Impact of a folic acid-enriched diet on urinary tract function in mice treated with testosterone and estradiol

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1Department of Comparative Biosciences, University of Wisconsin-Madison, Madison, Wisconsin; 2Department of Surgical Sciences, University of Wisconsin-Madison, Madison, Wisconsin; 3Department of Urology, University of Wisconsin-Madison, Madison, Wisconsin; 4Carbone Cancer Center, University of Wisconsin-Madison, Madison, Wisconsin; and 5George M. O’Brian Center of Benign Urology, University of Wisconsin-Madison, Madison, Wisconsin

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Keil KP, Abler LL, Allmann HM, Wang Z, Wang P, Ricke WA, Bjorling DE, Vezina CM. Impact of a folic acid-enriched diet on urinary tract function in mice treated with testosterone and estradiol. Am J Physiol Renal Physiol 308: F1431–F1443, 2015. First published April 8, 2015; doi:10.1152/ajprenal.00674.2014.—Aging men are susceptible to developing lower urinary tract symptoms, but the underlying etiology is unknown and the influence of dietary and environmental factors on them is unclear. We tested whether a folic acid-enriched diet changed urinary tract physiology and biology in control male mice and male mice with urinary dysfunction induced by exogenous testosterone and estradiol (T+E2), which mimics changing hormone levels in aging humans. T+E2 treatment increased mouse urine output, time between voiding events, and bladder capacity and compliance. Consumption of a folic acid-enriched diet moderated these changes without decreasing prostate wet weight or threshold voiding pressure. One potential mechanism for these changes involves water balance. T+E2 treatment increases plasma concentrations of anti-diuretic hormone, which is offset at least in part by a folic acid-enriched diet. Another potential mechanism involves neural control of micturition. The folic acid-enriched diet, fed to T+E2-treated mice, increased voiding frequency in response to intravesicular capsaicin infusion and increased mRNA abundance of the capsaicin-sensitive cation channel transient receptor potential vanilloid subfamily member 1 (Trpv1) in L6 and S1 dorsal root ganglia (DRG) neurons. T+E2 treatment and a folic acid-enriched diet also modified DNA methylation, which is capable of altering gene expression. We found the enriched diet increased global DNA methylation in dorsal and ventral prostate and L6 and S1 DRG. Our results are consistent with folic acid acting to slow or reverse T+E2-mediated alteration in urinary function in part by normalizing water balance and enhancing or preserving afferent neuronal function.

cystometry; mouse model; voiding; folic acid; epigenetics

BENIGN PROSTATIC HYPERPLASIA (BPH) is characterized by benign enlargement of the prostate. BPH is often associated with lower urinary tract symptoms (LUTS) that can include increased urinary frequency and urgency, increased nighttime urination, pain, weak stream, hesitancy, dribbling, incomplete emptying, and incontinence (25). Approximately 70% of men over age 70 experience BPH and/or LUTS (5).

Aging-related changes in plasma hormone concentrations are potential driving factors in the onset and progression of BPH and LUTS. Plasma testosterone concentration declines as men age, while estradiol concentration remains the same or even rises (13). Rodents treated with testosterone and estradiol (T+E2) to mimic this aging-associated hormonal milieu develop enlarged prostates, narrow prostatic urethras, and retain urine in a manner consistent with bladder outlet obstruction (4, 27).

Our group and others have been elucidating proliferative growth pathways in developing prostates with the goal of examining whether these pathways are activated inappropriately in aging men to drive BPH and LUTS (24). We recently showed that DNA methylation of the androgen receptor (Ar) controls the developing mouse prostate’s response to androgens, timing of ductal initiation, and quantity of ducts formed (17). Since androgenic signaling and new postnatal ductal growth are implicated in BPH, and since DNA methylation mediates changes in transcript abundance in the aging prostate and elsewhere (10, 14, 35, 37), we considered that aberrant DNA methylation patterns may contribute to BPH and LUTS.

DNA methylation is malleable and influenced by many environmental factors, including diet. This is relevant because dietary factors have been associated with BPH symptoms and bladder function (12). Folic acid is among a handful of dietary constituents that function as methyl donors for DNA methylation (38) and can increase or decrease DNA methylation in a gene- and cell type-specific fashion (3, 8). Furthermore, folic acid deficiency is associated with nocturnal bed wetting in children (1). It is added to most one-a-day and prenatal vitamins, occurs naturally in some foods, and is supplemented in others. Serum folate concentrations have risen ~2.5-fold in the US population since cereal grain fortification was initiated in 1998 (29). In addition, >40% of American men over age 60 take dietary supplements containing folic acid. This age group is a population at risk for developing BPH/LUTS, but it is unclear whether supplemental folic acid impacts urinary function in these men and whether dietary folic acid may be used as a tool to modify urinary function.

We characterized how dietary folic acid enrichment and T+E2 treatment influenced DNA methylation in mouse prostate, bladder, and L6 and S1 dorsal root ganglia (DRG) of neurons projecting to the bladder, prostate, and elsewhere. We also determined how these factors influence specific aspects of mouse urinary function and test the hypothesis that a folic acid-enriched diet delays or decreases severity of urinary dysfunction in T+E2-treated mice. Our results reveal that a folic acid-enriched diet is capable of modifying several potential contributors to urinary dysfunction in T+E2-treated mice. The diet protects against T+E2-mediated disruption of water balance, increases in bladder capacity and compliance, and sensitizes the voiding response to nociceptive stimulation.
Folic acid may mediate the latter action by augmenting afferent Trpv1 mRNA abundance in DRGs associated with afferent neurons that project to the bladder, prostate, and elsewhere. Together, these results indicate that dietary folic acid modifies urinary tract function in mice with obstructive voiding behavior.

MATERIALS AND METHODS

Mice. Sexually mature C57BL/6J nulliparous female mice were housed in polysulfone cages containing corn cob bedding and maintained on a 12:12-h light-dark cycle at 25 ± 5°C and 20–50% relative humidity. At least 2 wk before their first mating, female mice were placed on a base diet (control diet, Harlan Diet 2019, Harlan Teklad, Madison, WI) containing 4 mg/kg folic acid or fed the same base diet supplemented with folic acid to achieve a final concentration of 24 mg/kg folic acid (folic acid-enriched diet, Harlan Diet 120256). The folic acid-enriched diet provided 10 times more folic acid than the recommended daily requirement for mice (30). This 2-wk loading period was previously shown to be sufficient to increase maternal serum folic acid concentrations (28a) and induce changes in DNA methylation in offspring (38). Food and water were available ad libitum. Food and water bottles were weighed weekly to determine consumption. Dams were maintained on diets throughout lactation, and their offspring received the same diets after weaning and for the remainder of the experiment. Six-week-old male mice underwent sham surgery or surgery to subcutaneously implant testosterone (25 mg) and estradiol (2.5 mg; T2 implantation). All mice were randomized to carbachol was expressed as % maximal response to KCl, and this expansion. The maximal response of each tissue was determined as the peak to baseline pressure. Voiding was recorded for at least 1 h for each animal until a stable pattern was achieved. In some mice, capsaicin was added stepwise thereafter. Tissues were then washed with carbachol-free Krebs solution to return the tension to baseline, and a maximal contractile response was generated by adding 60 mM KCl to the baths. The maximal response to carbachol was expressed as % maximal response to KCl, and this normal value was normalized to bladder mass.

Cystometry. Mice were anesthetized with urethane (1.25 g/kg ip). A midline incision was made in the abdominal wall to expose the bladder. A purse string suture using 6-0 thread was inserted near the dome of the bladder. Catheters were created from PE-50 tubing, cut 2 mm shorter than the length of a 26-G syringe needle, and one end of the tubing was melted to create a cuff. The needle was run through the tubing and inserted through the apex and across the bladder wall. The needle was then removed and the purse string suture tightened, followed by two circumferential sutures to fix the catheter in place. The body wall and skin were sutured, and the animal was allowed to recover for 45 min before saline infusion. During experimentation, the animal was maintained in a supine position, and the cystometry catheter was connected to a three-way stopcock connected to an infusion pump and pressure transducer. Sterile saline was infused at a rate of 1.6 ml/h, and voiding was recorded for at least 1 h for each animal until a stable pattern was achieved. In some mice, capsaicin (30 μM diluted in saline) was instilled at a rate of 1.6 ml/h after mice reached steady-state voiding in response to saline infusion. Measurements were analyzed from at least six sustained voiding events per animal once a consistent pattern was achieved. Voiding events were subdivided into sustained voids, defined here as events where three or more drops of urine were produced, and drip voids, where only one or two drops of urine were produced. Parameters analyzed include intervoid interval, defined as the length of time between sustained voids; nonvoiding contractions, defined as a change in pressure greater than 5 mmHg not associated with expression of urine; maximal intravesical pressure associated with each voiding or nonvoiding contraction, defined as the peak to baseline pressure; void duration, defined as the time between maximal intravesical pressure and return to baseline pressure; threshold pressure, defined as pressure to elicit a voiding contraction and voiding event; capacity, defined as the interval multiplied by the saline infusion rate; compliance, defined as capacity divided by the change in pressure between voids; and leak point pressure, defined as pressure required to induce a void by applying pressure to the bladder filled to half-capacity as described previously (22).

Real-time quantitative PCR. Quantitative PCR (QPCR) was conducted as described previously (19) on bladder or DRGs from five mice per experimental treatment group using gene-specific primers for Trpv1

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mined by the ΔΔCt method as described previously (23) and normalized to peptidyl prolyl isomerase a (Ppia) abundance.

Statistics. Results are reported as means ± SE. Statistical analysis was performed using R version 2.13.1. Homogeneity of variance was determined using Levene’s test. For parametric data with two treatment groups, Student’s t-test was used to identify significant differences (P < 0.05) between groups. For parametric data with more than two treatment groups, one-way ANOVA, followed by Tukey’s honest significant difference (HSD), least significant difference (LSD), or a repeated measures ANOVA followed by pairwise t-tests using Holm correction were used to identify significant differences (P ≤ 0.05) between or among treatment groups. For nonparametric data with more than two treatment groups, the Kruskal-Wallis test was conducted. Contingency tables were analyzed using Fisher’s exact test.

RESULTS

Here, we characterize how dietary folic acid enrichment and T+E2 treatment influence prostate and urinary function. Increased dietary folic acid levels can be encountered throughout life. We modeled this exposure paradigm by enriching folic acid levels in male mice starting at conception and continuing through adulthood. Male mice were maintained on diets for 8 wk following T+E2 pellet implantation surgery to create four experimental groups: control diet sham, control diet T+E2, folic acid diet sham, and folic acid diet T+E2 (Fig. 1).

Influence of diet and T+E2 treatment on serum homocysteine concentration, mouse body mass, and food consumption over time. We tested whether a folic acid-enriched diet was sufficient to decrease plasma homocysteine concentration, a stable biomarker inversely related to plasma folic acid concentration. Serum homocysteine concentrations were significantly lower in 14-wk-old adult mice exposed to a folic acid-enriched diet in utero and through adulthood compared with mice receiving a control diet (Fig. 2A). T+E2 treatment alone did not impact homocysteine levels (results not shown). Therefore, the folic acid-enriched diet is sufficient to cause changes in methyl donor levels and may be responsible for observed changes in physiology.

Folic acid enrichment of the standard mouse diet has the potential to influence food palatability and therefore consumption by pregnant dams as well as their offspring. This could impact several modifiers of urinary function, including water consumption, body mass, and development of prostate, bladder, and other urinary tract tissues. To address these issues, we examined the number of prostatic ductal precursors (buds)

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**Fig. 1.** Experimental design. C57BL/6J dams were maintained on a control (4 mg/kg folic acid) or control diet supplemented with folic acid (24 mg/kg folic acid) for 2 wk before mating. Timed matings were performed, and the resulting male offspring were maintained on maternal diets through lactation, weaning, and adulthood. Body mass and food and water consumption were measured at weekly intervals starting at 3 wk of age and continuing through the end of the experiment at 14 wk of age. Six-week-old adult male mice underwent subcutaneous implantation of testosterone and estradiol (T+E2) pellets. Physiology and voiding function were determined 1 or 2 mo later at 10 and 14 wk of age, respectively.

**Fig. 2.** A folic acid-enriched diet decreases serum homocysteine concentration. A: male mice were maintained on a control or folic acid-enriched diet in utero and through lactation and adulthood. Serum homocysteine concentration was quantified by ELISA. Values are means ± SE. *Significant difference, P < 0.05; n = 4. Male mice were maintained on the maternal control or folic acid-enriched diet through lactation and weaning. Quantification of body mass (B), weekly food consumption (C), and weekly water consumption (D) from 3 to 6 wk of age is shown. Values are means ± SE. *Significant difference, P < 0.05; n ≥10/group.
formed in male mouse fetuses and found they were not affected by the maternal diet (20). We also found that the folic acid-enriched diet increased male mouse body mass at 4–6 wk of age (Fig. 2B). There was a significant main effect of time, treatment group, and an interaction of these variables on body mass after T+E2 implantation or sham surgery (Fig. 3A, Supplemental Table 2; all supplemental material for this article is accessible on the journal website). The folic acid-enriched diet and T+E2 treatment increased body mass at select stages of early adulthood, but these differences are not present at 14 wk of age when urinary function and prostate and urethral biology were assessed (Fig. 3A and B, Supplemental Table 1).

Food consumption increased equally over time in both diet groups at 3–5 wk of age (Fig. 2C). Following T+E2 implantation or sham surgery, there was a significant main effect of time and treatment group on food consumption (Fig. 3C, Supplemental Table 2). The folic acid-enriched diet decreased food consumption at 7–8 wk of age (Fig. 3C, Supplemental Table 1), but food consumption did not differ between diet groups from 11 to 14 wk of age, when most physiological measurements were made (Fig. 3D). Differences in body mass and food consumption diminished as mice aged; as a result, these factors do not likely account for all differences observed at later time points when most physiological measurements were made.

We did not observe a difference in water consumption between diet groups at 3–5 wk of age (Fig. 2D). There was a significant main effect of time, treatment group, and an inter-

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action of these variables on water consumption after surgery (Fig. 3E, Supplemental Table 2). T+E2 treatment increased water intake at select stages in both diet groups (Fig. 3E, Supplemental Table 1). The folic acid-enriched diet decreased water intake at select stages in sham mice and T+E2-treated mice (Fig. 3E, Supplemental Table 1). Most importantly, the folic acid-enriched diet decreased the absolute magnitude of water intake in T+E2-treated mice until 10 wk of age (Fig. 3, F and G). These results indicate that T+E2 treatment rapidly (within 2 wk) increased fluid intake and that the folic acid-enriched diet moderated the magnitude of this response.

A folic acid-enriched diet and T+E2 treatment changed water balance in mice. The fact that T+E2 increased fluid intake prompted us to examine whether urine concentration was also affected. Urine specific gravity and protein concentration were both decreased by T+E2 treatment (Fig. 4, A–C). Changes in water consumption are often associated with aberrant endocrine regulation. Diabetes mellitus, which is associated with diuresis and excessive thirst, is an unlikely contributor since fasting blood glucose concentrations were not different between groups (Fig. 4D). However, we found that T+E2 treatment in control diet mice increased serum ADH and decreased serum creatinine concentrations (Fig. 4, E and F) and that the folic acid-enriched diet protected against these changes (Fig. 4, E and F). Therefore, the folic acid-enriched diet may influence urinary function in T+E2-treated mice by normalizing water balance.

A folic acid-enriched diet reduced T+E2-induced abnormalities in voiding function. T+E2-treated mice exhibit abnormal voiding function (26, 27). We conducted the spontaneous void spot assay at defined intervals (baseline, 1 and 2 mo postsurgery) to test whether the time course of T+E2-induced changes in voiding function was further impacted by diet. There was a significant main effect of time and group and an interaction of these variables on total urine area, relative total urine area and relative area of primary void (Fig. 5, A and B, Supplemental Table 4). T+E2 treatment 1 mo postsurgery increased total urine area, primary void area, and decreased the percentage of urine area within corners in control-fed mice (Fig. 5, Supplemental Table 3); when normalized to body mass, the folic acid-enriched diet partially moderated these changes (Fig. 5, F–H). The only changes which persisted until 14 wk of age were those observed in urine area (Supplemental Table 3).

Changes in prostate DNA methylation accompany folic acid-enriched diet and T+E2 treatment. Changes in prostate and/or bladder growth and physiology could underlie the protective effects of a folic acid-enriched diet on specific aspects of urinary dysfunction in T+E2-treated mice. We first exam-
ined the prostate, which grows larger with T+E2 treatment (27). Since folic acid can influence DNA methylation, and since DNA methylation regulates prostatic growth in the mouse fetus (17), we also tested whether T+E2 treatment or the folic acid-enriched diet changes prostate or bladder global DNA methylation and prostatic wet weight. Prostate global DNA methylation was affected by the folic acid-enriched diet, but the observed effects were different among prostate lobes: DNA methylation was decreased by the folic acid-enriched diet in the anterior prostate, increased in the dorsal prostate, and unchanged in the lateral prostate (Fig. 6, A–C). While these effects of the folic acid-enriched diet were observed in both control and hormone-treated mice, the diet uniquely increased ventral prostate global DNA methylation in T+E2-treated mice (Fig. 6D). T+E2 treatment increased global DNA methylation in the bladder, and the folic acid-enriched diet protected against this increase (Fig. 6E).

T+E2 treatment increased bladder volume and wet weight of the prostate, seminal vesicle, and urethra in both diet groups at time of euthanasia (Fig. 7). Bladder wet weight increased in T+E2-treated mice fed the folic acid-enriched diet (Fig. 7). Normalization of prostate wet weight to body mass did not change relative differences between groups, except for relative lateral prostate weight. Relative lateral prostate weight was increased by T+E2 treatment in both diet groups, but among T+E2-treated mice relative lateral prostate mass was reduced with the folic acid-enriched diet compared with control diet group (Fig. 7I). We also confirmed that a folic acid-enriched diet does not cause overt changes in lower urinary tract histology (Fig. 7, J and K). These results provide evidence that
changes in prostate mass or lower urinary tract histology are unlikely to mediate the protective actions of folic acid on T/E2-induced voiding dysfunction.

A folic acid-enriched diet and T/E2 treatment alter bladder physiology. Since we observed changes in bladder global DNA methylation (Fig. 6E), we postulated that the folic acid-enriched diet could also impact bladder function in T/E2-treated mice. We conducted in vitro bladder bath studies and evaluated contractile responses to carbachol and potassium chloride. Responses were normalized to bladder strip wet weight to account for differences in bladder size between sham and T/E2 mice. T/E2 treatment in both diet groups decreased the maximal contractile response elicited by carbachol, but the carbachol potency (EC50) was the same across groups (Fig. 8).

Cystometry was performed on urethane-anesthetized mice to analyze bladder response to filling and emptying, end points requiring appropriate neural function. T/E2 treatment reduced the number of control-diet fed mice that elicited a voiding contraction during the monitoring period (2 h/mouse), and the carbachol potency (EC50) was the same across groups (Fig. 8). Cystometry was also used to measure bladder capacity and compliance, indicators of bladder remodeling and function. T/E2 treatment increased bladder capacity, and the folic acid-enriched diet protected against this increase (Fig. 9J). The folic acid-enriched diet also maintained bladder compliance at control levels even in the presence of T/E2 (Fig. 9K). Together, these results indicate that T/E2 treatment altered voiding parameters consistent with obstruction and the folic acid-enriched diet moderated at least some of these end points.

The inability of some control diet T/E2-treated mice to generate bladder contractions, and restoration of this function with a folic acid-enriched diet (Supplemental Table 1), could derive from treatment-related differences in afferent sensation. To test this hypothesis, we focused on capsaicin-sensitive TRPV1 receptor function. Capsaicin activates TRPV1 receptors, and ~60% of bladder afferent neurons are capsaicin sensitive (16). Capsaicin was delivered by intravesicular infusion to urethane-anesthetized mice and decreased the interval...
between sustained voids (consisting of 3 or more drops) (Fig. 10A). We next examined the total number of drip voids (1–2 drops) within the interval of six sustained voids for each group. Diet modified the T/E2-treated mouse response to capsaicin by increasing the frequency of drip voids over a six sustained void interval (Fig. 10B). Capsaicin also ameliorated the T+E2-induced increase in time for bladder pressure to return to baseline after a void in mice from both diet groups (Fig. 10C). These results indicate that capsaicin increases voiding frequency in all mice tested and that the folic acid-enriched diet
in T+E2 mice may sensitize to afferent stimulation and bladder filling.

A folic acid-enriched diet increased nociceptor transcript abundance in dorsal root ganglia of T+E2-treated mice. Changes in nociceptor abundance are one possible mechanism to explain the increase in voiding events observed with capsaicin. We found that mRNA abundance for the capsaicin-sensitive cation channel Trpv1 was greater in L6 and S1 DRGs from T+E2 mice receiving the folic acid-enriched diet vs. the control diet (Fig. 11A). Increased Trpv1 abundance could explain enhanced capsaicin sensitivity in folic acid-treated mice as shown in Fig. 10. Together, these results suggest increased bladder afferent stimulation may occur in T+E2 mice on the folic acid-enriched diet through increased Trpv1 expression.

We next examined global DNA methylation of L6 and S1 DRGs, which could potentially influence Trpv1 abundance. Although DRG global DNA methylation was affected by neither diet nor T+E2 treatment alone (Fig. 11B), it was uniquely increased by the folic acid-enriched diet in T+E2-treated mice (Fig. 11B). These results indicate the combination of a folic acid-enriched diet and hormone treatment changes DRG global DNA methylation and Trpv1 mRNA abundance.

**DISCUSSION**

Here, we reveal new end points of voiding dysfunction in T+E2-treated mice and show that a folic acid-enriched diet modifies voiding function in sham animals as well as in T+E2-treated mice. Consumption of a folic acid-enriched diet by control mice altered global prostate DNA methylation and decreased voiding leak point pressure. T+E2 treatment increased mouse urine output, time between voiding events, bladder capacity, and serum concentrations of ADH. Consumption of a folic acid-enriched diet moderated these T+E2 effects. The folic acid-enriched diet in T+E2-treated mice decreased urine output and time between voiding events, prevented an increase in serum ADH, bladder capacity and compliance, altered global DNA methylation in prostate, bladder, and DRG neurons, enhanced expression of Trpv1 in DRGs, and increased voiding in response to capsaicin. Overall, our results indicate that T+E2 treatment induces obstruction and urinary dysfunction and a folic acid-enriched diet is capable of moderating some of these changes. Whether the diet prevents, slows, or reverses T+E2-induced changes in obstruction and bladder decompensation is an area for future study. Our results argue against a reversal or inhibition of T+E2-induced increases in prostate wet weight as the primary mechanism of folic acid action since this end point was unaltered by diet, but reveal potential alternative mechanisms, including alterations in neural control of micturition and systemic water balance.

T+E2 treatment was shown previously to induce structural changes in the prostate and prostatic urethra that could potentially impair urinary flow and cause urinary obstruction. These structural changes were characterized by increased mouse prostate wet weight, increased ductal area within the periurethral region, and reduced urethral cross-sectional area (27). The physiological outcome of these changes with respect to mouse voiding function was not previously examined. Bladder outlet obstruction increases voiding pressure in men and rodents (2, 28, 31, 33). In the current study, a folic acid-enriched diet failed to moderate the T+E2-induced increase in prostate wet weight and threshold pressure (Figs. 7 and 9). Thus the effects of a folic acid-enriched diet in a T+E2 model of voiding dysfunction are not likely to be mediated by effects on prostate or urethral size. Whether a high folic acid diet prevents T+E2 induced urethral narrowing or fibrosis or mediates changes in myogenic and viscoelastic elements of the bladder is an area for future study.

Restoration of water balance in T+E2-treated mice consuming a folic acid-enriched diet is another potential mechanism by which the folic acid-enriched diet could moderate urinary dysfunction in T+E2-treated mice. We made the discovery that T+E2 treatment changes many aspects of systemic water balance and these changes are consistent with T+E2-induced nephrogenic diabetes insipidus. They include increased water consumption (Fig. 3, E–G) and urine production (Fig. 5A), decreased urine specific gravity (Fig. 4, A and B) and urine protein concentration (Fig. 4C), decreased serum creatinine (Fig. 4F), and increased serum ADH (Fig. 4E). Consumption of the folic acid-enriched diet protects against the T+E2-induced changes in serum creatinine and ADH (Fig. 4, E and F). Further investigation into how T+E2 treatment influences these end points is warranted, particularly if T+E2 treatment causes mice to become resistant to ADH challenge and if the folic acid-enriched diet alters the time course of this resistance. These results further indicate that T+E2 treatment may have direct effects on bladder function independently of changes in

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**Table 1. Mice eliciting a voiding contraction during a 2-h cystometry monitoring period**

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<th>% Total Mice Able To Elicit Voiding Contraction Over 2-h Period</th>
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<td>Control sham</td>
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<td>Control T+E2</td>
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<td>Folate sham</td>
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See the text for definitions.
Fig. 9. A folic acid-enriched diet protects against a T+E2-induced increase in intervoid interval and bladder capacity and compliance. Adult male mice were treated as described in Fig. 1. At 2 mo postsurgery, urethane-anesthetized mice were analyzed by cystometry. A: representative cystometrograms from animals in each treatment group which elicited voiding contractions. Insert: representative of the 30% of T+E2 mice on the control diet which did not elicit a voiding contraction during the 2-h monitoring period and instead showed a steady rise in pressure with no apparent voiding contractions. The following parameters were quantified from the remainder of mice: intervoid interval (B), time back to baseline after peak voiding pressure (C), leak point pressure (D), threshold pressure to elicit a void (E), drip voids (1–2 drops) over a 6-s sustained void (3 or more drops) interval (F), number of nonvoiding contractions over a 6-s sustained void interval (G), peak-to-baseline pressure of voiding contractions (H), peak-to-baseline pressure of nonvoiding contractions (I), bladder capacity (J), and bladder compliance (K). Values are means ± SE. *Significant difference, P ≤ 0.05; n ≥5/group.
prostate mass. This is supported by the fact that T+E2 treatment in female rats, which lack a prostate, have increased bladder capacity and altered histology compared with controls (7).

It is likely that changes in T+E2-treated mouse bladder function arise due to a combination of changes in the prostate as well as a direct effect on bladder tissue. Our results reveal bladder afferent sensitization as a potential mechanism by which the folic acid-enriched diet could improve T+E2-treated mouse urinary function. Chronic T+E2 treatment strikingly reduced detrusor cross-sectional thickness, caused bladder fibrosis (27), and reduced the percentage of mice eliciting a voiding contraction in response to intravesical saline (Table 1). Surprisingly, bladder strips from T+E2-treated mice remain capable of contracting in response to cholinergic stimulation in vitro, indicating at least some retention of functional activity (Fig. 8). These results are consistent with those observed with aging (32). We tested whether dietary folic acid enrichment retains or restores bladder sensitivity to filling in T+E2-treated mice uniquely (albeit temporarily) increased the frequency of drip voids in mice consuming the folic acid-enriched diet but not in mice consuming a control diet (Fig. 10B). These data indicate a potentially heightened afferent sensitivity in bladders of mice consuming the folic acid-enriched diet and raise questions about whether it would also augment urinary function in other mouse models of bladder outlet obstruction. It is also possible that a folic acid-enriched diet may protect against changes in lower urinary tract function induced by other insults (genetic or environmental) which converge upon bladder afferent sensitivity or detrusor function.

One interpretation of increased Trpv1 mRNA abundance in mice consuming the folic acid enriched- compared with the control diet is that it reflects differences in the stage of progression to bladder decompensation. Bladder obstruction, irritation, and hypertrophy have been shown to increase afferent sensitivity in animals and humans (6, 11, 31). However, downregulation of nicotinic acetylcholine receptors in sensory neurons occurs after bladder outlet obstruction (6). This downregulation is thought to happen as a decompensation mechanism for initial increased sensitivity. These data are important because they establish a time course: afferent hyperexcitability first, bladder decompensation second. It is therefore possible that the folic acid diet delays T+E2-induced bladder decompensation vs. the control diet. This notion is supported by the results showing that the folic acid-enriched diet moderates the T+E2-induced increase in relative urine area, intervoid interval, and bladder capacity and compliance (Figs. 5F and 9, B, J, and K) and increases the ability of T+E2 mice to elicit a voiding contraction (Table 1) and respond to capsaicin (Fig. 10).

Fig. 10. A folic acid-enriched diet enhances T+E2 bladder sensitivity to afferent stimulation. Adult male mice were treated as described in Fig. 1. At 2 mo postsurgery, capsaicin was administered by intravesicular infusion into urethane-anesthetized mice. The following parameters were determined: interval of sustained voids (3 more drops) before vs. after capsaicin infusion (A), number of voiding events defined as the number of drip voids (1–2 drops) occurring over the course of a 6-sustained void (3 or more drops) interval following capsaicin infusion (B), and time back to baseline after peak voiding pressure (C). Values are means ± SE.*Significant difference, P ≤ 0.05; n = 3/group.
We also identified T+E2- and folic acid diet-dependent changes in global DNA methylation of L6 and S1 DRG neurons and bladder. While we cannot speculate whether this change was specific to L6 and S1 DRG neurons, or occurred generally across all DRG neurons, these findings are significant since this is the first link between altered DNA methylation and T+E2-mediated changes in urinary function. DNA methylation and histone acetylation are known regulators of gene expression in rat nociceptive DRG neurons (15). Environmental stressors increase DNA methyltransferase 1 (Dnmt1) abundance uniquely in rat L6-S2 DRG neurons, which increases promoter DNA methylation of cannabinoid receptor 1 and decreases its expression (15). This relieves repression of TRPV1 and thereby increases TRPV1 expression and visceral pain sensitivity (15). Cannabinoid receptors and TRPV1 are important mediators of bladder afferent signaling, nociception, and voiding function (9, 36). Whether gene-specific DNA methylation and expression of cannabinoid and TRPV1 channels are affected by T+E2 and a folic acid-enriched diet and whether this is unique to L6 and S1 DRG neurons remains to be determined. Additionally, it has recently been shown that changes in chromatin structure are responsible for changes in Trpv1 expression (15), and the possibility that changes in chromatin structure are responsible for the changes in Trpv1 expression we observed is an area for future study (15). While the focus of this study was DNA methylation, folic acid on its own or through its ability to decrease serum homocysteine can also act as an antioxidant and can have beneficial actions on endothelial cell function and collagen deposition (29a). How these other end points contribute to urinary function and the timing of these events remain to be determined.

A new question that has emerged from this study is whether the folic acid-enriched diet moderates specific aspects of T+E2-induced urinary dysfunction by influencing urinary tract development or by interfering with T+E2 insult later in life. Whether exposure to a high folic acid diet in utero alone can act as a preventative or in adulthood alone can act as a treatment for urinary tract dysfunction remains to be determined.

REFERENCES


