Effects of chronic ischemia on bladder function in Watanabe heritable hyperlipidemic rabbits

Introduction and Objectives
With increasing number of elderly, lower urinary symptoms of these people cannot be negligible. In Japan, it has been reported that about 8.1 million people are suffering from overactive bladder (OAB). Various etiological factors have been suggested in terms of OAB, one of which is bladder ischemia derived from aging. A Watanabe heritable hyperlipidemic (WHHL) rabbit has been developed as an animal model for human familial hypercholesterolemia and has been widely used as a model of various organ ischemia and related diseases. In the present study, we examined the cystometric, histological, and pharmacological features of WHHL rabbits and evaluated the relationship between OAB and bladder ischemia.

Material and Methods
Firstly, we prepared 20 to 24-month-old WHHL rabbits (n=6), and age and sex-matched Japanese white rabbits (n=10) as controls, for evaluation of characteristics of WHHL rabbits. Secondly, we prepared 6-month-old (n=3), 12-month-old (n=3), and 24-month-old (n=5) WHHL rabbits and control rabbits (n=6) for evaluation of the influence of aging. In addition, 14 to 18-month-old WHHL rabbits (n=8) and control rabbits (n=8) were used in order to evaluate the release of ACh and ATP from bladder. Each rabbit was measured the volume and frequency of voiding for three days. Then, we exposed the rabbits' bladder under anesthesia and inserted a catheter into the bladder for cystometry. Cystometrograms were performed using constant infusion (1.5 ml/min) of saline into the bladder to elicit voiding. Saline voided from urethral meatus was collected and measured to determine the voided volume. Micturition pressure was also measured by a pressure transducer. After sacrificing the rabbit, we prepared bladder strips and suspended them in an organ bath filled with Krebs-Henseleit solution and isometric tension development was recorded. We investigated the effects of carbacol, KCl, and electrical field stimulation (EFS) on bladder strips. In addition, using the microdialysis technique, microdialysis probe was inserted into the bladder strip, and Ringer solution was perfused into the probe at a constant flow rate of 2.0 μl/min. Dialysate was collected during EFS (supramaximal voltage, 0.3 msec duration, 40Hz and 3 sec train) and during bladder strip stretch (0-40 mN resting tension). The amount of neuronal (EFS-induced) and non-neuronal (stretch-induced) releases of ACh and ATP in the dialysate fraction was measured by HPLC with ECD and luciferine-luciferase assay, respectively. To examine atherosclerotic plaque, we excised internal iliac artery which sectioned transversely and calculated internal area by measuring circumference of the inner wall of the arteries. Sections were stained with H&E for observation. For histological examination of bladder, we prepared paraffin blocks stained with H&E, and we also performed immunohistochemical stainings for S-100 protein and CGRP. In the latter specimen, mean nerve density score (MNDS) was calculated.
Results
In the WHHL rabbits, the number of micturition was higher and the voided volume was lower than in
the control. Cystometrograms of the WHHL rabbits showed premicturition contractions, shorter
interval of micturition, lower voided volume, and lower micturition pressure, as compared to the
control. The comparison among different age groups showed age-dependant changes in cystometric
findings. Older rabbits showed shorter interval of micturition, lower grade of micturitional pressure
and larger number of premicturition contractions. In the functional study, the contractions induced
by carbacol and EFS in younger WHHL rabbits significantly increased, whereas the responses
significantly decreased in older WHHL rabbits. The amount of neuronal ACh and ATP released from
the bladder strips was significantly lower in WHHL rabbits than in the control group under EFS. The
stretch-induced releases of non-neuronal ACh and ATP increased in a tension-dependent manner in
both groups, and the releases in WHHL rabbits were significantly higher than those from the control
group in a tension larger than 20 mN. We also studied the WHHL rabbits’ distal and proximal cross
sections of internal iliac arteries, and found significant atherosclerosis lesions and thickening of
media in both distal and proximal portions. Internal area was also calculated and the WHHL rabbits
showed significant cross sectional narrowing. Histological examination of the bladder showed that
urothelium became thinner and connective tissues in muscle layers increased as rabbits grew old.
With immunohistochemical stainings, the MNDS of S-100 protein positive neurons significantly
decreased and that of CGRP positive neurons significantly increased in the WHHL rabbits.

Conclusions
The present study elucidated one of the developmental mechanisms of OAB caused by ischemia and
aging. The data suggest that the WHHL rabbits showed detrusor overactivity, which might be caused
by bladder ischemia. And also it showed that the grade of ischemia advance with aging might result
in the decreased detrusor compliance and poor contractility. Our results suggest that strong
relationship among aging, histological ischemia and bladder dysfunction. The study also showed that
a WHHL rabbit is a useful animal model for evaluation of pathophysiology of overactive bladder and
for exploration of future treatment possibilities.