Prevention of deep tissue injury through muscle contractions induced by intermittent electrical stimulation after spinal cord injury in pigs

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Solis LR, Twist E, Seres P, Thompson RB, Mushahwar VK. Prevention of deep tissue injury through muscle contractions induced by intermittent electrical stimulation after spinal cord injury in pigs. J Appl Physiol 114: 286–296, 2013. First published November 21, 2012; doi:10.1152/japplphysiol.00257.2012.—Deep tissue injury (DTI) is a severe medical complication that commonly affects those with spinal cord injury. It is caused by prolonged external loading of the muscles, entrapping them between a bony prominence and the support surface. The entrapment causes excessive mechanical deformation and increases in interstitial pressure, leading to muscle breakdown deep around the bony prominences. We proposed the use of intermittent electrical stimulation (IES) as a novel prophylactic method for the prevention of DTI. In this study, we assessed the long-term effectiveness of this technique in pigs that had received a partial spinal cord injury. It is caused by prolonged external loading of the muscles, entrapping them between a bony prominence and the support surface. The entrapment causes excessive mechanical deformation and increases in interstitial pressure, leading to muscle breakdown deep around the bony prominences. We proposed the use of intermittent electrical stimulation (IES) as a novel prophylactic method for the prevention of DTI. In this study, we assessed the long-term effectiveness of this technique in pigs that had received a partial spinal cord injury. This study demonstrates, for the first time, that IES is an effective technique for preventing the formation of DTI in loaded muscles after spinal cord injury.

pressure ulcers; functional electrical stimulation; reduced mobility; muscle contraction

PRESSURE-RELATED DEEP TISSUE INJURY (DTI) is a type of pressure ulcer that is commonly prevalent in people with reduced mobility and sensation, such as individuals with spinal cord injury (7, 12, 51). It develops in muscles entrapped for prolonged durations between a bony prominence and a surface (9, 14, 37, 50). The entrapment causes muscle breakdown due to sustained mechanical deformation (6, 27, 28, 32, 34, 39), ischemia (19–21, 32, 59), and reperfusion injury (13, 32, 61). Because DTI develops deep in the tissue and without exhibiting early skin signs, it cannot be detected using routine skin inspections. Once skin signs become evident, extensive damage in the soft tissue underlying the skin would have already occurred. The resulting lesions often require surgical intervention and several months to heal. In the US, more than $11 billion is spent annually on treating pressure ulcers (2, 45).

Current interventions for the prevention of DTI consist of performing frequent repositioning movements to alleviate pressure from regions at risk (44, 63), the use of specialized wheelchair cushions and mattresses to reduce interfacial (skin surface) pressures (22, 40, 43), and a general improvement in nursing care, including better nutrition (18). Despite the advancements in clinical care and support surface technology, the incidence rates of pressure ulcers have not changed significantly since the 1940s (35).

Electrical stimulation for the prevention of pressure ulcers was suggested previously by Levine and colleagues (24–26). In their work, they found that muscle contractions produced by electrical stimulation reduce superficial pressure and increase blood flow. Bogie and colleagues (3–5), Liu et al. (30, 31), and Van Londen et al. (62) also reported similar results. Nonetheless, these studies had two limitations. 1) Testing the effectiveness of electrical stimulation in preventing the formation of pressure ulcers was not conducted, and 2) all advocated the use of continuous or near-continuous generation of muscle contractions to be effective, which limits the duration of deployment of electrical stimulation due to muscle fatigue.

Intermittent electrical stimulation (IES) is a novel electrical stimulation approach for the prevention of pressure ulcers. Electrical stimulation is applied for short durations (~10 s) every few minutes (~10 min) to loaded muscles (e.g., gluteus maximus muscles of the buttocks) to induce periodical contractions. These contractions produce postural shifts that mimic the subconscious shifts (fidgeting movements) performed by able-bodied individuals in response to discomfort while sitting or lying down. The brief contractions every few minutes greatly reduce the rate of muscle fatigue, allowing the use of the intervention throughout the day or night. We demonstrated in previous studies that each IES-elicted contraction significantly reduces and redistributes the levels of internal stress around bony prominences, and counteracts compression in the muscle (55, 56). In studies with human volunteers with or without spinal cord injury, we also showed that each IES-elicted contraction reduces interfascial pressure around bony prominences and increases oxygenation in the muscle for up to 15 min after each brief contraction (15, 53). In studies in rats, IES significantly reduced the extent of deep tissue damage.
caused by externally loading a muscle for a one-time duration of 2 h (8, 54). In these studies, IES was more effective in reducing the extent of DTI than periodical muscle unloading, which emulated the wheelchair pushup exercises prescribed to wheelchair users for preventing pressure ulcer formation (8).

The goal of the present study was to test, for the first time, the effectiveness of IES in preventing DTI formation in an animal model of spinal cord injury subjected to realistic levels of prolonged muscle loading. Studies were conducted in adult pigs with partial spinal cord injury leading to paralysis of one hindleg. Atrophied muscles in the paralyzed limb were loaded for 4 h every day for 1 mo, and the effect of IES in preventing the formation of DTI in the loaded muscles was assessed.

METHODS

Overview

Experiments were conducted in six adult Yucatan miniature pigs (weight 58.5 ± 7.4 kg; means ± SD) with approval from the University of Alberta Animal Care and Use Committee. All animals underwent a surgical procedure that hemisected the spinal cord at the second lumbar level (L-2), leading to paralysis of the left hindleg. The animals recovered from surgery for a period of 2 wk, after which an external load was applied over the superficial and middle gluteal muscles and biceps femoris muscle for 4 h every day, 4 days/wk, for 1 mo. The animals were randomly divided in two groups; one received IES to the loaded muscles during the loading hours, whereas the other did not receive IES. The effectiveness of IES in preventing the formation of DTI was assessed weekly through the use of magnetic resonance imaging (MRI).

Spinalization Surgery

Sedation was induced through intramuscular administration of a mixture of ketamine (22 mg/kg), glycopyrrolate (0.01 mg/kg), and xylazine (2.2 mg/kg). Anesthesia was induced and maintained through the use of isoflurane (2–3%). A 15- to 20-cm incision was made in the skin of the back, and the muscles covering the spinal column at the level of the L-2 vertebrae were separated to expose the spinal column. A laminectomy was then performed to expose the dorsal surface of the spinal cord. The dura mater was carefully opened using iridectomy scissors, and a mediolateral hemisection of the spinal cord was performed. The cord was then covered with a thin plastic film, and the muscles and skin were sutured shut in layers.

Postsurgical Recovery and Palliative Care.

The animals recovered individually in cages with padded flooring to prevent the formation of uncontrolled DTI or superficial pressure ulcers. Analgesia was induced and maintained through the use of isoflurane (2–3%). A 15- to 20-cm incision was made in the skin of the back, and the muscles covering the spinal column at the level of the L-2 vertebrae were separated to expose the spinal column. A laminectomy was then performed to expose the dorsal surface of the spinal cord. The dura mater was carefully opened using iridectomy scissors, and a mediolateral hemisection of the spinal cord was performed. The cord was then covered with a thin plastic film, and the muscles and skin were sutured shut in layers.

The rostral part of the sling, which supported the weight from the shoulders and head, was set slightly lower than the distal part. This allowed the pig to support itself with its front limbs. The time the pigs spent in the sling each day was gradually increased, starting with 1 h on the 1st day and ≤4 h by the end of the 1st week postsurgery. All of the pigs tolerated this procedure very well, remaining in place calmly and with minimal movement for the required time. Occasionally, the pigs would fall asleep, relaxing their front limbs and allowing the sling to support their weight fully.

Daily Loading Procedure

Two weeks after the spinalization surgery, the behavior (appetite and mood) of each animal returned to presurgery norms. During these 2 wk, most pigs learned to move around their kennel by utilizing their three intact limbs. The daily loading protocol entailed placing the pigs in the suspension sling for 4 h. To minimize the chance of random movements while in the sling, straps were used to attach the sling to the frame holding the sling, fixing the sling’s position in place (Fig. 1A). This constrained the pig’s body movement except for the head.

Loading was applied to the paralyzed limb through a 12 × 10 in. acrylic indenter (Fig. 1, A and B) over the superficial and middle gluteal muscles, as well as part of the biceps femoris muscle of the paralyzed limb, pushing the muscles against the iliac crest. To monitor discomfort or pain during the loading periods, close attention was given to the animals’ behavior, particularly vocalizations that are common when pigs experience pain or stressful situations. At no time during the loading periods did the animals vocalize; in fact, they often fell asleep during these periods.

The level of loading applied to the paralyzed side corresponded to 25% of each animal’s body weight (Fig. 2, A and C). In people with intact and injured spinal cords, 61 and 70% of body weight, respectively, is carried by both ischial tuberosities and thighs while sitting (15, 53, 56). Therefore, the applied loading on the paralyzed limb in
this study represented the average level of loading experienced by the tissue around each ischial tuberosity. The indenter was attached to a computer-controlled servo-motor (Danaher AKM23D; Danaher Motion, Washington, DC) mounted on the post of a drill press (Fig. 1A). The loading level was monitored through a force transducer (Interface SMT2–225; Interface) placed in-line with the servo-motor and indenter and through a thin pressure-sensing pad (PX200:36:36; XSensor Technology, Calgary, AB, Canada) placed between the indenter and the skin of the pig. Loading was applied for 4 h/day, 4 consecutive days/wk, for 4 wk. A piece of a 4-in.-thick Tempur-Pedic mattress was placed between the contralateral limb and the wall of the metal frame supporting the sling during the loading to prevent DTI formation in that limb.

**Application of IES**

Three of the six pigs were randomly assigned to receive IES throughout the duration of daily loading. Stimulation was delivered through a pair of electrodes placed on the skin (5 × 10 cm; Pure Care, Sherwood Park, AB, Canada) and a hand-held stimulator (BioStim NMS+ Stimulator; Biomedical Life Systems). The active electrode was placed over the motor point of the superficial gluteus muscle, whereas the return electrode was placed over the middle gluteus muscle. Stimulation consisted of trains of biphasic, charge-balanced, square pulses. Pulse width was 250 μs and pulse frequency 35 Hz. Pulse amplitude was adjusted individually for each pig to generate a visible fused contraction in the targeted muscle. The IES cycle consisted of 10 s of stimulation (“on” period) followed by a 10-min period of no stimulation (“off” period).

Force recordings (from the in-line force transducer) were used to monitor the quality of the contraction and indirectly assess muscle fatigue resulting from the use of IES over the 4 h of loading (Fig. 2, A, B, D, and E). The strength of IES-induced muscle contractions (based on the force produced by the push of the muscle belly against the indenter during the contractions) was assessed by monitoring the force generated during each IES on period (Fig. 2B) throughout a loading session. The force generated during the last 5 s of each on period of IES (Fig. 2D) was averaged and subsequently normalized to the force generated during the first IES-induced contraction for each session. The normalized forces from each session were averaged across all sessions for each pig, and a linear fit was estimated for all pigs (Fig. 2E).

**Assessment of DTI Through MRI**

The effectiveness of IES was evaluated weekly through MRI assessments of the pelvic region. Two assessments were acquired prior to the application of external loading, the first 1 wk prior to the spinalization surgery and the second 2 wk postsurgery. The two assessments allowed for comparisons of muscle mass before and after spinal cord injury and to ensure that no muscle injury developed unbeknownst to the researchers during the 2 wk of postsurgical recovery. The assessment acquired 2 wk postsurgery also served as a baseline measurement for subsequent assessments. Once the loading protocol was initiated, MRI assessments were obtained weekly (after 4 days of consecutive loading each week).

On the day of the MRI assessment, the animals were sedated using the same cocktail described in the surgical methods. A tracheal tube was then inserted to allow proper air flow, and intravenous catheters
were inserted in both ears. Anesthesia was maintained through bolus intravenous injections of propofol (2.5 mg/kg), which were adminis-
tered at a rate of 10 ml every 10 min. The animals were transpor-
ted to the Peter S. Allen MR Research Centre at the University of Alberta. Three different MRI sequences were utilized. Muscle morphology was assessed with a high-resolution 3D-gradient echo sequence with the following parameters: number of slices = 104, slice thickness = 2.5 mm, echo time (TE) = 2.33 ms, repetition time (TR) = 4.92 ms, field of view (FOV) = 400 × 400 mm, acquisition matrix = 640 × 640 pixels, in-plane resolution = 0.625 × 0.625 mm, and flip angle = 12°. A T2-weighted imaging was used to determine the presence of indicators of DTI in the muscle, such as edema and cell death, and provide a measurement of the extent of tissue injury (8, 54, 58). A T2-weighted spin echo sequence with fat suppression was used with the following parameters: number of slices = 10, slice thickness = 8 mm, TE = 70 ms, TR = 3000 ms, FOV = 283 × 400 mm, acquisition matrix = 272 × 384 pixels, and in-plane resolution = 1.04 × 1.04 mm. Additionally, a multiecho T2-weighted sequence was utilized to quantify the short and long transverse relaxation (T2) times of the middle gluteus muscle as well as their respective weightings contributing to the overall T2 signal. These short and long components have been associated with cellular and extracellular compartments, respectively (46, 47). The short T2 components are associated with the reduced mobility of water within the cells, whereas the long components are associated with relatively larger mobility of water in the vascular and interstitial spaces. The short and long T2 time components and their respective weightings, which reflect the different water pool volumes, were measured throughout the experimental protocol in all animals, including baseline (postsurgical assessment), prior to the initiation of the loading regime. The parameters for this sequence were as follows: number of slices = 20, slice thickness = 8 mm, 12 echo times with TE ranging from 23 to 353 ms in 30-ms increments, TR = 1,000, FOV = 266 × 400 mm, acquisition matrix = 256 × 384 pixels, and in-plane resolution = 1.04 × 1.04 mm.

Analysis of MR Images

Quantification of muscle atrophy. Muscle atrophy was determined by comparing the volume of the middle gluteus and biceps femoris muscles in the paralyzed leg before and 6 wk after spinalization. The muscles of interest were manually delineated in each of the slices obtained from the 3D-gradient echo MRI sequence, and the volume was calculated by summing the volume within the delineated region in each slice.

Detection of injury. Assessments of DTI through MRI analyses were performed by a researcher blinded to the treatment groups. Quantification of the extent of tissue injury in the middle gluteus muscle was performed through the analysis of the T2-weighted MR images using custom written Matlab (Mathworks, Cambridge, MA) scripts. The T2-weighted MR sequence is sensitive to the increase of more freely moving water in the tissue, serving as a good indicator of the formation of edema or scar resulting from cell death. For each MRI slice, two regions of interest were selected (Fig. 3); one delineated the middle gluteus muscle on the intact side of the pig (contralateral control limb), and the other delineated the muscle that received the daily loading on the paralyzed side (loaded limb). The signal intensity of each pixel in the region of interest of the loaded limb was compared with the average signal intensity of the region of interest in the contralateral control side. If the pixel had a signal intensity greater than the mean + 2 SD of the signal intensity on the contralateral control side, the pixel was considered to be injured tissue. The number of pixels with increased signal intensity from the loaded side was then calculated for each slice, and a measurement of total muscle volume exhibiting signs of DTI was obtained.

Biexponential fitting of multiecho MR images. Using custom-written scripts in Matlab, a region of interest was selected around the middle gluteus muscles on both the loaded and contralateral control limbs. A biexponential function was fitted to the MR signal from each pixel within these regions to quantify both the short and long T2 time components of the signal as well as their respective contributions (weightings) to the T2 times. The decaying MRI signal sampled over the 12 echoes, S(TE), can be represented as S(TEi) = c1 e^{-T2(1)/TEi} + c2 e^{-T2(2)/TEi}, where TEi is the echo time and c1 and c2 are the weightings of the short and long T2 components T2(1) and T2(2). Data were fitted with this equation using a nonnegative least squares method, using custom-written code in Matlab. Regions of interest were adjusted to avoid the inclusion of confounding tissues such as fat or fascia. The short and long T2 time components within the adjusted regions, as well as their respective weightings, were calculated for all individual pixels, and the results were averaged over the region of interest.

Histological Assessment of Tissue

Following the final MRI assessment, and under continued anesthesia (isoflurane 2–3%), muscle samples from the gluteus and biceps femoris muscles from both the loaded and contralateral control limbs were extracted. In addition, a muscle sample from the shoulder region was also extracted to serve as a naive, unaffected control sample (naive control). This additional sample was collected to ensure that the results from the contralateral control limb used as the reference for MR analyses were indeed indicative of healthy, undamaged muscle. All muscle samples were stored in 10% buffered paraformaldehyde fixative for processing at a later time. The animals were then eutha-
nized with an intracardiac injection of euthan (107 mg/kg pentobar-
bital sodium). Muscle samples were histologically processed for hematoyxin and eosin staining. The stained slides were analyzed by a researcher blinded to the experimental groups under a light microscope at ×10 and ×20 magnification and given scores from 0 to 4 based on the degree of cellular damage present in each slide, with 0 representing no signs of damage and 4 the most severely damaged. The slides were assessed according to cell shape, number of neutro-
phils, and amount of apoptosis and necrosis. Each slide was assessed three times to ensure an accurate rating.

Statistical Analyses

Reductions in muscle volume due to atrophy were assessed by comparing the muscle volume (middle gluteus and biceps femoris muscles) from each pig prior to the spinalization surgery with the volumes from the same muscles 2 and 6 wk postsurgery. A repeated-measures ANOVA was utilized to assess statistical significance. Time...
A one-way ANOVA was used to compare the strength of muscle contractions (represented by the push of muscle belly against the indenter) generated within the 1st hour of IES application against that during the last hour of IES application for all sessions of loading in the animals receiving IES. This comparison allowed for assessing the extent of muscle fatigue generated by IES during these sessions. Statistical comparisons of the muscle volume demonstrating increased signal intensity, indicating injury, in T2-weighted images in the loaded legs of all pigs were conducted using repeated-measures ANOVA. Time was used as the within-subjects factor and IES/no-IES as the between-subjects factor.

For the multiexponential T2-weighted analysis, the relative weighting of the T2 components c1 and c2 were assessed using a one-way ANOVA with three levels (contralateral limb, loaded limb with IES, and loaded limb without IES). The test was repeated for each time point and Tukey’s honestly significant difference post hoc test was used to identify which level(s) experienced significant differences. Only c2, the weighting of the long T2 component (i.e., the relative size of the extracellular volume), was considered in the analysis because the weighting of the short component, c1, is determined by the long component, c1 = 1 − c2. Contralateral limb data from the IES and no-IES groups were grouped because the conditions experienced by the contralateral limb in both groups were the same at all times.

Statistical comparisons between the different histological samples were conducted using a one-factor, four-level Kruskall-Wallis non-parametric test. The levels were considered to be the following four muscle groups: 1) naive control (samples from the shoulder), 2) contralateral control (samples from contralateral leg), 3) loaded no-IES (samples from loaded muscles that did not receive IES), and 4) loaded IES (samples from loaded muscles that received IES). A multiple-comparison test (Wilcoxon’s signed-rank test with a Bonferroni correction) was then performed to identify the specific group(s) with significant differences. A standard α of 0.05 was utilized for all statistical tests.

RESULTS

At the study end point, none of the pigs in either the control or IES groups had regained the ability to walk, and motor function in the paralyzed hindlimb was limited to that induced by reflexive action. All of the pigs underwent noticeable atrophy in the middle gluteus and biceps femoris muscles from the paralyzed limb as a function of time post-spinalization surgery. Two weeks after surgery and prior to the initiation of the external loading regime, the volume of these muscles was 7 ± 3% (mean ± SD; n = 6) smaller than that prior to surgery; however, the reduction was not statistically significant. By the time of the terminal MRI assessment (6 wk postsurgery), the loss of muscle volume had increased in all pigs to 19 ± 6% (mean ± SD; n = 6) of the presurgery volume. This reduction was statistically significant (P = 0.006). There was no significant difference in the degree of atrophy between the pigs that received IES and those that did not (P = 0.33).

External loading was equivalent to 25% of each pig’s body weight. Averaged over the entire contact area, this load was equivalent to 11.97 ± 2.57 kPa for all pigs. The strength of the contractions produced by IES was indirectly assessed by comparing force recordings obtained by the push of the muscle belly against the indenter during each muscle contraction within a loading session. The average strength of the contractions during the 1st hour of IES was 97.89 ± 1.03% (mean ± SE) of the force generated during the first contraction. During the last hour of IES, this strength decreased to 91.95 ± 1.49% (mean ± SE). Although this decrease was statistically significant (P < 0.0009), it was very small (<6%) over the 4-h period of loading and IES (Fig. 2E).

Assessment of Muscle Injury

Examples of regions showing increased T2 signal intensity in the loaded muscles of the paralyzed limb in a pig that received IES during the period of loading and a pig that did not receive IES are shown in Fig. 4 for 2 and 4 wk of loading. The use of IES significantly reduced the amount of injured tissue measured in the loaded muscles at all time points (P = 0.019; Fig. 6A). In the pigs that received IES, the extent of T2 hyperintensity was approximately five times less than the extent measured in the pigs that did not receive IES at all assessment points. In the pigs that did not receive IES, 23 ± 5% of the middle gluteus muscle volume in the loaded limb had measurable injury by the 2nd week of loading. By the 4th week of loading, this amount had increased to 49 ± 11% (Fig. 6A). Although there were no statistical differences between the volumes of T2 hyperintensity measured between weeks 2 and 4, the result was approaching significance (P = 0.07).

In comparison, the extent of injury measured in the pigs receiving IES was 5 ± 4% after 2 wk of loading and 8 ± 9% after 4 wk of loading (Fig. 6A). There were no statistical differences between measurements at any time point of loading (baseline, 2 and 4 wk) in this group (P = 0.63).

Changes to Intracellular and Extracellular Muscle Components by DTI

Examples of the distribution of the long T2 component weighting at baseline (postsurgical, preloading assessment) and 4 wk postloading are shown in Fig. 5 for one animal that received IES (Fig. 5, left) and one that did not receive IES (Fig. 5, right) during the period of loading. At baseline, there were no significant differences (P = 0.527) in the weighting of the long T2 component between the middle gluteus muscles of both hindlimbs in the IES (0.08 ± 0.02, mean ± SE) and no-IES (0.1 ± 0.02) groups. Moreover, the baseline weighting values of the long T2 component were similar (no statistical difference) between both animal groups (Fig. 6B).

Two weeks after the onset of the loading regime, the weighting of the long T2 component doubled to 0.22 ± 0.07 in the loaded middle gluteus muscle of the group that did not receive IES, whereas only a small increase was seen in the contralateral control limb (0.13 ± 0.02). In comparison, in the group that received IES, both hindlegs maintained similar weighting values for the long T2 component, increasing only slightly to 0.11 ± 0.02 and 0.12 ± 0.03, in the loaded and contralateral control limbs, respectively (Fig. 6B). The weighting of the long T2 component at this time point increased significantly in the loaded muscle of the group that did not receive IES compared with the same muscle in the contralateral limb (P = 0.05). This increase in the loaded muscles of the animals not receiving IES also neared significance relative to the loaded muscle in the pigs that received IES (P = 0.059). In the pigs receiving IES, there was no statistical difference between the long T2 component in the loaded and contralateral muscles (P = 0.946).

After 4 wk of loading, the weighting of the long T2 component in the middle gluteus muscle of the pigs that did not receive IES increased signal intensity, indicating injury, in T2-weighted images in the loaded legs of all pigs were conducted using repeated-measures ANOVA. Time was used as the within-subjects factor and IES/no-IES as the between-subjects factor.
receive IES remained elevated (0.22 ± 0.04). This elevation was again statistically significant ($P = 0.01$) compared with the contralateral limb (0.14 ± 0.01). It was also statistically significant ($P = 0.009$) relative to the loaded middle gluteus muscle in the pigs that received IES (0.10 ± 0.01). In contrast, there was no statistical difference between the weightings of the long $T_2$ component in the loaded and contralateral muscles of the pigs that received IES ($P = 0.761$).

**Assessment of Cellular Necrosis**

Tissue samples collected from the contralateral control limb in the groups with or without IES exhibited minimal signs of cellular damage (Fig. 7, top). In a majority of these samples (10 of 12), no damage was seen and normal cellular features were maintained, with no inflammatory cell infiltration into the interstitial space or within the cells. The same result was observed in the samples collected from the shoulder (naive control; not shown).

In the group that received IES, the majority of histological slides were very similar to those from the contralateral control limb (Fig. 7, bottom left). The highest score assigned to any of the samples in this group was 3, given to a single slide in the group. In this slide, the affected area demonstrated some infiltration of inflammatory cells into the interstitial space. There were also a few gaps in the cellular structure, with inflammatory cells in the space that used to be occupied by a

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**Fig. 4.** Comparison of the amount of DTI estimated in 1 pig from each group (IES and no-IES) after 2 and 4 wk of daily loading. Yellow arrows indicate the location of muscle regions considered to contain DTI (red regions).

**Fig. 5.** Comparison of the weighting of the long $T_2$ component in 1 pig from each group for both the loaded and contralateral control limbs. Color maps indicate the weighting for each pixel in the selected muscle regions. Long $T_2$ component = 1-$T_2$ short-component weighting.
muscle cell. Nonetheless, the median score for this group was zero (Fig. 8). In the group that did not receive IES, some degree of damage was seen across a majority of samples (10 of 12) from the entire group, with damage more pronounced in these samples compared with the other groups. These samples exhibited a larger number of neutrophil infiltrations into the extracellular volume fraction, was high in the pigs that did not receive IES, whereas in the pigs that received IES, the weighting of the short and long T2 components did not show significant changes over time compared with the measurements in the contralateral control muscles. This test provided a more direct measurement of the microscopic origin of the increase in the signal intensity in the T2-weighted images, indicating that the extracellular volume fraction was increased in the injured tissue, which is consistent with edema or cell death. The loaded middle gluteus muscle of the pigs that did not receive IES showed a doubling of the extracellular volume fraction from 10 to 22%, which agreed with the trends shown in the histological assessment of the muscle samples. Histology showed that the degree of cellular damage, with visible large increases in extracellular volume fraction, was high in the pigs that did not receive IES. In the pigs that received IES, cellular damage was comparable with that from muscle samples from the contralateral control limbs.

DISCUSSION

The goal of this study was to test, for the first time, the effectiveness of the novel IES approach in preventing the formation of DTI due to daily external loading of paralyzed muscles in animals with SCI. DTI was assessed using MRI techniques as well as through postmortem histological assessments.

Skin is a Poor Indicator of DTI

In the present study, none of the pigs from either group exhibited signs of damage in the skin surrounding the iliac crest, the bony prominence against which the external load was applied for the duration of the study. Despite the absence of damage at the skin level, significant muscle injury developed during the 4 wk of external loading in the pigs that did not receive IES. Currently, the primary method for detecting pressure ulcers is periodical skin inspections. Nonetheless, as demonstrated in this and other studies (37, 54, 56, 57), skin is a poor indicator of deep tissue health.

The difficulty in detecting DTI soon after its onset and during its stages of development compromises the effective deployment of early interventions and allows extensive tissue damage even in those animals with extensive muscle injury caused by the external loading.

Summary of Results

The use of IES significantly reduced the extent of DTI. By the 4th week of loading, T2-weighted MR imaging showed that the volumetric extent of injury in the loaded legs of the pigs that did not receive IES was nearly seven times larger than in the pigs that received IES. The multiecho T2-weighted MR analysis showed an increase in the weighting of the long T2 component in the loaded muscle of the pigs that did not receive IES, whereas in the pigs that received IES, the weighting of the short and long T2 components did not show significant changes over time compared with the measurements in the contralateral control muscles. This test provided a more direct measurement of the microscopic origin of the increase in the signal intensity in the T2-weighted images, indicating that the extracellular volume fraction was increased in the injured tissue, which is consistent with edema or cell death. The loaded middle gluteus muscle of the pigs that did not receive IES showed a doubling of the extracellular volume fraction from 10 to 22%, which agreed with the trends shown in the histological assessment of the muscle samples. Histology showed that the degree of cellular damage, with visible large increases in extracellular volume fraction, was high in the pigs that did not receive IES. In the pigs that received IES, cellular damage was comparable with that from muscle samples from the contralateral control limbs.
damage to develop untreated. Alternative techniques to skin inspection have been suggested to better detect and/or quantify the extent of a suspected DTI. These include the use of biomarkers measured from blood tests (16, 49) or sweat (11, 41, 42) or the use of imaging techniques such as MRI (54, 57, 59) and ultrasound (10, 36). Results to date have been promising in controlled research laboratory environments; nonetheless, limitations, including accuracy, specificity, availability, or cost, have prevented their clinical implementation for this purpose. In the present study, two different MRI sequences were successfully used to assess the development of DTI in the pigs. Although the current cost and availability of MRI scans may be prohibitive as an early detection and assessment technique of DTI in the immediate future, their high spatial resolution and ability to differentiate between different types of soft tissue make them ideal tools for assessing DTI progression in small studies.

**MRI Techniques are Sensitive Measures of Edema Formation and Associated DTI**

The development of a DTI in the muscle is associated with cellular death and the accumulation of fluid (edema). This leads to changes in the volume of freely moving water in the tissue, the extent of which we assessed using a T2-weighted MRI sequence. An increase in the signal intensity of T2-weighted MR images has been associated with the formation of acute edema in skeletal muscle, one of the earliest signs of an inflammatory response due to cellular damage in the tissue (8, 54, 58). Increased intensity in these images has also been associated with the amount of tissue injury (i.e., cell death) measured through postmortem histological assessments (54, 58). We further explored the tissue injury with biexponential T2 quantification to estimate the extracellular volume fraction, which can provide an indication of edema accumulation as the muscle’s cellular matrix breaks down due to injury. Such multiecho imaging has been used to identify short and long components in the signal from skeletal muscle, with the short component thought to be associated with cellular structures in the tissue and the long component associated with the extracellular space (23, 46–48). We showed a baseline weighting of ~0.1 for the longer T2 component, suggesting an extracellular volume fraction of 10% in uninjured muscle. Previous biexponential studies have shown similar weighting of the long T2 component in skeletal muscle (1, 17, 23, 38), which is also in general agreement with extracellular volumes measured with other methods, such as using extravascular contrast agents (60). The increase in the weighting of the long T2 component to >20% with DTI in the limbs with no IES is consistent with

![Fig. 7. Muscle samples from the contralateral control and loaded muscles from 1 pig in the IES and no-IES groups stained with hematoxylin and eosin. Arrows indicate location of high infiltration of inflammatory cells into the muscle tissue.](image)

![Fig. 8. Group results from tissue histological assessment. Results show median (square marker), 25th and 75th percentiles (box), maximum and minimum values (error bars), and outliers (*2 slices out of 12 in the group).*](image)
the histological results, where much larger extracellular volumes were directly visualized.

**IES May Be Effective in Preventing the Formation of DTI**

The use of IES significantly reduced the formation of DTI in the muscles entrapped against the iliac crest due to repeated external loading in all pigs that received IES compared with the pigs that did not receive IES. In all animals receiving IES, the amount of DTI measured through the T2-weighted images was significantly smaller than that measured in the animals that did not receive IES and was not different from measurements obtained at baseline. The multiecho imaging yielded similar results. In the pigs that received IES, there were no changes in the ratios between the weighting of the short and long T2 components of the muscle in the treated leg throughout the study, indicating that no changes occurred between the volumes of cellular and extracellular water content. Significant cellular death leads to the formation of edema, which is characterized by a more homogenous cellular environment (less differentiation in the water content and mobility between the 2 cellular spaces) and an increase in the weighting of the long T2 component. Therefore, the consistency in the ratio of cellular/extracellular water content suggests that in the IES group, no detectable damage developed in the tissue during the study. Moreover, the ratio of the cellular/extracellular water content was similar to that obtained from the contralateral limb used for control. In contrast, in the pigs that did not receive IES, there was a significant increase in the weighting of the long T2 component as early as the 2nd wk of loading.

The mechanisms underlying the effectiveness of IES in preventing the formation of DTI rely on the manner in which the stimulation is applied, generating muscle contractions that last a few seconds (~10 s) every few minutes (~10 min). This pattern mimics the natural repositioning movements that able-bodied individuals perform subconsciously when exposed to periods of prolonged sitting or lying down. In previous studies, we showed that IES works by effectively counteracting both mechanical (15, 53, 55, 56) and vascular (15, 53) factors that can lead to DTI development. This is the first study that demonstrates the long-term effectiveness of this novel approach in preventing the formation or substantially reducing the extent of DTI.

The degree of muscle atrophy resulting from spinal cord injury in the pigs in this study was less than that observed in our previous studies involving pigs with similar spinal lesions (55, 56). This could be due partly to the difference between studies in the original mass of the pigs before the spinal surgery. The pigs used in this study were on average 10 kg lighter than those utilized in our previous studies. A higher degree of atrophy (~50%) similar to the one encountered in humans with spinal cord injury and in our previous pig studies would result in higher levels of strain and stress in the loaded muscle for similar magnitudes of external loading (55, 56). Increased levels of strain and stress could in turn lead to greater extents of DTI in the control pigs (no IES) in this study. As for the pigs that received IES, our previous studies (55, 56) demonstrated that IES-induced contractions can effectively counteract the negative effects of external loading levels ≤75% of body weight, which is higher than the loading level used in this study. Therefore, we believe that IES would be effective even in more severely atrophied muscles.

Interestingly, the effects of IES are not dependent on muscle bulk (15, 56) and thus are not limited to those individuals who have undergone muscle buildup training after prolonged atrophy. Moreover, muscle fatigue was minimal throughout the duration of IES application in any loading session. This suggests that IES could be used throughout the hours of sitting or lying down without diminished effectiveness.

One potential problem that could affect the application of IES is the presence of a thicker layer of adipose tissue covering the target muscles, as seen in people who have obesity problems. This increase in the thickness of the adipose layer would require the use of higher amplitudes of electrical stimulation to induce a muscle contraction, which may become uncomfortable or even unbearable for some individuals if they retain sensation in the region. If a problem like this were to arise in practice, surface electrodes may not be the best way to apply IES, and alternatives like the use of implantable electrodes may need to be explored.

**Comparison of IES to Other Interventions for Preventing Pressure Ulcers**

In a previous study, we demonstrated that the active IES-induced muscle contractions in loaded muscles may in fact be more effective in reducing the extent of DTI than traditional prevention techniques involving complete unloading of the muscle (e.g., wheelchair pushups) (8). Loading encountered by a muscle while sitting or lying down deforms the muscles. Because atrophied muscles have reduced stiffness (33), the same level of loading induces larger deformations (28, 29, 55, 56). This increases the susceptibility of atrophied muscles to breakdown due to sustained loading and increases the chances of DTI formation. Even low levels of strain have been shown to increase the permeability of cell membranes, causing cell swelling that could potentially lead to cell death if not reversed (52). During the on phase of IES, active contractions are produced in the loaded muscle. This in turn periodically shortens the muscle and increases muscle stiffness, which reduces the extent of deformation. In addition to increasing muscle stiffness, the active contractions also periodically change the shape of the muscle, thus redistributing the levels of stress deep into the muscle and reducing their magnitudes in high-risk regions close to the bone (56). Therefore, each IES contraction is likely capable of “resetting” the mechanical properties in the muscle more effectively than passive unloading alone and preventing the muscle fibers from reaching a stage of irreversible deformation. The intermittent nature of the IES on/off cycle repeats these processes throughout the hours of IES use, thus potentially allowing the muscle to remain loaded for long periods of time without triggering the onset of DTI.

**Conclusion**

This study demonstrated that IES may be an effective prevention technique of DTI when applied daily to muscles that are subjected to prolonged external loads due to immobility. This paradigm-shifting approach may fill a critical gap in the interventions currently deployed for preventing pressure ulcers. Future studies will focus on identifying the best IES.
parameters (e.g., durations of on and off periods, stimulation amplitude, and frequency during the on period) for preventing DTI in preparation for clinical trials in human volunteers.

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AUTHOR CONTRIBUTIONS
L.R.S., P.S., R.B.T., and V.K.M. did the conception and design of the research; L.R.S., P.S., R.B.T., and V.K.M. performed the experiments; L.R.S., E.T., R.B.T., and V.K.M. analyzed the data; L.R.S., E.T., R.B.T., and V.K.M. interpreted the results of the experiments; L.R.S. and E.T. prepared the figures; E.T., R.B.T., and V.K.M. analyzed the data; L.R.S., E.T., R.B.T., and V.K.M. interpreted the results of the experiments; L.R.S. and E.T. prepared the figures; L.R.S. drafted the manuscript; E.T., P.S., R.B.T., and V.K.M. edited and revised the manuscript; R.B.T. and V.K.M. approved the final version of the manuscript.

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