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ORIGINAL ARTICLE

Impact on bone and muscle area after spinal cord injury

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Spinal cord injury (SCI) causes inactivation and consequent unloading of affected skeletal muscle and bone. This cross-sectional study investigated correlations of muscle and bone in spinal cord-injured subjects compared with able-bodied subjects. Thirty-one complete SCI paraplegics were divided according to the neurological level of injury (NLoI) into group A ($n = 16$, above thoracic 7 NLoI, age: 33 ± 16 years, duration of paralysis (DoP): 6 ± 6 years) and group B ($n = 15$, thoracic 8–12, age: 39 ± 14 years, DoP: 5.6 ± 6 years), compared with 33 controls (group C). All were examined with peripheral quantitative computed tomography at 66% of tibia length (bone and muscle area, bone/muscle area ratio). In able-bodied subjects, muscle area was correlated with bone area ($P < 0.001$, $r = 0.88$). Groups A and B differed significantly from the control group in terms of bone and muscle area ($P < 0.001$). In paraplegics, less muscle per unit of bone area (bone/muscle area ratio) was found compared with controls ($P < 0.001$). Bone area was negatively correlated with the DoP in the total paraplegic group ($r = -0.66$, $P < 0.001$) and groups A and B ($r = -0.77$, $P = 0.001$ vs $r = -0.52$, $P = 0.12$, respectively). Muscle area and bone/muscle ratio area correlations in paraplegic groups with DoP were weak. Paraplegic subjects who performed standing and therapeutic walking had significantly higher bone area ($P = 0.02$ and $P = 0.013$, respectively). The relationship between bone and muscle was consistent in able-bodied subjects and it was predictably altered in those with SCI, a clinical disease affecting bone and muscle.

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Introduction

Spinal cord injury (SCI) is associated with the development of rapid and severe bone and muscle impairment,[1–3] which is not only owing to a compromised biomechanical function but also has a central nervous system origin.[4,5] Moreover, it has been shown in numerous publications that the duration of paralysis (DoP) is actually related to the amount of bone and muscle loss in SCI.[6,7] So far, most published studies on muscle and bone loss in spinal cord-injured subjects have used dual-energy X-ray absorptiometry (DXA).[8,9] Few authors have published data for the study of long bones with peripheral quantitative computed tomography (pQCT) in SCI patients.[7,10–14] Peripheral quantitative computed tomography allows measurements of true volumetric densities with minimum exposure to X-rays and geometrical properties of bone, such as bone areas and cortical thickness. It also provides measurements of muscle area at selected sites, thereby enabling the calculation of bone area/muscle area ratio of long bones non-invasively.[15] Muscle and bone form a functional unit: if sufficient force (load) is applied to bone, a certain threshold strain in the bone is reached and it is synthesised; if muscle force is below a certain set point, for instance, muscles are immobilised or paralysed, bone tissue is lost.[16] Maximum muscle contractions impose the greatest load on bones, which leads the bone to change its geometry and its resistance through both modelling and remodelling mechanisms, and thus a linear relationship has been proposed between the cross-sectional area (CSA) of muscles and bone mineral content (BMC) in healthy individuals measured by pQCT.[17] Muscle CSA measured either by pQCT or magnetic resonance imaging has been proposed as a surrogate for muscle effectiveness or loading (force) rather than actual force in young and healthy individuals. With pQCT, it is obtained with a single slice at 66% of bone length (radius or tibia), where it is considered to be maximal. In subjects with SCI, it can be obtained as in able-bodied subjects and might be advantageous, because muscle CSA can be measured more precisely and it does not depend on motor function of the lower limbs, which is impaired in SCI.[17] With pQCT, positive relationships between

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muscles and bone geometry/density in healthy individuals and children with disabilities have been published.[18,19] With regard to cortical geometric properties, our previous publications revealed a significant increase of endosteal circumference in paraplegics, whereas periosteal circumference is comparable to that of healthy individuals. That increase leads to a significant reduction of cortical thickness in paraplegics.[10]

Moreover, the importance of the neurological level of injury (NLoI) and the influence of the DoP among spinal cord-injured paraplegic patients in areas of muscle and bone have not been adequately investigated.[20] Most studies were conducted with a small number of patients and mixed populations of paraplegics and tetraplegics.[21,22] All paraplegics with an injury in the thoracic region of the spinal cord are paralysed in the lower body or legs. Upper body strength depends on the NLoI; the lower the level, the stronger the upper body strength. However, the sympathetic nervous system may be compromised, especially in high-level injuries. There is evidence of the participation of the nervous system in skeletal development and bone turnover, and of a compromised muscle–bone function, which is not only biomechanical but also biochemical and neurogenic.[23,24] The importance of the NLoI among paraplegic patients is not fully explained, and there are no studies after separation of paraplegics according to the NLoI. The aim of this study was to analyse muscle and bone interactions in lower limbs of spinal cord-injured subjects with high and low NLoI, and investigate differences between them and in comparison with controls.

Results

Total paraplegic group

BMI values for our paraplegic population were significantly lower than for controls (23.9 ± 3 vs 26.12 ± 5, $P = 0.02$, respectively) (**Table 1**). In control subjects, muscle area was strongly correlated with bone area obtained from pQCT ($P < 0.001$, $r = 0.88$). The total paraplegic group had significantly less (cortical) bone area and muscle area compared with controls ($P < 0.01$ and $P < 0.001$, respectively; data not shown). Bone area was negatively strongly correlated with the DoP in the total paraplegic group ($r = -0.66$, $P < 0.001$) (**Figure 1**). Muscle area with the DoP and bone/muscle area ratio with the DoP correlations of the total paraplegic group were weak ($r = -0.12$, $P = 0.6$ and $r = 0.26$, $P = 0.2$, respectively) (**Table 2**). Paraplegics who used standing frames or long brace orthoses for standing or therapeutic walking independently of the NLoI had statistically significant higher bone area (mean 373.4 ± 68 vs 313.77 ± 65, $P = 0.02$ and mean 377.9 ± 59 vs 314 ± 69, $P = 0.013$, for standing and walking, respectively), and non-significant changes were found in muscle area values between paraplegics who performed standing or walking and wheelchair-bound paraplegics (**Table 3**).

High vs low paraplegic groups

Paraplegic groups A (high) and B (low) differed significantly from the control group (C) in muscle and bone area ($P = 0.001$ and $P = 0.01$, respectively). No significant differences were found between the two paraplegic groups. In paraplegics, bone/muscle area ratio was found to be higher (less muscle per unit of bone area) than in controls (mean ratio: 7 ± 2.15 high vs 6.25 ± 2 low vs 5.75 ± 0.51 controls, $P = 0.01$) (**Table 4**). On the other hand, muscle to bone area correlation in paraplegic groups was moderate to weak ($P = 0.245$, $r = 0.38$ and $P = 0.56$, $r = 0.176$ in high and low paraplegics, respectively). Bone area was negatively strongly correlated with the DoP in high paraplegics vs low paraplegics ($r = -0.77$, $P = 0.001$ vs $r = -0.52$, $P = 0.12$, respectively). In paraplegic groups, correlations of muscle area with DoP and bone/muscle area ratio with DoP were weak ($r = -0.29$, $P = 0.3$ vs $r = 0.08$, $P = 0.8$ and $r = 0.12$, $P = 0.67$ vs $r = 0.32$, $P = 0.36$ in high vs low paraplegics, respectively) (**Table 2**).

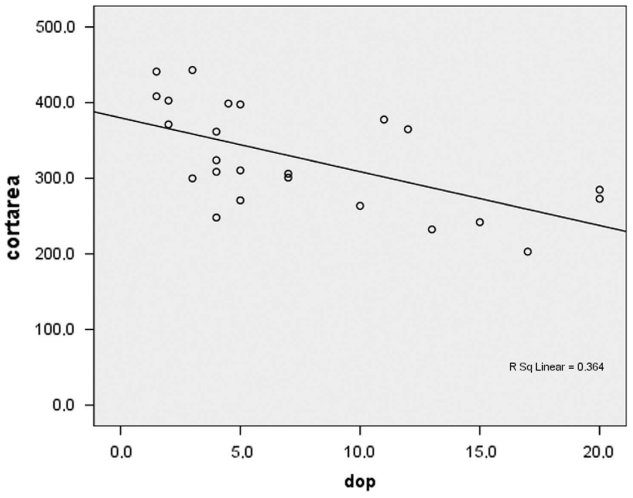

Figure 1 Schematic presentation of the correlation between bone area and DoP. A linear correlation between bone area (cortarea) and duration of paralysis (DoP) in 66% in the total paraplegic group was found to fit our data. Bone area was negatively correlated with the DoP in the total paraplegic group ($r^2 = 0.364$, $r = -0.66$, $P < 0.001$, bone area $= 379.9 - 7.12 \times$ DoP).

Table 1 Mean values, s.d. and statistical significances (*P*-value) of anthropometric data of all groups, and clinical parameters of both paraplegic groups

Demographics and clinical parameters of subjects	*Control group* n = *33* *mean* ± *s.d.*	*Group A* n = *16* *mean* ± *s.d.*	*Group B* n = *15* *mean* ± *s.d.*	*ANOVA* P-*value*
Age (years)	36.87 ± 18.9	32.88 ± 15.6	39.47 ± 13.81	0.370
Weight (kg)	81.36 ± 13	76.67 ± 17.12	76.67 ± 17.12	0.085
Height (m)	1.76 ± 0.05	1.77 ± 0.06	1.75 ± 0.10	0.676
BMI (kg m^{-2})	26.12 ± 5	22.94 ± 2.21	24.86 ± 3.50	0.02
Duration of paralysis (years)	—	6 ± 6	5.65 ± 5.8	0.87

Abbreviations: ANOVA, analysis of variance; BMI, body mass index. *P*-value < 0.05; group A: high paraplegia, group B: low paraplegia.

Table 2 Statistical significances and correlations of duration of paralysis and bone area, muscle area and bone/muscle area ratio in paraplegic groups

Duration of paralysis	*Group A*	*Group B*	*All paraplegics*
Bone area (mm^2)			
r	−0.768	−0.52	−0.66
P	0.001	0.12	0.001
Muscle area (mm^2)			
r	−0.288	0.08	−0.12
P	0.3	0.8	0.6
Bone area/muscle area %			
r	0.12	0.32	0.264
P	0.67	0.36	0.2

Correlations and statistical significances of measured variables with duration of paralysis. Statistical significances (*P*-value), *P*-value <0.05; Spearman's *r*: >0.6 high correlation; $0.3<r<0.6$ medium correlation; $r<0.3$ low correlation. Group A: high paraplegia (neurological level of injury (NLoI) > thoracic(T_7); group B: low paraplegia (NLoI: $T_7<$NLoI$<T_{12}$). All paraplegics: total paraplegic group including groups A and B.

Table 3 Mean values, s.d. and statistical significances (*P*-value) of bone-muscle variables of all paraplegic subjects, who performed standing or walking, independent of the neurological level of injury

Standing or *walking SCI subjects*	n	*Mean ± s.d.*	P-*value*
Standing			
Bone area (mm^2)			
No	18	313.77 ± 65	0.020
Yes	13	373.41 ± 68	
Muscle area (mm^2)			
No	19	5420.22 ± 1757	NS
Yes	17	4955.91 ± 1538	
Walking			
Bone area (mm^2)			
No	19	314.07 ± 69	0.013
Yes	12	377.90 ± 58	
Muscle area (mm^2)			
No	22	5268.66 ± 1726	NS
Yes	14	5094.59 ± 1583	

Abbreviation: SCI, spinal cord injury. Values of measured parameters in SCI subjects who performed walking or standing. *P*-value <0.05.

Discussion

The high correlation between the CSAs of bone and muscle supports the hypothesis that muscle loading is a leading variable determining bone strength in able-bodied subjects. Conversely, the relationship between bone and muscle was altered in those with SCI, a clinical disease affecting bone and muscle, compared with controls.

BMI ($kg\,m^{-2}$) values in paraplegics and controls were below values that signify obesity (BMI >27.8).[25] However, in our study population, we found lower values of BMI in paraplegics than in controls. According to the literature, anthropometric measures in SCI tend to underestimate fat percentage when compared with able-bodied individuals.[3] BMI is used as a measure of adiposity, but it does not distinguish between components of weight. An explanation of the lower values of BMI in our population could be the incidence of malnutrition in this population. Hypermetabolism, catabolism and accelerated nitrogen loss are well-recognised complications that occur after traumatic SCI, but this was not the case in our study because all paraplegics were in the chronic stage after SCI. However, it is open to question whether the cutoff points for underweight, normal, overweight and obese patients used in able-bodied populations can be applied to SCI subjects; more studies are needed to define cutoff points of obesity in SCI subjects and to analyse the impact of injury type and duration of injury on the extent of obesity. Anthropometric measures have been replaced by more sophisticated body composition technologies—that is, DXA—for a more precise quantification of fat.[26]

Table 4 Mean values, s.d. and statistical significances (*P*-value) of bone and muscle-measured variables between control and paraplegic groups

Bone and muscle-measured variables	n	*Mean ± s.d.*	P-*value*
Bone area (mm^2)			
Group A	16	316.44 ± 82	0.01
Group B	15	334.74 ± 56	
Control	33	370.48 ± 76	
Muscle area (mm^2)			
Group A	16	4835.53 ± 1573	0.001**
Group B	15	5743.43 ± 1638	
Control	33	6451.77 ± 1234	
Bone/muscle ratio (%)			
Group A	16	7.00 ± 2.15	0.01
Group B	15	6.25 ± 2	
Control	33	5.75 ± 0.5	

Abbreviation: NLoI, neurological level of injury. Bonferroni tests for the control group vs NLoI >7 (high paraplegic) group A, *P*-value <0.05, and control group vs $T_7<$NLoI$<T_{12}$ (low paraplegic) group B, **P-value <0.005. Group A: high paraplegia, group B: low paraplegia.

The mechanisms that underlie bone loss after SCI remain poorly elucidated and controversial. Disuse may have an important role, but factors that are independent of mechanical loading of the skeleton also appear to be important. Possible influential non-mechanical factors may include poor nutritional status, disordered vasoregulation, hypercortisolism (either therapeutic or stress related), alterations in gonadal function, endocrine disorders and neural factors.[27] According to Spungen *et al.*,[6] the predominant finding of SCI on bone is a large loss of bone during the first year of injury because of disuse osteoporosis. Biering-Sörensen *et al.*[28] demonstrated ongoing demineralisation 3 years after tibia trauma. According to Lazo *et al.*,[29] bone is progressively lost in SCI over a period of 12–16 months before stabilising. Bauman *et al.*[30] reported longer DoP-related loss of bone in the legs of monozygotic twins with chronic paraplegia in comparison with their able-bodied co-twins. The results in this pQCT study suggest a reduction of the tibia's (cortical) bone area in high and low paraplegics independently of the NLoI (27 vs 24%, in high vs low paraplegics, respectively) compared with controls during a mean DoP of ~5.5 years (6 ± 6 vs 5.6 ± 6, in high vs low paraplegics, respectively).

It can be assumed that it was probably the outcome of higher frequency of standing and increased mobilisation in the group with low paraplegia and the possible use of gait orthoses and standing frames that decelerated bone loss based on mechanostat theory through loading. This correlation holds also for pQCT for the CSA of diaphysis in this study. Bone (cortical) CSA was negatively strongly correlated with the DoP in the total

paraplegic group, a correlation that was stronger in high paraplegics. However, the application of sophisticated pQCT technology also highlighted the correlation in the low paraplegic group. This result neatly explains the 'paraplegic mechanostat theory', which is based on Frost's mechanostat theory:[16] despite the higher frequency of standing and increased mobilisation in the group of low paraplegics, the threshold or set point above and below which bone is, respectively, accrued or lost is not known and is regulated by various factors. The mechanostat theory describes a system in which a minimum effective strain, which is the lowest strain in the remodelling phase under which bone resorption exceeds formation, is essential for maintaining bone. If mechanical strains remain within a normal physiologic window (800–1500 μ strain), bone structure is maintained (remodelling). Unloading (disuse) reduces mechanical strains leading to increased remodelling in favour of bone resorption. In the overload zone (1500–3000 μ strain), new bone is added in response to mechanical requirement (modelling), leading to increased bone strength. In the pathological overload zone (>15 000 μ strain), bone is fractured. On the other hand, strains above the upper threshold of the remodelling window will increase formation over resorption.[16,31] Nevertheless, this explanation is quite simple considering the complex pathophysiology with regard to hormonal influences, the injury and the neurogenic factor in body composition of paraplegics.[3] All paraplegics were in the chronic stage, which suggests that the neurogenic factor coexists as an influential regulator.[10] After SCI, sympathetic activity is nearly or completely absent.[32] In a complete high-level SCI, changes in the autonomic nervous system are assumed to cause attrition of SCI bone, via changes in vascular tone and flow. With respect to the NLoI, Group A patients are susceptible to autonomic dysreflexia as a result of the damage to the nucleus of the sympathetic system. Sympathetic denervation in SCI would be expected to protect against bone loss; however, a reduction in sympathetic nervous system (SNS) activity does not explain the rapid bone loss that develops after SCI.[33]

Our results are in line with those of others who reported decline at the 86% of tibia diaphysis cortical CSA eventually reaching 65% of the non-SCI value and that tibia diaphyseal CSA does indeed reach a steady-state value in chronic SCI.[34] In another pQCT study, however, 21 individuals with chronic SCI (>7.6 years) who were followed up longitudinally over 30 months showed no significant cortical CSA decline at 38% of tibia.[11]

Disuse was thought to be the mechanism responsible for the skeletal muscle atrophy in paraplegics.[35] Actually, little is known regarding the nature and time frame of the influence of complete SCI on human skeletal muscle. Data exist from studies where different groups of a few subjects have been examined at different times in the early phase of paralysis. Muscles are significantly altered during the first 6 months after injury, according to studies conducted with paraplegics who noticed remarkable muscle atrophy between the first and the third month after injury, without further loss until the following 6 months.[36–38] Wilmet *et al.* found that the total muscle mass decreases by about 9.5% within 6 months, and the muscle mass of the lower limbs is reduced by 15.1% a year after the injury. In their longitudinal 1-year study, muscle mass was dramatically diminished during the first months after the injury in the lower limbs (in 15 weeks).[39] Using dual X-ray absorptiometry in a prospective study including six paraplegic legs of young men, the muscle mass decreased by 10.7%, whereas it increased significantly by 19.6% in the arms during the same period of time.[40] These results are different from our results as we found an increased reduction of muscle area compared with previous studies because of the higher duration of paralysis in our sample. According to our results, muscle area in 66% of the tibia's length was 39% and 34% lower in low and high paraplegics, respectively, versus controls within 5 years from injury. In a monozygotic twin study, where one brother had complete paraplegia and his twin brother was normal, the higher the duration of paralysis, the greater was the decrease in muscle mass.[41] However, all the studies mentioned above used DXA technology, mixed acute and subacute populations of paraplegics and tetraplegics or mixed paraplegic populations with spastic and flaccid paralysis in contrast to our study, which included only chronic paraplegic men.

In our chronic paraplegic population, SCI resulted in 39 and 34% reduction in muscle area in high and low paraplegics, respectively, compared with controls within 5 years from injury. A similar size reduction effect resulted in tibia's cortical bone area (27 and 24% lower, in high and low paraplegics, respectively), although the percentage difference was ~1.5 times higher in favour of muscle area. The more substantial difference in tibia muscle than bone suggests that SCI groups lost more muscle than bone after injury. This idea is further supported by the significantly higher ratio of (cortical) bone area to muscle area in both SCI groups compared with controls (**Table 2**). This discrepancy in the muscle–bone relationship in the two paraplegic groups is reflected by a very weak correlation of the tibia's cortical bone CSA and muscle area in 66% of the tibia's length. A major reason for loss of bone after SCI is the loss of voluntary muscle action on bone. A study of monozygotic twins, in which the relationship between lower extremity fat-free soft tissue mass and BMC was very weak in twins with SCI, suggests that the connection between muscle and bone in the lower extremities is lost when the muscle action associated with ambulation is gone.[41] This also explains our results for the weak correlation between muscle area and bone area. Loading is associated with ambulation, and normal physical function is critical to maintain bone and muscle integrity. Moreover, it is known that dynamic mechanical stimulation is more efficacious for bone formation, as under static loads bone cells become less responsive to stimuli. Most paraplegic subjects lose motivation during ageing in paralysis and do not among other things perform standing or walking, losing the indirect effects of loading in the legs.[10]

Sophisticated rehabilitation therapies were offered in hospitalised patients only. All subjects followed standard rehabilitation protocols for SCI after acute immobilisation including standing, walking with orthoses and regular use of standing frames in the hospital. Standing was continued after discharge from the hospital in all paraplegics, but only 40% of the subjects continued walking, mostly because of the unwillingness of the patient, rather than because of the functional level. Most of the patients followed therapy programmes (that is, physical therapy) in private at home or in physical therapy units, and quite a few in organised private or government rehabilitation facilities. Although the effect of passive loading, standing and therapeutic

ambulation in paraplegics is controversial, even during sitting in the wheelchair paraplegic limbs are exposed to some loading (forces). Immobility leads to a changing pattern of loading in the paralysed areas, which respond by alteration in skeletal structure,[10] and benefits in terms of bone mass from passive mechanical loading have been shown at the femoral shaft, but not at the hip joint.[42]

In the present study, the NLoI between paraplegic groups A and B was thoracic T_4 to T_7 vs T_8 to T_{12} (AIS-A), making the groups comparable in their physical abilities. Factors that might influence functional stability (associated medical complications, amount and nature of rehabilitation and individual factors such as age, activity level and so on) were controlled by the inclusion criteria. However, the potential to ambulate with KAFOs and elbow crutches is higher in subjects below T_7. A percentage of subjects (50%) in the high paraplegic group were not able to walk with KAFOs or with other walking devices because of their total inability to use trunk muscle, and they only performed therapeutic standing in frames. Paraplegics who used standing frames or long brace orthoses had statistically significant higher bone area (but not muscle area) independently of the functional level, meaning that even passive standing or therapeutic ambulation possibly has a positive effect on bone in chronic paraplegics. On the other hand, Eser *et al.*[43] found no correlation for passive standing training with bone status. Our failure to highlight a significant improvement in muscle area could lie in the small sample but mainly in our analysis, because we analysed these data independently of the NLoI (because of the small number of subjects).

In another study, mid-thigh muscle volume was moderately to strongly correlated with mid-femur cortical bone volume in men with SCI, and this relationship was linked with involuntary muscle spasms.[44] However, it has been reported that spasticity may be protective against bone loss in SCI patients, albeit without any preserving effect on the tibia.[13] In a longitudinal 1-year study, it was formulated that lean mass is better preserved in patients who develop spasticity. Other authors reached the same conclusion.[45] The latter study included patients with incomplete SCI. This conclusion, however, has been questioned by other researchers and has not been verified by studies involving patients with complete paraplegia.[7,13] Spasticity does not constitute a maintaining factor of the muscle system, so that either the myopathetic muscle does not recognise stimuli because of its degeneration or it recognises them wrongly.[46]

In the present study, all paraplegics were above the T_{12} level with various degrees of spasticity according to the Ashworth scale, which were not correlated with the examined parameters (data not shown). This result could be explained by the bone steady state. After a period of 16–24 months during injury, the bone metabolic process tends towards a new steady bone state, but bone mineral density at different regions continues to decrease and is inversely associated with the time of injury, which means continuous bone loss beyond the first 2 years after injury, reaching a new steady state at 4 (femur) to 7 (tibia) years.[10,13,28,47] In this study, paraplegics' muscles with a DoP of more than 5.5 years (see study sample) were already in a steady state according to the literature.[3] According to the aforementioned studies, this explains why bone loss in our sample was an ongoing biological phenomenon during the 5 years of paralysis required to reach the new steady state according to the paraplegic mechanostat when bone impairment was complete (that is, meaning also geometrical property alterations and not only volumetric bone mineral density).

This conclusion could be helpful in clinical practice, because it opens up a new perspective on how to manage bone and muscle loss in these subjects. We could interfere in the mechanostat process either on bone (mostly giving drugs) or on muscles through exercise protocols or various physical and mechanical means (that is, functional electrostimulation, vibration platforms and so on). The most important thing is to understand the optimal timing of this intervention. Because of the higher bone area/muscle area ratio in paraplegic groups compared with controls, the intervention should be started early to protect muscle loss, which tends to start sooner. By strengthening muscle (and bone) even in chronic paraplegics, we could interfere in the relationship, taking the bone area/muscle area ratio value closer to its normal value and reducing the fractures that are a life-threatening condition in this population. We need to declare some difficulties and limitations of this study. We recognise the possibility of increased mobilisation in some high paraplegics of our study population with walking orthoses or the possibility that low paraplegics will act like high paraplegics after the first years of paralysis, losing the effects of loading the skeleton.[10]

Conclusion

Current studies should focus on the intensification of muscle formation and maintenance in chronic SCI, because maintenance of muscles and muscle-deriving loading (force) are important for fracture prevention in this population. Muscles and bones act as a unit and are related tissues. Strategies that help paraplegics to bear weight, to stand or walk therapeutically should be added early on in the rehabilitation programme to gain benefits according to the muscles and bones.

Materials and Methods

Demographics

Sixty-four men were included in this study. Thirty-one had a complete (absence of sensory or motor function below the neurological level, including the lowest sacral segment) SCI according to the American Spinal Injury Association (ASIA) impairment scale (AIS-A).[48] All paraplegics (mean age 39.23 ± 15.76 years) were in chronic stage and at least 1.5 years post injury. As a chronic stage, we considered the neurological stabilisation and the absence of spinal shock. All were above thoracic (T) 12 NLoI. The total paraplegic group included subjects with an injury below T_4 and above T_{12} NLoI. We also separated paraplegic men into groups above and below T_7 NLoI. Group A included 16 ($n = 16$) high-SCI subjects T_4–T_7 NLoI, with a mean age of 32.88 ± 15.6 years and DoP of 6 ± 6 years, and group B included 15 ($n = 15$) low-SCI subjects T_8–T_{12} NLoI, with a mean age of 39.47 ± 13.81 years and DoP of 5.6 ± 6 years, in comparison with 33 ($n = 33$) healthy men as a control group. In **Table 1**, the anthropometric data and the clinical parameters of the study population are presented in detail.

Methods

SCI paraplegics were volunteers recruited from the Second Rehabilitation Department of the National Rehabilitation Centre 'EKA' in Athens (outpatients) and from the Greek Paraplegic Society after being invited to participate in clinical research undertaken by the Laboratory for Research of the Musculoskeletal System of Athens University. The control group also consisted of volunteers working in the laboratory and the hospital. The height of paraplegics was measured in supine position before the examination. The controls' height was measured with a wall-

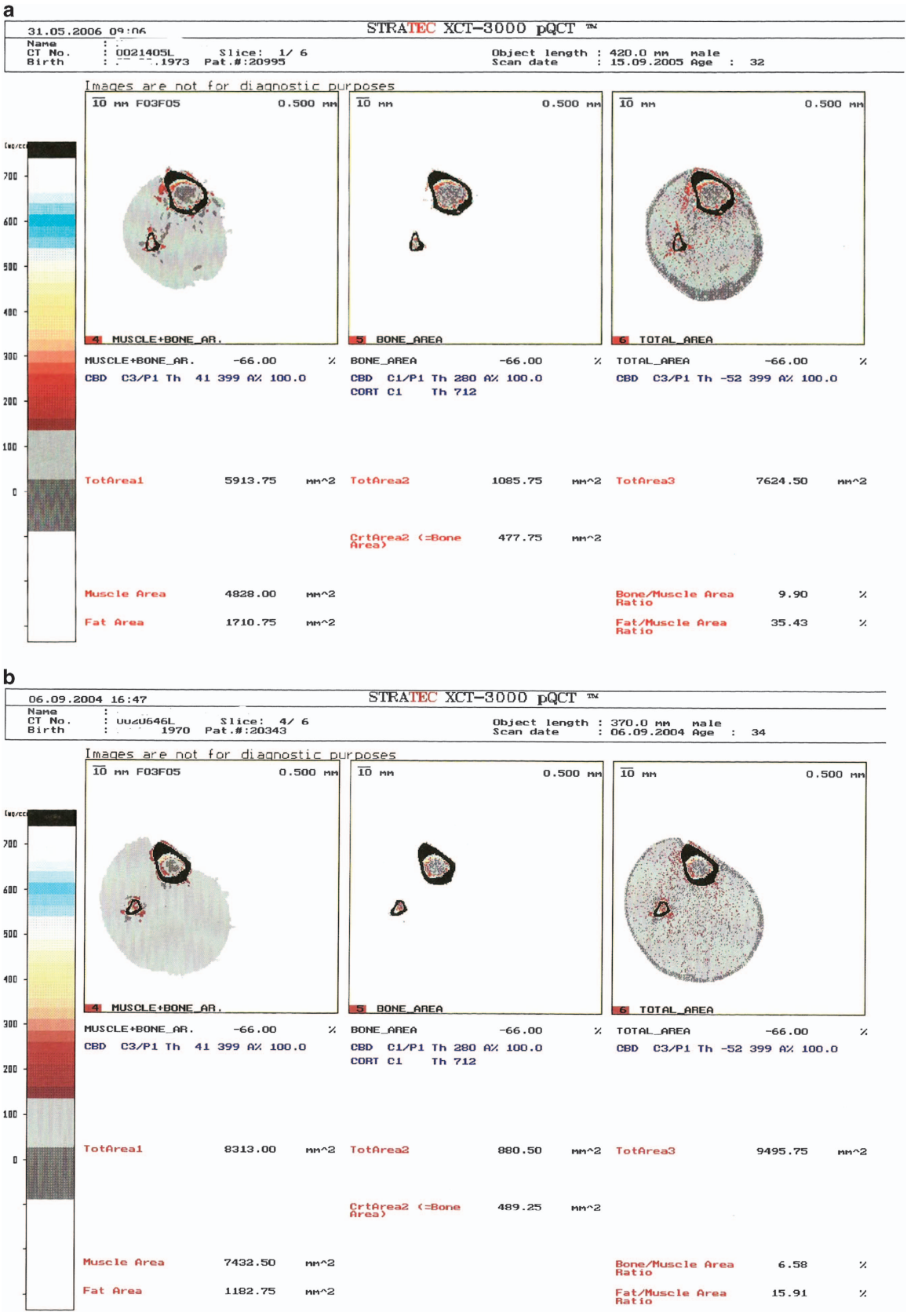

Figure 2 Peripheral quantitative computed tomography (pQCT) in 66% of the tibia in SCI vs control. Figures represent pQCT of the tibia slice (**a**) in spinal cord-injured subjects with paraplegia and (**b**) in controls (scanner XCT 3000, Stratec Medizintechnik).[15] Areas in black and red represent cortical and trabecular bone, respectively, whereas areas in grey represent fat. (**a**) pQCT of the tibia from a spinal cord-injured paraplegic thoracic $(T)_{12}$ 24-year-old man, slice: 66%. (**b**) pQCT of the tibia from a control subject, 30-year-old man, slice: 66%.

mounted ruler in the standing position. Weight of controls was measured on a standard weighing scale. Paraplegics' weight was measured in seating position in the wheelchair after subtraction of the wheelchair's weight. Body mass index (BMI) was calculated for each subject as follows: (BMI = weight (kg)/height $(m)^2$). All men were interviewed by a baseline personal data questionnaire based on anthropometric and clinical information. Anthropometric factors, including age, height, weight, BMI (in both paraplegic groups and controls) and clinical parameters, such as age at injury and DoP, were recorded for all paraplegics. Paraplegic subjects underwent interviews according to a baseline personal data questionnaire based on anthropometric and clinical information and clinical examination by the first author (YD) who defined the NLoI according to the international standards of the ASIA protocol and the ASIA impairment scale.[48] After the acute immobilisation period, all paraplegic subjects followed a rehabilitation programme including assisted standing exercises using various standing devices, standing frames and long leg brace orthoses in the rehabilitation hospital—that is, knee-ankle-foot orthoses (KAFOs), WALK-ABOUT and Advanced Reciprocating Gait Orthosis. All these gait orthoses were provided to the subjects according to the NLoI. Mobilisation included standing for 1 h every day in frames, and therapeutic walking was dependent on the functional capacity of the subjects. Standing was continued at home after discharge from the hospital for most subjects (>90%). Conversely, only 40% in both groups continued walking with long leg brace orthoses for more than 2 years. Independently of the neurological level of the lesion, some performed standing, therapeutic walking or both. In the present study, all paraplegics were above T_{12} NLoI with various degrees of spasticity. Spasticity was assessed on the Ashworth scale.[49] None of the spinal cord-injured subjects were under 25 years at the time of examination or suffered from heterotopic ossification or had had SCI during childhood or adolescence. We also excluded spinal cord-injured patients with chronic administration of drugs affecting bone and muscle metabolism, and coexisting diseases that impair bone tissue. Controls were considered healthy after physical examination and a comprehensive medical history review, which was free of any previous fracture, endocrine or metabolic bone disease, malignancy, drug abuse, alcoholism and hepatic or renal disorders.

We certify that all applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed during the course of this research. The protocol was designed according to the Declaration of Helsinki and approved by the Ethics Committee of Athens University. All subjects gave written informed consent to be included in this study. All men were examined by pQCT. Measurements were performed with a Stratec XCT 3000 scanner (Stratec Medizintechnik,[15] Pforzheim, Germany). Measurements were performed at the left tibia (one leg study). A single slice was taken at 66% of the tibia length proximal to the ankle joint so as to derive bone area to muscle area ratios at sites in which both areas are considered to be maximal. The pQCT parameters were measured from the shaft scans (tibia and fibula): bone cortical CSA, muscle CSA and bone/muscle ratio (**Figure 2**). Technical details are described elsewhere.[50,51]

Statistical analysis

All variables are represented by the number of patients (*n*), mean value (mean) and ss.d. Comparisons of variables among the three groups were performed with one-way analysis of variance and Bonferroni tests for pairwise comparisons. We used the Bonferroni test because the variances between groups were equal. Comparison of variables among the two paraplegic groups was performed with an analysis of covariance model controlling for age at injury and DoP, respectively. Correlations between pQCT parameters and DoP were examined using the Spearman's correlation coefficient because of violation of normality of the DoP variable. All tests were two sided; $P<0.05$ was defined as significant. All data analyses were performed with the Statistical Package for Social Sciences (version 10.0) software (SPSS Inc., Chicago, IL, USA).

Conflict of Interest

The authors declare no conflict of interest.

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