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ORIGINAL RESEARCH

High-Fat and High-Sucrose Diet Leads to Skeletal Muscle Loss and Bladder Dysfunction in Rat

Naoki Wada, Noriyuki Abe, Kotona Miyauchi, Shogo Makino, Hidehiro Kakizaki

Department of Renal and Urologic Surgery, Asahikawa Medical University, Asahikawa, Japan

Correspondence: Naoki Wada, Department of Renal and Urologic Surgery, Asahikawa Medical University, Asahikawa, Japan, Tel +81-166-65-2533, Email nwada@asahikawa-med.ac.jp

Purpose: In this study, we investigated skeletal muscle loss and bladder dysfunction caused by high-fat/high-sucrose (HFS) diet.

Methods: Twelve-week-old Sprague–Dawley (SD) female rats were fed on normal (Group N) or HFS (Group HFS) diet for 12 weeks. We conducted urodynamic investigation and pharmacologic in vitro. In addition, we measured gastrocnemius and tibialis muscle weight and protein concentration. The hypoxia-inducible factor (HIF)-1α and 8-hydroxy-2'-deoxyguanosine (8-OHdG) in the bladder were assayed.

Results: The urodynamic investigations revealed the significantly shorter intercontraction intervals and lower maximal voiding pressure in Group HFS than in Group N. Furthermore, the absolute and relative weights of the gastrocnemius muscle were found to be significantly lower in Group HFS than in Group N. The protein concentration of the gastrocnemius muscle was also significantly lower in Group HFS than in Group N. The absolute and relative weights of the bladder were also significantly lower in Group HFS than in Group N. The contractile responses of the bladder strips to electrical field stimulation and carbachol were significantly lower in Group HFS than in Group N. The HIF1α and 8OHdG in the bladder muscle were significantly higher in Group HFS than in Group N. The HFS diet reduced bladder capacity and contractility along with the loss of the gastrocnemius muscle.

Conclusion: HFS diet promotes bladder dysfunction similar to detrusor hyperreflexia with impaired contractility.

Keywords: high-fat, high-sucrose, bladder dysfunction, rat, skeletal muscle

Introduction

Several countries, including Japan, have already become super-aged societies. Frailty is a highly prevalent condition among the elderly and has been increasingly considered a crucial public health issue. Undernutrition, inaction, chronic inflammation, and metabolic abnormalities, including insulin resistance, are the contributing factors to sarcopenia, a common pathological condition among older people with frailty.[1] There are no established animal models representing fast muscle-dominated muscle loss, which is the characteristic change in sarcopenia. Animal models with insulin resistance or chronic inflammation can be used as substitutes for animals with aging-related sarcopenia.

Several previous studies in rats demonstrated that high-fat/high-sucrose (HFS) diet led to dynamic structural and inflammatory alterations of the skeletal muscle and accelerated the progression of sarcopenia by altering the postprandial stimulation of muscle protein synthesis.[2,3] The animals fed on HFS diet exhibited local inflammatory changes and decreased insulin sensitivity, which are similar to the molecular changes in humans with sarcopenia.[4] Changes in the lower urinary tract function of these animals are expected; however, this lacks information.

The structural and inflammatory alterations of the skeletal muscle may induce general or local oxidative stress reaction following ischemic change. We hypothesized that oxidative stress and ischemic change in the pelvis or the bladder in animals fed on HFS diet would induce bladder dysfunction. Thus, we investigated the change in the skeletal muscles and bladder function in rats fed on HFS diet.

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Methods

We conducted all procedures, and this animal research was approved by the Institutional Animal Care and Use Committee in Asahikawa Medical University. All procedures were also conducted according to National Institutes of Health guideline (https://grants.nih.gov/grants/policy/air/index.htm).

Animals

Twelve-week-old female Sprague–Dawley (SD) rats (body weight (BW): 200–226 g) were used and fed on normal (Group N; n = 18) or HFS (Group HFS; n = 15) diet for 12 weeks. HFS diet includes 40% of total energy (357 Kcal/100 g) as fat, 45% as sucrose, and 15% as protein (custom Diet #AIN-76, CLEA Japan, Inc., Shizuoka, Japan). Contrarily, normal diet includes 12% of total energy (356 Kcal/100 g) as fat, 59% as nitrogen-free extract, and 29% as protein (#CE-2, CLEA Japan, Inc., Shizuoka, Japan). The rats were housed on a 12:12 h dark/light cycle. Some of the rats (n = 10 in Group N and n = 9 in Group HFS) were allocated to the cystometric analysis, whereas the others (n = 8 in Group N and n = 6 in Group HFS) were allocated to the organ bath assay. Each experiment was conducted simultaneously. The gastrocnemius and tibialis muscles of all rats were harvested after each procedure and preserved at −80°C until the protein assay. The animals were euthanized via inhaled analgesia (isoflurane). After the cystometric analysis, the bladder was removed and divided into the bladder mucosa and muscle layers using microscissors under a microscope. The remaining bladder tissues from the organ bath assay were also used for the protein assay.

Cystometric Analysis

We evaluated bladder function via continuous filling cystometrograms (CMGs) under a conscious condition. After the animals were anesthetized with 1.5–2.0% isoflurane, we incised the lower abdomen and inserted a PE-50 tube (Clay-Adams, Parsippany, NJ, USA) into the bladder. After recovering from anesthesia, the rats were gently restrained in a cage (Natsume Seisakusho, Yushima, Tokyo, Japan), and the catheter was hooked up via a T shape cock to both pressure transducer and syringe pump. After the rats completely recovered from anesthesia, cystometry was performed with intravesical saline infusion (0.1 mL/min) to monitor stable bladder contractions for 120 min. After CMG became stable, we emptied the bladder using the catheter, and the CMG evaluation started via saline reinfusion. The CMG parameters included (1) intercontraction interval (ICI), defined as intervals between bladder contractions; (2) maximal voiding pressure (MP); and (3) post-void residual (PVR) withdrawn through the cystostomy catheter gravitationally after the end of micturition. The voided volume (VV) and bladder capacity were calculated using the following equation: VV = infusion rate (0.1 mL/min) × mean ICI (min) and bladder capacity = VV + PVR. The voiding efficiency (VE) (VV/bladder capacity × 100) was also calculated. The average of ICI and MP during 30 min was taken. PVR was measured at the end of continuous CMG. These parameters were evaluated using LabChart from AD Instruments (Colorado Springs, CO, USA).

Organ Bath Assay

The bladder was excised and weighed, and two full-thickness longitudinal strips of 2×8 mm were taken from each bladder body. The strips were mounted in isolated muscle baths containing Tyrode's solution (8.0 g/L NaCl, 0.05 g/L NaH_2PO_4-H_2O, 0.2 g/L KCl, 1.0 g/L $NaHCO_3$, 0.1 g/L $MgCl_2$-$6H_2O$, 1.0 g/L glucose, and 0.2 g/L $CaCl_2$ adjusted to pH 7.4 at 37°C) and equilibrated over 1 h. The contractile responses of bladder strips to three levels of electrical field stimulation (EFS; 2, 8, and 32 Hz, 20 volts), carbachol (20 μM), and potassium chloride (KCl; 120mM) were measured in this order in each group. After organ bath assay, the weight of each bladder strip was measured. The responses were recorded isometrically, and the maximal tensions normalized to strip weight and the response to KCl were compared. The average value obtained from two strips of each bladder was adopted as the maximal tension of each rat. These parameters were evaluated using LabChart from AD Instruments (Colorado Springs, CO, USA).

Protein Assay

We used an HIF1 Alpha (sandwich ELISA) ELISA Kit (LS-F4225, LifeSpan BioSciences, Inc., Seattle, WA, USA) and a Highly Sensitive ELISA Kit for 8-OHdG (KOG-HS10/E, NIKKEN SEIL Co., Ltd., Shizuoka, Japan) to measure the HIF1α and 8OHdG concentrations in the bladder muscle and mucosa, respectively. The assayed HIF1α and 8OHdG values were standardized with the whole tissue protein concentration measured using BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA). The protein contents of the gastrocnemius and tibialis muscles were standardized with each muscle weight after measuring the protein levels using BCA Protein Assay Kit.

Statistics Analysis

The results were expressed as means ± SEM. The Mann–Whitney *U*-test was employed for the statistical comparison between the two groups. $P < 0.05$ was considered to indicate statistical significance.

Results

Body and Tissue Weight

The BWs were similar in two groups at baseline (N: 202.0 ± 8.3 g; HFS: 204.8 ± 8.0 g) and 12 weeks after each diet (N: 305.0 ± 6.3 g; HFS: 309.1 ± 7.0 g) (Table 1). The tibialis muscle absolute weights and relative weight percent BW were 635.8 ± 14.5 mg and 0.22 ± 0.01 in Group N and 614.0 ± 9.2 mg ($P = 0.214$ vs Group N) and 0.21 ± 0.01 ($P = 0.077$ vs Group N) in Group HFS, respectively. The gastrocnemius muscle absolute weights and relative weight percent BW were 1071.1 ± 20.6 mg and 0.37 ± 0.01 in Group N and 1011.4 ± 9.4 mg ($P = 0.014$ vs Group N) and 0.34 ± 0.01 ($P = 0.016$ vs Group N) in Group HFS, respectively. The bladder absolute weights and relative weight percent BW were 85.1 ± 3.6 mg and 0.029 ± 0.001 in Group N and 69.9 ± 1.0 mg ($P < 0.001$ vs Group N) and 0.023 ± 0.001 ($P < 0.001$ vs Group N) in Group HFS, respectively (Table 1).

Cystometric Analysis

In Group HFS, the ICI was significantly shorter (176 ± 10 sec) than that in Group N (344 ± 51s) (mean differences 168 s [95% CI: 60, 276], $P = 0.004$) (Figures 1 and 2). The MP in Group HFS (15.7 ± 0.8 cmH_2O) was significantly lower than that in Group N (21.5 ± 1.7 cmH_2O) (mean differences 5.8 cmH_2O [95% CI: 1.9, −9.7], $P = 0.005$) (Figures 1 and 2). The VV and bladder capacity in Group HFS (0.29 ± 0.02 mL and 0.32 ± 0.01 mL) were significantly smaller than those in Group N (0.57 ± 0.09 mL and 0.62 ± 0.09 mL) (VV; mean differences 0.28 mL [95% CI: 0.10, 0.46], $P = 0.004$, bladder capacity; mean differences 0.29 mL [95% CI: 0.10, 0.49], $P = 0.004$). The PVR (N: 0.04 ± 0.01 mL; HFS: 0.03 ± 0.01 mL) (mean differences 0.01 mL [95% CI: 0.00, 0.04], $P = 0.253$) and VE (N: 93.0% ± 1.0%; HFS: 90.7% ± 2.8%) (mean differences 2.3% [95% CI: −3.9, 8.5], $P = 0.445$) were similar between the two groups (Figures 1 and 2).

Table 1 Body Weight, Muscle Weight and Bladder Weight in Rats Fed on Normal and HFS Diet

	Group N	Group HFS	Mean Difference, 95% CI	P value
Body weight				
Baseline (g)	202.0 ±8.3	204.8 ±8.0	−2.8 [−30.1, 24.6]	0.819
12-week later (g)	305.0 ±6.3	309.1 ±7.0	−4.1 [−23.0, 14.8]	0.667
Absolute weight				
Tibialis muscle (mg)	635.8 ±14.5	614.0 ±9.2	21.8[−13.3, 56.9]	0.214
Gastrocnemius muscle (mg)	1071.1 ±20.6	1011.4 ±9.4	59.7 [13.1, 106.2]	0.014
Bladder (mg)	85.1 ±3.6	69.9 ±1.0	15.2 [7.4, 23.1]	<0.001
Relative weight percent BW				
Tibialis muscle	0.22 ±0.01	0.21 ±0.01	0.01 [−0.001, 0.03]	0.077
Gastrocnemius muscle	0.37 ±0.01	0.34 ±0.01	0.03 [0.01, 0.06]	0.016
Bladder	0.029 ±0.001	0.023 ±0.001	0.006 [0.004, 0.008]	<0.001

Relative weight percent BW: the ratio of absolute weight to body weight

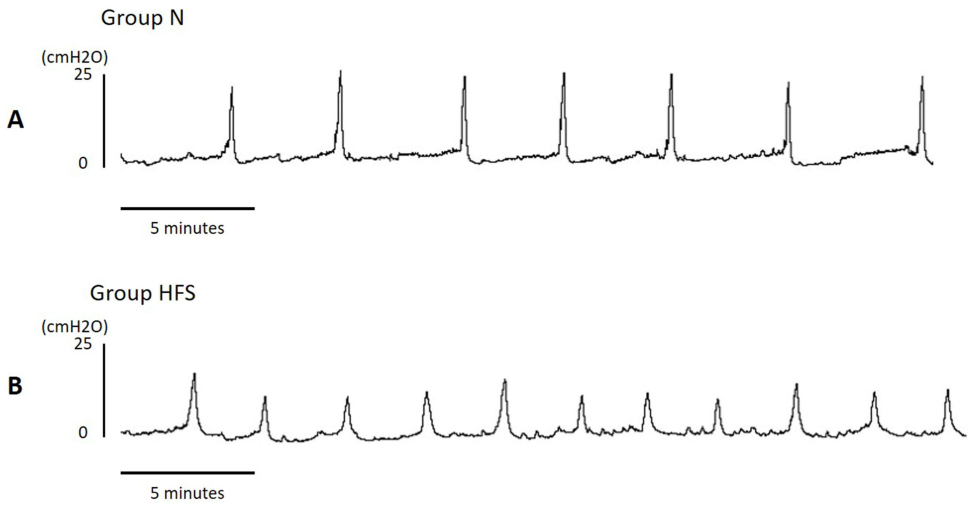

Figure 1 Representative cystometry traces in a rat fed on normal diet (**A**) and HFS diet (**B**). Compared with normal diet rat, the intercontraction interval was shorter and voiding pressure lower in HFS diet rat.

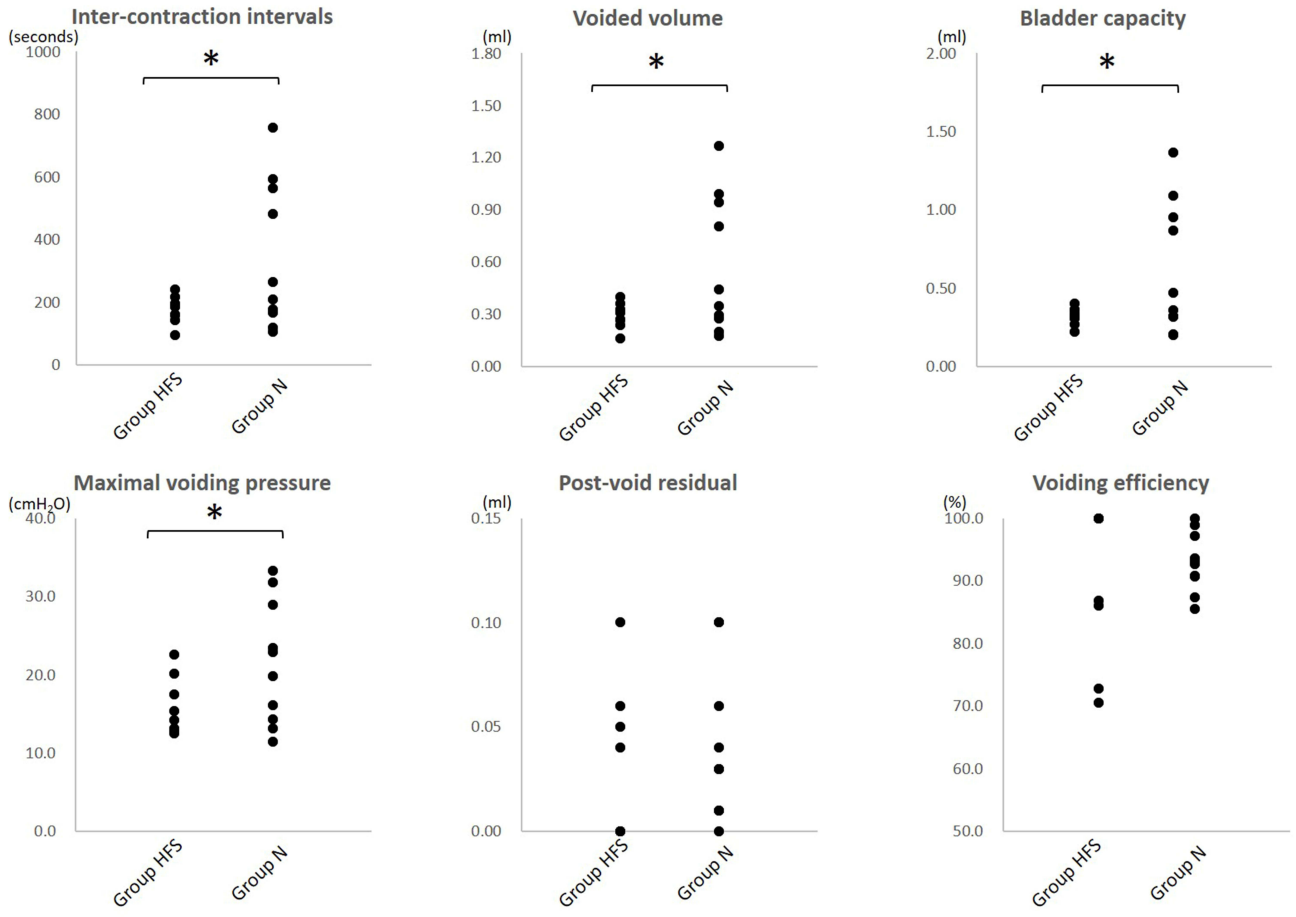

Figure 2 Analyses of the urodynamic parameters in rats fed on normal and HFS diets. N = 10 in Group N and N = 9 in Group HFS. The inter-contraction interval was significantly shorter in HFS diet rats than normal diet rats. The maximal voiding pressure was significantly lower in HFS diet rats. The voided volume and bladder capacity were significantly smaller in HFS diet rats. The post-void residual and voiding efficiency were similar between the 2 groups. *$P < 0.05$ using Mann–Whitney *U*-test.

Organ Bath Assay

The mean bladder strip weights were similar in two groups (N: 1.6 ± 0.1 mg; HFS: 1.7 ± 0.3 mg, $P = 0.745$). The contractile responses normalized to strip weight to 8 Hz (144.7 ± 13.4 vs 219.6 ± 24.0 g tension/mg, mean differences 74.9 [95% CI: 18.0, 131.7], $P = 0.012$) and 32 Hz (158.4 ± 14.6 vs 256.1 ± 25.3 g tension/mg, mean differences 97.7 [95% CI: 37.3, 158.1], $P = 0.003$) of EFS and carbachol (211.0 ± 25.5 vs 295.8 ± 29.9 g tension/mg, mean differences 84.8 [95% CI: 3.9, 165.6], $P = 0.041$) in Group HFS were significantly decreased than those in Group N. The contractile responses to 2 Hz of EFS (118.1 ± 13.9 vs 148.3 ± 16.7 g tension/mg, mean differences 30.2 [95% CI: −14.5, 74.8], $P = 0.177$) and KCl (177.9 ± 23.0 vs 244.0 ± 28.9 g tension/mg, mean differences 66.1 [95% CI: −9.8, 142.0], $P = 0.085$) in Group HFS were decreased compared with those in Group N without a statistical significance (Figure 3A). The contractile responses normalized to the KCL response to 32 Hz (92.7 ± 12.0 vs 108.5 ± 11.7 $\times 10^2$, mean differences 15.8 [95% CI: 5.4, 25.2], $P = 0.002$) in Group HFS was also significantly decreased than those in Group N (Figure 3B).

Protein Assay

The protein concentration of the tibialis muscle was similar in the two groups (HFS: 248.8 ± 11.8, N: 276.9 ± 29.4 mg/g muscle, mean differences 28.1 [95% CI: −43.3, 99.5], $P = 0.397$). The protein concentration of the gastrocnemius muscle in Group HFS (200.0 ± 5.5 mg/g muscle) was significantly lower than that in Group N (252.0 ± 19.2 mg/g muscle, mean differences 52.0 [95% CI: 6.0, 98.0], $P = 0.031$) (Figure 4).

In the bladder mucosa, HIF1α (N: 4.3 ± 1.2, HFS: 3.3 ± 0.8 ng/mg protein, mean differences 1.0 [95% CI: −2.0, 4.0], $P = 0.484$) and 8OHdG (N: 3.6 ± 0.6, HFS: 4.7 ± 0.8 ng/mg protein, mean differences −1.1 [95% CI: −3.1, 0.8], $P = 0.253$) were similar in the two groups. In the bladder muscle, HIF1α (N: 1.5 ± 0.2, HFS: 4.5 ± 1.1 ng/mg protein, mean differences −3.0 [95% CI: −5.5, −0.5], $P = 0.022$) and 8OHdG (N: 3.9 ± 0.6, HFS: 7.7 ± 0.7 ng/mg protein, mean differences −3.8 [95% CI: −5.7, −1.9], $P < 0.001$) were significantly higher in Group HFS than in Group N (Figure 5).

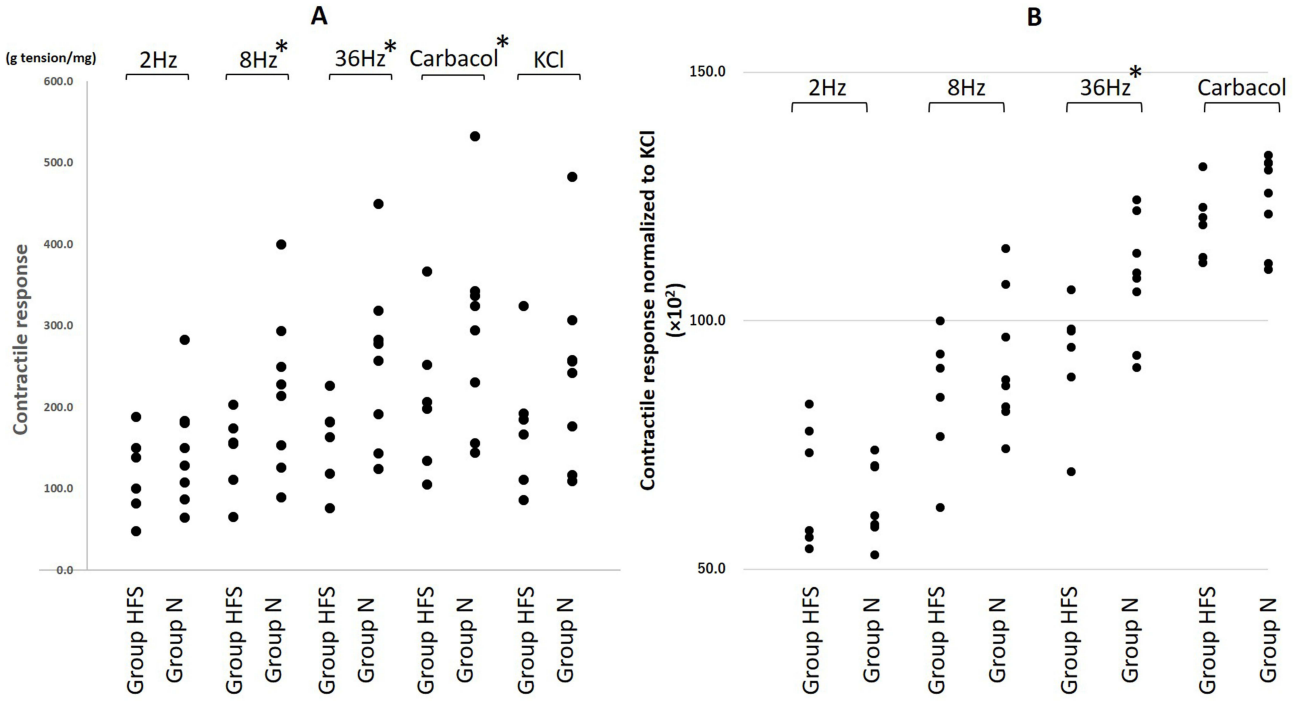

Figure 3 Maximal contraction of bladder strips normalized to strip weight (**A**) and the response to KCl (**B**) exposed to electrical field stimulation (2, 8 and 32 Hz at 20 volts), carbachol (20 μM), and KCl (120 mM) in normal and HFS diet groups. N = 8 in Group N and N = 6 in Group HFS. *$P < 0.05$ using Mann–Whitney *U*-test.

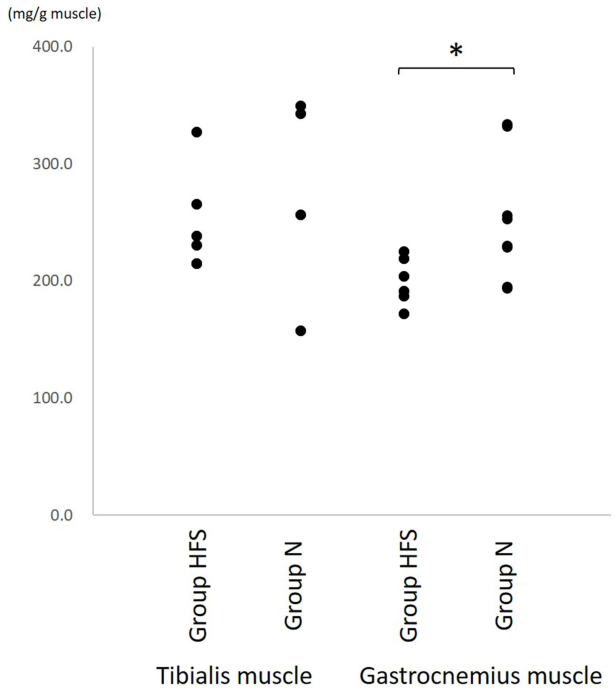

Figure 4 The protein concentration of the tibialis and gastrocnemius muscle. The protein concentration of the gastrocnemius muscle in Group HFS was significantly lower than that in Group N. *P < 0.05 using Mann–Whitney U-test.

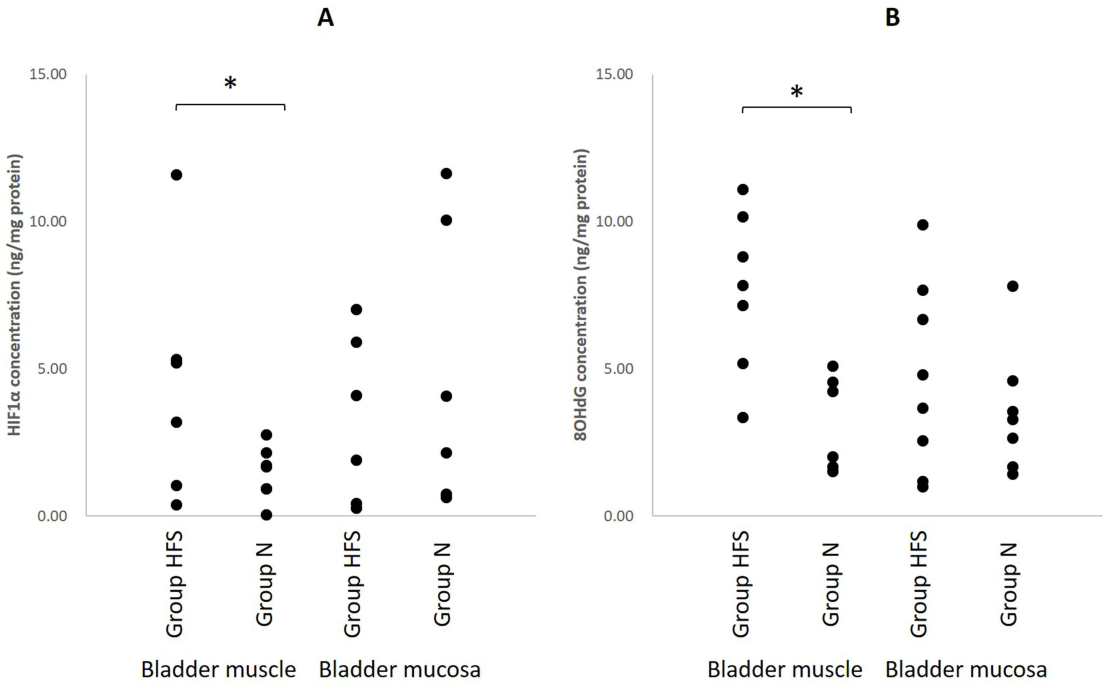

Figure 5 Bladder HIF1α (**A**) and 8OHdG (**B**) protein assay. HIF1α and 8OHdG in bladder muscle were significantly increased in HFS diet rats than in normal diet rats. N = 18 in Group N and N = 15 in Group HFS. *P < 0.05 using Mann–Whitney U-test.

Discussion

This study demonstrated that HFS diet led to loss of the gastrocnemius muscle, voiding interval shortening, and bladder contractile force reduction in rats. Because the HIF1α and 8OHdG levels in the bladder muscle were increased, oxidative

stress following bladder ischemia seemed to underlie these alterations of bladder function. HFS diet resulted in both skeletal muscle loss and bladder dysfunction similar to detrusor hyperreflexia with impaired contractility, which is most common in the elderly.

Aging is associated with a progressive loss of skeletal muscle mass and function, which is one of the causes of physical frailty in the elderly. Age-associated insulin resistance, low-grade inflammation, and oxidative stress reduce muscle mass.[1] Several studies demonstrated that the load of sugars, including glucose, fructose, and sucrose, are responsible for the development of insulin resistance, inflammation, and oxidative stress.[5,6] Gatineau et al reported that high-sucrose intake accelerated the progression of sarcopenia in rats by altering insulin sensitivity and muscle protein synthesis.[2] Furthermore, increased intramuscular fat deposits are positively correlated with insulin resistance and inflammatory changes within the muscle in the development of sarcopenic obesity.

In this study, the relative weight and protein concentration of the gastrocnemius muscle in HFS diet rats were lower than those of normal diet rats, although the mean BW was similar between the two groups. The mean BW and protein concentration of the tibialis muscle were also decreased in HFS diet rats without a significant difference compared with normal diet rats. The reason why the tibialis muscle was not significantly changed might be insufficient feeding period with HFS diet compared with the previous study.[2] Another explanation might be sex differences. In this study, we used female SD rats because the anatomy and function of the lower urinary tract are simpler than those of male rats to investigate the bladder function. Male rats were used in the previous research to investigate the change in the skeletal muscle after HFS diet.[2,3] In a study on humans, adipose infiltration to the skeletal muscle is different between males and females.[7] Furthermore, despite the HFS diet, a significant increase in BW was observed only in male rats, and female rats were resistant to enriched diets in some studies.[8,9]

Bladder ischemia is one of the key factors that induces bladder function deterioration. Previous studies demonstrated that bladder capacity and the contractile responses of bladder strips were decreased in atherosclerosis-induced chronic bladder ischemic model in rats and rabbits.[10–12] In this study, the increased HIF1α and 8OHdG in the bladder muscle of HFS diet rats indicate the oxidative stress after ischemic change in the bladder. The oxidative stress in the bladder induces the sensitization of the afferent nerve pathway, resulting in bladder overactivity.[13] The ischemia and oxidative stress affect the bladder nerves.[14,15] In the bladder ischemic models, the number of the bladder nerves was found to decrease. The oxidative stress and ischemic change in the bladder lead to the shorter contraction intervals and lower voiding pressure on CMG analysis as well as reduced contractile responses of bladder strips. These changes in bladder function discussed in previous studies are consistent with the results of the present study. Low inflammatory change in the bladder urothelium might be happened to be correlated with shorter intervals of voiding. In future study, histological examination of the bladder is warranted. We do not have any firm explanation for the decrease in bladder weight in HFS diet rats. According to previous studies, HFS diet increased the weight of some organs, such as liver and pancreas,[16] whereas it reduced the uterine and placental weights in pregnant rats.[17] Reduction of the blood supply to the bladder might influence the bladder weight, although no significant change was observed in bladder weight in the atherosclerosis-induced chronic bladder ischemic animal model.[10–12]

The greatest question is whether there is a direct association between skeletal muscle loss and bladder dysfunction following bladder ischemia in HFS diet rats. We speculated that the blood supply is decreased in the pelvis because of relative reduction of the skeletal muscle. However, there are not any data or literature to support this speculation. One reason might be that HFS diet is only the common factor to induce skeletal muscle loss and bladder dysfunctions. To date, there is no literature on the changes in bladder function of HFS diet animals. However, in other studies, frequent voiding and the decrease in detrusor contractile response to the cholinergic stimulation were observed in hypercholesterolemia rats.[18] Rats fed on fructose, but not sucrose, showed decreased detrusor contractile response to high KCl concentration and bladder overactivity and urinary frequency on CMG analysis.[19–21] Thus, HFS diet might induce bladder ischemia due to change in arterial sclerosis and bladder dysfunction, including bladder overactivity, urinary frequency, and reduced detrusor contraction independent of skeletal muscle loss.

A clinical study showed that psoas muscle volume was positively associated with bladder contractility in male patients.[22] The exact mechanism of this association remains to be elucidated. During the past couple of decades, it has been apparent that the skeletal muscle works as an endocrine organ, which can produce and secrete hundreds of

myokines that exert their effects in either autocrine, paracrine, or endocrine manners.[23] Recent literature demonstrated that the skeletal muscle produces myokines in response to exercise, which allow for crosstalk between the muscle and other organs, including brain, adipose tissue, bone, liver, gut, pancreas, vascular endothelium, and skin as well as the muscle itself.[23–26] Furthermore, some myokines such as myostatin negatively regulate myogenesis.[27] It is tempting to speculate that an increase or decrease in any myokines due to skeletal muscle loss might have any impact on bladder function. The effects of myokines secreted from the skeletal muscle on bladder function might be an interesting target in future research.

In HFS diet rats, the PVR and voiding efficiency were similar to those in normal diet rats despite the reduced voiding pressure and bladder contractility. Thus, the bladder function in HFS diet rats is compensated for by an unknown mechanism. One possible explanation might be related to the urethral function. Some species, including rats, have a pumping activity of the urethra to eliminate urine.[28] Detailed analysis using external urethral sphincter (EUS)–electromyogram (EMG) recordings in rats revealed rhythmic contractions and relaxations of EUS during voiding, referred to as EUS bursting.[28] Some research showed that in rats with spinal cord injury, the voiding efficiency was recovered and equal to normal rats through the EUS bursting activity.[29] Further evaluation of the urethral function of HFS diet rats is warranted.

Weight loss, but not sarcopenic obesity, is one of the key features of frailty after sarcopenia, inactivity, and undernutrition in the elderly. Sarcopenic emaciation animal models will be needed to investigate the pathophysiology of frailty in the elderly. The link between skeletal muscle loss and bladder dysfunction is poorly understood. We have a lot of problems to resolve to understand the underlying mechanisms. This study is preliminary but is the first report to describe the relationship between skeletal muscle loss and bladder dysfunction in HFS diet rats. In the future, it should be warranted to examine whether exercise and nutrition therapy might have therapeutic benefits to improve skeletal muscle mass and bladder function.

Conclusion

This is a preliminary but first attempt to elucidate the alterations of bladder function in HFS diet rats. HFS diet reduced bladder capacity and contractility with gastrocnemius muscle loss in rats. In addition, it led to the development of lower urinary tract dysfunction that is similar, at least in part, to detrusor hyperreflexia with impaired contractility.

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Disclosure

All authors do not have any conflict of interest to disclose.

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