CHARACTERIZING LOWER URINARY TRACT DYSFUNCTION
IN A PORCINE MODEL OF SPINAL CORD INJURY

by

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Abstract

Neurogenic lower urinary tract dysfunction after spinal cord injury results in severe morbidity and mortality. Consequences of neurogenic lower urinary tract dysfunction include urinary tract infections, incontinence, upper urinary tract deterioration, and reduced quality of life. Urodynamic studies are the current gold standard for characterizing neurogenic lower urinary tract dysfunction. Currently, there is a need for a large animal model of neurogenic lower urinary tract dysfunction for evaluation of the utility and safety of novel human-sized devices or treatments. In this thesis, the functional and morphologic changes of the bladder were characterized in a porcine model of spinal cord injury.

In Chapter 1, I provide background on spinal cord injury and the pathophysiology of neurogenic lower urinary tract dysfunction. I also discuss about the use of animal models for evaluation of human lower urinary tract diseases.

In Chapter 2, I pioneered a protocol to perform clinically relevant urodynamic studies in a porcine model of thoracic spinal cord injury. I identified that the pig’s lower urinary tract function is very similar to human bladder function before and after spinal cord injury.

In Chapter 3, I describe a protocol to implant radio telemetric devices into a porcine model to characterize physiologic bladder function and to evaluate the practicality of the system. I identified comparable detrusor pressure and external urethral sphincter activity recordings between the urodynamics and telemetry systems before and after spinal cord injury.
In Chapter 4, I evaluated the effects of chronic bladder drainage on the functional and histologic features of the bladder. I found high-risk urodynamic features in pigs that received chronic bladder drainage.

I have established a protocol to perform urodynamic studies in a porcine model of spinal cord injury to characterize neurogenic lower urinary tract dysfunction. The potential to perform repeated urodynamic studies in a single animal allows for investigation into the efficacy of treatment of therapies and devices over time. This scientific contribution will help bridge the gap between animal experimentation and human application for neurogenic lower urinary tract dysfunction after spinal cord injury.
Lay Summary

There is currently a need for a large animal model for the development and testing of novel human-sized devices that aim to improve urinary bladder function after spinal cord injury. Using a pig model of spinal cord injury, I have performed studies on the pig’s urinary bladder function to investigate if the pig’s urinary bladder function is similar to human bladder function after spinal cord injury. In my studies, I found similarities between human and pig urinary bladder function before and after spinal cord injury. In a parallel study, I demonstrated the use of implantable radio systems to wirelessly monitor the pig’s natural urinary bladder function from within their pens. With the establishment of large animal model of bladder dysfunction, we can take advantage of its size to evaluate the safety and utility of devices or therapies prior to application in humans.
Preface

In Chapter 1, I provide the background on normal lower urinary tract function and the consequences of spinal cord injury on lower urinary tract function. I also briefly discuss the use of rodent models of spinal cord injury to investigate neurogenic lower urinary tract dysfunction and the limitation with these animal models and why I characterized bladder dysfunction in a porcine model of spinal cord injury.

In Chapter 2, I conducted clinically relevant urodynamic studies to characterize the changes in the pig’s lower urinary tract function after spinal cord injury. I developed the protocol to perform urodynamic studies, sourced the equipment used and performed the experiments. Furthermore, I interpreted and analyzed the data, performed histologic quantification, and wrote the manuscript. A collaborative manuscript with Dr. Charles Hubscher, Dr. April Herrity and Dr. Max Boakye from the University of Louisville has been drafted and is currently being reviewed for publication at the time of thesis submission. The University of Louisville collaborators also performed urodynamic studies in pigs but with a lighter injury severity as well as without the use of procedural sedation during placement of the urodynamic catheters. Emily Deegan and Teresa Lim taught me the urodynamics procedure. Femke Streijger provided project supervision and edited the manuscript. Megan Webster, Shera Fisk, Nestor Chen, and Charlotte Morrison assisted with the experiments as well as performed behaviour training. Neda Manouchehri and Dr. Kim Kyoung-Tae performed the animal surgeries. Dr. Alex Kavanagh assisted with the interpretation of the urodynamic results, edited the manuscript, and advised on experimental design. Dr. Brian
Kwon and Dr. Lynn Stothers initiated the study, provided project support, and edited the manuscript.

In Chapter 3, I investigated the use of implantable telemetry systems by TSE systems (TSE Systems, Chesterfield, MO, USA) to remotely monitor the pig’s lower urinary tract function in a natural setting. I assisted with the design of the telemetry implants, performed telemetric recordings during urodynamic studies and also with the pigs in their pens, data analysis and interpretation, histologic evaluation, and wrote the manuscript. Femke Streijger provided project supervision and edited the manuscript. Megan Webster, Shera Fisk, Neda Manouchehri, Kitty So and Femke Streijger assisted with the design of the telemetry implants. Avril Billingsley, Alex Munro, Megan Webster, and Shera Fisk helped with telemetric recordings. Neda Manouchehri and Megan Webster performed the animal surgeries. Dr. Alex Kavanagh and Dr. Lynn Stothers assisted with the interpretation of the results, edited the manuscript, and advised on experimental design. Dr. Brian Kwon initiated the project, provided project support, and edited the manuscript.

In Chapter 4, I conducted investigations of the effect of varying lengths of bladder drainage on the spinal cord injured pig’s bladder urodynamic and histologic outcomes. Dr. Alex Kavanagh, Dr. Lynn Stothers, and I developed the idea. Kitty So designed the custom indwelling foley catheter. Shera Fisk trained the animals and prepared them for the experiments. Megan Webster, Shera Fisk, Nestor Chen, Alex Munro, and Avril Bilingsley assisted with the experiments. Neda Manouchehri performed the animal surgeries. Dr. Alex Kavanagh assisted with the interpretation of the results and advised on experimental design. Dr. Brian Kwon initiated the project and provided project support.
All animal experiments were conducted in accordance with the University of British Columbia Animal Care Committee (A16-0311). All procedures strictly adhere to the guidelines issued by the Canadian Council for Animal Care.
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List of Abbreviations

AD – autonomic dysreflexia
BD – bladder drainage
CIC – clean intermittent catheterization
cm H₂O – centimeters of water
CMG – cystometrogram
CNS – central nervous system
DLPP – detrusor leak point pressure
DSD – detrusor-sphincter dyssynergia
EUS – external urethral sphincter
Fr – French
H&E – hematoxylin and eosin
IPHFO – intraluminal pressure high frequency oscillations
IUS – internal urethral sphincter
LUT – lower urinary tract
ml/s – milliliters per second
NDO – neurogenic detrusor overactivity
NLUTD – neurogenic lower urinary tract dysfunction
Pabd – intraabdominal pressure
Pdet – detrusor pressure
Pdetopen – opening detrusor pressure
Pdet-Qmax – detrusor pressure at max flow
Pves – intravesical pressure
PVR – post-void residual
$Q_{max}$ – max urinary flow rate
SCI – spinal cord injury
UBC – University of British Columbia
UDS – urodynamic studies
UofL – University of Louisville
VCUG – voiding cystourethrography
Voided% – voided percentage
VV – voided volume
WPI – weeks post-injury
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Well, this concludes an important chapter of my life. The journey was arduous and filled with many challenges that I resented at the time. But looking back, I have grown so much from these experiences. Reminiscing of my time at the Kwon lab, I think one line can sum up what I have learned. *It is not what others say you are or who you are underneath, but it is what you do that defines you.*

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Dedication

This study is dedicated to all those who suffer from bladder dysfunction after spinal cord injury. I hope this tiny contribution of mine will help improve the quality of your lives in the very near future. To the next generation of scientific trailblazers and researchers, I hope you will be able use and further improve this model for posterity.
Chapter 1: Lower Urinary Tract Dysfunction after Spinal Cord Injury

1.1 Introduction

One of the most devastating physical impairments for a human to suffer is traumatic spinal cord injury (SCI). This sudden injury often results in profound disability with negative implications on all aspects of an individual’s life. It only takes one unfortunate and untimely accident to the spinal cord to flip a person’s entire life completely upside down.

On a global scale, the prevalence of traumatic SCI ranges from 280 to 906/million \(^1\). In Canada alone, approximately 86,000 individuals are living with SCI and this number is steadily increasing as there are 4,300 new cases of SCI each year \(^2\). These numbers are projected to increase to 121,000 individuals and 5,800 new cases each year by the year 2030 \(^2\). The most frequent cause of SCI in the younger population (age 20-29) is trauma from motor vehicle accidents \(^3,4\). In the senior population (age 65 and older), a majority of traumatic SCI results from falls \(^3,4\).

These individuals not only suffer from a loss of important bodily functions, but they also have to endure the emotional consequences of SCI such as depression, anxiety, despair, bitterness, and grief, all of which reduce quality of life \(^5\). In addition to these deficits, the economic burdens of SCI are enormous. It has been estimated in Canada that the lifetime economic burden per individual with traumatic SCI ranges from $1.5 million for incomplete paraplegia to $3.0 million for complete tetraplegia \(^6\). Over the past few decades, advances in medical, surgical, and
rehabilitative care has improved the survivability of this devastating injury. Take for example, thirty years ago, where the overall life expectancy of SCI patients ranged from a few to several months. In current times, individuals can expect to have a life expectancy of 25 to 30 years after the onset of injury depending on injury severity and location\textsuperscript{7,8}.

Although life expectancy after SCI has improved, advances in the management and treatment of the secondary consequences of SCI, such as bladder dysfunction, has remained relatively unchanged. In the 1970s, Lapides introduced clean intermittent catheterization (CIC) for management of the bladder\textsuperscript{9}. While CIC has been proven to be effective, quality of life is reduced since multiple trips to the bathroom are required every day. Inefficient storage and emptying of the bladder can lead to significant morbidity and mortality which is one of the key reasons why restoration of bladder function has consistently ranked as one of the most important health priorities in this population\textsuperscript{10,11}. Unluckily, current levels of research activity and emerging translational approaches do not reflect this importance\textsuperscript{12}.

Neurogenic bladder is a term used to describe bladder dysfunction in individuals with a neurologic condition. It is estimated that the frequency of neurogenic bladder in SCI individuals is 70-84\%\textsuperscript{13} and approximately 81\% of individuals with SCI will report at least some degree of impaired bladder function within 1 year after injury\textsuperscript{14}. To better understand the pathophysiology of neurogenic bladder after SCI, we will start by describing the anatomic features of the lower urinary tract (LUT) and how micturition (also known as voiding) in neurologically intact individuals occur.
1.2 Anatomical Features of the Lower Urinary Tract

The LUT consists of the urinary bladder and urethra. The urinary bladder is primarily made of smooth muscle and can be visually divided into three parts: the dome, the body, and the neck. The wall of the bladder is composed of four layers which are the urothelium, lamina propria (submucosal layer), detrusor muscle, and adventitia (Figure 1.1).

![Bladder anatomy and layers of the wall](https://example.com/bladder_anatomy.png)

*Figure 1.1 Bladder anatomy and layers of the wall. Adapted from Ajalloueian et al.*

The urothelium layer is the innermost layer and serves as the first responder of bladder defense against injuries and infections. Other functions of the urothelium include controlling permeability, immune responses, and cell-to-cell communication. The next outermost layer is the lamina propria which has been suggested to be essential in normal bladder function. The function of the lamina propria includes acting as a capacitance layer of the bladder wall, determining bladder compliance, and enabling adaptive changes to increasing volumes. In addition, the lamina propria may also have a central integrative role in signal transduction and be the source for the production of factors influencing the growth of both the urothelium and detrusor muscle. The detrusor muscle is primarily responsible for contracting during
micturition to push urine out of the bladder through the urethra which is the duct that urine exits out of.

There are two urinary sphincters located in the urethra which act as the gatekeepers for the storage and elimination of urine and they are termed the internal urethral sphincter (IUS) and external urethral sphincter (EUS). The IUS is located at the junction of the urethra with the urinary bladder. Although this muscle is continuous with the detrusor muscle, it is anatomically and functionally independent from it \(^{19}\). The IUS is innervated by the sympathetic nervous system and regulates involuntary control of urine flow from the bladder to the urethra. In men, the IUS also functions to prevent retrograde flow of semen into the bladder during ejaculation \(^{20}\).

The EUS is innervated by the somatic nervous system and provides voluntary control of the elimination of urine from the bladder to the urethra. The coordination between the IUS, EUS, and bladder is critical for continence. In a healthy individual, the sphincters should be contracted, and the bladder should be relaxed during storage of urine. When it is time to void, the sphincters should be completely relaxed, and the bladder should be contracting until it is completely empty. In **Figure 1.2**, the location of the IUS and EUS are shown (red circles) as well as the anatomic differences between the male and female LUT.
Figure 1.2 Anatomic Features of the Human Male and Female Lower Urinary Tracts. Adapted from Hill, 2015.
1.2.1 Neural Control of Micturition: Storage Phase

During the storage phase, the hypogastric nerve (sympathetic) releases noradrenaline which activates the Beta 3 receptors and causes the bladder to relax. In addition, the pudendal nerve (somatic) which innervates the EUS, releases acetylcholine, activating the alpha-1 receptors which causes the EUS to contract. Activation of the EUS is organized by urethral reflexes known collectively as the “guarding reflex.” This reflex is active during storage as well as during sudden increases in abdominal pressure either through coughing, sneezing, or laughing to prevent the involuntary emptying of the bladder. The kidneys produce urine which is transported through the ureters at a reported rate of 1 ml/min, up to a maximum of 5-10 ml/min towards the bladder. As the bladder is filled, the walls are stretched but the urine is retained at a low bladder pressure until it is time to void. The reported normal bladder capacity in humans ranges from 300-400 ml, although this can be variable between individuals. Information about bladder fullness are conveyed to the spinal cord by the pelvic (parasympathetic) and hypogastric (sympathetic) nerves.
1.2.2 Neural Control of Micturition: Voiding Phase

The voiding reflex in healthy individuals is under strict control, enabling individuals to decide if the place and time to void are socially acceptable. When there is approximately 300-400 ml of urine in the bladder, increased afferent firing of the bladder causes a conscious sensation of urinary urge. When the conscious decision to void is made, the pelvic nerve (parasympathetic) releases acetylcholine which stimulates the muscarinic 3 receptors of the bladder and causes the bladder to contract. The IUS is also relaxed via parasympathetic stimulation. Furthermore, this simultaneously activates the spinobulbospinal reflex pathways that pass through the pontine micturition centre in the brain which inhibits the sympathetic and pudendal outflow to the EUS, thus relaxing the EUS. When the voiding reflex is activated, urine exits the bladder via the urethra and this reflex remains active until the bladder is emptied. Figure 1.3 shows the efferent pathways of the lower urinary tract as well as the receptors and neurotransmitters involved in storage and voiding. Figure 1.4 shows the complex innervation of the bladder and the physiologic processes of storage and voiding.
Figure 1.3 Efferent Pathways of the Lower Urinary Tract. Adapted from Fowler et al. 26.
During the storage phase, the hypogastric nerve relaxes the bladder via the B3 receptors and the pudendal nerve contracts the EUS. When it is time to void, the pelvic nerve activates the spinobulbospinal reflex pathways (shown in blue) which passes through the pontine micturition centre. This reflex pathway simultaneously inhibits the hypogastric nerve and also activates the parasympathetic outflow to the bladder (green) as well as the urethral outlet (red) which results in appropriate contraction of the bladder and relaxation of the EUS until the bladder is emptied.
1.3 Sequela of Traumatic Acute SCI

SCI occurs from a sudden, traumatic impact on the spine that fractures or dislocates vertebrae. It can occur from motor vehicle accidents, gunshot wounds, falls, and sports injuries. SCI can be divided into two phases: the acute phase and the chronic phase.

Acute SCI can be further divided into a two-phase process: the first phase is the “primary mechanism of injury” and it is the initial structural damage to the spinal cord. This phase is characterized by damaged to the central gray matter with relative sparing of the white matter. Hemorrhage of the cord develops early and the disruption of blood flow can often result in local infarction. The primary mechanism of injury serves as the nidus for a complex biological cascade for the second phase of acute SCI which is known as the “secondary mechanism of injury.” This phase can occur over the time course of minutes to weeks and is characterized by ischemia-reperfusion, edema, excitotoxicity, macrophage infiltration, apoptosis, and other physiological consequences (Figure 1.5).
Treatment options for patients who suffer acute SCI are limited and primarily aim to mitigate the secondary pathologic processes. For instance, the augmentation of the mean arterial blood pressure to increase spinal cord perfusion is one such treatment option available \(^3\). The chronic phase of SCI can be summarized as the period when neurorecovery has plateaued. Assistive or compensatory approaches are often used to improve the individual’s quality of life. In the context of neurogenic lower urinary tract dysfunction (NLUTD), SCI essentially damages the communication between the brain and bladder. NLUTD can be considered a complication in both the acute and chronic phase of SCI and often requires pharmacologic interventions as well as consistent emptying of the bladder with catheters \(^3\).
1.3.1 Spinal Shock

In the days and weeks following acute SCI, a phenomenon termed “spinal shock” may occur. Spinal shock is not well-defined, and the usage of the term has been controversial. It has been used to describe the absence of spinal reflex activity below the level of the injury \(^{32}\). During this period, the bladder’s reflexive pathways are thought to be disrupted, resulting in an acontractile detrusor with urinary retention. This can lead to overdistension of the bladder if the bladder is not emptied appropriately. Overdistension of the bladder can result in urinary tract infections (UTI) (due to the stasis of urine), bladder ischemia, irreversible myogenic failure of the bladder wall, and potential bladder perforation \(^{33,34}\). The period of spinal shock has been suggested to vary widely from days to weeks and the return of the bulbocavernosus reflex can help to determine the end of spinal shock \(^{35,36}\). In 2004, Ditunno et al. proposed a four-phase model for spinal shock to succinctly explain this phenomenon using findings from basic neuroscience research (Table 1.1).

<table>
<thead>
<tr>
<th>Phase</th>
<th>Time</th>
<th>Physical Exam Finding</th>
<th>Underlying Physiologic Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0-1 day</td>
<td>Areflexia/Hyporeflexia</td>
<td>Loss of descending facilitation</td>
</tr>
<tr>
<td>2</td>
<td>1-3 days</td>
<td>Initial reflex return</td>
<td>Denervation supersensitivity</td>
</tr>
<tr>
<td>3</td>
<td>1-4 weeks</td>
<td>Hyperreflexia (initial)</td>
<td>Axon-supported synapse growth</td>
</tr>
<tr>
<td>4</td>
<td>1-12 months</td>
<td>Hyperreflexia, Spasticity</td>
<td>Soma-supported synapse growth</td>
</tr>
</tbody>
</table>

Table 1.1 Phases of Spinal Shock. Adapted from Ditunno et al \(^{37}\).
As shown in **Table 1.1**, phase 1 occurs immediately after injury and lasts for up to one day. During this period, there is a complete loss – or weakening – of all reflexes below the lesion site.

Phase 2 occurs over the next two days and is characterized by the return of some, but not all reflexes below the lesion site. One of the first reflexes that returns is the bulbocavernosus which has been used to mark the end of spinal shock. The bulbocavernosus reflex is mediated by the pudendal nerve and consists of the contraction of the bulbocavernosus muscle in response to squeezing the glans penis or clitoris\(^{38}\). In phase 3, deep tendon reflexes, which are muscle reflexes that respond to stretch, are restored. In addition, interneurons and lower motor neurons below the SCI begin sprouting, attempting to re-establish synapses. Finally, phase 4 is characterized by soma-supported synapse growth\(^{37}\).

In the field of urology, the end of spinal shock is also marked by the presence of neurogenic detrusor overactivity (NDO) events which is representative of the emergence of the new reflexive pathway of the bladder. NDO is characterized by the presence of involuntary detrusor contractions during the filling period of urodynamic studies (UDS)\(^{39}\). Initial UDS are typically delayed until the period of spinal shock resides, sometimes for up to 6 months\(^{40}\). While the period of spinal shock has been suggested to last from weeks to months, a recent study by Bywater et al. suggests that this may not necessarily be the case. Bywater et al., performed UDS on 54 patients during the first 40 days after SCI. They found that 63\% (34/54) of the patients demonstrated unfavorable urodynamic parameters such as neurogenic detrusor overactivity (NDO), detrusor-sphincter dyssynergia (DSD), and impaired bladder compliance. This study suggested that the reflexive pathways of the bladder return much early than previously thought. Moreover, this study highlights the importance of performing UDS as early as possible for early
intervention to improve prognosis \cite{41}. For instance, the recently published and updated Canadian Urological Association guidelines recommend that urologic evaluation should occur within 3-6 months after SCI \cite{42}.

1.4 Neurogenic Lower Urinary Tract Dysfunction

The LUT heavily depends on the central nervous system (CNS) to efficiently perform the simple functions of storing and emptying of urine. An ideal LUT contains a low-pressure bladder filling and storage, continence, and voluntary complete voiding \cite{43}. Disruptions or lesions to the CNS can lead to NLUTD. Akin to SCI, other neurological diseases such as Parkinson’s, spinal bifida, and even diabetes can result in NLUTD \cite{13}.

The degree of NLUTD depends on the level and the severity of the spinal cord injury. With respect to injury severity, there is some evidence suggesting that even those with incomplete SCI should be investigated thoroughly as these individuals can experience the same level of bladder dysfunction as those with a complete SCI \cite{44,45}.

The Madersbacher classification system characterizes NLUTD on the basis of detrusor and EUS activity depending on the level of the spinal cord lesion \cite{46,47}. Suprapontine lesions generally result in an overactive detrusor with a normal active EUS. Spinal cord lesions typically result in an overactive detrusor with an overactive EUS, also known as DSD. Finally, sacral lesions often result in an underactive detrusor with a normal active or underactive EUS (Figure 1.6). This classification system provides a general idea for physicians on how the LUT is likely to behave.
after SCI. However, newer systems such as magnetic resonance urography in combination with UDS have been proposed for the classification of NLUTD\textsuperscript{48}.

In the human SCI population, the Madersbacher classification generally works quite well to identify the NLUTD pattern. For instance, Weld and Dmochowski\textsuperscript{49} found that out of 196 patients with suprasacral injuries, 186 (94.9\%) demonstrated NDO and or DSD. Similarly, Kaplan et al.\textsuperscript{50} concluded that DSD was a common occurrence in human spinal lesions.

![Figure 1.6 NLUTD Patterns are Location Dependent. Adapted from Panicker et al.\textsuperscript{47}.](image_url)
1.5 Sequela of SCI on the LUT

The inability to perform the two simple functions of storing and emptying urine can result in some devastating consequences that can significantly reduce quality of life and in some cases, be life threatening. Common consequences of NLUTD after SCI include frequent UTI leading to sepsis, autonomic dysreflexia, upper urinary tract deterioration, and bladder or renal stone disease.\textsuperscript{42}

1.5.1 Urinary Tract Infections

Recurrent UTIs are a complicated and frustrating issue for individuals with SCI. Even in an age of antibiotics, UTIs remain difficult to diagnose, treat, and prevent in the SCI population. UTIs are classified as infections of any part of the urinary tract such as the bladder, urethra, ureters, and kidneys.\textsuperscript{51}

Outside of NLUTD, the gold standard for UTIs is the detection of the pathogen (bacteriuria) in the presence of clinical symptoms such as increased urinary frequency, dysuria, nocturia, lower back pain, cloudy urine and hematuria.\textsuperscript{52} A bacterial colony count of $10^3$ colony forming units per milliliter is considered the threshold for UTI.\textsuperscript{53} There is also a classification of uncomplicated and complicated UTIs, which is outside the scope of this thesis but nevertheless an important distinction to know.
In contrast, UTIs are diagnosed slightly different for individuals with SCI. The cause of UTIs in SCI patients is due to inefficient bladder emptying of the bladder and stasis of urine which promotes the colonization of foreign bacteria and impairs the phagocytic ability of the epithelial cells that line the bladder \(^{54}\). According to the Canadian Urological Association guidelines, a UTI can be diagnosed in an individual with NLUTD if their urine tests screens positive for all of the following: bacteriuria, pyuria, and clinical symptoms which include increased spasticity, malaise, lethargy or sense of unease, autonomic dysreflexia, and fever \(^{42}\). Unresolved UTIs can interfere with rehabilitation, lead to secondary urologic complications, and potentially lead to septicemia \(^{55}\). Furthermore, unique to the SCI population is a high prevalence of asymptomatic bacteriuria and it is strongly recommended not to treat this with antibiotics \(^{56}\).

The estimated overall rate of UTI in SCI patients is 2.5 episodes per patient per year and UTIs lead to most cases of septicemia with a death rate of about 15% \(^{54,57}\). UTIs are a burden to the healthcare system and each admission to the hospital due to UTI costs an average of $8000 to the Canadian healthcare system \(^{58}\). Current recommended treatment options for symptomatic UTI is a 7 to 10 day antibiotic treatment for those without a fever and for 14 days in patients with a fever \(^{59}\). Moreover, it is also recommended that SCI individuals practice CIC instead of utilizing indwelling catheters for the management of the bladder to prevent UTIs. Studies have shown that use of an indwelling catheter is associated with an increased frequency of symptomatic UTI \(^{56}\).
1.5.2 Autonomic Dysreflexia

Autonomic dysreflexia (AD) is a medical emergency for individuals with SCI characterized by an elevation in systolic blood pressure $\geq 20$ mm Hg from baseline in response to noxious or innocuous stimuli below injury level $^{60}$. It can also be presented as bradycardia and sweating, with hot flushes and occasional headaches $^{61}$. If left untreated, AD can lead to seizure, strokes, cardiac complications or even death $^{62,63}$. The trigger of AD is a noxious stimulus below the level of the lesion (generally below T6) which results in an activation of unopposed sympathetic activity $^{63}$. In the context of NLUTD, common causes of AD includes UDS, NDO, overdistension of the bladder, and even traumatic catheterization $^{62,64,65}$. Treatment of AD involves mitigating the triggering factor which would include reducing the frequency of NDO with a botox such as onabotulinumtoxina $^{60,66}$ and preventing overdistension of the bladder with appropriate catheterization.

1.5.3 Upper Urinary Tract Deterioration

Individuals with SCI are also at high risk for upper urinary tract deterioration and potentially irreversible renal dysfunction. Common causes of renal dysfunction in SCI patients include renal atrophy and scarring, hydronephrosis (swelling of the kidneys) and renal stone disease. Additionally, high resting detrusor pressures are a common culprit for upper urinary tract deterioration as these elevated pressures can be transmitted to the kidneys $^{42}$. Elevated detrusor leak point pressures (DLPP, defined as the lowest detrusor pressure at which urine leaks from the bladder in the absence of a detrusor contraction or increased abdominal straining) $^{67}$ can result
from NDO and impaired bladder compliance due to an alteration in bladder morphology after SCI. Current guidelines recommend to keep DLPP $< 40$ cm H$_2$O as recommended by Mcguire et al. However, this cut-off value was defined from a small cohort of children with myelomeningocele, and so while it has been utilized widely in the field, it may not actually be that applicable for SCI patients or for adults.

In a recent publication by Tarcan et al. recommended a lower cut-off value of 20 cm H$_2$O which demonstrated a higher sensitivity to predict upper urinary tract deterioration compared to 40 cm H$_2$O. In a clinical setting, renal imaging and UDS are necessary to identify these abnormalities before renal dysfunction becomes irreversible. Treatment typically aims to reduce NDO, UTIs, and transitioning patients from indwelling catheters to intermittent catheters as soon as possible.

### 1.6 Current Management Options for NLUTD after SCI

The aims of management of SCI patients with NLUTD are to maintain adequate storage and drainage in a low-pressure system, preserve renal function, reduce UTIs, and maintain continence by increasing bladder capacity. The aims must be balanced with the patient’s quality of life. In a review by McIntyre et al., they investigated the relationship between neurogenic bladder management techniques and quality of life among individuals with SCI. They found that SCI patients who could void normally had the highest quality of life ratings and this is followed by those who could void with assistance or perform CIC by themselves. The worst quality of life came with an indwelling catheter or when CIC by an assistant was required.
However, each bladder management methods comes with its own benefits and risks which must be accounted for and continuously re-evaluated during follow-ups.

1.7 Bladder Drainage via Clean Intermittent Catheterization

In 1972, Lapides et al. introduced CIC for management of neurogenic bladder and it has since become routinely performed and essentially the gold standard for bladder management. This procedure requires the patient or a care attendant to insert a catheter into the bladder every 4 to 6 hours, so that the amount of urine obtained each collection is generally no more than 500 ml. CIC has been associated with fewer complications compared to indwelling catheterization, including persistent catheter associated bacteriuria, damage to the urethra or bladder neck, creation of false passages, and stones in the bladder. The ideal individual for CIC has a low detrusor pressure at capacity, a minimum volume of 350-400 ml, an unobstructed urethra, and is compliant, understanding, continent, and cooperative with adequate hand function. Individuals that have limited extremity motor function, are female, or with limited functional bladder capacity (due to poor bladder compliance or NDO) may decide to use alternative catheter mechanisms such as indwelling or suprapubic catheterization.

1.7.1 Bladder Drainage via Indwelling Catheterization

Indwelling catheterization involves placement of a foley catheter either transurethral (indwelling) or through an artificial track between the lower abdominal wall and the bladder (suprapubic). With indwelling catheters, storage and emptying of the bladder can be easily controlled by the
switch of a simple catheter valve. Alternatively, the exposed catheter can be attached to a urine collection bag and emptied at least twice a day. In contrast to CIC, where the catheters are situated in the bladder for less than a few minutes, indwelling catheters can be in the bladder for up to 30 days or more, therefore, requiring less action from the individual. Indwelling catheters are typically used in individuals with acute SCI (during spinal shock) because it allows for precise monitoring of urinary output, especially when monitoring fluid balance is critical. Although the benefits and procedure of performing CIC are taught to patients, many revert to indwelling catheterization. Yavuzer et al. found that of 38 patients on CIC at discharge, 20 (52%) discontinued this method and reverted to indwelling catheterization. They reported that compliance with CIC was lower for women, for tetraplegics, and for those without complete injury. However, use of indwelling catheter has been shown to increase susceptibility to colonization with polymicrobial and dynamic bacteria at a rapid rate of 5-10% per day.

Therefore, it has been recommended that to routinely change the catheters every two to four weeks to reduce the risk of asymptomatic bacteriuria and UTIs. In addition, the catheter should be changed if a symptomatic UTI is suspected.

1.7.2 Anticholinergics and Botulinum Toxin A for Neurogenic Detrusor Overactivity

While catheter mechanisms help to prevent overdistension of the bladder by regularly emptying the bladder, some SCI individuals may have issues with incontinence caused by NDO. It has been suggested that in normal healthy individuals, the afferent pathway is mediated largely by alpha delta fibers which ultimately send information about the state of bladder fullness to the pontine micturition center via the periaqueductal gray matter. However, SCI results in a
disruption in these pathways, which results in the emergence of a different afferent pathway, one mediated by capsaicin-sensitive C-fibers. It has been proposed that this new afferent pathway drives a spinal segmental reflex pathway, causing NDO 79.

There appears to be an important interaction between the neuronal system and the uroepithelium specifically. The urothelium expresses various receptors including muscarinic, nicotinic, purinergic and transient receptor potential and also, releases various neurotransmitters (such as acetylcholine, adenosine 5′-triphosphate and nitric oxide) in response to mechanical and/or chemical sensory stimuli, thereby modulating activity of bladder afferent pathways 80. Among these receptors, the muscarinic receptors are arguably the most vital for bladder function.

A healthy human bladder tissue contains M2 and M3 muscarinic receptors in a 75-80: 20-25% ratio 81. The smaller population of M3 muscarinic receptors are responsible for contraction of the bladder 81 while the M2 muscarinic receptors have been suggested to mediate bladder contractions by reversing the Beta-adrenoreceptor-mediated relaxation82. After SCI, it has been suggested that there is an upregulation of M2 muscarinic receptors which may mediate contraction of the bladder 83. Therefore, anticholinergic medications, which block the action of acetylcholine, are typically used as the mainstay treatment for NDO 83. Similar to the issue with the cut-off value for DLPP, the body of evidence for the efficacy of anticholinergics based on randomized controlled studies is poor 84. Most recently, there has been a growing number of high quality studies demonstrating the efficacy of onabotulinumtoxinA in the treatment of NDO but further high quality studies are required 85–87.
1.8 Urodynamic Studies

The current gold standard for characterizing NLUTD after SCI is UDS. This is an umbrella term for a series of tests and procedures which yield quantitative bladder pressure and volume outcome data during both the filling (storage) and the voiding phases. Common tests and procedures include uroflowmetry, cystometry, pressure-flow studies, assessment of urethral closure, EUS electromyography (EMG), and voiding cystourethrography (VCUG). The goal of UDS is to provide the physician with objective data about the individual’s LUT and to identify any abnormalities in relation to what is known to be “normal.” As mentioned previously, the ideal normal LUT contains a low-pressure bladder filling and storage, continence, and voluntary complete voiding.

1.8.1 Uroflowmetry

Uroflowmetry is a non-invasive urodynamic investigation that measures the urinary flow rate. The units of urinary flow rate are expressed as milliliters per second (ml/s). The objective of uroflowmetry is to recreate the individual’s natural voiding pattern. In this test, the individual will void into a container that is placed on top of a flow meter. At the end of the test, the maximum urinary flow rate ($Q_{\text{max}}$), voided volume, time to maximum flow, post-void residual (PVR), and shape of the flow curve are recorded and analyzed.

Although there is great variation in uroflowmetry parameters in the non-symptomatic population, flow curves are generally repeatable and reproducible for the same patient. Healthy males aged
< 40 years usually have a $Q_{max}$ of > 25 ml/s and females usually have $Q_{max}$ of 5-10 ml/s more than males at a given bladder volume. A PVR volume less than 50 ml is considered to be normal, volumes greater than 200 ml is generally considered to be abnormal.

Finally, the shape of the flow curve should be bell-shaped. For different voiding disorders, distinct flow patterns will be observed. In Figure 1.7A, a normal bell-shaped flow curve is shown. A staccato-shaped flow is described as voiding curve with a varying flow rate (Figure 1.7B & C). It can occur with a weak or unsustained detrusor contraction but with a quiet EUS or with periodic bursts of EUS. An interrupted flow curve often has periods of no urine flow in the absence of EUS activity (Figure 1.7D). Prolonged and low amplitude flow curves with an absence of EUS activity are termed plateau shaped flow (Figure 1.7E). A tower-shaped flow curve is characterized as a short duration of urine flow with a high amplitude suggestive of detrusor overactivity (Figure 1.7F).
Figure 1.7 Flow Curve Patterns. Adapted from Schaeffer & Diamond, 2014.
1.8.2 Filling Cystometry and Pressure-Flow Study

Cystometry is a urodynamic procedure where a pressure catheter is placed into the bladder transurethral and saline is infused in a retrograde fashion to artificially create a fill-and-void cycle. This procedure can be done with EUS EMG and VCUG to visualize how the EUS behaves during filling and voiding. Setup for cystometry begins with the transurethral insertion of a urinary pressure catheter into the bladder to measure the intravesical pressure (Pves). Another pressure catheter is inserted into the rectum to measure the intraabdominal pressure (Pabd). The subtraction of Pabd from Pves provides the detrusor pressure (Pdet). EMG patch electrodes are placed perianal at 9 and 3 o’clock to measure the activity of the external anal sphincter which is thought to reflect the activity of the EUS 96 (Figure 1.8). The bladder is filled retrogradely with body temperature saline at a non-physiological filling rate to complete the test in a reasonable amount of time. The resting and filling pressures are monitored for abnormalities such as NDO (characterized by waveforms in Pdet with no changes in Pabd) and impaired bladder compliance (calculated as the change in volume/change in Pdet). The maximum cystometric capacity which is the greatest volume the bladder can hold before voiding is also measured. The graphical representation of a cystometry procedure is termed cystometrogram (CMG) or it can also be simply referred to as a urodynamics tracing.
During the voiding phase, urodynamic parameters such as DSD which is identified by an NDO event with concomitant EUS EMG activity during a leak or voiding attempt, voiding efficiency (volume output/volume infused * 100), DLPP, PVR, Pdet at Qmax, and Qmax are quantified. Poor prognostic features during UDS that requires appropriate treatment and follow up UDS to monitor treatment effect and the need for further treatment is summarized in Table 1.2.
### Urodynamic parameter

<table>
<thead>
<tr>
<th>Compliance</th>
<th>Low compliance (&lt; 20 ml/cm H$_2$O)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detrusor leak point pressure</td>
<td>Elevated (&gt; 40 cm H$_2$O)</td>
</tr>
<tr>
<td>NDO</td>
<td>Any degree</td>
</tr>
<tr>
<td>DSD</td>
<td>Any type</td>
</tr>
<tr>
<td>Vesico-ureteric reflex</td>
<td>Any grade</td>
</tr>
<tr>
<td>Bladder capacity</td>
<td>Reduced (&lt; 200 ml)</td>
</tr>
<tr>
<td>Sustained prolonged NDO</td>
<td>&gt; 75 cm H$_2$O</td>
</tr>
</tbody>
</table>

Table 1.2 Poor prognostic features on urodynamic studies. Adapted from Alsulihem & Corcos $^97$

### 1.8.2.1 Diagnosis of Detrusor-Sphincter Dyssynergia

UDS can play a critical role in diagnosing DSD in individuals with SCI. DSD has been associated with reduced bladder compliance, elevated upper tract pressures, VUR, hydronephrosis, and renal failure $^{98,99}$. Diagnosis of DSD can be made by examining the EMG activity on a CMG, the urethral pressure profilometry, or VCUG $^{100}$. On a CMG, DSD can be characterized increased EMG activity during a detrusor contraction (NDO) in the absence of Valsalva and Crede manoeuvres $^{101,102}$. Although EMG electrode patches are relatively easy to place on a patient for UDS and can provide general information about EUS EMG activity, interpretation may be limited since the patches are technically measuring EMG activity of the external anal sphincter. While it has been stated that the activity of the external anal sphincter can reflect the activity of the EUS in neurologically intact individuals $^{96,103}$, there is still concern
that this may not be applicable for the SCI population. Moreover, EUS EMG recordings are highly susceptible to movement artifacts and poor placement.

Alternatively, VCUG is a minimally invasive radiographic test that helps visualize how the bladder neck behaves during the filling and storage phase of the behaviour. This procedure helps the physician to diagnose vesicoureteral reflex (urine flows backwards to the kidneys) and the degree of DSD. It has been recommended that both modalities be used during UDS to limit the discrepancy because the diagnostic discrepancy between EMG and VCUG has been reported to range from 40% to 46% in humans. Interestingly, these studies also showed that males were more likely to be diagnosed using EMG whereas females were more often diagnosed by VCUG which further emphasizes the need to perform both EMG and VCUG during a UDS to accurately diagnose DSD.

1.9 Urodynamic Studies on Animal Models of SCI

Over the years, animal models of SCI have been utilized to better understand the fundamental biologic mechanisms and pathology of human SCI. The key advantage of using animal models is that it allows for strict control of the experimental conditions. Preclinical UDS have predominately used rodents such as rats, mice, and guinea pigs. This is mainly because of their small size, ease of maintenance, and short lifespan which allows researchers to simultaneously investigate the effects of aging. Other larger animal models such as rabbits, dogs, cats, monkeys, and pigs have also been utilized. Each animal model has its advantages and disadvantages, therefore, there is no species which can be crowned as the
“best” for all urologic investigations after SCI. However, out of all the available animal models, the rat’s bladder is arguably the most well-characterized by both *in vitro* and *in vivo* experiments.

The most common urodynamic test performed on anesthetized or conscious rodents is cystometry. The pressure catheter is typically placed in the bladder transurethral while the animal is under anesthesia. Alternatively, the pressure catheter can also be sutured into the dome of the bladder of the neck, tunneled subcutaneously, and, externalized through the skin in the back to perform cystometry in conscious animals. The most preferred anesthetic agent is urethane, because, although there are some changes in bladder and EUS function, the voiding reflex is not suppressed. The use of anesthetics in rodent students may undermine the translational value of the findings since UDS in humans are often performed without the use of anesthetics.

### 1.9.1 Urodynamic Findings in Rodent Models

Rodent models have played a crucial role in our understanding of LUT function, neural control of micturition, and SCI-induced changes from the acute to chronic stages. UDS have been performed in conscious, anesthetized, restrained, and decereberated rodents using a variety of methods and techniques including acute and subacute suprapubic and acute transurethral catheterization. With these techniques, it has been shown that the EUS plays an important role in efficient emptying of the rodent’s bladder. Four phases of micturition in male and female rodents has been previously described (Figure 1.9). At the start of micturition, there is an initial increase in the intravesical pressure (phase 1) followed by intraluminal pressure...
high frequency oscillations (IPHFOs, phase 2) and pulsatile flow of urine. The IPHFOs consist of a series of EUS openings and closings, which is believed to create a “milking action” of the urethra\textsuperscript{105}. At the end of micturition, there is a rebound increase in intravesical pressure, as the pulsatile flow ends (phase 3). In the final phase (phase 4), there is a rapid pressure decline to the baseline level seen before the micturition contraction.

\textbf{Figure 1.9 Four Phases of Rodent Micturition.} \textit{Adapted from Andersson et al.\textsuperscript{119}}

This bimodal pattern is similar to what has been observed in adult humans\textsuperscript{124,125}. In particular, the origin of the after-contraction/rebound pressure/postvoiding contraction that occurs at the end of micturition appears to be quite controversial. It is a phenomenon only seen during UDS and it has been suggested to be linked to detrusor overactivity, bladder outlet obstruction, and the
collapse of the bladder as it empties. However, its clinical significance has been suggested to be weak since it occurs after the end of voiding.

### 1.9.1.1 Limitations of Rodent Models for Neurogenic Lower Urinary Tract Dysfunction Investigation

Translation of findings from rodent studies to humans is limited in several ways. For instance, the difference in rodent and human bladders is one such limitation. The mouse bladder capacity is roughly 0.15 ml and the rat bladder capacity is approximately 1-2 ml, both of which are comparatively smaller than what the human bladder can hold (300-400 ml). This may make development and translation of novel-human sized devices that aim to improve bladder function after SCI challenging. Another important difference is that healthy rodents demonstrate bursting of the EUS during micturition whereas the human EUS remains relaxed during micturition.

On an EMG tracing, this is characterized by clusters of high-frequency spikes separated by low tonic activity which represents the pumping action of the EUS. The pumping of the rodents EUS appears to be important for efficient emptying of the bladder as it has been shown that abolishing the pumping activity of the EUS using neuromuscular blockers or pudendal nerve transections can reduce voiding efficiency. After SCI, rodents also demonstrate DSD which is characterized by bursting of the EUS during a detrusor contraction on a CMG. However, since uninjured rodents also demonstrate bursting during voiding, differentiating between DSD and normal rodent EUS bursting activity may be difficult. Ito et al., demonstrates this nicely in
Figure 1.10, where EMG bursting during the void is seen in both an uninjured (Figure 1.10A) and a spinal cord transected mouse (Figure 1.10B).

Figure 1.10 Bursting of EUS in an Uninjured and SCI mouse. Adapted from Ito et al. 108

1.10 Pigs for Lower Urinary Tract Disease Studies

There is currently a need for a large animal model of NLUTD for the testing of the safety and utility of novel-human sized devices such as indwelling pressure measurement devices and ultrasonic detection of bladder fullness 12. Pigs have been used increasingly in translational research because humans and pigs share many similarities such as size, physiology, anatomy, metabolic profile, and not to mention, a longer lifespan compared to rodents 134. Numerous
studies have suggested that the pig’s LUT can be a representative model for human LUT diseases\textsuperscript{118,135–138}. Anatomically, the presence of a horizontal slit-like urethral lumen and the transitional epithelium at the level of the bladder neck has been reported to be similar between the pig and the female human\textsuperscript{136}. Physiologically, female pigs demonstrate a drop in intraurethral pressure just prior to voiding and this resembles the drop in intraurethral pressure observed during voiding in healthy humans\textsuperscript{136,139}. Arguably, the most advantageous aspect of the pig is its large size which is suitable for the testing and translation of human-sized devices. For instance, human-sized telemetric devices have been previously implanted into pigs to examine physiologic voiding events\textsuperscript{140,141}.

Despite the translational advantages of using a pig model for LUT studies, there has only been so far one study that characterized the changes in the pig’s LUT function after SCI by Keller et al.\textsuperscript{142}. In this study, they investigated if sacral neuromodulation would ameliorate bladder function after SCI in a pig model. They reported that after a complete compression SCI, minipigs demonstrated one of two bladder phenotypes: a) persisting urinary retention and b) urinary retention followed by the emergence of DSD which is similar to the patterns found in humans with traumatic SCI\textsuperscript{143,144}.

1.10.1 Pig Model of Neurogenic Lower Urinary Tract Dysfunction after Spinal Cord Injury

The rodent model of SCI has been well-developed and established as there are numerous well-controlled injuries (contusion impactors, clip compression, dorsal hemisection) and detailed
outcome measures (Basso, Beattie, Bresnahan locomotor scale, cylinder rearing test, horizontal ladder test, catwalk)\textsuperscript{145}. However, the size of the rodent’s spinal cord and cerebrospinal fluid space is much smaller when compared to humans. This may underlie the lack of success of clinical trials for SCI therapies which otherwise have shown promise in preclinical rodent studies.

Lee et al., have previously described a large animal model of SCI using 20-25 kg Yucatan miniature pigs\textsuperscript{146}. The pig spinal cord (like the human spinal cord) is surrounded by a prominent layer of cerebrospinal fluid (\textbf{Figure 1.11}), making the pig a more clinically relevant animal model to test the biodistribution and effect of therapies applied extradurally or infused intrathecally.

\textbf{Figure 1.11} Magnetic Resonance imaging of a Sprague-Dawley Rat (A) vs. Yucatan Minipig (B) vs. Human (C). Adapted from Lee \textit{et al.}\textsuperscript{146}

The width of the porcine spinal cord (B) is close to a human spinal cord (C) and a prominent layer of cerebrospinal fluid surrounds the porcine and human spinal cord.
In this model, a laminectomy is performed on the T9-T12 vertebrae of anesthetized pigs to expose the spinal cord. A contusion SCI is induced with a 50 g weight drop at the T10 spinal level from a height of 20 cm to create a traumatic thoracic SCI. After the impact, an additional 100 g static weight is placed on top for 5 minutes of compression. This injury model generally leads to consistent and severe deficits in the hindlimbs of the animals. On the porcine thoracic injury behaviour scale, where 1 represents severe hindlimb deficit and 10 represents no hindlimb deficit, animals with a 20 cm injury severity are generally categorized with a porcine thoracic injury behaviour scale score of 1 to 3 which is described as varying degrees of hindlimb dragging. While this model has been utilized to investigate various aspects of spinal cord dysfunction since 2013, the deficits in bladder function were not characterized.
1.11 Research Questions

My research questions were “what are the functional and morphologic changes in the pig’s bladder after traumatic thoracic SCI? Furthermore, what are the similarities and differences between pig and human LUT function after SCI?”

Essentially, the bigger picture of this study was to establish a large animal model of NLUTD after SCI. The following chapter will describe the iterative process of developing a urodynamics protocol to characterize the functional changes in the pig’s bladder function after SCI. Moreover, in Chapter 3, I demonstrate the use of this large animal model of NLUTD for the investigation of a human-sized radio-telemetry transmitter to monitor the pig’s physiologic bladder function. Finally, in Chapter 4, I discuss and describe the findings from my attempt to create a more clinically relevant model by placing a permanent indwelling catheter in the pigs after SCI.
Chapter 2: Urodynamic Studies in a Pig Model of Spinal Cord Injury

2.1 Synopsis

In this chapter, I will describe the iterative process of developing and establish a protocol to perform UDS with clinically relevant urodynamics equipment in a pig model. There were numerous factors that had to be considered during the development process such as the voiding position, the effect of sedatives, the type of equipment to use, and the order in the steps to place the urodynamics equipment. I will present the urodynamic findings from both uninjured and SCI pigs from my study as well as the findings from the University of Louisville since we are writing a collaborative manuscript. Finally, I will discuss some of the challenges in the interpretation of the urodynamic findings.

2.2 Materials and Methods

The data in this study were acquired as part of a collaborative effort between UBC in Vancouver, British Columbia, Canada and UofL in Louisville, Kentucky, USA. This study involved work at both campuses using the shared UBC porcine model of SCI. All animal protocols and procedures were approved by the Animal Care Committees of UBC and UofL and were in accordance with both the Canadian Council and the United States Office of Laboratory Animal Welfare on Animal Care and Institutional Animal Care and Use Committee guidelines.
2.2.1 Animals and Experimental Design

To develop the CMG setup protocol, 44 female Yucatan minipigs were used (n = 35 with thoracic SCI, and n = 9 uninjured pigs). The animals were obtained from either Sinclair Bio-resources, Auxvasse, Missouri, USA or S&S farms, Ramona, California, USA. To characterize NLUTD in SCI minipigs, a comprehensive study in groups of animals with different SCI severities and injury levels was performed with an emphasis on examining the changes in detrusor pressure ($P_{det}$) and EUS EMG activity. Fifteen of these animals underwent contusion/compression SCIs at either the T2 (n = 9, 16.73 cm drop, 50 g midline contusion) or the T10 (n = 6, 20 cm drop, 50 g midline contusion) spinal level. After the initial weight drop contusion injury, an additional 100 g static weight was placed on top of the 50 g impactor to impart 5 min of compression (T2 animals had 120 minutes of compression). One additional T10 SCI group was included (n = 15) that had a 100 g weight drop contusion from 10 cm, followed by 5 min of compression with the same weight. The UBC Impactor described in 2013 was used at both UBC and UofL for inducing the contusion/compression injury. Four other pigs received a clip contusion-compression injury at the T3-T5 level, and one received a 40 cm contusion-compression injury severity with a 100 g weight at the T10 level. After SCI, the bladders were managed with an indwelling catheter that was inserted prior to the surgery and connected to a urine collection bag for 7 to 10 days. Afterwards, the catheter was removed, and the bladder allowed to drain spontaneously during the remaining study period. Further details of the injury, surgical procedure, post-operative veterinary monitoring, and care can be found in articles by Lee et al. and Kim et al.
The iterative development and implementation of a standardized CMG protocol in the pig model of SCI occurred in three experimental phases, with several protocol modifications being made to the setup procedure and initial training between the three:

*Experiment 1 – CMG recordings in anesthetized minipigs (pilot study).* Previous studies performing CMG’s on small animal models have been carried out successfully under anesthesia. Several pilot experiments were initially performed to evaluate the feasibility of performing CMG’s in anesthetized uninjured and SCI minipigs (n = 6) using different anesthetic drug combinations and doses. These drugs included propofol (8-20 mg/kg/hr) and fentanyl (22-45 mcg/kg/hr), either with or without bupivacaine (2 mg/kg), isoflurane (2%), and xylazine intramuscular (1 mg/kg). CMG’s in fully anesthetized conditions were obtained with the animals suspended in the air using a hammock-style sling.

*Experiment 2 – CMG recordings in awake SCI minipigs, with transient sedation during transurethral catheter placement into the bladder.* Previous reports have highlighted the potential effects of anesthesia on LUT function such as inhibition of the pontine micturition center and voluntary cortical control of the bladder, as well as suppression of detrusor contractions and the micturition reflex. Therefore, a protocol to perform CMG recordings in awake but partially restrained SCI minipigs (n = 15 of which n = 6 received T10 SCIs and n = 9 T2 SCI; study performed at UBC) was developed as the next step. A hammock-style sling was utilized to partially restrain the animals during filling cystometry (described in more details in the paragraphs below). Sedation (dexmedetomidine intramuscular; 0.05 mg/kg) was only used during placement of the transurethral urodynamic catheters. Dexmedetomidine
was reversed with atipamezole (0.2 mg/kg) given intramuscularly and the volume was adjusted based on the alertness of the animal since the sedative wore off with time. Each pig was sedated for no more than 90 minutes.

**Experiment 3 – CMG recordings in awake SCI minipigs without procedural sedation.** Lastly, a training protocol for repeated cystometry evaluations pre-/post-SCI was developed to eliminate the need of sedation during placement of transurethral urodynamic catheters. Differences in LUT function between pigs that received sedation and reversal pre-cystometry versus uninjured pigs that did not receive sedation with (suspended) or without the hammock-style sling (standing) were examined. For this experiment, n = 13 animals underwent contusion SCI (10 cm drop, 100 g midline contusion with 5 min of compression) at the T10 spinal level. This study was performed at UofL.

Injury parameters for animals in Experiment 1 (anesthetized) are summarized in Table 2.1. Cystometry equipment and injury parameters for animals in Experiment 2 (procedural sedation during urodynamic catheter placement) and Experiment 3 (no procedural sedation during urodynamic catheter placement) are summarized in Table 2.2.
One animal received a contusion-compression SCI. Four animals received a clip-compression SCI. One animal did not receive SCI or any sort of surgical procedure prior to CMG.

<table>
<thead>
<tr>
<th>Pig # (ID #)</th>
<th>Injury Level</th>
<th>SCI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (865)</td>
<td>T10</td>
<td>Contusion Compression: Height: 40 cm Force: 4081 Kdynes Displacement: 6.2 mm Impulse: 13.4 Kdynes*s Velocity: 2436 mm/s</td>
</tr>
<tr>
<td>2 (9088)</td>
<td>T4-T5</td>
<td>Clip: 50 g Compression: 1 min</td>
</tr>
<tr>
<td>3 (9196)</td>
<td>T4-T5</td>
<td>Clip: 80 g Compression: 5 min</td>
</tr>
<tr>
<td>4 (9125)</td>
<td>T3-T4</td>
<td>Clip: 50 g Compression: 5 min</td>
</tr>
<tr>
<td>5 (9207)</td>
<td>T3-T4</td>
<td>Clip: 30 g Compression: 10 min</td>
</tr>
<tr>
<td>6 (9109)</td>
<td>No-SCI</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Table 2.1 Experiment 1 (fully anesthetized CMG): Biomechanical Impact Parameters of the SCI for Each Animal.
<table>
<thead>
<tr>
<th>Description</th>
<th>Experiment 2 awake CMG sedated catheterization</th>
<th>Experiment 3 awake CMG non-sedated catheterization</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Injury level</td>
<td>T10</td>
<td>T9-T10, T10-T11</td>
</tr>
<tr>
<td>- Drop height (cm)</td>
<td>20</td>
<td>16.73</td>
</tr>
<tr>
<td>- Compression weight (g)</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>- Compression time (min)</td>
<td>5</td>
<td>120</td>
</tr>
<tr>
<td>Age at Time of Injury (days)</td>
<td>196 ± 21</td>
<td>165 ± 5</td>
</tr>
<tr>
<td>Bodyweight (kg)</td>
<td>30 ± 3</td>
<td>27 ± 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25 ± 1</td>
</tr>
<tr>
<td>Animal Consciousness during Catheterization</td>
<td>Sedated</td>
<td>Awake</td>
</tr>
<tr>
<td></td>
<td>[7/9]</td>
<td>[7/10]</td>
</tr>
<tr>
<td>- Size [Pves/Pabd] (Fr)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Animal Consciousness during CMG</td>
<td>Awake</td>
<td>Awake</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Standing or Suspended</td>
<td>Standing or Suspended</td>
</tr>
<tr>
<td>- Pre-SCI</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Suspended</td>
<td>Standing or Suspended</td>
</tr>
<tr>
<td>- Post-SCI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cystometry Equipment</td>
<td>Delphis, Triton (Laborie)</td>
<td>Lumax TS Pro (CooperSurgical)</td>
</tr>
<tr>
<td>Saline Infusion</td>
<td>Infusion pump</td>
<td>Gravity</td>
</tr>
<tr>
<td>Infusion Rate</td>
<td>1 ml/min per kg of body weight</td>
<td>34.5 ml/min</td>
</tr>
<tr>
<td></td>
<td>[range = 25-44 ml/min]</td>
<td></td>
</tr>
</tbody>
</table>

Table 2.2 SCI Protocol and Cystometrogram (CMG) Setup Differences between Experiments 2 and 3.

The table above highlights the differences in the details of the injury, setup protocol (sedation vs no sedation) and the cystometry equipment and settings used between the two experiments.
2.2.2 Hammock Style Restraining Unit and Training Regime

To ensure safe placement of the transurethral urodynamic catheters during cystometry (either with or without sedation), the pigs were acclimated to being suspended comfortably in a hammock-style sling with four leg openings inside a metal frame (Figure 2.1A).

Figure 2.1 (A) Cystometry Setup Design and (B) Placement of Cystometry Equipment

A hammock-style sling was placed into the middle of a metal frame (length: 152 cm, width: 68 cm, height: 88 cm). There were four small leg holes in the hammock-style sling for the animal. Once the animal was fitted into the sling, it was slowly elevated using a winch mechanism just before the sling touched the abdomen. This was done to give space for the animal to squat. The legs were loosely tied to the frame to prevent the animals from lifting their legs out of the sling. A harness worn by the pig was also tied to the frame to prevent the uninjured animals from jumping out of the sling. Both uninjured and SCI animals stood on all four limbs. SCI animals that had paralyzed hindlimbs were supported in an upright posture to promote positioning of partially extended hindlimbs beneath the body. The urodynamic pressure catheter was inserted transurethrally into the bladder. The abdominal pressure balloon catheter
was placed in the rectum. The catheters were subsequently taped to the side of the left buttock. EMG patches were placed in the perianal region at the 9 and 3 o’clock positions with a ground EMG placed on the bony protrusion of the left ankle.

This form of restraint required training for the animals to become accustomed to it. We developed a training regimen, which consisted of teaching the animal to learn target touching and accommodate to restraining and bladder catheterization procedures:

Stage 1 - Targeting. Minipigs were first trained to recognize their given names and to also look up at the trainers when their names were called. Second, they were trained to approach and touch a hand-held target (such as a small rubber ball fastened onto the end of a wooden dowel) using food, clicker and verbal cues; and third to follow the target in different directions. Successful completion of the given commands resulted in the animal hearing a click and receiving a food reward with verbal praise. The behaviour was considered mastered once the animal successfully responded to verbal commands including their name, “touch/target”, “stay”, and “follow”. Subsequently, the animals were target and clicker trained daily to freely enter and stay for 5-10 minutes in the metal frame with the sling on the ground. Once the animal mastered this task, Stage 2 restraint training began.

Stage 2 - Restraint Training. The next step in the training process was to acclimate the pigs to the sling restraint setup. The pigs were brought into the testing room and taught to walk into the sling restraint setup. Once the pig’s legs were in the holes of the sling, the sling was raised to one of two heights using the winch mechanism. The first sling height supported the pig’s weight and
allowed the hindlimbs to stand on the surface. The second sling height completely suspended the pig’s hindlimbs in the air. While restrained, positive reinforcement (food, petting, and verbal praise) was used to calm the animal. At the end of the session, the animal was hand-fed while the sling was lowered. Generally, 30 training sessions were needed until the maximum time of restraint was achieved (~10 minutes in duration).

Stage 3 – Handling and Restrain Training for Awake Transurethral Catheterization (Experiment 3 only). Potential challenges in placing urodynamic catheters in conscious, awake pigs without sedation could include discomfort, stress, and anxiety for the pigs during the procedure. To minimize stress and allow the catheterization procedure to be carried out safely, training began by suspending the pigs using a hammock-style sling and by desensitizing the pigs to manual touch, particularly around the vulva area. While suspended, the pig’s name and the “stay” command was given to the pig to focus on the trainer and remain still. When the animal became restless and agitated, a second trained animal attendant applied gentle but firm pressure onto the hips and shoulders until the animal stopped moving. As soon as the animal became still, the pressure was immediately released, and clicker was given followed by a food reward. On average, 9 training sessions were required to train the animals to acclimate to the handling procedures associated with catheterization without exhibiting behaviours indicative of fear or stress.
2.2.3 Cystometry Equipment and Setup

Urodynamic catheters were placed in either awake or sedated pigs suspended with the hammock-style sling. With the animal suspended in an upright position, feces were removed using digital stimulation to prevent defecation during testing. Using aseptic technique, a 7-Fr dual lumen catheter was passed transurethrally into the bladder for filling and to measure intravesical pressure (Pves). Another dual lumen catheter (9-Fr or 10-Fr) was passed into the rectum to measure abdominal pressure (Pabd). In situations where transurethral catheter insertion was difficult, an extra small KleenSpec LED speculum (Welch Allyn, New York, USA) was utilized to visualize the urethral opening (only in sedated pigs). Entry into the bladder was confirmed by the presence of urine flow. After successful placement of the transurethral catheter, the bladder was emptied, and a urine sample was collected for urinalysis for pre- and post-SCI pigs. The external pressure transducers were zeroed at atmospheric pressure using the level of the symphysis pubis as the reference height (Experiment 2 only). Two EMG electrode patches (Conmed, New York, USA) were subsequently placed on the skin around the external anal sphincter in the 9 and 3 o’clock positions which is thought to be a surrogate of EUS activity. The ground EMG patch was placed and taped onto either the bony protrusion of the ankle of the left hindlimb or onto the superior edge of the iliac crest (Figure 2.1B). The EMG data collected in this study were not filtered for analysis or presentation.

For animals that were sedated, atipamezole (0.1-0.2 mg/kg) was intramuscularly administered to reverse the sedation at a volume dependent on the alertness of the animal (typically quarter or half dosage). Filling of the bladder commenced once the animal recovered from sedation (all of
the following: eye blinking, eating from food bowl, noises, increased heart rate) which generally occurred within 8 to 13 minutes after the injection.

2.2.4 Cystometry Protocol

All awake cystometry evaluations (Experiment 2 post-sedation, and Experiment 3 without any sedation) were performed in an upright posture with or without sling support (with the latter allowing for a more natural squatting position during voiding). With the bladder drained and the pressure transducers zeroed, sterile saline was continuously infused into the bladder. It is important to note that infusion rates were equipment-dependent and thus differed between the two institutions. At UBC, body temperature saline solution (0.9%) was infused into the bladder via an infusion pump at a rate equivalent to the weight of the animal (1.0 ml/min per kg of body weight). At UoF, the bladder was filled with body temperature saline solution by gravity, with an average filling rate of 34.5 ml/min [range: 25-44 ml/min]. Saline infusion was stopped when a void or leakage of urine was visibly seen from the vulva of the pig. Urine was collected in a beaker placed on top of a uroflow meter placed underneath the pig to measure the flow rate of urine as well as volume of urine voided. At UoF, the flow rate of urine was not measured. Any residual volume was removed via the Pves catheter.

CMG recordings lasted between a period of 30–60 minutes from the start of the filling phase to the end of the voiding phase. Under awake conditions, animals were hand-fed using food as positive reinforcement. All procedures were performed between 9 a.m. and 2 p.m. to minimize the potential effects of circadian changes in voiding function\textsuperscript{161,162}. Following data collection, the urodynamic catheters and surface patch EMGs were removed. To prevent overdistension of the
bladder for post-SCI animals, manual expression of the bladder was performed with the Crede’s maneuver to ensure the bladder was at least partially emptied prior to transporting the animal back to the pen.

2.2.5 Definition of Urodynamic Outcome Measures

The CMG parameters studied during the filling phase were: $P_{ves}$, $P_{abd}$, detrusor pressure ($P_{det} = P_{ves} - P_{abd}$), cystometric capacity (maximum infusion volume reached at the end of filling) and EUS EMG activity. During the voiding phase, the maximum urine flow rate ($Q_{max}$), the detrusor pressure at maximum flow ($P_{det}.Q_{max}$), the detrusor pressure recorded immediately before the isovolumetric contraction ($P_{detopen}$), voided volume (VV), post-void residual (PVR), voided efficiency [VV/(PVR+VV) x 100%], and bladder compliance (change in bladder volume/change in $P_{det}$ during that change in bladder volume; expressed as ml/cm H$_2$O). The $P_{det}$ at which involuntary expulsion of water/urine occurred was considered the detrusor leak point pressure (DLPP). Neurogenic acontractile detrusor was defined by the absence of a detrusor contraction during voiding. Neurogenic detrusor overactivity (NDO) was characterized by involuntary detrusor contractions during the filling phase. We refrained from diagnosing DSD, since EUS activity was recorded with EMG patch electrodes without concurrent voiding cystourethrography. All parameters defined are in consonance with the metric units and definitions established by the International Continence Society (ICS) $^{67,163–165}$. For example, bladder compliance values less than 20 ml/cm H$_2$O were determined to be indicative of low bladder compliance.
2.2.6 Bladder Histology

At the end of the study, after being sedated deeply with Telazol (4–6 mg/kg, intramuscular), animals were euthanized with an intravenous overdose of sodium pentobarbital (120 mg/kg). The bladder was then removed and fixed with 10% formalin in 0.1 M phosphate buffer with a volume to tissue ratio of 20:1 for a minimum of 72 hours at 4°C within 30 minutes of harvesting. Subsequently, specimens were processed, and paraffin embedded by Wax-It Histology (Vancouver, BC, Canada). Tissues embedded in paraffin blocks were then sectioned (5um) and slides stained with hematoxylin and eosin (H&E) and Masson’s trichrome stain (Abcam, Toronto, Ontario, Canada). The slides were then examined for general morphologic and histopathologic changes using conventional light microscopy (ZEISS Axio Imager M2, ZEISS, Germany). Muscle-to-collagen ratio was quantified using Image J (National Institutes of Health, Bethesda, MD, USA) and averaged from two cross sections taken from the body region of the bladder. With the color threshold plugin, a threshold was set to only showing areas occupied by collagen (blue) or muscle (red), and subsequently binary images were made. The relative area (%) occupied by collagen and muscle was then calculated. Tissue thickness measurements were taken by drawing five lines across the tissue while ensuring the lines were perpendicular to the mucosa (using ZEN software; ZEISS, Germany). The length of the perpendicular lines was averaged and taken as the thickness (in millimeters, mm). This was then compared to control specimens taken from the bladder wall of Yucatan female pigs with an acute SCI (< 12 hours). Age at euthanasia, body weight, and bladder weight of these pigs are provided in Table 2.3. Not every bladder from a control or SCI animal was collected for histologic processing but their bladder weights were recorded.
The table above contains the age at euthanasia, injury level, body weight, and bladder weight from pigs whose bladder was processed for histologic examination. N.D. = not determined. A T-Test was performed to compare the differences in the histological parameters between the control and T2&T10 SCI animals. * significant difference between control and SCI age, body, and bladder weight, P < 0.05, ** P < 0.01, *** P < 0.001.

### Table 2.3 Age, Body weight, and Bladder weight of Pigs whose Bladder was Harvested for Histologic Analysis.

The table above contains the age at euthanasia, injury level, body weight, and bladder weight from pigs whose bladder was processed for histologic examination. N.D. = not determined. A T-Test was performed to compare the differences in the histological parameters between the control and T2&T10 SCI animals. * significant difference between control and SCI age, body, and bladder weight, P < 0.05, ** P < 0.01, *** P < 0.001.

#### 2.2.7 Statistical Analysis

All statistics were calculated using GraphPad (GraphPad Software, Inc., California, USA). The values are represented in mean ± SEM. T-tests were performed to compare CMG parameters between groups as well as for the age, SCI parameters, bladder weight, body weight, wall
thickness, % of muscle and collagen content, and muscle/collagen ratio of the detrusor. An ANOVA was performed to compare CMG parameters for Experiment 2 animals at 4, 8, and 10-17 weeks post-injury.

2.3 Results

2.3.1 Experiment 1: Cystometry in Fully Aneesthetized Uninjured Pigs

All anesthetized animals (n = 6) demonstrated inhibition of the voiding reflex and overflow incontinence. A CMG from an uninjured, propofol and fentanyl-anesthetized animal is depicted in Figure 2.2. At an infused volume of 950 ml, voiding was suppressed, and leakage occurs at a DLPP of 14 cm H$_2$O. Therefore, we proceeded with the development of a protocol in awake pigs, either initially sedated to facilitate placement of the urodynamic catheters (Experiment 2), or without the use of sedation (Experiment 3), which is also more analogous to how filling cystometry is performed in humans.
Figure 2.2 Cystometrogram from an Anesthetized, Uninjured Pig

After propofol (8-20 mg/kg/hr) and fentanyl (22-45 mcg/kg/hr) anesthesia, the Pves and Pabd catheters [computed detrusor pressure (Pdet) plotted] were both placed while the pig was supported in the sling. Filling cystometry was initiated with a starting detrusor pressure of 22 cm H₂O. There was a gradual rise in detrusor pressure over the course of bladder filling which commenced with a 25 ml leak at 950 mL of infused saline (DLPP = 14 cm H₂O). An increase in EMG activity was apparent just prior to and following the leak. There was no apparent voiding reflex. The fill rate was 23 ml/min and the total capacity was 950 ml. From top to bottom: intravesical pressure (Pves), abdominal pressure (Pabd), detrusor pressure (Pdet), electromyography (EMG), and volume infused (VH₂O).
2.3.2 Experiment 2: Awake Urodynamic Studies with Sedation During Catheterization

2.3.2.1 Procedural Observations

Most animals successfully learned targeting behavior and tolerated the awake CMG procedure very well. The pigs acclimated to the restraint procedure in approximately 30 sessions. Overall, 84 CMGs were attempted (38 pre-SCI and 46 post-SCI), with some animals having repeat CMGs pre- and post-SCI. In total, 64 CMGs (29 pre-SCI and 35 post-SCI) were analyzed, with 20 CMGs being excluded for a variety of reasons. In 8 cases we were unable to catheterize the bladder and in another 8 cases, the transurethral catheter fell out during the filling period or the animal voided due to movement. In 4 cases, the animals were deemed to have a concomitant UTI, as determined by a positive urinalysis test post-CMG with significant bacteriuria and pyuria along with visual observations of behaviors signifying pain, such as loss of appetite, quiet behaviour, as well as signs of fever, foul-smelling urine, cloudy urine, and hematuria 42.

2.3.2.2 Awake Cystometrogram in Uninjured Pigs

After the reversal of the sedation used to place the urodynamic catheters, the awake uninjured (non-SCI) animals had filling cystometry performed in either a suspended position using the hammock-style sling or in a standing/squatting position. Using this approach, two distinct voiding patterns were observed. The first pattern (n = 7; 9/29 CMG, 31%) was characterized by a marked detrusor contraction during the voiding phase (Figure 2.3B). The contraction of the detrusor continued and the EUS remain relaxed until voiding was complete.
Figure 2.3 Cystometrogram from an Uninjured Pig Demonstrating Voiding Pattern 1

(A) **Filling Phase.** This animal received sedation (dexmedetomidine) and reversal (atipamezole) prior to filling of the bladder. The fill rate was 37 ml/min and the total volume infused until the void was 890 ml. The change in Pdet was 5 cm H$_2$O over the entire filling cycle indicating good bladder compliance (dashed arrow). The shaded region highlights the zoomed-in view of the voiding phase shown in (B).
(B) Void. At the start of the void, a distinct detrusor contraction (arrow) is present. During the void, the detrusor continues to contract while the sphincter remains relaxed (arrowhead) until the void was complete. Near the end of the void, a rebound pressure (star) response occurs. At the end of the void, an increase in sphincter activity is seen signifying contraction of the sphincter (arrowhead). It is also important to note that the flow curve plateaus because the flow meter does not read measurements beyond 50 ml/s. From top to bottom: intravesical pressure (Pves), abdominal pressure (Pabd), detrusor pressure (Pdet), external anal sphincter electromyography (EMG), flow rate, and volume infused (VH₂O).

The second voiding pattern, (n = 12; 20/29 CMG, 69%), was characterized by a rapid increase in Pdet during the filling phase (Figure 2.4A) without a distinct voiding contraction during urine expulsion (Figure 2.4B). These animals typically demonstrated lower bladder compliance due to the bladder being filled far beyond its physiologic capacity, which at that point, the viscoelastic properties of the bladder are grossly distorted and do not necessarily reflect the “bladder compliance” during normal urine storage.
Figure 2.4 Cystometrogram from an Uninjured Pig Demonstrating Voiding Pattern 2

(A) Filling Phase. This animal received sedation (dexmedetomidine) and reversal (atipamezole) prior to filling of the bladder. The fill rate was 23 ml/min and the total volume infused until the void was 403 ml. From approximately 100 to 403 ml, reduced bladder compliance (dashed arrows) was observed (due to filling of the bladder beyond its physiologic capacity), characterized by a rapid rise in Pdet (↑ 25 cm H2O). The shaded region highlights the zoomed-in view of the voiding phase shown in (B).
(B) Void. Moments prior to the void, sudden movements from the pig caused spikes in the Pdet and EMG channels (X’s – movement artifact and not a true detrusor contraction). During the void, a distinct detrusor contraction was not observed (arrow) but the sphincter remained relaxed (arrowhead) throughout the duration of the void resulting in complete emptying of the bladder. At the end of the void, an increase in sphincter EMG activity (arrowhead) is observed indicating contraction of the sphincter. From top to bottom: intravesical pressure (Pves), abdominal pressure (Pabd), detrusor pressure (Pdet), external anal sphincter electromyography (EMG), flow rate, and volume infused (VH2O).

When comparing the CMG data between animals displaying voiding pattern 1 versus pattern 2 (irrespective of voiding position) the cystometric capacity was significantly greater in animals displaying voiding pattern 1 vs 2 (632 ± 92 ml vs. 429 ± 48 ml, respectively, p=0.04) (Figure 2.5). It is worth noting that 10 pigs (59%, 10/17) demonstrated capacities exceeding 400 ml which is considered to be the upper limit of the normative functional bladder capacity range (300-400 ml) in adult humans. Bladder compliance was highly variable (ranging from 6 to 442 ml/cm H2O) but there was a significant difference in compliance between animals displaying pattern 1 compared to animals displaying pattern 2 (115 ± 47 ml/cm H2O vs. 41 ± 10 ml/cm H2O, respectively, p=0.04). There was no significant difference in the voided % between the two voiding patterns (pattern 1: 96 ± 2% vs. pattern 2: 97 ± 3%, p=0.94). There were also no significant differences in Pdetopen, Pdet-Qmax, Qmax, and PVR.
Two distinct voiding patterns were observed: animals who had a clear detrusor contraction (pattern 1) and animals who had no clear contraction (pattern 2) after pre-cystometry sedation and reversal. These two distinct voiding patterns were seemingly dependent on the posture of the animal during cystometry, either standing/squatting or suspended in the sling. A T-Test was performed to compare the differences in the urodynamic parameters between animals that demonstrated voiding pattern 1 vs. 2 and animals that performed cystometry in a standing/squatting vs. suspended position. * significant difference between animals displaying voiding pattern 1 vs. pattern 2 or between animals that performed cystometry in a standing/squatting vs. suspended position, P < 0.05, ** P < 0.01, *** P < 0.001.

When comparing the CMG parameters between those animals in a standing/squatting position (n = 12; 19/29 CMG (66%); n = 7 displayed voiding pattern 1, n = 7 displayed voiding pattern 2, some animals demonstrated both voiding pattern 1 and 2) versus those were that were fully suspended (n = 5; 10/29 CMG (34%); n = 5 displayed voiding pattern 2), $P_{\text{detopen}}$ was significantly
lower in the standing animals compared to the suspended animals (23 ± 1 cm H$_2$O vs. 35 ± 6 cm H$_2$O, respectively, p=0.02). This may reflect the pressure being locally applied to the bladder by the sling itself during suspension. Furthermore, the cystometric capacity was significantly smaller in the fully suspended animals (609 ± 53 ml vs. 270 ± 21 ml, p=0.0001).

2.3.2.3 Awake Cystometrogram in Spinal Cord Injured Pigs (20-cm Drop)

A total of 15 animals received a thoracic SCI, of which 6 (40%) had a T10 injury and 9 (60%) had a T2 injury. Details on the injury and the biomechanical parameters related to the injury are provided in Table 2.4. With this injury severity, none of the animals were able to support their body weight during cystometry and the sling technique for supporting the hindlimbs and lower trunk was used.
Measures of age at surgery, body weight, and biomechanical impact parameters of the contusion injury for each animal. After SCI, biomechanical data acquired for each impact was collected and analyzed for peak force, impactor displacement from initial contact with the exposed dura, impulse (calculated as the integral of force with respect to time), and velocity at impact. N.D.: not determined. A T-Test was performed to compare the injury parameters between T2 and T10 SCI animals. * significant difference between SCI animals with injury at the T10 level vs. T2 level, calculated with a T-Test, P < 0.05, ** P < 0.01, *** P < 0.001.

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<td>13.1 ± 1.2</td>
<td>1862 ± 30*</td>
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Table 2.4 Experiment 2 Injury Parameters

Measures of age at surgery, body weight, and biomechanical impact parameters of the contusion injury for each animal. After SCI, biomechanical data acquired for each impact was collected and analyzed for peak force, impactor displacement from initial contact with the exposed dura, impulse (calculated as the integral of force with respect to time), and velocity at impact. N.D.: not determined. A T-Test was performed to compare the injury parameters between T2 and T10 SCI animals. * significant difference between SCI animals with injury at the T10 level vs. T2 level, calculated with a T-Test, P < 0.05, ** P < 0.01, *** P < 0.001.
NDO was observed in all T10 and T2 SCI animals, characterized by involuntary detrusor contractions during bladder filling (Figure 2.6). The onset of NDO was observed as early as 3 weeks after SCI (earliest time point CMG was performed) and was still observed at 17 weeks after SCI (latest time point CMG was performed). The amplitude and frequency of the contractions varied across SCI animals. NDO events started at approximately 53 ± 6% of the cystometric capacity (~295 ml on average).
Figure 2.6 Cystometrogram from a T10 SCI Pig at 17 Weeks Post-Injury Demonstrating Neurogenic Detrusor Overactivity (NDO) with Possible Detrusor-Sphincter Dyssynergia (DSD)

(A) Filling Phase. This animal received sedation (dexmedetomidine) and reversal (atipamezole) prior to filling of the bladder. The fill rate was 52 ml/min and the infused volume until the first leak was 1009 ml. At approximately 700 ml, NDO events (stars) which are characterized by a waveform appearance in the Pdet channel can be seen leading up to the first leak. During these NDO events, there are bursts of sphincter activity (arrowheads) which could possibly signify DSD. The shaded region highlights the zoomed-in view shown in (B).
(B) Leak. Possible DSD events prior to the first leak can be seen. At an infused volume of 1009 ml, the first leak of the study occurs during a possible DSD event resulting in incomplete emptying of the bladder. Two more subsequent leaks were captured in this study both of which appear to occur during a possible DSD event. From top to bottom: intravesical pressure (Pves), abdominal pressure (Pabd), detrusor pressure (Pdet), external anal sphincter electromyography (EMG), flow rate, and volume infused (VH₂O).

Analysis of the CMG parameters of T10 (10-17 weeks post-SCI) and T2 (12 weeks post-SCI) animals combined showed no significant differences in cystometric capacity post-SCI (Figure 2.7) compared to the uninjured group (646 ± 72 ml vs. 492 ± 46 ml, p=0.07).
All animals received a 20 cm drop contusion injury with 5 min of 150 g of compression at either the T10 (n = 3) or T2 level (n = 7). T10 animals received 5 minutes of compression and T2 animals received 120 minutes of compression. In addition, all animals received sedation (dexmedetomidine) and reversal (atipamezole) prior to filling of the bladder. As none of the animals were unable to support their body weight as a result of the injury severity, their hindlegs were weight-supported (toes have contact with the ground) or fully suspended using a custom-built sling. There were no significant differences between SCI animals with an injury at the T10 level vs. T2 level. Similar findings were found at the 3-4- and 7-8-week post-injury time points. A T-Test was performed to compare the differences in the urodynamic parameters between T2 and T10 SCI animals as well as between uninjured animals vs. T2&T10 SCI animals. * significant differences between all uninjured animals from Experiment 2 (n = 17) and the T2 + T10 SCI group, P < 0.05, ** P < 0.01, *** P < 0.001. Voiding in SCI animals occurred at a significantly lower \( Q_{\text{max}} \) \((8 \pm 3 \text{ ml/s vs. } 27 \pm 2 \text{ ml/s, } \text{p}<0.0001)\) compared to the uninjured group. In addition, the voided \% was significantly lower in SCI animals \((11 \pm 5\% \text{ vs. } 97 \pm 2\%, \text{p}<0.0001)\). As a result, SCI animals demonstrated a
significantly greater PVR compared to the uninjured group (593 ± 80 ml vs. 24 ± 10 ml, p<0.0001). Bladder compliance was not significantly different compared to the uninjured group (50 ± 9 ml/cm H₂O vs. 64 ± 17 ml/cm H₂O, p=0.59), nor was P_detopen/DLPP significantly different (24 ± 3 cm H₂O vs. 27 ± 2 cm H₂O, p=0.40). CMG’s performed at earlier time points (3-4 and 7-8 weeks), also revealed significantly lower voided volume (3-4 weeks: p<0.0001, 7-8 weeks: p<0.0001) and significantly greater post-void residual volume (3-4 weeks: p<0.0001, 7-8 weeks: p<0.0001) in the T2/T10 SCI animals relative to uninjured controls. Across time, there was no biological significance in the CMG parameters within the Experiment 2 SCI group.

2.3.3 Experiment 3: Awake CMG without Sedation during Catheterization

2.3.3.1 Procedural Observations

Using our training protocol, the pigs acclimated to the restraint procedure in approximately 9 sessions. All animals were successfully catheterized while awake and suspended in the sling without the use of sedation. Notably, all animals were tolerant and were also very cooperative during the awake catheterization procedure. A total of 32 CMGs (17 pre-SCI, 15 post-SCI) were conducted with an overall bladder catheterization success rate of 100%. One CMG recording session (3%) was not included in the analysis due to confirmation of a clinical UTI post-CMG. One (3%) other session data was not included due to the urinary catheter falling out or high interference of artifact in the cystometric profile, both due to animal movement. Overall, 30 CMGs were analyzed (17 pre-SCI, 13 post-SCI).
2.3.3.2 Awake Cystometrogram in Uninjured Pigs

All animals were able to void with a marked detrusor contraction (i.e. pattern 1) during all CMG sessions (n = 15; 17/17 CMG sessions, 100%) irrespective of voiding position (standing or squatting). Conversely, only 7/17 (41%) of the uninjured animals from Experiment 2 demonstrated this pattern. When comparing the CMG parameters between uninjured pigs from Experiment 2 and 3 displaying pattern 1, regardless of voiding position, the cystometric capacity of uninjured pigs from Experiment 2 was significantly greater compared to those in Experiment 3 (632 ± 92 ml vs. 301 ± 42 ml, p=0.001) (Figure 2.8).
Figure 2.8 Cystometrogram Parameters of Uninjured, Awake Animals without Procedural Sedation

Animals predominantly displayed a clear detrusor contraction during voiding (pattern 1). None of the animals received pre-cystometry sedation and reversal. There were no significant differences between animals in a standing/squatting vs. suspended position. VP1 = voiding pattern 1. A T-Test was performed to compare the differences in the urodynamic parameters between animals that performed cystometry in a standing/squatting position vs. in a suspended position as well as between all Experiment 3 and all Experiment 2 uninjured animals irrespective of voiding position. * significant differences between all Experiment 3 and all Experiment 2 animals that demonstrated voiding pattern 1, P < 0.05, ** P < 0.01, *** P < 0.001.
Furthermore, the $P_{detopen}$ was significantly lower by approximately 22 cm H$_2$O (24 ± 2 cm H$_2$O vs. 46 ± 5 cm H$_2$O, respectively, $p=0.003$). Voided % was not significantly different, with both experiments exhibiting voiding efficiencies of ~96% ($p=0.75$). Bladder compliance was significantly higher in Experiment 2 animals compared to those in Experiment 3 (115 ± 47 ml/cm H$_2$O vs. 23 ± 5 ml/cm H$_2$O, respectively, $p=0.01$). These differences were also observed in Experiment 2 animals that demonstrated voiding pattern 2.

No significant differences in the CMG parameters were observed between animals that voided in a standing/squatting position ($n = 8$) versus those were that were fully suspended ($n = 8$). One animal performed CMG in both a standing/squatting and fully suspended position.

### 2.3.3.3 Awake Cystometry in Spinal Cord Injured Pigs (10-cm Drop)

For the subsequent part of the experiment, the UofL team utilized the 100 g weight with a drop height of 10-cm followed by 5 min of compression with the same weight. Details on the injury and the biomechanical parameters related to the injury are provided in Table 2.5. $N = 2$ animals that had pre-injury cystometry performed had complications after SCI surgery and were euthanized.
Measures of age at surgery, body weight, and biomechanical impact parameters of the contusion injury for each animal. Measures of age at surgery, body weight, and individual biomechanical impact parameters of the contusion injury. After SCI, biomechanical data acquired for each impact was collected and analyzed for peak force, impactor displacement from initial contact with the exposed dura, impulse (calculated as the integral of force with respect to time), and velocity at impact. N.D.: not determined.

A T-Test was performed to compare the injury parameters between Experiment 3 and Experiment 2 SCI animals. * significant difference between SCI animals with a 10 cm drop, 100 g of compression vs. 20 cm drop, 150 g compression (Experiment 2, Table 4), P < 0.05, ** P < 0.01, *** P < 0.001.

Overall, 5/12 (42%) of the post-SCI animals demonstrated NDO at 11-13 weeks post-injury with NDO occurring at approximately 90 ± 4% of the cystometric capacity (~336 ml on average). There were no significant differences observed in cystometric capacity at 11-13 weeks after SCI compared to pre-injury values (503 ± 102 ml vs. 301 ± 42 ml, p=0.06). Similar to Experiment 2, SCI resulted in significantly elevated PVRs (353 ± 71 ml vs. 6 ± 4 ml, p<0.0001), and reduced voided% (28 ± 8% vs. 95 ± 3%, p<0.0001). Bladder compliance was not significantly different.

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Mean ± SEM

|                  | 208 ± 8 | 26 ± 1 | 1877 ± 112*** | 1.9 ± 0.2*** | 7.8 ± 0.4*** | 1010 ± 66*** |

Table 2.5 Experiment 3 Injury Parameters

Measures of age at surgery, body weight, and biomechanical impact parameters of the contusion injury for each animal. Measures of age at surgery, body weight, and individual biomechanical impact parameters of the contusion injury. After SCI, biomechanical data acquired for each impact was collected and analyzed for peak force, impactor displacement from initial contact with the exposed dura, impulse (calculated as the integral of force with respect to time), and velocity at impact. N.D.: not determined. A T-Test was performed to compare the injury parameters between Experiment 3 and Experiment 2 SCI animals. * significant difference between SCI animals with a 10 cm drop, 100 g of compression vs. 20 cm drop, 150 g compression (Experiment 2, Table 4), P < 0.05, ** P < 0.01, *** P < 0.001.

Overall, 5/12 (42%) of the post-SCI animals demonstrated NDO at 11-13 weeks post-injury with NDO occurring at approximately 90 ± 4% of the cystometric capacity (~336 ml on average). There were no significant differences observed in cystometric capacity at 11-13 weeks after SCI compared to pre-injury values (503 ± 102 ml vs. 301 ± 42 ml, p=0.06). Similar to Experiment 2, SCI resulted in significantly elevated PVRs (353 ± 71 ml vs. 6 ± 4 ml, p<0.0001), and reduced voided% (28 ± 8% vs. 95 ± 3%, p<0.0001). Bladder compliance was not significantly different.
versus pre-SCI (25 ± 6 ml/cm H₂O vs. 23 ± 5 ml/cm H₂O, p=0.89), nor was P_{detopen}/DLPP
significantly different (59 ± 12 cm H₂O vs. 46 ± 5 cm H₂O, p=0.26). While this SCI severity
resulted in obvious hindlimb impairments, 6/12 (50%) animals were able to have cystometry
performed in a stand/squat position with minimal support (physically holding the hindlimbs).
When comparing CMG parameters between those SCI pigs in a stand/squat position and those that
were fully suspended, the voided% was significantly higher in standing SCI pigs versus SCI
suspended animals (43 ± 12% vs. 11 ± 5%, respectively, p=0.002) (Figure 2.9).
Figure 2.9 Experiment 3 Cystometrogram Parameters of Spinal Cord Injured Pigs 11-13 Weeks Post-Injury

All animals received a 10-cm drop contusion injury with 5 min of 100 g compression between the T9-T10 level (n=10). None of the animals received pre-cystometry sedation and reversal. While this injury severity resulted in substantial hindlimb impairments, 4 out of 10 animals were able to perform cystometry in a standing/squatting position with minimal support (physically holding the hindlimbs). Cystometry in the remaining animals was performed in a suspended position using a custom-built sling. * significant difference between SCI animals and pre-SCI animals from experiment 3 or between SCI animals in a standing/squatting vs. suspended position, P < 0.05, ** P < 0.01, *** P < 0.001.
There were no significant differences in the CMG parameters between Experiment 2 (n = 10) and 3 (n = 6) suspended SCI animals at the 10-17-week time points. This could suggest that the use of procedural sedatives or injury severity may not have had a notable influence on overall CMG outcomes in chronic SCI animals.

### 2.3.4 Gross and Histological Features of the Bladder after Spinal Cord Injury

The wet weight of Experiment 2 SCI bladders (n = 23) was significantly increased compared with control animals (n = 21) (euthanized < 12 hrs post-SCI) (36 ± 2 g vs. 19 ± 1 g, p<0.0001). Furthermore, in those animals that underwent histologic analysis, the bladder wall in SCI animals (n = 10) was significantly thicker compared to control (n = 3) (5.5 ± 0.1 mm vs. 4.5 ± 0.1 mm, p<0.0001). All SCI animals, regardless if they had a T2 or T10 SCI, demonstrated noticeable detrusor hypertrophy (Figure 2.10).
Figure 2.10 Detrusor Hypertrophy in Spinal Cord Injured Bladders

Masson’s trichrome staining of the body of the bladder from a (A) control pig, (B) T2 SCI pig 12 weeks post-injury (WPI), and (C) T10 SCI pig 10 WPI. The smooth muscle tissue is stained in red and collagen is stained in blue.

There is marked detrusor muscle hypertrophy after SCI (both after T2 and T10 SCI) compared to the control. Moreover, there is a reduction of the submucosa (yellow arrows show the distance between the urothelium and the first suburothelial smooth muscle layer) in both the T2 and T10 SCI bladders compared to the control. There were no other apparent morphologic differences observed between pigs with a T2 or T10 SCI.

Dense patches of collagen (Masson’s Trichrome) within the detrusor layer were observed in a few cases, but significant fibrosis was not observed. The % of muscle was significantly increased in the bladder of SCI animals compared to controls (54 ± 1 % vs. 44 ± 1 % respectively, p<0.0001). However, the % collagen (41 ± 1 % vs. 43 ± 1 %, respectively, p=0.35) were similar between SCI and the controls. The muscle-to-collagen ratio (1.3 ± 0.05 vs. 1.0 ± 0.04, p=0.004) was significantly different between the SCI and control.

Hallmark features of SCI bladders included thinning of the lamina propria and mild to moderate chronic inflammation, identified by the presence of lymphoplasmacytic infiltration of the lamina
propria in H&E stained sections. Mild edema was observed in some cases. The presence of focal acute inflammation was also identified by the presence of neutrophils and eosinophils within the urothelium. Additionally, the urothelium of the SCI bladders showed more prominent folding compared to the control.

2.4 Discussion

The minipig model has shown great potential for urologic research. The anatomic and physiologic similarities of the LUT between humans and pigs have been well-described in several studies. Most recently, the minipig model was used for a NLUTD study by Keller et al., where they performed cystometry on post-SCI pigs to assess if sacral neuromodulation would ameliorate bladder dysfunction. In their study, they noted that catheterizing awake healthy minipigs was difficult. To circumvent this issue, the authors tried using sedatives (propofol or xylazine) to place the urodynamic catheters and then perform cystometry. However, they stated that this procedure gave “poor information” during CMG analysis and attributed this to the influence of the sedative agents.

In this collaborative study between UBC and UofL using the shared Yucatan minipig SCI model and the same impactor, we went through an iterative process to develop and adjust procedures to enable conscious, awake CMG without the need for sedation during catheterization. This achievement is accredited to the training protocol developed by our animal trainers. Our training and CMG setup protocol allows for reliable and reproducible assessments of LUT function in an
awake state with minimal restraints. Moreover, it also allows for us to longitudinally follow and characterize the changes in the pig’s LUT function before and after SCI.

Major findings in our study include demonstrating that during voids, the EUS of uninjured pigs is relaxed and this is similar to how the human EUS behaves during voiding. Following SCI, 15/22 (68%) pigs demonstrated NDO at the latest CMG time point (10-17 weeks post-injury) which parallels findings in humans with chronic traumatic SCI. The potential to perform longitudinal studies repeatedly in the same animal opens promising avenues to investigate NLUTD over time and in response to intervention.

2.4.1 Influence of Anesthetics on Lower Urinary Tract Function

The LUT is heavily dependent on the central nervous system to perform efficient storage and emptying of the bladder. Sympathetic activation of the internal urethral sphincter and pudendal stimulation of the EUS promote bladder continence. When the setting is appropriate and a conscious decision to void is made, voiding occurs via parasympathetic-mediated detrusor contraction and simultaneous EUS relaxation. Previous studies have shown that anesthesia can result in an inhibition of micturition, direct loss of coordination between the detrusor muscle and EUS as well as an imbalance of sympathetic and parasympathetic control of micturition. However, the influence of anesthetics and sedatives on CMG outcomes seem to be dependent on the class of the anesthetic agents as well as the depth of anesthesia. These findings appear to be congruent with the results from Experiment 1, where we investigated the influence of various anesthetics on bladder function and observed an inhibition of the voiding reflex which resulted in
overflow incontinence in uninjured and SCI pigs. Notable is that in small animal studies, urethane (for use only in terminal studies and not appropriate for use in large animals), is the anesthetic of choice, as it does not suppress contraction of the bladder. A comparison of the pressure curves in non-injured pigs that demonstrate voiding pattern 1 from Experiments 2 and 3 versus those of urethane-anesthetized female rats reveal similar patterns, including during the three phases of the voiding cycle. These phases include the initial rise in Pves, the plateau phase with high frequency oscillations which is when expulsion of urine occurs, and a third phase containing a rebound artifact and then a decrease in pressure.

2.4.2 Influence of Dexmedetomidine and Atipamezole on Lower Urinary Tract Function

CMG results from pigs in Experiment 2 may have been influenced by the sedative and reversal (dexmedetomidine and atipamezole). There was a higher likelihood of observing a greater cystometric capacity than the reported normative bladder range in humans (300-400 ml) in uninjured pigs that received sedation and reversal (10/17, 59%) compared to uninjured pigs that did not receive sedation (3/15, 20%). Moreover, bladder emptying (pattern 2) that occurred without contraction of the detrusor was only prominent (12/17 pigs; 71%) in pigs that were sedated and reversed, and did not occur in pigs that did not receive sedation (0/15 pigs; 0%). However, we acknowledge that our interpretation is complicated by the methodologic differences between Experiment 2 and 3, with discrepancies in the level and severity of the injury, sequela of SCI on LUT function, and the use of different cystometry equipment.

The effect of dexmedetomidine and atipamezole on LUT function have been previously investigated in rodent models. Ishizuka et al. reported that intrathecal and intra-arterial (close to
the bladder) administration of a single dose of dexmedetomidine in conscious rats reduced micturition pressure, bladder capacity and micturition volume; while atipamezole reversed the effects of dexmedetomidine. The same study also found a very similar result with systemic administration.

In another study, Harada et al. found that dexmedetomidine increased voiding frequency and overflow incontinence leading to bladder leakage, but the interpretation of this finding was complicated by the diuretic effect of dexmedetomidine. Diuresis by dexmedetomidine has also been reported in humans. Pigs in Experiment 2 typically voided approximately 12% more than what was infused during the CMG whereas pigs in Experiment 3 did not void more than what was infused suggesting there was diuresis in pigs that were sedated with dexmedetomidine. In the literature, other reported effects of dexmedetomidine on the LUT include reduced bladder sensation in humans and inhibition of bladder contractility in animals.

Harada et al. also reported that the rodent’s bladder capacity was decreased with dexmedetomidine, while atipamezole increased capacity. Therefore, suggesting that greater capacities in uninjured pigs may have been due to the effects of atipamezole. Overall, the combination of the dexmedetomidine and atipamezole may have resulted in diuresis, altered voiding patterns, and greater cystometric capacities in a proportion of Experiment 2 uninjured animals.

While we acknowledge the paucity of data describing CMG in animal models done with sedation, the aforementioned animal studies, studies in urethane-anaesthetized rats, and our data, highlight the potential influence of dexmedetomidine and atipamezole on the LUT function of
neurologically intact pigs. The variability in the results between previous studies and ours could be associated with differences in the experimental conditions such as, the species used, drug dosage, and the time between the exposure to the drug to when the animal voids or leaks. Taking all these potential issues into consideration, we plan in future studies to avoid using sedation such as dexmedetomidine for bladder catheterization, unless absolutely necessary.

2.4.3 Influence of Voiding Position on LUT Function

Among the multitude of variables to contemplate when interpreting CMG data, the voiding position is another important factor to consider. In humans, there has been conflicting and inconsistent results as to whether standing or sitting is more beneficial for an individual’s cystometric profile\textsuperscript{187}. The effect of voiding posture on the cystometry profiles of both pre- and post-SCI pigs was also considered. Suspended uninjured pigs demonstrated a smaller cystometric capacity compared to standing uninjured pigs in Experiment 2. Furthermore, suspended uninjured pigs demonstrated a higher $P_{\text{detopen}}$ and lower bladder compliance compared to standing uninjured pigs. If these findings were taken alone, this suggests that suspension with the hammock-style sling locally applied pressure onto the bladder resulting in an earlier void at a higher $P_{\text{detopen}}$ (due to the added pressure from the sling), a lower bladder compliance, and smaller cystometric capacities. Although differences were found in pigs that had been previously sedated (Experiment 2), there were no significant differences in the CMG parameters ($P_{\text{detopen}}$, bladder compliance, cystometric capacity) between suspended or standing uninjured animals in Experiment 3 suggesting that the voiding posture did not have an influence. Overall, these findings reinforce the
idea that sedatives may influence the interpretation of CMGs and should only be used if necessary, for CMG in animals.

2.4.4 Influence of the Actual CMG Testing Procedure on Lower Urinary Tract Functional Measures

Although the current gold standard for characterizing NLUTD includes conducting filling cystometry, it has been questioned whether such procedures can be used to characterize “normal” bladder function in the non-SCI condition. While in experimental studies, it would be only natural to perform CMG’s pre-SCI to establish the “normal baseline” state, it should be acknowledged that the act of performing CMG with transurethral urodynamic catheters and retrograde filling at rapid rates may not replicate “normal” function of the uninjured bladder. In our study, we observed large inter-animal variability in the CMG outcomes of uninjured pigs. These findings highlight the variability of cystometry, as also observed in healthy individuals. In 1999, Wyndaele reported wide variation in all CMG parameters from a total of 28 men and 10 women with no history, symptoms or signs of urologic disease. In the study, healthy volunteers demonstrated pathological signs such as altered flow patterns, low $Q_{\text{max}}$, large cystometric capacities, bladder overactivity and elevated PVR. Likewise, Leitner et al., found that 71% (30/42) of healthy participants without LUT symptoms demonstrated some sort of pathological finding, with DSD being the most common finding during CMG. Furthermore, the healthy participants also demonstrated larger bladder capacities during CMG compared to the capacities recorded in their bladder diaries.

These previous findings allude to the fact that CMG may not necessarily be a valid tool to define “normal” LUT function and variability may result due to the non-physiologic nature of the test.
For instance, we observed cystometric capacities in non-sedated uninjured pigs ranging from 122 to 811 ml. Note that we measured that the Yucatan minipigs void no more than 350 ml in their own pens (own preliminary data, not shown). Ideally, a second fill-and-void cycle should be performed at least a week apart to examine the degree of intra-animal variability. With everything considered, this may suggest that the comfort level of the pigs to void during CMG could influence the final CMG outcomes.

2.4.5 Return of the Bladder’s Reflexive Pathways in a Porcine Model of SCI

Following acute human SCI, it has been documented that there is an absence of the bladder’s reflexive pathways resulting in an acontractile detrusor and urinary retention for a period of time ranging from days to months after the onset of injury 36,188,189. During this period, the patient’s bladder is typically managed with an indwelling catheter to prevent overdistension of the bladder until reflexive voiding occurs 24. However, in a study by Bywater et al., 190 CMG investigations revealed an acontractile detrusor in only 20 of the 54 patients (37%) within the first 40 days after SCI but unfavorable CMG parameters in 34 (63%). The authors also found NDO in 32 patients, DSD in 25, maximum storage detrusor pressure greater than 40 cm H₂O in 17, vesicoureteral reflux in 3, and low bladder compliance (less than 20 ml/cm H₂O) in 1. Similarly, we observed NDO as early as 3 weeks post-injury in Experiment 2 animals which persisted until the end of the study (10 to 17 weeks post-injury). This suggests that the return of the reflexive pathways in the pig model of SCI may occur at 3 weeks after injury or potentially even earlier.

In the context of SCI, DSD typically occurs in patients with a suprasacral lesion 46,47. On a CMG recording, it could be argued that an increase in EMG activity with a concurrent NDO event is
indicative of DSD. Based on this definition, we observed DSD in 6/11 (55%) animals at 3-4 weeks post-injury, 4/10 (40%) animals at 7-8 weeks post-injury and 8/20 (40%) animals at 10-17 weeks post-injury. However, our assessment of DSD is limited by the use of EMG patch electrodes without simultaneous voiding cystourethrography. The EMG patch electrodes were placed perianal at 9’ and 3 o’clock sphincter and this is thought to reflect the activity of the EUS 90. However, Koyanagi et al. demonstrated using needle EMG electrodes, that there may be a dissociation between the EMG activity of the EUS and the external anal sphincter after acute SCI 104. This raises questions as to whether if the EMG activity of the external anal sphincter can truly be used as the reflection of the EMG activity of the EUS after SCI. Furthermore, previous studies on human patients have demonstrated that there is a diagnostic discordance for DSD of up to 40% when only one (EMG patches versus voiding cystourethrography) of these modalities is used101,191. These studies recommended that more cases of DSD can be diagnosed if both modalities are used simultaneously.

2.4.6 Bladder Trabeculation and Wall Thickening after SCI

Chronic SCI has been reported in other porcine 192 and rodent models 106,193 to result in increased bladder weight and wall thickness, highlighting the downstream consequences of SCI on the morphology of the bladder. Histologic evaluation of SCI bladder sections revealed increased markers of chronic inflammation in the submucosa. Specifically, plasmo-lymphocytic infiltration of the lamina propria was seen as early as 3 weeks post-injury. The retention of urine or the presence of foreign bacteria could have acted as a noxious stimuli and triggered chronic inflammation 194. Common morphologic observations in all SCI bladder sections was thinning of
the submucosa, detrusor hypertrophy, and thickening of the bladder wall independent of injury level, and CMG pattern (NDO vs. neurogenic acontractile detrusor).

Bladder trabeculation was also another common morphologic observation in the SCI bladders during histologic processing. It has been documented that after acute human SCI, bladder trabeculation is one of the earliest manifestations of NLUTD. Potential triggers for trabeculation can be a combination of bladder overdistension, increased outflow resistance, or elevated Pves. One hallmark of trabeculation is detrusor hypertrophy which is exactly what we observed in all of our post-SCI bladder sections.

The role of collagen in bladder filling has been documented previously, especially the importance of type III collagen fibers in bladder filling and maintaining compliance. “Non-compliant” bladders (neurogenic bladders) appear to be characterized by an increased pericellular infiltration of type III collagen fibers (fibrosis). However, we did not observe a difference in the % of collagen between post-SCI and the control pigs, suggesting there was no infiltration of collagen nor an alteration in the ratio of type I and III collagen fibers. It is possible that at this point of 10~17 weeks post-SCI, tissue changes are still evolving within the detrusor and so our histologic evaluation did not note any major changes in the collagen composition. We postulate that with more time, the bladders would have eventually progressed into a fibrotic state (with significant replacement of muscle with collagen) as this is more commonly observed in humans with chronic SCI. Further histologic work with immunohistochemistry is required to characterize the changes in the type I and III collagen fibers of the pig’s bladder after chronic SCI (> 1 year).
2.4.7 Limitations of the study

This porcine model of NLUTD was established by a collaboration between two independent institutions working on the same SCI model. While there was a unique strength in the collaboration and some observations were compatible between the two sites, there were also slight differences in the methodology and approaches.

First, different cystometry systems were used for Experiment 2 (fluid-filled pressure sensor catheters) and Experiment 3 (fiberoptic pressure sensor catheters). This complicates the comparison of the findings between the experiments, especially considering that these systems operate on different principles in terms of calibration. In fluid-filled systems, the pressure transducers are aligned with the ICS reference level (superior edge of the pubic symphysis) and zeroed to the atmospheric pressure. In contrast, fiberoptic systems are not calibrated to the ICS reference level and this has been suggested to result in variability in the initial detrusor pressure measurements.205

Secondly, the bladder filling rate must be controlled in a uniform and consistent fashion, ideally to the recommended rates by the ICS. The maximum physiologic filling rate is estimated to be the body weight in kg divided by four 206 which in this case would have been approximately, 5-10 ml/min for the pigs. Yet, there is a fine balance in choosing a rate that resembles the physiologic filling rate as well as completing the study in an acceptable time for the patient. Since rapid fill rates are not well-defined for pigs, we decided to make the infusion rate equivalent to the body weight at the time of testing to ensure the studies would be completed in a timely manner for
Experiment 2. However, in Experiment 3, the infusion rate could not be as tightly controlled because a gravity infusion system was used. This resulted in infusion rates ranging from 25-44 ml/min which could have resulted in non-linear filling of the bladder. As a consequence, CMG parameters such as bladder compliance \(^{207}\), detrusor pressure and contractility \(^{140,208,209}\), volume threshold to void \(^{210}\), and presence of NDO \(^{211}\) may have been affected by the uncontrolled fill rate producing increased variability. However, the exact influence of rapid and variable bladder filling on bladder function appears to be inconclusive in the literature therefore further investigation is required as future work.

### 2.5 Conclusions

Overall, with the establishment of these techniques for CMG, we contend that the Yucatan minipig model of SCI can serve as a valuable large animal model of NLUTD. The size of this animal is adequate for the development and testing of novel human-sized devices that aim to improve LUT function. A setup protocol for performing cystometry in awake, slightly restrained Yucatan minipigs that allows for repetitive and longitudinal evaluation of LUT function before and after SCI has been described. We do not recommend performing cystometry in anesthetized pigs as we have demonstrated how these drugs can inhibit the voiding reflex. Instead, we recommend performing cystometry in the pigs (either uninjured or post-SCI) without the use of sedation and reversal during catheter placement, unless absolutely necessary. This characterization of LUT function will allow for future studies to investigate different bladder management strategies post-SCI and the effect of SCI therapies, including neuromodulation on bladder function. Given the morbidity associated with LUT dysfunction after SCI, we hope that such a large animal model will
facilitate advances in urologic care that will ultimately improve the quality of life for persons living with SCI.
Chapter 3: Telemetric Monitoring of Porcine Bladder Function

3.1 Synopsis

As discussed earlier, the pig model allows for the evaluation of human-sized technologies, unlike the rodent. In a fitting manner, this chapter will discuss my findings from implanting a human-sized device in the model I described in Chapter 2. The goal of this study was to evaluate if this device could be used to remotely monitor the pig’s physiologic bladder function by comparing the telemetry data obtained urodynamics, the gold standard for characterizing NLUTD. I will introduce the custom implantable radio telemetry system that was developed alongside TSE Systems and also detail how the device was surgically implanted in the pigs. Following that, I will present the findings from my experiments that evaluated the congruency between the UDS and telemetry systems in both uninjured and SCI pigs. Furthermore, I will characterize the differences between voiding in a physiologic setting versus in a urodynamics setting.

3.2 Materials and Methods

The data acquired from this study was collected at the University of British Columbia (UBC), Vancouver, British Columbia, Canada. All animal protocols and procedures were approved by the Animal Care Committee of UBC and were in accordance with the Canadian Council on Animal Care.
3.2.1 Animals, Housing and Training

Female Yucatan minipigs (n = 6) were obtained from S&S Farms (age at SCI: 4-6 months, body weight at SCI: 25-30 kg) for this study. Seven weeks prior to telemetric transmitter implantation surgery, the pigs arrived at the facility and were group housed in an indoor pen that had access to another outdoor compartment. The pigs were fed 1.5% of their body weight twice a day and given ad-libitum access to water. After one week of acclimating to the facility, the animals began a 4 to 5-week sling training regimen to prepare for filling cystometry studies as previously described in Keung et al. (Keung et al., 2020, unpublished).

3.2.2 Experimental Design

The Stellar telemetric transmitter’s (#PPPBTA-XXL TSE Systems, Chesterfield, MO, USA) performance in the pigs was evaluated in two phases: 1) during filling cystometry and 2) weekly in-pen recordings. Pre- and post-SCI filling cystometry were performed the UBC porcine model of NLUTD after SCI (Keung et al., currently under review) and the congruency between the telemetry and urodynamic pressure catheters was assessed. Weekly in-person telemetric recordings were performed with the pre- and post-SCI animals freely moving in their pens to investigate the differences in voiding behaviour in a physiologic setting versus in a filling cystometry setting. A total of three experiments were performed with N = 2 used in each experiment to evaluate sensor performance and durability.
3.2.3 Stellar Telemetric Transmitter and Pressure Sensor Design

The main components of the Stellar telemetry system consisted of a transmitter unit with four independent channels which hosted up to three solid-state pressure-tipped catheters and a pair of EMG electrode leads (Figure 3.1). The transmitter itself also contained a microprocessor and an internal memory storage (1 GB). The system also came with a receiver antenna and Notocord-HEM software (NOTOCORD, Le Pecq, France) for data collection. The receiver antenna consisted of a single USB powered mobile unit connected to a laptop. Digital transmission range between the transmitter and the receiver antenna was 5 meters. The battery life of the transmitter provided at least 3 months of data collection depending on usage.
Figure 3.1 Main Components of the Stellar Telemetry System.

The Stellar telemetry system included the telemetric transmitter (Model PPPBTA-XXL, TSE Systems, Chesterfield, MO, USA) along with the intravesical pressure (Pves) and abdominal pressure (Pabd) catheters, and electromyography (EMG) leads bonded to the silicone suture plate.
Prior to implantation, the unit was calibrated in a closed system at several different temperature points to establish a pressure/temperature relationship with a final calibration to 37.5 degrees Celsius by TSE Systems. Detailed specifications of the telemetric system and all the different components that were evaluated is shown in Table 3.1.
### Transmitter Specifications

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<td>Battery life</td>
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<td>Temperature range</td>
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### Intravesical Pressure Catheter Specifications

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### Abdominal Pressure Catheter Specifications

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### Biopotential Electrodes Specifications

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### Receiver

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Table 3.1 Device Specifications of the UBC Stellar Telemetry Model PPBTA-XXL
Initial experiments were conducted using TSE’s original pressure sensors design (referred to as the “Standard” design, **Figure 3.2A & 3.3A**) to determine the quality of the pressure readings. Prompted by the results of the pilot studies, several design modifications were made to the pressure catheters and anchoring of the EMG leads in collaboration with TSE Systems to improve system-level performance and robustness.

Modifications to the design of the Pves catheter included thickening of the proximal and distal ends of the sensor housing, creating a “barbell” design for additional protection (**Figure 3.2B**). For the abdominal catheters, a secondary encapsulation was created around the glass-housed sensor with the aim to minimize drift and to protect the sensor from potential impacts during the animal’s movements. Three designs were tested: 1) abdominal “Balloon” sensor, in which the glass-housed sensor was encapsulated within a silicone gel-filled balloon (**Figure 3.3B**), 2) abdominal “Caged” sensor with the glass-housed sensor encapsulated by a silicone cage (**Figure 3.3C**), and 3) abdominal “Caged-Balloon” sensor using a combination of both the silicone gel-filled balloon and the silicone cage (**Figure 3.3D**).

The initial EMG anchor design was a thin mesh sheet attached to the EMG leads. This was later upgraded to a sturdier silicone suture plate (**Figure 3.4A and B**). A summary of the various Pves, Pabd, and EMG anchor designs evaluated in each animal is shown in (**Table 3.2**).
Figure 3.2 Intravesical Pressure Catheter Designs

(A) **Standard.** This design encapsulated the pressure sensor in medical grade glass. A stopper made of silicone was located 2 mm below the suture ring to prevent dislodgement of the catheter. The sensor was located near the distal end of the catheter tip. The length of the catheter tip was 2 cm and the width was 0.2 mm. The first and second iteration of the telemetric transmitter used this design. (B) **Barbell.** This design encapsulated the catheter tip with silicone and thickened the distal and proximal ends creating a “barbell” design. The sensor was located where the catheter tip was the thinnest. The length of the catheter tip was 2 cm and the width was 0.5 mm. The third iteration of the telemetric transmitter used this design.
Figure 3.3 Abdominal Pressure Catheter Designs

(A) **Standard.** This design encapsulated the pressure sensor in medical grade glass. The sensor was located at the distal end of the catheter tip. The length of the catheter tip was 0.5 cm and the width was 0.2 mm. The first iteration of the telemetric transmitter used this design. 

(B) **Balloon.** A latex balloon filled with silicone gel was used to encapsulate the pressure sensor housed in medical grade glass. The length of the catheter tip was 0.5 cm and the width was 1.5 cm. The second iteration of the telemetric transmitter used this design. 

(C) **Caged.** A cage made from silicone was surrounded the pressure sensor housed in medical grade glass. The length of the catheter tip was 0.5 cm and the width was 0.75 cm. The second and third iteration of the telemetric transmitter used this design. 

(D) **Caged-Balloon.** A latex balloon filled with silicone gel encapsulated the pressure sensor housed in medical grade glass with a silicone cage wrapping everything together. The length of the catheter tip was 0.5 cm and the width was 1 cm. The second and third iteration of the telemetric transmitter used this design.
**A. Mesh Sheet**

The EMG leads were bonded to a mesh sheet made from silicone. There was a 1.5 cm of separation between the two EMG leads. 2 cm of the distal ends of the EMG leads were exposed and looped to form a lasso. The first iteration of the telemetric transmitter used this design.

**B. Silicone Plate**

A plate made from silicone was bonded to the EMG leads. The silicone plate contained suture holes. There was a 1.5 cm of separation between the two EMG leads. 7 cm of the distal ends of the EMG leads were exposed and looped to form a lasso. The second and third iteration of the telemetric transmitter used this design.

---

**Figure 3.4 Electromyography Leads and Plate Design**

(A) **Mesh Sheet.** The EMG leads were bonded to a mesh sheet made from silicone. There was a 1.5 cm of separation between the two EMG leads. 2 cm of the distal ends of the EMG leads were exposed and looped to form a lasso. The first iteration of the telemetric transmitter used this design. (B) **Silicone Plate.** A plate made from silicone was bonded to the EMG leads. The silicone plate contained suture holes. There was a 1.5 cm of separation between the two EMG leads. 7 cm of the distal ends of the EMG leads were exposed and looped to form a lasso. The second and third iteration of the telemetric transmitter used this design.
<table>
<thead>
<tr>
<th>Animal</th>
<th>Pves Sensor</th>
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<td>Standard</td>
<td>Standard</td>
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<tr>
<td></td>
<td>Barbell</td>
<td>Balloon</td>
<td>Caged-Balloon</td>
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</tbody>
</table>

Table 3.2 Summary of Implant Designs for each Animal

For each iteration of the transmitter, different Pves and Pabd catheter designs were implanted and evaluated for performance and durability. Each new modification was prompted by the results from the previous experiment.

### 3.2.4 Transmitter Implantation Surgery

Animals were anesthetized with 2% isoflurane and placed in a supine position. A 10 cm abdominal incision rostral to the pubic bone was made to expose the bladder. A 5 cm subcutaneous pocket was created on the right flank to house the telemetric transmitter. With the transmitter housed in the right flank, the Pves catheter of was tunneled subcutaneously through the abdomen and inserted 2-3 cm into the bladder through a 0.5 mm incision made in the dome. The suture ring attached to the vesicular catheter was sutured securely into the dome of the bladder using 4-0 taper Prolene. A bladder leak test with 60 mL of saline was performed to ensure there were no openings in the bladder after inserting the Pves catheter into the dome. The Pabd catheter of the unit was tunneled subcutaneously to both sides of the peritoneal cavity and secured to the abdominal wall using 2-0 taper Prolene (Figure 3.5A).
In the first experiment, the distal silicone tubing of the EMG leads was pulled back to expose the fine steel wires, and using a pair of hemostat clamps, the steel wires were looped back to form a “lasso”. The leads and the silicone mesh sheet were then inserted from the anterior side of the bladder and tunneled underneath the pelvic bone alongside the urethra as far down as possible using fingers.

Later, the protocol for the surgical implantation of the EMG leads was adjusted. First, a 20-gauge needle was used to find the obturator foramen. Then, a pair of blunt hemostats was inserted into the hole and followed until the base of the bladder was reached. A feeding tube was attached to the tip of the hemostat and pulled back through the obturator foramen. Sutures that were attached to the holes of the EMG silicone plate were then fed through the feeding tube and the plate was pushed under to sit on the urethra but below the bone of the pelvis. Finally, a button was used to tie the sutures together to reduce tension and damage on the underlying musculature (Figure 3.5B). Throughout the implantation surgery, continuous monitoring of the telemetric recordings was performed to ensure the signals from the transmitter, solid-state pressure catheters, and biopotential EMG wire electrodes were being received by the antenna and functioning properly. After successful implantation, the subcutaneous pocket created for the implant, abdominal muscle, and fascia layers at the incision site were sutured closed using 2-0 taper PDS II suture. A schematic summarizing the implantation location of the transmitter, pressure catheters, and EMG leads is shown in (Figure 3.5C).
Figure 3.5 Implantation and Suturing of Pressure Catheter and Electromyography Sensor

(A) A small incision on the bladder wall was made and the bladder pressure sensor was inserted into the bladder. The suture ring of the bladder pressure catheter was sutured to the dome of the bladder. The abdominal pressure catheter was placed in the peritoneal cavity and sutured to the abdominal wall. (B) The EMG plate was placed underneath the pelvic bone alongside the urethra. A surgical button was used to secure and tie the sutures of the EMG plate together. (C) Schematic demonstrating the implantation location of the telemetric transmitter, bladder and abdominal pressure catheters, and EMG leads in a pig model.
3.2.5 Spinal Cord Injury Protocol

We have previously established a porcine model of SCI using a custom weight-drop apparatus \(^{145,146}\) and described in Chapter 1, section 1.10.1. The biomechanical impact parameters for each animal is shown in Table 3.3.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Injury Level</th>
<th>Age (Days)</th>
<th>Weight (kg)</th>
<th>Force (Zeroed)</th>
<th>Impulse (kdynes*s)</th>
<th>Distance (mm)</th>
<th>Velocity (mm/s)</th>
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</thead>
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<td>153</td>
<td>26.5</td>
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<td>4344</td>
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<td>26</td>
<td>3201.4</td>
<td>11.5</td>
<td>3.5</td>
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<td>9.9</td>
<td>3.5</td>
<td>1803</td>
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<td>3170.5</td>
<td>10.3</td>
<td>3.6</td>
<td>1807</td>
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<td>31.5</td>
<td>2893.6</td>
<td>10.1</td>
<td>3.1</td>
<td>1612</td>
</tr>
<tr>
<td>9047</td>
<td>T10</td>
<td>353</td>
<td>35.6</td>
<td>2997.8</td>
<td>10.2</td>
<td>3.3</td>
<td>1665</td>
</tr>
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<td><strong>Mean ± SEM</strong></td>
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<td><strong>238 ± 30</strong></td>
<td><strong>34 ± 3</strong></td>
<td><strong>3029 ± 30</strong></td>
<td><strong>10.6 ± 0.3</strong></td>
<td><strong>3.4 ± 0.1</strong></td>
<td><strong>1748 ± 35</strong></td>
</tr>
</tbody>
</table>

Table 3.3 Individual Biomechanical Impact Parameters

The table below summarizes the injury level, age and weight on the day of SCI, as well as the biomechanical impact parameters for all animals in this study. Distance represents the distance the impact tip travelled. Velocity represents the velocity the impact tip travelled.

3.2.6 Post-Operative Animal and Bladder Care

After implantation of the telemetric transmitter, animals were individually housed for 7 days. During this period, the animals received antibiotics (Baytril 10 mg/kg, once daily), an anti-inflammatory (Meloxicam 0.2 mg/kg, once daily) and an analgesic fentanyl patch (mg/kg/hr) was placed immediately after surgery. Fluid output and vitals were continuously monitored by animal care technicians for 24 hours. A recovery period of at least 1 month was given to the animals prior
to filling cystometry experiments. During this time, in-person recordings were performed to ensure the transmitter was still functioning. The telemetry system was determined to be calibrated properly if we observed equal pressure response in the Pves and Pabd channels during a vocalization event similar to a cough test in a human filling cystometry study \(^{212}\).

After SCI, the same animal surgical care protocol was performed. During this period, the bladder was managed using an indwelling Foley catheter that was placed during surgery. After 7-10 days, the bladder catheter was removed, and their bladders were allowed to drain spontaneously for the remainder of the study period. Daily visual inspection of the urine for signs of clinical UTIs was performed by the facility’s animal care technicians. The protocol for the diagnosis and treatment of clinically relevant UTIs was previously described in detail by Chapter 2.

### 3.2.7 Filling Cystometry

I have previously established a training plus setup protocol to perform conventional cystometry in awake, conscious female Yucatan minipigs (Chapter 2). Briefly, the animals were sling-trained for 4 weeks to acclimatize them to the sling and the testing room. On the day of cystometry, an intramuscular injection of dexmedetomidine (0.005 mg/kg) was given to the animal in its pen. Thirty minutes after the injection, the animal was transported to the testing room and transferred into a restraint sling. The vulva and the surrounding areas are cleaned with aseptic technique for the insertion of pressure catheters into the bladder and rectum to measure Pves and Pabd, respectively. EMG patch electrodes were placed perianal at 9 and 3 o’clock and the ground EMG patch was placed on the left knee. A flow meter was placed below the pig, and urine was caught
with a funnel by an attendant. Body temperature saline (0.9% NaCl) was infused into the bladder at a rate equivalent to the animal’s current weight (1 ml/min per kg) until the animal voided or leaked. In uninjured pigs, voiding was prompt when the pig squatted. To support the lower abdomen and hindlimbs of post-SCI pigs to a point where they are almost standing, a harness was attached to the pig and tied to the restraint cage with a hammock-style sling supporting the weight of the animal. Data was collected and recorded using UDS-120 (LABORIE, Montreal, Québec, Canada). Telemetric recording was performed concurrently during the test. In uninjured pigs, one fill and void cycle was recorded. Three or more fill and leak cycles were recorded for SCI pigs. At the end of the UDS, the equipment was detached, and the pigs were transported back to their pens. The bladders of SCI pigs were not drained to prevent physical damage to the telemetry Pves catheter.

3.2.8 Ambulatory Telemetric Measurements

Ambulatory recordings were performed with the animals in their pens twice a week up until SCI surgery. Each recording was conducted between 8 am and 1 pm. Two hours of recording was performed for each animal. During the recordings, the animals were separated into two adjacent pens. The receiver was placed outside the pen and the entire session occurred with concurrent video recording (Figure 3.6). Voiding in non-SCI pigs was prompted with squatting, with the start and end of the void marked by the attendant. In n = 2 animals, a free catch of voided urine was collected by using a specimen container attached to a long handle under the urine stream to measure the volume voided.
Figure 3.6 Setup for Weekly Telemetry In-Pen Recordings

During weekly in-person recordings, the receiver antenna was placed just outside the pen. Animals performed their natural behaviour during recordings. Concurrent video recording occurred during telemetric recordings. Telemetric signals were transmitted to the receiver antenna and data was recorded using Notocord-HEM (NOTOCORD, Le Pecq, France) on a computer. The start and end of the voids were marked in-person.

A different setup for post-SCI recordings was developed since the injury model resulted in lower body paralysis which made visualization of the urine stream difficult to trace. To help visualize the flow of urine, mats were placed inside the pen and covered with light colored blankets.
Recording required two personnel, one to mark down the time of urination/leakage and one inside the pen repositioning the animal when needed to allow visualization of urine leakage.

### 3.2.9 Bladder Histology

At the end of the study period, the animals were euthanized with an IV overdose of sodium pentobarbital (120 mg/kg), after being sedated deeply with Telazol (4-6 mg/kg) intramuscularly. Post-euthanasia, the telemetry transmitter, pressure catheters and EMG leads were dissected and extracted from the animal. The bladder was drained, weighed, then harvested and divided into the dome, body, neck, and urethra. Sections were placed into 10% formalin with a minimum volume to tissue ratio of 20:1 at 4°C for 72 hours within 30 minutes of harvesting. Sections were embedded into paraffin blocks and sectioned serially at 5 um by Wax-it Histology (Vancouver, BC, Canada). Slides were stained in hematoxylin and eosin \(^{166}\) and Masson’s Trichrome stain \(^{167}\). In particular, the area of the dome where the Pves catheter was inserted into was thoroughly examined for histopathologic features. Control bladders were taken from animals that had acute SCI (< 12 hours).

### 3.2.10 Data and Statistical Analysis

The telemetric-CMG parameters measured in the filling phase include Pves, Pabd, Pdet (Pves-Pabd) from the start of the fill to the start of the voiding phase. During the voiding phase, the amplitude of the detrusor contraction (if there was a distinct voiding contraction), voiding time,
and EUS EMG activity was examined. The quality of the recorded telemetric EMG data was assessed by visual comparison between the CMG and telemetric EMG traces.

In a restraint-free setting, changes in Pves, Pabd, and Pdet from the start of the fill to the start of the void/leak, along with the frequency of urination/leakage, and voiding time (for uninjured pigs) was analyzed. Volume of urine voided was measured and averaged for n = 2. After SCI, neurogenic detrusor overactivity (NDO) events were characterized as changes in the Pdet channels that had a waveform characteristic. Number of leakage events that were associated with an NDO event was quantified. We refrained from identifying detrusor-sphincter dyssynergia due to the absence of concurrent voiding cystourethrography and to avoid misdiagnosis.

Comprehensive analyses on telemetry and CMG data were only performed in animals that had functional telemetric Pves, Pabd, and Pdet readings. Signal dropouts were filtered by adjusting the output domain on the NOTOCORD-hem software. Telemetric pressure data were downsampled from 500 Hz to 10 Hz to match the frequency of the CMG pressure data for statistical analysis. In addition, pressures were zeroed to CMG baseline pressures. A moving average filter with an interval of 30 was applied to the data to filter out movement artifacts. Pressure and EMG data was not filtered for presentation of the figures. Statistical analyses were carried out using GraphPad Prism 8 (GraphPad Software, Inc., California, USA). A Pearson’s correlation coefficient was performed to compare the CMG and telemetry pressure data after time matching the CMG and telemetry pressure data. Correlation values less than 0.3 were considered to be very weak, values between 0.3-0.5 were weak, values between 0.5-0.7 were moderate, and values greater than 0.7 were strong. Results are presented as mean ± SEM. Differences were considered to be statistically significant at $P < 0.05$. 

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3.3 Results

3.3.1 General Health Observations after Implantation of Transmitter

Using our surgical implantation protocol, all telemetric transmitters were implanted successfully with minimal complications during the surgery. Acutely after implantation surgery, seromas developed but this did not lead to any major health issues. During the study period, there were no medical issues such as severe dermal infections at the site where the telemetric transmitter was placed. Transmitters migrated slightly from its original position in all animals during the study. N = 3 out of 6 post-SCI animals (50%) had a concomitant UTI in at least one post-SCI CMG recordings. However, no UTIs were detected prior to SCI in any of the animals after implantation of the transmitter therefore the presence of the Pves catheter was most likely not a contributing factor to the UTI.

3.3.2 Adjustments and Improvements in Pressure Sensor Designs

After implantation of the telemetric transmitter, there were issues with the durability of the several pressure sensor designs most likely attributed to the animal laying down while it was sleeping or from rough play. A summary of each sensor’s durability in each experiment is shown in Figure 3.7.
Figure 3.7 Intravesical (Pves) and Abdominal Pressure (Pabd) Sensor Longevity, Represented by Bars

Numbers indicate the beginning of each week after implantation. Red bars indicate duration of spinal cord injury (SCI). In general, the Standard Pves design was more durable compared to the Barbell Pves design. In terms of Pabd designs, the most robust designs were the Caged and Caged-Balloon lasting anywhere from 7 to 18 weeks after implantation. While the Standard Pabd design lasted for 12 weeks in one animal (*), signal drift was a major issue resulting in poor quality detrusor pressure readings.

3.3.2.1 Intravesical Pressure Sensor

While the Barbell design demonstrated the capability of monitoring changes in Pves, none of the sensors (0%) remained functional until the end of the study period and malfunctioned after 1 week and 7 week after implantation. The best results were obtained with the Standard design, where all four sensors (100%) remained fully functional until experimental termination (12-16-week study period).
3.3.2.2 Abdominal Pressure Sensor

One out of the two Standard sensors (50%) remained functional, whereas the remaining sensor malfunctioned 2 weeks after implantation. For the remaining sensor, however, signal quality was poor and noisy which affected the Pdet channel readings. Similarly, none of the Balloon sensors (0%) remained functional, and both malfunctioned 2 weeks after implantation. One of three Caged-Balloon sensors (33%) malfunctioned 7 weeks after implantation. The remaining sensors remained functional until experimental termination (week 16-18). The Caged design demonstrated the best results, with all three sensors (100%) remaining fully functional with satisfactory signal quality throughout the 16-18-week study period (4-11 weeks of SCI).

3.3.2.3 Electromyography Leads

There were no major issues with the EMG sensor becoming non-functional the study period in all animals. However, during extraction of the device from the animals, the surgical button used to fixate the position of curved silicone plate below the pubic bone was missing in n = 4 which suggested that the EMG silicone plate had shifted from its original placement which was also a major problem with the initial EMG plate mesh sheet design in n = 2. The exact timepoint when the plate shifted from its original position could not be identified on the recordings. However, since EMG recordings were optimal during pre-injury recordings, movement of the silicone plate most likely occurred during the period of SCI.
Overall, the best telemetric recording results obtained during filling cystometry were from the Standard Pves design, Caged and Caged-Balloon Pabd designs with the EMG leads bonded to a silicone plate. The following filling cystometry and weekly in-person recording results will be presented from n = 4 that received any of the listed designs.

### 3.3.3 Telemetry versus Conventional Cystometrogram Recordings

The total number of conventional CMG with concurrent telemetric recordings performed is summarized in (Table 3.4). In total, 22 CMG with concurrent telemetric were performed. 9/22 (41%) were performed in uninjured pigs and 13/22 (59%) were performed in post-SCI pigs at various timepoints after injury. 5/22 (23%) CMG were performed with the Standard Pves and Pabd sensors. 6/22 (27%) CMG were performed with the Standard Pves and Caged/Caged-Balloon Pabd sensors. Finally, 11/22 (50%) CMG were performed with the Barbell Pves and Caged/Caged-Balloon Pabd sensors.

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<tr>
<th>Experiment</th>
<th>Animal</th>
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<th>Pre-SCI after Implantation</th>
<th>2 Weeks Post-Injury</th>
<th>3/4 Weeks Post-Injury</th>
<th>8 Weeks Post-Injury</th>
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</table>

Table 3.4 Total Cystometry Performed in an Awake Uninjured and Spinal Cord Injured (SCI) Pigs

Summary table of the number of conventional cystometry performed. A total of 22 conventional cystometry with concurrent telemetric recordings was performed. X denotes studies with concurrent telemetric recordings. O denotes studies performed without concurrent telemetric recordings. * concomitant UTI during cystometry.
Animals that had a concomitant UTI during the CMG recording were not excluded for analysis since the purpose of this phase was to examine the congruency between the telemetry and CMG pressures.

### 3.3.3.1 Pre-Injury Recordings

Overall, pressure and EUS EMG recordings measured by the telemetric transmitter demonstrated comparable tracings with the conventional urodynamic pressure catheters and EMG patch electrodes in uninjured pigs. Correlation between the telemetry and Pves catheters was generally strong based on the coefficients. However, correlation between the telemetry and Pabd catheters ranged from very weak to strong when considering all animals. In addition, agreement between the two systems varied when measuring the change in pressures from the start of the fill to the start of the void. A representative tracing at 5 weeks post-implantation with the Standard Pves, Caged-Balloon Pabd, and EMG silicone plate is shown in Figure 3.8.
Figure 3.8 Comparison of Cystometrogram (CMG) and Telemetric Recordings during Filling Cystometry in an Awake, Uninjured Pig

From top to bottom: Detrusor pressure (Pdet), sphincter electromyography (EMG), flow rate (ml/s), and volume infused (VH2O). (A) Filling Phase. Prior to filling, this animal received sedation and reversal. During the filling phase, both the CMG and telemetric recordings demonstrated comparable detrusor pressure tracings. There is little change in Pdet during the filling indicative of good bladder compliance. The voiding phase occurs in the highlighted area (B). (B) Void. The void occurs at 889 ml. The amplitude of the contraction was quite similar between the two systems with the CMG measuring a ΔPdet of 16 cm H2O and the telemetry measuring a ΔPdet of 14 cm H2O.
During the void, there is quietening of EMG activity (arrowhead) in both systems with the telemetry picking up some movement artifact near the end of the void. At the end of the void, an increase in EMG activity indicates contraction of the EUS (arrowhead) followed by a postvoiding detrusor contraction (star) which is seen in both systems.

For animal 6502, the average CMG ΔPves (25 ± 2 cm H2O) was smaller compared to the telemetry Standard ΔPves (35 ± 3 cm H2O). Average CMG ΔPabd (3 ± 1 cm H2O) was also smaller compared to the telemetry Caged Balloon ΔPabd (12 ± 4 cm H2O). However, CMG ΔPdet (22 ± 2 cm H2O) was comparable to Caged Balloon ΔPdet (23 ± 4 cm H2O). There was a strong correlation between the telemetry and conventional CMG Pves catheters. At 5, 9, and 11 weeks post-implantation, the Pearson correlation coefficient for Pves was r = 0.77, P < 0.0001; r = 0.80, P < 0.0001; r = 0.84, P < 0.0001, respectively. However, the correlation between the telemetry and conventional CMG Pabd was very weak to weak. At 5, 9, and 11 weeks post-implantation, the Pearson correlation coefficient for Pabd was r = 0.34, P < 0.0001; r = 0.13, P < 0.0001; r = 0.37, P < 0.0001, respectively. Therefore, the correlation between telemetry and conventional CMG for Pdet ranged from very weak to strong (r = 0.71, P < 0.0001; r = 0.24, P < 0.0001; r = 0.61, P < 0.0001).

For animal 6504 at 4 weeks post-implantation, the ΔCMG Pves (30 cm H2O) was greater compared to the telemetry ΔStandard Pves (21 cm H2O) (r = 0.92, P < 0.0001). The ΔCMG Pabd (0 cm H2O) was smaller compared to the telemetry ΔCaged Pabd (5 cm H2O) (r = 0.80, P < 0.0001). ΔCMG Pdet (30 cm H2O) was greater compared to ΔCaged Pdet (16 cm H2O) (r = 0.85, P < 0.0001).
3.3.3.2 Post-Spinal Cord Injury

Telemetry and CMG Pves traces post-SCI was generally strong based on the coefficients for both animals. Pabd traces varied between the two systems and based on the coefficients, was very weak overall. In addition, confidence in the post-SCI EUS EMG recordings was low due to the fact that the plate had shifted from its original position during extraction of the implant from all animals. Shown in Figure 3.9 is a tracing from the same animal in Figure 3.8, but with a T10 SCI and at 3 weeks post-injury.
Figure 3.9 Comparison of Cystometrogram (CMG) and Telemetry Recordings during Filling Cystometry in a Spinal Cord Injured (SCI) Pig.

From top to bottom: Detrusor pressure (Pdet), sphincter electromyography (EMG), flow rate (ml/s), and volume infused (VH2O). At 3 weeks post-SCI, we can see a different urodynamic pattern emerges. (A) Filling Phase. Prior to the filling phase, this animal received sedation and reversal. During the filling phase, both the CMG and telemetric recordings demonstrated comparable detrusor pressure tracings. Neurogenic detrusor overactivity (NDO) events preceding the leak can be seen in (B).
(B) NDO and Leak. Distinct NDO events can be visualized prior to the first leak which occurs at an infusion volume of 963 ml. At the start of the leak, there is terminal detrusor overactivity. The amplitude of the contraction was also quite similar between the two systems with the CMG measuring a ΔPdet of 13 cm H₂O and the telemetry measuring a ΔPdet of 10 cm H₂O. There is inefficient emptying of the bladder as indicated by the poor flow (arrow). Both systems did not detect significant EMG activity during both the filling and leakage phase.

For animal 6502 at 3 weeks post-injury, the ΔCMG Pves (23 cm H₂O) was smaller compared to the telemetry ΔStandard Pves (31 cm H₂O), however the correlation was strong (r = 0.83, P < 0.0001). ΔCMG Pabd (-3 cm H₂O) was smaller compared to the telemetry ΔCaged Balloon Pabd (0 cm H₂O) but correlation was very weak (r = 0.11, P < 0.0001). ΔCMG Pdet (23 cm H₂O) was also smaller compared to ΔCaged Balloon Pdet (31 cm H₂O) (r = 0.74, P < 0.0001).

Animal 6504 had a concomitant UTI during the study. At 4 weeks post-injury, the ΔCMG Pves (30 cm H₂O) was smaller compared to the telemetry ΔStandard Pves (22 cm H₂O) (r = 0.79, P < 0.0001). The ΔCMG Pabd (0 cm H₂O) was comparable to the telemetry ΔCaged Pabd (1 cm H₂O) but there was a weak negative correlation (r = -0.38, P < 0.0001). ΔCMG Pdet (30 cm H₂O) was slightly greater compared to the telemetry ΔCaged Balloon Pdet (21 cm H₂O) but the correlation was also weak (r = 0.13, P < 0.0001).

3.3.4 Ambulatory Telemetric Recordings in Uninjured Pigs

In total, 105 voiding events were captured using telemetry in a restraint-free setting from N = 2 uninjured pigs. 56 voids (53%) were recorded for animal 6502 and 49 voids (47%) were recorded for animal 6504 during the 14-week recording period.
3.3.4.1 Uninjured Pig’s In-Pen Voiding Profile

Telemetry pressure recordings stabilized and changes in pressure became more consistent at approximately 3 to 4 weeks post-implantation. Detrusor overactivity induced by the implantation of the pressure catheter was not observed in any animals before SCI. A representative voiding detrusor contraction from an uninjured pig is shown in Figure 3.10A.
Figure 3.10 Telemetric Pressure and External Urethral Sphincter Electromyography (EUS EMG) Recordings in a Restraint-Free Setting with Natural Bladder Filling.

From top to bottom: Intravesical pressure (Pves), abdominal pressure (Pabd), detrusor pressure (Pdet), and external urethral sphincter electromyography (EMG). (A) Uninjured. At the start of the void, there is an increase in Pves, Pabd, and Pdet. The amplitude of the detrusor contraction was 27 cm H$_2$O. During the void, the increase in Pdet is sustained and the EUS is relaxed (arrowhead) during the void. Near the end of the void, there is a decrease in Pdet and an increase in EMG activity suggesting contraction of the EUS (arrowhead). Afterwards, there is a large postvoiding detrusor contraction. (B) Spinal cord injury (SCI). Following SCI, the same pig demonstrated leakage with neurogenic detrusor overactivity (NDO). Prior to the leak, a slight increase in Pves and Pabd is seen. The amplitude of the detrusor contraction was 14 cm H$_2$O. The leak occurs after the NDO event (star) and is short. During the leak, there is an increase in EMG activity (arrowhead), possibly signifying detrusor-sphincter dyssynergia. The pressures return to baseline after the leak.
3.3.4.2 Consistent Changes in Pressure Readings in Uninjured Pigs

12 weeks of in-pen recordings from animals 6502 and 6504 implanted with the second iteration revealed consistent changes in Pves, Pabd, and Pdet with small standard errors.

On average, the $\Delta \text{Standard Pves}$ was $29 \pm 1.0 \text{ cm H}_2\text{O}$, $\Delta \text{Caged Balloon Pabd}$ was $8 \pm 0.4 \text{ cm H}_2\text{O}$, and $\Delta \text{Pdet}$ was $21 \pm 0.7 \text{ cm H}_2\text{O}$ for animal 6502. The average voiding time was $8 \pm 0.4 \text{ sec}$. The frequency of micturition in a span of a 2-hour recording session ranged from 1 to 6 times. For animal 6504, $\Delta \text{Standard Pves}$ was $29 \pm 0.7 \text{ cm H}_2\text{O}$, $\Delta \text{Caged Pabd}$ was $8 \pm 0.5 \text{ cm H}_2\text{O}$, and $\Delta \text{Pdet}$ was $21 \pm 0.6 \text{ cm H}_2\text{O}$. The average voiding time was $6 \pm 0.3 \text{ sec}$. The frequency of micturition in a span of a 2-hour recording session ranged from 1 to 9 times.

On average, $\Delta \text{Caged Pabd}$ was $8 \pm 0.3 \text{ cm H}_2\text{O}$ and $\Delta \text{Caged Balloon Pabd}$ was $7 \pm 0.4 \text{ cm H}_2\text{O}$ for animal 9045. The average voiding time was $6 \pm 0.2 \text{ sec}$. The average voided volume was $135 \pm 6 \text{ ml}$. The frequency of micturition in a span of a 2-hour recording session ranged from 2 to 9 times. Similarly, there were consistent changes in Pabd for animal 9047. The average $\Delta \text{Caged Pabd}$ was $9 \pm 0.3 \text{ cm H}_2\text{O}$ and $\Delta \text{Caged Balloon Pabd}$ was $7 \pm 0.2 \text{ cm H}_2\text{O}$. The average voiding time was $9 \pm 0.3 \text{ sec}$. The average voided volume was $177 \pm 6 \text{ ml}$. The frequency of micturition in a span of a 2-hour recording session ranged from 4 to 12 times.

A table summary of the physiologic in-pen voiding parameters is shown in Table 3.5
<table>
<thead>
<tr>
<th>Animal #</th>
<th>ΔStandard Pves (cm H₂O)</th>
<th>ΔCaged Pab (cm H₂O)</th>
<th>ΔCaged Pdet (cm H₂O)</th>
<th>ΔCaged Balloon Pab (cm H₂O)</th>
<th>ΔCaged Balloon Pdet (cm H₂O)</th>
<th>Voiding Time (sec)</th>
<th>Voided Volume (ml)</th>
<th>Frequency of Micturition per 2 hours (times)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6502</td>
<td>29 ± 1.0</td>
<td>-</td>
<td>-</td>
<td>8 ± 0.4</td>
<td>21 ± 0.7</td>
<td>8 ± 0.4</td>
<td>-</td>
<td>1 to 6</td>
</tr>
<tr>
<td>6504</td>
<td>29 ± 0.7</td>
<td>8 ± 0.5</td>
<td>21 ± 0.6</td>
<td>-</td>
<td>-</td>
<td>6 ± 0.3</td>
<td>-</td>
<td>1 to 9</td>
</tr>
<tr>
<td>9045</td>
<td>-</td>
<td>8 ± 0.3</td>
<td>-</td>
<td>7 ± 0.4</td>
<td>-</td>
<td>6 ± 0.2</td>
<td>135 ± 6</td>
<td>2 to 9</td>
</tr>
<tr>
<td>9047</td>
<td>-</td>
<td>9 ± 0.3</td>
<td>-</td>
<td>7 ± 0.2</td>
<td>-</td>
<td>9 ± 0.3</td>
<td>177 ± 6</td>
<td>4 to 12</td>
</tr>
</tbody>
</table>

Table 3.5 Uninjured Pigs In-Pen Voiding Parameters.

Weekly in-person recordings revealed consistent changes in all pressure channels with small standard error. Voiding time was short, ranging from 6 to 9 secs. Most importantly, the voided volume recorded from 2 animals was low compared to bladder capacities seen in uninjured Experiment 2 animals from Chapter 2 which was on average about 600 ml.

3.3.5 Ambulatory Telemetric Recordings in Spinal Cord Injured Pigs

In total, 43 leakage events were captured from SCI pigs in a free-moving setting from N = 2 pigs. 20 leakage events (15%) were recorded for animal 6502 and 80% (16/20) of the leaks were associated with an NDO event. In contrast, 23 leakage events (17%) were recorded for animal 6504 where 57% (13/23) of the urination events were associated with an NDO event.

After SCI, pigs had impaired bladder emptying and leaks were occasionally associated with NDO. A leak was associated with an NDO event from a SCI animal at 4 weeks post-injury is shown in Figure 10B and compared against a void captured from the same animal before injury (Figure 10A). At the start of the leak, a slight rise in Pves and Pdet is observed whereas Pabd slightly decreases. The duration of the leak is short and occurs as Pves and Pdet begin to decrease. There
could also potentially be detrusor-sphincter dyssynergia as there is increased EMG activity during the NDO event.

3.3.5.1 Lower Detrusor Pressures during Leakage after Spinal Cord Injury

On average, four weeks of telemetry recording post-SCI revealed lower Pves, Pabd, and Pdet values with a slightly larger standard error compared to pre-injury values. For animal 6502, the $\Delta$Standard Pves was $12 \pm 2$ cm H$_2$O, average $\Delta$Caged-Balloon Pabd was $5 \pm 1$ cm H$_2$O, and average $\Delta$Pdet was $8 \pm 2$ cm H$_2$O. The frequency of leakage in a span of a 2-hour recording session ranged from 1 to 11 times. For animal 6504, the $\Delta$Standard Pves was $7 \pm 4$ cm H$_2$O, $\Delta$Caged-Balloon Pabd was $3 \pm 1$ cm H$_2$O, and $\Delta$Pdet was $4 \pm 3$ cm H$_2$O. The frequency of leakage in a span of a 2-hour recording session ranged from 1 to 14 times. A summary table of the results is shown in Table 3.6.

<table>
<thead>
<tr>
<th>Animal #</th>
<th>$\Delta$Standard Pves (cm H$_2$O)</th>
<th>$\Delta$Caged Pabd (cm H$_2$O)</th>
<th>$\Delta$Caged Pdet (cm H$_2$O)</th>
<th>$\Delta$Caged Balloon Pabd (cm H$_2$O)</th>
<th>$\Delta$Caged Balloon Pdet (cm H$_2$O)</th>
<th>Leakage Events Associated with NDO (events)</th>
<th>Total Leakage Events Captured (events)</th>
<th>Frequency of Micturition per 2 hours (times)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6502</td>
<td>12 ± 2</td>
<td>-</td>
<td>-</td>
<td>5 ± 1</td>
<td>8 ± 2</td>
<td>16</td>
<td>20</td>
<td>1 to 11</td>
</tr>
<tr>
<td>6504</td>
<td>7 ± 4</td>
<td>3 ± 1</td>
<td>4 ± 3</td>
<td>-</td>
<td>-</td>
<td>13</td>
<td>23</td>
<td>1 to 14</td>
</tr>
</tbody>
</table>

Table 3.6 Spinal Cord Injured Pigs In-Pen Voiding Parameters

Weekly in-person recordings revealed variable changes in all pressure channels. Changes in pressure after SCI were smaller compared to pre-injury pressures. Neurogenic detrusor overactivity (NDO) was observed and was associated with approximately 57% and 80% of the leaks for animal 6504 and 6502, respectively.
3.3.5.2 Comparison of Voiding Parameters during a Restraint-Free Void versus a Cystometrogram Void in Uninjured Pigs

There were differences in the pressure-flow parameters between the ambulatory recorded voids versus the CMG voids. In animal 6502, the amplitude of the detrusor contraction was $21 \pm 0.7$ cm H2O in an ambulatory setting. In comparison, from three CMG voids recorded, the $\Delta P_{\text{det}}$ was $11 \pm 3$ cm H2O. Animals 6504 did not demonstrate a distinct detrusor contraction during the void in both the CMG and telemetry systems.

Another major difference was that the voiding time was substantially shorter in the ambulatory setting versus during CMG. On average, the duration of urination was $8 \pm 0.4$ sec in the ambulatory setting whereas it was $26 \pm 0.6$ sec during CMG. This was a similar finding for animal 6504 (CMG: 44 sec, ambulatory: $6 \pm 0.3$ sec), animal 9045 (CMG: 43 sec, ambulatory: $6 \pm 0.2$ sec), and animal 9047 (CMG: 33 sec, 32 sec; ambulatory: $9 \pm 0.3$ sec).

3.3.6 Histopathologic Observations

Common histopathologic features observed in all SCI bladders with a telemetry Pves catheter implanted included mild to moderate chronic inflammation in the submucosa, detrusor hypertrophy, and increased bladder wall thickness in the dome and body regions of the bladder. A summary table of the injury level sustained, weeks post-SCI, age, body and bladder weight for at euthanasia each animal is shown in Table 3.7.
<table>
<thead>
<tr>
<th>Animal</th>
<th>Injury Level</th>
<th>Weeks Post-Injury</th>
<th>Age (days)</th>
<th>Weight (kg)</th>
<th>Bladder Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5042</td>
<td>T10</td>
<td>10</td>
<td>230</td>
<td>40</td>
<td>28</td>
</tr>
<tr>
<td>4344</td>
<td>T10</td>
<td>10</td>
<td>251</td>
<td>37</td>
<td>44</td>
</tr>
<tr>
<td>6502</td>
<td>T10</td>
<td>4</td>
<td>291</td>
<td>48</td>
<td>126</td>
</tr>
<tr>
<td>6504</td>
<td>T10</td>
<td>4</td>
<td>286</td>
<td>43</td>
<td>110</td>
</tr>
<tr>
<td>9045</td>
<td>T10</td>
<td>11</td>
<td>316</td>
<td>34</td>
<td>47</td>
</tr>
<tr>
<td>9047</td>
<td>T10</td>
<td>11</td>
<td>282</td>
<td>40</td>
<td>45</td>
</tr>
<tr>
<td><strong>Mean ± SEM</strong></td>
<td></td>
<td><strong>276 ± 13</strong></td>
<td><strong>40 ± 2</strong></td>
<td><strong>67 ± 17</strong></td>
<td></td>
</tr>
<tr>
<td>7914</td>
<td>(Control) T10 (&lt; 12 hrs)</td>
<td>N/A</td>
<td>157</td>
<td>27</td>
<td>24</td>
</tr>
<tr>
<td>7928</td>
<td>(Control) T10 (&lt; 12 hrs)</td>
<td>N/A</td>
<td>149</td>
<td>24</td>
<td>12</td>
</tr>
<tr>
<td>7932</td>
<td>(Control) T10 (&lt; 12 hrs)</td>
<td>N/A</td>
<td>152</td>
<td>22</td>
<td>12</td>
</tr>
<tr>
<td><strong>Mean ± SEM</strong></td>
<td></td>
<td><strong>153 ± 2</strong>*</td>
<td><strong>24 ± 1</strong></td>
<td><strong>16 ± 4</strong></td>
<td></td>
</tr>
</tbody>
</table>

Table 3.7 Summary Table of the Injury Level, Weeks Post-Injury, Age at Euthanasia, Body Weight, and Bladder Weight.

Control bladders were taken from animals with an acute SCI (< 12 hours). A t-test was performed between SCI and control. A T-Test was performed to compare the differences between the control and SCI group. * significant difference between SCI and control, P < 0.05, ** P < 0.01, *** P < 0.001. There were no significant differences between SCI and control bladder weight.
Thorough investigation into the area where the telemetry Pves catheter was inserted revealed focal ulceration with granulation tissue in animal 6504 and focal eosinophilic epithelial microabcesses in animal 9045. The remaining animals did not have any significant histopathologic observations in the same area. Differences in the degree of inflammation of the submucosa between control, SCI (12 weeks post-injury), and SCI (4 weeks post-injury) with telemetry Pves catheter insertion is shown in Figure 3.11.
Figure 3.11 Histopathologic Features of the Bladder after Spinal Cord Injury (SCI) and Implantation of a Telemetric Pressure Catheter.

(A) Control. Bladder section taken from a control animal and stained in H&E. Submucosa from the dome of the bladder is shown. Image taken at 10x demonstrating mild inflammation of the submucosa (red circle).  

(B) SCI. Bladder section taken from a SCI animal 12 weeks post-injury (WPI) and stained in H&E. Submucosa from the dome of the bladder is shown. Image taken at 10x demonstrating mild to moderate chronic inflammation of the submucosa (red circle).

(C) SCI and intravesical pressure catheter insertion. Bladder section taken from a SCI 4 WPI and stained in H&E. Submucosa from the dome of the bladder is shown. Image taken at 10x demonstrating focal ulceration of the urothelium (yellow arrow) with signs of acute and chronic inflammation along with granulation tissue (red circle). Only n = 1 had an ulcer near where the pressure catheter was inserted. The rest of the bladders that had a telemetry implant shared similar histopathologic features as (B).
3.4 Discussion

Radio-telemetry systems can provide real-time longitudinal monitoring of lower urinary tract function from an animal in restraint-free conditions with natural bladder filling. Previous studies have investigated the use of these studies in a pig model and shown the feasibility of using these systems 140,141. In this study, we designed and implanted a telemetric transmitter (TSE systems, Chesterfield, MO, USA) into female Yucatan pigs to evaluate their practicality to provide measurements of Pdet and EUS EMG activity in uninjured and SCI pigs. There were no detrimental health effects after implantation and the animals tolerated the devices very well during the study period. No major differences were observed when comparing the CMG parameters before and after telemetric implantation, however there was still uncertainty if the implantation of the telemetry Pves catheters influenced the voiding mechanics. Major findings include demonstrating comparable Pdet tracings and EUS EMG activity between the CMG and telemetry pressure tracings in both uninjured and SCI pigs. Moreover, telemetric recordings in a restraint-free setting revealed larger detrusor contractions and shorter voiding times compared to voids captured in a cystometry setting.

3.4.1 Influence of Telemetry Catheter Insertion into the Bladder

After the invasive implantation of the Pves catheter into the bladder, it took approximately 3 to 4 weeks for the recordings to stabilize and demonstrate consistent changes in pressure during voids. In the weeks following implantation, there were no detrusor overactivity events noted during weekly in-person recordings. This could imply that implantation of the Pves catheter into the dome
of the bladder caused minimal damage and irritation to the bladder. UTIs were also not noted after implantation further emphasizing that the implantation of the telemetry Pves catheter was minimally invasive.

### 3.4.2 Uninjured Pig’s Physiologic Voiding Profile

Overall, the results obtained from in-person recordings of uninjured pigs were within the expected physiological range of Pdet, when compared to another Yucatan minipig study by Zhichen et al. A distinct detrusor contraction during the void, followed by a larger postvoiding detrusor contraction at the end of the void, was also comparable to what other studies in minipig models have shown. Since this has been seen in other pig models, this postvoiding detrusor contraction phenomenon is likely be simply reflect how the pigs naturally void. Interestingly, a larger after-contraction was not typically observed during filling cystometry. Furthermore, the postvoiding detrusor contraction was not detected in all uninjured animals. In contrast, an postvoiding detrusor contraction was observed in all uninjured pigs consistently during weekly in-pen telemetry recordings. All these observations point to the fact that a larger postvoiding detrusor contraction is considered to be an uninjured female Yucatan minipig’s “physiologic” voiding profile. Alternatively, this larger postvoiding detrusor contraction could be attributed to the bladder pressing onto the pressure catheter as it collapses as this was what we discovered when we pressed the pressure catheter against the bladder wall during our bench top testing at euthanasia. The mystery of the postvoiding detrusor contraction remains unsolved but since it is not occurring when the animal is actively voiding, it is unclear how clinically significant it is, despite it overshadowing the actual voiding contraction in size.
3.4.3 Spinal Cord Injured Pig’s Physiologic Voiding Profile

In-person recordings on post-SCI pigs was challenging since leakage events were difficult to observe given the lower abdomen paralysis of the animals which obstructed the visualization of urine. In addition, leakage would sometimes occur from the animal dragging their lower abdomen across the floor. This made it especially difficult to identify and differentiate patterns of NDO and DSD from the noise created by the animal dragging their lower abdomen across the bedding. We have previously characterized the pig’s bladder function up to 17 weeks post-SCI with filling cystometry. We observed larger cystometric capacities and NDO in 68% of the SCI animals which may have been potentially induced by various aspects of our UDS protocol as we have previously discussed (Keung et al., 2020, unpublished). While NDO was observed in this study during weekly in-pen recordings using telemetry, this study would have benefitted from a longer duration of telemetry recording as the animals were only kept for 4 weeks after SCI.

3.4.4 Comparison between Telemetry and Urodynamics

Filling cystometry with concurrent telemetric recording studies revealed comparable pressure and EMG readings between the CMG and telemetry systems during the void. The presence of both the cystometry and telemetry catheter in the bladder did not appear to negatively influence bladder function. At the start of the filling, the telemetry Pves would occasionally increase or decreases but eventually stabilized during the test. We postulated because the telemetry catheters are temperature sensitive and because the temperature of the saline was lower than the body
temperature, (despite our attempts at keeping the temperature constant at 37 degrees Celsius with a water bath) this may have slightly altered the initial telemetry Pves readings.

Based on the Pearson correlation coefficients, the pressure readings between the CMG and telemetry Pves catheters were excellent. This is most likely attributed to the fact that both the urodynamic catheter and the telemetry catheter are measuring the pressure directly from within the bladder. However, correlation between the CMG and telemetry Pabd was generally very weak which could possibly be explained by the fact that the CMG Pabd was measured from the rectum and the telemetry Pabd was measured directly from within the pig’s abdomen. While studies have suggested the rectal or vaginal pressure measurements can be used as an indication of intra-abdominal pressures during urodynamic evaluation\textsuperscript{214–216}, these methods are still only estimates of intra-abdominal pressure. Moreover, evidence for using the rectal pressure as a surrogate of the intra-abdominal pressure appears to be extrapolated from a small study of 12 patients undergoing a laparoscopic tubal coagulation\textsuperscript{217}. Therefore, further studies between the relationship between rectal and intra-abdominal pressure is warranted.

In a CMG setup, the external anal sphincter acts as the surrogate for the EUS\textsuperscript{96}. Quantitative comparisons between the CMG and telemetry EMG data could not be performed because the telemetry EMG needed to be sampled at 500 Hz to retain its integrity and detail. Instead, qualitative comparisons were between the two systems was performed. We observed appropriate relaxation and contraction of the EUS in both systems when uninjured pigs voided which were marked by identifiable motor unit action potentials\textsuperscript{67}. Confidence in post-SCI EMG recordings was low due to two reasons. First, our injury model results in paralysis from the waist down in the pigs which
results in them dragging their hindlimbs. This can cause significant noise and movement artifacts in the EMG tracings. Second, during extraction of the transmitter and its associated components, the suture button used to secure to leads was missing in all the animals. This suggested that the leads had shifted from their original position. EMG readings in uninjured pigs did not appear to be affected since we observed appropriate relaxation and contraction of the EMG suggesting that the plate had not shifted yet. Since SCI resulted in dragging of the lower abdomen, this may have caused the suture button to loosen resulting in the shifting of the plate that held the leads.

3.4.5 Using Telemetry to Investigate the Effects of Sedatives

Our urodynamics setup utilizes a sedation and reversal protocol which has been a controversial issue in studies of lower urinary tract function. Recently, Soebadi et al., reported that their Gottingen minipigs voided infrequently after their anesthesia protocol which is similar to what was observed in our animals (Soebadi et al., 2019). Furthermore, Keller et al., reported that sedation with either propofol or xylazine intramuscular gave “poor information” about bladder function in uninjured pigs. As previously discussed in Chapter 2, a second voiding pattern where a distinct detrusor contraction was not observed during the void was only observed in uninjured animals that had sedatives and reversals implicating that sedative and reversals influenced voiding mechanics. In this study, we attempted to investigate the effects of sedation and reversal protocol on detrusor contractility by performing our sedation and reversal protocol uninjured animals in their pen. Telemetric recording was performed throughout the duration of the sedation, reversal, and during voiding. A detrusor contraction was still observed which suggests that our sedation and reversal protocol does not appear to inhibit detrusor contractility (own data,
not shown). Rather, it is the urodynamics procedure itself that may result in altered voiding mechanics in uninjured animals. To obtain a better understanding of the physiologic bladder volume and the volumes voided during urination, we collected urine from two uninjured animals. The voided volumes never exceeded 300 ml which is contrary to what we have observed during UDS for the animals in this study. We suspect that the nature of the urodynamics test (retrograde filling of the bladder, non-physiologic filling rate) may cause the uninjured pigs to feel uncomfortable and void in a non-physiologic way, altering bladder outcomes, similar to findings in healthy humans\(^9\),\(^{120}\).

### 3.4.6 Histopathologic Findings from Implantation of the Telemetry Catheter

At the end of the study period, there were no major complications with the extraction of the transmitter, pressure catheters and EMG leads. However, there was excessive tissue growth surrounding the wires of the pressure catheters and around the mesh ring on top of the dome of the bladder. There was no evidence of urate crystals found in or on the Pves catheter.

Histological evaluation of the bladder revealed similar pathologies as we have previously observed with other SCI animals (Keung et al., 2020, unpublished). This includes detrusor hypertrophy, presence of chronic inflammation, focal acute inflammation and goblet cells in the urothelium. Near the site of insertion of the Pves catheter, there was an ulceration for one animal, but this was not observed in the other animals. This ulceration may have been caused by the untreated UTI and may not necessarily have anything to do with the implantation of the Pves catheter since it was only observed in one animal.
3.4.7 Limitations

One major limitation of the study is that there are no good alternatives to monitor physiologic bladder function without an invasive tool (i.e. a pressure probe that is inserted into the lumen of the bladder). There is uncertainty as to exactly how much damage was caused from the implantation of the telemetry catheter and how this may have altered voiding mechanics. Another experimental limitation is that once a catheter became non-functional, there was no ability within our protocol to simply re-anaesthetize the animal and replace it. Different sensor designs were trialed to improve sensor performance and robustness, but as demonstrated, we frequently encountered sensor failure through this period of iterative technical development. In the future, we intend to utilize the best-performing designs that did not have major technical issues such as the Standard Pves catheter, Caged and Caged-Balloon Pabd catheters. Moreover, alternative surgical methods to secure the positioning of the EMG plate should be investigated to improve confidence in the post-SCI EMG recordings.

Another important limitation was that this study did not fully utilize the telemetry system to investigate what happens to the bladder after acute SCI. Telemetric recordings immediately after SCI would have provided further insights into the phenomenon of “spinal shock” and exactly when this period ends in the female Yucatan minipigs. Furthermore, recordings past 4 weeks post-injury would have been ideal for a comprehensive investigation into physiologic bladder function after SCI.
3.5 Conclusion

By performing conventional UDS with concurrent telemetric recording in awake uninjured and SCI pigs, I have shown the practicality of telemetry systems to monitor LUT function in a pig model of SCI. These systems can help capture the pig’s physiologic LUT function after SCI and provide new insights about bladder function. Telemetry systems can be used in adjunct with urodynamics to evaluate novel treatments or therapies in a physiologic setting. This will ultimately help bridge the gap between animal experimentation and human application to support NLUTD research.
Chapter 4: Chronic Bladder Drainage in a Porcine Model of Spinal Cord Injury

4.1 Synopsis

In this chapter, I will describe the findings from my preliminary investigation into the effect of varying lengths of chronic bladder drainage (BD) in a pig model of SCI. I will explain how we placed a custom permanent indwelling catheter in the pigs after SCI and also detail the differences in the urodynamic outcomes between SCI pigs that had one, 8, and 11 weeks of BD. Finally, I will discuss some of the challenges in the interpretation as well as how the findings in this study are relevant to humans living with SCI.

4.2 Materials and Methods

All animal protocols and procedures were approved by the Animal Care Committee of UBC and were in accordance with the Canadian Council on Animal Care. The animal care and animal veterinary technicians at the Centre for Comparative Medicine provided daily and routine care of all animals involved in this study.
4.2.1 Animals and Experimental Design

N = 4 animals were obtained from S&S farms, Ramona, California, USA. All pigs arrived at the facility and were group housed in an indoor pen that had access to another outdoor compartment. The pigs were fed 1.5% of their body weight twice a day and given ad-libitum access to water. Animals were housed for one week prior to the 4-week acclimation training program for UDS as previously described in Chapter 2. A contusion SCI (20 cm drop, 50 g midline contusion) at the T10 level was induced on all animals using the UBC porcine model of SCI \(^{145,146}\). All drop weights had an additional 100 g static weight placed on top for 5 min of compression.

During SCI surgery, a custom indwelling foley catheter was placed into the bladder. Afterwards, N = 2 received 8 weeks of BD and n = 2 received 11 weeks of BD. Animals that received 8 weeks of BD had UDS performed at pre-injury, 4, 8, and 12 weeks post-injury. Animals that received 11 weeks of bladder drainage had UDS performed at pre-injury, 2, 4, 8, and 11 weeks post-injury. At the end of the study, the bladders were extracted for histologic processing and examination.

4.2.2 Placement of a Custom Indwelling Foley Catheter

With the animals under general anesthesia during SCI surgery or awake after a UDS procedure, a 14 Fr indwelling foley catheter (Bard Medical, Covington, GA, USA) was placed into the animal’s bladder by a trained animal technician with sterile technique. Using the balloon channel, the balloon of the catheter was filled with 30 cc of saline. Next, the catheter was
clamped with a pair of hemostats to prevent the balloon from deflating. With the catheter clamped, the balloon and drainage ports were cut off using a pair of scissors. The exposed balloon channel was plugged with a stopper made from water-resistant epoxy to prevent the balloon from deflating after the hemostat was removed. The catheter was affixed inside the vagina by suturing the silicone tubing of the catheter to the wall of the vulva. To hide the catheter, the walls of vulva were sutured closed. The catheter was replaced after each UDS up until the complete removal of the catheter at the 8- or 11-week timepoint. Figure 4.1 shows the custom indwelling foley catheter.

Figure 4.1 Custom Indwelling Foley Catheter
The drainage and balloon ports of a 14 Fr foley catheter were cut off to expose the channels. After the balloon was filled with saline, the balloon channel was plugged with an epoxy plug.

After placement of the indwelling foley catheter, daily monitoring for the presence of urinary wet spots in the pens was performed to ensure the bladders were being drained continuously. In addition, the physical characteristics of the urine were observed for signs of UTIs (cloudy urine, malodorous urine, etc). In circumstances where the catheter was dislodged or a replacement was
required due to crystalline deposits in the drainage channel, a new indwelling foley catheter was placed in the awake animals as soon as possible.

4.2.3 Analysis of Urodynamic Parameters

Similar to Chapter 2, the urodynamic parameters studied during the filling period were: Pves, Pabd, Pdet (Pdet = Pves – Pabd), cystometric capacity (maximum infusion volume reached at the end of filling) and EUS EMG activity. During the voiding periods, Qmax, Pdet-Qmax, Pdetopen, VV, PVR, voided%, and bladder compliance (change in bladder volume/change in Pdet; expressed as ml/cm H2O). Compliance values less than 20 ml/cm H2O were determined to be indicative of low bladder compliance. In SCI pigs, neurogenic acontractile detrusor was defined by the absence of a detrusor contraction during voiding. NDO was characterized by involuntary detrusor contractions during the filling phase. DLPP was defined as the lowest detrusor pressure at which leakage is observed in the absence of either a detrusor contraction or increased abdominal pressure. Once again, I refrained from diagnosing DSD, since EUS activity was recorded with EMG patch electrodes without concurrent VCUG.

All parameters defined are in consonance with the metric units and definitions established by the ICS 39,221,222.
4.2.4 Histologic Analysis

The methods for histologic processing and quantification were previously described in Chapter 2. In short, the bladder was drained prior to extraction and the wet weight was recorded. Next, the bladder was divided into four anatomical regions: the dome, body, neck and urethra. The regions were fixed using 10% formalin in a 20:1 formalin to tissue ratio. Two sections from each region were embedded in paraffin blocks and stained in H&E and Masson’s trichrome. Images were captured using the ZEISS Axio Imager M2 (ZEISS, Germany) at 10x. Using ZEN software (ZEISS Germany), the distance of 5 lines drawn perpendicular to the mucosa were averaged for both bladder sections to measure the wall thickness. The percentage of muscle and collagen relative the cross-sectional area of the bladder was calculated using the color deconvolution plug-in and thresholding function on ImageJ (NIH, USA).

4.2.5 Statistical Analysis

A t-test was performed for statistical comparison of the urodynamic parameters between the different groups (pre-SCI, one, 8 and 11 weeks of BD). In addition, a t-test was also performed for comparison of the histologic findings (bladder wall thickness (mm), % of muscle, % of collagen) between animals that received 8 versus 11 weeks of BD. Histologic findings will be reported for each animal individually. Results will be presented as mean ± SEM.
4.3 Results

4.3.1 Baseline Voiding Profiles

All pre-injury UDS were successfully performed in all animals (n = 4) in this study (7/7 UDS, 100%). Animal 9045 had two pre-injury UDS performed and animal 9047 had three pre-injury UDS performed. A majority of the animals received sedation during catheter placement and performed UDS in a standing position (6/7 UDS, 86%). Animal 9047 performed one UDS in a fully suspended position (1/7 UDS, 14%).

In terms of voiding patterns, animal 7630 and 9045 demonstrated voiding pattern 2 (blunted detrusor contraction) during the void. In contrast, animal 7631 and 9047 demonstrated voiding pattern 1 (distinct detrusor contraction) during the void. Overall, the urodynamic tracings and parameters resemble previous findings from uninjured pigs in Experiment 2 of Chapter 2. A table summarizing the pre-injury urodynamic parameters is presented in Table 4.1.
Table 4.1 Cystometrogram Parameters for Uninjured, Awake Animals in Chronic Bladder Drainage Study

Two distinct voiding patterns were observed: animals who had a clear detrusor contraction (pattern 1) and animals who had no clear contraction (pattern 2). A T-Test was performed and no significant differences between animals displaying voiding pattern 1 vs. pattern 2 was observed.

4.3.2 Permanent Indwelling Catheter Complications

During the study period, the permanent indwelling catheter had to be replaced either due the catheter becoming dislodged or due to a catheter associated UTI. For animal 7630, the catheter was replaced a total of four times outside of UDS and for animal 7631, the catheter was replaced three times outside of UDS. For animal 9045, the catheter was replaced once outside of UDS and for animal 9047, the catheter was replaced twice outside of UDS. Frequent clinical UTIs outside and during UDS was a challenging issue in these animals compared to animals without chronic bladder drainage. Common bacterial species included *E. coli* and *Klebsiella pneumoniae* which are also common culprit of human UTIs 51. Animals that underwent UDS with a concomitant UTI were excluded from analysis. A UTI was defined by a positive urinalysis test post-UDS with significant bacteriuria and pyuria along with clinical symptoms such as loss of appetite, quiet behaviour, fever, foul-smelling urine, behaviours signifying pain, cloudy urine and hematuria 42.
Out of 12 post-SCI UDS performed in this experiment, three (25%) were excluded from analysis since a concomitant UTI was detected post-UDS. Specifically, animal 9045 and 9047 had a clinical UTI at the 4-week UDS timepoint and animal 9047 also had a clinical UTI at the 8-week UDS timepoint.

4.3.3 Injury Parameters

While all animals received the same contusion and compression injury using the UBC porcine model of SCI, the animals that received 8 weeks of BD received greater zeroed force (531.7 – 999.9) compared to animals that received 11 weeks of BD. Furthermore, the velocity at impact was also greater for animals that received 8 weeks of BD (118 – 183 mm/s) compared to animals that received 11 weeks of BD. There was also a noticeable difference in the age (92 – 210 days) and body weight (6.2 – 10.3 kg) between the two treatment groups with the animals that received 11 weeks of BD. These animals were older and heavier at the time of surgery compared to the animals that received 8 weeks of BD. A summary of the biomechanical impact parameters for each animal is presented in Table 4.2.
Table 4.2 Injury Parameters of Chronic Bladder Drainage Animals

The number of weeks of bladder drainage each animal (8 or 11 weeks) received is shown along with measures of age at surgery, body weight, and biomechanical impact parameters of the contusion injury for each animal. After SCI, biomechanical data acquired for each impact was collected and analyzed for peak force, impactor displacement from initial contact with the exposed dura, impulse (calculated as the integral of force with respect to time), and velocity at impact.

4.3.4 Lower Cystometric Capacity after 8 Weeks of Bladder Drainage

In total, 14 post-SCI UDS with BD were conducted. Three sessions (21%) were excluded from analysis due to a concomittant UTI. Therefore, 11 post-SCI UDS were analyzed. The urodynamic parameters for each animal in this study are shown in Table 4.3.
Table 4.3 Urodynamic Parameters of Spinal Cord Injured Pigs with 8 or 11 Weeks of Bladder Drainage

All animals received a 20 cm drop contusion injury with 5 min of 150 g of compression at either the T10 level (n = 4). As none of the animals were unable to support their body weight as a result of the injury severity, their hindlegs were weight-supported (toes have contact with the ground) or fully suspended using a custom-built sling. A T-Test was performed between animals that received 8 vs. 11 weeks of bladder drainage and between standing pre-SCI animals vs. post-SCI animals that received 8 or 11 weeks of bladder drainage. * significant difference between animals that received 8 vs. 11 weeks of bladder drainage, P < 0.05, ** P < 0.01, *** P < 0.001. # significant difference between pre-SCI animals from Experiment 2 that performed UDS in a standing position and post-SCI animals that received 8 or 11 weeks of bladder drainage, P < 0.05, **P < 0.01, *** P < 0.001.

Due to issues with concomitant UTIs during UDS at 4 weeks post-injury, comparisons between the UDS parameters of animals with 4 weeks chronic BD versus animals with only one week of BD was not performed.

The amplitude and frequency of NDO events varied in the animals with 8 weeks of BD. However, profound NDO events were observed in both animals that received BD after 8 weeks of BD compared to animals that only received one week of BD.
Furthermore, animals with 8 weeks of BD (n = 3) had a significantly lower cystometric capacity versus animals with only one week of BD (n = 10) at the 8 weeks post-injury UDS timepoint (168 ± 35 ml vs. 427 ± 46 ml, respectively, P = 0.01). In addition, animals with 8 weeks of BD demonstrated poor bladder compliance compared to animals with one week of BD (19 ± 16 ml/cm H₂O vs. 62 ± 14 ml/cm H₂O), however this difference was not statistically significant. There were no significant differences in the other urodynamic parameters. A CMG tracing between a T10 SCI animal that received one week of BD versus a T10 SCI animal that received 8 weeks of BD is shown in Figure 4.2.
Figure 4.2 Differences in Urodynamic Outcomes between One versus 8 Weeks of Bladder Drainage

On the left, a CMG from a T10 SCI animal with the standard one week of bladder drainage protocol at 8 weeks post-injury (WPI). On the right, is a CMG from a different T10 SCI animal with 8 weeks of bladder drainage at 8 WPI. Both animals received pre-cystometry sedation and reversal. During the filling phase, both animals demonstrated NDO. However, the animal that received 8 weeks of bladder drainage demonstrates profound NDO compared to the animal that had one week of bladder drainage. Furthermore, the cystometric capacity (taken from the volume infused until the first leak) is approximately 5 times greater in the animal that received only one week of bladder drainage compared to the animal that received 8 weeks of bladder drainage (519 ml vs. 146 ml).
4.3.5 Urodynamic Differences between 8 versus 11 weeks of Bladder Drainage

Removal of permanent indwelling foley catheter after 8 weeks of BD resulted in a substantially larger cystometric capacity at the 12-week UDS timepoint for both animals. At the 8-week UDS timepoint, animal 7630 and 731 had a cystometric capacity of 122 ml and 146 ml, respectively. At the 12-week UDS timepoint, animal 7630 had a cystometric capacity of 767 ml, approximately, a 6 to 7-fold increase. Similarly, animal 7631 had a cystometric capacity of 428 ml which is approximately, a 3 to 4-fold increase. Leaks occurred at low $P_{\text{det}}$ (< 40 cm H$_2$O) which is similar to animals that received one week of BD. Furthermore, the NDO events at the 12-week UDS timepoint did not occur early during the filling period as previously seen at the 4 and 8-week UDS timepoint. The urodynamic effect of removal of the indwelling foley catheter after 8 weeks in animal 7630 is shown in Figure 4.3.
Both animals received pre-cystometry sedation and reversal. At the 8 weeks post-injury (WPI) timepoint, animal 7630 demonstrated NDO and a low cystometric capacity of 33 ml. After removal of the permanent indwelling foley catheter, resulting in no bladder drainage for 4 weeks, the cystometric capacity increased by about 7-fold at the 12 WPI UDS timepoint (767 ml). Furthermore, NDO events were infrequent and appeared to predominately occur near the cystometric capacity.

Figure 4.3 CMGs from a T10 SCI Animal with 8 Weeks of Bladder Drainage at 8 and 12 Weeks Post-Injury
In contrast, both animals that received 11 weeks of BD demonstrated profound NDO with leakage at high $P_{\text{det}}$ (> 40 cm H$_2$O) at the final UDS timepoint (11 WPI). A representative tracing is shown in Figure 4.4.

Figure 4.4 CMGs from a T10 SCI Animal with 11 Weeks of Bladder Drainage at 8 and 11 Weeks Post-Injury

Both animals received pre-cystometry sedation and reversal. After 11 weeks of bladder drainage and at the 11 WPI UDS timepoint, profound NDO was observed with a very high DLPP (> 40 cm H$_2$O) compared to the 8 WPI UDS timepoint. Furthermore, cystometric capacity increased approximately 2-fold. There also appears to be more EMG activity which could be indicative of DSD but without VCUG, this diagnosis cannot be confirmed.
4.3.6 Detrusor Hypertrophy after 8 and 11 Weeks of Bladder Drainage

In general, histologic examination of H&E stained sections from the body of the bladder of animals that received 8 versus 11 weeks of BD revealed similar histopathologic findings between the two treatment groups. Signs of chronic inflammation such as lymphoplasmacytic infiltration of the lamina propria with focal lymphoid aggregates and neutrophils and eosinophils were a common finding in both treatment groups. These signs have also been found in SCI pigs that did not have a chronic indwelling foley catheter. However, focal epithelial lymphocytosis with rare epithelial neutrophils was found in all the BD pigs regardless of the length of drainage. This finding suggests irritation of the bladder epithelium potentially from direct contact with the catheter.

Pigs with chronic BD demonstrated detrusor hypertrophy similar to SCI pigs that had one week of BD. There were no significant differences in the amount of detrusor hypertrophy between those that received 8 versus 11 weeks of BD. However, the bladders that received 11 weeks of drainage appeared to be slightly thicker (animal 9047: 7.4 ± 0.5 mm; animal 9045: 7.5 ± 1.2 mm) compared to the bladders that received 8 weeks of BD (animal 7631: 7.2 ± 0.2 mm; animal 7630: 6.1 ± 0.2 mm). Sections from the body of the bladder for animals that received 11 weeks of drainage appeared to be quite varied (animal 9047: 42% muscle, 53% collagen; animal 9045: 50% muscle, 43% collagen). Animals that received 8 weeks of drainage also demonstrated some variability (animal 7631: 50% muscle, 41% collagen; animal 7630: 61% muscle, 36% collagen) (Figure 4.5).
Figure 4.5 Detrusor Hypertrophy and Notable Bladder Wall Thickening in Bladder Drained (BD) Bladders

From left to right: (A) control bladder (non-SCI), (B) T10 SCI with one week of BD at 10 weeks post-injury (WPI), (C) T10 SCI with 8 weeks of BD at 12 WPI, (D) T10 SCI with 11 weeks of BD at 11 WPI. All bladder sections were stained with Masson’s trichrome where red represents muscle and blue represents collagen. All post-SCI bladders regardless of bladder drainage treatment type, demonstrated detrusor hypertrophy with shrinkage of the submucosa tissue. Bladders with 8 or 11 weeks of bladder drainage had slightly thicker walls compared to SCI bladders with only one week of bladder drainage. There were no observable morphologic differences between 8 and 11 weeks of drainage, however, bladders with 11 weeks of drainage had the thickest walls out of all post-SCI bladders.

4.4 Discussion

The current protocol for management of the bladder after acute SCI in humans is to place an indwelling foley catheter to drain the bladder until the period of spinal shock resides to prevent overdistension of the bladder and UTI\(^{36}\). In some humans, the indwelling foley catheter can remain in the bladder for up to 40 days or more\(^ {41} \). Afterwards, clean intermittent catheterization
(CIC) is taught to patients and is currently the gold standard for post-SCI bladder management. With proper aseptic technique, CIC has been associated with fewer complications compared to indwelling foley catheters. However, this procedure requires sufficient upper extremity function or a care provider to assist in catheterizing the bladder every 4 to 6 hours depending on fluid intake. In addition, self-catheterization may cause discomfort in those with preserved urethral sensation. All these factors can impact the individual’s overall quality of life. Patients that do not feel comfortable with the idea of self-catheterizing themselves or those without sufficient upper motor function, may instead decide to opt with suprapubic or transurethral indwelling catheterization to manage their bladders and gain bladder independence.

In Chapter 2, I described the establishment of a protocol to perform urodynamics in conscious, awake minipigs before and after SCI with the ultimate goal to create a large animal model of NLUTD for use as a translational tool. However, our original protocol for managing the pig’s bladder after SCI may not necessarily reflect the management of the human bladder after SCI. Aside from the 7 days of indwelling foley catheterization after SCI, no further management was performed on the pig’s bladder and the bladders were allowed to drain spontaneously. In contrast, the human bladder is managed on a daily basis either via CIC or a suprapubic/indwelling catheter after SCI.

As previously shown in Chapter 2, UDS on SCI pigs with one week of drainage revealed low amplitude NDO, large cystometric capacities, and elevated PVR volume as early as 4 weeks post-injury. The detrusor pressure at leaks did not often reach what is considered to be harmful to human renal health (> 40 cm H2O). Furthermore, clinical UTIs were not common in pigs that
received the standard one week of BD during UDS or outside of UDS. However, clinical UTIs in humans with SCI is a common and frequent issue. It has been reported that 22% of patients with acute SCI develop UTIs during the first 50 days and annual UTI incidence in patients with chronic SCI is nearly 20%.\textsuperscript{224}

To make my model more clinically relevant, I wanted to examine the effects of varying lengths of chronic BD (8 versus 11 weeks) on the urodynamic and histologic outcomes in our pig model of SCI. Moreover, I wanted to if there were differences in the outcomes between pigs that receive chronic BD versus pigs that only receive one week of BD.

One major finding was that pigs 11 weeks of BD appeared to demonstrate more profound NDO at the final UDS timepoints compared to pigs with only one or 8 weeks of BD. 11 weeks of BD resulted in leakage at high P_{det} (> 40 cm H\textsubscript{2}O) in both animals at the final UDS time point. As previously mentioned, NDO with leakage at high P_{det} (> 40 cm H\textsubscript{2}O) are considered to be high risk features that require treatment and follow-up in humans.\textsuperscript{42} High DLPP in post-SCI pigs with 8 weeks or one one week of BD. N = 2 animals that received 8 weeks of BD did not once demonstrate leakage at P_{det} > 40 cm H\textsubscript{2}O. In Chapter 2, only two animals (13%) out of 15 T2/T10 animals that had one week of BD demonstrated leakage at P_{det} > 40 cm H\textsubscript{2}O at any post-SCI UDS timepoint. Symptomatic UTIs appeared to be more prevalent in animals that had chronic bladder drainage catheters compared to animals that only had one week of BD. In humans, the use of an indwelling catheter has been associated with an increased frequency of symptomatic UTI and bacteremia, and additional morbidity from non-infectious complications.\textsuperscript{76}
Histological evaluation revealed similar histopathologic features to pigs with only week of BD. This includes chronic inflammation, detrusor hypertrophy and thickening of the bladder wall. However, one prominent feature that was only found in bladders with chronic BD was infiltration of neutrophils in the urothelium. The presence of a chronic indwelling foley catheter may have resulted in physical abrasion of the urothelium of the bladder. In humans that have had long-term urethral catheterization, extensive damage to the urothelium of the bladder has been reported previously. Similar to post-SCI pigs with only one week of BD, there was detrusor hypertrophy with little to no indication of fibrosis. Since the bladders were extracted 11- or 12-weeks post-SCI, there may not have been enough time for fibrosis to develop, which contrasts findings by Fodistch et al. In their study, seven Gottingen minipigs underwent a complete spinal cord transection. The indwelling catheter placed during SCI surgery was removed 5 days after. Afterwards, the bladder was drained daily via CIC every 4 hours for up to 4 months after injury. Histologic evaluation with Masson-Goldner trichrome staining revealed significant loss of detrusor and a significant increase of collagen with a noticeable shift from type III to type I collagen which is indicative of fibrosis. While the findings from both studies are dissimilar, this may be attributed to the differences in the pig species used, histologic approach, and bladder management methods (chronic drainage versus CIC). Further studies in a pig model of SCI are required as there is currently limited literature that have investigated this topic.

A major limitation of this study is the very small number of animals (n = 4) with only 2 animals per group for each treatment type (8 weeks vs. 11 weeks of BD). Making interpretations and drawing conclusions from such a small sample size prevents the findings from being extrapolated. Moreover, a small sample size increases the chance of finding a false positive
meaning there could be a possibility that if we repeated this study in additional animals, we may not find profound NDO in other animals with 11 weeks of BD or substantial thickening of the bladder wall after chronic BD. However, the patterns of NDO and their distinctiveness of their spikes were striking. Another issue was that it was difficult to confirm with absolute certainty that the bladders were being properly drained during the entire duration of the study. In humans, it would be easy to check if the bladder is being fully emptied by using an ultrasound or by checking if the amount of water consumed matches the amount of urine outputted. Since the SCI pigs dragged their lower abdomen around, there was no other efficient and effective way to ensure the bladder was being properly drained on a consistent basis unless we used an ultrasound. Finally, I think further investigation is warranted to ensure reproducibility as well as to probe into the functional and histologic effects of removing the chronic drainage catheter after 8 or 11 weeks of drainage.

4.5 Conclusion

In this preliminary investigation, I explored the effects of chronic BD in a porcine model of SCI with the goal of making the model more clinically relevant. Results suggest that bladder management method and regimen can alter bladder function and morphology. Chronic BD after SCI resulted in profound NDO which was not observed in animals that had one week of BD. This study highlights the importance of bladder management after SCI, but further studies are required to solidify the findings.
Chapter 5: Conclusion

5.1 Summary

This thesis represents the integration of neurology and urology or also known as neuro-urology. It is primarily a subspecialty in the field of urology and receives comparatively less attention than, for example, onco-urology. However, NLUTD is highly prevalent and affects the lives of millions of people with neurological diseases. In the context of SCI, animal models have helped bridge the gap between experimentation and human application. To illustrate, rodent models such as rats and mice, have improved our understanding about the acute to chronic changes of the bladder after SCI. The popularity of rodents in SCI research is unsurprising due to their ease of handling and low cost. In a systematic review by Alhoseini et al., they found that among 2209 studies that investigated SCI using animals, 72.4% used rats and 16% used mice. Larger animal models such as dog, cats, primates, and pigs made up only 7.5% of the studies which reflects how much more is known about rodent bladder function after SCI versus larger animal models.

In an review article by Wheeler et al., they state that one of the current needs of SCI individuals is “dynamic” monitoring devices that provide them a better sense of what their surrogate sensations mean. Chronic monitoring of measures such as bladder pressure and volume would allow clinicians to monitor and fine tune treatments or develop new therapies. Nerve stimulation devices may help SCI individuals empty their bladders without the need of catheters, which is one of the top health priorities. The first step to help develop such monitoring devices and
also evaluate the safety and utility, would be to use an applicable animal model. While rodents could be used for this need, the size of the rodent bladder would make the development and translation of novel-human sized devices challenging. Another important consideration is that healthy rodent demonstrates bursting of the EUS during voiding. After SCI, the rodents also demonstrate bursting which makes interpretation and diagnosis of DSD difficult because healthy rodents also demonstrate bursting of the EUS.

The pig’s LUT function has been characterized previously and literature has stated there are similarities between human and pig LUT function. However, changes in pig’s bladder function after SCI have not yet been well explored. Thus, this thesis sought to characterize the functional and morphologic changes in the pig’s bladder after traumatic thoracic spinal cord injury to establish a large animal model of NLUTD after SCI.

5.2 Characterizing Neurogenic Lower Urinary Tract Dysfunction in a Porcine Model of Spinal Cord Injury

Through an iterative process, I developed a protocol to perform clinically relevant UDS in conscious, awake pigs. Using this setup protocol, I have demonstrated that uninjured Yucatan minipigs demonstrate silencing of the EUS during the void which is similar to how a healthy human EUS also behaves. After SCI, the pigs demonstrated NDO with potential DSD along with impaired voiding (elevated PVR, poor Qmax, reduced voided%) which is also similar to human bladder dysfunction after SCI. Histologic evaluation of SCI bladders revealed thickened bladder walls with detrusor hypertrophy when compared to the control. This finding is similar to acute
changes in the morphologic of the human bladder after SCI. Perhaps, if the study was extended beyond 17 weeks post-injury, fibrotic bladders may have been observed. Ultimately, using this setup protocol, I have demonstrated the potential to longitudinally track the changes in the pig’s bladder function on a monthly basis and consistently obtain interpretable urodynamic results.

Limitations of the study include the use of sedation to place the catheters for animals in Experiment 2. Sedatives may have influenced bladder function since one major observation was that uninjured animals that received sedation during catheter placement had much larger cystometric capacities compared to those that did not receive sedation. Moreover, animals that received sedation also displayed two different voiding patterns (distinct or no distinct detrusor contraction during the void). Our collaborators at the UofL, have demonstrated the ability to perform urodynamics in conscious, awake female Yucatan minipigs without the use of sedation and obtain consistent urodynamic data. This has major implications since it reflects how UDS are normally performed in humans. However, I still think sedation during catheter placement can be a practical option if awake catheterization cannot be performed.

All in all, an established large animal model of NLUTD can serve as a valuable translational tool to evaluate the safety and utility of potential treatments or devices. This model will be a valuable contribution to the field. For instance, treatments or devices that can reduce the frequency of NDO events or improve voiding efficiency could be one important outcome measure to evaluate the efficacy of such modalities. I hope one day this animal model will be able to help close the gap between animal experimentation and human application for the millions of individuals suffering from NLUTD.
5.3 Telemetric Findings in Uninjured and Post-SCI Pigs

One important limitation of urodynamics, which needs to be re-emphasized, is that it is an invasive procedure and interpretation of the results heavily relies on a snapshot of bladder function captured via a non-physiologic, retrograde filling of the bladder. The unnatural constraints of the urodynamics procedure can negatively influence urodynamic outcomes because the bladder may not behave as it would in a natural, physiologic setting. The simple act of voiding has a complex physiologic and mental component behind it. Studies in healthy humans have highlighted that the urodynamics test itself can reveal abnormalities in the standard outcomes even in what would be otherwise considered to be a “normal bladder”\textsuperscript{91,220}. For instance, Leitner et al., found DSD, a common finding in patients with a neurologic condition, in 77\% of healthy volunteers. If urodynamics has been shown to be uncomfortable even for humans, then one can infer that it would be quite uncomfortable for pigs. Therefore, a method that would allow us to monitor the pig’s natural detrusor and EUS function should be explored.

For this thesis, I investigated the use of implantable radio telemetry systems to monitor real-time measurements of Pdet and EUS EMG activity from the animals in their natural setting (housing pens). In close collaboration with TSE systems (Chesterfield, MO, USA), a custom implantable four-channel Stellar Telemetry transmitter was developed. The final transmitter consisted of one Pves catheter, two Pabd catheters, and a pair of EMG leads. Telemetric recordings were performed during UDS to evaluate the congruency between the two systems. Furthermore, recordings were also performed weekly in-person with the animals in their pens to analyze for differences between voids captured in a natural setting versus during a UDS.
The surgical implantation of the telemetric transmitter and its associated components was largely successful for all the animals with minimal complications. Pressure sensor performance and durability was satisfactory with most of the sensors designs tested. One major finding was that the Pdet and EMG recordings between the telemetry and urodynamics system were comparable in the same animal pre- and post-SCI. Based on the correlation coefficients, the telemetry and CMG Pves readings were strong. However, between the telemetry and CMG Pabd readings, the relationship ranged from very weak to strong based on the coefficients. The voiding time in a natural setting was substantially shorter compared to voids captured during a UDS. Furthermore, the amplitude of the Pdet contraction was slightly higher in a natural setting versus during UDS in one animal.

In hindsight, this study would have benefitted from a longer duration of recordings post-SCI. In total, the transmitter was implanted for 16-18 weeks in the pigs. The longest duration in the post-SCI state recorded was 10 weeks but the Pabd sensor was malfunctioning in both animals. So technically speaking, the longest recorded duration in the SCI state was 4 weeks. Thus, a great deal remains unknown about how bladder function post-SCI differs in a natural setting versus during UDS. Moreover, monitoring of detrusor and EUS function in the days following SCI was not performed as it was presumed that the detrusor would be areflexic due to spinal shock.36,37,41 There could be a possibility that NDO events were occurring during the acute period after SCI.

Another major uncertainty is if the implantation of the pressure catheter into the dome of the bladder negatively influenced bladder function. My only tool to evaluate and characterize bladder function prior to telemetry implantation was to perform UDS, but as previously
mentioned in Chapter 2, the urodynamics procedure itself can result in pathological findings in healthy individuals. Therefore, there did not appear to be a commercially available non-invasive tool that could monitor natural bladder function. However, early recordings after implantation of the transmitter did not reveal NDO events which would have been a telltale sign of bladder irritation. From this, it can be assumed that implantation of the Pves pressure probe itself did not have any major impact on bladder function.

Histological examination of the area around the catheter insertion revealed ulceration of the urothelium in one animal. This pathologic finding was not found in the other 5 animals and may have also been caused by the UTI the animal had prior to euthanasia. There were no other notable histopathologic differences between animals that received a telemetry catheter insertion versus those that did not. While there was substantial tissue growth over the implant and the associated components observed during extraction, there were no other concerning health consequences caused by the catheters or the implant.

Overall, I have demonstrated the practicality of using the pig model of NLUTD after SCI to evaluate the safety and utility of a human-sized telemetric device. An important point to make is that this device was not meant to be translated for human application. The main use of this device is to investigate the pig’s physiologic bladder function before and after SCI as well as to help us further understanding the influence of urodynamics on the LUT. The results from this study shed light as to how urodynamics can significantly alter bladder function. Essentially implying that urodynamics may not necessarily be the most reflective of physiologic bladder function. Yet, this does not discount the value of the diagnostic information urodynamics can
provide and I think telemetric devices should be used in adjunct with urodynamics in the pigs to better understand how the pigs void in different setups and settings. Future studies should utilize telemetric devices to evaluate the efficacy of treatments and other novel human-sized devices that aim to improve bladder dysfunction after SCI in a physiologic setting.

5.4 Findings from Chronic Bladder Drainage in SCI Pigs

As previously mentioned, individuals with SCI manage their bladders daily with CIC or indwelling/suprapubic catheter. While I have established a setup protocol to perform urodynamics in SCI pigs, the typical regimen of bladder management was not clinically relevant. Following SCI, the pig bladders were managed for only one week with an indwelling foley catheter. Afterwards, the bladders were allowed to drain spontaneously. This does not in any way resemble how the human bladder is managed after being discharged from the hospital. Therefore, I investigated the urodynamic and histologic effects of varying lengths of BD after SCI.

Two animals received 8 weeks of BD and another two received 11 weeks of BD. Although the number of animals in this investigation was low, there were several distinct urodynamic differences in those that received chronic BD (8 or 11 weeks) versus those that only received the standard one week of BD. In particular, animals with 8 weeks of BD (n = 3) had a significantly lower cystometric capacity versus animals with one week of BD (n = 10) at 8 weeks post-injury which has been reported to be found in humans that have an indwelling catheter because the bladder never fills completely. Removal of the indwelling catheter after 8 weeks resulted in
larger cystometric capacities (> 400 ml, upper range of normal bladder capacity) with less profound NDO at the 12-week UDS timepoint.

While animals with 8 weeks of BD demonstrated NDO, animals with 11 weeks of BD had even more profound NDO and high DLPP (> 40 cm H₂O) at the final UDS timepoint (11 weeks post-SCI). Such a high DLPP would be a red flag for humans given the potential that this would cause ureteral reflux, and would require treatment with follow up to preserve renal function 42.

Histologic evaluation revealed similar histopathologic features to post-SCI bladders that only received one week of BD such as detrusor hypertrophy and chronic inflammation. However, chronically drained bladders had thicker walls and infiltration of neutrophils into the urothelium which suggests that the catheter may have irritated the bladder wall. There were no histologic differences between bladders that received 8 versus 11 weeks of BD. This study highlights how different bladder management regimens after acute SCI can influence bladder function and, importantly, are a key methodologic consideration in such studies. With this knowledge in mind, the unresolved question is, “should all future studies in the pig have chronic BD after SCI?”

Based on the few numbers of animals that I have performed this study on, I think more studies need to be performed to fully answer this question. Another rationale for continuing to perform this study is that this will create a more clinically relevant model since humans manage their bladders daily.
An interesting aspect of using the porcine model of SCI is that one can argue that the SCI pigs technically perform daily bladder management by dragging their lower abdomen across the floor resulting in leakage from movement or stress incontinence. This is also somewhat similar to the Crede maneuver where manual pressure is applied to the abdomen at the location of the bladder in humans. However, this method of “bladder management” is not as efficient at emptying the bladder compared to the catheters. This is made evident from preliminary studies using a bladder ultrasound to measure physiologic post-void residual volumes of SCI pigs where they held anywhere from 91 to 563 ml of urine (preliminary data not shown) which is considered to be abnormal. The next steps would be to perform this study in more animals with varying lengths of bladder drainage (4, 8, 12 or even longer). Finally, I think it would also be important to probe into the periodically clamping the catheter after placement to see if the bladder can be “retrained” to store and void appropriately.
5.5 Final Thoughts

In conclusion, this thesis described the various experiments undertaken to characterize the functional and morphologic changes in the pig’s bladder function after SCI. This work serves as an important contribution to the field of SCI research because there is currently a need for a large animal SCI model of NLUTD for the evaluation of the safety and utility of novel human-sized devices that aim to treat NLUTD.

Through an iterative process, I have pioneered a protocol to perform clinically relevant UDS in conscious, awake pigs which can help identify the efficacy of treatments or therapies that aim to improve bladder dysfunction after SCI. Furthermore, I have demonstrated the use of this model by exploring other research avenues such as the use of implantable radio-telemetric devices and the effects of chronic BD. In the grander scheme of things, I hope this model will serve as a valuable translational tool for the SCI community to explore new avenues in NLUTD research to one day, improve the lives of millions affected by bladder dysfunction after SCI.
Bibliography


