182

Yoshiyama M[1], Mochizuki T[2], Nakagomi H[3], Miyamoto T[3], Kobayashi H[3], Zakoji H[3], Takeda M[3]
1. *Yumura Onsen Hospital,* ***2.*** *Yamanashi-Kosei Hospital,* ***3.*** *Department of Urology, University of Yamanashi Interdisciplinary Graduate School of Medicine and Engineering*

EVALUATIONS OF INCREASED VOIDING FREQUENCY IN MICE WITH DIABETES MELLITUS AT EARLY STAGE

Hypothesis / aims of study

Diabetic cystopathy is one of the well recognized complications of diabetes mellitus (DM) and may present various clinical symptoms and complaints such as overactive bladder, urge incontinence, decreased bladder sensation, underactive bladder and urinary retention. These manifestations are largely associated with progression of DM. Present studies using DM at *early* stage were conducted to examine if genetically mutated young DM mice have urological changes pertinent to complaints of human patients with early DM.

Study design, materials and methods

Female diabetic mice (+*Lepr*^{db} /+*Lepr*^{db} = *db/db,* n=15) (8-11 week-old, BKS.Cg-*Dock7*^{m} +/+ *Lepr*^{db}/J strain) and their age-matched mice with heterozygous mutation (*m*+/+ *Lepr*^{db} = *db/m*+*,* n=13) were used. For evaluating micturition *behaviour*, conscious mice were individually placed in newly-designed metabolic cages, which were improved for precise collection of voided urine, for consecutive 5 days, and data of voided urine and water-intake were continuously recorded into a computer. For CMG study, mice were decerebrated under sevoflurane anaesthesia and intravesical pressure was recorded *via* a PE-50 tube inserted into the bladder dome. CMG recordings were conducted in supine position of the animals under unanaesthetized conditions by continuously infusing saline (10 μl/min) at room temperature.

Evaluated parameters are: water intake (μl/day), voided urine volume (μl/day), urine volume/void (μl), voiding time/void (s), number of voids(/day), mean uroflow rate (μl/s) in metabolic cage study using conscious mice (i.e., micturition *behaviour*); and pressure threshold for inducing voiding contraction (PT, mmHg), maximal voiding pressure (MVP, mmHg), bladder compliance (BCP, μl/mmHg), voided volume (VV, μl), volume threshold for micturition (VT, μl), voiding efficiency (VE, %) in CMG study using decerebrate unanaesthetized mice (i.e., *reflex* micturition). Insensible perspiration rate (%) was calculated as [water intake (μl) – voided urine volume (μl)] x 100 / water intake (μl) in the metabolic cage study.

All values are expressed as mean ± S.E.M. Statistical analyses were made using unpaired *t*-test and $p < 0.05$ was considered significant (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

Results

Values in parameters associated with voluntary micturition *behaviour* in conscious mice and those associated with *reflex* micturition in decerebrate unanaesthetized mice are presented in Tables 1 and 2, respectively. Urine volume/void in conscious mice is considered to be relevant to VV in decerebrate unanaesthetized animals. No non-voiding contractions in bladder-filling phase were found during CMGs. Calculated insensible perspiration rate (%) of *db/db* mice (16.2 %) was markedly lower than that of *db/m*+ mice (40.2 %). Table 3 shows comparisons between *db/db* and *db/m*+ mice in weights of body, bladder and urethra.

Table 1 Voluntary micturition *behaviour* in conscious mice

	Water intake (ml)/day	Urine volume (ml)/day	Urine volume (μl)/void	Voiding time (s)	Number of voids/day	Mean uroflow rate (μl/s)
db/m+	3.6 ± 0.2	2.2 ± 0.2	157 ± 22	2.0 ± 0.1	15.2 ± 2.8	81.1 ± 10.6
db/db	8.2 ± 1.0**	6.9 ± 0.7***	202 ± 15	2.2 ± 0.2	34.5 ± 4.0**	103.6 ± 4.6

Table 2 *Reflex* micturition during CMGs in decerebrate unanaesthetized mice

	PT (mmHg)	MVP (mmHg)	BCP (μl/mmHg)	VV (μl)	VT (μl)	VE (%)
db/m+	8.7 ± 1.8	23.0 ± 1.3	19.9 ± 5.2	151 ± 17	162 ± 12	93.2 ± 3.9
db/db	4.6 ±0.4*	19.5 ± 2.8	59.2 ± 5.7*	240 ± 44	256 ± 43	92.9 ± 1.7

Table 3 Comparisons between DM mice and WT mice

	Age (week-old)	Body wt. (g)	Bladder wt. (mg)	Urethra wt. (mg)
db/m+	9.5 ± 0.4	21.0 ± 0.3	14.2 ± 0.5	3.8 ± 0.1
db/db	9.5 ± 0.3	42.8 ± 1.3***	18.7 ± 0.6***	3.8 ± 0.2

Interpretation of results

Behavioural study showed that conscious DM (*db/db*) mice have excessive water-intake and large amount of urine production, compared to those of *db/m*+ mice. However, insensible perspiration rate of *db/db* mice is much lower than that of *db/m*+ mice, leading to deterioration of polyuria. On the other hand, urine volume per void, voiding time and mean uroflow rate of *db/db* mice were comparable to those of *db/m*+ mice.

CMG study revealed that under decerebrate unanaesthetized conditions, *db/db* mice have lower bladder tone and larger functional bladder capacity than *db/m*+ mice do, suggesting that detrusor tone (myogenic) and bladder afferent activity (neurogenic or secondary to the myogenic change) during bladder-filling are already altered at early stage of DM. Both VE and MVP of *db/db* mice were comparable to those of *db/m*+ mice, indicating that functional efficiency in coordinated activity of bladder (contraction force) and urethra (relaxation) during voiding is preserved at this DM stage.

Eight to eleven week-old *db/db* mice are markedly obese, compared to *db/m+* mice. The *db/db* mice have heavier bladder than the *db/m+* mice do, whereas urethras of the *db/db* and the *db/m+* mice are same in the weight.

Concluding message

These studies suggest the possibilities that conscious DM mice at early stage have an urge to micturate during urine storage and/or to suddenly stop voiding urine before emptying the bladder and that the myogenic and neurogenic changes in the lower urinary tract function are already developed. Urinary frequency in the early DM mice may be caused by physical interception during bladder-filling and/or voiding due to obesity as well as by urging-sensations originated in the forebrain for initiating or terminating micturition. In addition, polyuria, but not bladder overactivity, affects frequent micturition in the early DM.

Specify source of funding or grant	**Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan: No. 22591787 (M. Yoshiyama) and No. 20390423 (M. Takeda)**
Is this a clinical trial?	**No**
What were the subjects in the study?	**ANIMAL**
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
Name of ethics committee	**University of Yamanashi Institutional Animal Care and Use Committee**