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Dietary macronutrients modulate hypertrophy and contractility of the detrusor in an experimental model of bladder obstruction

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ABSTRACT

Objectives: To investigate the effects of various diets on structure and function of the bladder in both normal and obstructed bladders of male Wistar rats.
Methods: Sham-operated rats and rats with experimentally-induced bladder outlet obstruction (BOO) were fed with standard rats' feed (control), High-carbohydrate (HCD), High-fat (HFD) and High-protein (HPD) diets. Feeding was continued for 4 weeks after BOO surgery. Bladder weight, detrusor contractility, Rho-Kinase (ROK) and Myosin Light Chain Kinase (MLCK) expressions were determined using standard methods.
Results: In comparison with control, bladder weight was increased in HFD (164 ± 9 mg), BOO (437 ± 21 mg), HFD-BOO (523 ± 19 mg) and HPD-BOO (268 ± 18 mg). Detrusor contractility was reduced in BOO and HFD-BOO. The ROK- I and II expressions were high in HCD-BOO and low in HPD-BOO but ROK-I was also elevated in BOO. However, MLCK increased only in HCD-BOO.
Conclusion: The results of the study reveal that diets with varying macronutrient compositions have variable effects on the bladder with and without obstruction. High-fat diets especially, affect detrusor morphology and function in both obstructed and unobstructed bladders.

1. Introduction

Bladder outlet obstruction (BOO) is a disorder common especially in older males which impacts negatively on the quality of life (QoL) of affected individuals. The aetiology of this condition could be traced to anatomic or physiologic causes and it often produces lower urinary tract symptoms (LUTS), although the degree severity of the LUTS it causes is highly variable and not predictable on the basis of a particular inciting aetiology [1]. BOO is almost synonymous with LUTS in men [2]. It has been shown that the prevalence of LUTS is directly linked, and increases linearly, with age [3,4].

Obesity and metabolic syndrome in individuals have been directly linked with a worsening of LUTS in conditions like BOO. Obesity, which is a major predisposing factor to metabolic syndrome, has a direct relationship with dietary intake, especially consumption of diets high in saturated fatty acids. Metabolic syndrome (MetS) is a conglomeration of different metabolic risk factors, which include visceral obesity, dyslipidemia, glucose intolerance, insulin resistance, and hypertension [5]. It has been recently suggested that MetS may play a critical role in the aetiology of LUTS, and dietary intake, which influences predisposition to metabolic phenotypes like MetS, has been proposed to have a relationship with onset, prevalence and severity of LUTS in BOO [6].

Diets have also been shown to have a strong association with changes in smooth muscle; with calcium-supplemented diets showing attenuating effects on cardiac smooth muscle hypertrophy [7]. Blueberry-rich diets have also been reported as suppressing α1-adrenergic receptor agonist-mediated contraction in vascular smooth muscle [8]. The detrusor muscle, a major constituent of the fibres making up the detrusor muscle of the bladder is predominantly constituted of smooth muscle fibres and we therefore hypothesized that dietary macronutrient composition of food could have an effect on detrusor smooth muscle hypertrophy and contraction in animals with normal bladders and those that have undergone experimentally-induced bladder outlet obstruction.

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2. Materials and methods

2.1. Animals

A total of 80 male albino rats of the Wistar strain were used in this study. The animals were obtained from the Animal House of the College of Medicine, University of Ibadan, Nigeria. All studies were approved by the University of Ibadan Animal Ethics Committee. The animals were divided into eight (8) dietary and BOO groups of ten animals each and were housed in well-aerated experimental animal cages, maintained under standard lighting conditions. They were acclimatised for 7 days prior to commencement of the grouping. During this period, they were fed on standard rat chow (Ladokun Feeds, Nigeria Limited®) and had access to clean drinking water. This study followed all guidelines in accordance with the International Ethical Norms on Animal Care and Use as contained in NIH publication 80-23, revised in 1985. In the final analysis, data from the male animals were utilised since a preliminary study in our laboratory had shown that female animals did not respond significantly to dietary changes within the experimental period [9].

2.2. Animal feed

Animal feeds were mixed from individual feed constituents in particular compositions for each of the dietary groups. The mixes were then pelletised to ensure even distribution of components and consumption by animals. The control diet was derived from standard rats' feeds commercially-propounded and sold by Ladokun Feeds®. Each of the experimental diets was formulated by altering the proportion of components supplying each macronutrient in the original standard feed. The diets were all designed under the close supervision of a nutritionist, and caloric compositions determined with a bomb calorimeter. The diets were designed so as to contain 20–25% total protein in order to provide the essential amino acids, in line with the recommendations of the American Institute of Nutrition [10]. Adequate nutritional requirements were ascertained and essential amino acids were added to the feeds to prevent under-nutrition. These were then fed to the animals as follows:

- - Group 1 : Sham-operated rats fed on normal rats' chow (26.5% protein, 40% carbohydrates, 29% fat, and 4.5% crude fibre)
- Group 2 : BOO animals fed on normal rats' chow
- Group 3: Sham-operated rats fed on a High Carbohydrate Diet (HCD) (20% protein, 58.5% carbohydrates, 17% fat, and 4.5% crude fibre)
- Group 4: BOO rats fed on a High Carbohydrate Diet (HCD) (20% protein, 58.5% carbohydrates, 17% fat, and 4.5% crude fibre)
- Group 5: Sham-operated rats fed on a High Fat diet (HFD) (22% protein, 13.5% carbohydrates, 60% fat, and 4.5% crude fibre)
- Group 6: BOO animals fed on a High Fat diet (HFD) (22% protein, 13.5% carbohydrates, 60% fat, and 4.5% crude fibre)
- Group 7: Sham-operated rats fed on a High Protein diet (HPD) (55% protein, 25.5% carbohydrates, 15% fat, and 4.5% crude fibre)
- Group 8: BOO animals fed on a High Protein diet (HPD) (55% protein, 25.5% carbohydrates, 15% fat, and 4.5% crude fibre)

The animals were fed for a period of 8 weeks, after which partial bladder outlet obstruction was induced in them surgically. Subsequently, they were fed for 4 weeks after induction.

2.3. Induction of bladder outlet obstruction

Twelve (12) hours prior to surgery, animals were fasted but allowed free access to drinking water. Anesthesia was induced by ketamine (75 mg/kg ip) and xylazine (15 mg/kg ip) (Merck™, USA). The bladder was approached through a lower midline incision, and the proximal urethra exposed. A 3-0 Novafil (monofilament polybutester; Davis & Geck, Wayne, NJ) ligature was placed around the urethra and tied in the presence of a steel rod placed in the lumen with a diameter of 0.9 mm. After the knot was tied, the steel rod was removed, the bladder repositioned, and the abdominal wall was closed.

2.4. Bladder weight determination

Animals were sedated with an intraperitoneal injection of 0.1 ml/100 g rat weight ketamine/xylazine cocktail containing 91 mg/kg ketamine and 9.1 mg/kg xylazine (Merck™, USA). The bladder was removed through an abdominal incision, emptied, and washed in ice-cold phosphate-buffered saline. After the bladder was removed, the animal was euthanized with sodium pentobarbital. The bladders were then weighed on an electronic organ weighing balance (CAMRY®, USA).

2.5. Detrusor contractile activity measurement

The bladder was opened longitudinally and three full thickness (with intact urothelium) strips from dorsal section of bladder body were placed in organ baths containing 15 ml Tyrode's solution, at 37 °C. The buffer containing the bladder strips was equilibrated with 95% O_2 and 5% CO_2. One end of each strip was connected to a force displacement transducer and change in muscle tension was measured and recorded on a polygraph (Indiamart®, New Delhi, India).

2.6. Rho-kinase polymerase chain reaction (PCR)

Total ribonucleic acid (RNA) from urethra and bladder (n = 10 each) was isolated utilizing the RNA easy Isolation Kit (Qiagen, Valencia, CA). Real-time quantitative polymerase chain reaction (RT-PCR) analysis [11] was used to assess the relative levels of Rho-kinases I and II mRNA in rat bladder tissue. About 2 mg of total RNA from each sample was treated with RQ DNAase 1 (Promega Biotechnology, Madison, U.S.A.) before being subjected to reverse transcription. The resulting cDNA was diluted to 100 ml and 5 ml was loaded to each PCR reaction. Reverse transcription and PCR reactions were performed according to the manufacturer's instructions (Applied Biosystems, Foster City, U.S.A.). Rho-kinase I and II sequence-specific amplification was detected with an increasing fluorescent signal of FAM reporter dye during the amplification cycle. Oligonucleotide primers and Taqman probes were designed using Primer Express software (Applied Biosystems) and were synthesized by Applied Biosystems. Sequences of forward primers, reverse primers, and probes are as shown in Table 1. Expression levels of each target gene were expressed relative to the housekeeping gene, β-actin.

Table 1
Primer sequences for Rho-Kinase I and II.

Rho-Kinase I	SEQUENCES
Forward primer	AGGCCTGTGCCAAACCTTT
Reverse primer	TGGTCCCTGTGGGACTTAACA
Taqman probe	CCGCCTGCCCTAGAGTGTCGAAGA
Rho-Kinase II	
Forward primer	CCCGATCATCCCCTAGAACC
Reverse primer	TTGGAGCAAGCTGTCGACTG
Taqman probe	CAACAAAACCAGTCCATTCGGCGGC

Table 2
Primer Sequences for Myosin Light Chain Kinase (MLCK).

MLCK	SEQUENCES
Forward primer	AATGGTGTTGCTGGAGATCGAGGT
Reverse primer	GCTGGATCAAATTGCGGTGGTTCA
GAPDH	
Forward primer	ATGATTCTACCCACGGCAAG
Reverse primer	CTGGAAGATGGTGATGGGTT

2.7. Myosin light chain kinase (MLCK) polymerase chain reaction (PCR)

Bladder and urethra were exposed through a lower abdomen midline incision and transection of the pubic symphysis. The bladder was separated from the urethra below the bladder neck. Both tissues were removed as a whole and were placed in ice-cold phosphate buffered saline (PBS). The mucosal and serosal layers of these tissues were mechanically separated from the smooth muscle layer. Bladder smooth muscle samples were kept in RNAlater (Inqaba Biotech, South Africa) for RNA isolation. Animals were euthanized at the end of the procedure. Total ribonucleic acid (RNA) from urethra and bladder (n = 10 each) was isolated utilizing the RNA easy Isolation Kit (Qiagen, Valencia, CA). All RNA were of high quality as indicated by a 2:1 ratio for 28S/18S ribosomal RNA and an optical density (OD) ratio of >1.9 for OD260/OD280. The isolated RNA (2.5 μg) was annealed to 0.4 μg of oligo-dT primer in a volume of 12 μl. The final volume was brought to 20 μl by adding 4 μl of 5X buffer, 2 μl of 0.1 M dithiothreitol (DTT), 1 μl of 10 mM deoxyribonucleotide triphosphate (dNTP), and 1 μl of SuperScript reverse transcriptase (Invitrogen, La Jolla, CA). After one hour of incubation at 42 °C, the RT mixture was incubated at 70 °C for 10 min to inactivate the reverse transcriptase. The cDNA library was diluted fivefold for real-time PCR by adding 80 μl of TE buffer. All reagents for SYBR Green real-time PCR, including the primers for rat MLCK were purchased from Applied Biosystems (Foster City, CA). Primer sequences for real-time PCR are presented in Table 2. The reactions were conducted in the Prism 7300 H T sequence detection system (Applied Biosystems) using a 96-well plate format. Cycling conditions included an initial phase at 95 °C for 3 min, 40 cycles at 95 °C for 15 s, and 55 °C for 60 s followed by a melting curve analysis at 95 °C for 15 s, 55 °C for 15 s, and 95 °C for 15 s. Real-time PCR results were analyzed by SDS 7000 software (Applied Biosystems) to determine the expression levels for genes of interest. Each measurement was performed in triplicate. Expression levels of each target gene were expressed relative to the housekeeping gene, β-actin.

2.8. Western blotting

Bladder tissue was homogenized in 15 ml RIPA buffer (50 mM Tris−HCl, pH 8.0, 150 mM NaCl, 0.5% sodium deoxycholate, 1% NP-40%, and 0.1% SDS) containing 1% complete EDTA-free protease inhibitor cocktail (Roche Diagnostics, Mannhein, Germany). The homogenate was centrifuged at 10,000 *g* for 15 min at 4°C and the supernatant taken. The total protein concentration of each supernatant was measured using Bio-Rad Dc protein assay reagents (Bio-Rad Laboratories, Hercules, CA, U.S.A.) using bovine serum albumin as standard. Each sample containing 100 μg of total protein was taken and mixed with 56 sodium dodecyl sulphate (SDS) sample buffer and phosphate buffered saline. After boiling for 10 min, samples were loaded onto Invitrogen NuPAGE 4–12% Bis-Tris Gel (Invitrogen, Carlsbad, CA, U.S.A.). The protein was separated by electrophoresis in a Novex gel box and blotted onto Invitrogen PVDF membranes. Membranes were blocked for 2 h with 5% dry non-fat milk in TBST buffer (20 mM Tris pH 7.5, 0.5 M NaCl, 0.1% Tween 20) and then incubated with a primary monoclonal antibody against Rho-kinase I (1:200 dilution; MFCD02264068, Merck KGaA, Darmstadt, Germany), Rho-kinase II (1:50 dilution; HPA007459, Merck KGaA, Darmstadt, Germany) and MLCK (1:300; R54520, BD Transduction Laboratories, San Diego, CA, U.S.A.) at 48 °C overnight. For normalization of the protein loading, a monoclonal antibody against β-Actin (MFCD00164531, Merck KGaA, Darmstadt, Germany) in 1:5000 dilution was used. The membranes were washed three times for 5 min each with TBST, and incubated for 1 h with 1:5000 HRP linked anti-rat Ig (GENA935, Merck KGaA, Darmstadt, Germany) in TBST containing 5% dry milk at room temperature. The membranes were then marked with a pen to identify the side of the bands and then incubated for 5 min with substrate (1:1 ratio, 250 μl: 250 μl) in the dark, during which time the C-digit scanner was switched on. The membrane was then read by facing it downwards (marked arrow facing down). The resulting images were analyzed with Image *J* software to determine the integrated density value (IDV) of each protein band normalized to the IDV of β-Actin.

2.9. Data analysis

Data obtained were expressed as mean ± standard error of mean (mean ± SEM). The significance of the results for dietary groups within each generation was evaluated using analysis of variance (ANOVA) and the means were compared using Tukey-Kramer Multiple comparison Test. $P < 0.05$ was regarded as statistically significant.

3. Results

Partial outlet obstruction of the rat bladder produces several significant changes in both morphology and function. The aims of this study were to investigate the effects of diet-induced metabolic phenotypes on the progression of bladder outlet obstruction in male wistar rats, while also considering their effects on the normal bladder. Animals with obstruction fed the standard rats' chow showed a significant increase ($P < 0.05$) in bladder weight in comparison with the control animals without obstruction, indicative of hypertrophy (Fig. 1). High fat diet (HFD)-feeding in rats resulted in increased bladder weight, even without obstruction. With obstruction, bladder weights in HFD-fed animals increased beyond normal obstruction. High carbohydrate (HCD) and High protein (HPD) diets reduced bladder weight in obstruction. In individual comparisons within each dietary group, the HFD- and HPD-fed animals both had an elevation in bladder weight in their respective obstructed groups in comparison with the unobstructed controls (Fig. 1).

Contractile responses of the bladder to electrical stimulation were significantly reduced ($P < 0.05$) in BOO by the 4th week when compared with the unobstructed control group (Fig. 2). This was also significantly decreased in HFD-fed animals, even without obstruction. HFD rats with BOO had a significant decrease in contractility, way beyond those observed in the obstruction control. The HPD-fed rats with BOO also exhibited a slight decrease in contractility in comparison to the unobstructed control by the 4th week, however, in comparison with the BOO animals, it showed a highly significant increase in conctractile activity (Fig. 2). The HCD reflected an increase in contractile responses in both unobstructed and obstructed bladders.

Highly elevated expression of Rho-kinase I was observed in obstructed animals fed on the control diet. Rho-kinase I expression was also upregulated in rats with obstructed bladders fed on the HCD. The HPD, on the other hand, reflected a decline in expression of the kinase. Rho-kinase II expression was upregulated in only the HCD animals with obstructed bladders. Animals with obstructed bladders fed on HPD showed downregulation of Rho-

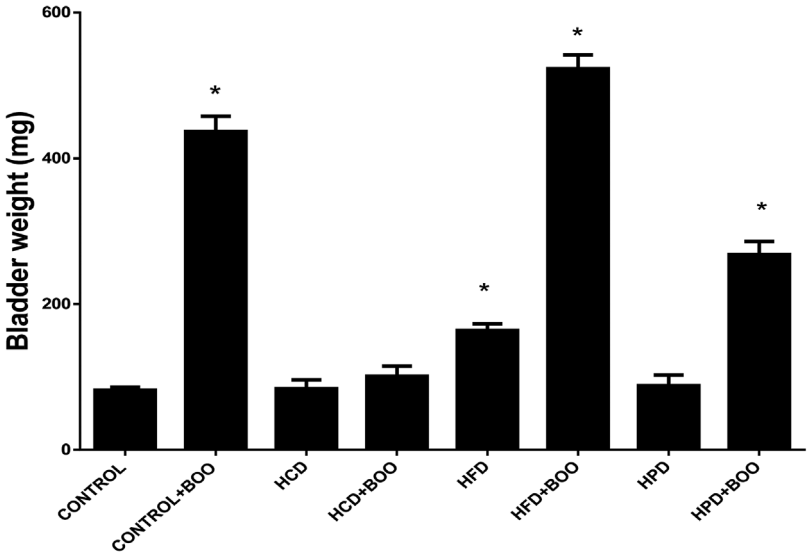

Fig. 1. Diet-induced changes in bladder weight in unobstructed and obstructed rats' bladders. Values are mean ± SEM for 10 animals per dietary group. $P < 0.05$.

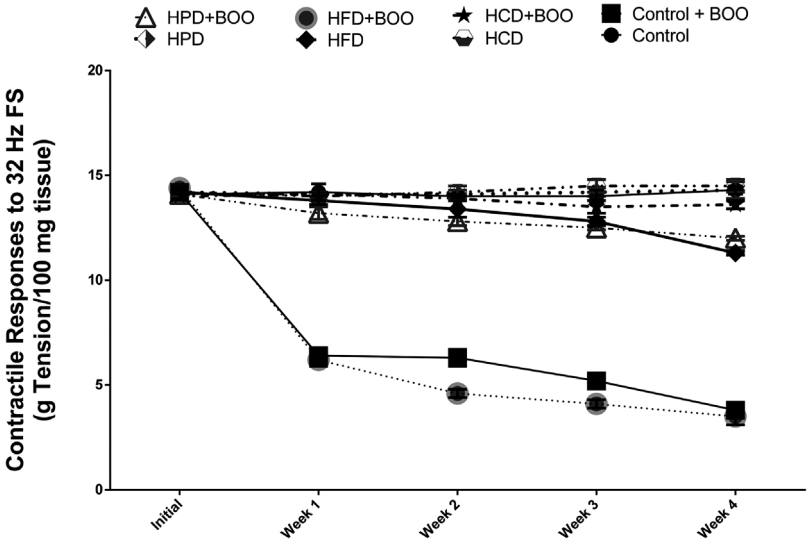

Fig. 2. Diet-induced changes in detrusor contractile activity in unobstructed and obstructed rats' bladders. Values are mean ± SEM for 10 animals per dietary group. $P < 0.05$.

kinase II expression. However, only obstructed animals fed on a HCD showed upregulation of Myosin Light Chain Kinase expression (Figs. 3–11).

4. Discussion

The smooth muscle of the bladder undergoes hypertrophy to compensate for, and generate, the increased pressures required to continue voiding processes in response to obstruction of the outlet of the bladder. Reports from several animal models of BOO have reported rapid, marked changes in morphology and function of the detrusor muscle of the bladder [12,13]. The results of this study indicate an increase in bladder weight in obstructed animals fed with the control diet, which confirms these findings. An elevation of bladder weight observed in animals fed with the HFD, even without obstruction, is further increased with experimentally-induced obstruction. HPD-fed animals also reflected an elevation in bladder weight in obstructed animals. Studies on bladder hypertrophy show that it is a consistent effect of obstruction in both animal models and in humans, and always results in an increase in the thickness and weight of the bladder [14,15]. Although bladder wall thickness could also increase with age in asymptomatic individuals, an increase in thickness has been directly and strongly associated with LUTS and BOO, and the degree of thickness seems to depend on the severity of obstruction [16,17], therefore the HFD and HPD-induced elevations suggest increased severity in obstruction which would result in worsening of LUTS in obstruction.

At some stages of growth in the hypertrophying rat urinary bladder, the synthesis of myosin is not in pace with the increase in smooth muscle volume. This results in lower myosin concentrations, and this has been correlated with decreased active force per muscle area in hypertrophic tissue [18,19]. In bladder outlet obstruction, therefore, there is a decrease in force per cross-sectional area in spite of the already-described increase in detrusor size. Our study confirms this finding; however, our experiments to determine contractile force of the detrusor in response to electrical stimulation revealed that the HFD-fed animals had a reduction in force generation even without obstruction (Fig. 2). In obstruction,

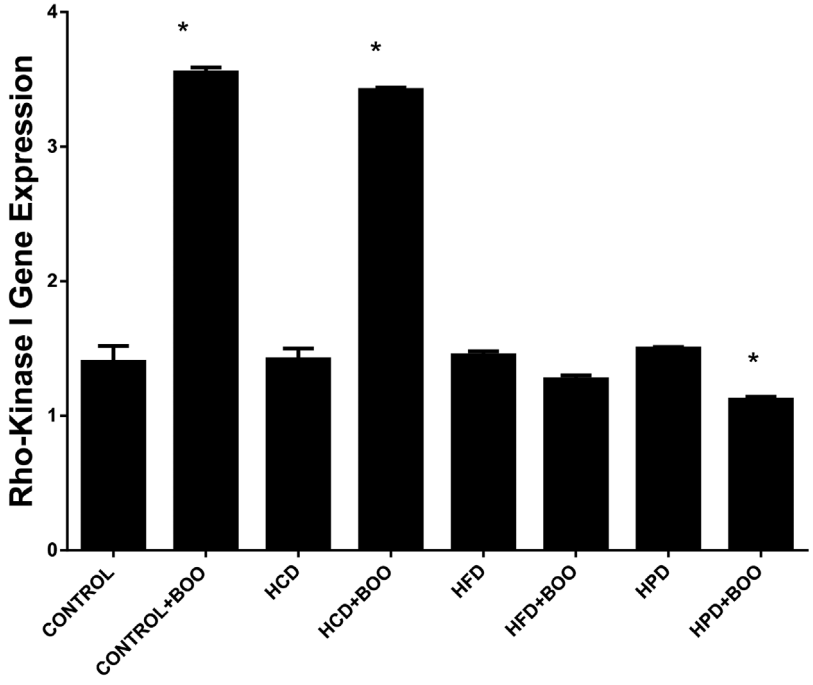

Fig. 3. Effects of diet-induced metabolic phenotypes on Rho-kinase I mRNA expression in both unobstructed and obstructed rats' bladders. Values are mean ± SEM for ten 12-week old male animals per dietary group; $P < 0.05$.
* = significant in comparison with control.

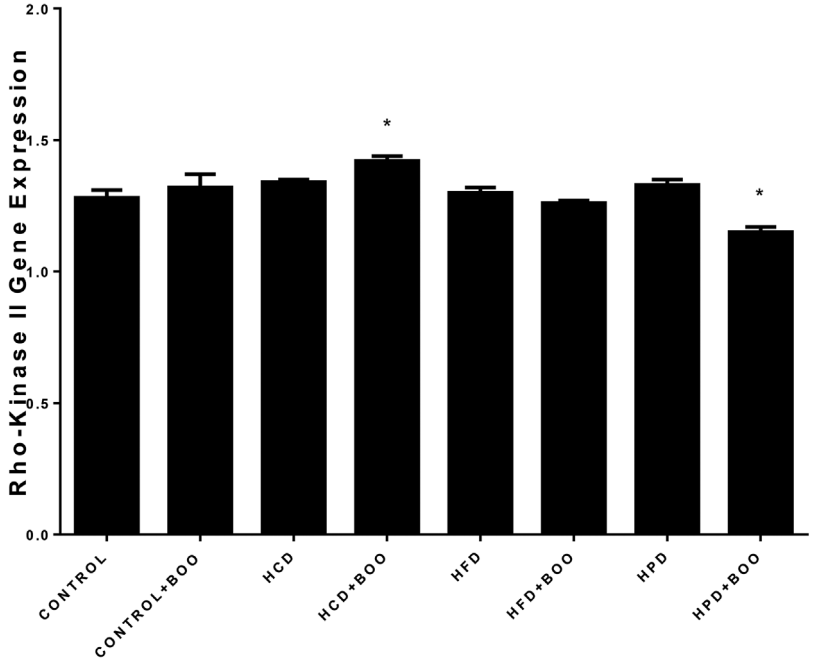

Fig. 4. Effects of diet-induced metabolic phenotypes on Rho-kinase II mRNA expression in both unobstructed and obstructed rats' bladders. Values are mean ± SEM for ten 12-week old male animals per dietary group; $P < 0.05$.
* = significant in comparison with control.

this animal group had a greater decrease in contraction than the obstructed control. This suggests that the HFD-induced changes in morphology result in a lessening of compensatory ability of the detrusor to expel urine against the obstruction, which could be adduced to a reduction in myosin content of the hypertrophying smooth muscle.

Rho-kinase I expression was elevated in the BOO control and HCD-BOO, while it was decreased by the HPD in obstructed animals. The expression of Rho-kinase II was only increased by the HCD in obstructed animals, while there was a decrease in HPD-BOO. Earlier studies have reported MLCK activity as unchanged in the compensatory stage in BOO [20]. This study confirms this report; MLCK, however, was increased in BOO animals fed on the HCD. Bing

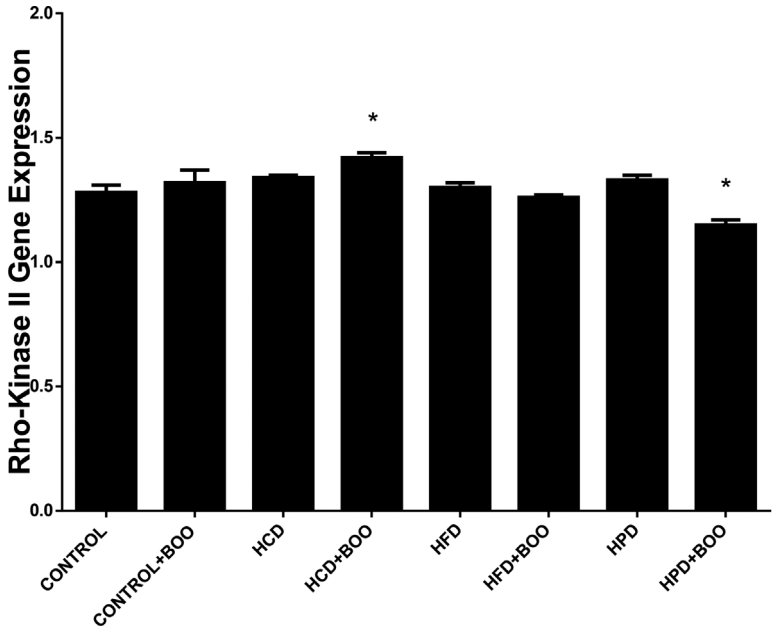

Figure 4: Effects of diet-induced metabolic phenotypes on Rho-kinase II mRNA expression in both unobstructed and obstructed rats' bladders

Values are mean±SEM for ten 12-week old male animals per dietary group; P<0.05
*** = significant in comparison with control**

Fig. 5. Effects of diet-induced metabolic phenotypes on Myosin light chain kinase mRNA expression in both unobstructed and obstructed rats' bladders. Values are mean ± SEM for ten 12-week old male animals per dietary group; $P < 0.05$.
* = significant in comparison with control.

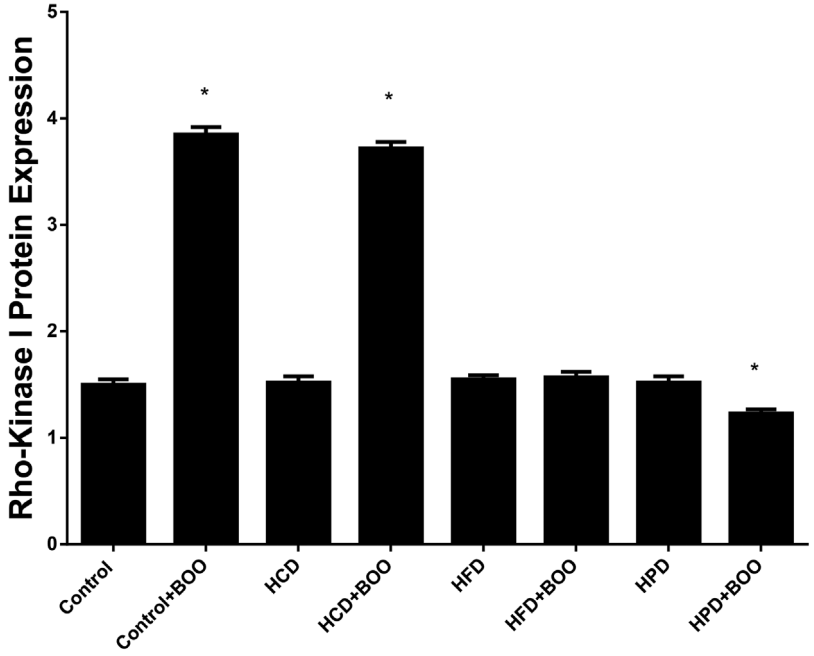

Fig. 6. Effects of diet-induced metabolic phenotypes on Rho-kinase I Protein expression in both unobstructed and obstructed rats' bladders. Values are mean ± SEM for ten 12-week old male animals per dietary group; $P < 0.05$.
* = significant in comparison with control.

and co-workers [21] also reported that ROK-I is overexpressed in BOO while ROK-II is unchanged, but Guven and his coworkers [22] reported that ROK-I decreases while ROK-II increases in expression in progression through the compensatory stage of BOO over time. The results of this study confirm the earlier reports on the expression of both Rho-kinase I and II by Bing and co-workers. The

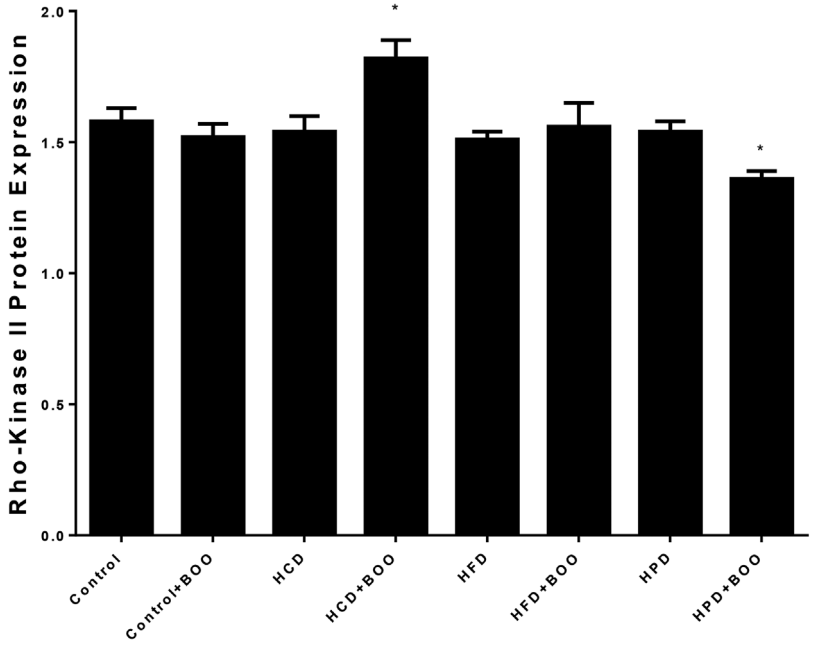

Fig. 7. Effects of diet-induced metabolic phenotypes on Rho-kinase II Protein expression in both unobstructed and obstructed rats' bladders. Values are mean ± SEM for ten 12-week old male animals per dietary group; $P < 0.05$.
* = significant in comparison with control.

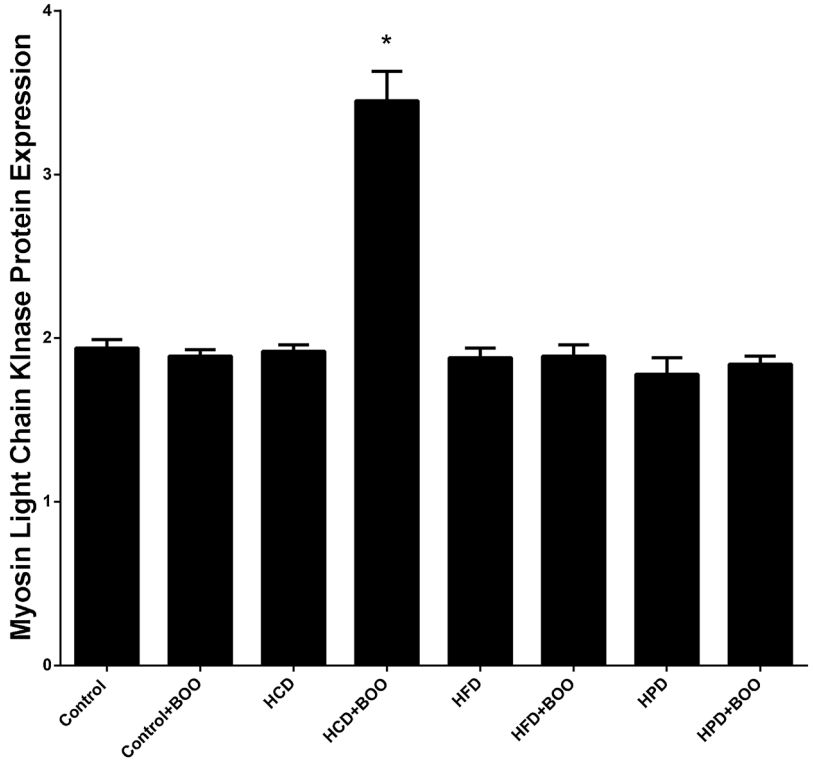

Fig. 8. Effects of diet-induced metabolic phenotypes on Myosin Light Chain Protein expression in both unobstructed and obstructed rats' bladders. Values are mean ± SEM for ten 12-week old male animals per dietary group; $P < 0.05$.
* = significant in comparison with control.

major signal responsible for activating the contractile apparatus in smooth muscle is an elevation of the intracellular calcium concentration. The increase in intracellular Ca^{2+} promotes its binding to calmodulin, which in turn activates the phosphorylation of myosin light chain (MLC) by myosin light chain kinase (MLCK), eventually resulting in smooth muscle contraction [23]. An increase in MLCK activity, as observed with HCD-feeding in this study, would result in increased detrusor contractile activity beyond normal (overactivity).

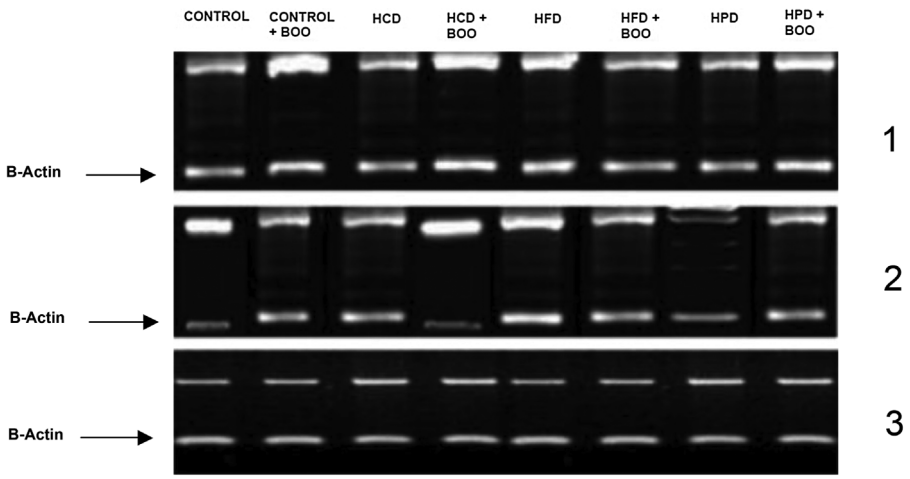

Fig. 9. Rho-Kinase I (1), Rho-Kinase II (2) and Myosin Light Chain Kinase (MLCK) (2) Expression respectively from PCR.

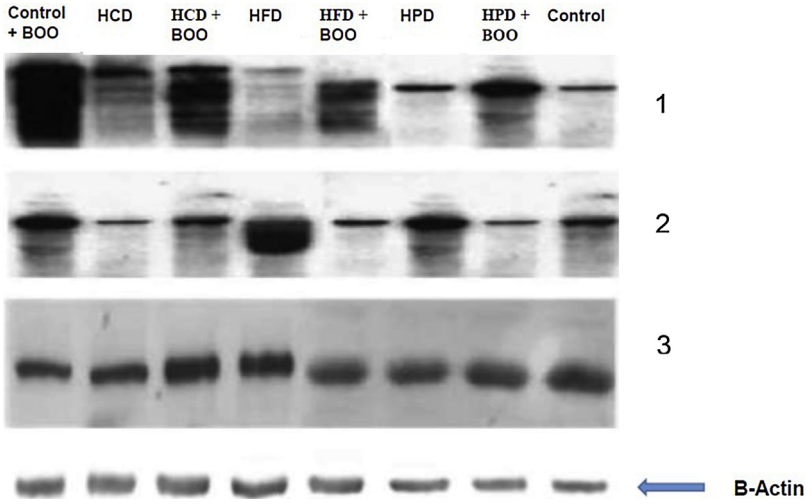

Fig. 10. Western Blots for Rho-Kinase I (1), Rho-Kinase II (2) and Myosin Light Chain Kinase (MLCK) (3) Expression respectively.

Secondary mechanisms have also been identified that can modulate contraction in smooth muscle independent of Ca^{2+}. Activation of excitatory G-protein coupled receptors results in contraction of smooth muscle without necessarily having an effect on intracellular Ca^{2+}, a process known as 'Ca^{2+}-sensitization'. The small GTPase Rho and one of its downstream effectors, Rho-associated kinase (Rho-kinase) have been shown to play important roles in this process. Activation of Rho-kinase causes phosphorylation, and therefore inactivation of smooth muscle myosin phosphatase, thereby preventing the dephosphorylation of MLC, leading to Ca^{2+}-sensitization of the smooth muscle [24]. The results from this study therefore suggest an important effect of HCD in animals with BOO. This diet caused an elevation in both MLCK and Rho kinase activity in obstructed bladders of these animals which would result in increased contractility and tone of their detrusor muscle. This would subsequently cause overactivity in these bladders, a direct effect of the compensation for obstruction and enhanced contractile response due to this compensation. High protein diet consumption in the animals however directly and indirectly affects smooth muscle compensatory contractile responses in BOO via their effects on both direct and indirect Ca^{2+} sensitization as has been described above.

In summary, dietary macronutrient content affects the bladder, especially in HFD where it enhances hypertrophy. Hypertrophy, with consequent smooth muscle/collagen imbalances, affects contractile responses of the bladder with high fat intake, even in unobstructed bladders. This is related to decreased myosin content of smooth muscle in spite of hypertrophy and hyperplasia. Consumption of HCD could however result in overactivity of the bladder as it enhances Ca^{2+} sensitization directly and indirectly through MLCK and Rho-kinase respectively, which would worsen LUTS in obstructed bladders. Further studies are indicated for confirmation of these mechanisms.

5. Conclusion

Dietary macronutrient content of food influences bladder function. Clinical management and treatment of bladder outlet obstruction might be improved if diet is taken into consideration during treatment. It is important to note that HFD consumption seems to have an adverse effect on the bladder even without obstruction, therefore limiting intake of fats might also decrease incidence of LUTS. Pathological bladder weight gain, similar to decompensation in BOO, reduces bladder function and compliance. Decreasing function, confirmed by decreased contractility of the detrusor muscle, would result in LUTS, causing underactivity and increased post-void residual urine volume. This would be important in obese individuals reporting with bladder dysfunction, as

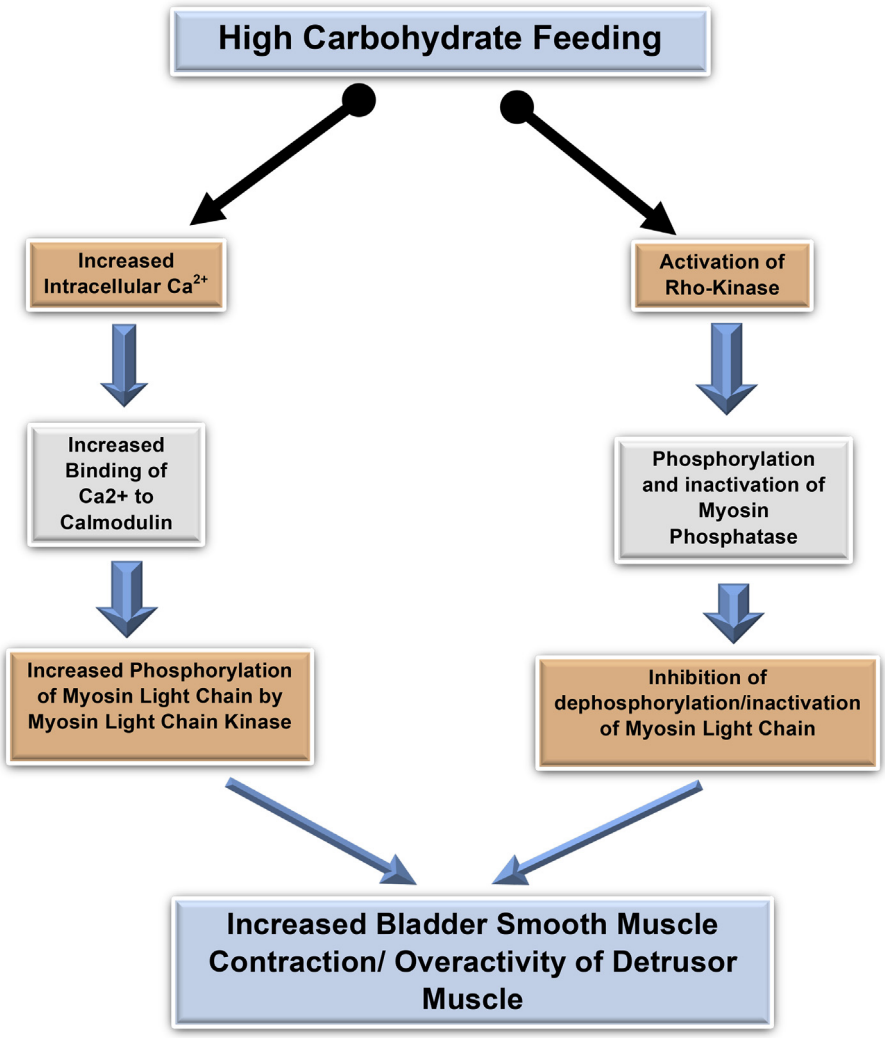

Fig. 11. Schematic showing relationship of diet with the Rho-Kinases and Myosin Light Chain Kinase.

these results suggest that a change in diet might have a beneficial effect in overall management. However, obese individuals taking a high-protein diet might also have increased severity of lower urinary tract symptoms in obstructed bladders.

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