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SHORT COMMUNICATION

Epidural stimulation for cardiovascular function increases lower limb lean mass in individuals with chronic motor complete spinal cord injury

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Abstract

Chronic motor complete spinal cord injury (SCI) results in paralysis and deleterious neuromuscular and autonomic adaptations. Paralysed muscles demonstrate atrophy, loss of force and increased fatigability. Also, SCI-induced autonomic impairment results in persistently low resting blood pressure and heart rate, among other features. We previously reported that spinal cord epidural stimulation (scES) optimized for cardiovascular (CV) function (CV-scES), which is applied in sitting position and does not activate the leg muscles, can maintain systolic blood pressure within a normotensive range during quiet sitting and during orthostatic stress. In the present study, dual-energy X-ray absorptiometry collected from six individuals with chronic clinically motor complete SCI demonstrated that 88 ± 11 sessions of CV-scES (7 days week^{-1}; 2 h day^{-1} in four individuals and 5 h day^{-1} in two individuals) over a period of ∼6 months significantly increased lower limb lean mass (by 0.67 ± 0.39 kg or 9.4 ± 8.1%; $P < 0.001$). Additionally, muscle strength and fatigability data elicited by neuromuscular electrical stimulation in three of these individuals demonstrated a general increase (57 ± 117%) in maximal torque output (between 2 and 44 N m in 14 of the 17 muscle groups tested overall) and torque–time integral during intermittent, fatiguing contractions (63 ± 71%; between 7 and 230% in 16 of the 17 muscle groups tested overall). In contrast, whole-body mass and composition did not change significantly. In conclusion, long-term use of CV-scES can have a significant impact on lower limb muscle properties after chronic motor complete SCI.

KEYWORDS
cardiovascular function, muscle hypertrophy, muscle properties, spinal cord epidural stimulation, spinal cord injury

1 | INTRODUCTION

Chronic motor complete spinal cord injury (SCI) results in paralysis and extensive neuromuscular and autonomic changes below the level of injury. Paralysed muscles demonstrate significant atrophy (Gorgey & Dudley, 2007; Kern et al., 2008), loss of force generation capacity (Thomas, Zaidner, Calancie, Broton, & Bigland-Ritchie, 1997), increased fatigability, and a shift toward the fast-fatigable phenotype (Shields, 1995). However, in spite of these deleterious adaptations, the paralysed skeletal muscle can be reconditioned

through training strategies that promote neuromuscular activation and force generation, with or without weight-bearing (Giangregorio et al., 2006; Shields & Dudley-Javoroski, 2007).

Spinal cord injury-induced autonomic impairment leads to blood pressure instability, which can manifest as persistently low resting blood pressure, bradycardia and dysrhythmias, autonomic dysreflexia and orthostatic hypotension, among others (Eldahan & Rabchevsky, 2018; Manogue, Hirsh, & Lloyd, 2017; Wecht & Bauman, 2018; Wecht, Weir, Martinez, Eraifej, & Bauman, 2016). We previously reported that arterial blood pressure can be modulated acutely with stimulation of the lumbosacral spinal cord (Aslan et al., 2018) and that spinal cord epidural stimulation (scES) parameters optimized for cardiovascular (CV) function (CV-scES) can maintain systolic blood pressure safely and effectively within 110–120 mmHg during quiet sitting and during orthostatic stress, without eliciting trunk or lower extremity muscle contraction as determined by visual inspection and surface EMG assessment (Harkema et al., 2018b). Daily use of CV-scES as an intervention to normalize systolic blood pressure led to mitigation of orthostatic hypotension and improved cardiovascular autonomic regulation during orthostatic stress, effects that persisted without the application of epidural stimulation (Aslan et al., 2018; Harkema et al., 2018a; Legg Ditterline et al., 2020).

Herein, we present new evidence that long-term use of CV-scES, which is applied in the sitting position and does not activate the leg muscles, can have a significant impact on lower limb muscle properties in individuals with chronic clinically motor complete SCI.

New Findings

- **What is the central question of this study?**
  Spinal cord injury results in paralysis and deleterious neuromuscular and autonomic adaptations. Lumbosacral epidural stimulation can modulate motor and/or autonomic functions. Does long-term epidural stimulation for normalizing cardiovascular function affect leg muscle properties?
- **What is the main finding and its importance?**
  Leg lean mass increased after long-term epidural stimulation for cardiovascular function, which was applied in the sitting position and did not activate the leg muscles. Leg muscle strength and fatigue resistance, assessed in a subgroup of individuals, also increased. These adaptations might support interventions for motor recovery and warrant further mechanistic investigation.

2 | METHODS

2.1 | Ethical approval

Research participants signed an informed consent for spinal cord epidural stimulation implantation, stimulation and physiological monitoring studies, which were conducted according to the standards set by the *Declaration of Helsinki* and approved by the University of Louisville Institutional Review Board. The study protocol entailed either 2 (IRB #13.0625; NCT02037620; $n = 4$) or 5 h (IRB #16.0179; NCT03364660; $n = 2$) of daily CV-scES (see below, 'Epidural stimulation for cardiovascular function' section) to maintain systolic blood pressure within a range of 110–120 mmHg.

2.2 | Participants

Six individuals with chronic, clinically motor complete and sensory complete or incomplete SCI were included in this study (Table 1). Before implantation of the epidural stimulator, the International Standards for Neurological Classification of Spinal Cord Injury (Burns et al., 2012) was used to classify each injury using the ASIA (American Spinal Injury Association) Impairment Scale (AIS).

TABLE 1 Characteristics of the research participants

Pub ID	Sex	Age (years)	Time between injury and surgery (years)	Injury level	AIS	Number of CV-scES sessions	Duration of CV-scES (h day^{-1})
B21	Male	31.0	6.9	C4	B	84	2
A41	Male	24.0	7.2	C4	A	85	2
A68	Male	35.0	3.8	C5	A	80	2
A80	Female	32.9	7.9	C6	A	108	2
A105	Male	33.7	10.0	C4	A	88	5
B47	Male	43.3	8.2	C4	B	80	5

Injury level is the neurological level of the lesion by AIS [American Spinal Injury Association (ASIA) Impairment Scale; Burns et al., 2012]. Individuals were enrolled in interventional studies that included daily sessions of either 2 or 5 h of spinal cord epidural stimulation aimed at normalizing blood pressure (CV-scES). Pub ID: publication identifier.

2.3 | Experimental protocol

Research participants were implanted with an scES unit between 3.8 and 10.0 years after SCI and were enrolled into interventional studies focused on the recovery of cardiovascular function.

Dual-energy X-ray absorptiometry (DXA) data were collected 6.3 ± 2.6 (Usual living) and 1.2 ± 0.8 months (Pre) before scES implantation in order to assess body composition data variability while research participants undertook their usual daily living. The DXA was then repeated 6.0 ± 2.6 months after scES implantation (Post-CV-scES) to assess the effects of cardiovascular epidural stimulation. Lower limb muscle strength and fatigue data were also collected from three of the four individuals enrolled in the study IRB #13.0625 at Pre and Post-CV-scES. This dataset could not be collected Post-CV-scES for participant A80.

2.4 | Spinal cord epidural stimulation implant

During the scES implantation procedure, a mid-line bilateral laminotomy was performed, typically at the L1–L2 disc space. An electrode array with 16 contacts (Specify 5-6-5 lead, Medtronic, Minneapolis, MN, USA) was placed into the epidural space at the mid-line. Electrophysiological mapping was performed after initial placement to optimize the location of the paddle electrode based on evoked responses recorded from bilateral surface EMG electrodes (Motion Lab Systems, Baton Rouge, LA, USA) placed over representative lower limb muscles. After the final placement of the electrode array, the electrode lead was tunnelled subcutaneously and connected to the neurostimulator.

2.5 | Dual-energy X-ray absorptiometry data collection and analysis

Dual-energy X-ray absorptiometry (Prodigy, GE Lunar, Encore v.16, ©2015, GE Healthcare, Chicago, IL, USA) was used to assess body mass and composition. In particular, whole-body percentage fat mass and lean mass and lower limb (regional) lean mass are reported in the present study. Participants were in the supine position, centred within the field of view of the scanner, with similar space laterally on both sides. The hands were placed at each side of the body with palms flat, avoiding overlap with the trunk and hips. A sheet was used to wrap the arms at the sides of the torso and assist with maintaining position throughout the entire period of the scan. Two straps were placed at the knees and ankles to keep the legs aligned in position and prevent any inappropriate overlap. The abdominal thickness was measured with callipers and the appropriate scan mode selected (thin, standard or thick). The whole-body measurements were estimated with the MirrorImage function for one participant (B47) because the DXA scan field was not sufficient to accommodate his full frame. Scans were scheduled at a consistent time of day for each individual. Participants were instructed to not exercise before the DXA scan in order to minimize fluid shift. All scans were performed by a certified radiology technician, and point typing and regions of interest were confirmed or appropriately adjusted by the same individual trained in DXA total body composition through the International Society for Clinical Densitometry.

2.6 | Lower limb muscle strength and fatigability

The experimental setting to assess lower limb muscle strength and fatigability characteristics has been described in detail previously (Arpin, Forrest, Harkema, & Rejc, 2019a). Briefly, neuromuscular electrical stimulation (NMES)-elicited isometric contractions of knee extensors, knee flexors and ankle plantarflexors of the left and right leg were assessed while subjects were seated on a dynamometer (Biodex, Shirley, NY, USA). The NMES (100 Hz stimulation frequency, 1 ms pulse width; Xcite Stimulator; Restorative Therapies, Baltimore, MD, USA) was delivered through two surface electrodes (Axelgaard Manufacturing, Fallbrook, CA, USA), which were consistently positioned across time points (Arpin et al., 2019a). Torque output was recorded by custom LabVIEW (National Instruments, Austin, TX, USA) software and sampled at 1 kHz.

The relationship between stimulation intensity and peak torque exerted (i.e. recruitment curve; Arpin et al., 2019a; Arpin, Ugiliweneza, Forrest, Harkema, & Rejc, 2019b) was examined to determine the stimulation intensity delivered to assess maximal torque output and fatigability. In particular, the stimulation intensity that generated the highest peak torque during the recruitment curve was used to assess maximal torque output, and the first intensity that was ≥80% of the highest peak torque was used for the fatigability protocol. Maximal torque output was assessed by eliciting three 3 s maximal stimulated contractions with 2 min rest in between. Muscle fatigability was assessed by eliciting 45 contractions consisting of 0.5 s duration of stimulation and 1 s rest period between muscle contractions.

All torque data were low-pass filtered at 10 Hz. The maximal torque output was defined by averaging the peak torque exerted among the three 3 s maximal stimulated contractions. Muscle work during the fatigability protocol was estimated by the sum of the torque–time integral (TTI) for the 45 NMES-elicited muscle contractions (Porcelli et al., 2016).

2.7 | Epidural stimulation for cardiovascular function

Individuals participated in 1–2 weeks of scES for spatial and temporal mapping of the electrode array to determine the parameters (i.e. electrode polarity, frequency, amplitude and pulse width) needed to increase systolic blood pressure to within 110–120 mmHg without activation of lower extremity muscles (Harkema et al., 2018b). In summary, parameters were changed systematically while beat-by-beat blood pressure [recorded from the index finger or thumb (Finapres

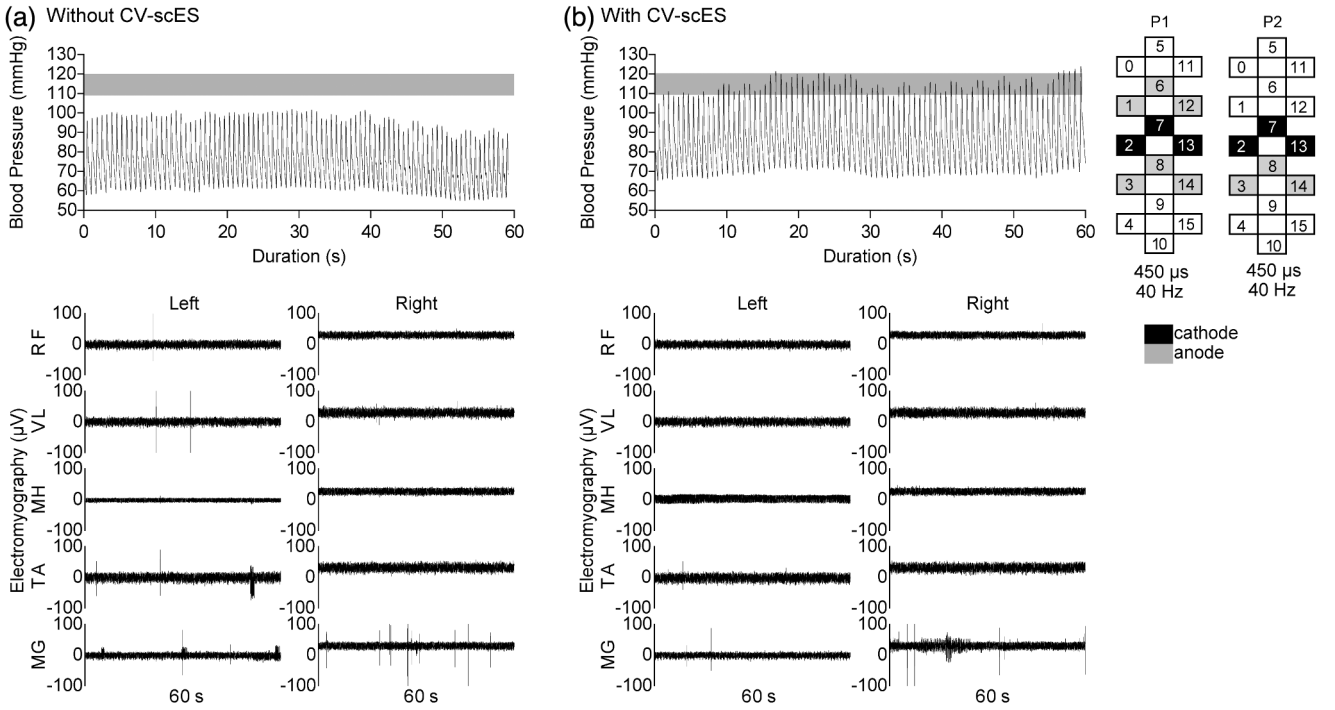

FIGURE 1 Blood pressure and EMG without CV-scES (a) and with CV-scES (b). Continuous data from research participant A105 illustrate blood pressure and EMG of the lower limbs during a mapping assessment to find spinal cord epidural stimulation (scES) parameters optimized for cardiovascular (CV) function (CV-scES). (a) Without CV-scES, blood pressure is low, without EMG activity of the lower limbs. (b) With active CV-scES, arterial blood pressure (in millimetres of mercury) increases and is sustained within the target range (110–120 mmHg) without EMG activity of the lower limbs. Right: representation of the scES electrode array, with parameters (i.e. stimulation pulse width, frequency and electrode polarity) used for CV-scES. The 16 electrodes in the array are numbered from 0 to 15; inactive electrodes are white, cathodes are black, and anodes are grey. Program 1 (P1) and program 2 (P2) are delivered sequentially by the same electrode array. Abbreviations: MG, medial gastrocnemius; MH, medial hamstring; RF, rectus femoris; TA, tibialis anterior; and VL, vastus lateralis

NOVA, Finapres Medical Systems, Amsterdam, The Netherlands)], ECG and surface EMG of the trunk and lower extremity muscles were recorded continuously. Bipolar EMG electrodes were placed bilaterally on the medial hamstrings, rectus femoris, vastus lateralis, gluteus maximus, tibialis anterior, soleus, medial gastrocnemius, rectus abdominis and oblique muscles. Blood pressure, heart rate and EMG data were acquired simultaneously at 2000 Hz and analysed offline using custom software (LabVIEW, National Instruments; and MATLAB, the Mathworks, Natick, MA, USA).

Parameters for CV-scES were considered successful if they met the following criteria: (i) they elicited a sustained increase in systolic blood pressure to between 110 and 120 mmHg from a hypotensive state for 2 h consecutively; (ii) they maintained systolic blood pressure within 110–120 mmHg for 3 days consecutively; and (iii) they did not elicit motor activity in the trunk or lower extremities (Figure 1).

Once CV-scES parameters were found, individuals used CV-scES to maintain their systolic blood pressure within the targeted range, while in the sitting position, for a total of 88 ± 11 days. Heart rate and blood pressure were monitored during CV-scES sessions, with modest adjustments to the stimulation amplitude as needed, to ensure that systolic blood pressure remained within the range during each session. If visible trunk or leg muscle activation was noted during a CV-scES session (i.e. because of long-term stimulation-promoted neural plasticity), stimulation parameters were immediately adjusted to meet the three criteria reported above.

2.8 | Statistical analysis

Outcomes at each time point (Usual living, Pre and Post-CV-scES) are summarized as the mean ± SD; changes between time points are summarized with the mean difference (Δ) and associated 95% confidence interval (CI). Statistical analysis was performed with mixed linear models regressing body mass, body composition and lower limb lean tissue outcomes against time points, controlling for the limb assessed (i.e. left or right). A random intercept for each participant was included to capture within-subject variability. To compare outcomes between Usual living, Pre and Post CV-scES, linear contrasts were built. All tests were two sided, and the significance level was set to 0.05. Statistical analyses were performed in SAS v.9.4 (SAS, Cary, NC, USA).

3 | RESULTS

Whole-body mass, lean mass and percentage fat mass did not change significantly throughout the experimental protocol. Whole-body mass

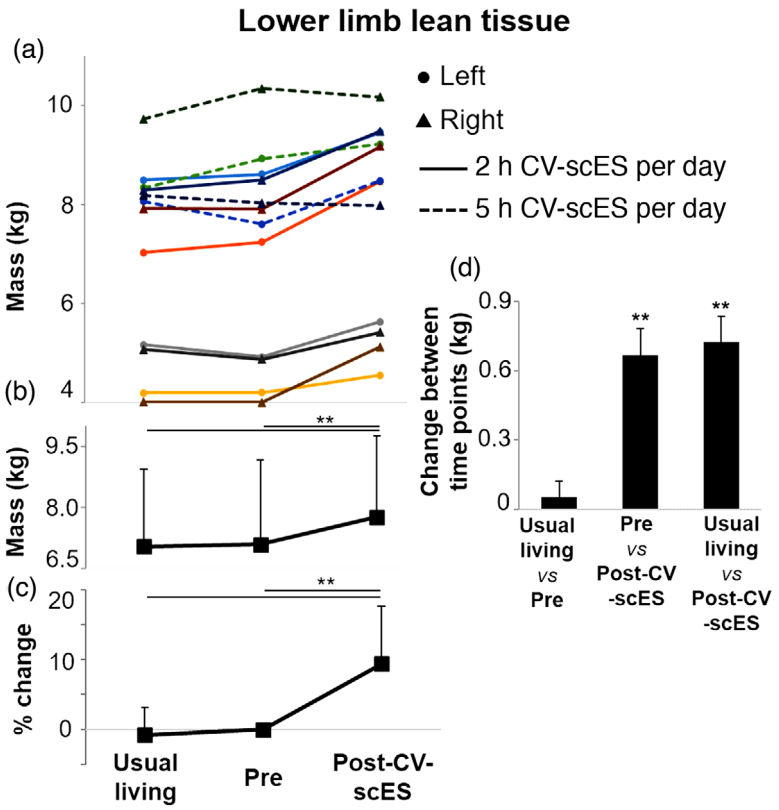

FIGURE 2 Lower limb lean mass assessed by dual-energy X-ray absorptiometry. (a) Individual data points for the left (circle) and right (triangle) lower limb assessed before epidural stimulator implantation (Usual living and Pre) and after the cardiovascular-specific epidural stimulation intervention (Post-CV-scES). The four individuals who received 2 h of CV-scES per session are represented by continuous lines, and the two participants who received 5 h of CV-scES per session are represented by dashed lines. Lower limb lean mass is represented as follows: (b) mean absolute value (±SD); (c) percentage (*vs* Pre) mean changes (±SD); and (d) mean difference between time points (±SEM). Differences were tested by mixed linear models; $^{**}P < 0.001$

at Usual living (72.7 ± 16.1 kg) did not change significantly when compared with Pre (72.8 ± 18.6 kg) and Post-CV-scES (77.6 ± 17.2 kg; $P = 0.142$). Whole-body lean mass followed a similar trend, with no significant difference across Usual living (45.7 ± 9.2 kg), Pre (45.7 ± 9.7 kg) and Post-CV-scES (48.1 ± 7.8 kg; $P = 0.742$). Likewise, the percentage fat mass remained constant across the three time points (34.6 ± 7.1% at Usual living, 33.9 ± 7.5% at Pre and 34.9 ± 6.5% at Post-CV-scES; $P = 0.740$).

Interestingly, lower limb lean mass assessed after CV-scES intervention (7.76 ± 2.00 kg) was significantly greater ($P < 0.001$) compared with Pre (7.10 ± 2.08 kg; Δ, 0.67 kg; 95% CI, 0.45–0.89 kg) and Usual living (7.04 ± 1.91 kg; Δ, 0.72 kg; 95% CI, 0.51–0.94 kg; Figure 2). Lower limb lean mass at Pre and Usual living were statistically comparable (Δ, 0.056 kg; 95% CI, −0.08 to 0.19 kg; $P = 0.740$). Additionally, visual inspection of individual data points suggested that participants who used CV-scES for 5 hdaily ($n = 2$) did not show trends that differed from individuals who used CV-scES for 2 h daily ($n = 4$; Figure 2a).

The increase in lower limb lean tissue (Figure 3a), which ranged between 10% (A41, left limb) and 17% (B21, left limb) in the three individuals whose muscle strength and fatigability were also assessed, coincided with a general increase in NMES-elicited maximal torque (57 ± 117% across all muscle groups and participants; Figure 3b,c). In particular, 14 of the 17 muscle groups tested overall (six muscle groups for participants A68 and B21, and five for A41) demonstrated higher maximal torque output after CV-scES, with increments ranging between 2 (subject A68, left knee flexors) and 44 N m (A41, right knee flexors).

After CV-scES, the torque–time integral elicited by NMES in fatiguing conditions generally increased as well (63 ± 71% across all muscle groups and participants; Figure 3d,e). In particular, 16 of the 17 muscle groups tested overall demonstrated increments ranging between 7 (subject B21, left plantarflexors) and 230% (subject A41, left plantarflexors).

4 | DISCUSSION

This study demonstrates that ~88 daily sessions of epidural stimulation aimed at normalizing cardiovascular function led to a significant increase in lower limb lean mass (+9.4%) in individuals with chronic motor complete SCI (Figure 2a–c). The negligible difference observed between Usual living and Pre in lower limb lean mass (0.8%) and in whole-body composition data supports the view that the intrinsic variability of these DXA outcomes was very limited and that the significant gain in leg lean mass after CV-scES was indeed attributable to the long-term epidural stimulation intervention. Additionally, muscle strength and fatigability data collected from a subgroup of individuals suggest that the observed muscle hypertrophy might coincide with improved muscle function, because both maximal torque output and muscle work generated in fatiguing conditions generally increased after CV-scES (Figure 3).

Previous research indicated that CV-scES modulates local efferent outflow of spinal sympathetic neurons; increased arterial blood pressure is immediate upon active stimulation, and increased pulse

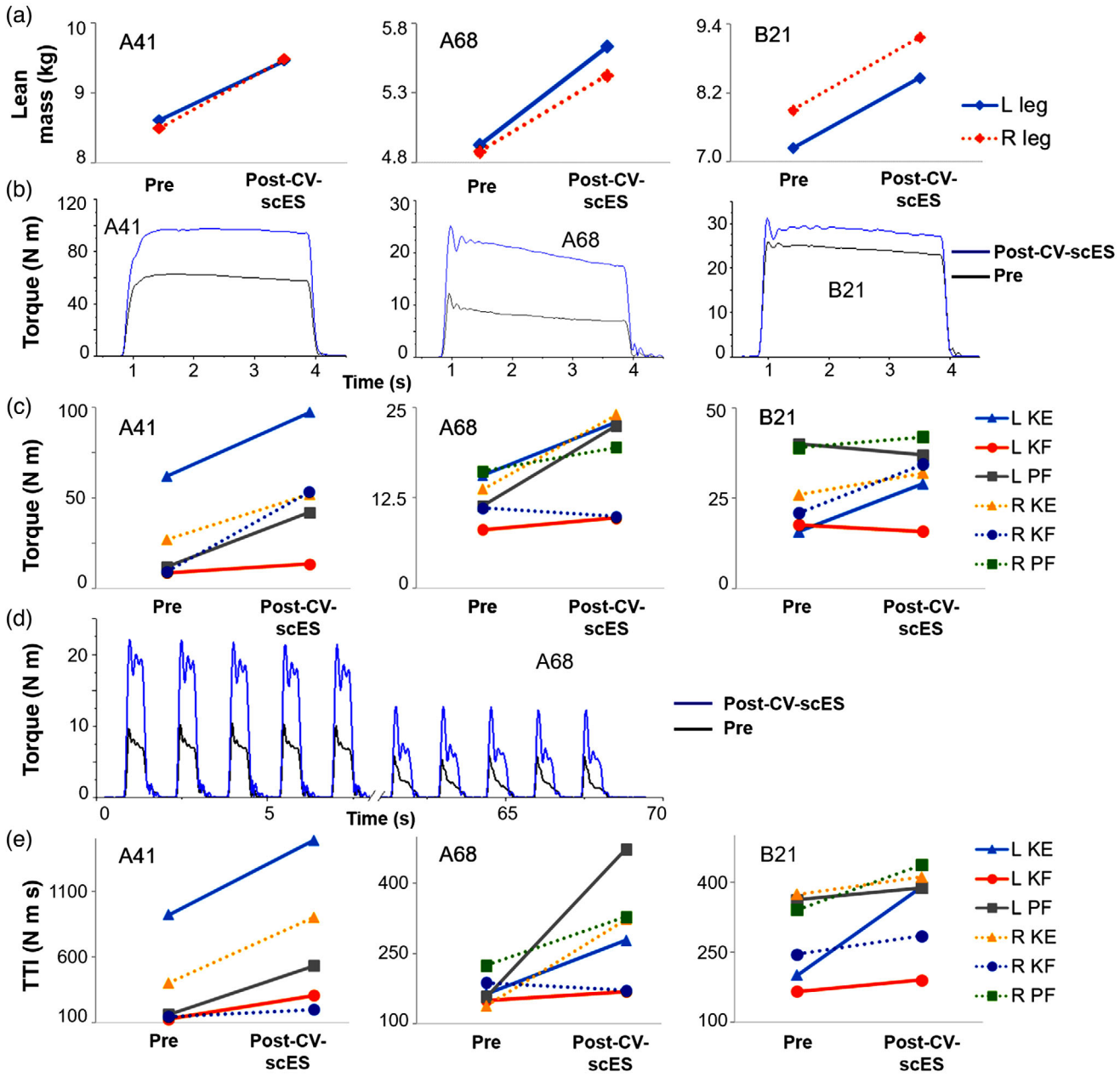

FIGURE 3 Lower limb lean mass and muscle torque data for research participants A41, A68 and B21. (a) Lower limb lean mass assessed before (Pre) and after the cardiovascular-specific epidural stimulation intervention (Post-CV-scES). (b) Representative torque output during maximal neuromuscular electrical stimulation (NMES)-elicited contractions of left (A41) and right (A68 and B21) knee extensors at Pre and Post-CV-scES. (c) NMES-elicited maximal torque output at Pre and Post-CV-scES of left (L; continuous lines) and right (R; dotted lines) knee extensors (KE), knee flexors (KF) and plantarflexors (PF). (d) Representative torque data for right knee extensors collected during the five initial and final muscle contractions of the fatiguing NMES protocol at Pre and Post-CV-scES. (e) Sum of torque–time integral (TTI) of the 45 muscle contractions included in the fatiguing NMES protocol. R PF was not tested in participant A41 because of a pressure sore on the foot sole

pressure that persists after CV-scES intervention suggests that venous return increases (Harkema et al., 2018a). However, these effects do not coincide with increased activity of supraspinal sympathetic reflexes (e.g. Mayer waves; Legg Ditterline et al., 2020). It is therefore likely that CV-scES increases arterial blood pressure by locally modulating efferent spinal sympathetic activity.

There is also evidence that the 16-electrode array surgically positioned to deliver scES is in proximity to both the spinal circuitry controlling the sympathetic system and the circuitry controlling posture and locomotion (Aslan et al., 2018). In fact, depending on the stimulation parameters applied, we have demonstrated facilitation of lower limb neuromuscular activation for standing (Mesbah et al., 2019; Rejc, Angeli, & Harkema, 2015), stepping (Angeli et al., 2018; Harkema et al., 2011) and voluntary movement (Angeli, Edgerton, Gerasimenko, & Harkema, 2014), blood pressure regulation without activation of leg muscles (Harkema et al., 2018a, b) or the concurrent

facilitation of lower limb muscle activation for standing and blood pressure regulation (Aslan et al., 2018). It is therefore conceivable that scES aimed at modulating blood pressure might also have impacted, to some extent, the lumbosacral circuitry controlling posture and locomotion because of the proximity between these neural structures, via neighbouring interneurons (Aslan et al., 2018) and/or other complex spinal neural connections (Etlin, Blivis, Ben-Zwi, & Lev-Tov, 2010; Nelson & Mendell, 1978; Sayenko, Angeli, Harkema, Edgerton, & Gerasimenko, 2014). However, CV-scES did not lead to lower limb neuromuscular activation as detected via surface EMG (Figure 1) and visual inspection.

Although future studies are needed to elucidate the mechanisms related to the observed lower limb muscle hypertrophy and improved function, it is plausible that CV-scES might have increased the excitability of spinal circuitry controlling lower limb muscles, which is substantially depressed after SCI in humans (Cote, Murray, & Lemay, 2017), either acutely (i.e. when scES is applied) and/or because of long-lasting stimulation-promoted neural plasticity (Harkema et al., 2018a). This increased excitability, in turn, might have contributed to muscle hypertrophy via neurogenic and/or mechanical factors. With respect to the first mechanism, the increased excitability of spinal circuitry might have potentiated the trophic effects of the motor innervation that prevents atrophy, to some extent, even in a paralysed muscle. In particular, the neuromuscular junction might have received an increased neural signalling to result in a larger release of calcium ions into the cell interior (Hofmann, 1980), which would increase muscle metabolism but still be insufficient to trigger a muscle contraction. An alternative or concurrent factor promoting leg muscle hypertrophy might have been an increased occurrence of spontaneous muscle contractions (Mayo, DeForest, Castellanos, & Thomas, 2017) when scES was not applied, following scES-promoted neural plasticity of the spinal circuitry. These increased spontaneous muscle contractions, in turn, might have provided the mechanical (muscle force generation and stretching) and metabolic stimuli required to trigger muscle hypertrophy processes (Mattle, Hess, Ludin, & Mumenthaler, 1991; Squecco, Kern, Biral, Rossini, & Francini, 2008; Wackerhage, Schoenfeld, Hamilton, Lehti, & Hulmi, 2019). Increased arterial blood pressure, whether systemic or local via myogenic reflexes, could also have contributed to these trophic mechanisms by supplying muscles with nutrients and oxygen necessary for protein synthesis (Joyner & Casey, 2015; Timmerman et al., 2010). Additionally, other contributing mechanisms related to changes in hormonal and inflammatory profiles cannot be excluded. It is also worth noting that the improved lower limb muscle properties promoted by CV-scES can be a relevant outcome to support concurrent interventions for the recovery of functional mobility after severe SCI. In fact, mobility can be limited by muscle weakness (Ploutz-Snyder, Manini, Ploutz-Snyder, & Wolf, 2002), even if lower limb motor control is sufficient.

In conclusion, this study demonstrated unexpected, positive lower limb muscle adaptations after long-term application of spinal cord epidural stimulation aimed at normalizing cardiovascular function in individuals with chronic clinically motor complete SCI. Further research is needed to understand the mechanisms related to these adaptations and their potential impact on lower limb functional recovery.

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COMPETING INTERESTS

None declared.

AUTHOR CONTRIBUTIONS

E.R., B.L.D. and S.J.H. contributed to the study conception and design. B.L.D., S.J.H., A.W., S.S. and E.R. contributed to data collection. B.U. contributed to the statistical analysis. E.R. and B.L.D. created the figures and wrote the first draft of the manuscript. All authors contributed to the analysis and interpretation of results and revised the manuscript. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

DATA AVAILABILITY STATEMENT

Data that support the findings will be made available through material transfer agreement upon request.

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