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Age- and region-related changes in the biomechanical properties and composition of the human ureter

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ABSTRACT

The ureter has been largely overlooked heretofore in the study of the biomechanics of soft biological tissues, although there has been significant motivation to use its biomechanical properties as inputs to mathematical models of ureteral function. Herein, we used histological analysis for quantification of collagen contents and thickness/area of ureteral layers, with concomitant geometrical analysis of zero-stress and no-load states, and inflation/extension testing to biomechanically characterize with the Fung-type model the ureters from cadavers. The effects of age and gender on the regional distribution of those properties were examined. Tissue properties did not differ ($p > 0.05$) between the left and right ureter. Regional heterogeneity was established that was profoundly age-related but seldom gender-related, based on the following evidence: 1) In younger subjects, the axial stress-circumferential strain curves of upper ureter were shifted to smaller stresses and model parameter a_2 representing axial stiffness was smallest ($p < 0.05$), i.e. upper ureter was the least stiff region axially; 2) upper ureter underwent axial stiffening with advanced age, evidenced by the increasing ($p < 0.05$) parameter a_2, and the stress-strain curves were uniformly exhibited along the ureter, evidenced by the non-varying ($p > 0.05$) parameters C, a_1, a_2, and a_4; 3) aging raised ($p < 0.05$) the collagen content of upper ureter to favor a near-uniform regional distribution; 4) wall thickness increased with age, unlike the opening angle and residual strains, reflecting the thickening of outer (muscular) vs. inner (mucosal) layers in aged subjects, with significant differences ($p < 0.05$) in some regions; and 5) gender affected little ($p > 0.05$) the opening angle and morphometry of no-load and zero-stress states.

1. Introduction

The ureters are paired tubular structures that propel urine produced by the kidneys to the urinary bladder. They are about 25–30 cm long and 2–3 mm in radius in the average adult, with their upper half located in the abdomen and their lower half located in the pelvic area. Histologically, they have thick walls consisting of the mucosa, the fibrous lamina propria, the muscular layer facilitating peristalsis, and externally of the adventitia (Wein et al., 2016). Surprisingly, the ureters have been largely overlooked in the study of soft tissue biomechanics, even though their conduit function is clearly mechanical, and there is clinical motivation to study their biomechanical properties given that they may be subject to iatrogenic or other types of mechanical trauma, e.g. trauma caused by motor vehicle accidents (Burks and Santucci, 2014; Morey et al., 2014). Additionally, the ureters may become mechanically obstructed by numerous conditions, e.g. kidney stones, tumors, infection, blood clots, in which case ureteral stents are inserted to keep them open, thereby restoring urine flow to the bladder and allowing the kidney to function normally (Smith et al., 2012).

Existing mathematical simulations of ureteral function in physiologic/pathophysiologic states and after clinical treatments (Waters et al., 2008; Vahidi et al., 2011; Vahidi and Fatouraee, 2012; Hosseini et al., 2013; Gómez-Blanco et al., 2016) are limited by oversimplified assumptions regarding the biomechanical properties and ignore residual strains, assumptions inconsistent with data from other soft tissues (Gregersen, 2002). Pioneering studies on animal ureters have been worthwhile to establish basic principles, i.e. the exponentially-shaped and pseudoelastic deformational response (Yin and Fung, 1971; Knudsen et al., 1994), and regional differences in that response as well as in residual strains (Hansen and Gregersen, 1999). Our group (Sokolis, 2012, 2014) recently reported inflation/extension data for rabbit ureter with reference to a properly-determined zero-stress state, fulfilling

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basic requirements for multiaxial characterization (Fung, 1993; Humphrey, 2002), but it is questionable whether these data may be extrapolated to the human condition. Because of the relative scarcity of human data (Boone and Smith, 1955; Rassoli et al., 2014), we initiated biomechanical studies in cadaveric tissue.

In this article, we report on the geometrical and biomechanical characterization based on the Fung-type model, using histological analysis as an adjunct study to assist with the quantification of layer-specific wall thickness and collagen content. We examined the effects of aging and gender on the regional distribution of those properties in an attempt to address physiological implications.

2. Material and methods

2.1. Human ureteral tissue and specimen preparation

Both ureters were harvested within 24 h of death from twelve subjects whose bodies had been preserved at 4 °C before autopsy at the Department of Forensic Medicine and Toxicology, Athens University Medical School. Subjects with important pathology of the urinary system were excluded; cadaveric data are listed in Table S1 (Supplementary material). The research protocol was approved by the Institutional Ethics Committee on Human Research. Informed consent was obtained from the relatives. All ureters were excised as long tubes, including the major calyces and a small part of the bladder, and stored in refrigerated saline within 48 h of harvesting until experimentation. Adherent tissue was trimmed and the ureters were subsequently divided into upper, middle, and lower regions (Fig. 1). Two tubular segments from each region, keeping clear of the renal pelvis and the bladder by 2 cm, were used in the experiments.

2.2. Biomechanical analysis

The biomechanical and histological methods are described elsewhere (Sokolis, 2012, 2014) as well as in Sections §S.2.1–S.2.3 (Supplementary material). Briefly, the ureters were cannulated at 100–120% of their no-load length while immersed in oxygenated (5% CO_2 in O_2) calcium-free Krebs solution (37 °C) with EGTA to abolish muscle tone. Five (preconditioning) inflation-deflation loops between 0–50 mmHg were performed on each length to minimize viscoelasticity and the inflating limb from an additional loop was used for analysis. At completion of the inflation/extension tests, two rings were removed from the midpoint of the ureteral segments to determine the zero-stress/no-load states.

The inflation/extension measurements and geometrical characteristics of zero-stress/no-load states were used to calculate biomechanical parameters, assuming that ureters are nonlinear, pseudo-elastic, anisotropic, incompressible, and homogeneous tubes subjected to finite deformation, whose radially cut-open state is stress-free. The theoretical formulation for a thick-walled and residually-stressed cylinder under intraluminal pressure and axial force is presented in standard textbooks of biomechanics, e.g. (Humphrey, 2002).

First, the diameter-pressure and force-pressure data were regressed with polynomial functions of 7th–9th order, as deemed appropriate, using MicroCal Origin v8.5 (OriginLab® Corp, Northampton, MA, USA), giving the same weight to the entire pressure range and each axial stretch. Polynomials were fitted to >1000 data points and the regressions accepted when correlation coefficients reached $r>0.95$. All biomechanical parameter calculations were done on the regressed data. The internal diameter d_i during inflation/extension was calculated from:

$$d_i=\sqrt{d_e^2-\frac{4A_0}{\pi\lambda_z}}, \tag{1}$$

where d_e was the external diameter and A_0 the cross-sectional area of the ureteral wall at the zero-stress state. The circumferential and axial stretch ratios λ_θ and λ_z were calculated relative to the zero-stress state as:

$$\lambda_\theta=\frac{s}{S},\quad \lambda_z=\frac{l}{L}, \tag{2}$$

where S was the zero-stress state circumference of the ureter and s that at the no-load or loaded states, and L and l were the *ex situ* and *in situ* lengths. The circumferential residual strains E_θ were determined at the internal and external wall boundaries using the Green strain definition:

$$E_\theta=\frac{1}{2}\left(\lambda_\theta^2-1\right). \tag{3}$$

Mean strain E_θ was calculated via mid-wall circumference at zero-stress and loaded states, given by $S=(S_e+S_i)/2$ and $s=(s_e+s_i)/2$. Mean values of normal stress components (2nd Piola–Kirchhoff definition) in the circumferential and axial directions S_θ and S_z were determined using the following equations:

$$S_\theta=\frac{Pd_i}{\lambda_\theta^2(d_e-d_i)},\quad S_z=\frac{4F+P\pi d_i^2}{2\pi\lambda_z^2\left(d_e^2-d_i^2\right)}, \tag{4}$$

where P was intraluminal pressure and F axial force.

To generate model parameters for future computational simulations of the ureter, the data from each specimen were characterized by the Fung-type model (Fung et al., 1979):

$$W=\frac{C}{2}(\exp Q-1),\quad Q=a_1E_\theta^2+a_2E_z^2+2a_4E_\theta E_z, \tag{5}$$

via parameters C (scaling factor) and a_1, a_2, and a_4, characterizing respectively tissue stiffness in the circumferential and axial directions and their interaction, as in (Sokolis, 2012, 2014) for rabbit ureter. Note that this model is applicable to the 3D boundary conditions of inflation/extension testing by direct enforcement of incompressibility (Humphrey, 2002). Pressure-diameter-force data ($\sim$4700) from 1–50 mmHg and all except the 100% axial stretch (to avoid negative axial forces associated with bending) were simultaneously fitted using a user-defined C-routine in MicroCal Origin and the least-squares Levenberg–Marquardt algorithm. The parameters were restrained by inequalities that ensured model convexity. Optimization was repeated for various

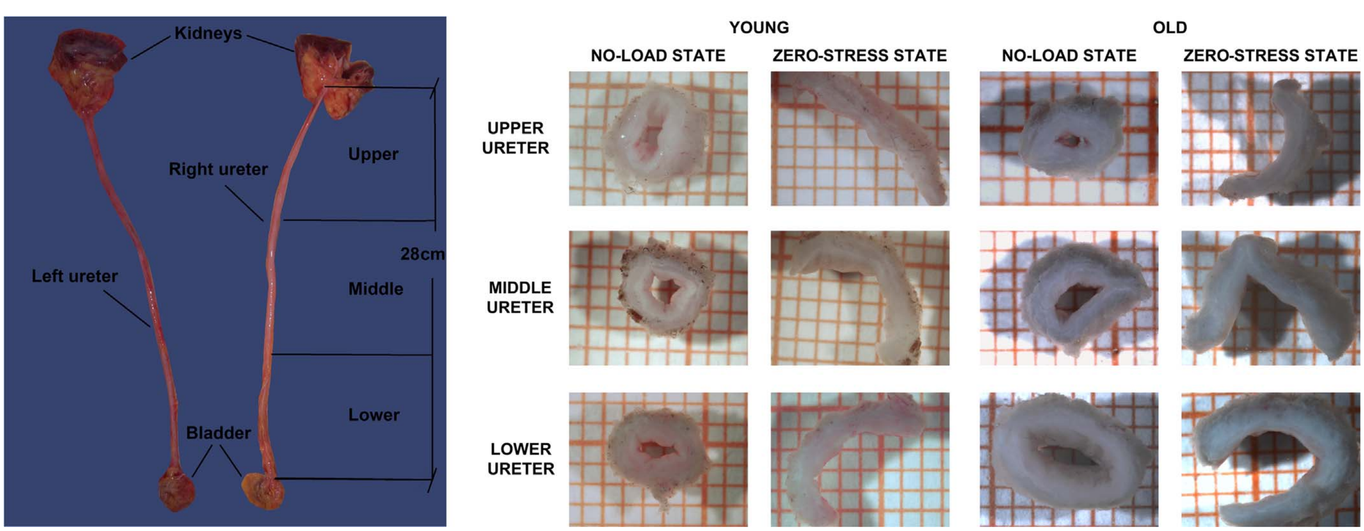

Fig. 1. Left panel: preparation of testing specimens; fresh intact ureters from a 68 y old female after cleaning adherent tissues. The upper, middle, and lower thirds are demarcated, from which straight tubular segments approximately 3 cm in length were removed for histology, inflation/extension testing, and determination of the no-load and zero-stress states. Right panel: representative photos of closed and radially-cut rings from the upper, middle, and lower ureter of a younger (25 y old) and an older subject (65 y old) at the no-load and zero-stress state. To enable calibration of dimensions, the rings were placed over millimeter paper.