Chronic activity-based therapy does not improve body composition, insulin-like growth factor-I, adiponectin, or myostatin in persons with spinal cord injury

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Spinal cord injury (SCI) induces dramatic changes in body composition including reductions in fat-free mass (FFM) and increases in fat mass (FM).

Objective: To examine changes in body composition in response to chronic activity-based therapy (ABT) in persons with SCI.

Design: Longitudinal exercise intervention.

Methods: Seventeen men and women with SCI (mean age = 36.1 ± 11.5 years) completed 6 months of supervised ABT consisting of load bearing, resistance training, locomotor training, and functional electrical stimulation. At baseline and after 3 and 6 months of ABT, body weight, body fat, and FFM were assessed using dual-energy X-ray absorptiometry, and fasting blood samples were obtained to assess changes in insulin-like growth factor-I (IGF-I), adiponectin, and myostatin.

Results: Across all subjects, there was no change (P > 0.05) in body weight, percent body fat, or FFM of the leg, arm, or trunk, whereas whole-body FFM declined (P = 0.02, 50.4 ± 8.4 to 49.2 ± 7.4 kg). No changes (P = 0.21–0.41) were demonstrated in IGF-I, adiponectin, or myostatin during the study.

Conclusions: Chronic ABT focusing on the lower extremity does not slow muscle atrophy or alter body fat, body mass, or regional depots of FFM in persons with SCI. Further, it does not induce beneficial changes in adiponectin, myostatin, or IGF-I. Alternative exercise-based therapies are needed in SCI to reverse muscle atrophy and minimize the onset of related health risks.

Keywords: Adipokines, Body fat, Exercise training, Fat-free mass, Paralysis

Introduction

Spinal cord injury (SCI) results in dramatic changes in body composition, physical function, and overall health status. Loss of lean body mass during the initial 6 months post-injury is equal to 9.5%,[1] and leg fat-free mass (FFM) declines as much as 15% after 1 year.[2] These changes result in persons with SCI maintaining 45–80% of muscle size versus age- and sex-matched able-bodied persons.[3] Spungen et al.[4] also reported greater fat mass (FM) at the arm, leg, and whole-body in persons with recent SCI compared to age-matched individuals. In addition, visceral adipose tissue is typically increased[5] which combined with rapid muscle atrophy increases risk of heart disease,[6] diabetes,[7] dyslipidemia,[6] and overall morbidity and mortality.

Exercise initiated soon after SCI seems to have beneficial effects on body composition by lowering FM and increasing FFM which may slow the loss of physical function as well as mitigate elevated risk of chronic disease attendant with muscle atrophy and fat accretion. In men and women with recent SCI, 3 or 6 months of functional electrical stimulation (FES) cycle ergometry prevented declines in whole-body, gluteal, and leg FFM versus controls or those performing isometric FES.[1] In men with complete, chronic SCI, 12 weeks of bilateral electrically stimulated resistance training of the thigh increased muscle cross-sectional area (CSA) by 35–39%.[8] Similar increases in muscle CSA were evident in a man with chronic SCI in response to 12

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weeks of electrically-stimulated resistance training of the knee extensors.[9] Chronic body weight-supported treadmill training enhanced lower extremity FFM in individuals with acute[10] and chronic SCI.[11] Approximately 12 hours/week of sports-based physical activity (tennis, basketball, swimming, etc.) for 4 weeks led to increased FFM and attenuated body fat in persons with chronic SCI.[12] Overall, participation in various modalities of exercise training, whether electrically stimulated or voluntary in nature, is effective to increase FFM and decrease FM in persons with SCI.

In addition, there is a growing use of activity-based therapy (ABT) targeting regions below the level of injury to improve standing, motor function, and locomotion.[13] This exercise modality is of high volume (>6 hours/week) and typically includes dynamic resistance training, FES, body weight-supported treadmill training, and load bearing and/or standing. Various components of ABT are used at the Kennedy Krieger Institute (Baltimore, MD), Shepherd Center (Atlanta, GA), and throughout the NeuroRecovery Network in the United States as well as abroad (University of Sydney) to aid in the rehabilitation of persons with SCI. ABT consisting of multiple exercise modalities as listed above has been shown to enhance motor function in persons with SCI[14] and more recently slowed bone loss in this population.[15] Yet, its energy cost (5.1–8.6 ml/kg/min as previously reported[16]) is lower than that shown for arm ergometry[17] which may potentially reduce its ability to alter comorbidities associated with SCI. To our knowledge, the effects of multimodal ABT upon deleterious changes in body composition attendant with SCI are unknown. If ABT is unable to modify metabolic dysfunction, additional exercise should be incorporated into this regimen to improve metabolic health in SCI.

Recently, insulin-like growth factor (IGF-I), myostatin, and adiponectin have been shown to be related to disease risk and health status through their effects on muscle mass and insulin action, which are diminished soon after SCI and contribute to metabolic dysfunction. IGF-I has a putative role in muscle growth and development[18] and has been reported to increase during acute exercise.[19] Prolonged endurance training typically reduces total IGF-I,[20] although no change[21] or small increases[22] have been reported, whereas resistance training seems to have a potentiating effect.[23] In rat muscle subjected to SCI, 5 days of locomotor training increased total IGF-I versus no exercise.[24] Myostatin is a negative regulator of muscle mass and is decreased in response to endurance training.[25] Plasma myostatin levels are also correlated with plasma glucose concentration in adults

with and without type II diabetes.[26] Adiponectin is produced in adipose tissue and has been reported to increase insulin sensitivity.[27,28] Circulating adiponectin levels are reduced in obesity and diabetes[29] and tend to increase with weight loss.[30] Although acute alterations in these proteins are relatively well-understood in the able-bodied, less is known about changes in these variables in response to prolonged exercise training, especially in individuals with SCI.

The primary aim of the current study was to examine changes in body composition in response to chronic multi-modal ABT, which is typically employed to improve locomotion and not indices of health status including FM and FFM, and unlike previous studies using FES[1] or electrically stimulated resistance training,[8,9] requires primarily voluntary muscle contraction. To better understand potential changes in cardiometabolic health through exercise training, IGF-I as well as myostatin and adiponectin were measured. To our knowledge, this is the first study to combine dual-energy X-ray absorptiometry (DXA)-derived changes in body composition with these markers in humans with SCI undergoing chronic ABT, and resultant information may help clinicians to identify optimal exercise regimens to improve body composition and attenuate risk of chronic disease in this population. Dietary intake was closely monitored, as this is typically lacking in studies[31] investigating changes in body composition in persons with SCI, and this variable has marked effects upon not only changes in FFM but also alterations in IGF-I in response to exercise training.[1,32] It was hypothesized that truncal and leg FFM would be significantly enhanced in response to ABT, with no change in body fat exhibited. In addition, we hypothesized that IGF-I would be increased, and myostatin decreased, in response to 6 months of training, with no change exhibited in adiponectin concentration.

Materials and methods
Participants
Adult men and women ($N = 17$) with SCI were recruited to participate in this investigation. Participant characteristics are demonstrated in Table 1. Eight individuals were identified as Caucasian, four as Hispanic, three as Middle Eastern, and two as African-American. To be eligible, subjects met the following inclusion criteria: completion of no formal ABT in the previous year, complete or incomplete SCI, injury level lower than C2, non-ventilator dependent, and physician's permission to engage in an intense exercise program. Eleven participants were within 1 year post-SCI. Prospective participants were excluded if they had completed ABT in the

Table 1   Participant baseline characteristics

Subject	Sex	Age (year)	Mass (kg)	Injury duration (year)	Injury level	Injury classification	Completeness	PA (hours/ week)
1	M*	19.0	53.9	0.6	C5	T	C	4.6
2	M[†]	29.0	73.4	2.9	T10	P	C	8.0
3	M	40.0	101.0	1.0	C4	T	C	9.0
4	M	36.0	80.1	0.5	T12	P	C	7.3
5	F	23.0	90.9	0.7	T12	P	C	13.0
6	M	23.0	79.1	0.3	C5	T	I	6.0
7	M	41.0	79.2	0.8	T3	P	C	4.7
8	M	30.0	61.4	9.3	C4	T	I	6.0
9	M	29.0	78.0	4.5	T12	P	I	10.0
10	F	23.0	55.5	0.6	T3	P	I	8.0
11	M[‡]	30.0	89.4	0.9	C5	T	I	12.0
12	M	42.0	67.0	0.2	L1	P	C	6.0
13	M	29.0	62.7	1.2	T8	P	I	8.0
14	M	43.0	91.3	0.9	T3	P	I	6.0
15	M	36.0	64.5	0.6	T3	P	I	8.0
16	M	49.0	83.2	10.0	C6	T	C	4.5
17	M	53.0	65.3	4.6	T11	P	C	10.0
	Mean ± SD	36.1 ± 11.5	72.0 ± 19.0	2.1 ± 2.9	NA	NA	NA	7.8 ± 2.9

M = male; F = female; T = tetraplegic; P = paraplegic; C = complete; I = incomplete; PA = hours per week of individualized ABT completed during this study.
*Withdrew from the study at 5 months to attend college out-of-state.
[†]Withdrew from the study at 1.5 months due to unrelated leg injury.
[‡]Withdrew from the study at 1 month due to family reasons.

preceding 12 months or were unwilling to abstain from additional exercise during the study, lacked the physical function to complete training or had excess pain, were taking medications that alter health status (such as testosterone, diabetic and/or cardiovascular drugs, and baclofen) other than calcium or vitamin D supplements, had medical conditions besides paralysis that alter body composition, such as diabetes or hyperthyroidism, were peri- or post-menopausal, or suffered an acute infection or illness. After providing their health-history via a brief survey, they provided informed consent to participate in the study which was approved by the University Institutional Review Board.

Design

Participants with SCI initiated 6 months of high-volume training at a local ABT rehabilitation center. During a single session at baseline and at 3 and 6 months, they underwent DXA scans to determine body composition of the whole body as well as the trunk and lower extremities. In addition, fasting blood samples were obtained, and a 4-day food log was completed. These assessments were performed a minimum of 16 hours after their last training session. Time of day was standardized within subjects across all trials. All training was supervised by experienced personnel and targeted regions below the level of injury. Compliance to training was monitored by staff at the facility on a daily basis.

Completion of ABT

Participants performed 2–3 hours/day of ABT targeting the lower extremities (80% for those with quadriplegia and 100% for paraplegia) a minimum of 2 days/week for 6 months at the facility. This regimen elicits intensities ranging from 5 to 8 ml/kg/minute, similar to circuit training and resistance exercise[33] yet lower than arm ergometry or wheelchair ambulation.[17] ABT promotes activation of the neurological levels located both above and below the injury level using rehabilitation therapies[13] and was previously shown[14] to enhance motor gains in persons with chronic SCI. Training was individualized for each client based on their baseline function, and each day, progression was instituted based on participant tolerance to training and level of adaptation. During the study, time performing active assistive exercises and passive gait training generally decreased while time performing resistance training and active gait training increased.

ABT consisted of the following modalities as recently described:[15] 1.5–2.0 hours/week of active assistive exercise,[34] 1.5–2.0 hours/week of upper/lower body and core resistance training, 1.5 hours/week of load bearing,[35] 30 minutes/week of arm/cycle ergometry,[35] 1.0–2.0 hours/week of gait training including assisted and unassisted walking[36] as well as body weight-supported mechanized elliptical training,[14] 10–30 minutes/ week of vibration training,[37] and 30–60 minutes/week of FES of the quadriceps, gluteals, and hamstrings.

Assessment of body composition

Participants were instructed to attend all sessions wearing clothing without metals. Initially, the subject was placed on the dual-energy X-ray absorptiometer (DXA software version 13.5, Lunar Prodigy Advance, GE Healthcare, Madison, WI, USA) for a few minutes to minimize the onset of muscle spasm. They were instructed to remain motionless and not talk during the scan, which was used to estimate whole-body and regional (arm, trunk, and legs) FM and FFM. Body weight (in kg) was calculated from the summation of FM, FFM, and bone mineral content (BMC). Body composition changes from 0 to 6 months of training were expressed in absolute units (kg) as well as using a mean percent change (%) score. Analyses were performed by the same technician who followed standard quality control procedures developed by the manufacturer. Intraclass correlation coefficients and coefficients of variation for whole-body and regional determinations of FM and FFM obtained in various individuals over separate days were equal to 0.98 and 0.99 and 0.7 and 0.8%, respectively. In addition, waist and hip circumference was obtained in the supine position according to standardized procedures[38] to allow determination of waist:hip ratio.

Assessment of IGF-I, adiponectin, and myostatin

Complete data were only obtained from 11 participants, as at one timepoint for three participants, blood samples could not be obtained. A fasting blood sample (10 mL) was obtained from the antecubital vein (21 G × 1.25 in BD Eclipse™ Vacutainer® Holder, Becton Dickinson and Company, Franklin Lakes, NJ, USA), and these samples were placed into a collection tube that was inverted five times to promote clotting. Tubes were then centrifuged for 10 minutes at 1200g (Fisher HealthCare Model 614B, Hanover Park, IL, USA). Serum aliquots were placed into 2 ml Eppendorf tubes and frozen at −80°C for later analysis using sandwich enzyme-linked immunosorbent assay (ELISA). The plasma IGF-I kit (IDS PLC, Scottsdale, AZ, USA) incorporated a sample pre-treatment to avoid interference from binding proteins. The assay used to measure total concentration of adiponectin (Alpco Diagnostics, Salem, NH, USA) uses a pre-treatment method to convert multimeric adiponectin to a dimer form. For the plasma myostatin ELISA (Cusabio, Hubei Province, China), a substrate solution is added to the wells and color develops in proportion to the amount of myostatin bound in the initial step. All samples were run in duplicate using ≤2 kits from the same batch, and the mean concentration was reported. Intra-assay

and inter-assay coefficient of variations were ≤5.9 and 5.4% for IGF-I, ≤5.4 and 5.3% for adiponectin, and ≤8 and 10% for myostatin. The sensitivity for these assays was 0.2–3.1 μg/l for IGF-I, 0.019 ng/ml for adiponectin, and 0.31 ng/ml for myostatin. Changes in these proteins were expressed in μg/l for IGF-I, μg/ml for adiponectin, and ng/ml for myostatin, respectively.

Assessment of dietary intake

Participants were required to complete a 4-day food log (including 2 weekend days) at baseline and at 3 and 6 months, and they were asked to maintain their dietary practices during the study. They were encouraged to actively report all food and drink ingested (including supplements) each day with specific instructions to describe the method of preparation, portion sizes, and brands where applicable. This information was reviewed during each visit and used to determine total caloric as well as macronutrient (fat, carbohydrate, and protein in g) and calcium (in mg) intake using a commercially available website (http://ndb.nal.usda.gov/ndb/foods/list).

Data analysis

Data are reported as mean standard ± deviation (SD) and were analyzed using SPSS Version 20.0 (IBM Corp, Armonk, NY, USA). Initially, normality of all variables was examined. Two-way (one within-subject factor representing training (0, 3, and 6 months) and one between-subject factor including injury completeness and duration of injury) analysis of variance with repeated measures was used to examine changes in all variables in response to training. The Greenhouse–Geisser correction was used to account for the sphericity assumption. If a significant F ratio was obtained, Tukey's *post hoc* test was used to identify differences between means. Partial eta-squared (η^2) was used as an estimate of effect size. Independent *t*-test was used to examine differences in percent change of various parameters across injury completeness/level and duration of injury, and the Pearson product moment correlation coefficient was used to examine pairwise relationships between variables. Statistical significance was established at P < 0.05.

Results

Training was well-tolerated by all participants, with a compliance rate equal to 100% for all required sessions. Nevertheless, three individuals did not complete 6 months of ABT due to moving out of state ($n = 1$), injury onset unrelated to the study ($n = 1$), and family issues that precluded training ($n = 1$), so complete

Table 2   Changes in body mass and body composition in response to 6 months of ABT

Parameter	0 month	3 months	6 months
Body mass (kg)	76.5 ± 13.0	77.4 ± 13.1	77.0 ± 13.1
WC (cm)	92.5 ± 14.6	92.6 ± 17.7	90.7 ± 15.0
WHR	0.91 ± 0.09	0.88 ± 0.09	0.89 ± 0.11
FFM (kg)	50.4 ± 8.4	50.3 ± 7.9	49.2 ± 7.4*
%BF	31.2 ± 12.9	32.0 ± 12.6	32.2 ± 11.8
LegFFM (kg)	14.5 ± 3.1	14.4 ± 3.2	14.3 ± 2.8
Leg%BF	34.7 ± 12.4	35.9 ± 11.9	36.4 ± 10.6*
TrunkFFM (kg)	24.4 ± 3.9	24.4 ± 3.6	24.0 ± 3.8
Trunk%BF	32.6 ± 14.2	33.3 ± 14.2	33.5 ± 13.4
ArmFFM (kg)	6.7 ± 2.2	6.9 ± 2.0	7.0 ± 2.0
Arm%BF	23.8 ± 13.6	24.0 ± 13.6	24.5 ± 13.7

WC = waist circumference; WHR = waist:hip ratio; FFM = whole-body fat-free mass; %BF = percent body fat; *P < 0.05 versus 0 month value.

body composition data are only reported for 14 individuals who completed 6 months of ABT.

Changes in body composition for all participants

Table 2 demonstrates changes in all parameters related to whole-body and regional body composition. Nonsignificant changes in most variables were revealed during the study, with the exception of whole-body FFM (P = 0.02, $\eta^2 = 0.25$) which declined during the 6 months study. In addition, a training × group interaction (P = 0.04) was revealed for FFM in that it declined by 2.7 kg in individuals with complete injury yet it was unchanged in those with incomplete injury. Leg%BF increased (P = 0.03, $\eta^2 = 0.26$) during the study from the 0 to 6 months value (P < 0.05) with no group interaction apparent (P = 0.38).

Individual changes in body composition

Fig. 1 shows individual changes in whole body and regional FFM during the study. There was large variability in alterations in FFM, as some subjects showed marked declines in FFM, whereas others showed a relative maintenance or increases in FFM.

Changes in body composition according to injury completeness, duration of injury, and severity

Whole-body FFM did not change in participants with incomplete injury (−0.04 ± 4.1%); however, FFM decreased (−4.8 ± 5.8%) in those with complete injury. Similar discrepant results, albeit nonsignificant interactions (P = 0.16–0.40), were revealed in response to ABT between complete and incomplete injury, for percent change in body fat (+3.0 ± 6.1% vs. +6.3 ± 9.5%), FFMleg (−4.9 ± 10.8% vs. +2.5 ± 7.1%), FFMtrunk (−2.6 ± 6.9% vs. −0.7 ± 3.2%), and
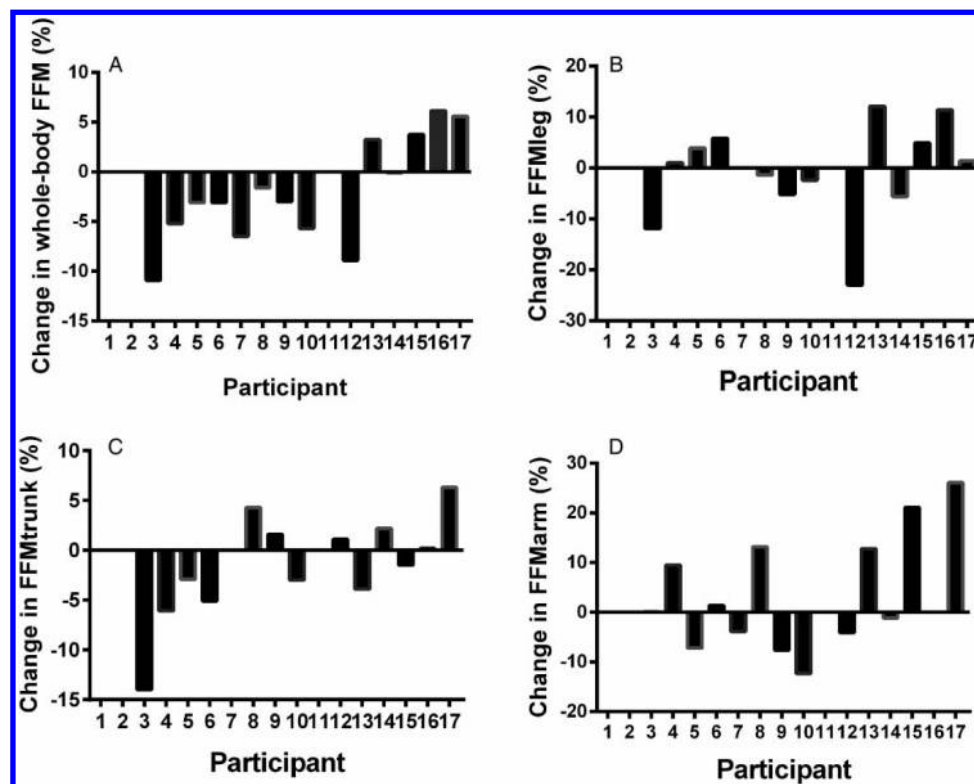
Figure 1   Individual changes in (A) whole-body FFM, (B) leg FFM, (C) trunk FFM, and (D) arm FFM in response to 6 months of chronic ABT in persons with spinal cord injury. Participants 1, 2, and 11 did not complete the study, and participants 8, 9, 13, 16, and 17 had chronic (>1 year) SCI.

Table 3   Differences (mean ± SD) in adiponectin, myostatin, and IGF-I concentration in response to 6 months of ABT

Parameter	Baseline	3 months	6 months
Adiponectin (μg/ml)	4.01 ± 0.96 (2.48–5.71)*	4.27 ± 0.97 (2.94–5.69)	4.09 ± 0.96 (2.70–5.19)
Myostatin (ng/ml)	2.54 ± 0.43 (0.92–2.82)*	2.50 ± 0.29 (0.80–2.74)	2.47 ± 0.36 (1.58–2.72)
IGF-I (μg/l)	53.1 ± 15.0 (31.0–74.0)*	55.2 ± 13.4 (35.3–75.3)	54.5 ± 11.9 (36.6–69.4)

*Range of values across the participants.

FFMarm ($+3.4 \pm 12.5\%$ vs. $+4.1 \pm 12.1\%$). When duration of injury was considered as a between-subject factor, with <1 year being classified as recent injury and >1 year equal to chronic, there were dissimilar changes in FFM observed for whole body ($-3.34 \pm 5.08\%$ vs. $1.08 \pm 4.02\%$, $P = 0.14$), the trunk ($-2.82 \pm 4.47\%$ vs. $2.08 \pm 4.43\%$, $P = 0.08$), arms ($0.94 \pm 9.43\%$ vs. $11.13 \pm 13.90\%$, $P = 0.13$), and legs ($-2.46 \pm 9.90\%$ vs. $1.73 \pm 7.42\%$, $P = 0.40$). There were no differences ($P > 0.20$ for training × group interaction) in responses between paraplegics and tetraplegics.

Changes in IGF-1 and myostatin

No change ($P = 0.41$) in IGF-I was demonstrated in response to ABT. Plasma levels of myostatin were unaltered ($P = 0.37$) in response to training, although a non-significant decrease was evident during the study. These data are given in Table 3.

Changes in adiponectin

No change in adiponectin was demonstrated in response to training ($P = 0.21$), although it was higher ($P = 0.04$) in individuals with incomplete versus complete injury. These data are given in Table 3.

Dietary intake

With the exception of protein intake expressed as a percentage of total calorie intake, which differed ($P = 0.03$) from 3 to 6 months, there was no change ($P > 0.05$) in any dietary-related parameter in response to training. These data are demonstrated in Table 4.

Discussion

The primary aim of the present study was to examine changes in FM and FFM in response to high-volume ABT in persons with SCI heterogeneous in age, sex, and injury completeness. In addition, determinations of plasma IGF-I, adiponectin, and myostatin were obtained as these have been reported to regulate muscle mass and overall health status in various populations. Despite high compliance to training across all participants, no change in body weight or body fat was exhibited, and training was unable to significantly modify FFM, as it declined in the majority of participants and as a group. Nevertheless, participants with

incomplete SCI, as well as those with injury duration greater than 1 year, showed maintenance or slight increases in whole-body FFM. In addition, results showed no change in IGF-I, myostatin, or adiponectin during the study. Ultimately, 6 months of ABT does not slow muscle atrophy in men and women with SCI or reduce body fat demonstrated in response to other training regimens,[8,12] although more robust changes were seen in individuals with chronic or incomplete injury. Alternative exercise modalities are needed to prevent deleterious changes in body composition in persons with acute, complete SCI.

Our results showing a small decline in FFM in response to ABT oppose data from multiple studies showing significant increases in muscle mass in SCI performing exercise training. Pacy *et al.*[39] revealed that in four men with paraplegia, 10 weeks of FES enhanced muscle area of the leg and decreased fat area. In patients approximately 14 weeks post-SCI, 3–6 months of FES cycle ergometry enhanced total body and leg lean body mass compared to control and isometric FES exercise,[1] in which the latter led to no change in lean body mass versus controls who lost 6.1–12.4% during the 6-month period. In men with complete, chronic SCI, 12 weeks of electrical stimulation-induced resistance training increased quadriceps femoris CSA by 35–39%[8] and 16–35% for the total thigh.[40] In this study,[40] DXA-derived estimates of leg FFM were also increased ($P < 0.05$), although no change in total body or trunk FFM

Table 4   Alterations in dietary intake (mean ± SD) in response to 6 months of ABT in men and women with SCI

Parameter	0 month	3 months	6 month
Calorie intake (kcal/day)	1780.9 ± 373.1	1735.8 ± 409.7	1785.8 ± 364.7
Carbohydrate (g)	213.2 ± 62.9	207.8 ± 56.5	224.6 ± 57.7
Lipid (g)	63.4 ± 14.5	61.9 ± 15.1	62.7 ± 15.9
Protein (g)	77.5 ± 23.2	86.5 ± 26.3	74.9 ± 24.0
Carbohydrate (%)	48.2 ± 9.2	48.2 ± 7.4	50.9 ± 10.4
Lipid (%)	32.6 ± 6.6	32.5 ± 5.5	31.5 ± 6.9
Protein (%)	17.4 ± 3.6	19.9 ± 4.6	16.6 ± 4.4*
Calcium intake (mg/day)	753.3 ± 349.9	698.5 ± 232.0	764.6 ± 342.4

*$P < 0.05$ from 3-month values.

was revealed which corroborates our findings (Table 2). Results from a case study in a man with chronic SCI demonstrated that 12 weeks of electrical stimulation-induced resistance training led to 12–43% improvements in leg CSA as well as reductions in intramuscular fat.[9] Similarly, 9 months of locomotor training in a man with chronic SCI elicited a 4% increase in leg FFM and 3.5% decrease in leg fat despite overall losses in whole body FFM and greater FM.[41] In contrast to the studies highlighted above describing beneficial effects of chronic training on FFM, our population was heterogeneous in injury completeness, duration, as well as sex and age, which may explain the lack of overall improvement in FFM. Moreover, the majority of studies in which increases in FFM occurred are characterized by electrically stimulated exercise, whereas in the present study ABT required primarily voluntary contraction. In addition, insufficient dietary intake does not seem to be an explanation, as participants' macronutrient intake (Table 4) was similar to that administered in the Gorgey *et al.*[40] study in which muscle hypertrophy was evident in response to training.

Individual data (Fig. 1) demonstrate marked variability in changes in whole-body and regional FFM that was not correlated to pre-training FFM/FM, duration of injury, protein intake, age, or frequency of physical activity. Closer examination of these data reveal that most participants who showed improved FFM were sedentary upon entering the study, so it would be anticipated that a new exercise stimulus such as ABT may promote FFM accretion, whereas those who typically lost FFM were habitual exercisers prior to the onset of SCI. However, no interaction (P > 0.05) was demonstrated for any outcome measure between those who were sedentary and habitually active. Despite no significant changes in leg FFM or muscle size in response to 4 months of FES-assisted walking in persons with chronic SCI, 7 of 17 patients (41%) exhibited increases in muscle area.[31] As SCI research is typically characterized by populations differing in age, sex, duration of injury, and injury level/severity, this is not unexpected. Data from able-bodied individuals exhibit discrepant responses to identical regimens of training whether it be resistance-based[42] or aerobic[43] in origin, so it is likely that similar individual differences in adaptation exist in SCI. Nevertheless, 15% reductions in leg FFM[2] after 1 year, and 12–24% reductions in muscle CSA[3] during the first 6 months after injury have been demonstrated, so our regimen could be seen as beneficial as it somewhat slowed the loss of FFM in our widely divergent cohort during a period when muscle atrophy is occurring.

However, there are a few factors which may explain the lack of significant changes in FFM seen in the present study. Although DXA has been reported[44] to provide an accurate measure of body composition in SCI, its sensitivity towards detecting small changes in FM or FFM is surpassed by magnetic resonance imaging (MRI). For example, MRI-determined mean CSA of visceral adipose tissue was reduced by 25%, and quadriceps muscle mass increased by 25–30%, in response to resistance training compared to no significant changes in estimates of whole-body FFM or FM/%BF determined by DXA.[40] Overall, no change in body weight or body composition in response to exercise training is frequently reported in the literature,[45] and can be viewed as a desirable outcome as SCI typically leads to enhanced body weight and body fat. In fact, findings from a recent systematic review[46] revealed that there is insufficient evidence regarding positive effects of exercise on body composition in this population. In our participants, FES comprised only 5.5% of time spent training, with greater participation in active assistive exercise (25%), load bearing (21%), dynamic resistance training (27%), and gait training (11%). It may be that these modalities do not require large enough forces to cause hypertrophy. Of these modalities, FES is frequently cited[1,3] as having the most significant effect upon body composition in SCI versus other modalities. However, ABT is designed to improve motor gains and achieve activation of the neurological levels both above and below the injury level[13] and does not solely target the leg muscles as does FES, so its inability to promote muscle hypertrophy is not surprising. Furthermore, its energy cost is less than other modalities typically performed in SCI. Overall, it appears that FES must be a more sizable component of exercise training in SCI if modifying deleterious changes in body composition is a primary outcome.

Serum IGF-I was reported to be 25% lower in adults with SCI compared to age-matched controls,[47] although in this study, men were older and had lived with SCI for a longer duration (~15 years) than in our cohort. In a cross sectional study, Chain *et al.*[48] demonstrated no difference in IGF-I between active and sedentary tetraplegics, although IGF-I was 25% higher in active compared to sedentary individuals. In addition, no difference in IGF-I was reported between able-bodied controls and men and women with acute SCI.[49] However, IGF-1 was strongly correlated to muscle size in men with complete SCI.[50] In the present study, IGF-I was unchanged with training although there was wide variability across participants. Milani *et al.*[51] reported marked variability in serum IGF-I, as it varied by more than 10% in greater than 50% of subjects

on repeated determinations over separate days. This surpasses the typical variation seen in most ELISA assays. A strong genetic influence (63%) on IGF-I has also been identified,[52] which suggests that interventions such as exercise training may have minimal effects upon circulating levels of this protein. Body composition also mediates IGF-I levels, as lower IGF-I has been reported in individuals with BMI $<20\,kg/m^2$ or $>35\,kg/m^{2,32}$ which was also revealed in our cohort as IGF-I tended to be higher in participants with BMI ranging from 20 to $30\,kg/m^2$ versus those with BMI $>30\,kg/m^2$ (data not reported). About 80% of IGF-I circulates in complex with IGF binding protein 3, yet only 1% exists in its free form.[53] In a previous study in which young women completed 8 weeks of intense physical training,[54] bioavailable IGF-I did not change, although total IGF-I was increased (P < 0.05) by 7% although this change is within the variation of most assays. Nevertheless, these findings were coincident with significant increases in FFM and attenuated FM which did not occur in the current study. As IGF-I is altered by a host of physiological factors and plays key roles in muscle mass[18] and bone health,[55] development of assay-specific norms has been emphasized[53] to better interpret patient results in diagnostic applications.

Compared to normal weight individuals, adiponectin levels are typically lower in the obese[30] as well as in persons with cardiovascular disease and/or diabetes.[56] For example, Jurimae et al.[57] demonstrated significantly lower adiponectin concentration in overweight (9.9 ± 3.1 µg/ml) compared to normal weight premenopausal women (14.4 ± 4.7 µg/ml), with adiponectin inversely related to parameters including BMI, body weight, and truncal fat. In a cross-sectional study in men with SCI,[58] adiponectin ranged from 2.9 to 37.2 µg/ml and was inversely associated with waist circumference and trunk fat, although in another study,[59] it was similar between able-bodied individuals and those with SCI, with this discrepancy being explained by similar body weight and BMI between subjects as well as different assays performed (ELISA versus RIA) across studies. In the present study, adiponectin concentration was similar in participants with greater than 30%BF compared to those with lower body fat.

Although acute exercise does not seem to enhance circulating adiponectin in most individuals with the exception of trained athletes,[57] both relatively brief (<12 weeks) and more prolonged training interventions (>12 weeks) have been shown to enhance plasma adiponectin. Twelve weeks of interval training in obese young females increased adiponectin which was coincident with significant reductions in body mass, %BF, and

insulin concentration.[60] In the SCI, only one study to our knowledge examined changes in adiponectin in response to training. Rosety-Rodriguez et al.[61] separated 17 men with paraplegia into a control or exercise (12 weeks of arm cranking) group. Results showed significant decreases in WC, leptin, and systemic inflammation with training, yet no change in adiponectin was revealed. In the current study, neither adiponectin nor body weight or body fat were altered in response to 6 months of ABT. This ABT regimen elicits relatively low energy expenditure[16] versus arm ergometry, and combined with no change in calorie intake (Table 4), occurrence of energy deficit leading to weight loss is unlikely. It may be that improvements in adiponectin are more likely to be revealed when body composition is altered through exercise and dietary interventions versus exercise alone.[62]

Previously, alterations in plasma myostatin have typically been examined in able-bodied individuals. Lakshman et al.[63] reported that myostatin was lower in older (7.0 ± 0.3 ng/ml) compared to young men (8.0 ± 0.4 ng/ml), and these values were threefold lower than previously reported values[25] ranging from 20 to 30 ng/ml. This variability has been attributed to different antigen–antibody combinations found in ELISA assays used to assess myostatin levels. In the Hittel et al. study,[25] 6 months of aerobic training in insulin-resistant men reduced myostatin and improved insulin sensitivity. However, there was no relationship (P > 0.05) between baseline myostatin and fat or FFM, which suggests that insulin sensitivity may be a more significant correlate of myostatin than body composition or muscle mass *per se*. In humans with chronic SCI who underwent greater than 6 years of soleus electrical stimulation training, results showed decreases in 10 mRNAs encoding for myostatin, and this was consequent with preservation in muscle force and CSA versus the untrained leg.[64] Our data showed that myostatin did not change during the study, but there was large individual variability as previously shown[25] which has been reported to be genetic in origin.[65] In addition, results from Louis et al.[66] showed that myostatin mRNA expression is reduced by 6-fold up to 24 hours after resistance exercise, so our low circulating levels of myostatin could be due to previous exercise performed in the afternoon prior to the blood sample, as completed by 35% of participants in the current study. Myostatin and IGF-1 share the same PI3K/Akt pathway with insulin, suggesting that they play similar roles in cell growth and metabolism. Ultimately, reducing myostatin expression via exercise may be important to promote adaptations to paralyzed muscle,[64] so it is merited to

identify an optimal dose and modality of exercise training targeting muscle mass in SCI, as it is presently unknown.

Type II diabetes is becoming far too common in persons with SCI. A recent study[7] demonstrated 60% greater risk of type II diabetes in a large sample of Canadians with SCI after adjusting for known risk factors (hypertension, smoking status, etc.). Similar prevalence was reported in adults with SCI living in the United States, who were 1.7 times more likely to have diabetes than adults who had experienced lower extremity fractures.[67] Consequently, management of this condition must be comprehensive since individuals with SCI have similar lifespan as able-bodied adults. Implementation of exercise including FES[3] and electrically stimulated resistance training[40] is effective to reverse muscle atrophy and improve insulin sensitivity in this population, which helps to enhance cardio-metabolic health and attenuate risk of diabetes. Scientists should continue to explore the optimal modality and dose of exercise needed to reduce the severity and the onset of comorbidities in SCI, as their findings will have tremendous impact on preserving health status of these individuals.

This study faces several limitations. Participants were heterogeneous in age, sex, and injury level/duration which may influence the results. Although they completed the same modality of training and minimum training frequency (≥ 2 days/week) and duration (≥ 4.5 hours/week), regimens were individualized for each client based on their tolerance to exercise, so were somewhat different across individuals. A non-exercising control group of persons with SCI was not recruited, so we are uncertain if our results are strictly due to the intervention. Measures of fasting insulin, insulin sensitivity, or maximal oxygen uptake were not obtained, which would have clarified potential changes in glucose control and cardiorespiratory fitness in response to exercise training. Basal metabolic rate was not measured, which would shed light on potential for fat or weight loss with exercise training. As typical caloric intake (Table 4) exceeded previously established values for basal metabolic rate in SCI (900–1500 kcal/day), it is not surprising that little changes in body mass were revealed. Nevertheless, our DXA-derived measures of FM and FFM were highly reliable as they showed little variation over repeated preliminary assessments in subjects with and without SCI. We reported alterations in body composition as percent change scores to overcome the large intersubject differences. Moreover, this study is strengthened by a relatively large sample size ($N = 14$) and measurement of various blood-based markers that complement the body composition data obtained from DXA. In addition, dietary intake was carefully monitored during the study to ensure that the implementation of chronic training rather than changes in food intake mediated the observed changes in our measures. Future studies should consider combining robust dietary interventions (reducing total caloric intake as well as intake of saturated fat and high-glycemic index sugars and increasing protein and unsaturated fat intake) with exercise training in this population to optimize potential changes in body composition.

Conclusion
In summary, 6 months of ABT in persons with SCI did not alter leg, arm, or truncal FFM, and a small decline in whole-body FFM was exhibited. Estimates of regional and whole-body FM were unchanged. Individuals with chronic or incomplete injury revealed more beneficial changes in body composition than those with acute or complete SCI. No change in IGF-I, myostatin, or adiponectin was revealed, which suggests that training was of insufficient stimulus to modify these markers soon after SCI. Future studies should use a more comprehensive regimen of exercise training including FES-assisted resistance training or cycle ergometry combined with ABT to examine if this paradigm has more positive effects on improving metabolic health and overall physical function in this population.

Disclaimer statements
Contributors
All authors participated in designing and initiating the study, the first author developed the manuscript and the remaining authors reviewed the manuscript for submission.

Conflicts of interest
None.

Ethics approval

None.

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