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Urine: Waste Product or Biologically Active Tissue?

Urinology Think Tank Writing Group^{*}, Jonathan Barasch¹, Tamara Bavendam², Lori Birder³, Tony Buffington⁴, Lysanne Campeau⁵, Roger Dmochowski⁶, Stephen M. Hewitt⁷, Warren G. Hill⁸, Deborah K. Hoshizaki², Jayoung Kim⁹, Jon Klein¹⁰, Jenna M. Norton², Patrick Seed¹¹, Joseph Williams¹², Weiqun Yu⁸, and Robert Star²

¹Department of Pathology and Cell Biology, Columbia University, New York, NY

²Division of Kidney, Urologic, and Hematologic Diseases, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD

³Department of Pharmacology and Chemical Biology, University of Pittsburgh, Pittsburgh, PA

⁴University of California Davis School of Veterinary Medicine, Davis, CA

⁵Department of Surgery, McGill University, Montreal, Quebec, Canada

⁶Department of Urology, Vanderbilt University School of Medicine, Nashville, TN

⁷Experimental Pathology Laboratory, Laboratory of Pathology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD

⁸Department of Medicine, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA

⁹Departments of Surgery and Biomedical Sciences, Cedars-Sinai Medical Center and University of California Los Angeles, Los Angeles, CA

¹⁰Department of Medicine and James Graham Brown Foundation in Proteomics, University of Louisville, Louisville, KY

¹¹Departments of Pediatrics, Molecular Genetics and Microbiology, and Surgery, Duke University, Durham, NC

¹²Department of Evolution, Ecology and Organismal Biology, Ohio State University, Columbus, OH

Abstract

Aims—Historically, urine has been viewed primarily as a waste product with little biological role in the overall health of an individual. Increasingly, data suggest that urine plays a role in human health beyond waste excretion. For example, urine might act as an irritant and contribute to symptoms through interaction with—and potential compromise of—the urothelium.

Methods—To explore the concept that urine may be a vehicle for agents with potential or occult bioactivity and to discuss existing evidence and novel research questions that may yield insight

^{*}Correspondence: Tamara G. Bavendam, MD, MS, NIH/NIDDK/KUH, 6707 Democracy Blvd, Office 6081, MSC 5458, Bethesda, MD 20892-5458, Phone: (301) 594-4733, Fax: (301) 480-3510, tamara.bavendam@nih.gov.

into such a role, the National Institute of Diabetes and Digestive and Kidney Disease invited experts in the fields of comparative evolutionary physiology, basic science, nephrology, urology, pediatrics, metabolomics, and proteomics (among others) to a Urinology Think Tank meeting on February 9, 2015.

Results—This report reflects ideas that evolved from this meeting and current literature, including the concept of urine quality, the biological, chemical, and physical characteristics of urine, including the microbiota, cells, exosomes, pH, metabolites, proteins, and specific gravity (among others). Additionally, the manuscript presents speculative, and hopefully testable, ideas about the functional roles of urine constituents in health and disease.

Conclusion—Moving forward, there are several questions that need further understanding and pursuit. There were suggestions to consider actively using various animal models and their biological specimens to elaborate on basic mechanistic information regarding human bladder dysfunction.

Keywords

biomarkers; exosomes; inflammatory response; interstitial cystitis; lower urinary tract symptoms; microbiome; overactive bladder; pelvic pain; urinary incontinence; urinary tract infection; urine

INTRODUCTION

The composition of urine reflects the generation of by-products of ongoing metabolism and the overall need to maintain solute and water balance in the body.¹ Historically, urine has been viewed primarily as a waste product with no biological role in the overall health of an individual, except perhaps as the “culture-media” for uropathic *Escherichia coli* (*E. coli*) during the course of urinary tract infections (UTIs). The examination of urine as a diagnostic tool of systemic health spans more than 8,000 years of human history.^{2,3} Johannes de Ketham published the urine wheel in 1491 to illustrate how the color and consistency of urine could be used to determine a diagnosis.⁴ Macroscopic examination of urine (uroscopy) was performed throughout antiquity and middle ages; urine was visually examined, smelled, and even tasted to determine the internal state of the body. Microscopic examination of urine developed during the 19th and 20th centuries, and in 1933, R.F. Pitts developed urine markers to determine how the kidney functions.⁵

During the past few decades, urine is viewed as a convenient source for biomarkers that might provide insights to human health. Investigators have used sophisticated methods such as mass spectrometry to identify and catalogue concentrations of exosomes, cells, proteins, and other small molecules in urine to identify constituents that might predict or indicate the presence of diseases such as urinary incontinence (UI), overactive bladder, interstitial cystitis/bladder pain syndrome (IC/BPS) and UTIs.^{6–11} The current status of urine biomarkers for IC/BPS recently has been reviewed; to date no urine biomarker has been determined to be diagnostic.¹²

Notwithstanding, urine may play roles in human health beyond waste excretion. Anecdotal reports suggest that lower urinary tract symptoms (LUTS) such as frequency, urgency and pain/discomfort vary considerably during the course of a day and may be provoked by

certain types of fluids, food, and circumstances that alter urine composition.¹³ For example, some IC/BPS patients have reported that symptoms improve with increased water intake and/or after elimination of specific fluids and foods from their diet. Variability in IC/BPS patients' pain symptoms appears to correlate with urinary concentration, and conversely, may be relieved by urinary dilution,^{14,15} but apparently not by ingestion of acidic foods when presented blindly to study participants.^{14–16} These observations suggest that urine might act as an irritant and contribute to symptoms through interaction with—and potential compromise of—the urothelium.^{17,18}

NIDDK'S URINOLOGY THINK TANK

To explore the concept that urine may be a vehicle for (potential) bioactive agents and to discuss existing evidence and novel research questions that may yield insight into such a role, the National Institute of Diabetes and Digestive and Kidney Disease (NIDDK) invited experts in the fields of comparative evolutionary physiology, basic science, nephrology, urology, pediatrics, metabolomics, and proteomics (among others) to a *Urinology Think Tank* meeting on February 9, 2015. The meeting discussion was framed around the following questions:

- Is there evidence that molecules in the urine play an active role in bladder homeostasis, dysfunction, or disease?
- Does urine allow for communication from the kidney to the bladder?
- Is it worthwhile to pursue the idea of urine as a biologically active fluid?
- What data are needed and what types of studies are necessary to determine whether urine is an active fluid?

This report reflects ideas that evolved from this meeting and current literature, and presents speculative, and hopefully testable, ideas about the functional roles of urine constituents in health and disease.

THE CONCEPT OF URINE QUALITY AND POTENTIAL EFFECTS ON THE UROTHELIUM

Based on meeting discussion, the concept of urine quality emerged. Much like how water quality may affect the ecologic health of a body of water, urine quality is proposed as a potential factor contributing to bladder health. Specifically, the authors define urine quality as the biological, chemical, and physical characteristics of urine, including the microbiota,^{19–21} cells,²² exosomes,^{23–26} pH, metabolites,^{7,27} proteins,^{28,29} and specific gravity^{30–32} (among others). These features might interact in complex ways with one another and the bladder and/or kidney to potentially affect health. A challenge in assessing urine quality is to determine the source of its constituents. The metabolites, exosomes, and proteins might be filtered through or secreted by the kidney, or might be released from the urothelium at any level from the renal pelvis to the urethra.

Existing evidence supports the notion that urine quality—or more specifically molecules in the urine—may affect the function of the urothelium (Figure 1). The urothelium is more than a barrier between urine and the underlying bladder tissue; it also serves as a functional layer that may modulate bladder function. Urothelial function in molecular transport is demonstrated by solute and water movement, and is supported by the presence of numerous functional receptors and channels (e.g., aquaporins, urea transporters, ion channels, mineralocorticoid receptors).³³ Urothelial cells express many hormone receptors, including purinergic receptors. Adenosine triphosphate (ATP) is released into the urinary space upon bladder stretch.³⁴ Secretion of other molecules by the urothelium also may occur during filling-emptying cycles, which may stabilize the filling process and improve vascular perfusion.^{33,35–38} In addition, the urothelium is sensitive to the excretion of drug metabolites and can affect other sensory functions.^{34,39} Growth factors, including epithelial growth factor (EGF) and fibroblast growth factor receptor (FGFR), also may regulate different urinary epithelia, given the high level of exposure to toxins and the mechanical demands on the bladder.⁴⁰ Several growth factors such as NGF, BDNF and VEGF can be released following inflammatory triggers and directly act on their associated receptors.⁴¹

MICROBIOTA OF THE BLADDER AND URINE QUALITY

A potential role for the microbiota is perhaps the most likely of all proposed aspects of urine quality according to current evidence, and this includes studies of microbiota in the urine of healthy patients.^{42–44} This evidence emphasizes a new perspective for the role of urine in bladder health: urine as a culture media. The discovery of microbes in the healthy bladder adds to the complexity of understanding bladder health and development of LUTS and raises key questions about the potential role of microbiota in the urine: might urine microbiota influence the composition and quality of urine, and does the composition and quality of the urine in turn influence the microbiota? Might “healthy” urine microbiota be protective, providing a degree of homeostasis in response to the normal variation in urine composition? Might pathological changes in the microbiota directly or indirectly affect urine quality and bladder function? Although there are clear examples for uropathogenic *E. coli*, little is known about whether changes in the microbiota positively or negatively affect the growth of uropathogenic *E. coli* or other species. Evidence suggests that a specific urinary microbiome is associated with bladder health, and that the urinary microbiota may be altered in patients with urologic cancers, IC/BPS, neurogenic bladder dysfunction, sexually transmitted infections,⁴⁴ as well as in individuals with spina bifida (Patrick Seed, personal communication) or urgency UI^{45,46} compared with healthy individuals and contribute to pathogenesis of these diseases.

The interface between the microbiota and the interior wall of the bladder presents opportunities for the microbiota and urine to affect bladder function through interaction with the urothelium (Figure 1). Pathogenic microbes in close proximity to the bladder surface might compromise its barrier function and initiate a series of perceived or actual symptoms. For example, an in vitro study of the canine bladder showed that UTI resulted in decreased tight junction integrity in the urothelium and resulted in epithelial death and inflammatory responses.⁴⁷

Interaction between the microbiota and urothelium might in turn affect detrusor muscle activity and ultimately influence bladder function/dysfunction. The detrusor muscle remains relaxed during bladder filling to allow the bladder to store urine, and contracts during micturition to release urine. Filling or stretch on the bladder wall elicits release of signaling and chemical molecules that can modulate the activity of both afferent nerves and the muscular components of the detrusor. Mechanisms have been described by which the urothelium influences bladder function/dysfunction via the detrusor muscle. For example, studies using both animal and human bladder strips have consistently shown that release of urothelial mediators (both inhibitory and excitatory) can regulate the smooth muscle contractility.^{48,49} In addition, changes in the underlying microvasculature and extracellular matrix can not only alter the bladder architecture but impact urothelial-cell interactions in bladder health and disease.⁵⁰

The microbiota also might influence more distant structures of the urinary tract. Bladder infection stimulates gene expression in the kidney,⁵¹ suggesting a signaling mechanism from bladder to kidney. A specific cell type appears to be activated in UTI, the intercalated cell, which is otherwise known for H⁺ excretion.⁹ These studies suggest that the kidney might regulate the microbiome of the urogenital system and contribute to its defense.^{9,52,53}

Other components of urine quality may influence the microbiota and modulate relationships between the microbiota and bladder health. Urine has been shown to inhibit expression of the virulence factors in uropathogenic *E. coli*,⁵⁴ the most common cause of community-acquired UTI. We speculate that changes in diet might affect the composition of the urine to affect the bladder microbiota. For example, ingestion of cranberries (juice and extract) has been studied for prevention of bladder infections, with conflicting results.⁵⁵ The proposed mechanism of action is that proanthocyanidin decreases the adhesiveness of *E. coli* to uroepithelia cells.⁵⁶ In another example, patients with diabetes or those with glucosuria induced by medications⁵⁷ have increased risk of UTIs, potentially a result – at least in part – of higher glucose concentrations in urine promoting growth of uropathic *E. coli*.⁵⁸ In these and other examples, urine may affect the ability of uropathic *E. coli* to colonize the bladder by influencing expression of genes that produce the Type 1 pili, which enable adhesion to the urothelium.⁵⁴

OTHER POTENTIAL COMPONENTS OF URINE QUALITY

Other potential components of urine quality are less well studied. Yet, data are available to merit further exploration. A precedent has been set for the idea of nutritionally derived urinary substances influencing pain symptoms; however, the required blinded, randomized, controlled trials to demonstrate causal links have not been conducted. Food gluten neuropathy and its dietary management has been associated with urinary symptoms, implicating gluten metabolic byproducts, immunological/allergic reactions, or a shared system of neuronal development and function between the gut and bladder.^{59–65} It is well established that caffeine increases bladder muscle activity,⁶⁶ and carbonation or spicy and acidic flavors reportedly trigger symptoms, especially in patients with IC/BPS.^{67–69} Additionally, It is possible that exosomes might mediate communication from the kidney or

bladder to other cells, perhaps by endocytosis, transferring content and altering cellular behavior as a means of communication.^{70–72}

Limitations

Gaps remain in our understanding of the influence(s) of urine constituents on healthy and diseased urothelium. This is partially due to differences in experimental approaches, which range from in vitro studies through studies of healthy animals subjected to various induced injuries, to investigations of human beings with naturally-occurring diseases studied at variable times during the course of their disease.

The potential roles of ingested substances on urine composition and subsequent influences on the urothelium remain unclear. For example, studies have reported that consumption of some foods influence symptoms in patients with IC/BPS.⁷³ To date, however, these investigations appear to have relied on patient reports, so participants already may have known what foods they were “supposed” to respond to. The authors of a recent review concluded that, “The need for double-blind, placebo-controlled research to evaluate the role of foods and beverages on pain and urinary symptoms is warranted.”⁷³ The blinded food trial is a well-developed methodology in allergy medicine⁷⁴ that might be applied to the study of the effects of foods on the urine and urothelium. One such study was conducted as part of a Master’s thesis (reported in abstract form only) 25 years ago.⁷⁵ Fisher et al concluded from their study of the effects of ingestion of acidic foods on urinary pH and symptomatology in patients with IC/BPS that, “exclusion of acidic foods from the diet of IC pts (sic) is not indicated.”¹⁶

While human data from well-controlled trials is the gold standard for investigating the effect of dietary components on patient symptoms, an alternative approach would be to use rodent models to evaluate micturition readouts such as void spotting and cystometry. Advantages of this approach include the ability to administer controlled doses (e.g., using defined concentrations added to drinking water or food gels), controlling the time of exposure to substances, collection of urine that can be analyzed – either by metabolic cage or simpler urine collection systems – and studies could examine effects in both males and females. More complex study designs would allow the dissection of interactions between disease (e.g., UTI) and diet. Of course, if dietary components are shown to affect micturition parameters in rodents, then the bladders can be removed and assayed further for changes in permeability, morphology, presence of immune cells, and inflammatory markers, to name a few.

Normalization of measured concentrations of biomarkers in the urine also presents significant challenges. Urinary biomarkers are frequently reported as a normalized ratio to urinary creatinine concentration [UCr] to control for variations in urine flow rate. Unfortunately, [UCr] varies for a wide variety of reasons, including age, sex, diet, kidney function, intra-individual variability over time, etc.⁷⁶ Additionally, the concentration of creatinine in urine is approximately 100 times that in the blood,⁷⁷ so changes in bladder permeability might reduce [UCr], further confounding interpretation of results. Ingested markers such as para-amino benzoic acid,⁷⁸ and external markers such as lithium also have been used, with variable results.⁷⁹ Internal markers have a variety of limitations,⁸⁰ and

external markers do not appear to have been explored extensively. Given these limitations, methods for normalizing urine constituent concentrations will need to be validated for each population under study. Standardizing the concentrations of a substance with reference to urine osmolality is a possibility; however, just like UCr may suffer from the confounding effects of kidney diseases.

We also currently lack standard, well-validated protocols that are widely used across organizations for the collection, fractionation, storage, and shipping of urine, although the U.S. Food and Drug Administration provides guidelines for bioanalytical method evaluation that could provide useful guidance for developing such protocols.⁸¹ One recent development worthy of further investigation for some compounds may be dried urine spots.^{82,83}

Future Directions

In this report on the proceedings of the *Urinology Think Tank* meeting held in February, 2015, the authors aimed to summarize current researched-based knowledge on urine biology. Moving forward, there are several questions that need further understanding and pursuit. What is considered normal/healthy urine? What is the biological meaning of urine in our body? How can urine-based molecular signatures be interpreted? What are the origins of these molecules, and what are their target tissues? How do gender differences influence urine composition in various pathological conditions compared to healthy controls? The NIDDK-funded, Multi-Disciplinary Approach to the Study of Pelvic Pain (MAPP) network studies have begun to provide novel basic and mechanistic findings in this field by carefully examining the clinophysiology of urine obtained from patients with urologic chronic pelvic pain syndrome. To elaborate on this basic mechanistic information regarding human bladder dysfunction, there were suggestions to consider actively using various animal models and their biological specimens.

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Figure 1.

Potential Influence of Urine Quality on Bladder Health through Interactions with the Urothelium: a Theoretical Model. Several components within (e.g., microbiota, metabolites, proteins, exosomes) and characteristics of (e.g., specific gravity, chemical composition) the urine may influence bladder health via interaction with the urothelium. Additionally, many of these components and characteristics may influence *one another*.