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Original Article

Hylonome

Lower-extremity muscle atrophy and fat infiltration after chronic spinal cord injury

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Abstract

Background: Atrophy and fatty-infiltration of lower-extremity muscle after spinal cord injury (SCI) predisposes individuals to metabolic disease and related mortality. **Objectives:** To determine the magnitude of atrophy and fatty-infiltration of lower-extremity muscles and related factors in a group of individuals with chronic SCI and diverse impairment. **Methods:** Muscle cross-sectional area and density were calculated from peripheral quantitative computed tomography scans of the 66% site of the calf of 70 participants with chronic SCI [50 male, mean age 49 (standard deviation 12) years, C2-T12, AIS A-D] and matched controls. Regression models for muscle area and density were formed using 16 potential correlates selected *a priori*. **Results:** Participants with motor-complete SCI had ≈32% lower muscle area, and ≈43% lower muscle density values relative to controls. Participants with motor-incomplete SCI had muscle area and density values that were both ≈14% lower than controls. Body mass (+), tetraplegia (+), motor function (+), spasticity (+), vigorous physical activity (+), wheelchair use (-), age (-), and waist circumference (-) were associated with muscle size and/or density in best-fit regression models. **Conclusions:** There are modifiable factors related to muscle size, body composition, and activity level that may offer therapeutic targets for preserving metabolic health after chronic SCI.

Keywords: Spinal Cord Injury, peripheral Quantitative Computed Tomography (pQCT), Muscle Density, Muscle Cross-sectional Area, Body Composition

Introduction

Individuals with SCI are at increased risk of developing secondary complications, such as cardiovascular disease, type II diabetes, and osteoporosis, and often experience these conditions at an earlier age as compared to their able-bodied peers[1]. Secondary complications, which are often treatable, are a major contributor to premature mortality and a disproportionally high use of healthcare resources by individuals with SCI[2,3]. Furthermore, both the life expectancy and the number of older adults sustaining a SCI are increasing, and consequently it is expected that the prevalence of chronic diseases among individuals with SCI will grow[4].

Skeletal muscle plays a vital regulatory role in maintaining metabolic and bone health, and lower-extremity muscle atrophy is a major contributing factor to metabolic dysregulation after SCI[5]. The accumulation of adipose tissue within and between the muscle groups of the lower-extremities can be up to four times greater in those with SCI compared able-bodied controls[6-8]. Adipose tissue deposition in skeletal muscle is closely linked with chronic inflammation, glucose intolerance, impaired serum lipid and lipoprotein levels, and decreased strength and mobility in many clinical populations including SCI[9]. Additionally, an inverse relationship between fatty-infiltration of muscle and bone quality has emerged in other populations including older adults[10], and those who have suffered a stroke[11]; suggesting that muscle quality, in addition to muscle size or strength, can affect bone health in those with disability.

Declines in lower-extremity fat-free mass after SCI are well-documented[12] however, examinations of lower-extremity

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muscle size and fatty-infiltration are limited to studies using small and relatively homogeneous samples. Only two studies[7,13] have documented muscle atrophy and fatty-infiltration in individuals with incomplete SCI despite their innate neuroplasticity and preservation of motor function. Furthermore, only three studies[6,14,15] have reported muscle size and quality of individuals with chronic SCI, and no study has reported correlates associated with muscle atrophy or fatty-infiltration after complete or incomplete SCI of any duration. As a result, our current understanding of how muscle changes with chronic SCI may not be generalizable across the diverse population of individuals with SCI, and the factors related to muscle adaptations remain largely unknown.

It is unclear how much skeletal muscle adaptation after SCI is inevitable and how much is preventable by addressing modifiable factors. There is a need for studies of muscle status across a large sample of individuals with SCI diverse with respect to gender and impairment, so that we can begin to understand the modifiable and non-modifiable correlates of adverse muscle changes post-SCI. As muscle size and density may be amenable to rehabilitation interventions such as electrical stimulation-assisted walking[16], cycling[17], or muscle strengthening[18], there is a need to identify whom to target with these interventions.

Reductions in lower-extremity muscle size and quality have been reported in small-scale studies usually after acute SCI[6,7,19,20]; however, the degree of atrophy and fatty-infiltration of lower-extremity skeletal muscle after chronic SCI has not been well documented. Therefore, the purpose of this study was two-fold. Our first goal was to characterize the degree of atrophy and fatty-infiltration of lower-extremity skeletal muscle in a large group of SCI participants with diverse impairment compared to matched able-bodied controls. Our second goal was to create multivariate models of muscle area and density to better understand the factors associated with muscle atrophy and fatty-infiltration after long-term paralysis or paresis.

Methods

SCI participants

A secondary analysis of baseline data from a two-year longitudinal study being conducted at the University of Waterloo and the Lyndhurst Centre, Toronto Rehabilitation Institute, University Health Network was performed[21]. Men and women ≥18 years of age with spinal cord impairment [C1-L2, American Spinal Injury Association Impairment Scale (AIS) A-D] of sudden onset (<24 h) were included in this study. Participants were at least two-years post injury prior to enrolment. Participants were excluded if they had (a) a current or prior known condition, other than paralysis, known to influence bone metabolism including oral glucocorticoid use ≥ three months, malignancy, and known liver disease or malabsorption condition; (b) a body mass ≥122 kg; (c) planned to become pregnant or were pregnant at enrolment; and (d) contraindications to pQCT including bilateral lower-extremity metal implants or severe hip and knee flexion contractures.

Past and current medical history, demographic, lifestyle, and impairment data were obtained via participant interview and chart abstraction. Participants' neurological level of injury and AIS classification were determined by a physiatrist (BCC) using the International Standards for Neurologic Classification of SCI. To isolate the effect of voluntary muscle activation on muscle status, the calf (ankle dorsiflexors, long toe extensors, and plantar flexors) lower-extremity motor score (cLEMS) of the leg scanned was used in the analyses. Supine height, body mass, and waist circumference (measured at the lowest-rib) were also recorded. Spasticity was assessed using the self-report Penn Spasm Frequency and Severity Scale[22], and the subscore for the leg scanned was used in the analyses. Participant's mobility status was classified dichotomously as using (or not using) a wheelchair for community mobility.

Vitamin D

Blood samples were drawn using a Vacutainer system (BD Vacutainer, Becton, Dickinson and Company). The collected samples were placed on ice for immediate analysis. Participants fasted for at least twelve hours prior to blood collection. Serum 25(OH)D was determined with a chemiluminescent immunoassay using the Diasorin LIAISON (Diasorin S.p.A.) which exhibits 100% cross-reactivity for both 25(OH)D2 and 25(OH)D3 to estimate the total 25(OH)D circulating in the body[23].

Physical activity level

Physical activity was assessed by the Physical Activity Recall Assessment for People with Spinal Cord Injury (PARA-SCI). The PARA-SCI is a valid and reliable physical activity recall questionnaire to assess the type, frequency, duration and intensity of physical activity performed by those with SCI using a wheelchair as their primary mode of mobility[24]. The average minutes per day of mild, moderate, vigorous, and total physical activity were calculated and included in this cross-sectional analysis.

Able-bodied controls

Control participants were objectively selected from pooled pQCT studies conducted at the University of Saskatchewan[25,27]. To be eligible for selection, participants were recreationally active and free of musculoskeletal health disorders. Able-bodied controls were matched to SCI participants for age, gender, and height using a propensity score algorithm[28]. One able-bodied control was included for each SCI participant.

pQCT assessment

Muscle cross-sectional area (cm^2) and muscle density (mg/cm^3) were calculated from pQCT scans of the 66% site of the tibia measured from distal to proximal, starting at the inferior border of the medial malleolus. This site was chosen because it is the region of the calf with the largest circumference and muscle cross-sectional area[29]. Images were acquired using

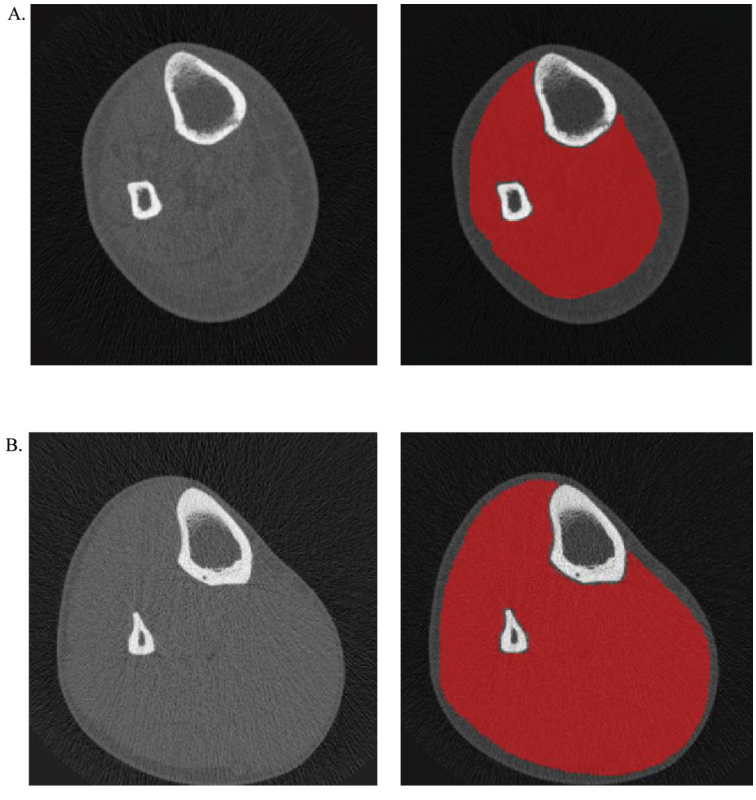

Figure 1. An illustration of watershed-guided muscle segmentation of the 66% site of the calf of a male with complete paraplegia (A) and corresponding able-bodied control (B).

a Stratec XCT 2000 scanner (Stratec Medizintechnik) with Stratec software version 5.50. The right tibia was scanned except in cases of severe spasticity or other contraindications, such as the presence of metal or fracture. A slice width of 2.2mm was used for all SCI and able-bodied scans. Pixel sizes of 0.5*0.5 mm and 0.4*0.4 mm were used for scans of SCI and control participants, respectively. All pQCT scans of SCI participants were acquired by the same X-ray technician using the same scanner.

Tissue segmentation was performed using sliceOmatic software, version 4.3 (Tomovision). Our group has previously published a detailed description of this technique[30]. Briefly, muscle was defined as the soft tissue beneath the fascia boarder separating the muscle and subcutaneous fat compartments (Figure 1). Tissue was segmented with the aid of a watershed algorithm and manually corrected for watershed spillover. We found that this method has tighter re-resting limits compared to threshold-based analysis techniques, especially for individuals with a high degree of fatty infiltration[30]. Density values are reported in mg/cm^3 based on a calibration using a hydroxyapatite-equivalent European forearm phantom. Scans that had severe movement artifacts were excluded from the analysis based on the visual scale reported by Blew et al.[31]. All pQCT analyses were performed by a single investigator (CM). Prior to analysis, pQCT scans were randomized and blinded to the assessor.

Statistical analysis

Descriptive statistics were used to describe participants' muscle status and demographic, anthropometric, and impairment characteristics. Categorical variables are presented as counts (n) and percentage (%), and continuous variables are presented as means [standard deviations (SD)]. All statistical analyses were performed on SAS 9.2 software (Cary, North Carolina).

Independent t-tests were used to test for significant differences in age, height, and body mass between SCI cases and controls, and paired t-tests were used to compare the muscle area and density of individuals with complete and incomplete SCI to controls. Independent t-tests were also used to compare differences in muscle area and density between individuals with complete and incomplete SCI. Multiple linear regression analyses were used to identify correlates of muscle area and density. Separate models for muscle area and muscle density were created for the entire SCI sample, and sub-samples separated by motor-completeness of injury (i.e., AIS A and B versus C and D). Potential correlates selected *a priori* included: age (years), gender[32], height (cm), body mass (kg), waist circumference ($</\geq 94$ cm)[33], injury duration (years), age at injury (years), calf-muscle (L4, L5, S1) lower-extremity motor-score (cLEMS, /15), level of injury (tetraplegia/paraplegia)[34], wheel-

	All participants	Participants with motor-complete SCI	Participants with motor-incomplete SCI	Able-bodied controls
# Participants (%)	70	45 (64.2%)	25 (35.7%)	70
Sex, n (%)				
Female	20 (28.6%)	13 (28.9%)	7 (28.0%)	20 (28.6%)
Male	50 (71.4%)	32 (71%)	18 (72%)	50 (71.4%)
Age [years (SD)]	48.8 (11.5)	45.5 (9.8)	54.9 (12.0)[a]	47.4 (13.8)
Duration of Injury [years (SD)]	15.5 (10.0)	17.8 (10.0)	11.4 (8.8)[a]	
Age at Injury [years (SD)]	33.7 (14.7)	28.9 (12.4)	42.4 (14.9)[a]	
Level of Injury, n (%)				
Tetraplegia	36 (51.4)	22 (48.9%)	14 (56%)	
Paraplegia	34 (48.6)	23 (51.1%)	11 (44.0%)	
Height [cm (SD)]	174.5 (10.3)	173.7 (10.5)	176.0 (9.8)	173.8 (8.5)
Body Mass [kg (SD)]	80.1 (18.5)	78.6 (19.2)	82.9 (14.6)	85.0 (15.0)
Waist Circumference [cm (SD)] †	97.4 (14.8)	96.6 (15.0)	98.9 (14.6)	
25(OH)Vitamin D [nmol/L (SD)]	87.8 (35.0)	89.2 (32.9)	85.6 (38.9)	
AIS, n (%)				
A	42 (60.0%)	42 (93.3%)	-	
B	3 (4.2%)	3 (6.7%)	-	
C	10 (14.2%)	-	10 (40.0%)	
D	15 (21.4%)	-	15 (60.0%)	
Lower Extremity Motor Score [/50 (SD)]	11.0 (15.8)	-	29.1 (12.5)	
Physical Activity [min/day (SD)]≠				
Mild	121 (133)	130 (150)	105 (100)	
Moderate	86 (114)	81 (135	94 (71)	
Vigorous	25 (35)	26 (36)	24 (35)	
Total	232 (210)	237 (245)	223 (139)	

† Indicates n = 68 due to missing data
≠ Indicates n = 58 due to missing data

[a] Significant difference between motor-complete and incomplete groups (p<0.05)
SCI: spinal cord injury; SD: standard deviation; AIS: American Spinal Injury Association Impairment Scale

Table 1. Participant characteristics.

chair use (yes/no)[20], serum 25(OH)D (nmol/L)[35], lower-extremity spasm frequency and severity (/7)[36], mild, moderate, vigorous, and total daily minutes of physical activity (min/day)[37]. Correlates found to be independently significant at p<0.20 in bivariate regression analyses were entered into multi-variable regression models using manual model selection based on R^2 and C(p) statistics[38]. A minimum of ten observations for each independent variable were included to avoid over-fitting the models[39].

Results

Participant characteristics

Of the 409 individuals approached for this study, 274 were unreachable, deceased, or declined to participate. Following screening, 70 consenting adult men (n=50) and women (n=20) were eligible for study participation. Participants had mean (SD) duration of injury of 15.5 (10.0) years, and age of 48.8 (11.5) years (Table 1). Forty five individuals had motor-complete injuries. Fourteen of the 25 individuals with incomplete SCI used a wheelchair for ambulation. There were no differences in the age, height, body mass or gender frequency between SCI cases and controls (p<0.05, Table 1).

Muscle area and density

pQCT scans from five participants were not obtained because participants either missed their appointment (n=1), died after study enrollment but before pQCT scan acquisition (n=1), or had severe lower-extremity spasticity or a calf circumference which exceeded the size of the gantry opening, precluding accurate positioning for scan acquisition (n=3).

Individuals with SCI had significantly smaller calf-muscle area and density values compared to matched controls (Table 2). Participants with motor-complete SCI had on average 34.7 (20.2) cm^2 [43.4 (23.0) percent] lower muscle area, and 22.7 (13.6) mg/cm^3 [31.9 (19.4) percent] lower muscle density than their matched control (both p<0.001). Participants with motor-incomplete SCI had mean muscle area and muscle density values that were 13.0 (22.3) cm^2 [14.4 (28.7) percent] and 10.0 (9.4) mg/cm^3 [14.3 (13.2) percent] lower than controls, respectively (both

	Complete SCI	Incomplete SCI	Able-bodied controls
N	39	25	64
Muscle Area (cm^2)	43.1 (16.7)[a,c]	65.5 (19.3)[a,c]	78.0 (12.1)
Muscle Density (mg/cm^3)	48.8 (14.1)[a,c]	60.2 (9.2)[b,c]	71.0 (2.3)

[a] *Indicates significant difference between SCI and controls (p<0.001)*
[b] *Indicates significant difference between SCI and controls (p<0.01)*
[c] *Indicates significant difference between complete and incomplete SCI (p<0.001)*

Table 2. Means and standard deviations of muscle cross-sectional area and density of SCI and able-bodied participants.

a. Model 1: Full Cohort, n=64			
Variable	**Parameter Estimate**	**Standard Error**	**p**
Body Mass (kg)	0.62	0.08	<0.001
Paraplegia	-5.35	3.00	0.079
cLEMS (/15)	1.11	0.45	0.016
Wheelchair Use	-12.88	5.58	0.025
R-Square for model=0.70, p<0.001			
b. Model 2: Motor-Complete SCI, n=36			
Variable	**Parameter Estimate**	**Standard Error**	**p**
Body Mass (kg)	0.62	0.11	<0.001
Spasticity (/7)	2.07	1.31	0.125
Vigorous Physical Activity (min/day)	0.06	0.06	0.264
R-Square for model=0.57, p<0.001			
c. Model 3: Motor-Incomplete SCI, n=25			
Variable	**Parameter Estimate**	**Standard Error**	**p**
Body Mass (kg)	0.72	0.13	<0.001
Wheelchair Use	-16.45	4.58	0.002
R-Square for model=0.69 p<0.001			
Note: cLEMS: calf-muscle lower-extremity motor score			

Table 3. Best-fit multivariate regression models for muscle area.

p<0.01). Notably, standard deviations in muscle area and density appeared to be greater in those with both complete and incomplete SCI compared to controls, indicating variability in the degree of muscle atrophy and fat infiltration after SCI.

Determinants of muscle area

The best-fit multivariate models were able to explain 57-70% of the variation in muscle area. Having a lower body mass, being paraplegic, having a lower cLEMS, experiencing a lower frequency and severity of muscle spasms, participating in fewer minutes of vigorous physical activity, and using a wheelchair for ambulation were associated with having a smaller muscle area (Table 3). Muscle area was strongly linked to body mass after complete and incomplete SCI. Wheelchair use had the greatest relative contribution to muscle area models, such that using a wheelchair for ambulation was associated with an estimated 12-17 cm^2 smaller muscle area. Paraplegia, spasm frequency and severity, and vigorous physical activity level improved the fit of the regression models; however, these variables were not statistically significant (p>0.05) suggesting a marginal impact on muscle area.

Determinants of muscle density

The best-fit multivariate models were able to explain 34-41% of the variation in muscle density. It was observed that having a lower cLEMS, experiencing a lower frequency and severity of lower-extremity muscle spasms, performing fewer minutes of daily vigorous physical activity, having an older age, using a wheelchair for ambulation, and having a larger waist circumference were associated with having a lower muscle density (Table 4). Having a waist circumference ≥94 cm and using a wheelchair for ambulation were both associated

a. Model 1: Full sample, n=54			
Variable	**Parameter Estimate**	**Standard Error**	**p**
cLEMS (/15)	0.84	0.45	0.068
Spasticity (/7)	2.28	0.90	0.015
Vigorous Physical Activity (min/day)	0.09	0.04	0.055
Wheelchair Use	-9.62	5.56	0.090
Waist Circumference (≥94cm)	-8.21	3.22	0.014
R-Square for model=0.41, p=<0.001			
b. Model 2: Motor-Complete SCI, n=36			
Variable	**Parameter Estimate**	**Standard Error**	**p**
Age (years)	-0.36	0.21	0.095
Waist Circumference (≥94cm)	-9.73	4.01	0.021
Spasticity (/7)	2.74	1.49	0.075
R-Square for model=0.36, p=0.001			
c. Model 3: Motor-Incomplete SCI, n=25			
Variable	**Parameter Estimate**	**Standard Error**	**p**
Age (years)	-0.27	0.13	0.053
Wheelchair Use	-8.84	3.16	0.011
R-Square for model=0.34 p=0.009			
Note: cLEMS: calf-muscle lower-extremity motor score			

Table 4. Best-fit multivariate regression models for muscle density.

with an estimated 8-10 mg/cm^3 lower muscle density in the best-fit models. Motor-score, vigorous physical activity level, and age improved the fit of the regression models; however, these variables did not reach statistical significance ($p>0.05$) which suggests a lesser impact on muscle density.

Discussion

This study highlights the degree of muscle atrophy and fatty-infiltration of skeletal muscle in a cohort of individuals with chronic SCI and diverse impairment. Participants with complete SCI had 43% and 32% lower muscle area and density values relative to their controls, respectively. For those with incomplete SCI, muscle area and density values were 14% lower relative to controls. Additionally, the variation in muscle area and density differences between SCI cases and controls indicated that not all individuals experience the same degree of lower-extremity muscle atrophy and fatty-infiltration after chronic SCI. Many of the correlates of muscle area or density were those that were related to muscle function and activity level, suggesting that maintaining or increasing muscle activity may prevent adverse changes in muscle area and density over time after SCI.

Previously, our understanding of the true magnitude and variability in atrophy and fatty-infiltration of muscle has been limited to studies with small, homogeneous samples. After chronic motor-complete SCI, muscle area values relative to controls were comparable to those reported in the acute stage of injury using magnetic resonance imaging (MRI), where decreases in gastrocnemius and soleus muscle areas were 54% and 68% of those of controls[19]. On average, muscle areas among individuals with incomplete SCI were 14% lower than controls, a difference that is smaller than was previously reported[7,20]. It is possible that this difference is attributable to functional improvements in the chronic stage of injury or to methodological differences between the two technologies (pQCT vs. MRI). However, it is also plausible that the observed disparity is the result of the heterogeneity in motor function among the incomplete SCI population. If the latter is true, our results highlight the importance of understanding the factors related to the degree of atrophy and fatty-infiltration in the incomplete SCI population.

Multiple regression models were able to explain between 57-70% of the variation in muscle area, and 34-41% of the variation in muscle density using 16 variables selected *a priori*. We observed that body mass, level of injury, motor-score, spasticity, and wheelchair use were associated with muscle area, and motor-score, spasticity, vigorous physical activity level, wheelchair use, age, and waist circumference were associated with muscle density in best-fit models. Our models of muscle size and quality after SCI suggest that muscle status is largely driven by muscle function or activity, and body size and composition; variables that may represent therapeutic intervention targets. For example, one could theoretically aim to preserve or increase muscle activity and maintain a healthy body composition in the sub-acute stages of injury to avoid metabolic implications in the chronic stage of SCI.

Few studies have explored factors related to muscle atrophy and fatty-infiltration that may inform preventative or treatment strategies for metabolic diseases. Using dual-energy X-ray absorptiometry, Spungen et al. reported that duration or level of injury, advancing age, and completeness of injury are related to percent fat-free mass in a large sample of males with complete and incomplete chronic SCI[34]. Additionally, spasticity[15,36,40] and wheelchair use[20,41] have been linked to improved muscle size in small scale investigations. Previous studies have been limited by the number of correlates examined and the impairment and demographic diversity of study samples, and consequently have been restricted in their ability to comprehensively examine multi-factorial relationships regulating muscle status. The current study incorporates an extensive list of potential correlates related to muscle including both inherent and modifiable factors in a sample of individuals with diverse SCI impairment.

Muscle size is a large component of total body mass, and therefore it is not surprising that body mass was strongly associated with muscle area. Notably, body mass and muscle area were associated in those with both complete and incomplete SCI, suggesting that body mass is the main determinant of calf-muscle area despite chronic lower-extremity paralysis or paresis.

Measures of muscle function (cLEMS) and wheelchair use were associated with both muscle area and density for all SCI cases, and in subgroup analyses. Previously, intermittent bouts of ambulation have shown to improve muscle size in those with incomplete SCI[42,43] whereas bouts of passive standing with vibration did not[44]. Lower muscle area values have been observed in both wheelchair dependant and ambulatory individuals relative to matched able-bodied controls approximately one-year after incomplete SCI[20]. However, wheelchair users exhibited significantly greater plantar flexor muscle atrophy compared with the dorsiflexors, and a greater degree of atrophy in the medial gastrocnemius muscle compared to non-wheelchair users[20]. Parameter estimates from the current study indicate that wheelchair use had the greatest impact on muscle area and density, suggesting that the maintenance of muscle function, particularly through upright ambulation in those individuals with incomplete SCI who have residual motor function, may contribute to the prevention of muscle loss, and indirectly prevent secondary metabolic complications.

We observed that muscle activation in the form of physical activity and involuntary spasticity was associated with improved muscle size and quality. Exercise has shown to increase insulin sensitivity and attenuate inflammatory responses that may be implicated in increased skeletal muscle lipid deposition[45-48], and spasticity scores have been reported to be associated with a larger upper and lower leg muscle area after chronic SCI[15,40]. Furthermore, spasticity has been indirectly linked to the prevention of intramuscular adipose tissue accumulation[40]. Externally eliciting muscle activity though electrical stimulation therapy has been successful in increasing lower-extremity muscle size and decreasing fat infiltration after chronic complete SCI[18]. Therefore, therapeutic strategies that promote muscle activity through physical activity participation that are supplemented with electrical stimulation therapy for those without voluntary muscle contraction may improve muscle health after chronic SCI.

Waist circumference has been used widely as a surrogate marker of obesity and has been shown to be a strong predictor of total body fat and visceral adipose tissue in the able-bodied population[49-51]. After SCI, waist circumference has been suggested as a favourable bed-side assessment tool for ectopic adiposity and cardiovascular disease risk[52-54]. Recently, a waist circumference of ≥94 cm has been reported as an SCI-specific threshold to best predict a positive outcome of >10% risk on the Framingham 30-year risk score[33]. Parameter estimates from the current study indicate that having a waist circumference ≥94 cm is related to having a 8.2 mg/cm^3 lower calf-muscle density; a clinically important change as a difference in calf-muscle density of only 5 mg/cm^3 has been observed between those with and without diabetes[55]. Waist circumference could be used as an accessible tool to assess metabolic health after chronic SCI; however, further research incorporating additional markers of metabolic function are needed to verify its predictive validity.

It is unclear why paraplegia was associated with a lower muscle area. This was unexpected as higher level injuries are related to impaired mobility, body composition, and autonomic function[34,56,57]. While our sample had an even distribution of individuals with complete and incomplete tetraplegia and paraplegia, those with tetraplegia tended to have a higher cLEMS compared to those with paraplegia (mean ± SD: 3.4±5.1 *versus* 1.7±3.9). However, cLEMS was controlled for in the regression model suggesting that there may be other mechanisms underlying the observed association. The study results suggest that having paraplegia *versus* tetraplegia is an indicator of having a smaller lower-extremity muscle size; however, additional work with a larger sample is needed to verify this finding.

Limitations

Due to resolution limitations of pQCT, muscle area was defined as the soft-tissue area contained within the facia border of the calf-muscle group, and therefore includes the area of adipose and other non-contractile tissues. Studies using MRI technology have demonstrated that not accounting for inter- and intramuscular adipose stores may overestimate thigh-muscle area values by up to six percent[7]. Given the degree of atrophy observed after SCI, it is likely that this amount of overestimation would have a limited impact on the study implications. Secondly, individuals with SCI often experience lower-leg edema and venous pooling, and it is unclear what effect fluid shifts have on pQCT-derived muscle area and density values. It is possible that an increased fluid content in the lower-leg could result in an overestimation of muscle area and density[58]. Thirdly, there were potential determinants of muscle status that we did not account for such as periods of immobilization or disuse (e.g., due to casting or prolonged bed-rest), nutritional intake, or presence of chronic inflammation. Lastly, the greatest diagnostic yield from muscle density measurement

may be in highlighting risk-factors related to dyslipidemia, glucose intolerance, and diabetes. The most clinically relevant limitation of this study was that we were unable to report the direct association between muscle density and glucose tolerance or dyslipidemia after chronic SCI. Although previous observational studies have examined the relationship between fatty-infiltration, pQCT-derived muscle density, and metabolic disease in other populations[55,59,60], the direct relationship between muscle density and glucose tolerance has not previously been reported in the SCI-community.

Summary

Individuals with chronic complete and incomplete SCI display reductions in lower-extremity calf muscle area and density relative to their able-bodied peers; however, lower-extremity muscle atrophy and fatty-infiltration are quite variable across individuals with chronic-SCI, and occur to a much greater extent in individuals with motor-complete injury than motor-incomplete injury. Regression models identified body mass, motor-score, wheelchair use, spasticity, vigorous physical activity participation, age, and waist circumference as potential clinical predictors of muscle status. Our research offers insight into SCI-specific metabolic disease risk profiles to aid in the treatment and detection of metabolic disease, and the modifiable correlates identified may offer targets for future therapeutic interventions.

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