatively correlated with grams of total daily beverage consumption based on the BEVQ at Time 1 and Time 2 ($r = -0.202$ and $r = -0.238$,

respectively; $p < 0.05$).[104] Likewise, USG was negatively correlated with BEVQ water intake (grams) at Time 1 and Time 2 ($r = -0.236$, $p < 0.05$ and $r = -0.319$, $p < 0.01$, respectively).[104] Self-reported water and total beverage intake did not differ between the BEVQ and food intake records $p > 0.05$.[104] Daily consumption (grams) of water, total beverages, and sugar-sweetened beverages determined by the BEVQ Time 1 were correlated with reported intake determined by the dietary intake record (all $r_s > 0.45$).[104]

Menstrual cycle monitoring. The lead investigator tracked all participants' menstrual cycles using a coded calendar system, to determine the approximate date of their menses to begin.

Ambient conditions. The daily high air temperature (°C) and low humidity (%) during data collection days are reported using the National Weather Service Climate Report. The participants resided in a desert climate, possibly influencing hydration outcomes if the data were collected in the hotter months.[6]

Fluid and urination diary. The fluid diary (4-time points x 5 minutes) collected all fluids, fluid types (sweetened-sugar beverages, caffeinated beverages, milk, yogurt, gelatins, smoothies), amounts, and consumption time. Likewise, the urination diary (4-time points x 5 minutes) collected the frequency, urination length, and time of day of urination and if a menstruation product was changed at a urination time (yes/no). Four additional yes/no questions were asked whether participants engaged in sexual activity, wiped back-to-front, wore non-cotton underwear/restrictive clothing, and washed hands before touching genital areas per data collection day. The fluid intake and urination diaries were adapted and modified from Hooten et al. (2018)[7] and given to the participants in paper formats.

Estrogen (estradiol) hormone testing. Estrogen concentration were measured via salivary estradiol. Estradiol is five and 10 times more potent than estrone and estriol, respectively, and is the preferred estrogen to measure in premenopausal females, as this is the predominant estrogen during the reproductive years.[94] Salivary estradiol was measured on their phase 1 days 3 and 6 during each condition. The morning saliva sample represents the peak level of daily hormone production[94]; the participant self-administered the saliva collection adhering to the protocol instructions with a research member present on days 3 and 6 during the exchange of the diaries and urine sample materials (standardized time between 5:30-9:30 AM). Toothbrushing, eating, smoking, and drinking (except water) one hour before sample collection was not allowed to reduce the risk of contaminating the saliva sample with blood or foreign particles.[94] Immediately after collection, saliva samples were stored in the freezer at or below -20°C. The saliva assay kits, SalivaBio Collection Aid (Salimetrics, Carlsbad, CA, USA), measured estradiol and were analyzed by Arizona State University College of Health Solutions Recharge Center's laboratory personnel. The SalivaBio's Passive Drool saliva collection method increases participant's compliance, preserves the sample's integrity and is on-field accessible. The salivary estradiol level ranges for premenopausal females in the "follicular phase" are between 0.9-2.5 (pg/mL)[98] though were expected to be lower with collection occurring during phase 1 (early follicular). The estradiol samples were used as an objective measure of phase 1 occurring.

Urine sample collection and analyses. To ensure urine was free of most germs and blood for result accuracy, participants used a "mid-stream clean-catch" collection

technique for the first morning and afternoon (4:00-8:00 PM) urinations (washing hands, inserting a new tampon [cup or noting if a participant does not use an inserted product], using a disposable sanitary towelette, and urinating two seconds before collecting the sample; 4 tampons and sanitary towelettes were provided per collection period). All urinations were collected in separate sterilized 4oz containers. Each container was designated with the participant's ID number, date, and time of urination. On days 3 and 6, the urine samples (along with the diaries) were collected at the laboratory or picked up at the participant's residence. The first morning (days 3 and 6) and afternoon (4:00-8:00 PM, days 2 and 5) urine samples were used for hydration status, as these represent the most to least concentrated urine, respectively.[68] Previous works have also demonstrated that afternoon urine samples are comparable to a 24-hour sample hydration status in free-living healthy adults.[69,73] Urinalysis and cultures were performed on the first-morning urine samples, as potential abnormalities detected are most accurate for uropathogenic bacterial activity.[105]

The collected first morning and afternoon urine samples were measured at 20°C±2°C (Traceable thermometer, Fischer Scientific, ±0.2°C accuracy) for all hydration assessments, standing 2-3 hours in the laboratory following refrigeration for next day analyses. Likewise, first-morning urine samples were measured at the same temperature for urinalysis and cultures. Immediate hydration, urinalysis and cultures was not feasible given the varying schedules of participants; thus, all measurements were standardized as listed above to eliminate discrepancies in the protocol. Participants were instructed to refrigerate all first morning and afternoon urine samples. For each first morning and afternoon urine sample, 0.5 mL of urine was placed via pipette into a digital handheld refractometer (4410 [PAL-10S] Digital USG Refractometer, Atago, Tokyo, Japan), measuring USG to assess

hydration per the manufacturer's instructions. Duplicate measurements were averaged, and if there was a ≥0.005 variation between the two measurements, a third measurement was performed using the median. The USG cut-off value was <1.020 to be euhydrated for first-morning and afternoon samples.[31,75]

Urinary reagent test strips and cultures evaluated the first-morning samples' urinary tract health (days 3 and 6). The reagent test strips (Multistix 10SG, Siemens Medical Solutions, Malvern, PA, USA) were dipped into the urine sample cup to record nitrite and leukocyte esterase, adhering to the manufacturer's instructions.[105] The reading time for nitrites is one minute and two minutes for leukocyte esterase. Cut-off values for a possible positive UTI result are trace or more of leukocyte esterase and nitrite (+).[105] Leukocyte esterase has a 73–84% specificity and an 80–92% sensitivity, and nitrites are 96–99% specificity and sensitivity of 41-57% for UTIs and are done as a screening tool before cultures within clinical settings.[1] No leukocyte esterase or nitrite were found present in any morning urine samples nor UTIs clinically diagnosed in cultures.

Urine cultures examined uropathogenic bacterial activity (colony count and bacterial type). Urine droplets were smeared in petri dishes (MacConkey's/Blood agar plate) via sterilized 1μL inoculation loop (Renon Lab) and incubated at 37°C for 24 hours. Biochemical reactions and a 40X-2500X LED microscope (AmScope, Irvine, CA, USA) were used to determine colony count and bacterial type per lab manual instructions. A midstream urine culture in premenopausal females of at least 10^3 cfu/mL uropathogens with ≥1 UTI symptom or $\geq 10^5$ cfu/mL uropathogens is considered a UTI diagnosis.[1,7] Common uropathogens include E. Coli, S. saprophyticus, Enterobacilli species, Enterococci, and

Candida.[1] Normal perineal flora are Lactobacilli, Corynebacterium, Staphylococci, and Streptococci.[1]

UTI Symptoms Assessment Questionnaire (UTISA). The UTISA (1 minute) is a 14-item self-administered questionnaire to determine the symptom severity and impairment of a UTI.[107] The questionnaire regards seven common symptoms (urination frequency and urgency, pain or burning sensation when urinating, passing small amounts of urine, pain or pressure in lower abdomen/pelvic area, pain or pressure in the lower back, and blood in urine) and is rated on a Likert scale ranging from 0 (did not have) to 3 (severe) (range: 0-3).[107] Similarly, these symptoms are scored on how bothersome they are on a Likert scale ranging from 0 (not at all) to 3 (a lot) (range: 0-3).[107] The UTISA demonstrates good convergent validity with high correlations between similar domains of the King's Health Questionnaire (all $r_s > 0.40$) and divergent validity with small correlations between unlike domains (all $r_s < 0.15$).[107] Discriminant validity was excellent when between UTISA scores with a clinical evaluation of UTI symptoms performed by the investigator at baseline and subsequent visits.[107] The UTISA was administered on day 16 of months 1 and 2 menstrual cycles to report whether UTI symptoms arose during the 6-15 days after the onset of phase 1 of their menstrual cycle, based on the findings of Hooten et al.'s (1996)[82] time frame of most UTI clinic visits.

Research Questions and Outcomes

Question 1: What are the fluid intake behaviors and lifetime UTI history of premenopausal Reserve Officers Training Corps (ROTC) cadets?

Primary Outcomes: Mean daily total fluid intake and lifetime UTI history

Question 2: Will increased water intake reduce found uropathogenic bacterial activity of underhydrated menstruating premenopausal females?

Primary Outcome: Uropathogenic bacterial activity

Secondary Outcomes: Total fluid intake, urination frequency, and USG

Sample Size Calculation and Statistical Analysis

Samples size calculation. To determine the required sample, the effect size from Hooten et al.'s (2018) findings that evaluated the effect of increasing 1.5 L of daily water on the occurrence of cystitis episodes for 12 months in premenopausal women suffering from recurrent UTIs were used. Hooten et al. (2018)[7] reported a 48% decrease in UTI episodes in the intervention group and a 9.6% decrease for the control group. The uropathogenic bacterial activity count was not recorded, rather a positive or negative UTI found was noted. The current study used similar procedures, yet it (i) was smaller in size, (ii) was shorter in duration, (iii) collected data during menstruation, (iv) targeted a healthy female population (no recurrent UTI), (v) and examined uropathogenic bacterial activity if found as the primary outcome variable. Currently, no research has examined increased fluid intake on uropathogenic bacterial activity among premenopausal women, with or without a UTI, as a primary outcome. Therefore, a more conservative estimate of 15% difference between the control and intervention group with a 1.5% population estimate of the standard deviation was used. An a priori calculation using G*Power 3.1 determined the sample size for N= 13 participants allowing for 0.05 correlation coefficients between repeated measures with an alpha of 0.05 to obtain 80% power.[108] Recognizing the small sample size for the primary outcome (uropathogenic bacterial activity colony count), an a priori calculation for the secondary outcomes, specifically total fluid intake, was calculated. G*Power 3.1

determined the sample size for N= 9 participants allowing for 0.05 correlation coefficients between repeated measures with an alpha of 0.05 to obtain 80% power.[108] The two calculations gave a relative estimate the sample size should be between 9-13 participants. Using the a priori sample size calculation for the primary outcome and expecting an attrition rate of 15%, recruitment aimed for a total of 15 underhydrated menstruating premenopausal females.

Descriptive statistical analyses. The descriptive statistics summarizes pre-screening and personal characteristics data (BEVQ, lifetime UTI history, demographics, occupation, family planning, sex, and hygienic practices, lifestyle behaviors, PSS-4, estradiol levels, and UTISA), and reported these outcomes as mean±standard deviations (mean±SD), frequencies (n), and percentages (%). Similar outcomes are reported for the fluid and urination diaries, USG, uropathogenic bacterial, ambient conditions, and menstrual cycle length. Outcome variables with non-normal distributions were transformed (natural log) to meet a normal distribution. Chi-square and Mann-Whitney U tests examined descriptive data differences for UTI history and study randomization sequence order.

Specific aims statistical analyses. To address question one's aim and hypotheses, Chi-square analyses examined distribution differences (i) lifetime UTI history (UTI history vs. no UTI history), (ii) BEVQ total daily monthly average fluid intake category (<l.5, between 1.5-2.19, and ≥2.2 L/day), and (iii) and lifetime UTI history and BEVQ total daily monthly average fluid intake categories. The effects sizes are expressed as Cramer's V; values range from 0-1 (small= 0.10; medium= 0.30; and large= 0.50).[109] The total number

of women completing the BEVQ and lifetime UTI history questionnaires were used given the small number of ROTC cadets.

Concerning the primary outcome of second question, the planned general linear model (GLM) and binary logistic regression model (BLRM) analyses on uropathogenic bacterial colony count and growth could not be employed. Minimal growth occurred; only four cultures produced uropathogenic bacterial activity from four participants. Given the unexpected growth, it is difficult to establish the effects of the intervention on the primary outcome. Rather, the four individuals with uropathogenic bacterial activity were analyzed as separate cases.

For the secondary outcomes (total fluid intake, urination frequency, and USG), GLM were employed to determine whether total fluid intake and urination frequency increased while USG decreased between the control and intervention conditions on days 2-3 and 5-6.[110] The effects sizes are expressed as partial eta squared (η^2); values range from 0-1 (small= 0.01; medium= 0.06; and large= 0.14).[109] The washout period between 14-28 days, depending on the participant's menstrual cycle length, was more than sufficient to eliminate carryover effects, and the study was not a behavioral change intervention, reducing sequence effects. Spearman's Rho correlations determined the relationship's strength and direction between total fluid intake, urination frequency, total urination length (s), and USG on data collection days 2-3 and 5-6 per condition, reporting the 95% confidence intervals (CI) for effect sizes.

All hydration data from randomized participants included in the analysis used the intent-to-treat principle to preserve randomization and reduce bias,[111] using the sequence sample's mean outcomes. Data were entered into the Statistical Package for Social Sciences

(SPSS) Version 27 (IBM, Armonk, NY, USA). All results were deemed significant using an alpha of $p < 0.05$.

CHAPTER 4

RESULTS

Study Participants

Question one. Fourteen Army (n= 8, 57%) and Navy (n= 6, 43%) ROTC cadets completed the BEVQ and lifetime UTI history questionnaires in September 2021 (question one; What are the fluid intake behaviors and lifetime UTI history of premenopausal Reserve Officers Training Corps (ROTC) cadets?). Additional analysis to examine distribution frequency with all women completing the BEVQ and lifetime UTI history and intervention study samples can be seen in Tables 1-2.

Question two. Participant enrollment and follow-up occurred between September-December 2021. Enrollment targeted first ROTC cadets, then uniformed servicewomen, followed by a general female population meeting the inclusion criteria (question two; Will increased water intake reduced found uropathogenic bacterial activity of underhydrated menstruating premenopausal females?). A total of 111 women were screened for participation: this included 14 ROTC cadets, and 97 women not within the ROTC or uniformed services. Thirty-four women were eligible for pre-enrollment, of which 20 were excluded to owing an unresponsiveness or unwillingness to participate. Fourteen women completed pre-enrollment and were randomized to intervention of control conditions at the start of the study; one participant dropped out before data collection began (see Figure 9 illustrating the consort diagram highlighting the study recruitment and data collection flow).

A total of 13 premenopausal regular (menstrual cycle length 21-35 days without confirmed ovulation) menstruating females (age 22.15±4.06; height cm 163.61±7.99; weight kg 59.14±11.8; race 77% White; ethnicity 15% Hispanic) participated in the study. Twelve

(92%) identified being full-time university students, nine (69%) stated to be sexually active within the last 12 months, and seven (54%) reported being in a relationship. Five (38%) had a UTI history; all reported one past UTI incident. The pre-enrollment 4:00-8:00 PM urine sample found an overall mean±SD for USG of 1.022±0.002. The reported mean±SD menstrual cycle length and active bleeding days was 26.31±3.09 (range, 21-33) and 4.92±1.04 (range, 3-7) days, respectively. Five (38%) were currently on a hormonal birth control (combined pill). Among sexually active participants (n= 9), other family planning practices reported include condom (67%) and contraceptive diaphragm (11%) use; nine (100%) urinated and six (67%) washed after sexual activity. Regarding hygienic practices, nine (69%) used tampons, four (31%) did not always wash hands before touching the genital area, and two (15%) did not always wipe front-to-back after toileting. One (7%) reported holding urine to avoid using toilets.

All (100%) were non-tobacco users, with 50% (n= 13) reported consuming alcohol. Additional lifestyle behaviors included a mean±SD of 7.00±5.67 hours of physical activity per week, 7.15±1.07 hours of sleep per night with a 3.85±0.69 quality sleep score (range, 1-5), and a perceived stress level of 8.62±2.02 (range, 0-16). The study sample was healthy with no significant comorbidities or medications influencing hydration status. Randomization resulted in an unequal split, with four participants randomized to the AB sequence and nine to the BA sequence, though not statically significant, $\chi^2(1)= 1.923$, $p= 0.166$, V= 0.404. Likewise, no differences in family planning, sex, and hygienic practices, lifestyle behaviors, and perceived stress was found for UTI history and study randomization sequence order splits, all $p_s > 0.05$. Additional total sample and split based on UTI history and study

randomization sequence order regarding family planning, sex, and hygienic practices, lifestyle behaviors, and perceived stress can be seen in Tables 3-4.

BEVQ and Lifetime UTI History

For ROTC cadets, the overall mean±SD for self-reported total monthly average fluid beverage intake was 1.75±0.65 L/day. The mean±SD for water, sweetened-sugar, and alcohol monthly average fluid beverage intake were 1.25±0.57, 0.35±0.29, and 0.03±0.05 L/day, respectively. Sixty-four percent (n= 9) of ROTC cadets reported a daily average beverage fluid intake of <2.2 L/day within the last month, not meeting the US National Academies of Sciences, Engineering, and Medicine daily fluid beverage recommendation.[5] Forty-three percent (n= 6) of ROTC cadets reported a daily average beverage fluid intake within the last month <1.5 L/day and are considered low-volume drinkers based on the European Food Safety Agency recommendation.[7] In addition, two (14%) ROTC cadets reported a lifetime history of a UTI diagnosis and consumed a total monthly average fluid beverage intake within the last month <1.5 L/day.

Table 1 provides the mean±SD for the BEVQ and n (%) for lifetime UTI history split for the total population, ROTC cadets, and study intervention sample. Given the small ROTC cadet sample, the inclusion of all women (N= 111) who completed the BEVQ and lifetime UTI history questionnaire was used to examine the distribution frequency in total daily average fluid intake categories (<1.5, 1.5-2.19, and ≥2.2 L/day) and lifetime UTI history (yes/no). Chi-square analysis did not find a significant difference between those with (52%) and without (48%) a UTI history, χ^2 (1)= 0.225, p= 6.350, V= 0.036. A significant difference was found among the number of women within each BEVQ total daily average fluid intake categories (<1.5 L/day, 52%; 1.5-2.19 L/day, 27%; and ≥2.2 L/day, 21%), χ^2

(2)= 18.541, p< 0.001, V= 0.043. Similarly, chi-square analysis found a significant association between the fluid intake categories and UTI history, χ^2 (2)= 6.063, p= 0.046, V= 0.234, signifying that the number of women with a lifetime UTI history was less with a higher fluid intake (see Table 2).

Table 1

Average Daily Beverage Fluid Intake and Lifetime UTI History

	Total (N= 111)	**ROTC Cadets (n= 14)**	**Study Participants (n= 13)**
BEVQ		mean±SD	
Total (L/day)	1.58±0.75	1.75±0.65	0.84±0.30
Water (L/day)	0.97±0.56	1.25±0.57	0.48±0.29
Sweetened-sugar (L/day)	0.33±0.37	0.35±0.29	0.23±0.19
Alcohol (L/day)	0.09±0.20	0.04±0.05	0.03±0.05
Lifetime UTI History		n (%)	
Yes	58 (52%)	2 (14%)	5 (39%)
No	53 (48%)	12 (86%)	8 (61%)

Note. All percentages rounded to the nearest whole number.

Table 2

Distribution of Total Average Daily Beverage Fluid Intake and Lifetime UTI History

	<1.5 L/day	**1.5-2.19 L/day**	**≥2.2 L/day**	**Total**
Lifetime UTI History		n (%)		
Yes	32 (55)	19 (63)	7 (30)	58 (52)
No	26 (45)	11 (37)	16 (70)	53 (48)
Total	58 (52)	30 (27)	23 (21)	111 (100)

Note. N= 111 participants completing the BEVQ and lifetime UTI history questionnaires. All percentages rounded to the nearest whole number. χ^2 (2)= 6.063, p= 0.046, V= 0.234.

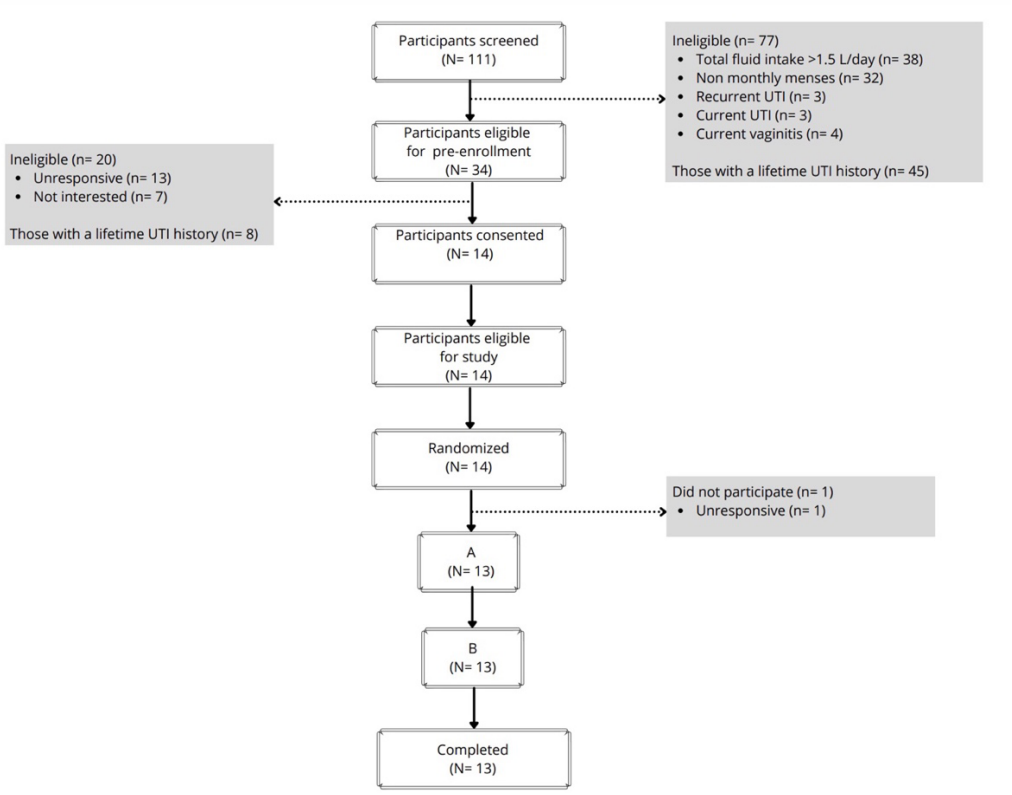


Figure 9. Consort diagram. Randomization order sequence (A= Intervention; B= Control). The consort diagram illustrates the completion rate at each study stage.

Table 3

Family Planning, Sex, and Hygienic Practices, Lifestyle Behaviors, and Perceived Stress by Lifetime UTI History

	Total (N= 9)	**UTI (n= 4)**	**No UTI (n= 5)**
Family Planning Practices		n (%)	
Use contraceptive diaphragm	1 (11)	1 (25)	0 (0)
Use condom	6 (67)	2 (50)	4 (80)
Use spermicide	0 (0)	0 (0)	0 (0)
Sex Practices			
Had multiple partners within last month	0 (0)	0 (0)	0 (0)
Urinate after sexual activity	9 (100)	4 (100)	5 (100)
Wash after sexual activity	6 (67)	2 (50)	4 (80)
	Total (N= 13)	**UTI (n= 5)**	**No UTI (n= 8)**
Hygienic Practices		n (%)	
Do not always wipe front-to-back after toileting	2 (15)	2 (40)	0 (0)
Hold urine to avoid using toilets	1 (7)	1 (20)	0 (0)
Wear nylon/latex underwear, tights, pantyhose	3 (23)	1 (20)	2 (25)
Use feminine hygiene sprays, wipes, soaps	0 (0)	0 (0)	0 (0)
Use douches	0 (0)	0 (0)	0 (0)
Do not always wash hands before touching genital area	4 (31)	2 (20)	2 (25)
Use tampons	9 (69)	5 (100)	4 (50)
Lifestyle Behaviors			
Use tobacco products	0 (0)	0 (0)	0 (0)
Consume alcohol	6 (47)	5 (100)	1 (13)
		mean±SD	
Hours of physical activity per week	7.00±5.67	6.44±3.77	7.90±8.35
Hours of sleep per night	7.15±1.07	7.25±1.17	7.00±1.00
Quality of sleep	3.85±0.69	3.75±0.71	4.00±0.71
Perceived stress	8.62±2.02	8.25±1.75	9.20±2.49

Note. All percentages rounded to the nearest whole number. All dichotomous questions are displayed as 'yes' responses. Family planning and sex practices answered by those only sexually active within the last 12 months. Higher scores are indicative of better quality of sleep and higher levels of perceived stress

Table 4

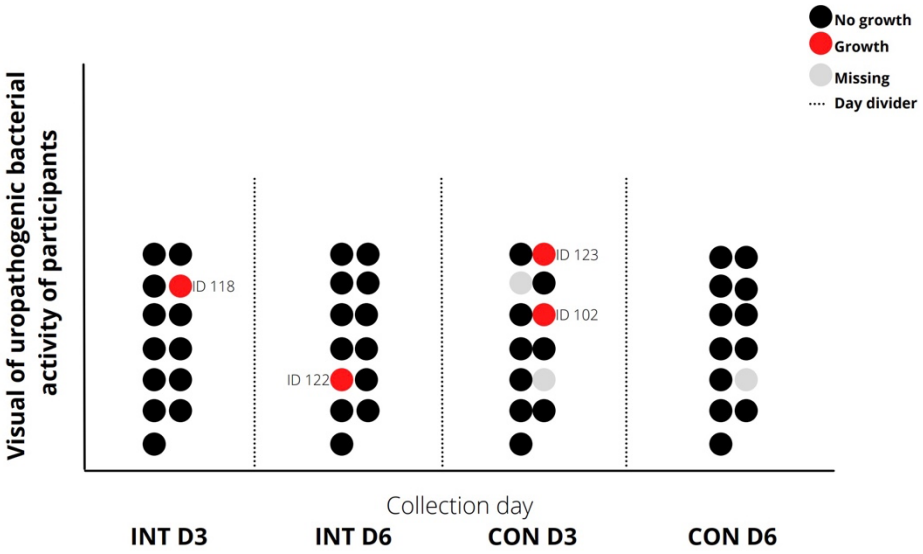
Family Planning, Sex, and Hygienic Practices, Lifestyle Behaviors, and Perceived Stress on the AB and BA Sequences

	Total (N= 9)	**AB sequence (n= 3)**	**BA sequence (n= 6)**
Family Planning Practices		n (%)	
Use contraceptive diaphragm	1 (11)	1 (33)	0 (0)
Use condom	6 (67)	1 (33)	5 (83)
Use spermicide	0 (0)	0 (0)	0 (0)
Sex Practices			
Had multiple partners within last month	0 (0)	0 (0)	0 (0)
Urinate after sexual activity	9 (100)	3 (100)	6 (100)
Wash after sexual activity	6 (67)	2 (67)	4 (67)
	Total (N= 13)	**AB sequence (n= 4)**	**BA sequence (n= 9)**
Hygienic Practices		n (%)	
Do not always wipe front-to-back after toileting	2 (15)	0 (100)	2 (22)
Hold urine to avoid using toilets	1 (7)	1 (25)	0 (0)
Wear nylon/latex underwear, tights, pantyhose	3 (23)	1 (25)	2 (22)
Use feminine hygiene sprays, wipes, soaps	0 (0)	0 (0)	0 (0)
Use douches	0 (0)	0 (0)	0 (0)
Do not always wash hands before touching genital area	4 (31)	2 (50)	2 (22)
Use tampons	9 (69)	2 (50)	7 (78)
Lifestyle Behaviors			
Use tobacco products	0 (0)	0 (0)	0 (0)
Consume alcohol	6 (47)	2 (50)	4 (44)
		mean±SD	
Hours of physical activity per week	7.00±5.67	9.88±8.47	5.72±3.91
Hours of sleep per night	7.15±1.07	7.50±0.58	7.00±1.22
Quality of sleep	3.85±0.69	4.00±0.00	3.78±0.83
Perceived stress	8.62±2.02	9.25±2.87	8.33±1.66

Note. Randomization order sequence (A= Intervention; B= Control). All percentages rounded to the nearest whole number. All dichotomous questions are displayed as 'yes' responses. Family planning and sex practices answered by those only sexually active within the last 12 months. Higher scores are indicative of better quality of sleep and higher levels of perceived stress.

Uropathogenic Bacterial Activity

Figure 10. presents the uropathogenic bacterial activity per participant at each data collection day (see Tables 5-6 to view data in table format). Forty-nine urine cultures were performed (missing due to participants not collecting urine samples, n= 3). Thirty-one percent (n= 4) participants had uropathogenic bacterial activity growth; limited to one culture for each participant (total cultures, n= 4). Combining the day 3 and day 6 for the intervention, two cultures (7%, N= 26 cultures) found uropathogenic bacterial activity; intervention days 3 and 6 each had one culture. Similarly, for the control day 3 and day 6, two cultures (8%, N= 23) with uropathogenic bacterial activity were found, both witnessed on control day 3. No UTIs diagnoses were recorded at follow-ups; those reporting UTI symptoms on the UTISA were related to that of menstrual cycle symptoms (blood in urine, n= 3; pelvic pain, n= 1; and lower back pain, n= 2). The following paragraphs highlight these participants— those with cultures having uropathogenic bacterial growth, examining individual history and fluid, urination, and hygienic behaviors the day before the uropathogenic bacterial activity was found.



F***igure 10.*** Uropathogenic bacterial activity per data collection day. Abbreviations (INT= Intervention; CON= Control; D= Day). Each circle represents a participant, with the organization of participant exact per collection day. Found uropathogenic bacterial activity is noted in red with the participant's identification (ID) number. ID 118, Enterobacter sp. (2,000 cfu/mL). ID 123, Enterobacter sp. (2,000 cfu/mL). ID6: ID 122, E. coli (34,000 cfu/mL). ID 102, S. saprophyticus (12,000 cfu/mL).

Participant ID 118. Participant 118 (age, 25; height cm, 168.30; weight kg, 59.40; race, White; ethnicity, non-Hispanic) identified as a full-time university student, in a relationship, and sexually active within the last 12 months. Habitual family, sex, and hygienic practices (yes responses) included urinating after intercourse, washing hands before and after touching the genital area, and tampon use. Her pre-enrollment 4:00-8:00 PM urine sample found a USG of 1.020 and self-reported a BEVQ total average fluid intake of 1.15 L/day. In addition, she reported a mean menstrual cycle length and active bleeding days of 33 and five days, respectively. Participant 118 was on hormonal birth control (combined pill) and had a UTI history (n= 1). The individual was healthy with no significant comorbidities or medications influencing hydration status.

Uropathogenic bacterial activity (Enterobacter sp.; 2,000 cfu/mL) was found on the first-morning urine sample culture on intervention day 3. A total fluid intake of 2.16 L and urination frequency of nine were reported on the fluid-urination diaries on intervention day 2; USG 4:00-8:00 PM on intervention day 2 and first-morning day 3 urine samples were 1.009 and 1.012, respectively. No deviations from the urine collection protocol or storage were noted. Participant 118 reported not engaging in sexual activity, wiped front-to-back for all urinations, washed hands before touching the genital area for all encounters, and wore tight-restrictive clothing within 24-hour of sample collection. Other non-pathogenic bacterial activity (common vaginal flora; 8,000 cfu/mL) was found and was the predominate bacterium. Therefore, it is highly plausible the uropathogenic bacterial activity growth reported was due to contamination, and not found in the urethra or urinary tract.

Participant ID 123. Participant 123 (age, 18; height cm, 165.80; weight kg, 49.20; race, White; ethnicity, non-Hispanic) identified as a full-time university student, in a relationship, and not sexually active within the last 12 months. Habitual family, sex, and hygienic practices (yes responses) included holding urination to avoid toileting. Her pre-enrollment 4:00-8:00 PM urine sample found a USG of 1.022 and self-reported a BEVQ total average fluid intake of 0.63 L/day. In addition, she reported a mean menstrual cycle length and active bleeding days of 30 and five days, respectively. Participant 123 was not on hormonal birth control and had no UTI history. The individual was healthy with no significant comorbidities or medications influencing hydration status.

Uropathogenic bacterial activity (Enterobacter sp.; 2,000 cfu/mL) was found on the first-morning urine sample culture on control day 3. A total fluid intake of 1.45 L and urination frequency of five were reported on the fluid-urination diaries on control day 2;

USG 4:00-8:00 PM on control day 2 and first-morning day 3 urine samples were 1.008 and 1.017, respectively. No deviations from the urine collection protocol or storage were noted. Participant 123 reported not engaging in sexual activity, wiped front-to-back for all urinations, did not wash hands before touching the genital area for all encounters, and did not wear tight-restrictive clothing. Other non-pathogenic bacterial activity (common vaginal flora; 9,000 cfu/mL) was found and was the predominate bacterium. Therefore, it is highly plausible the uropathogenic bacterial activity growth reported was due to contamination, and not found in the urethra or urinary tract.

Participant ID 102. Participant 102 (age, 19; height cm, 162.80; weight kg, 81.28; race, White; ethnicity, non-Hispanic) identified as a full-time university student, single, and not sexually active within the last 12 months. Regular family, sex, hygienic practices (yes responses) included wiping back-to-front, wearing tight-restrictive clothes, and tampon use. Their pre-enrollment 4:00-8:00 PM urine sample found a USG of 1.020 and self-reported a BEVQ total average fluid intake of 0.86 L/day. They reported a mean menstrual cycle length and active bleeding days of 32 and four days, respectively. Participant 102 was not on hormonal birth control and had no UTI history. The individual was healthy with no significant comorbidities or medications influencing hydration status.

Uropathogenic bacterial activity (S. saprophyticus; 12,000 cfu/mL) was found on the first-morning urine sample culture on control day 3. A total fluid intake of 1.86 L and urination frequency of nine were reported on the fluid-urination diaries on control day 2; USG 4:00-8:00 PM on control day 2 and first-morning day 3 urine samples were 1.005 and 1.027, respectively. Deviations from the urine collection protocol were noted; deviating from the protocol handling and storage instructions of providing the sample to the investigator

within an hour of collection or refrigerating the sample if unable to give the research team within the hour. Participant 102 reported not engaging in sexual activity, did not wipe front-to-back for all urinations, washed hands before touching the genital area for all encounters, and did not wear tight-restrictive clothing. Other non-pathogenic bacterial activity (common vaginal flora; 2,000 cfu/mL) was found and was not the predominate bacterium. Therefore, it is plausible the uropathogenic bacterial activity growth reported was that found in the urethra or urinary tract, though colony amount may be less (see Figure 11).

Participant ID 122. Participant 122 (age, 28; height cm, 171.10; weight kg, 66.20; race, Asian; ethnicity, non-Hispanic) identified as a full-time university student, in a relationship, and sexually active within the last 12 months. Regular, family, sex, and hygienic practices (yes responses) included condom use, urinating after intercourse, wearing tight-restrictive clothing, and tampon use. Their pre-enrollment 4:00-8:00 PM urine sample found a USG of 1.023 and self-reported a BEVQ total average fluid intake of 1.05 L/day. In addition, they reported a mean menstrual cycle length and active bleeding days of 28 and four days, respectively. Participant 122 was not on hormonal birth control and had a UTI history (n= 1). The individual was healthy with no significant comorbidities or medications influencing hydration status.

Uropathogenic bacterial activity (E. coli; 34,000 cfu/mL) was found on the first-morning urine sample culture on intervention day 6. A total fluid intake of 5.12 L and urination frequency of nine were reported on the fluid-urination diaries on intervention day 5; USG 4:00-8:00 PM on intervention day 5 and first-morning day 6 urine samples were 1.015 and 1.022, respectively. Deviations from the urine collection protocol were noted; deviating from the protocol handling and storage instructions of providing the sample to the

investigator within an hour of collection or refrigerating the sample if unable to give the research team within the hour. Participant 122 reported not engaging in sexual activity, did not wipe front-to-back for all urinations, did not wash hands before touching the genital area for all encounters, and did not wear tight-restrictive clothing. Other non-pathogenic bacterial activity (common vaginal flora; 12,000 cfu/mL) was found and was not the predominate bacterium. Therefore, it is plausible the uropathogenic bacterial activity growth reported was that found in the urethra or urinary tract, though colony amount may be less (see Figure 12).

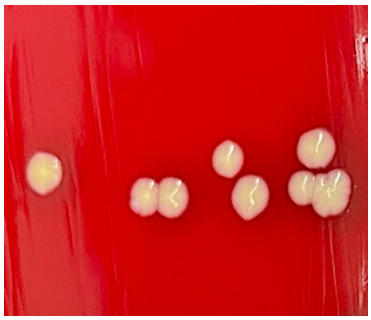


Figure 11. S. saprophyticus. Participant ID 102 control day 3 uropathogenic bacterial activity colony count (12,000 cfu/mL).

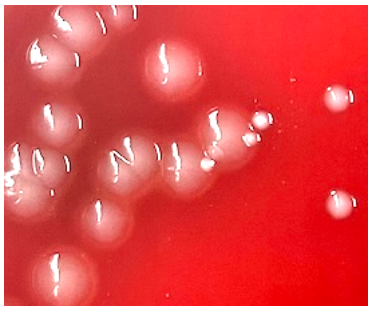


Figure 12. E. coli. Participant ID 122 intervention day 6 uropathogenic bacterial activity colony count (34,000 cfu/mL).

Table 5

Uropathogenic Bacterial Activity Day 3 per Period on the AB and BA Sequences

	AB sequence			BA sequence	
	Intervention (n= 4)	**Control (n= 3)**		**Intervention (n= 9)**	**Control (n= 8)**
Bacterial Activity			n (%)		
Colony growth	1 (25)	1 (33)		0 (0)	1 (13)
Colony growth category					
Insignificant (1,000-9,000 cfu/mL)	1 (100)	1 (100)		0 (0)	0 (0)
Moderate (10,000-99,000 cfu/mL)	0 (0)	0 (0)		0 (0)	1 (100)
			n		
Colony growth count (cfu/mL)	2,000	2,000		0	12,000
			n (%)		
Compromised sample	0 (0)	0 (0)		0 (0)	1 (100)
Contaminated sample	1 (100)	1 (100)		0 (0)	1 (100)
Bacteria Type					
Enterobacter species	1 (100)	1 (100)		0 (0)	0 (0)
E. coli	0 (0)	0 (0)		0 (0)	0 (0)
S. saprophyticus	0 (0)	0 (0)		0 (0)	1 (100)

Note. Randomization order sequence (A= Intervention; B= Control). All percentages rounded to the nearest whole number. All dichotomous questions are displayed as 'yes' responses. One participant missing culture for Control Day 3 from each sequence. All samples reported negative for urinalysis strips of leukocytes and nitrites. Compromised samples are defined as participants deviating from the protocol handling and storage instructions of providing the sample to the investigator within an hour of collection or refrigerating the sample if unable to give the research team within the hour. Contaminated samples refer to samples that have one+ organisms identified and above >10,000 cfu/mL. A <10,000 cfu/mL with identified bacteria is normal, though still attributable to common introital, vaginal, and urethral flora. No UTI symptoms or diagnoses reported for UTISA follow-ups.

Table 6

Uropathogenic Bacterial Activity Day 6 per Period on the AB and BA Sequences

	AB sequence			BA sequence	
	Intervention (n= 4)	**Control (n= 4)**		**Intervention (n= 9)**	**Control (n= 8)**
Bacterial Activity			n (%)		
Colony growth	0 (0)	0 (0)		1 (11)	0 (0)
Colony growth category					
Insignificant (1,000-9,000 cfu/mL)	0 (0)	0 (0)		0 (0)	0 (0)
Moderate (10,000-99,000 cfu/mL)	0 (0)	0 (0)		1 (100)	0 (0)
			n		
Colony growth count (cfu/mL)	0	0		34,000	0
			n (%)		
Compromised sample	0 (0)	0 (0)		1 (100)	0 (0)
Contaminated sample	0 (0)	0 (0)		1 (100)	0 (0)
Bacteria Type					
Enterobacter species	0 (0)	0 (0)		0 (0)	0 (0)
E. coli	0 (0)	0 (0)		1 (100)	0 (0)
S. saprophyticus	0 (0)	0 (0)		0 (0)	0 (0)

Note. Randomization order sequence (A= Intervention; B= Control). All percentages rounded to the nearest whole number. All dichotomous questions are displayed as 'yes' responses. One participant missing culture for Control Day 6 from the BA sequence. All samples reported negative for urinalysis strips of leukocytes and nitrites. Compromised samples are defined as participants deviating from the protocol handling and storage instructions of providing the sample to the investigator within an hour of collection or refrigerating the sample if unable to give the research team within the hour. Contaminated samples refer to samples that have one+ organisms identified and above >10,000 cfu/mL. A <10,000 cfu/mL with identified bacteria is normal, though still attributable to common introital, vaginal, and urethral flora. No UTI symptoms or diagnoses reported for UTISA follow-ups.

Total Fluid intake, Urination Frequency, and USG

Tables 7-8 presents the descriptive results for total fluid intake, urination frequency, and USG organized by sequence order (additional participant's individual total fluid intake, urination frequency, and USG outcomes can be seen in Appendix G and H). When combining data collection days 2 and 5 per intervention (INT) and control (CON) condition for the total sample, the mean±SD for total fluid intake was INT 2.99±1.05 and CON 1.85±0.89, resulting in a significant increase (62%) from the control to intervention condition, $p < 0.001$, $\eta^2 = 0.459$.

The total sample mean±SD for total fluid intake per data collection day was INT D2 3.12±0.96, INT D5 2.86±1.12, CON D2 2.11±0.90, and CON D5 1.59±0.80. A significant increase (48%) for day 2 from the control to intervention condition was found, $p = 0.007$, $\eta^2 = 0.501$. A significant increase (80%) for day 5 from the control to intervention condition was found, $p = 0.009$, $\eta^2 = 0.475$. No significant differences between group sequences yielded between conditions or days, meaning there was no sequence order effect, all $p_s \geq 0.259$, η^2 range (0.001-0.114).

Three (23%) participants did not meet the recommended 2.2 L/day of total fluid intake on intervention day 5. Four (31%) and three (23%) participants consumed >2.2 L/day of total fluid intake on control day 2 and day 5, respectively. Two (15%) reported a higher total fluid intake on control day 2 than their intervention day 2; two participants had higher total fluid intake on control day 5 than their intervention day 5. Most consumed a higher total fluid intake on day 2 for both conditions. Water was the dominant beverage choice for all participants.

All (100%) participants urinated at least five times on intervention days 2 and 5. Three (23%) participants urinated less than five times on control days 2 and 5. When combining data collection days 2 and 5 per INT and CON condition for the total sample, the mean±SD for urination frequency was INT 8.08±1.96 and CON 6.53±2.39, resulting in a significant increase from the control to intervention condition, p= 0.003, η^2= 0.301. The total sample mean±SD for urination frequency per data collection day was INT D2 8.62±2.22, INT D5 7.54±1.56, CON D2 7.00±2.74, and CON D5 6.07±1.98. A non-significant increase for day 2 from the control to intervention condition was found, p= 0.072, η^2= 0.244. A non-significant increase for day 5 from the control to intervention condition was found, p= 0.012, η^2= 0.421.

The afternoon days 2 and 5 USG values were lower than the first-morning days 3 and 6, regardless of condition. Similarly, all conditions for days 2 and 5 4:00-8:00 PM urine samples had a USG value <1.020, with the control condition days reporting higher values than the intervention days. The USG values for first-morning days 3 and 6 for all conditions were variable, ranging above and below 1.020. All first-morning intervention days 3 and 6 had lower USG values compared to the control days (see Table 9).

The total sample mean±SD for the combined afternoon USG values were INT 1.011±0.007 and CON 1.016±0.007, resulting in a significant decrease in urine concentration from the control to intervention condition, p= 0.002, η^2= 0.323. The total sample mean±SD for afternoon USG per data collection day was INT D2 1.009±0.005, INT D5 1.013±0.008, CON D2 1.013±0.006, and CON D5 1.019±0.007. A non-significant decrease in urine concentration for day 2 from the control to intervention condition was

found, p= 0.106, η^2= 0.203. A significant decrease in urine concentration for day 5 from the control to intervention condition was found, p= 0.008, η^2= 0.459.

The total sample mean±SD for the combined first-morning USG values were INT 1.015±0.007 and CON 1.020±0.006, resulting in a non-significant decrease in urine concentration from the control to intervention condition, p= 0.023, η^2= 0.190. The total sample mean±SD for first-morning USG per data collection day was INT D3 1.015±0.006, INT D6 1.015±0.008, CON D3 1.019±0.006, and CON D6 1.020±0.006. A non-significant decrease for day 3 in urine concentration from the control to intervention condition was found, p= 0.097, η^2= 0.212. A non-significant decrease for day 6 in urine concentration from the control to intervention condition was found, p= 0.139, η^2= 0.173.

Spearman's rho correlations examined the relationships between total fluid intake, urination frequency, total urination length (s), and USG on data collection days 2-3 and 5-6 per condition. A significant moderate positive correlation was found for control day 2 (r= 0.793, p= 0.001, 95% CI [0.415, 0.938]) between urination frequency and total urination length, signifying increases in urination frequency led to greater total urination length in seconds. No other significant correlations were found between hydration measures nor conditions.

Estradiol, Sex and Hygienic Behaviors, and Ambient Conditions

Tables 7-8 presents the descriptive results for additional non-primary outcomes of salivary estradiol levels, sex and hygienic behaviors, and ambient conditions organized by sequence order. When combining data collection days 3 and 6 per condition for the total sample, the mean±SD for salivary estradiol (pg/mL) was INT 0.77±0.25 and CON 0.87±0.36, the low levels indicating participants were menstruating (phase 1, "follicular

phase" salivary estradiol levels between 0.9-2.5 pg/mL). No significant differences between conditions, days, and sequences were found, all $p_s > 0.05$.

Regarding sex and hygienic behaviors, one (7%) participant engaged in sexual activity on control day 2 (AB sequence, n= 0; BA sequence, n= 1) and control day 5 (AB sequence, n= 1; BA sequence, n= 0); three (23%) engaged in sexual activity on intervention day 5 (AB sequence, n= 1; BA sequence, n= 2). All AB sequence participants (100%) wiped front-to-back for all urinations, while three BA sequence participants did not, regardless of condition or collection day. Half (50%) of AB sequence participants and over 60% of BA sequence participants did not wash their hands before wiping and/or changing menstrual products during each collection day. Non-cotton underwear and/or tight restrictive clothing were worn by 50-75% of AB sequence and 11-33% of BA sequence participants during collection days. No significant distribution frequencies for sex and hygienic behaviors were found between conditions, days, or sequences, all $p_s > 0.05$.

The mean±SD daily high temperature (°C) and low humidity (%) per condition was INT 29.50±2.39 °C and 17.00±6.75 % humidity and CON 30.50±3.77 °C and 14.92±4.09 % humidity, indicating temperatures and humidity were. No significant differences were found between conditions, days, and sequences, all $p_s > 0.05$. Of note, an AB sequence participant during intervention day 2 collected data during rainy weather, accounting for a daily low humidity of 44% and contributing to the high group sequence average of 23.50±14.61 % humidity. However, it did not appear to impact fluid intake when reviewing their data.

Table 7

Hydration, Estradiol, Behaviors, and Conditions Day 2-3 per Period on the AB and BA Sequences

	AB sequence		BA sequence	
	Intervention (n= 4)	**Control (n= 4)**	**Intervention (n= 9)**	**Control (n= 8)**
Fluid Diary	mean±SD			
Total (L)[a]	3.47±1.24	1.60±0.63	2.97±0.91*	2.33±0.99
Water (L)	3.13±1.59	1.17±0.49	2.29±0.53	1.62±1.34
Sweetened-sugar (L)	0.01±0.03	0.31±0.32	0.18±0.32	0.56±0.31
Alcohol (L)	0.25±0.50	0.05±0.10	0.09±0.19	0.00±0.00
Urination Diary				
Urination frequency	9.75±1.71	7.75±3.10	8.50±2.14	7.00±2.67
Total urination length (s)	98.25±10.66	72.00±18.02	99.50±42.87	79.63±32.88
Average urination length (s)	10.18±0.88	9.94±3.28	15.15±10.81	11.52±2.49
Menstrual product change frequency	5.25±2.06	5.00±2.16	3.56±1.51	3.44±1.67
USG				
Day 2 4:00-8:00 PM	1.008±0.001	1.015±0.007	1.009±0.005	1.012±0.007
Day 3 first-morning[b]	1.020±0.007	1.016±0.002	1.013±0.005*	1.021±0.007
Ambient conditions				
High temperature (°C)	30.14±1.06	28.20±3.58	28.76±2.31	30.80±3.46
Low humidity (%)	23.50±14.61	13.75±2.36	16.22±3.90	14.89±6.23
Estradiol				
Day 3 first-morning pg/mL	0.71±0.18	0.56±0.24	0.82±0.32	1.04±0.36
Behaviors	n (%)			
Engage in sexual activity	0 (0)	0 (0)	0 (0)	1 (13)
Wiped back-to front during urinations	0 (0)	0 (0)	3 (33)	5 (63)
Washed hands before wiping and/or changing menstrual product	2 (50)	2 (50)	6 (67)	7 (88)
Wore non-cotton underwear and/or restrictive clothing	2 (50)	2 (50)	3 (33)	1 (13)

Note. Randomization order sequence (A= Intervention; B= Control). All percentages rounded to the nearest whole number. All dichotomous questions are displayed as 'yes' responses. One participant missing fluid and urination diaries for Control Day 2 from the BA sequence. One participant missing urine samples for Control Days 2-3 from each sequence. One participant missing salivary sample for Control Days 3 from each sequence. [a]Intervention condition significantly different control condition for BA sequence, $p = 0.027$. [b]Intervention condition significantly different control condition for BA sequence, $p = 0.043$.

*$P < 0.05$

Table 8

Hydration, Estradiol, Behaviors, and Conditions Day 5-6 per Period on the AB and BA Sequences

	AB sequence		BA sequence	
	Intervention (n= 4)	**Control (n= 4)**	**Intervention (n= 9)**	**Control (n= 8)**
Fluid Diary	mean±SD			
Total (L)[a]	2.32±0.72	1.54±0.94	3.10±1.28*	1.61±0.90
Water (L)	2.07±0.49	1.16±0.69	2.60±1.10	1.12±0.74
Sweetened-sugar (L)	0.23±0.22	0.15±0.18	0.24±0.40	0.31±0.29
Alcohol (L)	0.00±0.00	0.23±0.36	0.00±0.00	0.04±0.11
Urination Diary				
Urination frequency[b]	7.50±1.29*	6.50±1.29	7.88±1.55	5.88±2.42
Total urination length (s)	72.00±21.23	9.38±2.25	97.38±53.13	11.64±5.65
Average urination length (s)	9.56±2.04	9.07±2.42	15.86±12.30	11.82±5.62
Menstrual product change frequency	3.00±2.45	2.75±2.22	2.67±1.12	2.25±1.04
USG				
Day 5 4:00-8:00 PM[c]	1.011±0.010*	1.018±0.012	1.012±0.008	1.019±0.005
Day 6 first-morning	1.010±0.008	1.024±0.009	1.016±0.006	1.020±0.005
Ambient conditions				
High temperature (°C)	30.97±3.36	28.75±2.88	29.30±3.06	31.85±4.66
Low humidity (%)	16.75±5.74	15.25±3.50	15.00±8.50	15.33±5.75
Estradiol				
Day 6 first-morning pg/mL	0.83±0.09	0.83±0.46	0.73±0.27	0.84±0.32
Behaviors	n (%)			
Engage in sexual activity	1 (25)	1 (25)	2 (22)	0 (0)
Wiped back-to-front during urinations	0 (0)	0 (0)	3 (33)	3 (38)
Washed hands before wiping and/or changing menstrual product	2 (50)	2 (50)	7 (78)	6 (75)
Wore non-cotton underwear and/or restrictive clothing	2 (50)	3 (75)	1 (11)	1 (13)

Note. Randomization order sequence (A= Intervention; B= Control). All percentages rounded to the nearest whole number. All dichotomous questions are displayed as 'yes' responses. One participant missing fluid and urination diaries for Control Day 5 from the BA sequence. One participant missing urine samples for Control Days 5-6 from the BA sequence. One participant missing salivary sample for Control Day 6 from the BA sequence. [a]Intervention condition significantly different control condition for BA sequence, $p = 0.012$. [b]Intervention condition significantly different control condition for AB sequence, $p = 0.034$. [c]Intervention condition significantly different control condition for AB sequence, $p = 0.027$.

*$P < 0.05$

Table 9

Urine Specific Gravity Categories per Data Collection Day on the AB and BA Sequences

	AB sequence		BA sequence	
	Intervention (n= 4)	**Control (n= 4)**	**Intervention (n= 9)**	**Control (n= 8)**
USG Day 2			n (%)	
≥1.020	0 (0)	1 (33)	0 (0)	1 (13)
<1.020	4 (100)	2 (67)	9 (100)	7 (88)
<1.013	3 (75)	1 (33)	7 (78)	4 (50)
USG Day 3				
≥1.020	2 (50)	0 (0)	1(11)	5 (63)
<1.020	2 (50)	3 (100)	8 (89)	3 (38)
<1.013	2 (50)	0 (0)	4 (44)	1 (13
USG Day 5				
≥1.020	1 (25)	2 (50)	3 (33)	4 (50)
<1.020	3 (75)	2 (50)	6 (67)	4 (50)
<1.013	2 (50)	1 (25)	5 (56)	1 (13)
USG Day 6				
≥1.020	0 (0)	2 (50)	4 (44)	5 (63)
<1.020	4 (100)	2 (50)	5 (56)	3 (38)
<1.013	2 (50)	1 (25)	1 (11)	1 (13)

Note. Randomization order sequence (A= Intervention; B= Control). USG categories defined: ≥1.020, underhydrated; <1.020 euhydrated; <1.013 optimally euhydrated. All percentages rounded to the nearest whole number. USG days 2 and 5 derived from afternoon 4:00-8:00 PM urine samples and days 3 and 6 from first-morning urine samples. One participant missing urine samples for Control Days 2-3 from each sequence. One participant missing urine samples for Control Days 5-6 from the BA sequence.

CHATPER 5

SUMMARY AND DISCUSSION

The study sought to 1) assess the fluid intake behaviors and lifetime UTI history of ROTC cadets and 2) examine the effects of increased water fluid intake on the uropathogenic bacterial activity of underhydrated menstruating premenopausal females. Based on the BEVQ, more than half of all ROTC cadets self-reported not consuming an average total daily beverage fluid intake of 2.2 L/day. Over a third were considered low-volume drinkers, intaking less than 1.5 L/day,[7] and supporting the previously limited sources documenting fluid intake behaviors among active-duty women.[10,19,50] In addition, the lifetime history of UTI incidents was low, with 85% (n= 12) not having been diagnosed. Regarding study question two, only four cultures detected uropathogenic bacteria from four participants, with no patterns between conditions or days, making it difficult to determine the effectiveness of the intervention. All (100%) adhered to the additional 1.89 L of water consumption on data collection days, yet some may have over-compromised habitual intake. Total fluid intake significantly increased during the intervention than the control condition and for both days 2 and 5. Similarly, urination frequency significantly increased, and afternoon USG values decreased when drinking the additional water for both conditions, though morning USG values varied.

BEVQ and Lifetime UTI History

The few ROTC cadets with a lifetime UTI history could have been attributed to the low participation of cadets completing the questionnaire. Furthermore, the cadets were younger (between 18-22 years) and had not served active-duty. The occupational demands of actively serving could comprise fluid intake and urination behaviors,[10,19,50] possibly

contributing to the higher UTIs witnessed among active-duty military women.[14,15] Though the two ROTC cadets with a UTI past were low-volume drinkers,[7] the total number of premenopausal women completing the questionnaires were included to better determine the distribution frequency in total daily average fluid intake categories (<1.5, 1.5-2.19, and ≥2.2 L/day) and lifetime UTI history (yes/no). Those consuming less fluids were more likely to have a UTI history, similarly found among active-duty military,[10,50] non-military,[57] custodial,[58] and nursing student[60] women. These findings are contrary to Remis et al. (1987),[61] who did not find volumes of fluid to protect from a UTI in college women. The current study for question one included 111 females, with a relatively even split between those with (52%) and without (48%) a lifetime UTI history compared to an uneven group of 43 women with a UTI diagnosis against 149 upper respiratory infection and 227 gynecology visit female patient controls in Remis et al. (1987) and most being euhydrated, possibly contributing to the opposing findings.[61]

Uropathogenic Bacterial Activity

Comparison to the number of uropathogenic bacterial colonies with Hooten et al. (2018) is unavailable, as colony count was not reported[7] nor in other fluid intake interventions documented in Fasugba et al. (2020),[32] Perrier et al. (2020),[2] and Scott et al.'s (2020)[99] reviews. The lower levels of estrogen inhibiting the growth of essential bacterium to maintain normal vaginal pH and weakening the defense-like barrier to prevent the colonization of pathogenic bacteria, which occurs during menses, is well-documented.[9,76-81] Furthermore, increasing urine void frequency and volume via fluid intake to facilitate the flushing of bacteria and thus reduce concentration in the urinary tract is the mechanism proposed by Hooten et al. (2018).[2,7] Therefore, low-volume female drinkers are expected to

have higher pathogenic bacterial concentrations, and perhaps more so at the end of their phase 1 menstrual cycle.

Pathogenic bacteria colonization begins with the ability to adhere, and some strains can invade, to receptive epithelial cells in the vaginal, periurethral, and urethral environments and ascend to the bladder, leading to a UTI.[1,114-125] It's possible the vaginal and periurethral defenses were resilient enough during phase 1 to prevent colonization in the urethral area.[9,76-81] Several studies have compared the pathogenic bacteria of periurethral swabs and urine samples of prepubescent girls and premenopausal women, with and without a UTI history, to predict UTI onset.[114-124] The presence of a pathogen in the periurethral area by itself is not a factor for further colonization in the urethra and bladder; instead, the combination of defective local defenses, receptivity of epithelial cells, and pathogen's ability to adhere and possibly invade epithelial cells increases colonization.[114-125] Thus, the current sample could have i) experienced marginal alterations in vaginal and periurethral local defenses and ii) had estrogen levels high enough during phase 4 to provide subsequent lingering protection when menstruating. Daily salivary estradiol samples were not gathered for all menstrual cycle phases nor vaginal and periurethral swabs during menstruation. Furthermore, an additional urine sample collected at menstrual cycle phase 2 (approximately days 10-13 on a 28-day cycle) when estrogen is at its highest[9] and thus favoring pathogenic adhesion may have been more appropriate to detect growth in the urethral area than in phase 1[82-86]; thus, urine samples at phases 1 and 2 are recommended, as this was not conduct for this study.

Based on the samples' UTI history, their risk of a UTI may not have best represented the target population of interest. The study defined those at risk of a UTI as with or without a lifetime UTI history. Due to slow recruitment, eight participants had no

UTI history, and those reporting a lifetime UTI history all had one past incident; the occurrence during their life was not gathered. The isolated to none past UTI incidents most likely did not place these women at a high enough risk of fostering an environment for pathogenic bacteria to colonize within the urethra. As 44-70% of women will develop a UTI within a year after the initial onset[7], the quick succession is partly due to the defense-like barrier being weakened[114,116,122,125] and the low estrogen at menstrual cycle phase 1[9] perchance compounds that[76-81] for those menstruating. Therefore, including those with a UTI history and a recent UTI diagnosis would have included a population at greater risk of a recurrence. A study design focusing on the primary prevention of a recurrent UTI hypothesizing those at risk of a recurrent UTI as having a UTI diagnosis within the last 30 days but with no UTI symptoms or diagnosis within the previous 12 months may have found uropathogens and determined intervention effectiveness.

Total Fluid intake, Urination Frequency, and USG

Underhydration was defined as having a BEVQ monthly daily average total fluid intake of <1.5 L/day and pre-enrollment 4:00-8:00 PM USG of ≥1.020. The BEVQ (range; 0.35-1.45 L/day) and pre-enrollment 4:00-8:00 PM USG (range; 1.020-1.025) values indicated participants to be low-volume drinkers[2], yet the fluid diaries and USG values during participants' control condition suggested most to be just above or below 1.5 L/day and three consuming the 2.2 L/day minimum. Self-reported data is subject to under- and overreporting[126]; thus, a fluid frequency questionnaire reflecting the previous month rather than a 24-hour collection to establish habitual fluid intake was utilized.[127] The BEVQ is validated against dietary intake records, yielding no differences in the self-reported water and total beverage intake between the BEVQ and food intake records $p > 0.05$.[104]

Furthermore, the daily consumption (grams) of water, total beverages, and sugar-sweetened beverages were correlated with reported intake by the dietary intake record (all $r_s > 0.45$).[104] Compared to a spot 12:00-5:00 PM urine sample, USG negatively correlated with total daily beverage gram consumption of the BEVQ at time one and time two ($r = -0.202$ and $r = -0.238$; $p < 0.01$).[104]

The BEVQ, while appropriate for initial screening and excluding high-volume fluid consumers, may not have been precise enough to determine individuals with daily fluid intake fluctuations that hover around the 1.5 L/day cut-off value. Olzinski et al. (2019) found substantial variation in USG values between test days of female collegiate athletes.[128] When classifying female athletes' hydration status, some were consistently high, some consistently low, and roughly one-third were reported to be above and below the USG underhydration ≥1.020 cut-off value during the two measurement days.[128] Therefore, it is probable the study included females that are part of the group that fluctuates their fluid intake and thus average around the cut-off, and not regularly within a low-volume consumption group. Additionally, the self-reporting on the BEVQ and spot pre-enrollment USG may not have aligned with fluid behaviors of their phase 1 menstrual cycle, as these were gathered during phases 2-4.

A pre-enrollment phase 1 menstrual cycle consisting of a 3-day fluid diary and 24-hour urine collection, the protocol used by Hooten et al. (2018),[7] gathering two weekdays and one weekend day, if possible, could have improved accuracy of documenting fluid habits specific to this phase rather than a spot afternoon urine sample and be compared to pre-screening BEVQ at phases 2-4. A similar hydration collection approach of a 3-day fluid diary, 24-hour urine, and BEVQ during the intervention could have compared daily

and monthly fluctuations, though was not conducted. Lastly, two participants with the highest fluid intake recorded in the diaries, regardless of condition, spoke fluent English as a second language and identified as international students; they could have misinterpreted the imperial units on the BEVQ.

During the study, the days of the week menses began and collection during the Fall season may have disrupted habitual fluid intake.[129-133] Most participants' phase 1 menstrual cycle days occurred during the week and weekend days and on the University's homecoming, Halloween, and Thanksgiving weeks. Haines et al. (2012) found adults 19-50 years old (N= 9,900) had significantly greater energy intake of alcohol, fat, and protein on the weekend than weekdays.[129] Furthermore, Haines et al. (2012),[129] Ma et al. (2006),[130] and de Castro (1991)[131] reported caloric intake to be highest in the Fall, peaking in November. While these studies did not specifically report fluid outcomes, increased total fluid intake is highly probable.[129-132] When taking the BEVQ, participants reflected on the past month of August and September 2022. Therefore, the week-weekend schedules, season, and celebratory events could have boosted habitual fluid intake behaviors. Hence, the season in and of itself could have impacted individuals from being categorized as low-volume drinkers (<1.5 L/day) in Summer to consuming between 1.50-2.19 L/day regularly in Fall, theoretically having enough urination volume and frequency to rid pathogenic bacteria from the urethral environment. Lastly, three participants reported traveling, and one participant rucking (walking with weight; Army standard is 12 miles in three hours carrying 60-100 lbs. of gear on them) on data collection days, influencing fluid behaviors.

Furthermore, USG values during the control condition were ≤1.020 for most participants. The USG values for days 2 and 5 4:00-8:00 PM and days 3 and 6 first-morning

samples varied considerably. Discrepancies in USG could have been affected by blood[112], as four participants had noticeable traces in their samples and diet and supplements use, which was not gathered.[5,6] Finally, the lack of association between hydration markers was surprising[73-75] though can be attributed to the reasons above, subjective participant recording, and the 4:00-8:00 PM urine samples perchance influenced by a quick succession of fluid intake, leading to hyperhydration before collection.[112,113] Taking on the responsibility of managing a sample within this time frame may have modified fluid-urination behaviors to resemble a "good research participant" to ensure the specimen was collected.

Strengths and Limitations

The study sought to explore the relationships between hydration, menstruation, and uropathogenic bacterial activity to improve the prevention and prevalence of UTIs witnessed among active-duty military women. This study was among the first to i) examine increased fluid intake as primary prevention and ii) consider the implications of menstrual cycle phases among a premenopausal female population. Notable strengths include conducting a randomized control study and collecting self-reported and objective physiological data to compare hydration, menstruation, and urinary tract health. Yet, the study is not without its limitations.

Including that mentioned in sections B-C, generalizability to active-duty military women is partial, as the recruitment of ROTC cadets and women in service was low. Expanding to a general female population was required to meet the study sample requirements, and while occupations differ, physiological comparisons can still be inferred and generalized to a non-military population. Like most crossover studies, the sample size was small, with data from 13 participants analyzed. Furthermore, true randomization (dice

roll) instead of alternating between sequences determined order, resulting in unequal groups. Jointly, the small sample and group sequence sizes can impact observed power and outcomes, as power decreased within sequence groups and participant's outcomes influencing means. Thus, the effect sizes and descriptive data are beneficial in highlighting meaningful and individual changes. Participants did not record fluid-urination diaries nor collect urine samples for the phase 1 entirety; thus, adherence to fluid intake protocols for days 1, 3, and 4 and implications on uropathogenic bacterial activity are unknown. The inclusion of all phase 1 menstrual cycle days for collection, while invaluable, would have significantly constrained supplies and participants' compliance. Dietary intake was not gathered. Consequently, total fluid intake did not include water content in foods, which accounts for approximately 20% of total fluid intake.[5,6] Relatedly, a 24-hour urine collection was not used, missing total urine output and volume and volume per urination to compare against the fluid-urination diaries. The 24-hour urine collection could have better explained the lack of associations among the hydration markers, though introducing an additional participation burden.

A midstream clean-catch approach and urine culture is the clinical standard to evaluate bacteria in the urinary tract and diagnose a UTI.[1] Participants received both verbal and written instructions to follow this approach. Trace amounts of pathogens could have been present in the urethra but not fully adhered to the uroepithelium, with the midstream clean-catch protocol riding the lining of early colonization.[114] Before using the midstream clean-catch approach, the sample collecting the initial three-five seconds and another collecting the mid-stream sample could have helped uncover the initial stages of uropathogenic bacterial activity. However, including a second urine collection increases

costs, participation burden, and concerns of sample preservation integrity when conducted in a low-resource applied laboratory facility, witnessed in this study.[134] Also, a wider inoculation loop of 10μL rather than 1μL could have better caught possible uropathogens in the urine collection cup as the 10μL loop is wider[134]; increasing incubation time to more than 24-hours does not have an additional detection advantage.[135]

The study duration was approximately 60 days, concentrating on the immediate (24-hour) and short-term (96-hour) effects of increased water intake at a single menstrual cycle phase 1 compared to a control. However, this may not have allowed sufficient time to capture changes in uropathogenic bacterial activity. [7,39-42,43,64,107] Previous fluid intake and fluid-educational interventions on UTI symptoms or diagnosis outcomes spanned six to 18 months.[7,39-42,43,64,107] Similarly, studies examining bacterial activity in the periurethral and urethral environments collected samples daily, every-other-day, or weekly for four to 12 months.[115,116,122] Intervention duration of at least six menstrual cycles may yield more informative results on the effects of increased fluid intake on uropathogenic bacterial activity. Unfortunately, study design and implementation were confined to the limited personnel, resources, monetary, and time available amidst the COVID-19 pandemic.[136,137] Though within a short period, the study gathered substantial self-reported (BEVQ, fluid-urination diaries, personal characteristics) and physiological (USG, urethral bacterial activity, salivary estradiol, and ambient conditions) data.

Finally, this study examined the immediate (24-hours) and short-term (96-hours) effects of increased water (+1.5 L) intake on the uropathogenic bacterial activity of regularly menstruating women. While total fluid intake considerably increased on a group level, uropathogenic growth was negligible. Future research should explore underhydrated (total

fluid intake ≤1.0 L/day and USG >1.20) active-duty military females at risk of a recurrent UTI diagnosis for an extended (+6 months) prospective increased water intake (meeting a total fluid intake of ≥2.2 L/day) intervention in (i) deployed and non-deployed settings, and/or non-military women (i) suppressing menstruation, (ii) with variations in hormonal birth control on all menstrual cycle phases, and (iii) experience menstrual cycle irregularity (Oligomenorrhea) or disorder (Premenstrual Dysphoric Disorder). Those with recurrent UTI do not fall under an acute uncomplicated UTI category (the focus of this study), as persistence may be attributed to an abnormal structure and non-functioning urinary tract, pathogenic-invaded vaginal cells, or deficient estrogen levels[1,125,138,139]; thus, needing specialized treatment (surgery, physical therapy, host-penetrating medication, topical estrogen) and not within the scope of this study.[1,125,138,139]

Clinical and Practical Implications

UTIs will continue to affect military women's health and service obligations negatively,[14,15] yet preventative measures of consuming a total fluid beverage (mainly water) intake of ≥2.2 L and urinating 5-7 times per day can support an optimal hydration status (24-hour USG >1.013) to help reduce the frequency of onset and severity of symptoms.[1,2,7,10,12,13,64] Though the results of increased fluid intake (+1.27 L from CON) on uropathogenic bacterial activity during phase 1 of the menstrual cycle are inconclusive, education within military medicine concerning hydration, menstruation, and urinary tract health is still encouraged, especially for women with a high UTI history or recurrent UTI diagnosis and consuming very low volumes of fluid.[12,13,64] All servicewomen should be required to attend the Women's Health Promotion Program (WHPP) while at recruit training (boot camp) and pre-deployment readiness to learn how to prevent and care for a

UTI correctly.[64] Teaching women self-hydration assessments (tracking daily total fluid intake, urination frequency, and urine color)[2,7] and how estrogen fluctuations during menstrual cycle phases impact vaginal flora[9] may promote positive drinking and urination behaviors, especially during menstruation.

In addition to education, clinicians may ask servicewomen about their daily total fluid intake, urination frequency, and UTI history during medical visits to help identify those at greater risk. Women visiting military clinics for a suspected UTI are encouraged to report if they have a regular monthly menstrual cycle and, if so, approximately what day of their menstrual cycle they began to experience symptoms. For those at risk or who have recurrent UTI with or without regular menses, evaluating urinary tract structure and function, vaginal intracellular health, and estrogen levels may determine if more intensive treatment is needed.[1,125,138,139] Thus, surgery, experimental host-penetrating antibiotics, and topical estrogen, in addition to good hydration practices, are required.[1,6,7,125,138,139]

Leadership can motivate military women to engage in proper hydration practices by making fluids accessible, encouraging urination relief breaks, and providing urinary healthcare kits.[6,64] Considering environmental differences, carrying a collapsible-water bladder and chlorine and iodine tablets to improve taste and odor will promote increased drinking behaviors.[6] However, it is encouraged to drink small quantities of fluid frequently to safeguard against overdrinking, gastric discomfort, and hyponatremia.[6,18] Perrier et al. (2020) suggests five to seven urinations within a 24-hour[2]; thus, taking three to five relief breaks within an active eight-hour workday is reasonable. Urinary healthcare kits could include urine strip tests, mobile urination devices, cleansing materials, menstruation products, over-the-counter vaginal medications, and hydration reminder sensors, such as Ulla (blinking pattern

developed by the Navy), to attach to water bottles. Such efforts by leadership and kits could positively influence servicewomen's hydration needs and urinary tract health, whether at home base, offsite training, or deployed.[14,15,64]

Future Research

While the study did not yield expected findings, it found that participants were able to significantly increase total fluid intake, leading to higher urination frequency and lower USG values. Further, the study serves as a pilot intervention for future works to increase the experimental designs' scientific rigor and robustness. To expand hydration as primary prevention to UTI, it is suggested to target a higher risk group with stricter hydration markers. Premenopausal females with a regular menstrual cycle at risk of a recurrent UTI as having a UTI diagnosis within the last 30 days but with no UTI symptoms or diagnosis within the previous 12 months may be better candidates. The BEVQ with an additional 3-day fluid-urination diary along with a 24-hour urine collection during a pre-enrollment phase 1 menstrual cycle (and during the intervention for later comparisons) could determine low-volume drinkers (≤1.0 L/day) with a high USG value of >1.020.

A crossover design has the advantage of serving as one's control. However, it is smaller in sample size, increases participation drop-out, and is challenging to conduct for extended prospective studies.[140] A randomized control trial (RCT) matching similar key characters (UTI history, fluid-urination behaviors, and menstrual cycle length and bleeding days) with one in the control and experimental conditions may be more efficient and appropriate for long-term observation of increased fluid intake on uropathogenic bacterial activity. Accordingly, a 6-month trial gathering multiple phase 1 menses 3-day fluid-urination behaviors and 24-hour urine collections and uropathogenic bacterial activity at phases 1 and

2 can help determine if the intervention successfully prevented a recurrent UTI diagnosis after the initial onset.

Of note, many urine sample cultures captured bacteria found in normal vaginal flora, and participants had difficulties delivering first-morning urine void samples to lab personnel and facilities within an hour or refrigerating samples at their residences until scheduled deliveries. Providing participants with the Peezy midstream collection device[141] and portable insulated coolers may reduce post-bladder microbial contribution and maintain sample integrity, especially when conducting applied research. An in-house study option at a military command will also allow easier tracking and sample collection, especially for a longer duration. Mail-in urine sample strips to examine estrogen instead could capture the lowest levels immediately after awakening and with dried strips having a 30-day shelf life.[142,143] Lastly, embedding the study within military medical clinics will increase active-duty military participation and record how the occupational and situational uniquely impact fluid-urination behaviors.

CHAPTER 6

CONCLUSION

In summary, this study surveyed a small number of ROTC cadets and added non-uniformed services participant's fluid behaviors and lifetime UTI history. A significant increase in fluid intake between conditions were found. Furthermore, with a small healthy female sample, uropathogenic bacterial activity was found; however, the sample was not at high enough UTI risk to determine the effects of the increased fluid intake intervention as primary prevention. Minimal uropathogenic bacterial activity growth occurred overall, and fluid intake was often just above or below 1.5 L during the control conditions, accounting for high urination frequencies and low USG values, in contrast to that reported on the BEVQ pre-enrollment USG values found. These results may have been driven by the study samples' risk of a UTI based on their fluid intake behaviors, hydration status, and UTI history to not best represent the target population of interest.

Overall, the results suggest the need for a narrower primary prevention UTI definition, stricter inclusion hydration criterion, and protocols and tools more suitable when examining possible uropathogenic bacterial activity in the urinary tract. Also, with a sufficient fluid intake resulting in five or more urine voids may not have yielded a buildup of uropathogenic bacterial activity with this specific healthy population. Future studies should explore the threshold of sufficient vs. insufficient fluid intake on uropathogenic bacterial activity using a RCT, matching participants in the control and experimental conditions, and collecting 3-day fluid-urination diaries over several phase 1 menstruation cycles, using a female population to generalize physiology outcomes to active-duty military women if necessary.

Though the intervention results were undetermined, military women's hydration, menstruation, and urinary tract health remain prominent health concerns. Efforts to assess their fluid consumption and urination behaviors during menstruation and UTI risks are warranted.

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APPENDIX A

STUDY RECRUITMENT FLYER

FUN Health Study
Female Urogenital Nutrition Health Study
Volunteers
Females between the ages of 18-34 years.
Details
Help me better understand your nutrition and urinary tract health needs! Undergo a brief pre-enrollment visit and you may qualify for a nutrition intervention focusing on urinary tract health.
The following will be collected:
- Body Measurements
- Urine & Saliva Samples
- Fluid & Urination Diaries
- 3 questionnaires
Scan the QR code to see if you qualify! →
Participation is voluntary. Volunteers may receive up to a $50 gift card!
Interested? Contact: Kaila Vento at kvento@asu.edu
Conducted by the Athleat Field Lab at CHS
Supported by the ASU GPSA
Approved by the ASU IRB

APPENDIX B

PRE-SCREENING QUESTIONAIRE

Female Urogenital Nutrition (FUN) Health Study

Thank you for your interest in the Female Urogenital Nutrition (FUN) Health Study!

Please answer the following questions to determine whether you meet the study's eligibility criteria. Its fulfillment will take a few minutes.

For additional questions, please feel free to contact Kaila Vento at kvento@asu.edu or (619) 987-7595.

Very Respectfully,
Kaila A. Vento

Female Urogenital Nutrition (FUN) Health Study- Pre-Screener

Instructions: Please select the answer corresponding to the given question.

1. Are you a female between 18-34 years age?
    - **Yes**
    - **No**

2. Are you currently or plan to be in the next three months pregnant or breastfeeding?
    - **Yes**
    - **No**

3. Are you currently taking antibiotics?
    - **Yes**
    - **No**

4. Are you currently taking medications (i.e., diuretics) to treat blood pressure, heart failure, liver disease, or kidney disease or are diagnosed with diabetes?
    - **Yes**
    - **No**

5. Are you currently on hormonal replacement therapy?
    - **Yes**
    - **No**

6. Are you currently experiencing vaginal inflammation, visible redness, itchiness, and/or discomfort?
    - **Yes**
    - **No**

7. Are you currently experiencing an urgent need to urinate, frequent small urine passes, blood in urine, and/or a burning sensation when urinating?
    - **Yes**
    - **No**

8. Have you ever been diagnosed with a urinary tract infection (UTI) (i.e., an infection in any part of your urinary system, which includes your kidneys, bladder, ureters, and urethra; urge to urinate, burning sensation when urinating, passing frequent small amount of urine, cloudy urine, pink/red urine (blood), strong-smelling, pelvic pain)
    - **Yes**
    - **No**

9. Have you been diagnosed with 3 or more UTIs within the last 12 months or 2 or more UTIs within the last 6 months?
    - **Yes**
    - **No**

10. Is your menstrual cycle regularly occurring with a length between 21-35 days (i.e., from the onset of bleeding to the beginning of the next menstruation)?
    - **Yes**
    - **No**

11. Are you currently on a hormonal contraceptive?
    - **Yes -> move to question 12**
    - **No -> you are done with this questionnaire**

12. If yes, please select the hormonal contraceptive you currently use.
    - **Hormonal intrauterine device (IUD) (i.e., Mirena®, Kyleena®, Liletta®, and Skyla®)**
    - **Non-hormonal copper intrauterine device (IUD) (i.e., ParaGard®)**
    - **Implant (i.e., Implanon® and Nexaplanon®)**
    - **Injection (i.e., Depo Provera®)**
    - **Contraceptive patch (i.e., Ortho Evera®)**
    - **Vaginal ring (i.e., NuvaRing®)**
    - **Progestin-only pills (POP) (i.e., Camila, Errin, Heather, Jencycla, Jolivette, Nor-QD, Nora-BE, Orthoa Micronor)**
    - **Combined oral contraceptives ("the pill") (i.e., Alesse, Apri, Aranelle, Aviane, Azurette, Beyaz, Caziant, Desogen, Enpresse, Estrostep Fe, Gianvi, Kariva, Lessina, Levlite, Levora, Loestrin, Lybrel, Mircette, Natazia, Nordette, Ocella, Low-Ogestrel, Lo Ovral, Ortho-Novum, Ortho Tri-Cyclen, Previfem, Reclipsen, Safyral, Seasonale, Seasonique, TriNessa, Velivet, Yasmin, and Yaz)**

13. Have you changed hormonal contraceptives within the last 3 months?
    - **Yes**
    - **No**

APPENDIX C

BEVERAGE INTAKE QUESTIONAIRE (BEVQ)

Beverage Questionnaire

Instructions:
In the past month, please indicate your response for each beverage type by marking an "X" in the bubble for "how often" and "how much each time"
1) Indicate how often you drank the following beverages, for example, you drank 5 glasses of water per week, therefore mark 4-6 times per week
2) Indicate the approximate amount of beverage you drank each time, for example, you drank 1 cup of water 2 times per day, therefore mark 1 cup under "how much each time"

Subject ID

Date

	HOW OFTEN (MARK ONE)							HOW MUCH EACH TIME (MARK ONE)				
Type of Beverage	**Never or less than 1 time per week (go to next beverage)**	**1 time per week**	**2-3 times per week**	**4-6 times per week**	**1 time per day**	**2+ times per day**	**3+ times per day**	**Less than 6 fl oz (3/4 cup)**	**8 fl oz (1 cup)**	**12 fl oz (1 1/2 cups)**	**16 fl oz (2 cups)**	**More than 20 fl oz (2 1/2 cups)**
Water	O	O	O	O	O	O	O	O	O	O	O	O
100% Fruit Juice	O	O	O	O	O	O	O	O	O	O	O	O
Sweetened Juice Beverage/Drink (fruit ades, lemonade, punch, Sunny Delight[a])	O	O	O	O	O	O	O	O	O	O	O	O
100% Vegetable Juice (V8[b], etc.)	O	O	O	O	O	O	O	O	O	O	O	O
Whole Milk	O	O	O	O	O	O	O	O	O	O	O	O
Reduced Fat Milk (2%)	O	O	O	O	O	O	O	O	O	O	O	O
Low Fat/Fat Free Milk (Skim, 1%, Buttermilk, Soymilk)	O	O	O	O	O	O	O	O	O	O	O	O
Soft Drinks, Regular	O	O	O	O	O	O	O	O	O	O	O	O
Diet Soft Drinks/Artificially Sweetened Drinks (Crystal Light[c])	O	O	O	O	O	O	O	O	O	O	O	O
Sweetened Tea	O	O	O	O	O	O	O	O	O	O	O	O
Coffee, with cream and/or sugar (includes non-dairy creamer)	O	O	O	O	O	O	O	O	O	O	O	O
Tea or Coffee, black, with/ without artificial sweetener (no cream or sugar)	O	O	O	O	O	O	O	O	O	O	O	O
Non-alcoholic or Light Beer	O	O	O	O	O	O	O	O	O	O	O	O
Beer, Ales, Wine Coolers	O	O	O	O	O	O	O	O	O	O	O	O
Hard Liquor (shots, rum, tequila, etc.)	O	O	O	O	O	O	O	O	O	O	O	O
Mixed Alcoholic Drinks (daiquiris, margaritas, etc.)	O	O	O	O	O	O	O	O	O	O	O	O
Wine (red or white)	O	O	O	O	O	O	O	O	O	O	O	O
Meal Replacement Shakes/Protein Drinks (Slimfast[d], shakes, etc.)	O	O	O	O	O	O	O	O	O	O	O	O
Energy Drinks (Red Bull[e], Rockstar[f], Full Throttle[g], etc.)	O	O	O	O	O	O	O	O	O	O	O	O
Other (list):	O	O	O	O	O	O	O	O	O	O	O	O
Other (list):	O	O	O	O	O	O	O	O	O	O	O	O

Virginia Polytechnic Institute and State University, 2008

Figure. The Beverage Intake Questionnaire used to evaluate initial validity and reliability. Scoring instructions are available from the corresponding author upon request. [a]Sunny Delight Beverages Co, Cincinnati, OH. [b]Campbell's Soup Company, Camden, NJ. [c]Kraft Foods, Inc, Northfield, IL. [d]Unilever, Englewood Cliffs, NJ. [e]Red Bull, Fuschl am See, Austria. [f]Rockstar Energy Drink, Las Vegas, NV. [g]Coca-Cola Company, Atlanta, GA.

APPENDIX D

PERSONAL CHARACTERISITCS QUESTIONNAIRE

Female Urogenital Nutrition (FUN) Health Study

Thank you for you participating in the Female Urogenital Nutrition (FUN) Health Study!

Please answer the following personal characteristics questions. Its fulfillment will take a few minutes.

For additional questions, please feel free to contact Kaila Vento at kvento@asu.edu or (619) 987-7595.

Very Respectfully,
Kaila A. Vento

Female Urogenital Nutrition (FUN) Health Study- Personal Characteristics

Instructions: Please select or fill in the answer corresponding to the given question.

Demographics

1. How old are you?_____

2. What is your race?
    - **Black/African American**
    - **Asian**
    - **White/Caucasian**
    - **Native American/Alaska Native**
    - **Native Hawaiian/Pacific Islander**

3. What is your ethnicity?
    - **Hispanic/Latino/Spanish origin**
    - **Not Hispanic/Latino/Spanish origin**

4. What is your current marital status? Please select one.
    - **Married**
    - **Living with a significant partner**
    - **In a relationship**
    - **Divorced or separated**
    - **Never married**
    - **Single**
    - **Other____**

5. What is your current occupation?_____

6. Please select whether you identify with any of the following organization/statuses.
    - **Reserve Officer Training Corps (ROTC)**
    - **Police**
    - **Fire**
    - **Military veteran**
    - **Security**
    - **Parks & Recreation**
    - **Lifeguard**
    - **Other uniformed services____**
    - **None**

Menstrual Cycle

1. When was the first day of your last period (i.e., the onset of bleeding)? Please provide the Month and Day._____

2. On average, how many days do you experience bleeding?_____

3. On average, what is the length in days your menstrual cycle (i.e., from the onset of bleeding to following start of your following period)?_____

Family Planning, Sex, and Hygienic Practices

1. Please select all *family planning practices* you currently use.
    - **Contraceptive diaphragm**
    - **Condom**
    - **Spermicide**

2. Please select all *sex practices* engaged in.
    - **Sexually active within the last 12 months**
    - **Had more than one partner in the last month**
    - **Urinate after intercourse**
    - **Wash genital area before and after sexual activity**

3. Approximate last date of sexual intercourse (can write N/A or Prefer not to answer)? _____

4. Please select all *hygienic practices* you currently use.
    - **Do not always wipe front to back after toileting**
    - **Hold urine to avoid using toilets**
    - **Wear nylon/latex underwear, tights, pantyhose**
    - **Use feminine hygiene sprays/wipes/soaps**
    - **Use douches**
    - **Do not always wash hands before touching genital area**
    - **Use tampons**

Lifestyle Behaviors

1. Do you currently use tobacco products?
    - **Yes**
    - **No**

2. Do you currently consume alcohol?
    - **Yes**

- **No**

1. On average, how many hours of sleep do you get?_____

2. On average, what is the quality of your sleep?
   - **Terrible**
   - **Poor**
   - **Fair**
   - **Good**
   - **Excellent**

3. On average, how many hours of all physical activity (i.e., training, heavy lifting, digging, aerobics, fast bicycling, swimming, walking, yoga, recreation) do you spend per week?_____

Perceived Stress Scale- 4 (PSS-4)

1. Within the last month, how often have you felt that you were unable to control the important things in your life?
   - **Never**
   - **Almost never**
   - **Sometimes**
   - **Fairly often**
   - **Very often**

2. Within the last month, how often have you felt confident about your ability to handle your personal problems?
   - **Never**
   - **Almost never**
   - **Sometimes**
   - **Fairly often**
   - **Very often**

3. Within the last month, how often have you felt that things were going your way?
   - **Never**
   - **Almost never**
   - **Sometimes**
   - **Fairly often**
   - **Very often**

4. In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?

   - **Almost never**
   - **Sometimes**

- **Fairly often**
- **Very often**

APPENDIX E

FLUID AND URINATION DIAIRES

Fluid Consumption Diary

Thank you for participating in the Female Urogenital Nutrition (FUN) Health Study!

Please record all drinks consumed during the 24-hour day (i.e., beverage, quantity, and context) in the given diary below. Its fulfillment will take a few minutes of your day.

Below are guidelines to complete the diary correctly:

- The diary has been prepared to record in detail what you drank for a 24-hour day.
- Please record all drinks consumed, regardless of whether it was at home or not.
- Each consumed beverage should be entered on a separate line. Please include any type of beverage: juice, water, tea, beer, etc.
- For each moment of the day, you can enter several types of consumed beverages.
- Please record a systematic diary - if possible - without leaving it for the end of the day.

How to Fill in Diary

For each beverage consumed, please record the following information:

1. Time of day
2. Type of drink
3. Beverage packaging
4. The amount of beverage consumed

Please see instructions Sections A-C columns to correctly record beverage type, drink package, and amount consumed.

For additional questions, please feel free to contact Kaila Vento at kvento@asu.edu or (619) 987-7595.

Very Respectfully,
Kaila A. Vento

Beverage Type - Column A

In column A of the diary, please enter the number in front of the kind of beverage that you drank.

WATER

1. Tap water
2. Bottled non-carbonated mineral water (including the water drank for the study – if you are in the intervention group)
3. Bottled carbonated mineral water
4. Water from distributor\big bottle

HOT DRINKS

5. Coffee
6. Hot chocolate, Cacao, Cappuccino
7. Tea (black)
8. Fruit tea
9. Green tea
10. Other Tea

MILK DRINKS

11. Milk (full\skimmed)
12. Buttermilk, kefir
13. Drinking yoghurt
14. Other milk drinks

SUGARED BEVERAGES

15. 100% fruit juice
16. Thick mashed juice, usually based on carrot or banana (i.e., Kubus, Pysio)
17. Vegetable juice (i.e., tomato)
18. Fruit nectar
19. Fresh juice one or two-days (i.e., Marvit)
20. Fresh juice prepared at home (squeezed)
21. Compote
22. Fruit non-carbonated drinks
23. Iced Tea
24. Iced Coffee
25. Energetic drinks (i.e., Red Bull)
26. Sports drinks (i.e., Gatorade, Powerade)
27. Carbonated cola type drink
28. Other carbonated drink – fruit, tonic, orangeade (i.e., Fanta, Sprite)

DIET BEVERAGES

29. Fruit non-carbonated drinks
30. Diet Iced Tea
31. Diet Iced Coffee
32. Diet Energetic drinks (i.e., Red Bull)
33. Diet Carbonated cola type drink
34. Other diet carbonated drink – fruit, tonic, orangeade (i.e., diet Fanta, diet Sprite)

ALCOHOL

35. Beer
36. Wine
37. Aperitif, Cocktail, Liqueur, Drink
38. High percentage alcohol (i.e., vodka, cognac, whisky)

FLUIDS REQURING A SPOON

39. Yogurt
40. Gelatin
41. Ice cream, frozen yogurt, gelato
42. Porridge
43. Pudding

OTHER

44. Other

Drink package – Column B

Please enter the code for the packaging corresponding to the drink you entered on columns A and B.

TETRA PACK

1. Tetra pack 0.2 L
2. Tetra pack 0.25 L
3. Tetra pack 0.33 L
4. Tetra pack 0.5 L
5. Tetra pack 1 L
6. Tetra pack 1.5 L
7. Tetra pack 2 L

PLASTIC BOTTLE

8. Plastic bottle 0.25 L
9. Plastic bottle 0.33 L
10. Plastic bottle 0.5 L
11. Plastic bottle 0.7 L /0.75 L
12. Plastic bottle 1 L
13. Plastic bottle 1.5 L
14. Plastic bottle 2 L
15. Plastic bottle 5 L

GLASS BOTTLE

16. Glass bottle 0.2 L
17. Glass bottle 0.25 L
18. Glass bottle 0.33 L
19. Glass bottle 0.5 L
20. Glass bottle 0.66 L
21. Glass bottle 0.75 L
22. Glass bottle 1 L

CAN

23. Can 0.33 L
24. Can 0.5 L

OTHER

25. Other

Amount of drank drink – Column C

Please enter the quantity of beverage consumed at a given moment in liters for example:

- If you drank a can of cola => please write **0.33 L**
- If you drank a glass of water from the 1.5 L bottle => please write **0.25L**

To help you, you will find below a table of various sizes commonly used:

	**Standard glass – 0.25 L**
	**Mug – 0.25 L**
	**Cup of coffee – between 0.1 and 0.2 L**
	**Glass of vodka –40 mL**
	**Glass of wine – between 0.15 L to 0.25 L.**

Please fill in the diary as described above. When entering the beverages consumed, you should consider the day and the different moments of the day.

THANK YOU FOR YOUR HELP AND COOPERATION!

Date (DD/MM/YYYY): I__I__I/I__I__I/I__I__I__I__I

Time of day Enter the time of consumption in AM/PM	**Type of drink** Enter the code type of beverage (See A)	**Drink package** Enter the code for type of packaging consumed if applicable (See B)	**Amount consumed** Enter the quantity consumed drink at that time of the day in Liters (See C) For example: 0.25 L (a glass) 0.33 L (can of soda) 0.20 L (less than a teacup)
Example 7:30 AM	**9**	**N/A**	**0.25 L**

Urination Diary

Thank you for participating in the Female Urogenital Nutrition (FUN) Health Study!

Please record all urinations during the 24-hour day in the given diary below. Start recording urinations in the morning after discarding the first voided sample and proceed with the recording for 24 hours, including the first voided urine on the following morning. Additionally, please time the length of each urination with a watch or phone. Its fulfillment will take a few minutes of your day.

Below are guidelines to complete the diary correctly:

- The diary has been prepared to record in detail urinations for a 24-hour day.
- Please record all urinations and lengths, regardless of whether it was at home or not.
- Each urination should be entered on a separate line.
- Please record a systematic diary - if possible - without leaving it for the end of the day.

How to Fill in Diary

For each urination, please record the following information:

1. Time of day
2. Frequency
3. Length
4. Menstruation Product

Additionally, at the end of the 24-hour urination recording, please answer the four yes/no questions below the diary.

Please see instructions Sections A-C columns to correctly record urination frequency and menstruation product change.

For additional questions, please feel free to contact Kaila Vento at kvento@asu.edu or (619) 987-7595.

Very Respectfully,
Kaila A. Vento

Frequency - Column A

In column A of the diary, please enter the urination frequency number.

Example: #1

Length- Column B

In column B of the diary, please enter the urination length in seconds. Please use a watch or phone to record the seconds.

Example: 14 seconds

Menstrual Product - Column C

In column C of the diary, please enter YES/NO whether you changed a menstrual product (i.e., tampons, sanitation pad/line, cup).

Example: No

Date (DD/MM/YYYY): I__I__I/I__I__I/I__I__I__I__I

Time Enter the time of urination in AM/PM	**Frequency** Enter the urination frequency count (See A)	**Length** Enter the urination length in seconds	**Menstruation Product** Enter whether a menstruation product was changed (See B)
Example 5:30 AM	**1**	**14 seconds**	**No**

Directions: Please **circle Yes/No/Prefer not to answer** to the following sexual and hygienic practices within the last 24-hours.

1. Did you engage in sexual activity (i.e., intercourse) **Yes/No/Prefer not to answer**
2. Did you wipe back-to-front during any urinations? **Yes/No**
3. Did you wash your hands before wiping and/or changing menstruation product? **Yes/No**
4. Did you wear non-cotton underwear and/or restrictive clothing? **Yes/No**

Urination Collection Instructions

Thank you for participating in the Female Urogenital Nutrition (FUN) Health Study!

Please collect the following urine voids:

- Afternoon urine void (first void between the hours of 4:00-8:00 PM)
- Following first morning urine void (first void after wakening)

Below are guidelines to record and store all urine samples correctly:

- Please use the appropriate labeled collection container specified for the urination sample.
- Follow the Midstream Clean Catch Urine Collection Instructions attached and materials provided.
- Store each urination sample in the refrigerator until exchanging samples and materials to the research team.

How to Record Urination Collection

For each urination sample, please record the following information on the container cap:

1. Time of urination

Please see the Midstream Clean Catch Urine Collection Instructions to correctly collect and store each urine sample.

For additional questions, please feel free to contact Kaila Vento at kvento@asu.edu or (619) 987-7595.

Very Respectfully,
Kaila A. Vento

Midstream Clean Catch Urine Collection Instructions

Introduction

What is a Midstream Clean Catch Urine Specimen?

A midstream clean catch urine collection is a method of obtaining a urine specimen that is free of most germs that normally are found on the skin of your urinary area.

Why is this type of collection requested?

The clean catch method is used to avoid contaminating the urine sample with bacteria that are normally present in the urethra and appear in a voided urine sample. It is used for a routine urinalysis, urine culture, or other urine tests that require uncontaminated urine for accurate results.

Instructions:

1. Wash hands thoroughly with soap and warm water.
2. Insert a new tampon to stop the flow (provided in tote bag).
3. Separate the skin folds around the urinary opening for collection.
4. Open the towelette packet and wash the urinary opening and the surrounding areas from front to back. Discard the cloth in the waste basket (provided in the tote bag).
5. Void into the toilet for a few seconds and then stop. Wait until the urine stream is well established before stopping.
6. Place the empty collection container into the path of the stream (provided in the tote bag).
    - Check that the appropriate labeled collection container specified for the urination sample is being used.**
7. Restart the urine stream and collect in sterile container to catch the middle portion of your flow, filling the cup at least halfway.
8. Urinate remaining volume into the toilet.
9. Tightly screw the cap on the container and avoid touching inside of container.
10. Make sure the container is appropriately labeled with your ID number, date, and urination time.
11. Store the urination sample in the refrigerator until exchanging samples and materials to the research team.

For additional questions, please feel free to contact Kaila Vento at kvento@asu.edu or (619) 987-7595.

APPENDIX F

URINARY TRACT INFECTION SYMPTOMS ASSESSMENT (UTISA)

Instructions: Thank you for participating in the Female Urogenital Nutrition (FUN) Health Study! Please read the following text and complete the associated questions.

Please indicate whether you have had the following symptoms/problems in the last 10 days and how severe they were: *(Please circle one number for each symptom)*				**SYMPTOMS**	If you have experienced these symptoms/problems in the last 10 days, please indicate <u>how bothersome</u> they were: *(Please circle one number for each symptom)*			
Did not have	Mild	Moderate	Severe		Not at all	A little	Moderately	A lot
0	1	2	3	Frequency of urination (going to the toilet very often)	0	1	2	3
0	1	2	3	Urgency of urination (a strong uncontrollable urge to pass urine)	0	1	2	3
0	1	2	3	Pain or burning when passing urine	0	1	2	3
0	1	2	3	Not being able to empty your bladder completely/passing only small amounts of urine	0	1	2	3
0	1	2	3	Pain or uncomfortable pressure in the lower abdomen/pelvic area	0	1	2	3
0	1	2	3	Lower back pain	0	1	2	3
0	1	2	3	Blood in your urine	0	1	2	3

If any symptoms occurred, did you seek a healthcare provider for a suspected urinary tract infection (UTI)?

- **Yes**
- **No**

APPENDIX G

PARTICIPANTS INDIVIDUAL RAW HYDRATION OUTCOMES

AB Sequence

Participant ID	ID2	ID5	CD2	CD5
108 **BEVQ total fluid intake: 0.77 L** **Pre-USG: 1.025**				
Total fluid intake	5.15 L	1.71 L	0.88 L	0.53 L
Urination frequency	10	6	6	6
Urination length total (s)	94 s	66 s	87 s	70 s
Urination length average (s)	9.40 s	11.00 s	14.50 s	11.67 s
USG PM (4-8)	1.007	1.022	1.022	1.032
USG AM (first-morning, day 3 [below day 2] and day 6 [below day5])	1.025	1.013	1.016	1.034
118 **BEVQ total fluid intake: 1.15 L** **Pre-USG: 1.020**				
Total fluid intake	2.50 L	2.78 L	1.65 L	1.18 L
Urination frequency	9	9	12	8
Urination length total (s)	98 s	96 s	80 s	55 s
Urination length average (s)	10.88 s	10.67 s	6.67 s	6.88 s
USG PM (4-8)	1.009	1.006	1.015	1.008
USG AM (first-morning, day 3 [below day 2] and day 6 [below day5])	1.012	1.001	1.014	1.018
123 **BEVQ total fluid intake: 0.63 L** **Pre-USG: 1.022**				
Total fluid intake	3.59 L	3.09 L	1.45 L	1.70 L
Urination frequency	12	8	5	5
Urination length total (s)	113 s	80 s	46 s	53 s
Urination length average (s)	9.42 s	10.00 s	9.20 s	10.60 s
USG PM (4-8)	1.007	1.005	1.008	1.014
USG AM (first-morning, day 3 [below day 2] and day 6 [below day5])	1.022	1.015	1.017	1.021
125 **BEVQ total fluid intake: 0.77 L** **Pre-USG: 1.021**				
Total fluid intake	2.59 L	1.70 L	2.40 L	2.75 L
Urination frequency	8	7	8	7
Urination length total (s)	88 s	46 s	75 s	50 s
Urination length average (s)	11.00 s	6.57 s	9.38 s	7.14 s
USG PM (4-8)	1.018	1.015	NA	1.023
USG AM (first-morning, day 3 [below	1.010	1.010	NA	1.010

day 2] and day 6 [below day5])				

BA Sequence

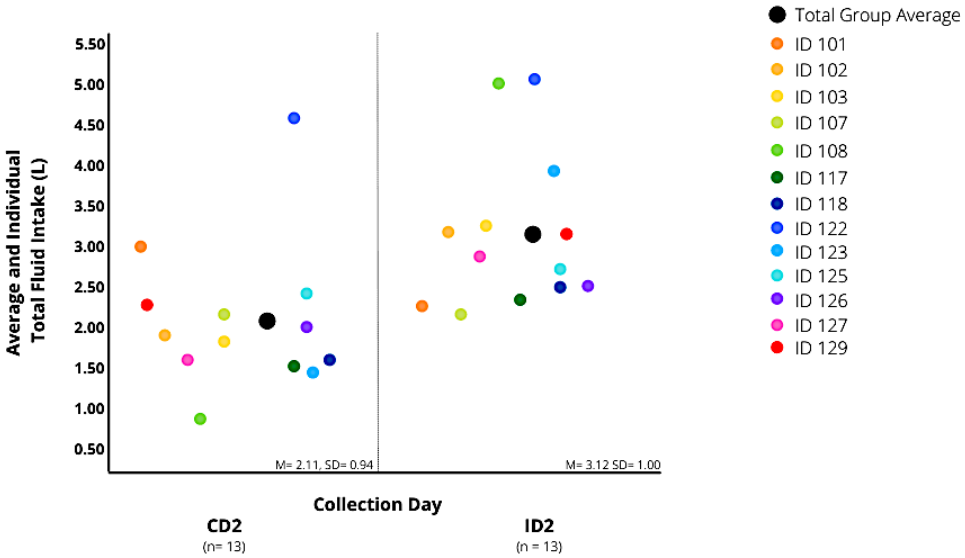
Participant ID	ID2	ID5	CD2	CD5
101 **BEVQ Total fluid intake: 1.45 L** **Pre-USG: 1.021**				
Total fluid intake	2.25 L	2.00 L	3.00 L	1.50 L
Urination frequency	6	7	4	5
Urination length total (s)	63 s	67 s	54 s	55 s
Urination length average (s)	10.50 s	9.57 s	13.50 s	11.00 s
USG PM (4-8)	1.009	1.011	1.025	1.022
USG AM (first-morning, day 3 [below day 2] and day 6 [below day5])	1.010	1.021	1.029	1.024
102 **BEVQ Total fluid intake: 0.86 L** **Pre-USG: 1.021**				
Total fluid intake	3.13 L	2.37 L	1.86 L	0.25 L
Urination frequency	8	7	9	4
Urination length total (s)	47 s	44 s	85 s	34 s
Urination length average (s)	5.88 s	6.29 s	9.44 s	8.50 s
USG PM (4-8)	1.011	1.021	1.005	1.026
USG AM (first-morning, day 3 [below day 2] and day 6 [below day5])	1.010	1.019	1.027	1.022
103 **BEVQ total fluid intake: 1.15 L** **Pre-USG: 1.022**				
Total fluid intake	3.20 L	2.45 L	1.85 L	1.50 L
Urination frequency	9	8	6	5
Urination length total (s)	100 s	116 s	65 s	41 s
Urination length average (s)	11.11 s	14.50 s	10.83 s	8.20 s
USG PM (4-8)	1.005	1.012	1.008	1.024
USG AM (first-morning, day 3 [below day 2] and day 6 [below day5])	1.006	1.016	1.023	1.026
107 **BEVQ total fluid intake: 0.90 L** **Pre-USG: 1.021**				
Total fluid intake	2.20 L	4.20 L	2.20 L	1.30 L
Urination frequency	8	7	10	8
Urination length total (s)	59 s	58 s	90 s	43 s
Urination length average (s)	7.38 s	8.29 s	9.00 s	5.38 s
USG PM (4-8)	1.014	1.004	1.014	1.016

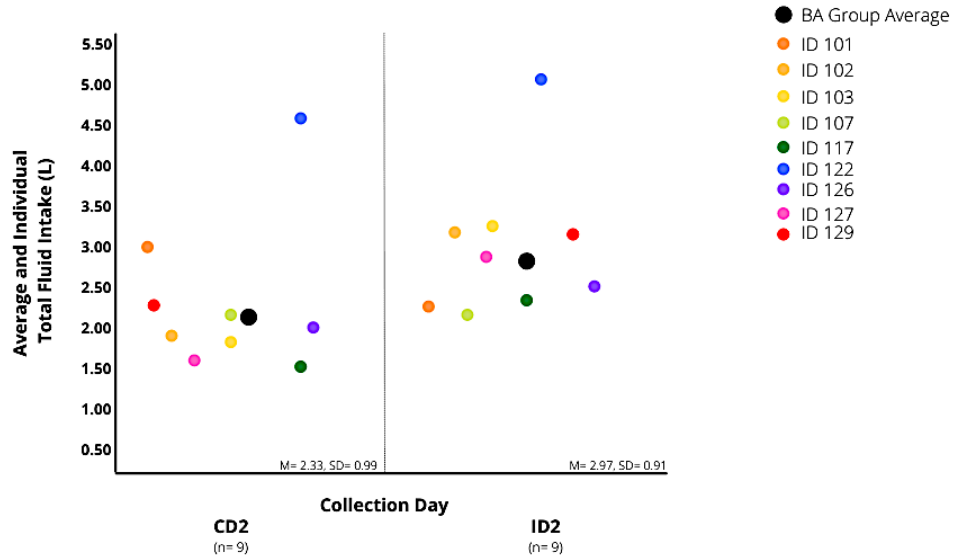
USG AM (first-morning, day 3 [below day 2] and day 6 [below day5])	1.015	1.016	1.019	1.021
Participant ID	**ID2**	**ID5**	**CD2**	**CD5**
117 **BEVQ total fluid intake: 0.53 L** **Pre-USG: 1.023**				
Total fluid intake	2.38 L	4.83 L	1.50 L	NA
Urination frequency	5	5	4	NA
Urination length total (s)	205 s	229 s	49 s	NA
Urination length average (s)	41.00 s	45.80 s	12.25 s	NA
USG PM (4-8)	1.007	1.022	NA	NA
USG AM (first-morning, day 3 [below day 2] and day 6 [below day5])	1.018	1.032	NA	NA
122 **BEVQ total fluid intake: 1.05 L** **Pre-USG: 1.023**				
Total fluid intake	5.13 L	5.21 L	4.68 L	3.25 L
Urination frequency	8	9	8	9
Urination length total (s)	175 s	213 s	132 s	209 s
Urination length average (s)	21.88 s	23.67 s	16.50 s	23.22 s
USG PM (4-8)	1.008	1.015	1.003	1.013
USG AM (first-morning, day 3 [below day 2] and day 6 [below day5])	1.014	1.022	1.009	1.012
126 **BEVQ total fluid intake: 0.64 L** **Pre-USG: 1.024**				
Total fluid intake	2.34 L	1.89 L	2.00 L	2.50 L
Urination frequency	11	11	10	9
Urination length total (s)	122 s	115 s	110 s	106 s
Urination length average (s)	11.09 s	10.45 s	11.00 s	11.78 s
USG PM (4-8)	1.002	1.004	1.010	1.012
USG AM (first-morning, day 3 [below day 2] and day 6 [below day5])	1.009	1.013	1.021	1.021
127 **BEVQ total fluid intake: 0.35 L** **Pre-USG: 1.021**				
Total fluid intake	2.88 L	2.72 L	1.55 L	1.36 L
Urination frequency	6	6	3	4
Urination length total (s)	100 s	83 s	26 s	66 s
Urination length average (s)	16.67 s	13.83 s	8.67 s	16.50 s
USG PM (4-8)	1.005	1.006	1.015	1.018

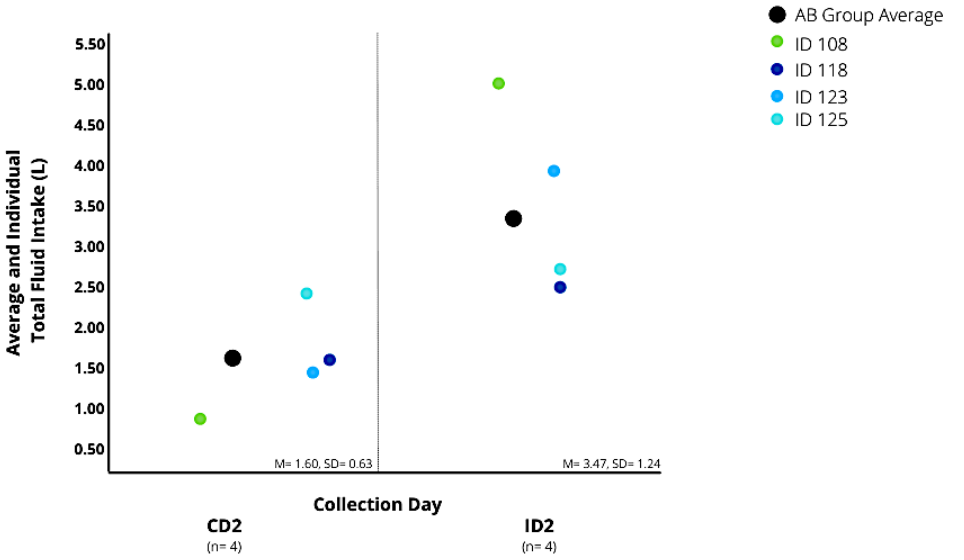
USG AM (first-morning, day 3 [below day 2] and day 6 [below day5])	1.022	1.008	1.025	1.018
Participant ID	**ID2**	**ID5**	**CD2**	**CD5**
129 **BEVQ total fluid intake: 0.73 L** **Pre-USG: 1.020**				
Total fluid intake	3.25 L	2.25 L	2.35 L	1.20 L
Urination frequency	12	8	6	3
Urination length total (s)	130 s	83 s	75 s	30 s
Urination length average (s)	10.83 s	10.38 s	12.50 s	10.00 s
USG PM (4-8)	1.019	1.026	1.015	1.018
USG AM (first-morning, day 3 [below day 2] and day 6 [below day5])	1.019	1.025	1.015	1.013

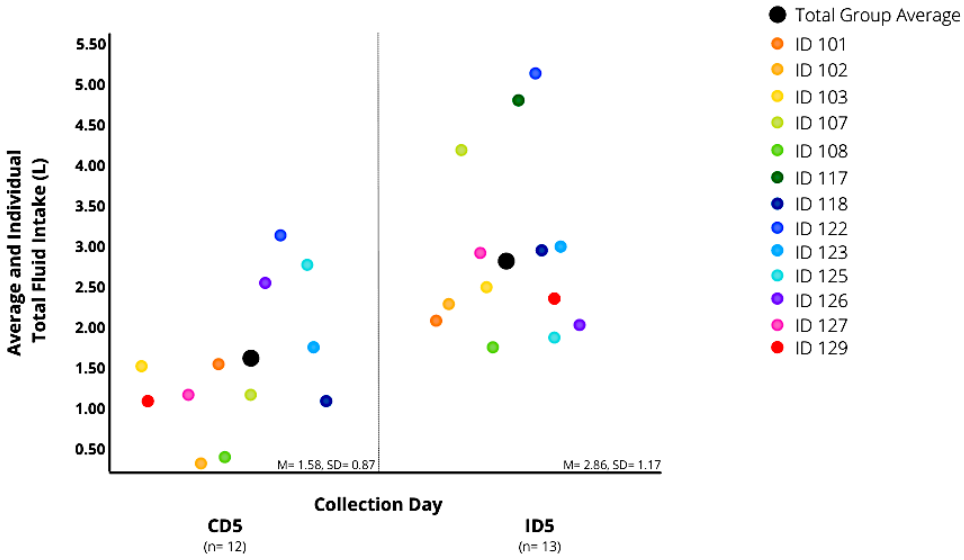
APPENDIX H

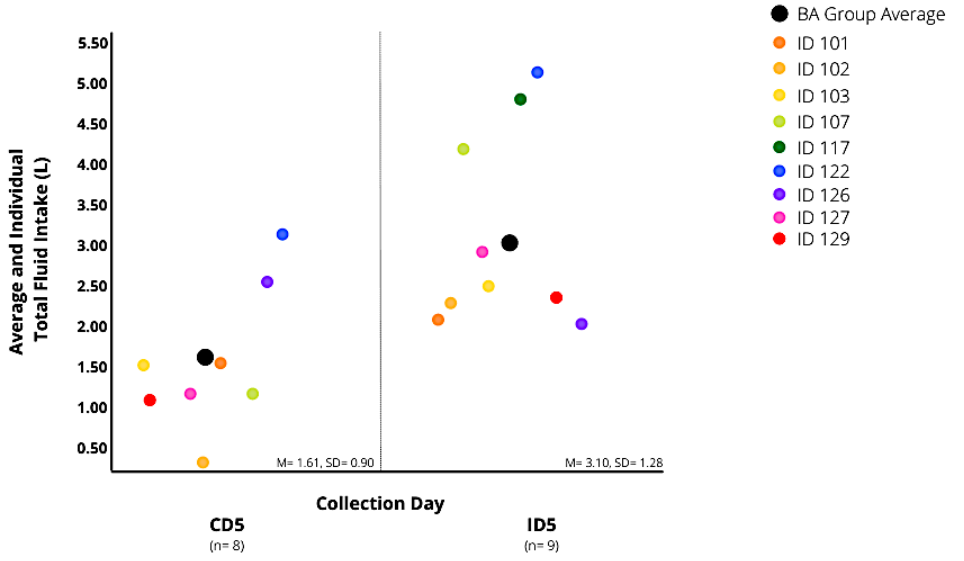
TOTAL AND GROUP SEQUENCE AVERAGE AND INIDIVIDUAL PARTICIPANTS INDIVIDUAL RAW HYDRATION OUTCOMES

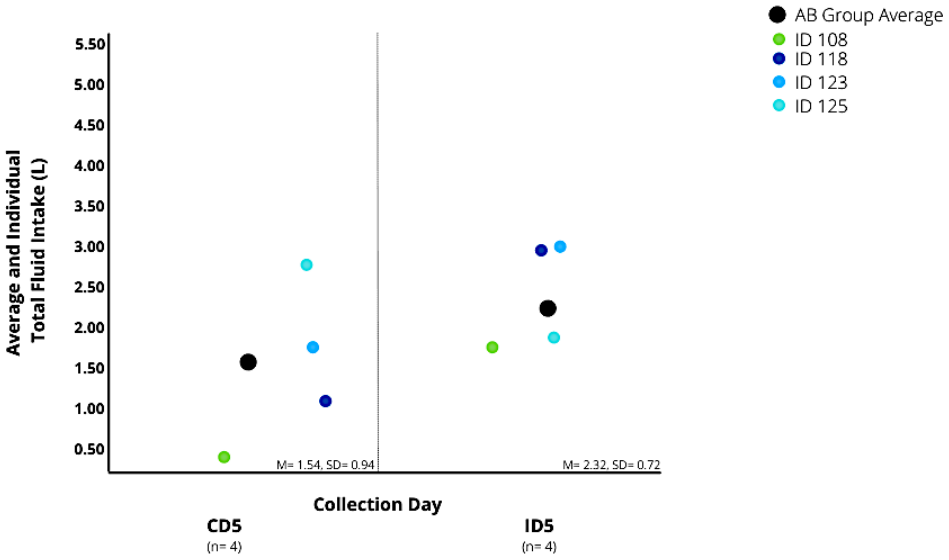

Total Group Average
ID 101
ID 102
ID 103
ID 107
ID 108
ID 117
ID 118
ID 122
ID 123
ID 125
ID 126
ID 127
ID 129
Average and Individual Total Fluid Intake (L)
5.50
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
M= 2.11, SD= 0.94
M= 3.12 SD= 1.00
Collection Day
CD2
(n= 13)
ID2
(n = 13)

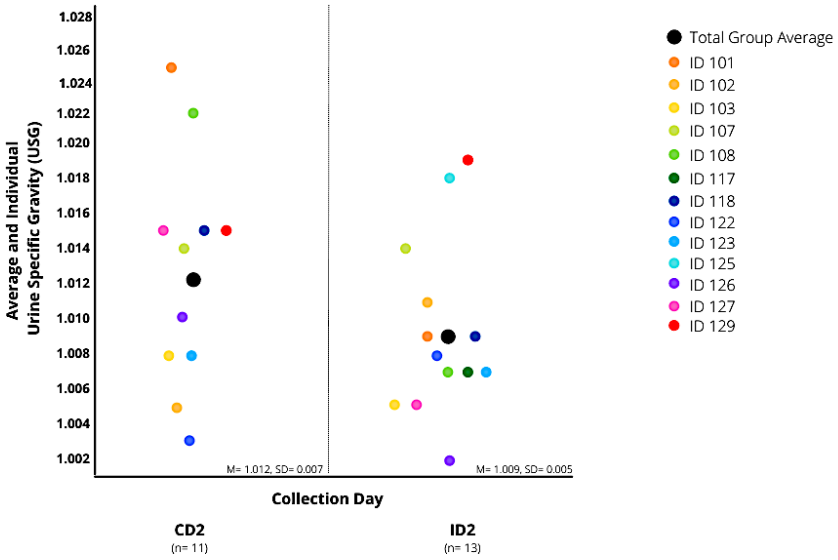

BA Group Average
ID 101
ID 102
ID 103
ID 107
ID 117
ID 122
ID 126
ID 127
ID 129
Average and Individual Total Fluid Intake (L)
5.50
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
M= 2.33, SD= 0.99
M= 2.97, SD= 0.91
Collection Day
CD2
(n= 9)
ID2
(n= 9)

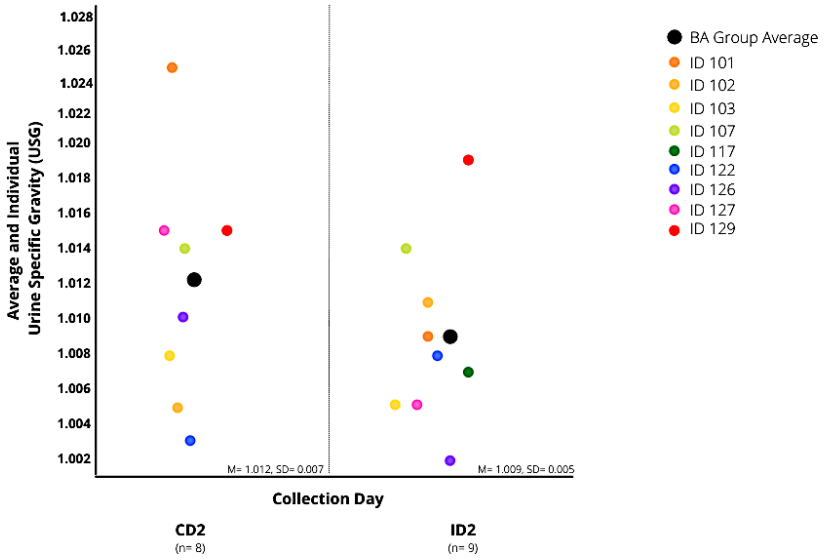

AB Group Average
ID 108
ID 118
ID 123
ID 125
Average and Individual Total Fluid Intake (L)
5.50
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
M= 1.60, SD= 0.63
M= 3.47, SD= 1.24
Collection Day
CD2
(n= 4)
ID2
(n= 4)


Total Group Average
ID 101
ID 102
ID 103
ID 107
ID 108
ID 117
ID 118
ID 122
ID 123
ID 125
ID 126
ID 127
ID 129
Average and Individual Total Fluid Intake (L)
5.50
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
M= 1.58, SD= 0.87
M= 2.86, SD= 1.17
Collection Day
CD5
(n= 12)
ID5
(n= 13)


BA Group Average
ID 101
ID 102
ID 103
ID 107
ID 117
ID 122
ID 126
ID 127
ID 129
Average and Individual Total Fluid Intake (L)
5.50
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
M= 1.61, SD= 0.90
M= 3.10, SD= 1.28
Collection Day
CD5
(n= 8)
ID5
(n= 9)


AB Group Average
ID 108
ID 118
ID 123
ID 125
Average and Individual Total Fluid Intake (L)
5.50
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
M= 1.54, SD= 0.94
M= 2.32, SD= 0.72
Collection Day
CD5
(n= 4)
ID5
(n= 4)


Total Group Average
ID 101
ID 102
ID 103
ID 107
ID 108
ID 117
ID 118
ID 122
ID 123
ID 125
ID 126
ID 127
ID 129
Average and Individual Urine Specific Gravity (USG)
1.028
1.026
1.024
1.022
1.020
1.018
1.016
1.014
1.012
1.010
1.008
1.006
1.004
1.002
M= 1.012, SD= 0.007
M= 1.009, SD= 0.005
Collection Day
CD2
(n= 11)
ID2
(n= 13)


Average and Individual Urine Specific Gravity (USG)
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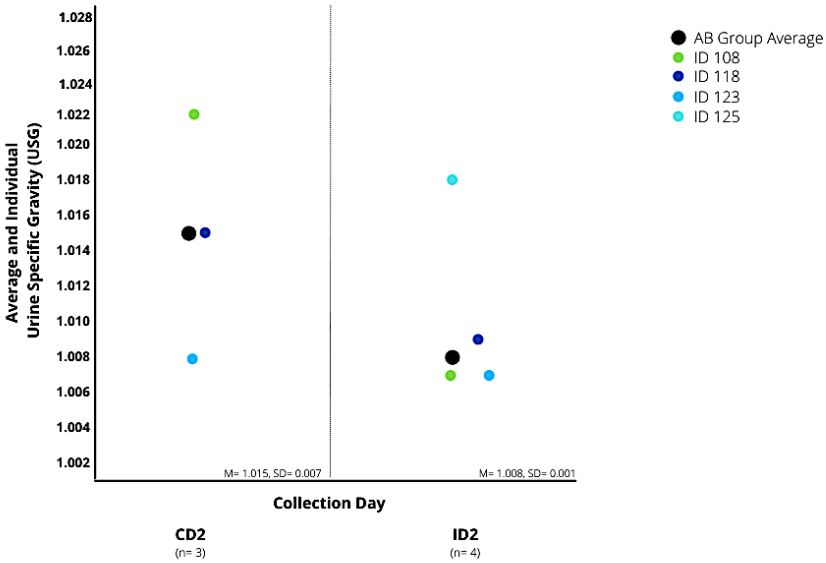
1.026
1.024
1.022
1.020
1.018
1.016
1.014
1.012
1.010
1.008
1.006
1.004
1.002
M= 1.012, SD= 0.007
M= 1.009, SD= 0.005
Collection Day
CD2
(n= 8)
ID2
(n= 9)
BA Group Average
ID 101
ID 102
ID 103
ID 107
ID 117
ID 122
ID 126
ID 127
ID 129


Average and Individual Urine Specific Gravity (USG)
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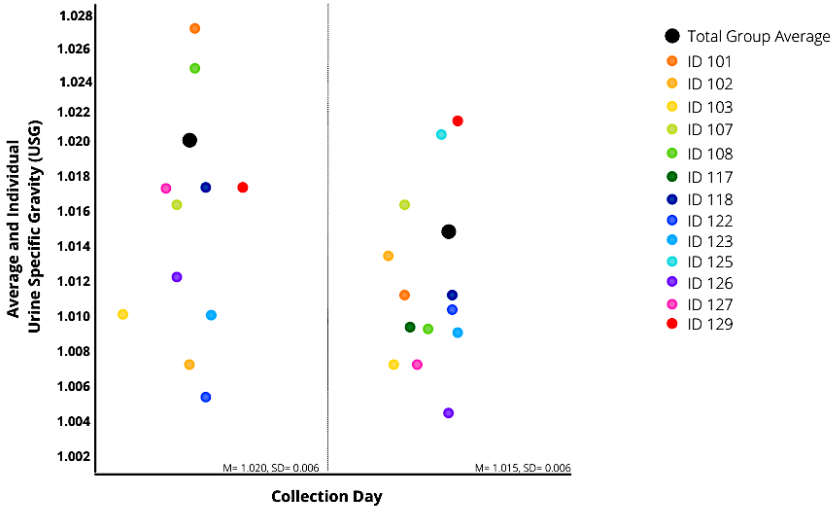
1.024
1.022
1.020
1.018
1.016
1.014
1.012
1.010
1.008
1.006
1.004
1.002
M= 1.015, SD= 0.007
M= 1.008, SD= 0.001
Collection Day
CD2
(n= 3)
ID2
(n= 4)
AB Group Average
ID 108
ID 118
ID 123
ID 125


Average and Individual Urine Specific Gravity (USG)
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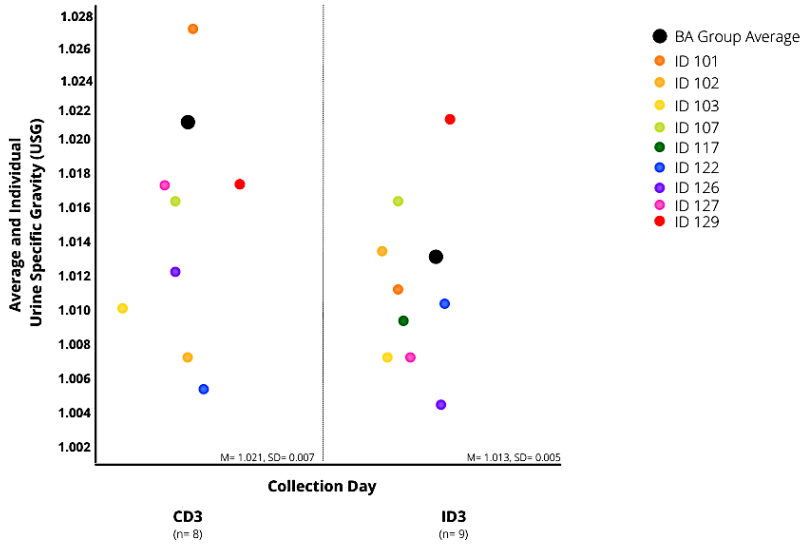
1.026
1.024
1.022
1.020
1.018
1.016
1.014
1.012
1.010
1.008
1.006
1.004
1.002
M= 1.020, SD= 0.006
M= 1.015, SD= 0.006
Collection Day
CD3
(n= 11)
ID3
(n= 13)
Total Group Average
ID 101
ID 102
ID 103
ID 107
ID 108
ID 117
ID 118
ID 122
ID 123
ID 125
ID 126
ID 127
ID 129


Average and Individual Urine Specific Gravity (USG)
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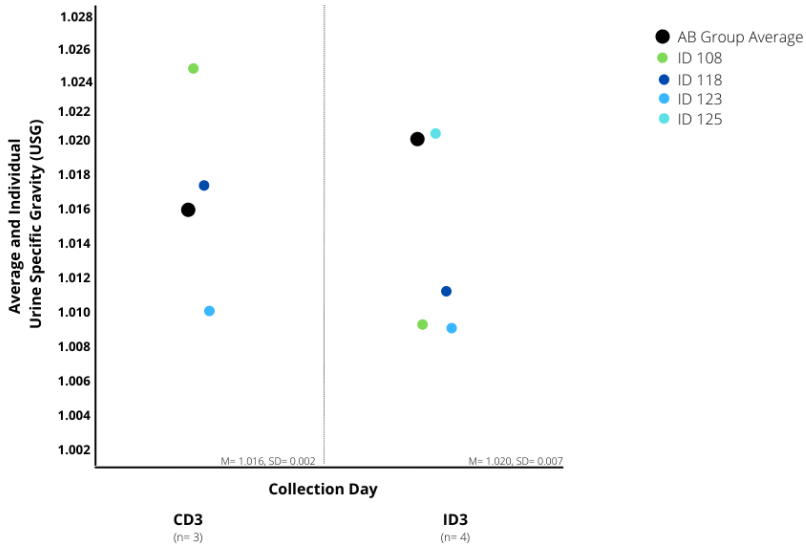
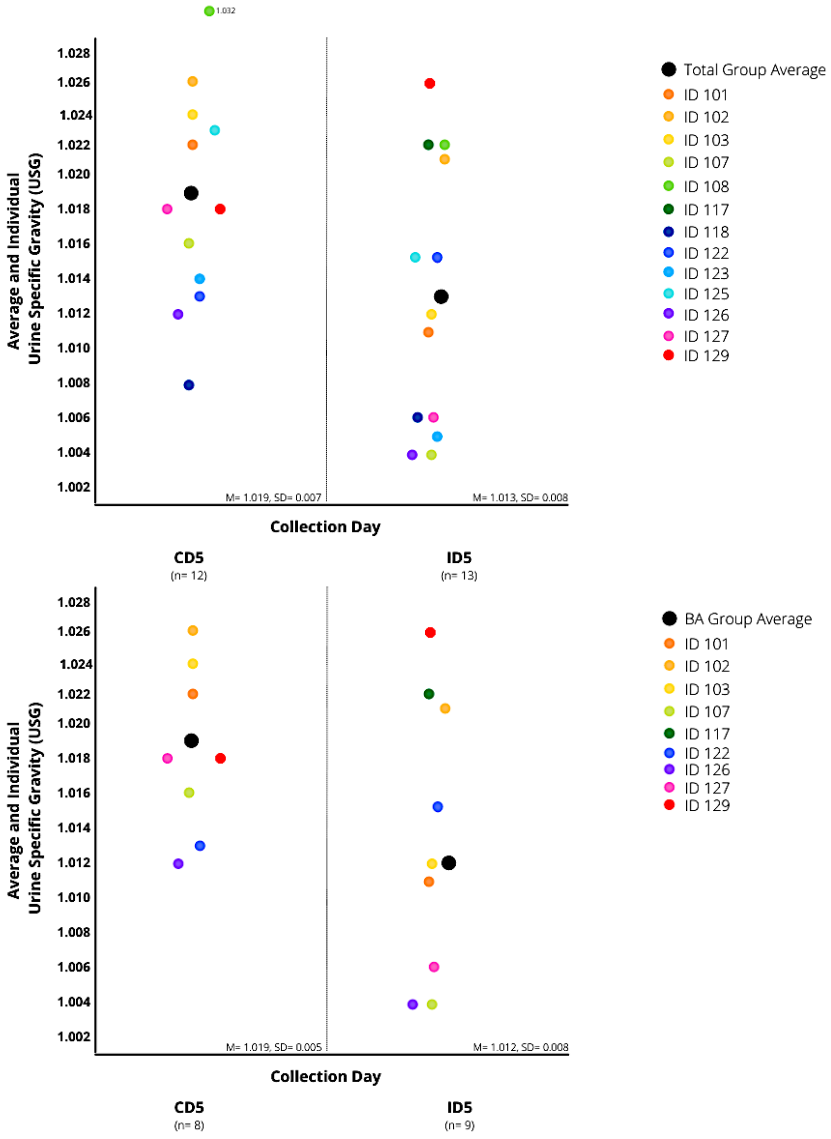
1.026
1.024
1.022
1.020
1.018
1.016
1.014
1.012
1.010
1.008
1.006
1.004
1.002
M= 1.021, SD= 0.007
M= 1.013, SD= 0.005
Collection Day
CD3
(n= 8)
ID3
(n= 9)
BA Group Average
ID 101
ID 102
ID 103
ID 107
ID 117
ID 122
ID 126
ID 127
ID 129


Average and Individual Urine Specific Gravity (USG)
1.028
1.026
1.024
1.022
1.020
1.018
1.016
1.014
1.012
1.010
1.008
1.006
1.004
1.002
M= 1.016, SD= 0.002
M= 1.020, SD= 0.007
Collection Day
CD3
(n= 3)
ID3
(n= 4)
AB Group Average
ID 108
ID 118
ID 123
ID 125


1.032
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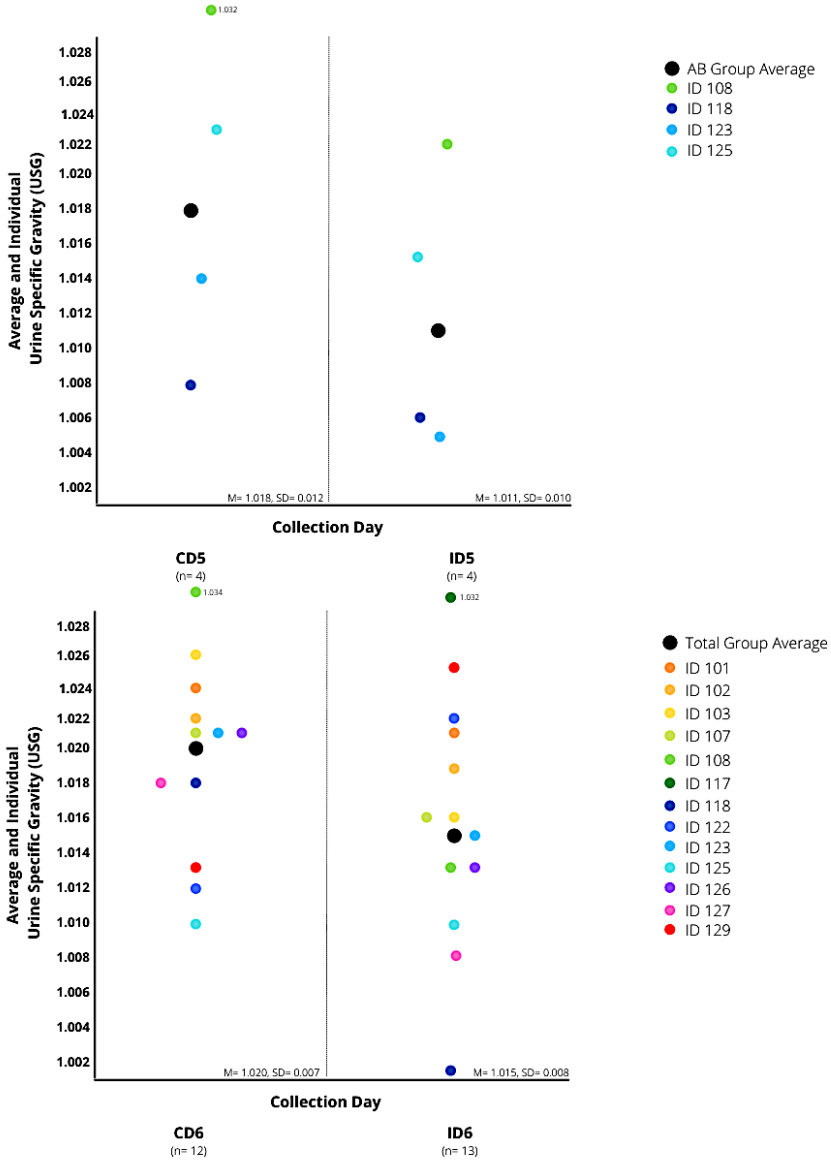
1.026
1.024
1.022
1.020
1.018
1.016
1.014
1.012
1.010
1.008
1.006
1.004
1.002
Average and Individual Urine Specific Gravity (USG)
M= 1.019, SD= 0.007
M= 1.013, SD= 0.008
Collection Day
CD5
(n= 12)
ID5
(n= 13)
Total Group Average
ID 101
ID 102
ID 103
ID 107
ID 108
ID 117
ID 118
ID 122
ID 123
ID 125
ID 126
ID 127
ID 129
1.028
1.026
1.024
1.022
1.020
1.018
1.016
1.014
1.012
1.010
1.008
1.006
1.004
1.002
Average and Individual Urine Specific Gravity (USG)
M= 1.019, SD= 0.005
M= 1.012, SD= 0.008
Collection Day
CD5
(n= 8)
ID5
(n= 9)
BA Group Average
ID 101
ID 102
ID 103
ID 107
ID 117
ID 122
ID 126
ID 127
ID 129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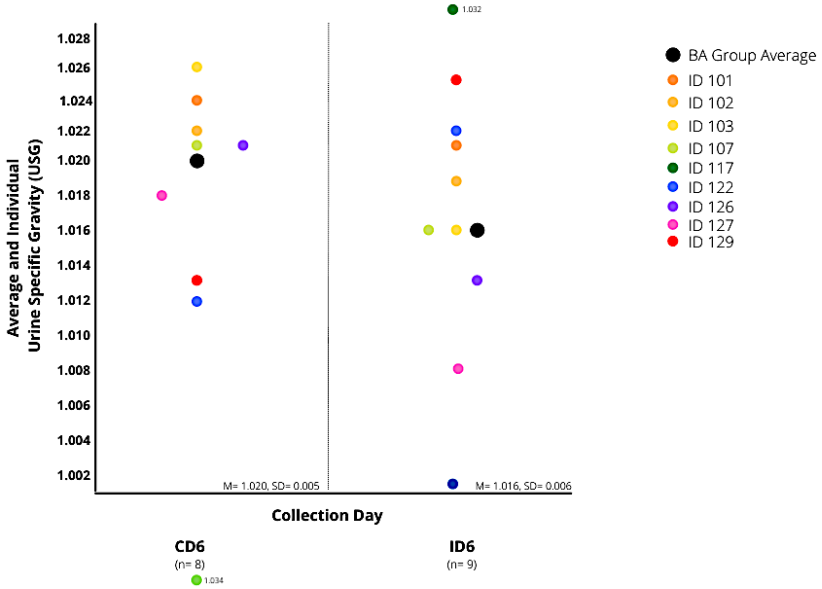
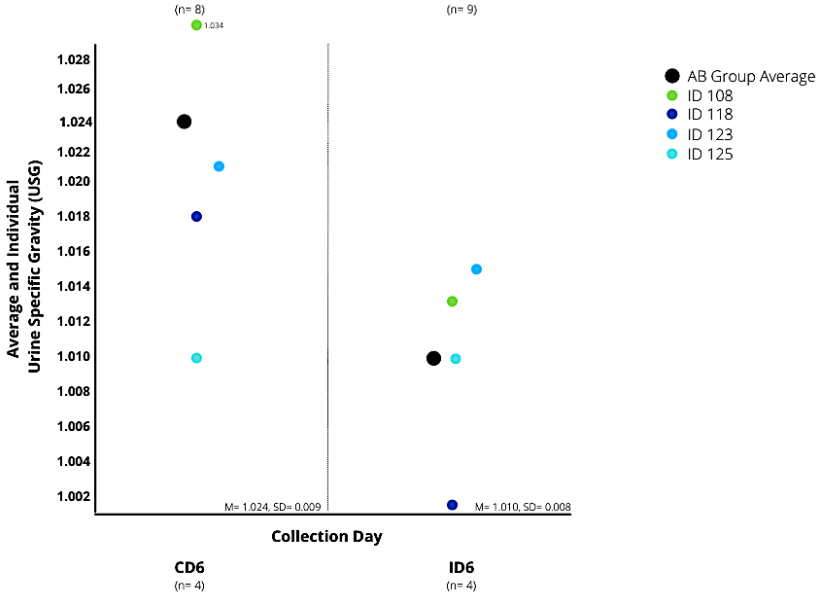
1.026
1.024
1.022
1.020
1.018
1.016
1.014
1.012
1.010
1.008
1.006
1.004
1.002
Average and Individual Urine Specific Gravity (USG)
M= 1.018, SD= 0.012
M= 1.011, SD= 0.010
Collection Day
CD5
(n= 4)
ID5
(n= 4)
AB Group Average
ID 108
ID 118
ID 123
ID 125
1.034
1.032
M= 1.020, SD= 0.007
M= 1.015, SD= 0.008
CD6
(n= 12)
ID6
(n= 13)
Total Group Average
ID 101
ID 102
ID 103
ID 107
ID 108
ID 117
ID 118
ID 122
ID 123
ID 125
ID 126
ID 127
ID 129


Average and Individual Urine Specific Gravity (USG)
1.032
1.028
1.026
1.024
1.022
1.020
1.018
1.016
1.014
1.012
1.010
1.008
1.006
1.004
1.002
M= 1.020, SD= 0.005
M= 1.016, SD= 0.006
Collection Day
CD6
(n= 8)
ID6
(n= 9)
BA Group Average
ID 101
ID 102
ID 103
ID 107
ID 117
ID 122
ID 126
ID 127
ID 129


Average and Individual Urine Specific Gravity (USG)
1.034
1.028
1.026
1.024
1.022
1.020
1.018
1.016
1.014
1.012
1.010
1.008
1.006
1.004
1.002
M= 1.024, SD= 0.009
M= 1.010, SD= 0.008
Collection Day
CD6
(n= 4)
ID6
(n= 4)
AB Group Average
ID 108
ID 118
ID 123
ID 125

APPENDIX I

IRB APPROVAL

APPROVAL: MODIFICATION

Floris Wardenaar
Nutrition
(602) 827-2841
Floris.Wardenaar@asu.edu

Dear Floris Wardenaar:

On 9/24/2021 the ASU IRB reviewed the following protocol:

Type of Review:	Modification / Update
Title:	Female Urogenital Nutrition Health Study
Investigator:	Floris Wardenaar
IRB ID:	STUDY00014289
Funding:	Name: Graduate College (GRAD)
Grant Title:	None
Grant ID:	None
Documents Reviewed:	• Form-Bioscience-Protocol_FUN.docx, Category: IRB Protocol; • FUN Health Study Consent Form.pdf, Category: Consent Form; • FUN Health Study Personal Demographics.pdf, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions); • FUN Health Study Pre-Screener.pdf, Category: Screening forms; • FUN-Health Pre-Screener Consent.pdf, Category: Consent Form; • recruitment_material_24_09_2021.pdf, Category: Recruitment Materials; • recruitment_material_24_09_2021_flyer.pdf, Category: Recruitment Materials;

The IRB approved the modification.

When consent is appropriate, you must use final, watermarked versions available under the "Documents" tab in ERA-IRB.

In conducting this protocol you are required to follow the requirements listed in the INVESTIGATOR MANUAL (HRP-103).

REMINDER - All in-person interactions with human subjects require the completion of the ASU Daily Health Check by the ASU members prior to the interaction and the use of face coverings by researchers, research teams and research participants during the interaction. These requirements will minimize risk, protect health and support a safe research environment. These requirements apply both on- and off-campus.

The above change is effective as of July 29th 2021 until further notice and replaces all previously published guidance. Thank you for your continued commitment to ensuring a healthy and productive ASU community.

Sincerely,

IRB Administrator

cc: Kaila Vento
Stavros Kavouras
Floris Wardenaar
Alexis Koskan
Carol Johnston
Kaila Vento