N (G)-nitro-L-arginine methyl ester (L-NAME), a nitric oxide synthase inhibitor, diminishes oxidative damage in urinary bladder partial outlet obstruction

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Running title: L-NAME diminishes oxidative damage in partial bladder outlet obstruction

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ABSTRACT

Partial bladder outlet obstruction (PBOO) results in cellular damage due to ischemia and reperfusion injury. Our study seeks to establish how early this damage can occur, and the role that nitric oxide may play in its pathophysiology. Surgical partial bladder outlet obstructions (1, 3, and 7 days) were performed on male New Zealand white rabbits. Half of the animals were pre-medicated for 3 days with L-NAME, an inhibitor of nitric oxide synthase prior to obstruction. Bladder weight increased with duration of PBOO, but was significantly lower at 3 and 7 days in animals treated with L-NAME as compared to their untreated counterparts. Contractile function decreased progressively with PBOO duration. At 1-day post obstruction, bladder contractility was significantly lower in the L-NAME rabbits than in the untreated rabbits. At 3 and 7 days, contractility of the L-NAME bladders was equal or higher than the untreated bladders. The level of hypoxia at 1 day following obstruction was significantly higher in the L-NAME treated animals than in the untreated controls, but equal at 3 and 7 days obstruction. Increased nitrotyrosine was seen by Western blot in all obstructed animals. However, the amount was significantly less in the L-NAME treated animals at 3 and especially 7 days. Nerve density decreased progressively following obstruction; however, it decreased to a significantly lesser degree in the L-NAME treated bladders than in the untreated groups. These results suggest that L-NAME pretreatment enhanced ischemic damage at 1 day following obstruction; but protected the bladder from NO generated free radical damage at the later time periods by inhibiting the generation of nitrotyrosine.

Key Words: bladder outlet obstruction, ischemia- reperfusion, nitric oxide, rabbit
INTRODUCTION

Partial outlet obstruction of the urinary bladder is one of the major pathological effects of benign prostatic hyperplasia in men. It has been demonstrated that ischemia followed by reperfusion is an etiology for the progression of bladder dysfunction associated with partial bladder outlet obstruction in a variety of animal models (8, 2, 18). Cyclic episodes of ischemia/ reperfusion (I/R) lead to both direct ischemic damage, and the generation of free radicals, both reactive nitrogen species and reactive oxygen species (RNS, ROS), which result in membrane and sub-cellular organelle damage.

The initial structural response to partial outlet obstruction in the rabbit is a rapid remodeling of the bladder wall that includes urothelial and serosal hyperplasia and smooth muscle hypertrophy. It has been demonstrated that significant changes in blood flow occur within bladder tissue very early in this process. Lieb et al (14) demonstrated that, as early as one day following obstruction there is a significant increase in blood flow to the bladder, which is caused by NO-stimulated vascular relaxation. Schroeder et al (18) demonstrated that a decrease in blood flow resulting from I/R is correlated with decreased contractile function. In this study we hypothesize that ischemia and I/R are induced very early post obstruction, and that both processes may be related to nitric oxide (NO) and the generation of RNS and subsequent free radical damage.

Nitric oxide (NO) can be produced from exogenous sources, such as nitrovasodilators or endogenously from L-arginine by one of several isoforms of nitric oxide synthase. NO has been implicated as a nonadrenergic noncholinergic inhibitory neurotransmitter that mediates relaxation activity in the lower urinary tract (4, 1). It is
also known to be a powerful and ubiquitous regulator of vascular tone, and can lead to reperfusion-based injury. Nitric oxide related tissue injury is likely to be partially due to peroxynitrite, a relatively long lived, strong oxidant that is generated by the near diffusion limited reaction between nitric oxide and superoxide(11). A major product from the reaction of peroxynitrite with proteins is nitrotryosine (12). Using antibodies that specifically recognize nitrotyrosine, extensive protein nitration has been detected in human coronary artery atherosclerotic lesions (3) and in acute lung injury (10, 13). Nitrotyrosine has been demonstrated to be a marker of free radical damage due to reactive nitrogen species and will be used in our study as a marker of reperfusion-based injury.

To investigate further the role of I/R injury in bladder function in patients with BPH, we employ an animal model of New Zealand white male adult rabbits with surgical PBOO to investigate this theory. The location and severity of this damage as it evolves over time will be characterized in the immediate post-obstructive period using several modalities. It is likely that NO performs a key role in the pathophysiology of this process, and it is hypothesized here that substances that decrease reperfusion following ischemia will mitigate damage in this setting. L-Nitro-Arginine-Methyl-Ester (L-NAME), which acts as a competitive inhibitor of Nitric Oxide Synthase (NOS) due to its structural similarity to arginine - the substrate of NOS, may act as such an agent.

**MATERIALS AND METHODS**

**Surgical Procedure.** Young New Zealand white male rabbits (3-5kg; 15-20 weeks) were obtained from Milbrook Breeding Labs, Amherst, MA. Animals were
divided into two groups. One group was pre-medicated with L-NAME by implanting an
osmotic pump at an infusion rate of 6 mg/kg/day in the subcutaneous tissue behind the
rabbit’s neck, and allowing one week for equilibrium to reach adequate serum levels of
L-NAME and the infusion was continued during the entire experimental period.
The other group of animals received no pre-medication. For both groups, surgical
bladder outlet obstruction was performed under general anesthesia with
ketamine/xylazine. Animals were catheterized with an 8Fr Coude tip catheter prior to the
procedure. The bladder was identified, and a 3-O silk suture was tied around the
catheterized bladder neck with care taken to avoid damaging the vesical vessels. The
tension of the suture was standardized with all obstructions performed by the same
surgical team. Following the procedure, the catheter was removed. At one, three and
seven days postoperatively four animals from each group were euthanized with
pentobarbital. Two hours prior to euthanasia, 2 ml hypoxypen-1 (20mg/kg body
weight) was administered subcutaneously to the rabbits. The bladder was harvested from
each animal and placed in Tyrode’s solution (a physiological medium of dextrose,
calcium chloride, sodium bicarbonate, potassium chloride, magnesium chloride, and
sodium phosphate). The peri-vascular fat and connective tissues were removed and the
bladders weighed. Three full thickness bladder strips were obtained from the
supratrigonal dorsal aspect of each rabbit bladder for contractile studies. Two full-
thickness strips were isolated and fixed in formalin for histology and
immunohistochemistry. The balance of the bladder body was separated into muscle and
mucosa, flash frozen in liquid nitrogen and used for protein extraction and biochemical
assays. Non-obstructed control animals, both with (n=4) and without (n=4) exposure to L-NAME were euthanized and had tissue isolated for study in an analogous manner.

**Physiologic Studies.** Bladder strips were mounted in a 30ml physiologic medium of Tyrode’s solution with dextrose (1mg/ml) and gassed with 95% O₂, 5% CO₂ at 37°C. Two grams of tension was applied to each strip and the strip equilibrated for 15 minutes. The maximal response to electrical field stimulation (2, 8 and 32 Hz, 80V, 1ms duration, 15 seconds train) was determined. Strips were then exposed successively and independently to carbachol (20 μM) and potassium chloride (120mM) and the contractile response determined. Peak tension was recorded for each of the different stimuli. The concentrations in these experiments were based on the results of dose response studies showing that they produced maximal responses in normal and obstructed rabbit bladder strips. Between additions of pharmacological agents, each strip was washed three times with fresh buffer at 10 minutes intervals.

**Protein isolation and sodium dodecyl sulfate- polyacrylamide gel electrophoresis (SDS- PAGE) and western blotting.** Frozen bladder tissues (100 mg) were ground to a fine powder in a mortar cooled in liquid nitrogen and homogenized in buffer containing 20% glycerol, 50 mM Tris –HCl (pH 6.8), 0.5% (v/v) Tween- 20 and protease inhibitors (0.5mM PMSF, 2μM pepstatin, 2μM antipain and 0.1mg/ml trypsin inhibitor). After adding SDS (final concentration, 1%), the sample was boiled for 4 min, and centrifuged at 10,000 rpm for 15 min. Protein concentration in the supernatant was measured using the Pierce BCA protein assay kit. Equal amounts (20 μg) of total protein from control, obstructed and L-NAME treated rabbit bladders were loaded on 12% SDS – PAGE gels and transferred to Immobilon – P membranes with Towbin buffer [25mM Tris , 192 mM
glycine and 20% (v/v) methanol]. Membranes were blocked with 5% nonfat milk in 0.05% Tween-20 in PBS for 1h at room temperature and then incubated with primary antibody, monoclonal antibody to nitrotyrosine (Alexis Biochemicals, San Diego, CA). After treatment with the primary antibody, the membranes were washed with TBST buffer (20mM Tris, 500 mM NaCl, 0.05% Tween 20) and incubated with secondary antibody (goat anti-mouse Ig G at 1:10000). Substrates were visualized by using echochemiluminescence (Amersham Pharmacia Biotech, Buckinghamshire, England) and by exposing the membranes to autoradiographic films (Kodak X-O MAT). Band intensities were scanned and analyzed with a Kodak Image Station 440CF and a Kodak ID Image Analysis Software (Scientific Image System, Rochester, NY). Standard curves were constructed to establish that the protein concentrations used for analysis fell within the linear portion of the curves.

**Histologic Studies.** Full thickness specimens were cut from the bladder, placed in OCT compound (TBS, Durham, NC), snap frozen in liquid nitrogen and stored at -70C. Transverse sections 5 microns thick were then cut on a Microm HM505E cryostat at -25C and mounted on Bond Rite treated slides (Richard Allan Scientific, Kalamazoo, MI). The frozen sections were air dried for 1 hour, fixed in ice cold acetone for 10 min, then air dried again. Prior to staining the sections were soaked in 2 changes of PBS for 15 min each to remove the OCT. Another section of full thickness bladder tissue from each specimen was fixed in 10% neutral buffered formalin for 4-8 hrs, routinely processed and embedded in a paraffin block. Before embedding, the square section of tissue was sliced into 2-3 strips and each was embedded on edge to provide a cross-sectional view of bladder after microtome sectioning. Five-micron thick sections were cut from each block
and mounted on positively charged slides, deparaffinized in xylene, and used for the various immunostaining studies.

**Immunostaining.** Tissue sections were incubated with the mouse monoclonal antibody to nitrotyrosine and another set with hypoxyprobe primary antibody on the Ventana ES automated immunostainer (Ventana Medical Systems, Tucson, AZ, USA) using an avidin-biotin and dianinobenzidene (DAB) chromagin detection followed by a light hematoxylin counterstain. The detection kit from Ventana was used with the dilutions, times and temps set by the supplier, except for the following: Initial blocking done with serum-free protein block (DAKO, Carpinteria, CA,) for 12min. Nitrotyrosine primary antibody was applied for 1 hour room temp at a dilution of 1:50 and hypoxyprobe primary antibody at a dilution of 1:100 in antibody diluent (DAKO, Carpinteria, CA). The secondary antibody in the kit is substituted with biotinylated goat anti-mouse predilute (Lab Vision, Fremont CA). After immunostaining, all slides were dehydrated through graded alcohols and then mounted with Permount (Fisher Scientific, Pittsburgh, PA) mounting medium.

**Neurofilament staining:** Nerve damage is investigated through immunohistochemistry evaluating for the presence and density of neurofilaments within the axons of bladder nerves. Tissue sections were stained with the mouse monoclonal antibody anti-neurofilament clone 2f11 (Neomarkers, Fremont, CA) on the Ventana ES automated immunostainer (Ventana Medical Systems, Tucson, AZ) using an avidin-biotin and dianinobenzidine(DAB) chromagin detection followed by a light hematoxylin counterstain. 100µd of neurofilament primary antibody was applied for 32 min at a dilution of 1:20 in antibody diluent (DAKO, Carpinteria, CA). The secondary antibody
in the kit is substituted with biotinylated goat anti-mouse predilute (Lab Vision, Fremont, CA). After immunostaining, all slides were dehydrated through graded alcohols and 2 changes of xylene then mounted with Permount (Fisher Scientific, Pittsburgh, PA) mounting medium.

**Nerve calculations.** The nerve data including size and quantity was acquired from the stained cross sections using Image Pro Plus (Media Cybernetics, Silver Springs, MD) image analysis software. Images for analysis were acquired using a Spot CCD color digital camera linked to an Olympus BX-60 microscope. The transverse section of each specimen was captured in 4 frames at 100X magnification. The nerves in each image were highlighted using the Image Pro Plus software by manually selecting the pixel value within the stained nerves using the color cube based tool in the count/size application of the program. The area measurement was then selected for all the nerves highlighted and the data saved to Excel and later compiled and compared for all the groups.

**Statistical Analysis.** A student t-test was used to calculate difference between means in the contractile function and bladder weights obtained in this experiment. Statistical significance was judged by a p-value of less than 0.05.

**RESULTS**

There was no significant difference in bladder weight between those control animals pre-mediated with L-NAME and those not. There was a progressive increase in bladder weight among the obstructed animals. However, in those animals pre-mediated with L-NAME, there was a significantly smaller increase in bladder weight at both three and seven days of PBOO (Figure 1).
In general, there was a progressive (decrease in contractile response to all forms of stimulation with duration of obstruction. In regard to the contractile responses to field stimulation, for all frequencies of stimulation, after one day of obstruction, those animals treated with L-NAME had significantly poorer contractile function as compared with those with PBOO alone. This trend reversed at three and seven days of PBOO, with the L-NAME group contracting better than the PBOO alone group which shows significant difference at 32Hz (Figure 2). Similarly, for both carbachol and KCl (Figure 3) after one day of obstruction, those animals treated with L-NAME had significantly poorer contractile function as compared with those with PBOO alone. This trend reversed at three and seven days of PBOO, with the L-NAME group contracting better than the PBOO alone group.

There was a progressive and significant increase in the expression of nitrotyrosine over the 7 day period of obstruction. In contrast, at the 3 and 7 day time periods, the level of nitrotyrosine in the L-NAME treated obstructed rabbits decreased to control levels, providing strong evidence that NO-generated RNS is involved in the response to obstruction (Figures 4 A&B).

Immunohistochemistry also demonstrated increased expression of nitrotyrosine in obstructed rabbits. Again, there was significantly less staining of nitrotyrosine in L-NAME treated groups (Figure 5). The presence of nitrotyrosine in the PBOO rabbit bladders indicates that oxidants derived from nitric oxide such as peroxynitrite are generated during bladder outlet obstruction and may be involved in its pathogenesis. Using hypoxprobe-1, there was no evidence of hypoxia in the control groups. Following PBOO, at day one there was minimal staining in the urothelium only. After
three days, there was moderate staining in the urothelium as well as light staining in the vascular endothelium. At day seven, there was intense staining in the urothelium and vascular endothelium, as well as small amounts of hypoxia in the muscularis (Figure 6 A-D). When examining the samples from animals treated with L-NAME, differences arose at day one of obstruction; there was significantly greater staining of the urothelium as compared to those with PBOO alone, as well as significant vascular endothelial uptake. No differences were seen at days three and seven with L-NAME versus PBOO alone (Figure 6 E&F).

In the neurofilament studies, the control animal tissue demonstrated staining of nerve fibers of all sizes throughout the submucosa and muscularis. There were no changes seen following one day of obstruction. At three days, over half of small nerve tracts were absent but large nerve bundles were still present in similar number as controls. By day seven, there was no small nerve tract staining, and there was a small decrement in the number of large nerve tracts (Figure 7). At each time point, the nerve density of the L-NAME treated groups was significantly higher than their nontreated counterparts (Figure 8). Thus, treatment with L-NAME provided significant protection of the nerves, which are the most sensitive subcellular structures to ischemic and reperfusion damage.

DISCUSSION

In this study, we investigated the effects of L-NAME, a NOS inhibitor on the response to partial bladder outlet obstruction mediated injury in rabbits at 1, 3, and 7 days following obstruction. We found a striking time course of effect with L-NAME reducing
bladder function one day after obstruction, but enhancing bladder function at 3 and 7 days.

We found that L-NAME negatively affected bladder contractile properties and histological appearance one day after surgical obstruction. This has never been studied previously. It is well recognized that blood flow generally speaking is reduced after partial bladder obstruction. However, in the first day after obstruction, there is an NO stimulated increase in blood flow that is thought to mediate the maintenance of function and the bladder hypertrophy that begins at this time. This is confirmed by histological studies that demonstrate no significant hypoxia or neural injury. Our study uses L-NAME to block NO. Our findings of decreased function and increased ischemia in L-NAME treated rabbit confirm the previous findings that NO serves to help maintain function during the first day after obstruction. An alternative cause of bladder dysfunction 1 day post obstruction in L-NAME treated animals may be inducible NO synthase (iNOS) expression. By day 1 of PBOO, iNOS is not likely to be expressed and L-NAME will inhibit constitutive endothelial NO synthase(eNOS) and neuronal NO synthase(nNOS). This would decrease blood flow and exacerbate ischemia decreasing contractility as observed. The implications of our study are significant in that they give further confirmation that blood flow is a key mediator of function after partial bladder outlet obstruction.

We also found that L-NAME blockade of NO helped to prevent deterioration of bladder function 3 and 7 days after obstruction. This paradoxical finding has never been reported previously. However, our findings are entirely consistent in this area. Not only field stimulation (which reflects nerve function), but also carbachol and KCl (that reflect
muscarinic receptor stimulation and smooth muscle contraction) responses were all similar. In addition, our histological studies of nerve staining supports that nerves are more intact when L-NAME is used during obstruction. However, we believe that the explanation is found by studying the response to ischemia and reperfusion.

In the contractile studies, L-NAME significantly enhanced the contractile dysfunctions to all forms of stimulation at 1 day, but showed improved contractile responses in comparison to untreated obstructed bladders at 3 and 7 days. Previous studies (14) demonstrated that PBOO resulted in a significant NO-stimulated increase in blood flow at 1 day, which returned to control levels at 3, and 7 days post obstruction. This increase in blood flow would be expected to compensate for the ischemia mediated by the obstruction. In the L-NAME treated rabbits, this increase in blood flow would have been reduced or eliminated and can account for both the increased hypoxia observed at the 1 day period of obstruction, and the enhanced contractile dysfunctions observed at this time. However, the L-NAME would also be expected to reduce or eliminate the RNS generated by the increased NO with the resulting reduction in RNS-stimulated generation of nitrotyrosine and reduction in membrane damage. This can account for the improved contractile function at 3 and 7 days, and also the higher nerve densities at these time periods.

Partial bladder outlet obstruction causes an increase in bladder mass primarily due to smooth muscle hypertrophy and hyperplasia of the urothelium and interstitial fibroblasts (16, 6). The increased wall thickness and wall tension results in cyclical ischemia/reperfusion during and subsequent to each voiding contraction (8, 9). Cyclical I/R lead to an increase in free Ca\(^{2+}\) that activates Ca\(^{2+}\)-dependent hydrolytic enzymes
such as calpain, phospholipase A$_2$ and endonucleases. The reactive oxygen and nitrogen species generated subsequently result in characteristic damage to proteins, phospholipids and DNA that eventually disrupt cellular function.

We have shown that these I/R initiated events begin in transient hypoxic foci within the bladder wall during the bladder’s initial response to obstruction and that RNS generation would also result from this cyclical I/R. There may be several stimuli for NOS expression in response to bladder obstruction. Mechanical stretch is known to induce NOS expression. Hence the increased pressure toward the bladder that accompanies bladder outlet obstruction may cause production of NOS in detrusor smooth muscle cells. Oxidatively modified proteins have been used as early markers of oxidative stress in a variety of physiologic and pathophysiologic processes. Damage to proteins by reactive oxygen and nitrogen species affects the function of enzymes, receptors and signal transduction mechanisms. Nitrotyrosine, generated upon reaction of the reactive nitrogen species, (ONOO$^-$) with protein tyrosine residues was associated with neuronal ischemic injury and numerous neurodegenerative diseases. Antibodies specific to nitrotyrosine can measure this biomarker of oxidative stress. We have found an overexpression of nitrotyrosine in obstructed rabbit bladders when compared to the controls.

In conclusion, there is clear evidence of early cellular damage following PBOO. Hypoxia, which may be extrapolated to ischemia, occurs in bladder detrusor smooth muscle tissues causing severe damage to nerves, and the related contractile response to field stimulation. It has been shown that nitric oxide exerts a positive influence at one day of obstruction by increasing the blood flow to the bladder tissue, but subsequently
causes reperfusion based injury due to free radical damage. Pre treatment with L-NAME significantly enhanced the ischemic damage at 1 day of obstruction but significantly inhibited the generation of nitrotyrosine and resulted in a remarkable preservation of nerve density, which indicates that L-NAME reduces free radical damage associated with PBOO. Further studies will be directed towards proteomic methods, which can identify specific proteins nitrated in outlet obstruction and the complex effect of NO on biologic systems.
GRANTS

Supported in part by Yamaneuchi USA Research Foundation, the Office of Research and Development Medical Research Service, Department of Veteran’s Affairs, and by NIH grant RO-1-DK 067114.
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FIGURE LEGENDS

Fig. 1. Effect of partial outlet obstruction and premedication with L-NAME on bladder weight. Each bar is mean ± SEM of 4 animals. * = significantly different from control, x = significantly different from L-NAME, p< 0.05.

Fig. 2. Effect of partial outlet obstruction and premedication with L-NAME on contractile response to field stimulation (2, 8 and 32 Hz respectively). The bars represent mean plus or minus SEM of 4 control and 4 obstructed rabbits, either with or without pretreatment with L-NAME. * = significantly different from L-NAME treated groups, p< 0.05.

Fig. 3. Effect of partial outlet obstruction and premedication with L-NAME on contractile response to carbachol and KCl. The bars represent mean plus or minus SEM of 4 control and 4 obstructed rabbits, either with or without pretreatment with L-NAME. * = significantly different from L-NAME treated groups, p< 0.05.

Fig. 4. Western blots analyses of nitrotyrosine expression. A, equal amounts of total extractable proteins (20μg) from control, obstructed and obstructed premedicated with L-NAME rabbit bladder smooth muscles were separated by electrophoresis, transferred to membrane and probed with antibody specific to nitrotyrosine, as described. Lane 1, control which is normal rabbit without obstruction. Lane 2, 3 and 4, obstructed for 1 day, 3 days and 7 days respectively. Lanes 5, 6 and 7, obstructed for the representative days after giving L-NAME. Note the overexpression of nitrotyrosine in 7 days obstructed groups, the expression of which is downregulated in L-NAME treated groups. B, Average expression of nitrotyrosine in the different samples. Results are shown as plus or minus SEM in 4 rabbits. * = significantly different from control, x = significantly different from no treatment, p< 0.05.
Fig. 5. Rabbit bladder tissue sections immunostained with nitrotyrosine. A, control with diffuse staining throughout bladder detrusor smooth muscle (marked as m) B, 7 day obstructed rabbit bladder tissue section showing significant increase in expression of nitrotyrosine. C, L-NAME treated 7 day obstructed group showing staining similar in density to the control groups. All figures in 200X magnification.

Fig. 6. (A-F) Hypoxyprobe staining of the bladder tissue sections. A, control B, 1 day obstructed C, 3 days post obstruction D, 7 days post obstruction. These are from rabbits obstructed only without giving L-NAME. There was no observable hypoxia in control as well as 1 day of obstruction. Areas of focal hypoxia are seen in the 3 day obstructed groups(C). By 7 days, hypoxic sites were seen in the proliferated, thickened mucosa (black arrows) and in the smooth muscle compartment (blue arrows). E and F. Focal hypoxia following obstruction in L-NAME treated animals at 1 day after obstruction. Black arrows point to the vascular epithelium which is hypoxic. Blue arrows point to the hypoxic areas in the detrusor smooth muscle compartments. All figures in 100X magnification.

Fig. 7. Neurofilament images from control, 3 days and 7 days obstructed rabbit bladder tissue sections. A, The control animal bladder tissue section showed staining of nerve fibers of all sizes throughout the muscularis(black arrows). There were no noticeable changes in 1 day post obstruction and hence not shown. B, At three days, large nerve bundles were still present in similar numbers as controls (black arrows), while over half of the small nerve tracts were absent. C, At day seven, there were no nerve tract staining in the tissue sections. D, there was only one large nerve tract seen in the muscularis(black arrows). All figures in 200X magnification.
Fig. 8. Graph showing the protective effect of L-NAME in nerve damage occurring due to PBOO. The nerve count progressively declined over the 7 day period. At each time point, the nerve density of the L-NAME treated groups were significantly higher than their non treated counter parts. * = significantly different from L-NAME treated groups, p< 0.05.
L-NAME diminishes oxidative damage in partial bladder outlet obstruction

Fig. 1

![Bar chart showing bladder weight in non-treated and L-NAME treated groups over different days of obstruction.](image)

Fig. 2

![Bar chart showing contractile responses at different frequencies (2 Hz, 8 Hz, 32 Hz) in non-treated and L-NAME treated groups over different days of obstruction.](image)
L-NAME diminishes oxidative damage in partial bladder outlet obstruction

Fig. 3

Contractile Responses (gm)

![Graph showing contractile responses for different conditions.](image)

Control | Obstructed
--- | ---
Carbachol | KCl

Fig. 4

A. 1 2 3 4 5 6 7

![Image of a western blot.](image)

B. Optical Density (mOD)

![Bar chart showing optical density for different conditions.](image)
Fig. 5

Fig. 6
L-NAME diminishes oxidative damage in partial bladder outlet obstruction

Fig. 7

A

B

C

D

Fig. 8

Nerve Density (nerves/mm²)

Control 1 Day 3 Day 7 Day

Obstructed

No treatment L-NAME

*