Influence of High Fat Diet Feeding for 20 Weeks on Lower Urinary Tract Function in Mice

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Objectives: We investigated the possible changes in lower urinary tract function in mice fed a high fat diet (HFD).

Methods: Male C57BL/6J mice were divided into two different feed groups: normal diet (ND) and HFD (n = 16 in each). The body weight, blood glucose level and voiding frequency/volume (FV) relations (for 24 h) were measured every 4 weeks. At 25 weeks old, blood pressure and heart rate, cystometry and isolated detrusor smooth muscle function were measured. After the experiments, serum fat level was measured.

Results: The body weight and blood glucose level of the HFD group were significantly higher than those of the ND group after 9 weeks old. In the FV measurements, the mean voided volume was not significantly different between the two groups, although voiding frequency, total voided volume and water intake volume in the HFD group were significantly lower than those in the ND group. At 25 weeks old, the mean heart rate in the HFD group was significantly higher than that in the ND group, but no significant difference in the blood pressure was observed. None of the cystometric parameters analyzed showed significant differences between the two groups. The contractile response to either carbachol or high K+ was not significantly different, whereas the contractile response to electrical field stimulation was significantly higher in the HFD group. In the HFD group, the mean total cholesterol level was significantly higher.

Conclusion: The present results suggest that HFD-feeding for 20 weeks in mice unlikely affects bladder function even though it induced diabetes, hyperlipidemia and tachycardia.

Key words diabetes mellitus, diet, mice, urinary bladder

1. INTRODUCTION

Metabolic syndrome is a combination of medical disorders, including insulin resistance, glucose intolerance, hypertension, and hyperlipidemia in the same individual, and is associated with a high risk for type 2 diabetes, cardiovascular disease (CVD) and subsequent premature mortality.1,2 It has been reported that the prevalence of metabolic syndrome is about 10–20% in middle-aged US and Asian populations.3–5 More recently, metabolic syndrome and type 2 diabetes are known to be risk factors not only for CVD but also for lower urinary tract symptoms (LUTS).6,7 A number of observational studies revealed that lower urinary tract dysfunction, including LUTS in men, overactive bladder (OAB), and stress and urge incontinence in women are associated with obesity and/or diabetes.8–11

As we can speculate, healthy diet and lifestyle prevent these disorders.12 In contrast, dietary fat intake often has been claimed as being responsible for increase in adiposity. Human studies have shown that high fat diets (HFD, >30% of energy from fat) can easily induce obesity.13–17 Diets rich in fat not only induce obesity in humans but also make animals obese.18–20 In both rats and mice a positive relationship has been found between the level of fat in the diet and body weight or fat gain.21–24

Therefore, in the present study, we focused on the relationship with lower urinary tract (LUT) function and metabolic syndrome induced by diet and investigated LUT function in mice fed a HFD.

2. METHODS

2.1. Animals and experimental group

Thirty-two adult male C57BL/6J mice were used. The mice were maintained under standard laboratory conditions with a 12:12-h light : dark cycle and free access to food pellets and tap water. The protocol was approved by the Animal Ethics Committees of The University of Tokyo Graduate School of Medicine and was in line with National Institutes of Health (NIH) guidelines for the care and use of experimental animals.

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Received 17 May 2012; revised 25 June 2012; accepted 3 July 2012.

DOI: 10.1111/j.1757-5672.2012.00172.x
All the mice (4 weeks old) were obtained from CLEA Japan, Inc. (Tokyo, Japan), and fed a normal diet (ND) for 1 week. After measurement of body weight, blood glucose and voiding frequency/volume (FV) relations as described below at 5 weeks old, the mice were divided into two different feeding groups: ND (N = 16) and HFD (N = 16, Fig. 1a). ND and HFD were obtained from CLEA Japan, and these diets contained different ingredients and fatty acids as indicated in Table 1. Every 4 weeks, body weight and blood glucose level, which were measured from tail vein using a disposable glucose test sensor (Glutest, Sanwa Kagaku Kenyusho Co. Ltd, Tokyo, Japan), were measured at around 13:00 hours and voiding FV relations were measured until 21 weeks old. At 25 weeks old, cardiovascular system relations, cystometry (CMG) and isolated detrusor smooth muscle contractile function were measured. After the experiments, bladder weight was measured and blood serum was collected to measure serum fat level. The animals were then killed by an overdose of anesthesia.

2.2. FV measurement (every 4 weeks, 5-21 weeks old)

Mice were placed without any restraint in a metabolic cage (MCM/TOA-UF001-006, Mitsubishi Chemical Medicine Corporation, Tokyo, Japan) for 24 h to adapt to the environment. This metabolic cage has a specially designed net that allows liquid (urine) to pass separately from feces, so that the voided urine volume can be measured precisely (Fig. 1b). After adaptation for 24 h, voided volume, voiding frequency and water intake volume were recorded continuously on a data acquisition program (Power Lab; AD Instruments, Sydney, NSW, Australia) for 24 h (start from 9:00 hours). Mice were allowed free access to water and food during recording.

2.3. Cardiovascular system relations (heart rate and blood pressure) measurement (at 25 weeks old)

At around 13:00 hours, the mouse was placed in a restraint cage in a warm (38 °C) condition for approximately 2–3 min, then heart rate and blood pressure were measured in conscious animals by tail-cuff plethysmography (BP-98A-L; Softron, Tokyo, Japan). The values were measured three times for each mouse and the average value was calculated.

2.4. CMG measurement (at 25 weeks old)

Mice were anesthetized with 30 mg/kg intraperitoneal pentobarbital sodium. A polyethylene catheter (Clay-Adams PE-50; Parsippany, NJ, USA) was inserted into
the bladder through the dome and secured. After the operation, each mouse was housed single in a cage.

Continuous CMG was performed in conscious rats 4 days after surgery. Each mouse was placed without any restraint in a metabolic cage (MCM/TOA-UFO01-006) for at least 2 h to adapt to the environment. The bladder catheter was connected to a pressure transducer (DX-100; Nihon Kohden, Tokyo, Japan) and microinjection syringe pump (KDS100; Muromachi, Tokyo, Japan) by a three-way tap. Saline at room temperature was continuously infused into the bladder at a rate of 0.01 mL/min. Basal pressure (BP; cmH2O), micturition threshold (MT; cmH2O), peak micturition pressure (PP; cmH2O), and voided volume (VV; mL) were recorded continuously on a data acquisition program (PowerLab). Bladder capacity (BC; mL) was calculated as intercontraction interval × saline infusion rate into the bladder. All parameters were averaged for 30 min after the pressure curves were stabilized.

2.5. Isolated detrusor smooth muscle contractile function measurement (at 25 weeks old)

The mouse was killed and the bladder was removed. The bladder strip was equally separated (approximately 1 × 1 × 5 mm) into bladder body longitudinally. Isolated detrusor smooth muscle strip was transferred to 5-mL organ baths containing Krebs solution (see below for composition) maintained at 37 °C. The Krebs solution was bubbled with a mixture of 95% oxygen and 5% CO2, giving a pH of 7.4. The strip was attached at one end to a tissue holder and at the other end to a force displacement transducer (Type 7923; NEC San-Ei Instruments Ltd, Tokyo, Japan). Data were recorded and analyzed by data acquisition program (PowerLab). The strip was stretched giving a pH of 7.4. The strip was attached at one end to a force displacement transducer (Type 7923; NEC San-Ei Instruments Ltd, Tokyo, Japan). After the equilibration period (approximately 2 h), the experiment was started by exposing the strips to a 124 mM potassium (K+) Krebs solution. After washing out the K+ Krebs solution, contraction was evoked using the muscarinic cholinergic agonist carbachol (CCh; 10−8 to 10−5 M; Wako Chemical Co., Tokyo, Japan). After the final concentration of CCh had been added, the strip was washed and left undisturbed until baseline tension was regained. Frequency-response relations (2, 10 and 20 Hz) were then recorded with electrical field stimulation (EFS; pulse-width: 0.8 msec, 50 V, duration: 5 sec, stimulation interval: 1 min).

The Krebs solution consisted of sodium chloride (NaCl), 118 mM; potassium chloride (KCl), 4.7 mM; calcium chloride (CaCl2), 2.5 mM; sodium bicarbonate (NaHCO3), 12.5 mM; potassium dihydrogen phosphate (KH2 PO4), 1.2 mM; magnesium sulfate (MgSO4), 1.2 mM; and glucose, 5.55 mM (Wako Chemical Co.). To make the 124 mM K+ Krebs solution, NaCl was replaced with an equimolar amount of KCl.

2.6. Serum fat (total cholesterol and triglyceride) level measurements (at 25 weeks old)

Whole blood (approximately 0.5 mL) was harvested from the caudal vena cava just prior to sacrificing the animal. The blood sample was transferred to a test tube and left at room temperature for 1 h, and then centrifuged at 3000 × g for 10 min (4 °C) to separate the serum. The collected serum was stored and frozen (−80 °C) until measurement. To measure the fat level, each kit (TCHO-PIII for total cholesterol and TG-PIII for triglyceride, FUJIFILM, Tokyo, Japan) was used according to the manufacturer’s instructions.

2.7. Statistical analysis

All data are expressed as the mean ± SEM. Results were analyzed using unpaired Student’s t-test or Mann-Whitney U-test comparison each groups. P < 0.05 was considered statistically significant.

3. RESULTS

3.1. Body weight and blood glucose level

The body weight and blood glucose level of the HFD group were significantly higher than those of the ND group after 9 weeks old (Fig. 1d,e); in particular, body weight was twofold at 25 weeks old. The body shape of the HFD mice was also larger than the ND mice (Fig. 1c).

3.2. FV measurement

On analysis of FV measurement during the total 24 h voiding frequency, total voided volume and water intake volume in the HFD group were significantly lower than in the ND group throughout the observation period after 9 weeks old. However, in these periods the mean voided volume was not significantly different between the two groups, although the value tended to increase with aging in both groups (Fig. 2). During the dark cycle, similar tendencies were observed in all of these four parameters (Fig. 3). During the light cycle, these parameters showed a similar tendency except the mean voided volumes at 13 and 17 weeks old, which were significantly lower in the HFD than in the ND group (Fig. 4).

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Fig. 2 Parameters of frequency/volume (FV) measurement during total 24 h.

Fig. 3 Parameters of frequency/volume (FV) measurement during the dark cycle.
3.3. Cardiovascular system relations (heart rate and blood pressure) measurement

The mean heart rate in the HFD group was significantly higher than that in the ND group. However, in all of the blood pressure parameters (systolic, diastolic and mean blood pressure), no significant differences were found between the two groups (Table 2).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal diet</th>
<th>High fat diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (bpm)</td>
<td>657.56 ± 10.90</td>
<td>727.44 ± 7.96</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>115.33 ± 2.99</td>
<td>117.97 ± 3.10</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>77.26 ± 2.59</td>
<td>79.47 ± 3.22</td>
</tr>
<tr>
<td>Mean blood pressure (mmHg)</td>
<td>89.76 ± 2.46</td>
<td>92.19 ± 3.02</td>
</tr>
<tr>
<td>Bladder weight (mg)</td>
<td>37.25 ± 2.40</td>
<td>40.81 ± 2.02</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>62.86 ± 2.91</td>
<td>178.87 ± 17.35</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>96.79 ± 12.00</td>
<td>86.47 ± 18.69</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM. No significant differences were found between normal diet and high fat diet groups.

3.4. CMG measurement

None of the cystometric parameters analyzed (basal pressure, micturition threshold, micturition pressure, bladder capacity, voided volume and residual volume) showed significant differences between the two groups (Table 3).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal diet</th>
<th>High fat diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal pressure (cmH2O)</td>
<td>7.54 ± 2.75</td>
<td>5.45 ± 1.59</td>
</tr>
<tr>
<td>Micturition threshold (cmH2O)</td>
<td>18.55 ± 4.26</td>
<td>9.25 ± 1.73</td>
</tr>
<tr>
<td>Peak pressure (cmH2O)</td>
<td>39.41 ± 4.62</td>
<td>33.54 ± 1.20</td>
</tr>
<tr>
<td>Bladder capacity (mL)</td>
<td>0.153 ± 0.042</td>
<td>0.137 ± 0.027</td>
</tr>
<tr>
<td>Voided volume (mL)</td>
<td>0.146 ± 0.042</td>
<td>0.116 ± 0.027</td>
</tr>
<tr>
<td>Residual volume (mL)</td>
<td>0.008 ± 0.011</td>
<td>0.021 ± 0.009</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM. No significant differences were found between normal diet and high fat diet groups.

3.5. Isolated detrusor smooth muscle contractile function measurement

The contractile response to either carbachol or high K+ was not significantly different between the two groups, whereas the response to EFS was significantly higher in the HFD group than in the ND group (Fig. 5).
3.6. Serum fat (total cholesterol and triglyceride) level and bladder weight

The mean total cholesterol level was significantly higher in the HFD group than in the ND group, while the mean triglyceride level was not significantly different between the two groups (Table 2). The bladder weight of the HFD group tended to be greater than that of the ND group, but this difference was not statistically significant (Table 2).

4. DISCUSSION

In the present study, continuous feeding of a HFD containing a large amount of fatty acids and large number of calories in mice resulted in larger body weight and higher blood glucose level compared with ND feeding, even though the HFD group appeared to eat less of the diet (ND mouse approximately 3 g/day, HFD mouse approximately 1.5–2 g/day). However, water intake volume was smaller in the HFD mice throughout the observation period, which might have been influenced by the smaller amount of diet eaten. At the end of experiment (25 weeks old), serum total cholesterol level was higher in the HFD group, but there was no significant difference in triglyceride level between the two groups. This discrepancy between the results of total cholesterol and triglyceride may have occurred because of the different populations of the ingredients in the HFD diet used: large amount of monounsaturated fatty acid and smaller amount of polyunsaturated fatty acid. Regarding the cardiovascular relations parameters, the mice fed the HFD did not show hypertension, although they had a higher heart rate. The short observation period may reflect this minor change in cardiovascular relations. Based on the results mentioned above, we believe that the mice fed the HFD in the present study had some characteristics of metabolic disorders, which fulfills a diabetic factor associated with hypercholesterolemia (a hyperlipidemia factor) but without hypertension.

We investigated LUT function in HFD fed mice. In the FV measurements until 21 weeks old, the most remarkable differences between the two different feeding groups demonstrated in both light and dark cycles were lower number of micturition, smaller total voided volume and total water intake. It is likely that less water intake caused less total urine output and less frequent voiding, as the mean voided volume was not different between the two groups even though small variation was noted during the light cycle. In addition, we found no significant differences in any of the cystometric parameters analyzed.

Fig. 5 Contractile responses of the isolated detrusor smooth muscle strips taken from mice fed a high fat diet (HFD) and those fed a normal diet (ND). (a–c) Response to carbachol (CCh), electrical field stimulation (EFS), and high K⁺, respectively, on numeric force value (mN/g). (d,e) Response to CCh and EFS, respectively, on relative percentage of the high K⁺ value.
between the two groups. In contrast to the negligible LUT dysfunction demonstrated in the present HFD-feeding mice, Rahman et al. reported that more of the rats fed a HFD (consisting of 2% cholesterol and 10% lard) for 6 months showed bladder overactivity than the controls in conscious CMG measurement, although there was no significant difference in the glucose or triglyceride levels between the two groups. It has been demonstrated that streptozotocin-induced diabetic rats have increased bladder capacity (threshold volume), contraction duration, voided volume and residual volume, suggesting impairment of bladder sensation and voiding dysfunction in those rats. Some epidemiological studies showed similar controversy in the relationship between LUTS and metabolic disorders in humans. Although there is experimental limitation (HFD feeding for only 20 weeks) in the present study, such a controversial relationship between LUTS and metabolic disorders in clinical studies may link to our findings.

The present in vitro functional study on detrusor contractility showed that the contractile response to either carbachol or high K+ was not significantly different between the two groups, whereas the response to EFS was significantly higher in the HFD group than the ND group at higher frequency stimulations (10 and 20 Hz). In general, acetylcholine (ACh) and other neurotransmitters, including adenosine triphosphate (ATP), are released as co-transmitters from the nerve ending of the parasympathetic nerves during voiding. These co-transmitters may affect the contractile response to EFS. Therefore, transmitters other than ACh may have contributed to the higher response to EFS in the HFD mouse detrusor. In fact, high percentage (about 70%) of atropine-resistant component of EFS-induced contraction even at 30 Hz in the mouse detrusor has been reported. However, such changes in neurotransmitter release may not have a major role in in vivo voiding function, as no significant impairment of voiding function on the present in vivo CMG investigation was demonstrated.

Although the present study has some limitations, including sample size, duration effect, diet effect and detrusor overactivity observed, we conclude that HFD-feeding for 20 weeks in mice unlikely affects LUT function, even though it induced diabetes, hyperlipidemia and tachycardia. Further studies with a longer follow-up are needed to overcome the limitations of the present study and to determine the influence of metabolic disorders on LUT function.

Acknowledgment

The authors express their gratitude for the excellent technical assistance provided by Toshiki Homma (Kissei Pharmaceutical Co. Ltd) in the performance of analysis of fat levels. The present study was supported by the Kanzawa Medical Research Foundation (NA Grant) and a Grant-in-Aid for Scientific Research (YI; Grant No. 40159588, NA; Grant No. 80595257), from the Ministry of Education, Culture, Sport, Science and Technology of the Japanese Government.

Disclosure

The authors of this paper have no financial or commercial interests to disclose.

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