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ORIGINAL ARTICLE

Bladder urinary oxygen tension is correlated with urinary microbiota composition

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Abstract

Introduction and hypothesis Presence of microbial communities (microbiota) in an organ system depends on environmental factors, such as oxygen availability. We describe a novel technique to measure bladder urine oxygen tension (BUOT) in ambulatory women and use that technique to compare BUOT values to female urinary microbiota and participant urinary signs and symptoms.

Methods Ambulatory female urogynecology patients presenting for clinical care who were willing to undergo transurethral catheterization underwent BUOT determination with a non-invasive flow-through oxygen sensor. To detect urinary microbiota in the bladder, 16S rRNA gene sequencing was performed on catheterized urine. Multivariate statistical analyses were performed to examine potential correlations among BUOT, urinary microbiota compositions and clinical variables.

Results Significant variation in BUOT existed between individuals (range: 0.47–51.5 mmHg; median: 23.1 ± 13.5). Microbiota compositions were associated with BUOT ($p = 0.03$). BUOT was significantly lower in urines that were nitrite negative on dipstick analysis ($p = 0.0001$) and in participants who answered yes to having urinary leakage on the validated Urinary Distress Inventory ($p = 0.01$).

Conclusions BUOTs can be measured in ambulatory women. For urogynecology patients, a wide range of values exist. BUOT may be associated with the presence of urinary microbiota and resultant signs and symptoms.

Keywords Human microbiome · Urinary bladder · Oxygen tension

Introduction

Oxygen is a required element for cellular metabolism and is essential to human survival. Oxygen delivery throughout the body is dependent on the cardiac output, hemoglobin concentration and vascular network. Many comorbidities that affect blood flow to the body, such as tobacco abuse, hypertension and diabetes, can theoretically affect delivery of this critical nutrient, contributing to disease.

The kidney is supplied by a vast network of vessels and receives approximately 25% of the cardiac output with every heartbeat [1]. The oxygen in the kidney diffuses into the urine, and urinary oxygen tension can be measured. Publications from the 1960s describe the use of invasive monitoring devices in humans; those authors found that the oxygen tension of urine is fairly constant under normal conditions, decreasing progressively in value from the kidney (34–58 mmHg) down the ureters and to the bladder (19–44 mmHg). Urinary oxygen tension was dependent on the patient's level of hydration,

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inhaled oxygen content and administration of diuretic or vasopressor agents [2, 3].

Whether the presence of vascular comorbidities affects delivery of oxygen to the bladder is not well established, although bladder ischemia in the context of its relation to lower urinary tract symptoms (LUTS) has been investigated in a few small trials [4–6]. Given the human body's great ability to compensate for subtle variations in conditions, it is also possible that bladder urinary oxygen tension (BUOT) does not have as strong of a correlation with urinary signs or symptoms as it does with the bladder's resident urinary microbial communities (microbiota) [7]. Since the initial report that the bladder urine is not sterile, a series of studies have provided convincing evidence that the female urinary bladder does indeed contain resident microbiota, termed the female urinary microbiota (FUM) [7–13].

Since some microbes are exquisitely sensitive to oxygen tension, the FUM of women with different BUOTs may differ in the composition of its members. Thus, the first step in this investigation is to determine whether the BUOT and FUM compositions are associated. In the era of a known FUM, we hypothesize that a relationship exists between BUOT and microbiota composition. Using a non-invasive oxygen sensor, we describe BUOT ranges in female urogynecology patients, determine if a relationship exists between BUOT and microbiota composition and ascertain if BUOT is associated with urinary signs or symptoms.

Methods

Clinical procedure

After Institution Review Board approval, eligible participants were recruited from the urogynecology practice at a tertiary care medical center. Eligibility criteria included any female participant ≥ 18 years old who was willing to undergo transurethral catheterization—a procedure commonly performed in our clinic for evaluation of urogynecology disorders. Patients were excluded if they were pregnant, non-English speaking or had an indwelling Foley catheter. Demographics, medical history, oral temperature, pulse oximetry and pelvic examination were recorded. Urine dipstick testing was performed to assess for presence of blood, nitrites and leukocytes. Participants completed two validated questionnaires assessing urinary symptoms. The six-question Urinary Distress Inventory Short Form (UDI-6) addresses presence and bother of urinary leakage and obstructive symptoms. The Urinary Tract Infection Symptom Assessment (UTISA) is a 7-domain, 14-question survey of symptoms commonly associated with an acute urinary tract infection (UTI) [14, 15].

Once a participant had signed the consent form, a catheterized urine sample was obtained with the oxygen sensor attached to the catheter. For the majority of the women in this study, first voided and then catheterized urine samples were obtained to measure their post-void residual (PVR) for their clinical care. We also performed a dipstick analysis, and urine that was concerning for a urinary tract infection or microscopic hematuria was sent to the clinical laboratory for clinical care. Any remaining urine was available for study analysis. Only our new patient visits and postoperative visits require a PVR measurement; thus, some of the enrolled women did not require a PVR measurement, and therefore they were catheterized with a full or semi-full bladder. A non-invasive flow-through oxygen sensor with a compact fiber optic oxygen transmitter (Precision Sensing; Regensburg, Germany) was used for BUOT measurement. With this device, values were temperature compensated for each participant. The sensor was attached by a stopcock to the distal end of a standard 14F straight catheter. The proximal end of the catheter was then inserted into the urethra by the investigator. As the urine flowed by the sensor, the oxygen tension readings were obtained at 3-s intervals until the bladder was empty. Urine was collected into a sterile specimen cup for analysis.

Given the need to account for ambient atmospheric oxygen contamination in the sensor system, BUOT was defined as the lowest value obtained if the readings plateaued. Bladder urine volume had to be sufficient to wash out the high levels of atmospheric oxygen levels (ambient atmospheric oxygen levels are 160 mmHg). BUOT was measured every 3 s. A value was considered plateaued if it was < 2% lower than the previous value. Bladder oxygen tension values that did not reach such a nadir were considered to be an inaccurate representation of the BUOT, and the participant was excluded from subsequent analyses. Women were also excluded from subsequent analyses if they did not have enough urine left over after their clinical sample was obtained (~ 5 ml). The determination of whether a BUOT reading had plateaued was made at the time of the data analysis. The statisticians who made this determination were blinded to the demographics, clinical measures (other than BUOT) and urinary microbiota results. BUOT measurements were reported as an average of the three lowest readings obtained.

Sample analysis

An aliquot of the urine sample was labeled and sent to the clinical microbiology laboratory for processing. An additional aliquot of urine was frozen with 10% AssayAssure (Sierra Molecular Corporation, Incline Village, NV) for subsequent 16S ribosomal RNA (rRNA) gene sequencing. Samples were extracted in batches using custom lysing buffer and enzymes and the Qiagen blood and tissue kit. A two-step PCR amplification of the variable region 4 (V4) of the gene was performed using universal primers 515F and 806R. Subsequent library preparation and sequencing was performed on an

Ilumina MiSeq at Loyola Genomics Facility. Technical replicates negative and positive controls were obtained for confirmation of sequencing results. This procedure was previously described in detail [16]. A third aliquot of the urine sample was immediately analyzed by expanded urinary quantitative culture (EQUC), which was performed as a quality assurance measure of sequencing. The protocol for this culture procedure has been published previously [10].

Quality control and de-multiplexing of multiplex sequence data were done with onboard MiSeq Control software and MiSeq Reporter (current version: 2.1.43). Then, the *mothur* pipeline, with its protocols for MiSeq-generated 16S rRNA gene sequence data, was used to combine paired end reads and remove contigs of incorrect length, contigs containing ambiguous bases and chimeric sequences [17]. Taxonomic classification at the genus level was performed using *mothur*'s built-in RDP Classifier with its default parameter. To correct the uneven sequencing depth among different urine samples, a random subsampling of 5000 reads per sample was performed for all the subsequent microbiota analyses. Samples with < 5000 reads were classified as undetectable by this method.

Statistical analysis

To explore the potential correlation between BUOT and individual bacterial abundance, we attempted standard linear regressions. However, further examination revealed that BUOT typically does not display a linear relationship with the abundance of individual bacteria (e.g., a bell-shaped tendency can be observed in scatter plots of BUOT versus bacterial abundance). Therefore, linear regressions are not suitable for the analysis. Instead, we applied the canonical correspondence analysis (CCA) test, which is robustly used in ecology to identify correlations between species abundance and environmental gradients with non-linear relationships. In our project, the environmental gradients refer to the different BUOT measurements. The CCA test was performed using the R package *vegan* to examine the correlation between BUOT and urinary microbiota compositions. Pearson/Spearman/Kendall's correlation tests, t-test and Wilcoxon signed-rank test were used to explore the correlation between BUOT and various clinical variables, such as age, menopausal status, prolapse stage, dipstick analysis and validated questionnaires [18]. Multiple test correction was performed using the Benjamini-Hochberg procedure [19].

Results

We enrolled 115 participants, who were primarily Caucasian with a mean age of 62.5 (range 24–91) years. In 111 (96.5%) of the participants, we obtained bladder oxygen levels; we failed to obtain levels for four participants because of technical issues with the sensor. Sixty-two (56%) of these 111 participants reached the pre-specified plateau of BUOT readings, which was based on the lowest reading being within 2% of the previous reading. The mean urine volume obtained at the time of catheterization (with the oxygen sensor attached to the catheter) was 114 ml for 62 participants who had a BUOT reading that plateaued and 49 ml for the 52 participants who did not ($p < 0.0001$). The lowest BUOT value also varied between the groups; for those who did and did not plateau, the BUOT was 23 and 31 mmHg, respectively ($p = 0.037$).

Of the 62 participants with a BUOT that plateaued, 34 had ≥ 5000 sequencing reads and were included in further statistical analysis. Table 1 compares the participant demographics, clinical data and BUOT measurements for the group with sufficient sequencing reads ($N = 34$) and those without ($N = 28$). There were no clinically significant differences in demographics, patient history, relevant medication use, symptom scores or stage of prolapse. The only variable in the dipstick analysis that varied was that 100% of women who did not have sufficient sequencing reads had negative leukocyte esterase ($p = 0.005$). Pulse oximeter readings, BUOT and catheterized urine volumes also did not vary between the two groups of participants.

A significant variation in BUOT existed among the 34 participants with sufficient sequencing reads, with a range of 0.47 to 51.5 mmHg (median = 23.1, SD = 13.5). Figure 1 displays a histogram of frequency of oxygen tensions (in mmHg) among the 34 participants analyzed. Figure 2 shows the bacterial community compositions, with each participant's urine microbiota composition organized by increasing BUOT. Using the CCA test on genus level data, we found that BUOT is significantly associated with microbiota composition ($p = 0.03$).

BUOT was associated with one clinical sign, namely the presence of nitrites in urine dipstick analysis. BUOT values were significantly lower in those participants with nitrite-positive urine ($p = 0.0001$). Analysis of BUOT and clinical urinary symptoms, as measured by the UTISA and UDI-6, reveals that BUOT values are significantly lower in women who report presence of urinary leakage, as specified in the fourth question of the UDI-6 ($p = 0.01$) (Fig. 3). There was no significant relationship between BUOT and the other clinical variables collected.

Discussion

This study demonstrates that BUOT can be measured in ambulatory women using an inexpensive straight catheter stopcock and disposable sensor. However, the technique requires a urine volume sufficient to wash out the atmospheric oxygen (160 mmHg) and allow the decreasing BUOT values to plateau. Thus, not all women who participated in this clinical trial had sufficient urine volumes and were thus excluded from

Table 1 Demographic and clinical measures of study population

	Final participants ($N = 34$)	Obtained accurate BUOT but not in final analysis ($N = 28$)	p value
Age (years) (range)	65 (32–88)	65 (41–85)	0.898
BMI (SD)	29.4 (6.9)	28.8 (6.3)	0.720
Ethnicity (n, %)			
Caucasian	27 (79.4%)	23 (82.2%)	1[a]
Other	7 (20.6%)	5 (17.8%)	
High blood pressure (n, %)	16 (47.0%)	8(28.6%)	0.101[a]
Smoker (n, %)	3 (8.8%)	4(14.3%)	0.210[a]
Diabetes (n, %)	5 (14.7%)	2(7.1%)	0.594[a]
Postmenopausal status (n, %)	26 (76.5%)	26 (92.9%)	0.162[a]
Hormone therapy use (n, %)	5 (14.7%)	4 (14.3%)	1[a]
Incontinence medication (n, %)	6 (17.6%)	1 (3.6%)	0.180[a]
Stage of prolapse (n, %)			
0–1	13 (38.2%)	14 (50.0%)	0.501
2	4 (11.8%)	6 (21.4%)	0.495
3–4	6 (17.6%)	2 (7.14%)	0.397
Pessary use	2 (5.9%)	2 (7.14%)	1[a]
UDI-6 Score (SD)	40.1 (30.4)	35.8 (25.2)	0.486[b]
UTISA score (SD)	0.31 (0.8)	0.56 (1)	0.2883[b]
Urine dipstick analysis			
Leukocytes (n, %)			
Negative	24 (70.6%)	28 (100%)	0.005[a]
Trace or small	4 (11.8%)	0	
Moderate	2 (5.9%)	0	
Large	3 (8.8%)	0	
Nitrites			
Positive	6 (17.6%)	2 (7.14%)	0.407
Blood			
Negative	5 (14.7%)	5 (17.8%)	1[a]
Trace-small	24 (70.6%)	20 (71.4%)	1[a]
Moderate-large	3 (8.2%)	3 (10.7%)	1[a]
Bladder urine oxygen tension (SD) mmHg	23.1 (13.9)	24.0 (12.7)	0.515[b]
Pulse oximeter reading % saturation (SD)	96.9% (2.3)	95.9% (9.3)	0.426[b]
Catheterized volume ml (SD)	124 (94)	103 (45)	0.938

[a] Chi-squared

[b] Wilcoxon signed rank test

further analyses. In our study, mean void urine volumes of ~110 ml were more likely to result in a BUOT that plateaued, whereas mean void urine volumes of ~50 ml were unlikely to plateau. In fact, as bladder volumes increased by 55%, BUOT values decreased by 35%. This supports our rigorous methodology, which required that BUOT values plateau. We contemplated ways to "wash out" the oxygen measuring system with sterile saline or water, but decided against such strategies because we were concerned that we might introduce bacterial contamination.

We also found that BUOT varies among women, displays a relatively wide range of oxygen tensions and is significantly associated with urinary microbiota composition. While oxygen tension levels in the bladder are dependent on many variables, such as oxygen levels in the kidney, hydration status and inhaled oxygen, this is the first study to report a relationship between the oxygen levels and the urinary microbiota. This finding is not surprising, as the urinary microbiota include bacteria that require oxygen for survival (aerobes), bacteria for which oxygen is toxic (obligate anaerobes) and a range of bacteria with varying oxygen tolerances (facultative anaerobes) [10]. Clinical medicine has focused on single microbial species as a cause of disease or health. In actuality, microbes do not live in isolation; they exist as part of larger

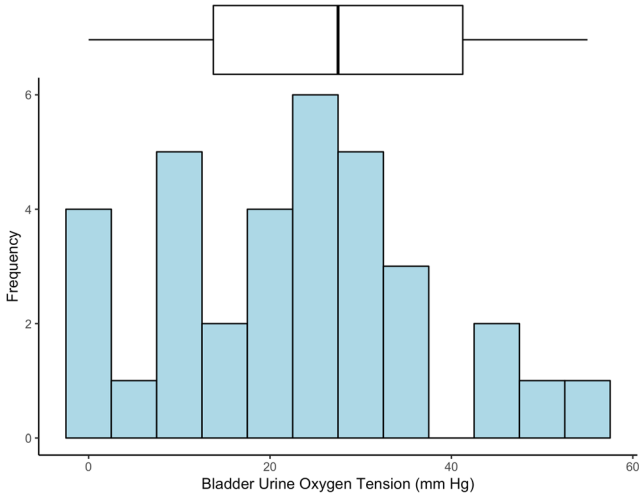

Fig. 1 Histogram of BUOT frequencies ($N = 34$)

microbiota whose compositions are shaped by environmental factors. Evidence that a relationship exists between microbiota and BUOT can now facilitate future exploration into how these microbial communities are influenced by BUOT and how those communities impact health and disease.

This work also demonstrates that BUOT may be associated with some urinary signs and symptoms. Specifically, lower BUOT was associated with the presence of nitrites on dipstick analysis and presence of urinary leakage. Clinically, nitrites are found in the urine of patients whose urine contains high enough levels of bacteria to reduce urinary nitrates to nitrites [20]. This can be seen in urine dipstick analysis of patients with UTIs. The urinary pathogens causing presence of nitrites also may lead to lower BUOT, which is supported by our findings of higher relative abundance of *Escherichia coli* in participants with lower BUOT (Fig. 2).

Women with urinary tract infections often report transient worsening of their urinary symptoms, including urgency, frequency and leakage. As a result, it is clinically plausible that a lower BUOT is associated with a higher abundance of *E. coli* and the symptom of urinary leakage. In an innovative study in Greece in the late 1990s, the voided urine of patients with culture-proven *E. coli* UTIs had significantly lower oxygen tension than the voided urine of healthy patients. Oxygen tension also was found to be decreased in patients with influenza and increased in patients with chronic renal failure [21]. Our current study only measured BUOT in a small number of ambulatory urogynecology patients. The observed association between lower BUOT, urinary leakage and the presence of *E. coli* would best be studied in women with and without urinary symptoms because not all *E. coli* strains are pathogenic.

Our study did not show significant correlations between vascular comorbidities (smoking, hypertension and diabetes) and either lower BUOT tension or more severe urinary symptoms. This was a correlation hypothesized to exist with advanced vascular disease, as chronic ischemia-related bladder dysfunction has been cited [4]. This lack of correlation is most likely due to the small sample size and low rate of advanced comorbidities in the participants of this study. Given the participants were well enough to present to an outpatient clinic, their disease may not have been advanced enough (severity not quantified); conversely, it may support the human body's ability to compensate for such disease. A large survey study of over 2000 men and women in Austria explored the association between presence of vascular risk factors (diabetes, hypertension, hyperlipidemia, tobacco abuse) and LUTS using a symptoms questionnaire. They found that while none of these vascular diseases alone were independently correlated to LUTS, there was an association between the number or vascular comorbidities and presence of LUTS [22].

Strengths of this study include use of a urethral catheter with a flow-through oxygen sensor device attached, which

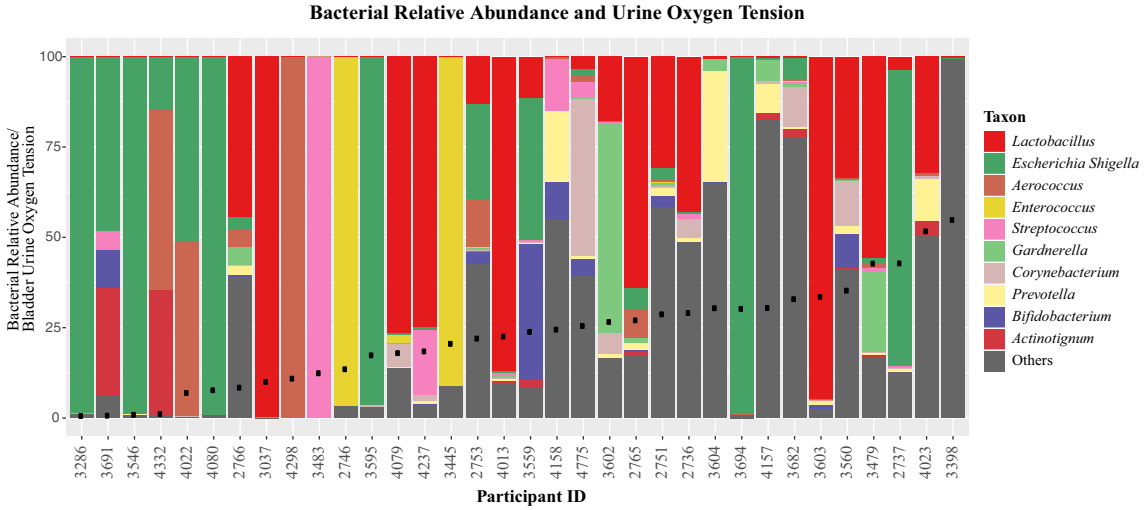

Fig. 2 Relative abundance of bacterial genera in each participant, as determined by 16S rRNA gene sequencing and organized by ascending BUOT (black dots). "Others" includes all detected minor genera

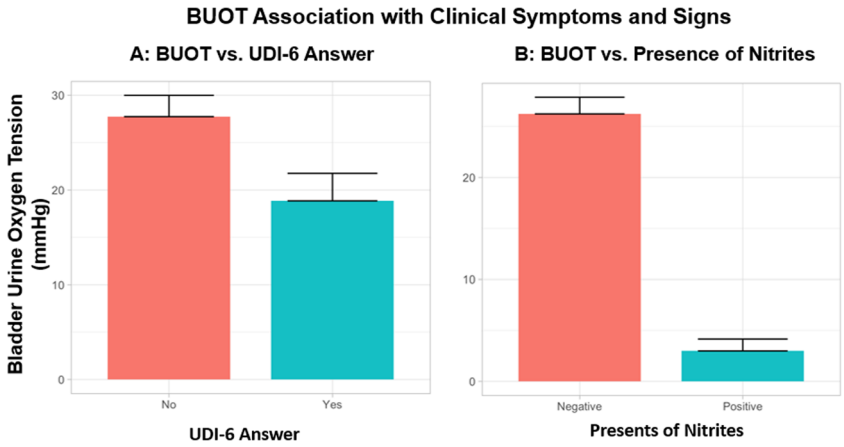

Fig. 3 BUOT association with clinical symptoms and signs. **a** BUOT is significantly higher in patients who answered "No" to the 4th question in the Urinary Distress Inventory-6: "Do you leak small drops of urine?" **b** BUOT is significantly higher in participants whose urine was nitrite-negative on dipstick analysis

was critical for two aspects of the study. First, this method allowed us to avoid post-bladder contamination (urethra, vulva and vaginal). Second, it allowed for a closed system to reduce atmospheric oxygen (160 mmHg) as the urine flowed past the sensor. The novel and less invasive nature of the technique allowed measurement of BUOT in ambulatory women rather than requiring anesthesia and ureteral catheters as performed in some of the earlier studies. The BUOT values obtained in our study are similar to the values obtained in the 1960s by Leonhardt and Landes [2, 3]. The most frequent BUOT values between studies were nearly identical (20–35 mmHg in our data versus 25–35 mmHg in the 1960s literature). Although the range obtained in our study was wider (0.46–51.5 mmHg vs. 19–44 mmHg), this was likely due to our ability to detect lower values with the closed-system device compared with voided samples. Additionally, the major taxa in the sequencing data were also found to be culturable (data not shown), indicating that our results reflect live endogenous bacteria in bladder.

One limitation of this study was the relatively small sample size. Only 55% of women who had credible BUOT measurements had sufficient sequencing reads to be considered for analysis. Also, at the start of this study, we were not aware that women needed to have a minimum of ~110 ml of urine to obtain an accurate reading. As a result, our use of women who had previously voided prior to undergoing BUOT measurement resulted in a large number of samples that could not undergo microbiota analysis. Thus, our future studies of BUOT will enroll women with a comfortably full bladder. We also recruited a specific population—urogynecology patients—who by being seen in such a clinic are more likely to have urinary symptoms than women enrolled from the community. However, all-comers of this patient population were included, no matter their diagnoses or symptom profile. Future studies with larger sample sizes including non-urogynecological populations with well-characterized demographics and urinary symptoms may provide insight into the relationship between the BUOT and its impact on the bladder microbiota.

Overall, this is the first study to demonstrate that BUOT variation exists in ambulatory women and may be significantly related to both urinary microbiota and urinary signs and symptoms.

Compliance with ethical standards

Conflicts of interest MB Shannon: none. R Limeira: none. D Johansen: none. X. Gao: none. H Lin: none. Q Dong: none. AJ Wolfe: received research grants from Astellas and Kimberly-Clark. ER Mueller: received research grants from Astellas, is consultant for Butler-Snow/Ethicon and sits on a board for Boston Scientific.

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