

## Alterations in the contractile phenotype of the bladder: lessons for understanding physiological and pathological remodelling of smooth muscle

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- Introduction
- Alterations in bladder contractility with normal development
- Assessment of bladder function in genetically modified mice
- A whole organ perspective on the bladder's response to pBOO
  - Clinical observations
- Experimental observations
- *In vivo* measures of pBOO in a rabbit model
- Relating muscle strip studies to whole organ function
- Myosin light chain phosphorylation
- Regulation of myosin light chain de-phosphorylation
- Alterations in the thick contractile filaments
  - SM1 and SM2 isoforms
  - SM-B and SM-A isoforms
- Regulatory light chain
- Alterations in the thin contractile filaments
- Alterations in tension transfer complex

### Abstract

The contractile properties of the urinary bladder are changed by the conditions of normal development and partial bladder outlet obstruction. This change in the contractile phenotype is accompanied by changes in the regulatory cascades and filaments that regulate contractility. This review focuses on such changes during the course of normal development and in response to obstruction. Our goal is to discuss the experimental evidence that has accumulated from work in animal models and correlate these findings with the human voiding phenotype.

**Keywords:** bladder • outlet obstruction • myosin • smooth muscle • hypertrophy • remodelling

### Introduction

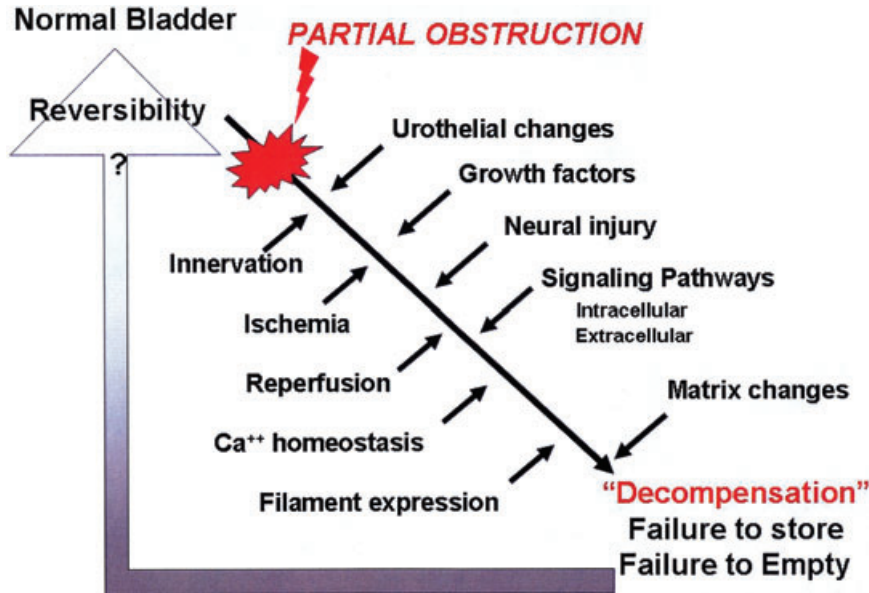
The urinary bladder serves to store urine at low pressures, and then in the appropriate time and place and under the coordinating control of the central and peripheral nervous system empty its contents to near completion. To effectively expel 95% of its stored volume, this system must be able to (1) generate intravesical pressure, (2) relax the bladder neck (a complex arrangement of smooth muscle under control of the autonomic nervous system) and (3) relax the striated external sphincter (which is under control of the somatic nervous system). Upon emptying its contents, the bladder then begins to slowly fill, a process that calls for suppression of the neural input that triggers contraction and a relaxation of the smooth muscle fibres during slow stretch to allow for low-pressure storage. Once the bladder has filled to its capacity, the

sensation of the need to void is triggered, and the voiding cycle is initiated again. When the lower urinary tract is functioning normally, the average adult will empty their bladder six to eight times per day and be capable of a 3- to 4-hr interval free of the need to void. This voiding cycle will be affected by the process of normal development, stress, aging and disease.

Patients will present with lower urinary tract symptoms (LUTS) that fall across a spectrum of severity such as urgency, frequent urination, nocturia or a slowing of the urinary stream; in some instances, these may progress to incontinence. The combined cost of caring for patients with these conditions in the United States alone has increased to over US\$ 10 billion annually [1]. The most common diseases affecting the lower urinary tract are

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**Fig. 1** A flow diagram demonstrating some of the proposed changes that may occur in the bladder in response to partial outlet obstruction that culminate in the 'decompensated bladder' as defined by a bladder that is non-compliant and fails to generate the power necessary to empty.

obstetrical trauma resulting in stress incontinence in women, and the obstruction that results from benign prostatic hyperplasia (BPH) in men. It is clear that a better understanding of the pathophysiology of the LUTS that arise secondary to partial bladder outlet obstruction (pBOO) or contribute to stress urinary incontinence would allow for implementation of better pharmacological approaches to management of these conditions.

The urinary bladder is composed of several distinct anatomic components and contains multiple cell types. The innermost layer or rathenium is an epithelial surface that rests on a basement membrane and serves as a barrier to prevent absorption of urine. Below this urothelial layer is found the lamina propria, a layer that is rich in capillaries as well as a heterogeneous cell population composed of fibroblasts, myofibroblasts and a smaller population of cells that stain positive for c-kit known as the interstitial cells of Cajal. Moving beyond this layer, one finds bundles of long and slender smooth muscle cells encased in sheaths of extracellular matrix. An outer serosal layer is also noted and is composed primarily of what are thought to be fibroblasts.

This microanatomic complexity allows the bladder to meet its functional needs, but it also makes the bladder a difficult organ to study in response to pathological conditions such as pBOO. It is clear that pBOO can affect multiple components of the bladder wall such as the microcirculation, extracellular matrix, nerves and muscle (Fig. 1). Studying the effects of pBOO on smooth muscle contractile function alone is challenging because of the confounding effects that are exerted by simultaneous changes taking place in the extracellular matrix and innervation.

It is important to define the scope and limitations of this review, which will focus on smooth muscle contractility in the bladder under normal and pathological conditions. Once force is generated by the intracellular contractile filaments, it must be transmitted to the cell membrane and in turn to the extracellular matrix. This force

transfer across the membrane to the extracellular matrix elements is of particular importance in smooth muscle, which must exert its forces within a hollow viscus. In contrast, force generated by skeletal muscle is transferred to bone by tendons. The tension transfer mechanism coupling actin filaments to a protein complex consisting of talin, vinculin, sarcoglycan and fibronectin is essential to normal force generation; diseases such as muscular dystrophy or hollow visceral myopathy which affect this complex will also exhibit smooth muscle pathophysiology [2, 3]. Because adenosine-5'-triphosphate (ATP) is required for the generation of force, mitochondria indirectly contribute to force generation. Deficiencies in mitochondrial function have been noted in experimental models of pBOO [4, 5], and patients with mitochondrial storage deficiencies may present with urinary retention [6]. It is important to understand these molecular changes, which ultimately affect the bladder's ability to generate power. Human urodynamic pressure flow studies show a loss of power in severe outlet obstruction. Although these patients are often capable of generating a significant intravesical pressure, the bladder smooth muscle loses its ability to shorten and does so at a much slower rate. The result is a loss of power ( $P_{max} \times \text{Volume/time}$ ), which ultimately results in an elevated post-void residual urine. This review will focus on the generation of active contractile force by the major intracellular filaments and those that terminate at the cell membrane.

It is also important to group the clinical conditions and their associated experimental models for the purpose of such a review. One benefit of studying normal development in bladder smooth muscle function is that there are significant shifts with aging that have been well-documented clinically. Documenting these developmental changes has clinical relevance, but also offers the opportunity to study a system free of the problems that are introduced by a surgically induced model of partial outlet obstruction. However, the majority of this review will focus on the response of

the bladder to partial outlet obstruction with a correlation between the human condition and the findings observed experimentally. In the past, the only experimental approach to this condition was the use of surgically induced partial obstruction. Although such models offer an opportunity to study bladder wall hypertrophy over a 2- to 4-week time frame that makes such a model economically viable, such an approach leads to multiple changes in all cell populations within the bladder wall making it difficult to tease apart all the confounding variables. In recent years, the use of transgenic, knockout and more recently inducible genetic variants such as Cre-Lox strains open up the possibility for experimental studies of bladder wall hypertrophy in the absence of surgical intervention.

## Alterations in bladder contractility with normal development

Clinical urologists have observed that voiding pressures are elevated at birth, and decline during the first year of life. This hypercontractility of the human detrusor was most pronounced in male infants, and in that subset of patients with vesicoureteral reflux [7]. As these patients were studied by 1 and 2 years of age to monitor them for resolution of their reflux, the voiding pressures were also observed to drop back towards normal levels. Normal male neonates also demonstrate elevated voiding pressures [8]. Male neonates with vesicoureteral reflux may void with pressures in excess of 150 cm H<sub>2</sub>O [9] and those without reflux voided with pressures in excess of 80 cm H<sub>2</sub>O. By age 2 these males were voiding with a pressure of 40 cm H<sub>2</sub>O, which remains stable until the onset of BPH in mid-life. The clinical relevance of this data is that such declines in voiding pressures were also correlated with the 'spontaneous' disappearance of the reflux. These clinical observations lead investigators to question whether similar changes might be taking place in experimental models of normal development.

Using an *in vitro* whole organ preparation operating under conditions, Keating *et al.* [10] demonstrated that bladders from 3-day-old and 1-week-old rabbits generated higher intravesical pressures under isometric conditions in response to electrical field stimulation as well as direct stimulation with bethanechol. These pressures declined such that there were minimal differences noted between the 4- and 8-week time points. It was also noted that in a Ca<sup>2+</sup> free buffer, neonatal bladder smooth muscle rapidly lost its contractility in contrast to that observed in mature bladder smooth muscle [11]. Using a Ca<sup>2+</sup> replenishment protocol, the neonatal bladder muscle achieved maximal force generation at much lower Ca<sup>2+</sup> concentrations and these findings were confirmed with FURA-2 measurements showing a rapid rise in intracellular cytosolic [Ca<sup>2+</sup>] in the neonatal as opposed to the mature bladder smooth muscle [11]. Probing for the voltage-operated calcium channel using the PN-200 radioligand revealed that there was no developmental shift in the expression of this calcium channel [12]. Further work using nifedipine and ryanodine as pharmacological probes suggested that one major difference in bladder smooth muscle contractility might be found in how Ca<sup>2+</sup> is handled by the sarcoplasmic retic-

ulum [13]. Significant up-regulation in two key components of the sarcoplasmic reticulum [the ryanodine channel and the Sarcoplasmic Endoplasmic Reticulum ATPase (SERCA)] has been identified during normal development of the urinary bladder [14, 15].

Developmental changes in bladder smooth muscle were also examined in a murine model. Wu *et al.* [16] were able to demonstrate that neonatal smooth muscle was capable of generating increased force when corrected for cross-sectional area when compared to the mature group. Newborn bladders generated significantly more tension in response to KCl (43.3 *versus* 17.4 mN/mm<sup>2</sup>) and bethanechol (40.6 *versus* 11.9 mN/mm<sup>2</sup>) and both groups were equally affected by application of the Rho-activated kinase (ROK) inhibitor Y-27632, suggesting that there was no developmental shift in the ROK-mediated modulation of contractile force. Developmental changes have also been reported in the expression of the smooth muscle myosin heavy chain isoforms SM1 and SM2, which arise from an alternative splicing mechanism near the carboxy terminus. These investigators did note that SM1 mRNA decreased from 60% at birth to 50% at 12 weeks and the expression of SM1 protein decreased from 72.5% at birth to 50% by 3 weeks and it remained stable at 12 weeks. During this time, the total myosin expressed per gram protein remained stable. The authors concluded that the SM1 myosin heavy chain isoform that is thought to contribute to the optimal assembly of myosin filaments may have a role in the decline in force production seen in the normal aging murine bladder. Developmental shifts from the SM1 to SM2 myosin heavy chain isoforms have been observed in rabbit bladder smooth muscle [17] and in vascular smooth muscle [18].

In a subsequent paper, Ekman *et al.* studied developmental changes in murine bladder muscle and observed a lower peak force was developed in the neonatal group as compared to the mature group [19]. Their work differed in that the bladder mucosa was stripped away from the mature bladders, although left intact in the neonates; this may well explain the difference noted with the paper by Wu *et al.* [16] in which the urothelium was left intact in both age groups. Another important difference is that Wu *et al.* corrected the force generated to account for the muscle fraction within the strips studied using histological cross sections. The work of Ekman *et al.* also demonstrated a shift in the sensitivity of the neonatal bladder smooth muscle to extracellular Ca<sup>2+</sup> repletion [19]; in findings analogous to those reported in rabbit bladder smooth muscle strips [11, 13], the neonatal murine smooth muscle generated higher forces at lower Ca<sup>2+</sup> concentrations. This enhanced sensitivity to extracellular Ca<sup>2+</sup> was also noted in the permeabilized fibre preparation leading these investigators to conclude that the sensitivity of the filaments to Ca<sup>2+</sup> might be altered as part of normal development. These authors also studied myosin light chain phosphorylation, which was increased in the neonates, and light chain dephosphorylation, which was diminished in this group relative to the mature bladder smooth muscle. The decreased rate of dephosphorylation in the neonatal bladders was associated with a lower expression of the phosphatase regulatory subunit MYPT-1 in newborn tissue [19], which could serve to explain the greater sensitivity of the filaments to Ca<sup>2+</sup> repletion.

## Assessment of bladder function in genetically modified mice

Within the past 15 years, the number of genetically modified mice available for study has proliferated rapidly, and a number of interesting voiding phenotypes have been described. One limitation of such an approach is that the mutation is often globally expressed right from the start of conception, which means that to be available for study such a mutation must not be embryonically lethal. The problem with embryonic lethality is potentially solved by using a Cre-lox strategy that allows for temporal activation of the mutation or inducing transgenic overexpression with a doxycycline sensitive promoter. Current promoters are available to drive Cre or Tetracycline Activator protein expression to the urothelium or smooth muscle of the bladder (although the current generation of smooth muscle promoters do not restrict expression to just the bladder). Such approaches also have their potential for confounding variables due to compensatory changes in gene expression. As an example, Burnett *et al.* described voiding dysfunction in the nNOS knockout mouse [20], in which the gene deletion was not targeted to the urinary tract [21]. Subsequently, Sutherland *et al.* pointed out these mice were also noted to be thirstier, and had a vigorous diuresis that they attributed to changes in the central nervous system [22]. Despite these limitations, genetically altered mice offer an opportunity to understand how a change in one gene may shift the voiding phenotype in the absence of surgical manipulation.

Another example of how anticipated findings from one model do not always translate to the genetically modified strain is seen in our studies of SERCA. In a rabbit model of partial outlet obstruction, we identified a decline in SERCA mRNA [23] and protein expression both by Western blotting and functional assays [24] in a manner analogous to that described in the cardiac literature for aortic stenosis. This down-regulation leads us to believe that we would identify functional changes in the SERCA2 knockout mouse bladder, which existed only in the heterozygous state (the homozygous recessive is embryonically lethal). In fact, our *in vitro* whole organ studies could not identify a difference in bladder function between the  $\pm$  and  $\pm$  genotypes [25] when studied in the absence of pBOO. This was similar to what was described by Periasamy *et al.* who noted that despite deletion of 50% of SERCA expression, peak cardiac pressures were unchanged, although there was a decline in the rate of pressure generation ( $dP/dt$ ) [26]. These investigators subsequently created a surgical model of partial aortic stenosis, and noted that the  $\pm$  mice were more likely to develop congestive heart failure [27]. In contrast, when we subjected the SERCA2 mice to pBOO, we noted that there was diminished bladder wall mass, improved *in vitro* whole organ function, a shift in myosin isoform expression back towards that seen in control tissues, less DNA synthesis, and less activation of the calcineurin pathway in the  $\pm$  group when compared to their  $+/+$  littermates [25]. Such a finding might be explained by the global nature of this mutation, which when acting over the life of the animal would be expected to lead to secondary compensatory

changes. Although transgenic models are very useful for the study of smooth muscle function, there is always a potential for confounding effects, and hence the warning that the findings are tissue- and cell-type specific [28].

Two separate strains of transgenic mice have been used to study the dynamics of SM1- and SM2-induced overexpression targeted primarily to bladder and aorta by use of the smooth muscle actin promoter [29]. Overexpression of the transgenic SM1 insert could be assessed by immunoblot probing for the additional flagging c-myc sequences contained within this transgene. The SM2 isoform was identified by a different V5 expression tag. Despite ample overexpression of these transgenic isoforms, which were readily identified by immunoblot analysis of their added flag markers, the total ratio of SM1/SM2 remained virtually unchanged. This would suggest that the smooth muscle cells within these tissues will put compensatory mechanisms into play to maintain this ratio. In addition, despite this transgenic overexpression of these isoforms, the total myosin heavy chain expression was not increased. Significant increases in the ability of the bladder to generate force were observed in the SM1 transgenic mice; in contrast, strips from the SM2 transgenic mice produced 20% less force. These investigators also reported substantial differences in the ability of the bladder smooth muscle from these transgenic mice to redevelop force after a quick release. The SM1 transgenic bladders had faster ( $1.8 \pm 0.3$  sec.), whereas the SM2 had slower ( $7.1 \pm 0.5$  sec.) rates of force redevelopment. These authors concluded that the carboxyl terminal isoforms of the myosin heavy chain may indeed impact upon the rate of force generated by smooth muscle, and that their expression is tightly regulated.

An alternative approach to the study of the role of the SM2 myosin heavy chain isoform was taken by Chi *et al.* [30], who succeeded in knocking out its expression thus leaving the mouse dependent upon only the SM1 isoform. Although the  $\pm$  mice were phenotypically normal, the  $-/-$  mice died within 30 days of birth with the phenotype showing marked dilation of the GI tract, bladder distention and significant dilation of the upper urinary tract. Despite the deletion of SM2, there was also a concomitant loss of SM1 expression and this in turn affected the assembly of the thick filaments. It is of note that again in a genetic model, there appears to be tight control over the SM1 and SM2 ratio. Despite these changes in the SM1 and SM2 isoforms, urinary bladder strips from the SM2  $-/-$  mice developed an increased contraction in response to KCl depolarization or M3 receptor stimulation with carbachol when compared to their wild-type littermates. There were no differences noted between the genotypes as to the degree of phosphorylation of the regulatory light chain (MLC<sub>20</sub>). It is intriguing that the loss of the SM2 isoform accompanied by the down-regulation of the SM1 isoform leads to bladder smooth muscle with hypercontractility. This would suggest that under normal conditions, SM1 and SM2 actually serve to negatively modulate force development. Although these *in vitro* assays focused on peak force generation, it may well be that what SM1 and SM2 allow for is a more robust thick filament assembly, and an enhanced ability to maintain long-term sustained muscle tone which in turn would be a critical property for any of these hollow viscera.

Non-muscle myosin isoforms can also play a role in force generation in bladder smooth muscle as was shown by Lamounier-Zepter *et al.* [31] in a neonatal mouse strain with a homozygous deletion of smooth muscle myosin heavy chain. The bladder smooth muscle from the  $-/-$  genotypes was shown to be capable of slowly responding to KCl by generating a tonic contraction. In contrast, the  $+/+$  genotypes responded to KCl stimulation with both phasic and tonic contractions. An inhibitor of MLCK was tested, and found to diminish the phasic contraction in the  $+/+$  genotypes but had no effect in the  $-/-$  strain; this leads the authors to suggest that the initial phasic contraction was highly dependent upon KCl induced rises in  $Ca^{2+}$  triggering the MLCK smooth muscle myosin heavy chain pathway. Both  $+/+$  and  $-/-$  strains of bladder smooth muscle responded to PDBu stimulation which suggested a role for protein kinase C triggering a contraction *via* the non-muscle myosin heavy chain filaments. Pre-incubation of these strips with the PKC inhibitor Ro-32-0432 completely abolished these tonic contractions in both genotypes.

Smooth muscle myosin heavy chain also has two additional isoforms SMA and SMB, which arise from an alternative splicing mechanism near the amino terminus. The B isoform that predominates in normal bladder is associated with a higher velocity of contraction, which can be correlated with its higher rate of ATP consumption. The slower B isoform makes its appearance following bladder outlet obstruction, which results in severe hypertrophy. The role of the N-terminal isoform shift has also been assessed using the approach of targeted gene deletion [32]. Babu *et al.* succeeded in deleting exon-5B (specific for SM-B) in the gene for the heavy chain of smooth muscle myosin and were able to show a complete loss of the SM-B myosin isoform. This smooth muscle was left entirely dependent upon the SM-A isoform; furthermore, there were no associated shifts in the carboxy terminal SM1 and SM2 myosin isoforms. These investigators noted significant declines in the peak force generated by bladder and mesenteric vascular smooth muscle, as well as highly significant declines in the rate at which tension is re-established following a quick release of 5%. Hypolite *et al.* using this same strain of mice demonstrated that the bladders from the SM-B  $-/-$  weighed more, and demonstrated a higher level of PKC- $\alpha$  as well as increased levels of CPI-17, which would help to explain the higher levels of myosin light chain phosphorylation and force generation [33]. The difference reported in force generation between these two studies is probably explained by the method of stimulation applied to the tissues. In the paper by Babu *et al.*, a dose-response curve with KCl was utilized; whereas in the study by Hypolite *et al.*, the tissues were challenged with a high KCl Tyrodes buffer. These findings in a pure model of gene deletion become significant as we describe the contractile phenotype that results from murine pBOO.

Murine genetic manipulations have also been utilized in the study of the role played by Myosin Light Chain Kinase (Mlck) in the initiation and modulation of smooth muscle contractility. Given the central role of this enzyme, the strategy utilized was to generate Mlck floxed mice in which exons 23–25 were flanked by loxP sites [34]. This mutation was then targeted to smooth mus-

cle by crossing the floxed mice with a smooth muscle directed Cre mouse; the mutation was then induced by treating the offspring with tamoxifen to induce the deletion at specific time points. Mice receiving tamoxifen died within 17 days of induction of the mutation. In this study, which focused on gut, the major phenotypic alteration was severe intestinal dysmotility with a dilation of the digestive tract and a reduction in food intake and faecal output. Isolated muscle strips showed a loss of force development and a diminished phosphorylation of the myosin regulatory light chain in response to either KCl induced depolarization or direct agonist stimulation with acetylcholine. The authors noted that there was also a markedly abnormal and distended bladder observed in these mice as well as changes in their voiding patterns. Although these results are not necessarily surprising, they serve to confirm the concept that MLCK represents a central step in the pathway to force generation in smooth muscle that cannot be bypassed; whereas, other pathways to force contraction may emerge following outlet obstruction, they cannot replace this key step in the cascade. This murine model is also analogous to the hollow visceral myopathy syndrome, which also proves to be fatal.

This same group also used transgenic methods to further delineate the time course of the transients and activation of the CaM-induced activation of Mlck [35]. This was accomplished by inserting two fluorescent markers within the catalytic site which when activated, resulted in a physical separation of these two markers. The resulting conformational change was then measured by shifts in fluorescence signalling, that is the fluorescence ratio energy transfer (FRET). This approach leads to several observations: (1) with stimulation there was a rapid rise in measured cytosolic  $Ca^{2+}$  and an initial rise in the FRET unaccompanied by a change in force; (2) force production lagged but ultimately rose to expected levels. In a recent publication focused on further studying, this time course in response to electrical field stimulation using bladder smooth muscle strips, these authors report a latency for the onset of a rise in  $[Ca^{2+}]_i$  of 55 msec. and of a change in FRET of 65 msec. [36]. The absolute value of these measures rose again as the regulatory light chain was phosphorylated and force was developed. The latencies for RLC phosphorylation and force generation were 100 and 109 msec., respectively. The authors felt that the delayed temporal response between RLC phosphorylation and the actual rise in force production is a reflection of the inertia that must be overcome due to elastic elements within the tissues. These investigators also noted that there were no changes in the phosphorylation of the two Mlck regulatory proteins MYPT1 and CPI-17 or of paxillin, a membrane bound protein involved in tension transfer. The authors concluded that neural stimulation leads to a rapid rise in cell  $[Ca^{2+}]_i$ , Mlck activation and RLC phosphorylation which suggest that 'a tightly coupled  $[Ca^{2+}]_i$  signaling complex serves as the main mechanism leading to contraction' [36]. It is essential to keep in mind that these elegant studies have been carried out in transgenic mice in the absence of pBOO; as we shall describe in our next section, the role of MYPT1 and CPI-17 are altered by the obstructive phenotype.

# A whole organ perspective on the bladder's response to pBOO

## Clinical observations

In response to pBOO, the human bladder can adapt by undergoing compensatory hypertrophy defined as a bladder that has developed increased mass [37], wall thickness and generates higher pressures to overcome the increased outlet resistance with a non-existent or minimal post-void residual urine. The most common form of outlet obstruction remains BPH; this slow growth of the prostate over many years leads to a gradual narrowing of the urethral lumen. Although the process of BPH is silent, the symptoms of this condition are primarily those affecting the bladder. These patients present with voiding symptoms, which include urgency, frequency, slowing of the urinary stream, straining to void and nocturia all of which ultimately affect the patient's quality of life. So long as there is a good compensatory response by the bladder, a patient can have a nearly symptom-free existence. However, when the demands outstrip the bladder's adaptive response, contractile performance becomes less efficient, urinary frequency increases, voided volumes drop and the bladder becomes dilated with resulting elevated post-void residuals. Sullivan and Yalla described the concept of the detrusor reserve that they established using detailed urodynamic studies on elderly male patients with BPH [38]. In making this measurement, they placed a catheter within the bladder lumen, and measured the peak-voiding pressure with a balloon inflated to occlude the bladder outlet—this in effect defined the peak isometric pressure. Then they deflated the balloon and measured the peak pressure as the patients voided around the catheter. They defined the detrusor reserve pressure as the difference between the maximal pressure generated under isovolemic conditions and the maximal pressure generated during voiding. The higher the detrusor reserve, the greater was the likelihood that the patients would void to completion with a minimal residual urine. However, as the detrusor reserve pressure dropped, the post-void residual increased. As the residual urine increases, resting storage pressures increase, and this eventually leads to the development of hydronephrosis, which if left untreated can lead to renal compromise. Such a situation is highly analogous to the cardiac response to aortic stenosis in which the myocardium first undergoes hypertrophy and, despite wall thickening, pumps effectively with a near normal ejection fraction. However, over time the ventricle dilates, the ejection fraction diminishes and the heart functions less efficiently resulting in congestive heart failure [39–41].

In general if these patients present early on in their course of illness, medical intervention can alleviate the symptoms. At present this might be in the form of  $\alpha$ -blockers to diminish prostatic resistance, but the pharmacological options for these patients remain limited. For those with more advanced obstruction, surgery often relieves the symptoms; however, up to 30% of patients will have persisting complaints of LUTS, despite successful surgical correction of the anatomic obstruction [42, 43]. Finally it must

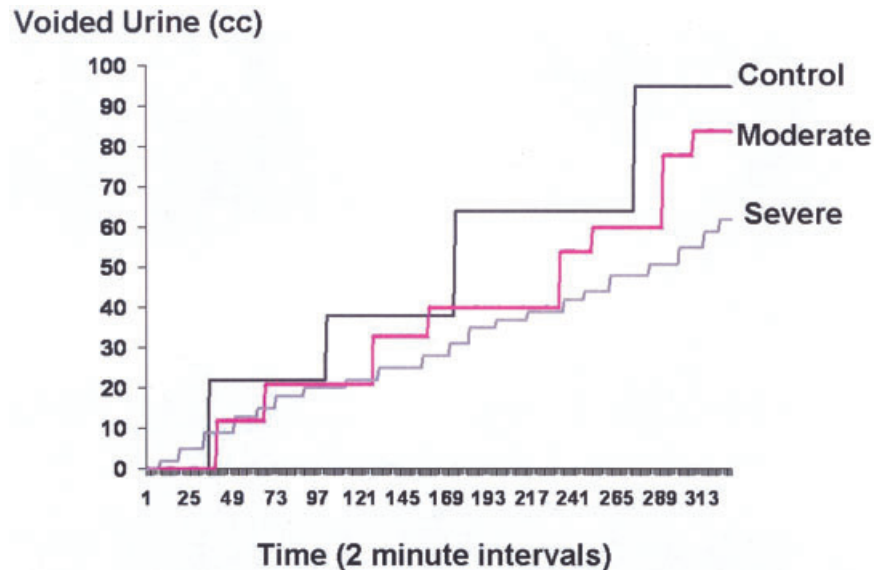
also be noted that bladder wall hypertrophy also develops in patients with neurogenic bladders secondary to either spinal cord injury or spina bifida. This is due to the presence of striated sphincter dysinergia, which results in bladders that empty under high pressures, and begin to develop an increased post-void residual. The progressive bladder wall fibrosis that develops, places the upper tracts and kidneys at risk. For this group of high-risk patients, new therapies are needed that target the pathways we will discuss in this review.

## Experimental observations

Numerous experimental models of pBOO have been developed over the past 40 years in multiple species each of which has a unique set of advantages and limitations. The most common model in use for many years has utilized the surgical induction of pBOO in the New Zealand white rabbit [23, 44, 45]. Its benefits include a larger size that allows for technical ease in performing the surgery to create pBOO as well as its surgical reversal. The rabbit model is limited by its lack of genetic variants. In contrast, the mouse model offers a growing number of transgenic and knockout strains for study [25, 46], and allows for a well-established genomic platform to allow for microarray studies of gene expression [47]. The primary disadvantage of the murine model of pBOO is the steep learning curve needed to master the microsurgical procedures used. The large size of a single rabbit bladder allows for efficient distribution of tissues from the same obstruction to several investigators making this an effective model for multidisciplinary research teams. In the end however, what investigators wish for in any experimental model of pBOO is to try and recapitulate the human condition. Thus, successful pBOO in an animal model should result in urinary frequency, diminished voided volumes and increased post-void residual urine. In the end, all findings made in muscle strips or even single cells should be able to relate back to the whole organ system and explain the fundamental steps that lead to failure to empty.

Much has been written about the use of cell culture as a means to study smooth muscle systems, but this must be tempered by the realization that once *in vitro*, a phenotypic shift or de-differentiation occurs in these cells. As an example, one can identify clear-cut changes in the quantity as well as the isoform expression of bladder smooth muscle myosin heavy chain gene expression [48]. This instability in myosin heavy chain isoform expression leads these investigators to develop a cell line with a stable expression profile of the myosin heavy chains [49], which may then be used to answer a focused question as we will demonstrate later on. In summary, the use of cultured cells offers the benefit of a more 'homogeneous' cell population in which to test a specific hypothesis. The application of this technique will be highly dependent upon the preservation of expression of the genes and proteins of interest once the cells are placed in culture, and as of now cannot fully replace the use of a live animal model.

**Fig. 2** *In vivo* voiding pattern analysis over a 12-hr period showing the normal voiding pattern compared to that seen with moderate and severe hypertrophy. Note that severe bladder hypertrophy is characterized by frequent urination of small volumes.



## ***In vivo* measures of pBOO in a rabbit model**

The consequences of pBOO have been well-characterized in the rabbit model of pBOO using voiding pattern analysis, and *in vivo* videourodynamics. Placing the animal in a metabolism cage with a digital scale, which read at 2-min. interval, offers a means to non-invasively assess the voiding phenotype following pBOO. pBOO consistently leads to increase in voiding frequency as shown in Figure 2, and these parameters correlated nicely with the gain in bladder mass.

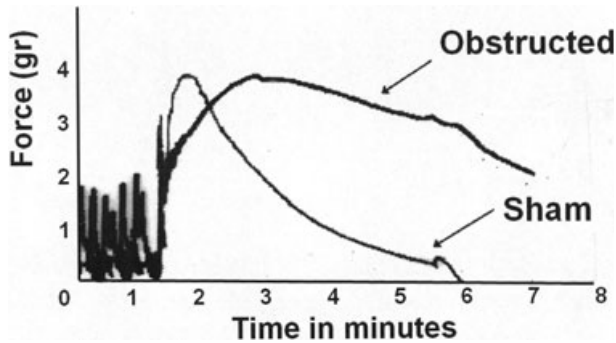
Using this same rabbit model, Stein *et al.* correlated the non-invasive voiding pattern data with an *in vivo* videourodynamic assessment of bladder function after pBOO [50]. Any rise in bladder pressure was monitored by fluoroscopy to confirm whether the rabbit was voiding. Fluoroscopic monitoring also allowed for an accurate determination of the post-void residual urine. Voiding pressures increased following pBOO, as would be expected given the increase in urethral resistance in a manner analogous to that described by Sullivan and Yalla [38]. As bladder mass increased, the voiding pressures also increased, but at the expense of a very large increase in post-void residual urine which was very accurately determined by aspiration with fluoroscopic confirmation. Unlike the human study of Sullivan and Yalla, we were unable to occlude the outlet in the rabbit to determine detrusor reserve. It must be pointed out that in a subset of bladders with the greatest increase in bladder mass and an elevated baseline cystometry pressure immediately prior to voiding, the sum of the residual and voided urine exceeded the infused volume. This is an experimental manifestation of a clinically observed phenomenon in patients with posterior urethral valves [51, 52] and is felt to reflect a post-obstructive diuresis, which

in turn suggests that pBOO in this experimental model can lead to similar upper urinary tract changes.

In summary, these data suggest that despite obvious limitations of such an acute model, surgically induced pBOO can recapitulate over a shorter time frame the following essential findings observed in human beings with pBOO: (1) an increase in bladder mass, (2) an increase in voiding frequency, (3) a drop in voided volumes, (4) increased voiding pressures, (5) a significant rise in post void residual urine in a subset of these bladders, (6) upon reversal of the outlet obstruction, residual urinary frequency was seen in 30% of the rabbits [53] and (7) evidence that at the extremes of pBOO, the upper tracts begin to sense increased resting bladder pressures as manifested by the post-obstructive diuresis.

## **Relating muscle strip studies to whole organ function**

If one is to relate the findings from experiments carried out using muscle strips to whole organ function, the study details must be examined closely. The most critical element is the strip size, and the presence or absence of the urothelium. A small strip may be isolated, and a segment of muscle bundles teased out to study in a high-end myograph. Such a strip will perform differently, which a larger strip harvested from the full thickness of the bladder wall. The traditional approach taken using such large strips often showed a loss of isometric tension following pBOO [24]. In contrast, smaller isolated fibres from this same population of bladders studied using a Guth myograph demonstrated normal force generation, but declines in velocity that could be measured in such a system [54]. It is important to note that there is often heterogeneity



**Fig. 3** Typical tracings obtained from muscle strips taken from a sham control bladder and a bladder following pBOO. The peak tension achieved is similar, but with pBOO the rate at which the tension is generated is much slower and the tension is sustained for a longer period and there is a rise in the phasic contractions in the absence of any stimulation.

within the bladder wall [55] and hence it can be useful to study several strips from different locations, if size allows. The most common tracings obtained under conventional isometric conditions show that although the peak tension achieved is similar for control and pBOO tissues, the tension is generated more slowly in the pBOO strips, and the force is maintained for a longer time frame (Fig. 3).

## Myosin light chain phosphorylation

For many years it has been accepted that a critical element in the initiation of smooth muscle contractile force is the phosphorylation of the 20 kD myosin light chain [56, 57]. The timing of this phosphorylation with respect to the rise in cell calcium and the conformational changes induced by the  $\text{Ca}^{2+}$ -calmodulin complex when it binds to myosin light chain kinase provide additional evidence in support of this pathway [35, 36]. This established relationship leads investigators to probe for activation of the myosin light chain phosphorylation in bladder smooth muscle following outlet obstruction. Studies by Cher *et al.* demonstrated that with pBOO there was no change in the peak values of myosin light chain phosphorylation induced by pBOO, although they did identify changes in the expression of the SM1/SM2 isoforms [58]. In addition, their data suggested that when corrected for total cross-sectional muscle area and strip size, there was no loss of force generation with pBOO, leading them to conclude that there were only minimal changes in the muscle itself, and that it was the deposition of extracellular matrix elements that changed the voiding phenotype. Their findings were also supported by Su *et al.* [53], who demonstrated that muscle strips from obstructed rabbit bladders demonstrated increased sensitivity and force generation in response to the cumulative addition of KCl or carbachol. The peak myosin phosphorylation between the two groups did not differ, but there was a substantial

rise in basal myosin light chain phosphorylation. The maximal velocity of contraction ( $V_{\text{max}}$ ) in response to KCl stimulation was 10-fold lower following pBOO. This would suggest that pBOO results in smooth muscle hypertrophy that allow for higher output of force as measured under isometric conditions, but at the expense of reduced cross-bridge cycling rates that lead to a lower velocity of contraction when the muscle strip is allowed to shorten. These findings are relevant because although the obstructed bladder muscle may be capable of generating force, it does so with less efficiency, and with a much slower velocity of contraction, with a resulting loss of power.

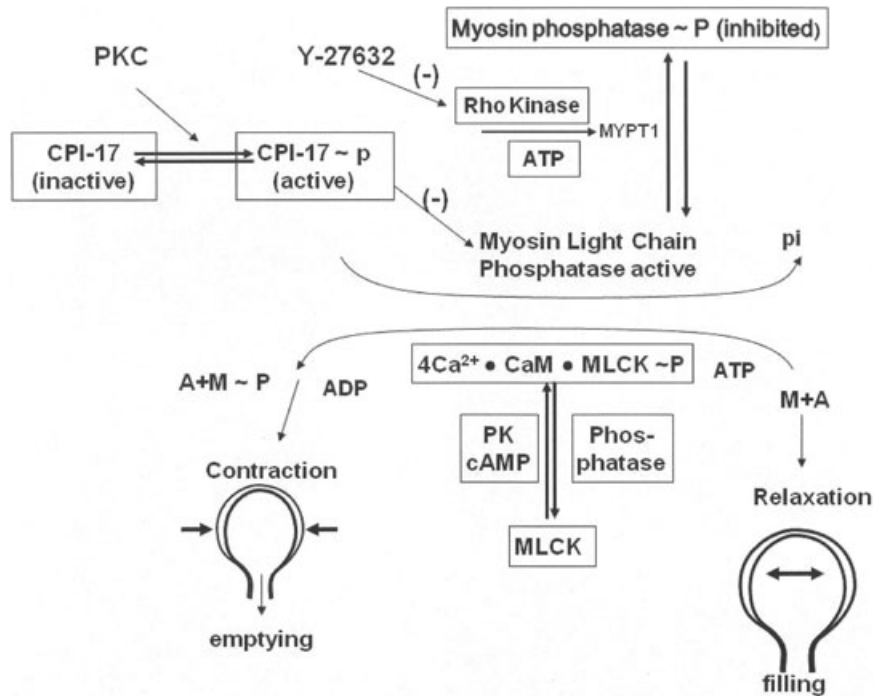
Stanton *et al.* using a skinned fibre preparation also demonstrated that there was no difference in peak force generated between the smooth muscle from control or pBOO groups [59]. In this preparation, there are also no differences in the degree of myosin light chain phosphorylation across the range of  $\text{Ca}^{2+}$  concentrations. However, there was a substantial difference in the  $\text{Ca}^{2+}$  dose-response curves noted, with the smooth muscle from the pBOO group requiring a substantially higher  $\text{Ca}^{2+}$  concentration to achieve similar force. The authors concluded that there was a substantial  $\text{Ca}^{2+}$  de-sensitization that occurred with the transition to bladder decompensation following pBOO. Although this  $\text{Ca}^{2+}$  de-sensitization mechanism can be demonstrated *in vitro* using the sophisticated analysis made possible by a skinned fibre preparation that allow for better control over individual variables, it is important to remember what might also be happening in the tissues *in vivo* where many changes are occurring simultaneously. Given the prior studies showing a loss of SERCA activity, protein expression and gene expression [23, 24], one might postulate that the loss of sarcoplasmic reticulum proteins that regulate cytosolic  $\text{Ca}^{2+}$  homeostasis are happening *in conjunction* with the  $\text{Ca}^{2+}$  de-sensitization of the filaments, which result in a less effective shortening of the muscle fibres and lead to decompensation as manifested by an increasing residual urine. Evidence suggests that with significant pBOO there is dysregulation of basal cytosolic  $\text{Ca}^{2+}$  as shown by the data suggesting activation of the calcineurin pathway.

## Regulation of myosin light chain de-phosphorylation

Initial studies of MLCK regulation focused on its activation by the  $\text{Ca}^{2+}$ -calmodulin complex; it is now apparent that a complex regulatory system of kinases and phosphatases controls the state of MLCK phosphorylation and activity, which we have summarized in a simplified form in Figure 4 [60]. Phosphorylated MLCK is dephosphorylated by an MLCK phosphatase, whose activity has been assayed in severe bladder wall hypertrophy and found to be diminished [61]. This low MLCK phosphatase activity is significant when one considers the shift from a phasic contraction in control tissue to a more tonic contraction seen in pBOO (Fig. 3). It can also explain the increase in the basal level of myosin light chain phosphorylation seen following pBOO [54].



**Fig. 4** The regulation of myosin light chain kinase and myosin light chain phosphatase. The action of phosphorylated CPI-17 serves to inhibit the activity of myosin light chain phosphatase activity. Similarly, the actions of Rho Kinase serve to phosphorylate the myosin light chain phosphatase rendering it inactive. This would explain why the addition of the Rho Kinase inhibitor Y-27632 would lead to a faster decline in tension in muscle strips pre-contracted with bethanechol. This pathway also offers an explanation for why PKC activation leads to an increase in force generation which it does indirectly by inhibiting myosin light chain phosphatase activity.



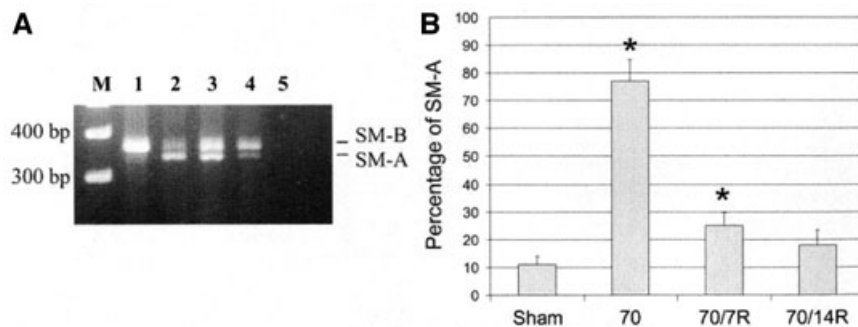
The small GTPase Rho-Kinase is capable of phosphorylating myosin light chain phosphatase, which serves to inhibit its action [62]. This in turn leads to increases in the amount of phosphorylated myosin light chain, resulting in prolonged actin myosin ATPase activity and force generation. In a study of pBOO using the rabbit model, Bing *et al.* [61] demonstrated that the ROK inhibitor Y-27632 increased the relaxation of pre-contracted muscle strips from pBOO *versus* sham bladders. These authors also demonstrated overexpression of the ROK- $\beta$  (but not the ROK- $\alpha$ ) isoform in pBOO leading them to conclude that the ROK-mediated pathway plays a role in the high degree of force maintenance and slow relaxation seen following pBOO. In contrast, Guven and Levin using a chronic model of rabbit pBOO found an increase in the expression of ROK- $\alpha$  and a minimal increase in ROK- $\beta$  [63]. Such differences in outcomes between these two research groups might be explained in part by the duration of pBOO as well as the use of metabolic monitoring of the void patterns, which allows for analysis of bladders that are deemed to be truly decompensated.

More recent studies by Wang and Moreland also suggest a role for Rho-kinase in phosphorylating myosin light chain phosphatase, thus leading to its inactivation [64]. Using phosphorylation, site-specific antibodies they were able to measure Thr(38)-CPI-17 and Thr(696)/Thr(850)-MYPT1 in response to carbachol stimulation. Phosphorylation of Thr(38)-CPI-17 rose with force in response to carbachol stimulation and was reduced by a PKC inhibitor. There was a high baseline phosphorylation of

Thr(696)/Thr(850)-MYPT1, but only Thr(696) phosphorylation increased during carbachol stimulation, and this increase could be eliminated by inhibitors of PKC or Rho kinase. These authors concluded that the changes in these pathways could account for the high degree of basal myosin light chain phosphorylation that is consistently observed following pBOO.

Chang and Chacko also suggested a role for the PKC pathway leading to diminished phosphorylation of CPI-17 in those bladders that were deemed to be decompensated [65]. This loss of phosphorylated CPI-17 would in turn lead to increased myosin light chain phosphatase activity which in turn would lead to less overall myosin light chain phosphorylation and force development. Immunostaining studies showed a co-localization of the phosphorylated CPI-17 and PKC in the smooth muscle and fewer signals were noted from those bladders, which were deemed to be decompensated. They concluded that the PKC pathway served to modulate bladder contractility under normal conditions as well as in cases of pBOO with compensated function; in contrast, the PKC regulatory pathway appeared to be impaired in those bladders with decompensated function.

In summary, data from several sets of experiments suggest that the elevated basal myosin light chain phosphorylation that is observed following pBOO may be related to a loss of myosin light chain kinase phosphatase activity as well as an increase in Rho-kinase activity. The loss of the PKC pathway following pBOO should also be placed in the context of the work cited earlier by



**Fig. 5** Analysis of myosin heavy chain (MHC) mRNA transcripts using RT-PCR. **(A)** Primers were used to amplify across the region of mRNA containing the MHC SM-A/SM-B alternative splice site. The expected sizes of the RT-PCR products were 338 bp for SM-A and 359 bp for SM-B. Lanes 1–4 were amplified from urinary bladder smooth muscle (SM) total RNA obtained from sham-operated (Sham), 7-day obstructed (70), 7-day obstructed/7-day reversed (70/7R) and 7-day obstructed/14-day reversed rabbits (70/14R), respectively. Lane 5: negative

control lacking RNA. Lane M: 100-bp DNA ladder run as a standard. **(B)** Bar graph showing the average expression of SM-A in the different samples. Values are means  $\pm$  S.E. obtained from separate determinations from at least five different rabbits. \* $P < 0.05$  compared with sham (from Ref. [67] with permission).

Lamounier-Zepter *et al.* [31], who demonstrated a role for the PKC pathway in maintain the tonic phase of bladder contraction; this tonic phase was abolished when strips were incubated with the PKC inhibitor Ro-32-0432. These experiments would suggest that following pBOO, the muscle adapts to its new demands by losing MLCK phosphatase activity, which would allow for an enhanced ability to maintain force during the tonic contraction. This benefit however is offset by the loss of PKC activity. Is it possible that in severe decompensation, induction of PKC activity could restore enough of the tonic contraction to enhance bladder emptying?

## Alterations in the thick contractile filaments

### SM1 and SM2 isoforms

Several studies show that the expression of the SM1 isoform of myosin heavy chain is increased over the SM2 isoform following pBOO [44, 46]. Using the rabbit model of pBOO, Wang *et al.* observed a rise in the expression of the SM1 isoform relative to that of the SM2 isoform using SDS-PAGE electrophoresis to characterize the protein expression [66]. Through the use of RT-PCR they demonstrated that the mRNA transcript for SM2 was decreased following pBOO. Following reversal of pBOO, the mRNA and protein expression for the SM1 and SM2 isoforms reverted back to normal ratios. Their data suggested that obstruction-induced hypertrophy resulted in a down-regulation of the pathways controlling expression of the myosin SM2 isoform. These authors also reported a decline in force generation that correlated with this shift in the myosin isoforms following pBOO.

These findings in the model of pBOO must be reconciled with the findings reported in the SM2 knockout mouse in which the SM2  $-/-$  mice developed an increased force in response to KCl depolarization or carbachol stimulation when compared to their

wild-type littermates with no differences noted in the phosphorylation of the myosin regulatory light chain. It may well be that the ratio of SM1 and SM2 allow for a more robust thick filament assembly, and an enhanced ability to maintain long-term sustained muscle tone which in turn would be a critical property for any of these hollow viscera. Under normal unobstructed conditions, the loss of these thick filaments would translate into better force generation as seen in the knockout. However, in response to obstruction, there is a net overall increase in the SM1 and SM2 isoforms and the thicker myosin heavy chain filament serves to negatively modulate force generation. It must of course be kept in mind that pBOO induces multiple shifts in the large and small contractile filaments, so the loss of force generation is also a reflection of these other changes as well.

### SM-B and SM-A isoforms

In bladders with pBOO, a major shift takes place in myosin heavy chain mRNA expression away from the fast B isoform seen in normal tissue to the much slower A isoform [67]. The importance of this observation is underscored by its reproducibility in several models of pBOO, including the mouse [25, 46] and rat. It is important to note that when the bladders are segregated into categories based upon the degree of hypertrophy and the *in vivo* voiding measures of void pattern analysis or videourodynamics, the greatest shift to the A isoform is seen in those bladders with the highest mass, and the highest post-void residual urine [67]. Furthermore, this shift to the A isoform reverted back to normal when the obstruction was reversed by removal of the ligature and the bladder was allowed a 2-week recovery (Fig. 5).

The loss of the SM-B and gain in the SM-A mRNA isoform must be correlated with the observed physiology in the pBOO model as well as with the SM-B knockout phenotype. Using a rabbit model of pBOO, Su *et al.* described an increase in the force and a 10-fold drop in the velocity of shortening in those bladders developing severe hypertrophy [54]. These findings also fit with the observations of Hypolite *et al.*, who described increased force

developing in the SM-B  $-/-$  knockout mouse along with an enlarged and overdistended bladder [33]. The drop in velocity reported by Su *et al.* [54] also match the differences in rates of force regeneration described in the quick release experiments by Babu *et al.* [32]. The application of *in vivo* cystometry to further probe for the effect of SM-B deletion will add additional insight as to the impact of this genotype on whole organ function. The clinical relevance of this preceding discussion is that although the obstructed bladder muscle may be capable of generating force, it does so with less efficiency, and a significantly slower velocity of contraction, with a loss of power that could be a major contributing factor to the rising post-void residual urine.

The loss of SM-B and the resulting shift towards bladder decompensation make this a potential therapeutic target. We began to suspect that one potential pathway to bladder decompensation might involve the calcineurin pathway originally described by Crabtree in lymphocytes [68]. The phosphatase calcineurin is activated by a slow and sustained rise in basal cytosolic calcium, which in turn triggers the de-phosphorylation of the Nuclear Factor of Activated T cells (NFAT). The de-phosphorylated NFAT then crosses the nuclear membrane to function as a transcription factor. Although originally described in lymphocytes, this pathway is in fact ubiquitous as a means of transducing subtle ligand, stretch or stress-induced changes in cell calcium into changes in gene expression. This pathway is of clinical relevance because calcineurin serves as the target for the immunosuppressive drugs Cyclosporine A (CSA) and FK-506. In a rabbit model of pBOO, we initially described a rise in atrial natriuretic factor (ANF) mRNA in the bladder wall which was in turn suppressed when the rabbits were treated with CSA [69]. In our early studies we relied upon ANF mRNA as a surrogate marker for activation of the calcineurin pathway as this has been well-described in the cardiac literature. We have also shown this in the rabbit model using gel shift assays. We have also demonstrated this to be the case in a murine model of pBOO also using gel shift assays [25] as well as an NFAT-luciferase reporter construct. In both the rabbit and murine models of pBOO, the administration of CSA in randomized trials lead to a diminished bladder mass and a shift away from the SM-A isoform back towards the more favourable SM-B isoform. In the model of pBOO in the SERCA2  $\pm$  mouse, the diminished expression of NFAT in the gel shift assays correlated with a predominance of the SM-B isoform mRNA [25].

This shifting of SM-A expression back towards the more favourable SM-B isoform mRNA by the administration of CSA is of mechanistic as well as translational interest. It has been shown that smooth muscle myosin heavy chain isoforms are the result of alternative splicing [70, 71]. Further evidence in favour of the alternative splicing hypothesis comes from single-cell PCR from stomach smooth muscle where both A and B isoform mRNA were expressed together [72]. These investigators noted that the percentage of the B isoform was not identical even within similar regions of the stomach. Such an approach reflects a sampling of the smooth muscle populations at a particular moment in time and

several questions can be raised. Is it possible that each smooth muscle myocyte has already been set to produce a certain ratio of the A and B isoforms, and that with pBOO, these different cell populations expand at differing rates? Or is it possible that in response to pBOO, bladder wall hypoxia is induced, which leads to a shift in individual cell splicing modulated by multiple transcription factors such as NFAT and Hypoxia Inducible Factor (HIF)? Another alternative possibility is that following pBOO, the rate of myosin heavy chain synthesis is increased, and the splicing is rate sensitive [73, 74].

## Regulatory light chain

It has also been shown that with pBOO, changes are induced in the regulatory light chain. With 7 days of pBOO, a significant rise in expression of mRNA coding for the MLC17<sub>b</sub> isoform was observed [67]. This shift partially reversed back towards normal, following a 14-day recovery period. The protein expression of MLC17<sub>b</sub> as determined by two-dimensional gel electrophoresis followed a similar trend over the same time frame.

## Alterations in the thin contractile filaments

In addition to these findings in myosin heavy chain isoform expression, alterations in the expression of the thin filaments caldesmon and calponin have been described following pBOO.

The role of caldesmon in smooth muscle function has been recently reviewed [75]. It is known to bind actin, myosin, tropomyosin, as well as calmodulin, and it inhibits the actin-myosin ATPase. It is currently thought of as a filament that binds to the major contractile filaments in a manner that acts as a brake. Once released from these filaments, the muscle shows an enhanced ability to generate force. Using the rabbit model of pBOO, Burkhard *et al.* noted a marked increase in the expression of the *l*-caldesmon [44] as did Zhang *et al.* [76]. A similar finding was noted in a rat model of pBOO [77]. In these studies it was noted that in normal bladder the *h*-caldesmon isoform predominates, but with pBOO there is a marked shift towards the *l*-caldesmon isoform, which was associated with a diminished velocity and efficiency of contraction. This leads Shukla *et al.* [78] to consider whether overexpression of the *h*-caldesmon isoform could restore the contractile phenotype. To answer this question, investigators relied upon a cell line established from smooth muscle isolated from a rabbit subjected to pBOO. This cell line was characterized by having a stable expression of myosin heavy chain isoforms [49] as well as a pre-ponderance of *l*-caldesmon. Using a cDNA transfection strategy, Shukla *et al.* created an overexpression

of *h*-caldesmon, which they were then able to correlate with alterations in filament assembly as well as an improved *in vitro* contractile phenotype as measured by the rate of cell contraction in response to agonist stimulation [78]. These authors concluded that the thin filament *h*-caldesmon serves to modulate the interaction between actin and myosin, and its restoration lead to improvements in cell morphology and function *in vitro*. Picking up from this theme, Deng *et al.* were able to show using siRNA techniques that the silencing of caldesmon expression lead to less incorporation of actin into the intermediate filament structures [79]. Following successful caldesmon silencing there were much fewer actin filaments surrounding myosin as determined by electron microscopy, suggesting that this protein plays an important role in the structure of the cytoskeleton. The regulation of caldesmon is complex as further illustrated by the findings in the SM-B knockout mouse in which caldesmon expression is decreased in bladder smooth muscle in the absence of any obstruction [80]. Further clarification of caldesmon's role in the regulation of bladder contractility will come as the voiding phenotype of this knockout mouse is characterized [81].

Other thin filament regulatory proteins are expressed in smooth muscle and serve to modulate the actin–myosin interaction and these include h1-calponin as well as  $\alpha$ -tropomyosin. The expression of these proteins at the mRNA and protein level has been demonstrated to increase following pBOO and these changes were localized to the detrusor smooth muscle myocytes using double immunofluorescence microscopy [82]. Matthews *et al.* studied the h1-calponin knockout mouse and noted that in bladder smooth muscle, there was no evidence for any changes in excitation contraction coupling, but there was a significant rise in the velocity of contraction in these knockout mice compared to their wild-type littermates [83]. Because these studies were also carried out in a permeabilized fibre preparation, it was possible for these investigators to add back exogenous calponin, and this in turn lead to a decline in velocity back to that seen in the wild-type mice. These authors also noted that the h1-calponin knockout mice had an associated 25–50% decline in  $\alpha$ -actin expression. Studies have been also performed in double knockout mice for SM-B and h1-calponin, which show an increased velocity of bladder contraction and peak force when compared to the SM-B knockout alone [84]. However, the extrapolation of these findings to the situation arising from pBOO alone is made more complicating by the fact that there is also a decline in smooth muscle  $\alpha$ -actin levels as well as a rise in *h*-caldesmon levels in these double knockout mice which must also contribute to the observed physiology. The complex interactions and tight regulation of this family of filaments will challenge investigators hoping to control one variable at a time in an attempt to tease out the 'pure effect' of a particular filament.

## Alterations in tension transfer complex

Much literature concerning the pathophysiology of bladder outlet obstruction has focused on the role of the extracellular matrix, which serves to restrict the muscle's contractile performance as well as the filaments and other intracellular machinery that leads to force generation. However it is clear that once force is generated by the filaments, an essential function of the cytoskeleton is to transfer this energy to the cell membrane and then out to the extracellular matrix. Evidence is accumulating that some of the damage following pBOO may be occurring at the level of the cell membrane junction where the contractile fibres end in a tension transfer complex that allows for force to be transmitted beyond the cell to effect shortening. This junctional complex has been best characterized in striated muscle for it is the large molecule dystrophin that is mutated in muscular dystrophy. However many of these same proteins from the sarcolemmal complex will also be identified in smooth muscle. The first molecule from this junctional complex to be studied within the urinary bladder following pBOO was beta-sarcoglycan, which serves to transfer tension generated by the intracellular contractile filaments to collagen type IV which envelops the outer wall of the cell membrane. Using a rabbit model of pBOO, Wei *et al.* demonstrated a rapid loss of expression of the beta and gamma sarcoglycans with a partial recovery following surgical reversal of the pBOO [85]. Using the same rabbit model of pBOO, Macarak *et al.* demonstrated immunohistochemical shifts in the structural relationship between the expression of beta-sarcoglycan and collagen type IV which suggested a significant disruption had taken place within the tension transfer apparatus had taken place [86]. It was noteworthy that these histological shifts could be correlated with the degree of bladder dysfunction observed after pBOO. Further studies using ultrastructural techniques to characterize the nature of this tension transfer apparatus as well as studies of pBOO in knockout mice will help to further clarify the role of this complex in the detrusor response to outlet obstruction.

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## Conflicts of interest

The authors confirm that there are no conflicts of interest.

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