Effects of exercise training on urinary tract function after spinal cord injury

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Hubscher CH, Montgomery LR, Fell JD, Armstrong JE, Poudyal P, Herrity AN, Harkema SJ. Effects of exercise training on urinary tract function after spinal cord injury. Am J Physiol Renal Physiol 310: F1258–F1268, 2016. First published March 16, 2016; doi:10.1152/ajprenal.00557.2015.—Spinal cord injury (SCI) causes dramatic changes in the quality of life, including coping with bladder dysfunction which requires repeated daily and nightly catheterizations. Our laboratory has recently demonstrated in a rat SCI model that repetitive sensory information generated through task-specific stepping and/or loading can improve nonlocomotor functions, including bladder function (Ward PJ, Herrity AN, Smith RR, Willhite A, Harrison BJ, Petruska JC, Harkema SJ, Hubscher CH. J Neurotrauma 31: 819–833, 2014). To target potential underlying mechanisms, the current study included a forelimb-only exercise group to ascertain whether improvements may be attributed to general activity effects that impact target organ-neural interactions or to plasticity of the lumbosacral circuitry that receives convergent somatovisceral inputs. Male Wistar rats received a T9 contusion injury and were randomly assigned to three groups 2 wk postinjury: quadrupedal locomotion, forelimb exercise, or a nontrained group. Throughout the study (including preinjury), all animals were placed in metabolic cages once a week for 24 h to monitor water intake and urine output. Following the 10-wk period of daily 1-h treadmill training, awake cystometry data were collected and bladder and kidney tissue harvested for further tissue assessments. Our initial findings to include bladder dysfunction which requires repeated daily and nightly catheterizations. Life-long urological care is required for SCI individuals, impairment has an enormous impact on the quality of life (2, 3, 26). Life-long urological care is required for SCI individuals, yet most efforts treat symptoms but do not improve intrinsic function (68, 80). Bladder management requires intermittent catheterization throughout the day/night to avoid incontinence, bladder overdistention (which can create high pressure and reflux to the kidneys), inflammation, infections, and autonomic dysreflexia. Despite bladder dysfunction being a high priority for SCI individuals, the focus of health care professionals is on rehabilitation aimed at optimizing mobility and the remaining musculoskeletal function. Locomotor training (LT) has emerged as a safe and effective therapy for post-SCI motor deficits with many benefits (cardiovascular function, strength, mobility) (9, 23, 43, 52, 84). Recent animal studies, however, have shown that LT post-SCI also improves bladder function (46, 90), a finding consistent with a few reports from human SCI studies (42, 48, 75). For example, a recent study from our laboratory (90) has shown, using a spinal contusion model in adult male rats, functional gains of lower urinary tract function as assessed with terminal urodynamic measures after 12 wk of daily LT for a period of 60 min/day. The interaction of lower limb musculature with the bladder and its sphincter has been observed sporadically over the years, as far back as 1933, in both humans and animal studies (20, 53, 74). Flexor and extensor reflexes can be modulated by the state of bladder filling and voiding in normal humans and those with central nervous system damage (64). In humans with spasticity, the general pattern is that detrusor contractions precede limb flexor spasms (69). This vesicosomatic relationship involving lumbosacral reflex circuitries could contribute to the enhancement of bladder function with LT.

The multisystem functional gains with task-specific training have generated multiple novel hypotheses regarding potential underlying mechanisms. With respect to the improvements in bladder function with 60 min/day of stepping on a treadmill using body weight support and manual facilitation in a natural position (90), the current study was designed to expand upon our initial findings to include 1) non-weight-bearing stepping with a forelimbs-only exercise group; 2) collection of weekly metabolic cage data to monitor SCI-induced persistent polyuria (overproduction of urine); and 3) further tissue assessments that include the structural integrity of the bladder wall and the impact of training on the upper urinary tract (kidneys).

The bladder wall itself has been shown to be more compliant after spinal transection, and the extracellular matrix components largely determine its mechanical properties (36). Collagen and elastin, two major connective tissue proteins, provide tensile strength and elasticity and are implicated as being directly responsible for the mechanical changes in the bladder wall of SCI rats (83), so their quantification will give a more accurate picture of potential bladder composition remodeling. Long-standing detrusor sphincter dyssynergia with chronic SCI, even with careful bladder management, may lead to vesicoureteral reflux due to high bladder pressures from overdistention and complications that include kidney infections, pyelonephritis, and hydronephrosis (4, 13, 59). Bladder infections from multiple daily catheterizations may also spread to the kidney. Chronic kidney disease is highly prevalent in the SCI population, with complications that include decreased

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glomerular filtration rate and renal plasma flow (30, 60, 73). For the current study, the expression of two proteins indicative of tissue turnover in the kidneys, transforming growth factor-β (TGF-β; a fibrogenic growth factor implicated in the pathogenesis of renal scarring) and cluster of differentiation molecule 11b (CD11b; an adhesion molecule which promotes cell-cell adhesion between leukocytes and leucocyte-endothelial cells in inflammation), were assessed to determine whether cell adhesion between leucocytes and leucocyte-endothelial cells in inflammation), were assessed to determine whether improved bladder function with LT could also reduce the kidney’s susceptibility to post-SCI complications. TGF-β promotes fibrogenesis, cell apoptosis, and tissue healing and suppresses excess cellular proliferation, differentiation, and immunity (66). Although increased expression of TGF-β is considered a valuable marker in determining fibrosis with kidney disease (1, 11, 38, 94, 95), exacerbation of the immune response or autoimmunity has been reported in association with the absence or decreased expression of TGF-β (37), and the immune system is known to be impacted in persons with kidney disease (1, 11, 38, 94, 95), exacerbation of the immune response or autoimmunity has been reported in association with the absence or decreased expression of TGF-β (37), and the immune system is known to be impacted in persons with SCI (61). Also, damage to kidney tissue in ischemia-reperfusion injury can be mitigated by blocking CD11b (71) as it contributes to epithelial injury, inflammation, and fibrosis (32).

METHODS

All experimental procedures were conducted according to National Institutes of Health guidelines, and protocols were approved by the Institutional Animal Care and Use Committee at the University of Louisville School of Medicine.

A total of 55 adult male Wistar rats (Harlan Sprague Dawley, Indianapolis, IN), weighing initially ~250 g, were individually housed in an animal room with a 12:12-h light-dark cycle. They had ad libitum access to water and food (Laboratory Rodent Diet).

SCI

The Infinite Horizon (IH) impactor device (Precision Systems and Instrumentation, Fairfax Station, VA) was used to make a clinically relevant contusion injury (225 kdyne) at the T9 spinal level of 48 rats (7 additional rats served as sham surgical controls). This impact produces a moderate to severe incomplete SCI. Procedures for SCI followed our previously published protocols (41, 49, 50, 91). Briefly, animals were anesthetized with a mixture of ketamine (80 mg/kg) and xylazine [10 mg/kg intraperitoneally (ip)] and 0.1-ml supplemental doses were given ip as needed to maintain a deep surgical level of anesthesia. Vital sign monitoring included heart rate with a rodent stethoscope, respiratory rate, and ventilator status with a small animal oximeter (Starr Life Sciences), body temperature with a rectal thermometer, and anesthetic depth with corneal, palpebral, pedal, and pinna reflexes as well as tail pinch.

For the spinal contusion, the cord was exposed at the T9 level via removal of the overlying vertebral lamina, and the IH impactor force-driven device (76) was used to make the contusions (no dwell time). After the injury, a piece of thrombin-soaked Gelfoam was placed into the vertebral defect, the surrounding musculature and subcutaneous tissue were sutured in layers with 4-0 monofilament, and the skin was closed with Michel clips (removed 7 days po). All rats were injected subcutaneously with 0.5 ml of dual penicillin (penicillin G coupled with Procaine, PenJect, Butler Schein, Columbus, OH) as a general prophylactic and recovered in a temperature-controlled environment. In the days immediately following SCI, all animals were also given subcutaneous injections of ketoprofen (Ketofen, 2.5 mg/kg, Fort Dodge Animal Health, Fort Dodge, IA) for analgesia (twice a day for 2 days) and 5 mg/kg gentamicin (Genta-Fuse, Butler Schein, Dublin, OH) to prevent bladder infections (once per day for 5 days).

The urinary bladder was initially emptied by manual crede every 8 h until the micturition reflex occurred automatically (spontaneously triggered by contact with cage bedding or otherwise without caretaker assistance), 6–10 days after injury (29, 51). Residual urine was collected and measured three times over a 24-h period for every rat on the days immediately postinjury and recorded in the animals’ recovery log. Only the maximum single daily urine volume (typically the AM volume after lights on, i.e., after the active phase during which more water tends to be consumed) was used for analysis as this value is most reflective of the largest bladder capacity. Note that the amounts obtained at a 3- to 4-day time point are reflective of the initial peak bladder dysfunction immediately post-SCI with higher volumes indicative of a more severe injury (91). These data along with a 2-wk time point score of overground locomotion using the 21-point Basso-Beattie-Bresnahan (BBB) open-field locomotor test (6) (as previously described in detail) (90, 91) were set aside for end-of-study post hoc analysis to check for pretraining group differences in terms of lesion severity. Training was initiated 14 days postinjury after animals were randomly divided into three study groups (as described below). This delayed time frame was chosen because I) rehabilitation efforts initiated too early after SCI have been shown to be detrimental as they can exacerbate secondary injury cascades (79) and 2) 2 wk are beyond the period of SCI-induced plasticity of sacral parasympathetic bladder reflex pathways (18, 19, 86) which can occur up to 10 days (29, 51).

Treadmill Training

SCI rats were randomly divided into 3 groups of 16 each 2 wk postinjury: two groups followed a step-training regimen over a treadmill belt assisted by a body weight support system (Exer-3R treadmill, Columbus Instruments) (90, 91), and a nontrained control group used no body support. All groups were harnessed with a cloth rodent vest (Robomedica, Mission Viejo, CA) with spring scales clipped onto both ends of the vest for weight support as needed. The quadrupedal-trained group (n = 16; SCI + QT) stepped on the treadmill with all four limbs, bearing weight on the hindlimbs with manual facilitation below the level of injury (90), with the spring scales self-adjusted to allow for quadrupedal stepping and complete paw placement. The forelimb-only trained group (n = 16; SCI + FT) had their hindlimbs slightly elevated from the surface of the treadmill using the spring scale (non-weight bearing; similar to arm crank exercise in human SCI) so that they only stepped with their forelimbs. The nontrained control group (n = 16; SCI) was also harnessed on the treadmill for the equivalent 1-h time frame but was not stepped. Seven additional rats, used as noninjured controls (surgical shams: spinal cord exposed like the other groups but no contusion), were handled weekly but remained in their home cages for the duration of the study.

Given the time necessary for the daily step training of each rat, the current study was done in 4 groups of 12 rats each over a period of ~2 yr. Each set of 12 comprised 4 SCI + QT, 4 SCI + FT, and 4 SCI only control rats. In a given hour session, one SCI + QT and one SCI + FT rat were on the 15-inch-wide treadmill belt with one nontrained SCI rat in a harness next to it. Thus 4 h of training were done in total per day, with 15-min breaks every hour between groups. Each set of 12 rats took part in the study for a total of ~14 wk (acclimation period, preinjury testing, SCI, 10 wk of training/testing starting 2 wk post-SCI, awake cystometry at the end of training immediately before euthanasia and tissue removal). A time line is provided in Fig. 1.

Before SCI and post-SCI pretraining, all rats were exposed to the treadmill environment for at least two half-hour sessions. Training was initiated on schedule at 14 days after injury for all rats, given that there were no signs of infection or other complications arising from the surgery (a rare occurrence). The SCI + QT and SCI + FT groups of animals were at first placed on the treadmill in the harness vest for 20 min, beginning at a slow speed. The session time was gradually increased from 20 min to the target of 58 min over the course of the first 7 training days as described previously (90). All rats on the

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treadmill were monitored by an experienced investigator at all times. However, to help provide adequate afferent feedback related to stepping for the SCI+QT group (similar procedure done with locomotor training in humans), the experimenter manually assisted for plantar paw placements with full toe extension and no ankle rotation. Independent stepping was encouraged when the rats had achieved better coordination, stability, and absence of hindlimb dragging. Although sessions are terminated early for any animals showing signs of stress (for example, diarrhea, porphyrin stains in eyes, or irregular breathing pattern) or a session skipped if any abrasions from training were observed on the paw or skin (until healed, as noxious input can interfere with spinal learning) (39), there were no such instances in the current study.

**Voiding Behavior**

Voiding behavior was assessed weekly with a six-station Columbus Instruments Comprehensive Lab Animal Monitoring System (CLAMS). The CLAMS unit and corresponding software (Oxymax for Windows, version 4.83) was used to collect 24-h urination data and food and water intake for all groups of rats. Animals were placed in cages once a week throughout the experiment (including preinjury, the 2 wk post-SCI, and throughout the 10-wk training period) for 24-h data collection periods with food and water ad libitum. Following each 24-h testing session, each metabolic cage was disassembled, cleaned, and reassembled for the next session.

**End of Study Awake Cystometry**

Filling cystometry (nonstop transvesical) experiments were conducted in conscious rats (see Refs. 92 and 93 for justification) after ~10 wk of training (or equivalent time frame) for all groups. For catheter placement (47, 54, 63), a 1.5-cm midline abdominal incision was made under brief gas anesthesia (2% isoflurane). The catheter (PE-60 tubing), with previously heated tip to form a collar of elastin and collagen) using ELISA. Western blots were done to assess connective tissue proteins that provide tensile strength and elasticity (elastin and collagen) using ELISA. Western blots were done to assess the expression of two proteins in the kidney whose presence are indicative of tissue stress or damage (TGF-β and CD11b). Spinal cord tissue containing the lesion area (T8–T10) was also removed and immersed in 4% paraformaldehyde for at least 48 h, followed by a 30% sucrose/phosphate buffer solution with 1% sodium azide for at least 24 h and until the tissue was cut transversely.

Following the cystometry recordings, rats were overdosed with the ketamine/xylazine mixture and immediately perfused with heparinized saline followed by a solution of 30% RNAlater (Ambion, Grand Island, NY) in a 1 mg/ml phosphate buffer solution for tissue retrieval (spinal cord lesion site, kidney and bladder tissue). The bladder was removed, blotted dry, and weighed. The left kidney was also removed and along with the bladder placed in a 100% solution of RNAlater for 24 h at −20°C and then flash frozen in liquid nitrogen and stored at −80°C. As described below, bladder tissue was used for measuring connective tissue proteins that provide tensile strength and elasticity (elastin and collagen) using ELISA. Western blots were done to assess the expression of two proteins in the kidney whose presence are indicative of tissue stress or damage (TGF-β and CD11b). Spinal cord tissue containing the lesion area (T8–T10) was also removed and immersed in 4% paraformaldehyde for at least 48 h, followed by a 30% sucrose/phosphate buffer solution with 1% sodium azide for at least 24 h and until the tissue was cut transversely.

After procedures for the fourth and final set of 12 rats were completed, all collected tissues (coded to maintain blindness of experimenter to group identity) were processed together (see procedures for ELISA and Western blots below). After all experiments were complete, the data were decoded and separated into groups (sham, SCI, SCI+QT, SCI+FT) and analyzed using SigmaStat. One-way ANOVA or one-way repeated measures ANOVA with a significance level of P < 0.05 was used followed by the Holm-Sidak method for pairwise multiple comparisons according to previously published protocols (90, 91).

**ELISA for Elastin and Collagen**

The amount of collagen and elastin present in bladder tissue was quantified using a collagen assay and elastin assay (Biocolor, Northern Ireland, UK). For collagen analysis, frozen bladder tissue (kept at −80°C) was thawed and placed in an acid-pepsin solution overnight at 4°C to make the collagen soluble. A 100-μl sample containing the tissue or three reference standards was added to a 1.5 ml microcentrifuge tube, and 1 ml of Sircol Dye reagent was added to each tube. Following 30 min on a shaker, tubes were centrifuged for 10 min and then drained leaving the collagen at the bottom of each tube. An acid-salt wash was then applied to remove any remaining dye. A 250-μl solution of alkali reagent was then added to each tube and vortexed for 5 min. Two hundred microliters of standard, blanks, and
samples were then pipetted into individual wells of a 96-well plate, which was then read at 555 nm using a Spectramax Plus microplate reader (Molecular Devices, Sunnyvale, CA) to determine the tissue collagen content.

For elastin analysis, bladder tissue was placed in 0.25 M oxalic acid and heated to 100°C for 1 h. This was done twice for each sample. A 100-μl volume of sample, standard, or blank was added to a 1.5 ml microcentrifuge tube followed by 100 μl of elastin precipitating reagent. After 15 min, tubes were centrifuged and drained leaving the α-elastin at the bottom of each tube. A 1-ml volume of dye was then added to each tube and placed on a shaker for 90 min. Tubes were then centrifuged and drained again. Then, 250 μl of dye dissociation reagent was added to each tube and vortexed. Each tube was then revortexed 10 min later. An amount of 200 μl from each tube was then placed in a 96-well plate, which was read at 513 nm using the Spectramax Plus microplate reader to determine the tissue elastin content.

Western Blots for TGF-β and CD11b

Expressions of the proteins TGF-β and CD11b were analyzed with Western blotting following our previously published protocols (40). The left kidney was sectioned (to include both cortex and medulla in the sample) on an ice tray and homogenized in ice-cold lysis buffer (50 mM Tris-HCl (pH 8.0), 200 mM NaCl, 50 mM NaF, 0.3% Triton X-100, 1 M DTT, 1 M benzamidine, 100 mM Na-orthovanadate, 100 μM PMSF) and protease inhibitor (78425, Halt protease inhibitor single-use cocktail, Thermo Scientific). For the protein assay, the Bio-Rad protein assay reagent was used. Protein estimation was done in a photometer at 590-nm absorbance. Samples were loaded with 4× loading buffer (dye) by adjusting the proportion according to the value derived from the estimation. Gels were run in mini-protein gel tanks (Bio-Rad mini-protein tetra system) in 1×.5-mm precast gels (45-1083, mini-protein TGX gels, Bio-Rad) at 100 V for 1.5 h. The running buffer was 1× Tris-glycine-SDS buffer. A protein ladder (161-0374, Precision plus protein standards, dual color, Bio-Rad) was used for the band-level detection. Protein was transferred overnight at 30 A on nitrocellulose membranes (162-0115, Bio-Rad) in the transfer buffer (1× Tris-glycine-methanol-SDS buffer). Membranes were then blocked in 3% nonfat dry milk, a TBST solution was applied overnight, and then the membranes were washed the next day with TBST. The membranes were then cut at the level of 75 kDa, the upper portion was incubated in rabbit anti-CD11b (75476, Abcam), and the lower in rabbit anti-TGF-β (3711, Cell Signaling). The membranes were incubated overnight in primary antibody at 1:1,000 dilution (diluted in TBST and 3% nonfat dry milk solution) on a mechanical shaker in a cold room. The following day, membranes were incubated in horse-radish peroxidase (HRP)-conjugated anti-rabbit secondary antibody (7074, Cell Signaling) for 1 h at room temperature after washing in TBST buffer three times for 5 min each on a mechanical shaker. The membranes were treated with HRP antibody detection reagent (HyGlo chemiluminescent) and exposed to autoradiography film (HyBlot autoradiography film, Denville Scientific). β-Actin (4967S, Cell Signaling) was used as a loading control. After exposure for principal proteins, the membranes were stripped and reprobed with rabbit anti-β-actin antibody at 1:1,000 dilution.

All the samples were run at least three times, and the bands from all tests were analyzed by inverse densitometry using ImageJ 1.47 (version 1.47, National Institutes of Health) according to our previous protocols (40). Each individual value was normalized by subtracting the background and dividing the value with corresponding β-actin values (minus background). The values obtained from the ImageJ program were then analyzed for the various group comparisons. For the calculation, final raw values for the proteins were obtained after subtracting the background and dividing the result with the loading control. Each value obtained from the calculation for SCI and training groups was normalized with the mean value of shams (i.e., sham controls were set to a value of 1). Statistical analysis included one-way ANOVA with Tukey’s honest significant difference post hoc t-tests. All values are expressed as means ± SE. A P value of ≤0.05 was considered statistically significant.

Histology

The lesion site tissue was sectioned on a cryostat (Leica CM 1850) at 30-μm-thickness and stained with Luxol fast blue and cresyl violet (Kluver-Barrera method) per established protocols (41, 90). Spot Advanced software (Diagnostic Instruments, Sterling Heights, MI) and a Nikon E400 microscope were used to obtain measurements for quantification of the lesion epicenter (based on total lesion area) as previously described (41, 91). The percent white matter sparing was determined by dividing the white matter remaining at the epicenter by the average area of white matter present in more intact sections. The intact area of white matter for a given region is estimated by averaging measures from two sections 2 mm rostral and two 2 mm caudal to the epicenter. To compare white matter sparing with bladder function, a multiple regression analysis was performed.

RESULTS

A total of 46 male rats were randomly divided into 3 groups (SCI, SCI+ QT, SCI+ FT) at 2 wk postinjury (2 of the initial 48 died due to complications following the injury). Post hoc analysis of the group data for contusion parameters (kilodyne force and impactor displacement), 4-day urine volume, and 2-wk BBB locomotor score reveals no significant differences with respect to the injury itself and functional outcomes between the groups before the initiation of training (Fig. 2).

Data from the weekly metabolic cage CLAMS system was generated by Oxymax computer software and saved for post hoc analysis. To avoid potential week-by-week variability, 24-h data obtained from two separate time points were averaged for analysis, including two baseline measures preinjury, each of the 2-wk measures postinjury/pretraining, two midway training time points (weeks 3 and 4), and the last two training week time points [weeks 8 and 9-week 10 (12 post-SCI) was the terminal assessment week]. The data for total 24-h urine volume, average volume per void, total number of voids in 24 h, and total 24-h water intake are presented in Fig. 3. A representative example of the 24-h micturition cycle for an SCI and an SCI+ QT rat at four different time points (before injury, after injury, midtraining time point, late in training time point) is provided in Fig. 4. Note that the SCI-induced increase in production of urine after injury was not due to an increase in water intake (compare with preinjury control values for all groups in Fig. 3), as we have shown previously (91). The CLAMS data, when considered in their entirety, suggest that the higher volume per void for the SCI control group after the 9-wk training period could be a compensation for the higher 24-h production of urine (polyuria) for that group. The terminal awake cystometry data (Fig. 5) are consistent with these findings, as the maximum amplitude of contraction was significantly higher for only the SCI nontrained group relative to shams, i.e., a higher void volume requiring a larger bladder contraction to empty (#, Fig. 5). Note, however, that the trained SCI animals as a group were also significantly different from the nontrained SCI animals (**P < 0.01), but, individually, only the SCI+ FT group was significantly different (*P < 0.01). No other differences were found between groups relative to shams (intercontraction interval and peak pressure; not
No differences were found in bladder weight and the ratio of elastin-to-collagen between SCI groups, although bladder weight was significantly higher for all groups at 12 wk post-SCI ($P < 0.05$; Fig. 6).

Expression of the proteins TGF-β and CD11b were analyzed with Western blots using the left kidneys from shams and the three SCI groups. For TGF-β, two different bands were detected: one protein band located at 25 kDa and the other at 50 kDa. No differences were found in bladder weight and the ratio of elastin-to-collagen between SCI groups, although bladder weight was significantly higher for all groups at 12 wk post-SCI ($P < 0.05$; Fig. 6).
kDa. These bands represent mature TGF-β and TGF-β precursors (known to produce a band between 45 and 65 kDa), respectively, per the manufacturer’s datasheet. The 25-kDa bands (mature) of TGF-β were analyzed, and a comparison between the expressions in sham vs. SCI (nontrained) animals revealed a significant decrease in kidney TGF-β levels following chronic SCI (Fig. 7B). For the SCI+QT and SCI+FT trained groups, the protein levels were not significantly different from shams ($P > 0.05$). In contrast, the kidney CD11b levels (location at ~160 kDa per the manufacturer’s datasheet) increased in density at the 12-wk postinjury time point (SCI) and remained significantly higher, relative to shams, in the SCI+QT/SCI+FT training groups (Fig. 7C).

Histological analysis of white matter sparing at the lesion epicenter as well as estimates of the total lesion volume revealed no significant differences between the three groups of SCI rats. A summary of the mean data is presented in Fig. 8. Total lesion volume ranges for the three groups were 9.1–15.6 mm$^3$ (SCI), 9.6–14.7 mm$^3$ (SCI+QT), and 9.0–14.9 mm$^3$ (SCI+FT). Epicenter percent white matter sparing ranges for the three groups were 4.5–32.4% (SCI), 6.1–42.2% (SCI+QT), and 5.9–44.1% (SCI+FT). To further explore the possi-

Fig. 4. Twenty-four-hour micturition cycle. Shown are representative total 24-h urine events measured with the CLAMS system for 2 rats, 1 from the SCI control group (A) and 1 from the SCI+QT group (B). Each plotted line represents a different time point of the experiment; preinjury baseline (○); 2 wk postinjury (pretraining; △); 6 wk postinjury (4-wk posttraining time point in B; ●); 10 wk postinjury (8-wk post-training time point in B; □). Each individual symbol represents a single urine event that is a cumulative total over the 24-h period. The horizontal bar represents the 12-h phase when the housing facility lights are off (active period). Note that the majority of urine events occur during the active phase.

Fig. 5. Terminal awake cystometry. Shown are raw recordings of fill/void cycles from each group of animals. In A, 5 full fill/void cycles are shown for a QT animal. In B, a representative example of 1 fill/void cycle (note the scale bar) is provided for each group (different QT animal from A), and the maximum amplitude values are shown (mmHg). The group means of the averaged data is graphed in C. Significant group differences are shown relative to shams (#) and relative to nontrained SCI animals (*, **). Values are means ± SE.
bility of relationships between injury severity and bladder function, a multiple regression analysis was done, and no relationship was found \((P > 0.05)\) between epicenter white matter sparing and bladder outcomes (including voided volume) within each training group, and the training effect is independent of lesion variability.

**DISCUSSION**

The results of the present study demonstrate an impact of exercise training on multiple post-SCI-induced impairments related to various aspects of urinary tract function using metabolic cage frequency-volume measurements of voiding and awake cystometry. Improvements in both the SCI+QT as well as the SCI+FT groups implicate underlying mechanisms beyond repetitive sensory information from the hindlimbs driving spinal network excitability of the lumbosacral urogenital neural circuitry. Furthermore, preliminary evidence for the potential impact of exercise training on the upper urinary tract in addition to the lower urinary tract underscores overall health benefits of activity-based training on the entire urinary system within the SCI population.

**SCI-Induced Polyuria**

Metabolic cage data indicate that following SCI, mean 24-h urine volume and the average volume per void increased in all groups of animals, a finding consistent with our previous study demonstrating SCI-induced polyuria for a wide range of spinal lesion severities (mild to severe) (91). After 10 wk of activity-based training, total urine volume and average volume per void were significantly lower in both the SCI+QT and SCI+FT groups relative to the SCI group, although the trained group values were still significantly above preinjury baseline. The further increase in average void volume at the later time points could be reflecting better efficiency (i.e., lower residual volumes), which would be consistent with the significantly higher maximum contraction amplitudes for the SCI rats relative to the sham animals as revealed with terminal cystometry. Note that the metabolic cage procedure that was done weekly does not give a measure of residual volume. Future studies would need to involve a time course for awake cystometry with a chronically implanted bladder catheter to further address these novel findings. The benefits regarding the duration of intense physical activity with 60 consecutive min of training (current and previous data on locomotor and nonlocomotor systems including the bladder) (90) but not 30 min of stepping as we have shown previously for SCI-induced polyuria (91), are consistent with a study on locomotion using a rat spinal transection model showing dependence of functional recovery on the number of repetitions of the weight-bearing stepping activity (15).

Potential mechanisms for improving SCI-induced bladder dysfunction with activity-based training include general exercise effects that impact target organ-neural interactions or potential plasticity of the spinal bladder reflex circuitry (such
as changes in the properties of afferent neurons in the bladder receptors and/or cell bodies and/or terminals within superficial laminae of the dorsal horn. For example, one likely contributor to the observed SCI-induced polyuria and subsequent alterations in bladder function is hypernatremia (a decrease in the level of serum sodium), which can often occur due to high water content in the blood (55). Hypernatremia is often associated with hyposmolality (a decrease in blood osmolality). One of the easiest ways for the body to increase the level of sodium and increase plasma osmolality is to increase water excretion through urination. Several studies have shown that hypernatremia develops following SCI (10, 33, 70). The trigger that the body uses to increase water excretion in response to hypernatremia and/or hyposmolality is to decrease the release of antidiuretic hormone (ADH; also known as vasopressin) from the posterior pituitary gland (neurohypophysis). When plasma ADH levels are low, the body excretes more water. Importantly, a number of studies involving able-bodied human subjects indicates that exercise can increase plasma ADH levels (45, 87, 88). Given that exercise and LT are rehabilitative therapies that many SCI individuals receive, there is potential for improvement in urological function as long as sufficient amounts are provided [recall that our animal data indicate improvements with 60 but not 30 min (91) per day of intense training]. Our initial data with human SCI research participants (at least 2 yr postinjury) indicate significant improvements in bladder function (as assessed with cystometry) following 80 sessions of LT or LT plus stand training for 60 min/day 5 days/wk (48). Note that if polyuria following SCI is triggered by hypernatremia and/or hyposmolality, then there should be a decrease in the plasma ADH levels post-SCI. Recent data from our laboratory using male rats with a T9 contusion indicate that serum ADH levels decrease significantly 2 wk after severe SCI (unpublished observations), which is consistent with our metabolic cage data showing significantly elevated total 24-h urine volume at 2 wk (Fig. 3). Further studies are in progress. Anecdotally, SCI individuals often report limiting fluid intake to decrease the number of catheterizations over a 24-h period. However, this lack of fluid intake can lead to other metabolic consequences (77). Although polyuria is common after SCI (56), few studies have investigated the mechanisms underlying this condition.

An additional potential mechanism for the observed changes in urological function with LT could involve somato-pudendal reflex interactions such as the flexor and extensor reflex circuitry interactions with external urethral sphincter reflexes as described previously by Tai et al. (81). Motor and autonomic output of the spinal cord is driven in large part byafferent input and local or propriospinal circuitry emphasized after SCI conditions (7, 12, 17, 34, 44), which create the potential for interactions and the triggering of some plasticity within the lumbosacral circuitry to the bladder. For example, multiple sensory inputs from the periphery during locomotion, particularly limb loading (28) and stepping rate (31), provide information to these networks to improve stepping (16, 21, 22, 24, 25). These interactions, however, would not explain the improvement observed with the forelimb exercise group of rats receiving 60 min of daily training. It is conceivable, however, that intense repetitive forelimb exercise with sufficient spinal network excitability via residual supraspinal input induces a net improvement in functional reorganization of the lumbosacral neural circuitry (14, 58, 67, 78). In individuals with an incomplete spinal cord injury, there is some facilitation of the lower extremity muscles in those subjects when walking with reciprocal arm swing vs. without (walking while holding on to parallel bars) (8, 85). Specifically, the presence of some residual intact long propriospinal interenlargement pathways that mediate interlimb coordination for locomotor function in the rat (72) could induce adaptive changes to neural networks within the lumbosacral spinal cord, such as those controlling the bladder. However, given the severity of injury (22.0 ± 2.7% white matter sparing at the epicenter for the forelimb-trained SCI group) and the fact that there was little or no hindlimb activity in the FT group during training and no air-stepping, the probability for the occurrence of such interactions is likely low. Also note that the animals with the most spared white matter did not have the best recovery of bladder function. Thus, although load-bearing on the hindlimb has been shown to be of critical importance for stepping (82), other systemic factors may be vital contributors for improving bladder function with task-specific training based rehabilitation.

Kidney Findings

The results from the present study demonstrate that after a clinically relevant spinal contusion injury, there is a significantly lower level of TGF-β expression in the kidney (NT group) relative to shams. Since TGF-β controls T cell activation and abolition of TGF-β causes gradual infiltration of leukocytes into multiple organs (37, 62, 65), our finding may indicate the presence of an altered immune response in the kidneys during the chronic phase after SCI. In addition to lower TGF-β levels, there was a corresponding rise of CD11b expression in the kidney after chronic SCI (SCI group relative to shams). TGF-β has a known inhibitory effect on CD11b expression (5), which may explain the post-SCI increase in CD11b activity. Note that activation of macrophages, which play a role in both injury and repair in kidney tissue (27), is
required for the synthesis of TGF-β, so a rise in CD11b expression may also indicate activation of endogenous macrophages for tissue homeostasis.

In both SCI training groups, the level of TGF-β expression was not significantly different from the levels in surgical sham animals, indicating the possibility of immune homeostasis and maintenance of renal health (normalized glomerular filtration rate and kidney function) and decreased susceptibility to infection, which would impact the quality of life for the SCI population. Exercise has been shown to reverse negative immune alterations (35), including after SCI (61), but the time since injury and the ideal starting point of training as well as the duration and intensity (57, 89) need further consideration to optimize the most effective strategy not just for urinary tract function but functional recovery in general.

Perspectives and Significance

Bladder dysfunction after SCI is rarely studied in experimental animals yet is overwhelmingly the most significant concern for those suffering from SCI, and urological complications result in significant morbidity and mortality. Importantly, even small improvements in bladder function can have a tremendous impact on these individuals’ continual health and quality of life. Results from this animal study provide some initial clues about potential underlying mechanisms regarding our findings on the effects of activity-dependent plasticity induced by LT after chronic SCI on nonlocomotor systems (i.e., bladder function).

Conclusions

Our studies to date indicate that activity-based training can influence urological outcomes, which are of great importance to persons with SCI. The positive benefits of exercise on bladder function post-SCI are likely indirect. These novel findings suggest that physical activity after SCI could translate to significant quality of life gains.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


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