

# Change in Neuroplasticity-Related Proteins in Response to Acute Activity-Based Therapy in Persons With Spinal Cord Injury

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**Background:** Activity-based therapy (ABT) focuses on regaining motor and sensory function below the level of the lesion in persons with a spinal cord injury (SCI). This is accomplished through repetitive training of specific motor tasks. Research has shown that ABT may increase neuroplasticity in the rat and human spinal cord. **Objective:** The primary aim of this study was to examine acute alterations in neuroplasticity-related proteins during ABT in persons with SCI. **Methods:** Volunteers were current participants in an ABT program and consisted of 12 men and 3 women (age,  $31.8 \pm 10.9$  years) with chronic SCI (injury duration,  $63.9 \pm 54.4$  months). A single 2-hour bout of ABT consisted of standing load bearing, body weight-supported treadmill training, whole body vibration, and functional electrical stimulation. Blood samples were obtained at baseline and immediately after completion of each modality to determine serum levels of brain-derived neurotrophic factor (BDNF), prolactin, and cortisol. **Results:** One-way analysis of variance (ANOVA) with repeated measures was used to examine differences in proteins over time. Results revealed baseline levels of BDNF ( $2.37 \pm 1.41$  ng/mL) that were lower than previous research has demonstrated in persons with SCI. No change in BDNF or cortisol was found, although prolactin was significantly reduced in response to ABT. **Conclusion:** Despite the length of the bout, acute changes in BDNF were not observed. Whether different intensities or modalities of ABT may promote acute increases in serum BDNF in individuals with SCI remains to be determined and further study is merited. **Key words:** BDNF, cortisol, exercise, prolactin, rehabilitation, spinal cord injury

Recent research demonstrates that exercise after spinal cord injury (SCI) may increase neuroplasticity in the rat and human spinal cord.<sup>1,2</sup> However, the mechanisms explaining this neuroplasticity are still relatively unexplored. Data from animal studies suggest that neurotrophic factors released during exercise may be a contributing factor. One such protein is brain-derived neurotrophic factor (BDNF), which is found in the brain and periphery. It promotes synaptic plasticity and neuronal growth in noninjured animals and alters motoneuron survival in animals with SCI. Increased levels of BDNF were demonstrated in healthy adults exercising at 55% and 75% maximal workload ( $W_{max}$ ),<sup>9</sup> as well as in individuals with multiple sclerosis performing 30 minutes of moderate

cycling. Exercise-mediated alterations in BDNF seem to be intensity-dependent, as no change in BDNF was shown during mild exercise versus more intense workloads. In addition, prolactin has been identified as a possible mediator of neuroplasticity in the spinal cord as it serves as a marker of serotonergic activation during exercise.<sup>12</sup> In a previous study, prolactin was increased ( $P < .05$ ) in response to time trial arm ergometry in SCI athletes, yet mild exercise did not induce changes in prolactin. Whether this discrepant response of prolactin to exercise occurs in other individuals with SCI remains to be determined.

Exercise also tends to increase cortisol release that may impact neurogenesis via an inhibition of growth in the hippocampus and/or through regulation of BDNF. Nevertheless, it has been

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**Table 1.** Subject physical characteristics

Subject	Sex	Age, years	Height, cm	Mass, kg	SCI		Injury level	ASIA LEMS
					duration, mo	AIS score		
1	M	32	182	74.5	146	B	C5/6	0
2	M	18	178	68.0	16	B	C5/6	0
3	M	22	186	80.0	40	C	C6/7	12
4	M	29	175	86.5	18	C	C4	15
5	F	29	160	59.0	21	A	T12/L1	0
6	M	19	178	56.4	36	A	T10/11	0
7	M	28	168	95.0	69	B	T7/9	0
8	M	24	178	68.0	36	C	T6	12
9	M	37	168	77.0	24	A	C5/6	0
10	M	31	168	59.7	36	A	C5	0
11	M	53	175	63.7	180	C	C5	10
12	M	57	180	63.7	42	C	C8	24
13	F	24	174	54.0	96	B	T2/3	0
14	M	34	187	61.5	42	C	C5/6	2
15	F	32	170	59.5	156	A	C5/6	0
Mean $\pm$ SD		31.3 $\pm$ 11.1	175.1 $\pm$ 7.4	68.4 $\pm$ 11.9	63.9 $\pm$ 54.4			5.0 $\pm$ 7.4

Note: AIS = American Spinal Injury Association Impairment Scale; C = cervical; F = female; L = lumbar; LEMS = lower extremity motor score; M = male; mo = month; T = thoracic.

suggested that this enhanced stressor response may have little effect upon neuroplasticity; as no correlation has been revealed between BDNF and cortisol, further study is warranted.

It is not known whether acute exercise induces significant changes in neurotrophins in men and women with SCI, as only one study has examined this issue. Rojas-Vega et al required previously trained elite male athletes ( $\text{VO}_2\text{max} = 34.5 \text{ mL/kg/min}$ ) with SCI from T4-T12 to complete a 42-km hand-biking time trial during which blood samples were obtained to measure concentrations of BDNF, cortisol, and prolactin.<sup>13</sup> Results demonstrated increased ( $P < .05$ ) BDNF with initiation of exercise, although postexercise values were similar to baseline. Postexercise measures of cortisol and prolactin were also higher ( $P < .05$ ) than baseline levels. The population that was studied is relatively unique and exercise performed is atypical of common practice in the rehabilitation of persons with SCI, so it remains to be determined whether similar acute increases in neurotrophins would be revealed in less active individuals completing different exercise modalities.

Activity-based therapy (ABT) consisting of high-volume training focusing on the core and lower extremities has recently been identified as an

effective technique to promote functional recovery in this population.<sup>17-19</sup> The primary aim of this study was to examine acute changes in BDNF, cortisol, and prolactin during a prolonged bout of ABT in men and women with SCI. Individual changes in these proteins were also examined to better understand how different rehabilitation modalities may affect neuroplasticity. These data have the potential to impact the neurorehabilitation of persons with SCI. It was hypothesized that exercise would elicit significant acute changes in BDNF and cortisol as previously demonstrated in this population.<sup>13</sup>

## Methods

### Participants

Inclusion criteria for the present study were men and women age 18 to 60 years with chronic SCI (>12 months) at C2 or lower, an American Spinal Injury Association Impairment Scale (AIS) score of A, B, or C, and current participation in ABT at the facility. Exclusion criteria included ventilator dependence, use of medications other than those for bowel or bladder issues, and onset of other neurological disease including traumatic brain

injury. Participants were free of known cardiac, pulmonary, or metabolic disease. Their physical characteristics are shown in **Table 1**. Written informed consent was obtained from all subjects, and the experimental procedures were approved by the university institutional review board. We certify that all applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed during the course of this research.

### Design

Dressed in a T-shirt and shorts or pants, participants were tested between the hours of 12:00 p.m. and 4:00 p.m. after a 2-hour fast. Participants also abstained from physical activity for 24 hours before the session. A catheter was placed into the antecubital vein, and a resting blood sample was obtained. Then the participants initiated a single bout of ABT consisting of approximately 30 minutes each of 4 exercise modalities, load bearing (LB), body weight-supported treadmill training (BWSTT), whole body vibration (WBV), and functional electrical stimulation (FES). The selection of these modalities was based on their use in previous research that demonstrated functional changes from prolonged exposure<sup>17-23</sup> and on the ability to isolate stimulus to the lower extremities preventing upper extremity neuromuscular response. Exercise sequence was nonrandomized and based on minimizing transfer time between modalities as well as preventing possible premature fatigue (FES last). This 2-hour session was overseen by staff at the ABT facility. Blood samples were obtained immediately at the completion of each modality.

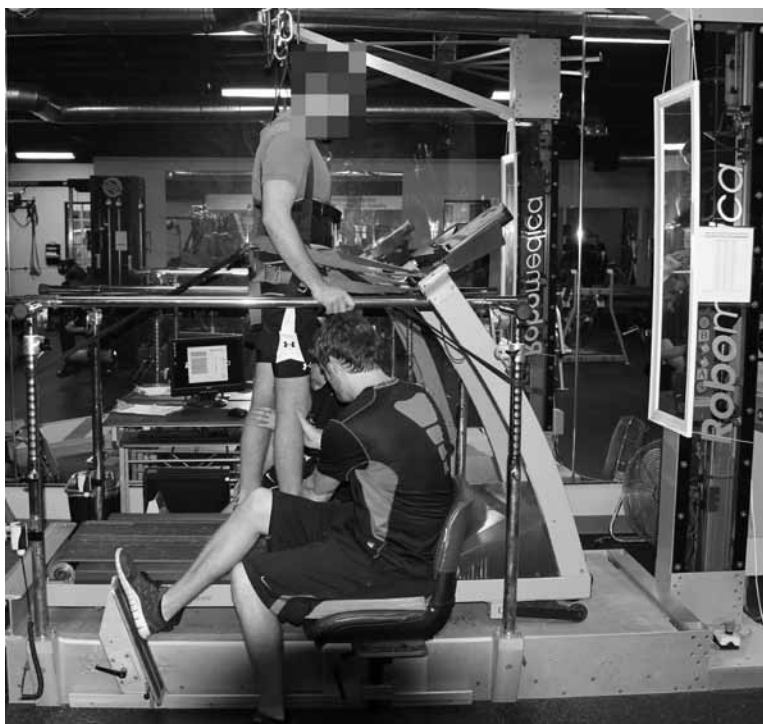
### Activity-based therapy

Initially, participants performed LB in a standing frame (Evolv Glider; Altimate Medical Inc., Morton, MN) in which they were raised into a standing position with the hip and knee joints extended to 175° (**Figure 1**). Once in this position, they were instructed to relax their upper body while a staff member manually manipulated the standing frame handles to enable a rhythmic gliding leg movement. The staff member moved

the handles at a pace of 90 beats/min following a metronome. This activity was continuously performed for 30 minutes. Next, participants were placed in a harness (BonMed, Bonn, Germany) and were lifted into position by the BWSTT device (Robomedica Inc., Mission Viejo, CA) (**Figure 2**). Sixty percent of participants' body weight was supported by the machine, and the pelvis was secured to reduce body sway via 4 rubber straps attached to the harness and the device. Treadmill speed was equal to 0.54 m/s. This speed was selected to eliminate trunk and upper extremity involvement during walking. Assistance with leg movement was provided by 2 staff members who were trained to move the legs to simulate normal gait kinematics. Participants performed ten 2-minute bouts of exercise interspersed with 1 minute of rest. During WBV, participants were suspended over a vibration platform (pro5; Power Plate Acquisitions LLC, Northbrook, IL) via a vertical winch and harness (**Figure 3**). Each participant was positioned with the hip and knee joints flexed to 30°. The harness was secured to the front and rear to stabilize the pelvis. The feet were secured to the platform with a strap so that the soles of the feet continually remained in contact with the platform. The feet and knees were placed 25 cm apart using 2 foam blocks and 2 straps. The vibration platform was set to vibrate at 35 Hz with an amplitude of 2 mm (vertical displacement). Participants performed fifteen 1-minute bouts of vibration interspersed with 1 minute of rest. Last, while seated in a wheelchair, participants' legs were attached to a recumbent FES cycle (RT-300; Restorative Therapies Inc., Baltimore, MD) (**Figure 4**). The gluteus maximus, hamstrings, and quadriceps muscles were stimulated bilaterally with surface electrodes (PALS Platinum; Axelgaard Manufacturing Co., Fallbrook, CA). All participants began the session with stimulation equal to 140 mA, pulse width 250  $\mu$ s, and frequency 33.3 Hz. Ergometer cadence was set at 45 rpm with a resistance of 1.0 Nm. Stimulation increased to tolerance from 0 to 100% of 140 mA during the bout. If a participant was unable to tolerate 140 mA, stimulation was set at the maximum tolerable level, which ranged from 36 to 140 mA. Participants performed a 2-minute



**Figure 1.** Load bearing using the standing frame (LB). The participant's legs were moved by a trained staff member.



**Figure 2.** Body weight-supported treadmill training (BWSTT) set up.



**Figure 3.** Whole body vibration (WBV) set up. The participant was secured in a harness with the knees and hips flexed at 30.



**Figure 4.** Functional electrical stimulation (FES) on RT300 bicycle.

warm-up followed by 27 minutes of stimulation and then a 1-minute cool down. Transfer times between modalities were recorded and ranged from  $465.2 \pm 131.5$  s after standing frame to  $557.3 \pm 175.2$  s between BWSTT and WBV to  $734.4 \pm 206.0$  s between WBV and FES, respectively.

### **Blood sampling**

Blood samples (10 mL) were obtained pre exercise and immediately after cessation of each modality. A catheter (21 G x 1.25 in BD Eclipse with Vacutainer holder) was inserted into the antecubital vein to continuously draw blood using a 3-way stopcock and syringe. There was a 10-minute lag time between catheter insertion and the first pre-exercise blood draw to reduce any neuroendocrine response to this stressor.<sup>24</sup> Sterile saline was flushed into the stopcock continuously to keep it patent. Whole blood was extracted into 5 mL serum separator tubes (Vacutainer SST; Becton Dickinson, Franklin Lakes, NJ) and allowed to clot for 30 minutes at 23°C. Subsequently, samples were centrifuged at 8,500 rpm for 2 minutes (StatSpin Express 2; Iris Sample Processing, Inc., Westwood, MA) at 23°C, and serum was aliquoted into sterile 2 mL cryogenic tubes (CryoGen; Globe Scientific Inc., Paramus, NJ) and frozen at -80°C until analysis.

### **Determination of neurotrophins**

Frozen serum aliquots were thawed, and cortisol, BDNF, and prolactin were measured with multiplex bead ELISA assays. For BDNF and prolactin, 25  $\mu$ L of serum was assayed in triplicate using a Milliplex MAP Human Pituitary Magnetic Bead Panel 2 (EMD Millipore, Billerica, MA), according to the manufacturer's directions. For cortisol, 250  $\mu$ L aliquots of serum were acidified in trifluoroacetic acid and subjected to solid phase extraction. Twenty-five  $\mu$ L of extracted serum was subsequently used for cortisol measurements with a Milliplex MAP Steroid/Thyroid Hormone Magnetic Bead Panel. Assays were run on the Luminex Magpix instrument using XMAP technology (Luminex, Austin, TX) and analyzed with Milliplex Analyst software (VigeneTech Inc., Carlisle, MA). BDNF, cortisol, and prolactin data are expressed as ng/mL.

The intra-assay and interassay coefficient of variations were equal to 10% and 10% for cortisol and BDNF and 15% and 10% for prolactin, respectively. Due to some incomplete samples, change in cortisol throughout the entire bout was obtained for only 12 participants: 4 with paraplegia and 8 with tetraplegia.

### **Data analysis**

Data are reported as mean  $\pm$  SD and were analyzed using IBM SPSS version 20.0 (IBM, Armonk, NY). A one-way analysis of variance (ANOVA) with repeated measures was used to determine differences in BDNF, prolactin, and cortisol during exercise, with group (tetraplegia vs paraplegia) used as a between-subjects factor. If a significant *F* ratio was obtained, Tukey's post hoc test was used to identify differences between means. The Greenhouse-Geisser correction was used to account for the sphericity assumption of unequal variances across groups. Effect size was computed as partial eta-squared ( $\mu^2$ ). The Pearson product moment correlation coefficient was computed to identify relationships between variables. Statistical significance was set at  $P < .05$ .

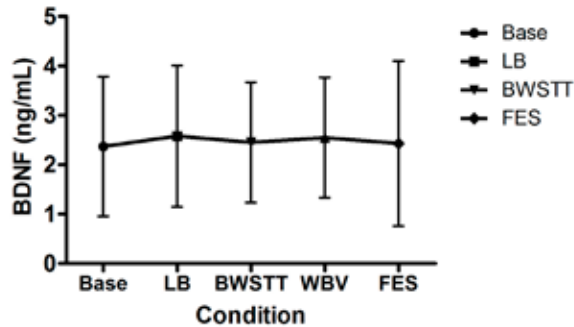
## **Results**

### **Alterations in BDNF**

Baseline BDNF (mean  $\pm$  SD) was equal to  $2.37 \pm 1.41$  ng/mL (0.27-5.26 ng/mL) across participants. Data revealed no effect of exercise on BDNF,  $F(4, 52) = 0.14$ ,  $P = 0.97$ . Similarly, there was no Group x Exercise interaction,  $F(4, 52) = 0.63$ ,  $P = 0.64$ . These data are demonstrated in **Figure 5**. Only 6 of 15 participants demonstrated increases in BDNF from baseline to post exercise, and the mean percent change was equal to  $17.8 \pm 52.7\%$ , respectively. Univariate ANOVA revealed that overall BDNF levels were higher ( $P < .05$ ) in persons with paraplegia versus tetraplegia.

### **Alterations in prolactin and cortisol**

Prolactin was significantly different in response to exercise (main effect),  $F(4, 52) = 5.21$ ,  $P = .001$ ,  $\mu^2 = 0.29$ . In addition, a Group x Exercise

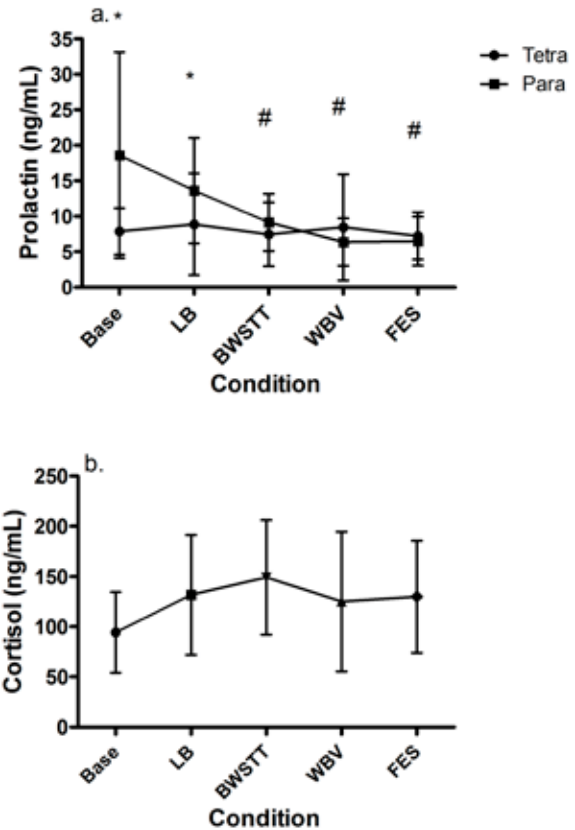


**Figure 5.** Change in brain-derived neurotrophic factor (BDNF) in response to activity-based therapy in persons with spinal cord injury. Base = baseline; BWSTT = body weight-supported treadmill training; FES = functional electrical stimulation; LB = load bearing; WBV = whole body vibration.

interaction was demonstrated,  $F(4, 52) = 4.59$ ,  $P = .003$ ,  $\mu^2 = 0.26$ . These data are shown in **Figure 6a**. Post hoc analyses revealed that prolactin was greater ( $P < .05$ ) in participants with paraplegia versus tetraplegia at baseline as well as after load bearing. Within participants with paraplegia, prolactin decreased ( $P < .05$ ) from baseline to all modalities with the exception of load bearing, respectively. Mean change in prolactin was equal to  $-26.2 \pm 40.1\%$  (range,  $-80.3\%$  to  $96.9\%$ ) across participants. There was a nonsignificant trend ( $P = .07$ ) for cortisol to increase during exercise,  $F(4, 52) = 2.32$ ,  $\mu^2 = 0.19$ . Data also revealed no Group  $\times$  Exercise interaction,  $F(4, 52) = 1.58$ ,  $P = .20$ . These data are shown in **Figure 6b**. Percent change in cortisol concentration from baseline to after exercise was equal to  $82.9\% \pm 121.1\%$  and ranged from  $-53.4\%$  to  $294.2\%$ , respectively, across participants. Eight of 15 individuals showed marked increases in cortisol during exercise.

#### Correlation analyses

Change in BDNF was unrelated ( $P > .05$ ) to predictors including injury duration or AIS lower extremity motor score, but it was positively associated with baseline BDNF concentration,  $r = -0.52$ ,  $P = .048$ . Baseline prolactin was negatively associated with percent change in prolactin in



**Figure 6.** Change in (a) prolactin and (b) cortisol in response to activity-based therapy in persons with spinal cord injury. Base = baseline; BWSTT = body weight-supported treadmill training; FES = functional electrical stimulation; LB = load bearing; Para = paraplegia; Tetra = tetraplegia; WBV = whole body vibration. \* $P < .05$  between persons with paraplegia and tetraplegia; # $P < .05$  from baseline in persons with paraplegia.

response to exercise,  $r = -0.48$ ,  $P = .07$ . Percent change in cortisol was significantly ( $P = .02$ ) related to baseline cortisol levels,  $r = -0.73$ .

#### Discussion

ABT has been shown to induce recovery of function below the level of injury<sup>17-23</sup>; however, the exact mechanisms mediating this recovery have yet to be discovered. It has been theorized that one of the components may be an increase in neurotrophin levels within the central nervous

system due to increased physical activity.<sup>25</sup> The primary aim of this study was to assess acute alterations in neuroplasticity-related proteins in a heterogeneous group of men and women with chronic SCI performing one 2-hour bout of ABT (not including transfer times). To our knowledge, only one other study examined acute exercise-induced changes in neurotrophins in humans with SCI, and the population was comprised of elite wheelchair athletes completing arm ergometry time trial exercise that is above the level of spinal cord lesion.<sup>13</sup> Data from the present study demonstrated no change in BDNF or cortisol, although prolactin was significantly reduced in response to exercise that was designed to target muscles below the injury level.

One explanation for the lack of change in BDNF in response to ABT in the present study is its low to mild intensity. A recent study by Harness and Astorino revealed that energy expenditure during a similar regimen of ABT ranged from 1 to 3 metabolic equivalents (METs),<sup>26</sup> which is approximately 20% to 50%  $\text{VO}_{2\text{max}}$  in the SCI population studied. Recent studies in healthy adults revealed that low to moderate intensity exercise (10 minutes at 140 W) did not alter BDNF concentration,<sup>16</sup> although significant increases were exhibited at higher intensity equal to 75%  $\text{W}_{\text{max}}$ .<sup>9</sup> Similar results were also demonstrated by Ferris et al in active men and women.<sup>11</sup> Overall, BDNF concentration seems to exhibit a dose-response pattern in that relatively mild workloads do not induce BDNF; whereas, higher intensity aerobic exercise seems to increase BDNF release.<sup>27</sup>

However, neither acute nor chronic resistance training at relatively high loads (80% one repetition maximum [1-RM]) induced changes in BDNF in healthy young men and women.<sup>28</sup> This latter result opposes typical acute changes in BDNF observed in response to aerobic exercise, whose force demand is lower than that inherent to resistance training. Yet, resistance training is characterized by brief bouts of activity interspersed with recovery, so the overall energy expenditure is lower versus aerobic exercise. This is similar to the activities performed in the present study where bouts of exercise were interspersed with recovery during transfer times between equipment.

The observed baseline concentrations of BDNF (**Figure 5**) are dramatically lower than values reported in male athletes with SCI ( $37.2 \pm 19.8$  ng/mL) as well as healthy adults ( $18.1 \pm 1.1$  ng/mL),<sup>11,13</sup> individuals with multiple sclerosis ( $4.4 \pm 0.5$  ng/mL),<sup>10</sup> diabetes ( $\sim 18.0$  ng/mL),<sup>29</sup> and depression ( $24.4 \pm 6.1$  ng/mL).<sup>30</sup> In contrast, Floel et al reported relatively low concentrations of BDNF ( $1.5 \pm 0.5$  ng/mL) in healthy older adults.<sup>31</sup> Zoladz et al reported that fitness level may mediate BDNF induction, as plasma BDNF was 3-fold higher in athletes versus untrained individuals.<sup>32</sup> Whether this low concentration is due to blunted release of BDNF or increased rate of clearance remains to be determined. Recently, BDNF's role in energy metabolism has been identified as it promotes lipid oxidation via activation of AMP-activated protein kinase.<sup>33</sup> Astorino and Harness showed low rates of lipid oxidation during exercise in men and women with SCI recruited from this facility,<sup>34</sup> which may be partially mediated by low baseline BDNF levels as well as lack of induction of BDNF with acute ABT exercise.

Goekint et al have speculated that peripheral blood draws may not be able to always detect BDNF release from the brain in response to exercise.<sup>28</sup> Moreover, there is widespread variability in procedures followed to obtain and analyze blood samples for BDNF, including variations in ELISA kits, use of plasma or serum samples, and/or duration of sample storage, which may explain dissimilar determinations of BDNF across studies.<sup>35</sup> In the Rojas-Vega study, there was no description of pre-exercise diet or medication use that could have influenced their reported results.<sup>13</sup>

In healthy adults and men with SCI, data demonstrate exercise-induced increases in prolactin that are explained by homeostatic disturbances in acid-base balance.<sup>13,36</sup> In pregnant women, moderate cycling did not induce changes in prolactin<sup>37</sup> and, similar to our results, it was attenuated ( $P < .05$ ) in response to exercise. Given the relatively low energy expenditure and perturbation characteristic of modalities performed in the present study, our opposing findings showing a decrease in prolactin with exercise initiation seem plausible. Despite very similar baseline concentrations across studies



(8.5-18.6 ng/mL in the current study vs 9.0-10.0 ng/mL in Rojas Vega et al),<sup>36</sup> our population was comprised of men and women with SCI differing in injury completeness, level and location, and exercise tolerance compared to Rojas-Vega's quite homogeneous sample of elite male athletes with SCI localized from T4-T12. As chronic exercise training has been revealed to enhance neuroplasticity,<sup>38</sup> it may be that athletes have a greater ability to induce prolactin in response to acute exercise compared to nonathletes, especially when the exercise is above moderate intensity.

In the present study, cortisol increased in response to exercise, but this change failed to reach significance ( $P = .07$ ). Many studies conducted in healthy adults and in men with SCI revealed increases in cortisol after completion of intense exercise.<sup>9,13,16</sup> Ratamess et al demonstrated that increases in cortisol may be promoted when exercise induces lactate accumulation,<sup>39</sup> which low to moderate intensity exercise does not do. Fitness level and age seem to affect changes in cortisol release in response to exercise. Trained athletes tend to have high levels of resting cortisol compared to untrained individuals as well as a blunted response to exercise.<sup>40</sup> In the present study, there was a significant inverse correlation between baseline cortisol and percent change with exercise. It is not clear whether acute increases in cortisol impair neuroplasticity; despite in vitro data showing that cortisol release inhibits birth of new cells,<sup>41</sup> it seems unlikely in humans as increases in cortisol with exercise are relatively transient.<sup>16</sup> A long-term ABT study comparing changes in cortisol and other neurotrophins serving as indices of neuroplasticity would help to resolve this issue.

### Study limitations

There are a few limitations to this study. First, the exercise performed in this study is typically implemented at facilities providing specialized rehabilitation to persons with SCI, and this exercise is of greater volume than most treatment options used in this population. In addition, changes in neuroplasticity-related proteins were measured during a single bout of ABT and not after chronic exercise training. Due to the goal of

strictly targeting the lower extremities, this study may not be applicable to a more intense protocol that involves trunk and upper extremity activation. All participants were in the chronic stage of SCI, so it is unknown whether a single bout of ABT would elicit significant acute increases in BDNF in individuals with subacute injury. Finally, all participants were regularly participating in an ABT program, so this single bout of exercise may not have provided an adequate stimulus to elicit an acute neurotrophic response.

### Conclusion

ABT is becoming more prevalent in the field of neurological rehabilitation. As ABT gains acceptance, it is becoming paramount to understand the underlying mechanisms that may affect change in neural function below the lesion level. Neurotrophic response to activity has been postulated as one of these mechanisms. This study is the first to examine BDNF response in nonathletic individuals with SCI participating in an ABT protocol focusing strictly on the lower extremities and is the first to document baseline serum BDNF levels in this population. The results of the current study do not support an increase in serum BDNF concentrations in response to acute ABT consisting of 4 lower extremity modalities. It remains to be determined whether different intensities or modalities of ABT may promote acute or chronic increases in serum BDNF in individuals with SCI.

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