Meagre Effects of Disuse on the Human Fibula Are Not Explained by Bone Size or Geometry

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Keywords: Mechanoadaptation, loading, pQCT

Abstract

Purpose: The fibula supports only a small and highly variable proportion of shank compressive load (-8 to +18%), and little is known about other kind of stresses. Hence, whilst effects of habitual loading on tibia are well-known fibula response to disuse is difficult to predict.

Methods: Therefore, we assessed fibular bone strength using peripheral quantitative computed tomography (pQCT) at 5% increments from 5%-90% distal-proximal tibia length in nine participants with long-term spinal cord injury (SCI, age 39.2±6.2y, time since injury 17.8±7.4y) representing a cross-sectional model of long-term disuse, and in nine able-bodied counterparts of similar age (39.6±7.8y), height and mass.

Results: There was no group difference in diaphyseal fibula total bone mineral content (BMC) (P = 0.22, 95% CIs -7.4%--13.4%, and +10.9-+19.2%). Site by group interactions (P < 0.001) revealed 27% and 22% lower BMC in SCI at 5% and 90% (epiphyseal) sites only. Cortical bone geometry differed at mid and distal diaphysis, with lower endocortical circumference and greater cortical thickness in SCI than able-bodied participants in this region only (interactions both P < 0.01). Tibia bone strength was also assessed; bone by group interactions showed smaller group differences in fibula than tibia for all bone parameters, with opposing effects on distal diaphysis geometry in the two bones (all P < 0.001).

Conclusions: These results suggest that the structure of the fibula diaphysis is not heavily influenced by compressive loading, and only mid and distal diaphysis are influenced by bending and/or torsional loads. The fibula is less influenced by disuse than the tibia, which cannot satisfactorily be explained by differences in bone geometry or relative changes in habitual loading in disuse. Biomechanical study of the shank loading environment may give new information pertaining to factors influencing bone mechanoadaptation.

Mini Abstract

Fibula response to disuse is unknown; we assessed fibula bone in spinal cord injury (SCI) patients and able-bodied counterparts. Group differences were smaller than in the neighbouring tibia which could not be explained by bone geometry. Differential adaptation of the shank bones may indicate previously unknown mechanoadaptive behaviours of bone.

Introduction

The human fibula contributes with the tibia to support and transmit loads through the shank. However, the fibula only supports -8% to +19% of shank compressive load, with its contribution increasing in plantarflexion and eversion [1] and with increasing load magnitude [2]. In addition, it makes a minor (~10%) contribution to torsional stiffness of the shank [3]. Whilst bone mineral content (BMC) (an indicator of bone's compression strength) is broadly similar along fibula's length, there is greater site variance in bending and torsional strength [4]; in all modes of loading strength is lowest in proximal diaphysis. This suggests that bending and torsional loading may have a greater influence on fibula bone strength than compression. Whilst fibula stresses have not been measured directly, the fibula has a far smaller cross-sectional area than the tibia, and only 20-25% of the BMC of the larger bone [5]. This is supportive of a more minor role of the fibula in force transmission in the lower leg than that of the tibia, but this contribution does not appear to be trivial. This is evidenced by the 25-40% increase in tibia cross-sectional area (CSA) following fibula removal [6]. This may not be solely a direct compensation to support the the same stress distribution within the leg, but could also reflect the altered mechanical environment caused by the structural change to a single supporting shaft.

At any rate, the fibula still appears to retain mechanoadaptive capacity. Athletes have greater fibular strength than sedentary peers, with greater adaptation observed in hockey players (where substantial dorsiflexion and eversion could be expected during acceleration and turns) than runners [7]. A more dramatic example is the case of fibulae transplanted to replace the tibia, which can increase their size several-fold [8]. However, it is unknown to what extent

fibula shape and structure is influenced by habitual loading. Study of participants in disuse conditions (*i.e.* where habitual shank loading through locomotion is absent) would reveal the extent of this influence.

Spinal cord injury (SCI) patients represent an extreme case of lower limb musculoskeletal disuse in humans. The traumatic origin of the injury does not present any confounding influence associated with disuse conditions arising from infectious disease or conditions directly affecting bone metabolism. Although effects on *e.g.* nerve and blood supply do occur, evidence that upper limb BMC in SCI patients is similar to [9] or even greater [10] than in controls/at baseline suggests that these factors cannot account for group differences in bone strength.' Accordingly, BMC in the epiphyseal regions of the tibia in long-term SCI patients is around 50% lower than age and size-matched able-bodied males [11], and 20% lower in diaphyseal regions. A study found lower fibula BMD in SCI patients than able-bodied counterparts [12], although only trabecular bone in the ultra- distal epiphysis (4% site) was examined.

Examination of fibula bone strength indicators throughout its length in SCI patients and able-bodied counterparts would provide valuable evidence for effects of disuse on human fibula. Magnitude of compressive, bending and torsional strength deficit in SCI patients should reveal the extent to which these modes of loading contribute to fibula bone strength in healthy, ambulatory males. Bone strength loss in response to disuse is greater in the lower than upper limbs [13]. However, the frequency and intensity of muscular loading during habitual activities differs between the upper and lower limbs [14] hence disuse conditions will not represent a similar departure from habitual loading. In comparison, the tibia and

fibula experience the same frequency of loading and whilst tibia is more heavily loaded the relative difference in loading magnitude should be similar.

Therefore, it is hypothesised that whilst disuse will be associated with lower BMC in the fibula of SCI patients than able-bodied counterparts, the group differences will be similar in relative terms *i.e.* as a percentage to those observed in the tibia. In addition, that due to the lower habitual loading in proximal fibula diaphysis (evidenced by low compressive and bending/torsional strength relative to other regions [4]) effects of disuse will be less pronounced in this region.

Methods

This analysis examines fibula bone structure indicators from peripheral quantitative computed tomography (pQCT) scans. Analysis of those indicators in tibia in spinal cord injury (SCI) patients and able-bodied counterparts [11] has previously been conducted and published. In addition, the previous article describes in full the experimental protocols including recruitment of participants.

Participants

Nine male participants with paraplegia due to spinal cord injury (eight complete lesions with ASIA score A, one incomplete with flaccid paralysis with ASIA score B) were recruited through National Governing Bodies for wheelchair tennis, basketball and hand cycling and included in this study.

SCI and control group age (39.2±6.2y and 39.6±7.8y), height (1.79±0.04m and 1.77±0.03m) and body mass (76.9±9.0kg and 77.0±9.4kg) were similar ($P > 0.4$ in all cases). SCI participants were 21.4±5.8y at time of SCI. Time since SCI was at least nine years in these participants, and bone loss following SCI appears to reach a steady-state after 3-8 years [10]. Nine male non-SCI participants were recruited amongst members of staff at the Alsager campus of Manchester Metropolitan University, and paired with an SCI participant of similar age and body size. None reported any musculoskeletal disorder or major disease and all were involved in regular physical activity including running, cycling and football. All SCI patients included were physically active before their injury, meaning that either their occupation required physical labour or that they took part in competitive sports in their leisure time. Recruitment of able-bodied participants also aimed at identifying individuals who matched

their SCI counterparts in this respect. The study was approved by the Ethics Committee of Manchester Metropolitan University, and all participants gave written informed consent prior to participation.

pQCT Scanning

pQCT scans (XCT 2000, Stratec Medizintechnik GmbH, Pforzheim, Germany) of the self-selected dominant leg were taken at 5% increments of tibia length (measured from the medial malleolus to the palpated medial knee joint cleft) from 5%-95% tibia length in all participants. As the XCT2000 cannot scan the whole lower leg, ten scans (5%-50%) were taken using the distal tibia endplate as reference and ten (50%-95%) using proximal endplate as reference – the average of calculated values from the two 50% scans was used. Pixel size of 0.5mm edge length, slice thickness of 2mm and scan speed of 40mm.s^{-1} were used. The fibula was not visible at 95% site - hence only scans from 5%-90% sites were examined using v6.00 of the software supplied with the machine. Given the need to examine all images in the same way, a number of peeling thresholds were examined. A threshold of 120mg.mm^{-3} was found to accurately separate bone and soft tissue throughout all analysed scans, as due to the thin cortex in SCI patients in distal and proximal sites higher thresholds resulted in portions of the bone cross-section being excluded from analysis. This low threshold will lead to overestimation of total bone CSA by around 5% [15] but accurate assessment of BMC. A threshold of 650mg.mm^{-3} was then used to distinguish cortical bone with peeling mode 1 – this threshold has previously been shown to accurately assess cortical geometry [16]. Therefore the focus of these analyses are primarily on BMC and cortical geometry.

From the output resulting from the Automated Analysis function in the software, a number of

bone measurements were recorded. These were total BMC (vBMC.tot, $mg.mm^{-1}$), cortical BMC (vBMC.ct, $mg.mm^{-1}$), total bone cross-sectional area (Ar.tot, mm^2), cortical bone area (Ar.ct, mm^2) and cortical bone mineral density (vBMD.ct, $mg.mm^{-3}$). Trabecular bone is commonly examined in the inner 45% of bone total area. However, due to high cortical thickness to bone CSA ratio in the fibula only at the 90% site could trabecular bone be examined. Whilst at the majority of sites in both groups cortical thickness was greater than 2.5mm, cortical thickness at 5% and 90% sites in a number of participants was less than 1mm, or double the voxel edge length which would lead to inaccurate assessment of cortical bone geometry. Therefore for the 10% to 85% sites only values for cortical thickness ($Ct.Th_{der}$, mm) and endocortical circumference (EcC, mm) obtained from a ring model were recorded, as was polar moment of inertia (MI_p, mm^4). Accuracy of cortical BMD assessment is impaired when cortical thickness is less than 2mm [17]. Therefore, cortical BMD was only assessed at 15% to 85% sites where all individuals had cortical thickness greater than 2mm. In addition, a correction was applied to cortical BMD values to account for the partial volume effect [15]. To permit comparison of effects of disuse on tibial and fibular bone, these variables were also assessed at all sites using the same analysis parameters. Precision data for fibula pQCT scans has not previously been reported. We therefore analysed twenty-five pairs of scans repeated seven days apart that were obtained using the same machine by the same operator for a previous study [13]. These scans were obtained using a similar protocol in epiphyseal (4%), metaphyseal (14%) and diaphyseal (38%) sites allowing assessment of precision across sites. Short-term error was similar to that previously reported for tibia scans [18] – values for vBMC.tot were less than 0.6% at all sites, whilst only Ar.tot at the 4% site (2.12%) and EcC (1.74%) and I_p at the 14% site (1.59%) had values over 1.5%.

Statistical analyses:

Linear mixed effect (LME) models - with group (SCI/control), site (5%-90%) and group*site interaction as fixed effects and subject as random effect - were examined using the R statistical environment (version 3.1.2, www.r-project.org). Group*site effects indicate where magnitude of group differences varies between sites – where these interactions occur, the 'lme' function in R provides details of the location and significance of these site-specific effects. Residual plots were examined to ensure homoschedascity of residuals. In able-bodied participants, total BMC was lowest at 80 and 85% sites, and bending/torsional stiffness lowest from 70%-80% sites. Therefore 80% was set as null variable for investigation of site and group*site interaction effects with each of the other seventeen sites as it would reveal differing influences of SCI upon compressive and bending/torsional stresses. In addition, the 80% location site represents a diaphyseal site with a high proportion of total BMC being cortical bone. Hence within group*site analysis, the comparisons with ultraproximal and ultradistal sites would reveal differential effects of disuse on epiphyseal and diaphyseal bone. Baseline characteristics of SCI and control groups were compared using independent t-tests. To compare disuse effects on tibia and fibula, a further LME model was constructed incorporating fixed effects of group, site and bone (tibia/fibula) in addition to interactions. Previous work has demonstrated that disuse-related bone loss is greater at sites with a larger endocortical circumference [11, 19]. This suggests that a greater cortical surface area may allow for greater rates of bone breakdown. Therefore, to assess whether differences in absolute or relative cortical surface area could explain differences in disuse effects between tibia and fibula, relationships between magnitude of group difference in BMC and endocortical circumference and surface:volume ratio (endocortical circumference divided by cortical area) were also examined for each bone. For all analyses,

effects were considered significant at $P < 0.05$; data are reported as mean ± 95% confidence interval.

Results

<Table 1 about here>

<Figure 1 about here>

Effects of disuse on fibula bone measures

There were significant effects of site on every measured bone parameter (all $P < 0.001$, Table 1). However, as anatomical variation within the fibula has been previously described [4] and was not a main focus of the study these effects are not discussed. There was no main effect of group on total or cortical BMC (both $P > 0.2$) – however, interactions revealed significant group difference at 5% and 90% sites only for total BMC (both $P < 0.001$, Figure 1a) with values 22 and 27% lower in SCI. No significant effect of group or group*site interaction for cortical CSA or cortical BMD (all $P > 0.2$) was observed. Whilst there was a tendency for lower endocortical circumference in SCI ($P = 0.08$), differences in total CSA were not significant ($P = 0.26$). To ensure that lack of observed group effects in total CSA were not attributable to the low peeling threshold used, analysis was repeated using a peeling threshold of 650mg.mm^{-3} at 10% to 85% sites where a thicker cortex allowed use of a higher threshold. Whilst this resulted in lower total CSA in both groups, there was little effect on relevant group differences and no significant effects of group or group*site were observed (both $P > 0.7$). Site*group interactions revealed smaller endocortical circumference in SCI from 20-25% and 35-55% sites (Figure 2b, all $P < 0.05$). These differences did not result in a main group effect on cortical thickness ($P = 0.9$), although cortical thickness was greater at 25% and 45-50% sites in SCI (all $P < 0.05$). Whilst these geometrical differences between groups resulted in ~5% lower torsional MI in SCI, this was not significant ($P > 0.5$ in all cases).

Adjustment for body size (tibia length x body mass) did not affect . Examination of trabecular BMD at the 90% site only showed that values were 41% lower ($P = 0.005$) in SCI patients (105±17mg.mm^{-3}) than in able-bodied participants (179±58mg.mm^{-3}).

<Figure 2 about here>

Tibia/Fibula Comparison:

Significant bone*group effects were observed for all measured bone variables (all $P < 0.001$), with group differences more pronounced in the tibia than fibula. Total tibial BMC deficit in SCI patients was 21-50% dependent on site (Figure 3a). Observed advantages in distal fibula diaphysis cortical thickness in SCI were in contrast to lower values throughout tibia length (Figure 3b). Similar opposing effects were observed in endocortical circumference; whilst distal fibula diaphysis values were lower in SCI than able-bodied counterparts, tibia values were greater in SCI (Figure 3c).

<Figure 3 about here>

Further analyses were performed to investigate whether cortical bone geometry could explain site and bone-specific effects of disuse in diaphyseal sites. Whilst endocortical circumference was greater in the tibia, surface:volume ratio (endocortical circumference divided by cortical area) was lower than in the fibula at all sites (both $P < 0.001$). In the tibia, significant associations were observed between group BMC differences (calculated both as absolute and percentage differences between age and body size-matched SCI/control pairs),

and surface:volume ratio (both P <0.001) but not unadjusted endocortical circumference (Figure 4). In the fibula, only associations with surface:volume ratio but not unadjusted endocortical circumference were observed. In all cases, observed relationships were substantially stronger than those observed for total BMC suggesting that bone geometry is more powerful predictor of bone loss than bone mineral.

<Figure 4 about here>

Discussion

The aim of this study was to investigate the effects of disuse on bone structure throughout fibula length, and to compare these effects to those observed in the tibia. At all sites, group differences between SCI and able-bodied counterparts in fibula bone parameters were less than that in tibia in contrast to the hypothesis, whilst contrasting group differences in cortical thickness and endocortical circumference were observed. Only at distal and proximal epiphyses were substantial group differences in BMC evident. In distal and mid diaphysis, the cortex was narrower and thicker in SCI patients, although this did not result in significant group differences in torsional stiffness. This is in partial agreement with the hypothesis that effects of disuse would be greater in distal than proximal fibula.

Effects of disuse on the fibula are site-specific

Previous studies have examined the effects of regular exercise on fibular bone structure [7, 20, 21], and of disuse on trabecular BMD in distal fibula [12]. This is the first study to examine effects of disuse on bone strength indicators throughout fibula length. Strikingly, nearly two decades post-injury there was no difference in diaphyseal BMC between SCI patients and able-bodied counterparts with confidence intervals for paired comparisons encompassing zero at 10%-85% sites in Figure 3. However, BMC at proximal and distal epiphyses was 22% and 28% lower in SCI. There are several factors which may contribute to the site-specific pattern of bone loss observed in this study. The epiphyses are particularly rich in trabecular bone, which is highly-metabolically active and lost in addition to that in the cortical compartment. Although trabecular bone could only be examined at one proximal epiphyseal site, there was a difference of over 40% between groups in BMD in favour of non-

SCI suggesting substantial trabecular bone loss. Endocortical circumference is greatest in the epiphyses; a transitional zone of bone adjacent to the endocortical surface appears to be predisposed to disuse-related bone losses [22]. Accordingly, endocortical circumference correlates strongly with the magnitude of bone loss in the tibia [11, 19], supported by similar associations in fibula in the current study.

Compressive strength is dependent upon bone mass, whilst bending and torsional strength also depends upon bone geometry. Fibula diaphysis cortical geometry but not mass was significantly affected by disuse, which can be taken as an as a further evidence of the relatively greater importance of bending and torsional as opposed to compressive loads in the fibular shaft [4]. These interpretations are based on the established Mechanostat theory, which suggests that BMC and geometry are adapted to regulate habitual strains [23]. Recent evidence suggests that fibula bending and torsional strength follows a 'W' shape throughout its length, perhaps in order to prevent site-specific risk of fracture and store energy [4]. The site-specific differences throughout fibula length (lower mass proximally and distally in SCI and differences in cortical geometry in the distal shaft) appear to be those required to blunt this characteristic shape *i.e.* for fibula to lose its phylogenetic adaptation to habitual loading. As with observations in diaphyseal BMC, there may be some minor effects of disuse and from 60%-75% sites group differences in bone inner and outer geometry were less than 5%.

Effects of disuse are greater in the tibia than the fibula

Whilst effects of disuse on fibula were observed, at all sites group differences were less pronounced than in the tibia. Contrasting effects of disuse on bone geometry in the two bones were also observed. This appears to result from the absence of BMC loss in fibula, and

suppression of the usual age-associated expansion in bone area. These discordant effects of disuse were observed despite the two bones' close proximity, and the subsequent assumption that shank unloading would result in a similar relative change in habitual loading in each bone.

Clearly, the fibula is far less responsive to disuse than the tibia in contrast to the hypothesis, although the reasons are unclear. One consideration is the level of habitual loading; the fibula only carries up to 18% of the total shank compressive load dependent on joint angle and load magnitude [1, 2]. Whilst little is known currently about the muscular forces acting upon the two bones during movement, the far greater BMC in the tibia [5] is supportive of greater habitual load than in the fibula. However, it could be assumed that the relative change in habitual loading *i.e.* the stimulus for mechanoadaptation in disuse would be similar or greater in the fibula than tibia due to the lower proportion of shank load that it supports in lower magnitude loading [2].

Bone loss in response to disuse in humans appears to only last for a few years [10] due to apoptosis of osteoclasts caused by low habitual loading [24], therefore the rate of bone resorption is important. A large endocortical circumference would permit greater bone loss from the highly active subendocortical zone [22], and indeed endocortical circumference explains 98% of site variance in tibia bone loss following SCI [11]. Tibia endocortical circumference is 2-3 times greater than that in the fibula [11], which might in part explain greater absolute bone loss. However, relative bone loss *i.e.* percentage change is likely more related to surface:volume ratio than endocortical circumference; supported by stronger associations with the former observed in both bones. Greater surface:volume ratio in the

fibula should result in greater relative group differences although in reality the opposite is true. Hence, in combination lower absolute but not relative mechanoadaptive stimulus (habitual loading) and capacity (bone geometry) are evident in the fibula. This cannot explain the lower relative response to disuse observed in this bone.

It may be that there is a minimum 'design' for bone, whereby genetic determinants pre-program bone morphology in the absence of substantial loading. Long bone formation and cross-sectional growth occurs *in utero* even in neurological conditions where movement and associated loading is absent [25], and development continues in children following spinal cord injury [26]. Therefore habitual loading of the fibula may not be sufficient to provoke substantial adaptation from this base design, resulting in an absence of disuse effects once this loading is removed. Indeed, it does not seem that the fibula is inherently unresponsive to mechanoadaptive stimuli; evidenced by its dramatic growth when transplanted to replace an excised tibia [8]. In addition, that fibula bone strength is greater in athletes than controls [7, 20]; although observed effects are smaller than those observed in the tibia [21]. Instead, it may be that unknown elements of the loading environment such as the relative contributions of compressive, bending or torsional stresses or even strain rate may differ substantially between the two bones.

The fibula is attached to the tibia by ligaments and an interosseous membrane, and its acutely angled distal articulating surface does not substantially overlap the talus in the transverse plane. Fibular force transmission may therefore be influenced by lengthening of the connective tissues reducing peak strain and strain rate (known to be important for bone mechanoadaptation [27, 28]). This is in contrast to the tibia, where both proximally and

distally large flat epiphyseal surfaces allow direct transmission of joint forces. Studies investigating the shank loading environment would help explain dischordant adaptation in the tibia and fibula. Together with interventions aimed at manipulating shank loading, this work could provide valuable information on the importance of mechanoadaptative stimuli variables.

Limitations

The scans examined were taken originally to examine the tibia, and hence sites are indicated with reference to tibia rather than fibula length. In addition, the fibula extends distally around 5-10% further than the tibia so the most distal portion of fibula was not examined. However, it is clear that the majority of fibula which was imaged appears little affected by disuse in comparison to the tibia. The study of spinal cord injury patients was cross-sectional and so group differences (or lack thereof) may be attributable to factors other than different activity levels. In order to minimise this risk, effort was made to match participants well by age, mass and height (factors known to influence bone size and strength). Verification of these findings in an interventional study would be ideal, but may prove difficult due to the time taken for measurable changes in bone geometry to occur. Even in long bed rest studies of 90 days, tibial diaphyseal BMC loss is only ~10% of that observed in SCI [13] therefore such studies are likely not powered to detect cortical geometry changes observed in this study.

As existing data was used, *a priori* sample size testing was not performed; therefore some minor effects of disuse on diaphyseal BMC and proximal diaphyseal geometry may occur which this study was underpowered to detect. However, from group differences in BMC

from 15% to 80% sites and bone geometry from 65% to 80% sites were all less than 5%. Given that long-term SCI patients represent an extreme disuse case, it is highly likely that the variance in fibula bone strength within the general population attributable to differences in habitual loading is much smaller than these values. Whilst the thin fibula cortex observed distally and proximally could affect cortical bone measurements, observed differences were only evident in regions where cortical bone thickness was thick enough to allow accurate assessment of bone density and geometry. It is possible that lower bone turnover in SCI patients may affect mineralisation and in turn affect bone measurements; however effects of SCI on mineralisation are unknown. Although adjustment was not made for multiple testing in this study, the most striking results are the lack of detectable group effects in the fibula, and greater group differences in epiphyseal than diaphyseal BMC and in the tibia than fibula. Statistical adjustment via *e.g.* a conservative Bonferroni adjustment would not affect non-significant group differences, and for fibula BMC group*site interactions and all group*bone effects *P* was less than 0.001 therefore results would have still been below set significance level after adjustment.

A detailed history of physical activity of SCI participants pre-injury was not collected. However, the main persisting benefit of physical activity appears to be an advantage in total bone area [29], which cannot be increased via physical activity in adulthood in the epiphysis [30]. Therefore that tibia proximal and distal epiphyseal total bone area was similar between SCI and able-bodied participants [11] is supportive of a similar physical activity level in both groups through to adulthood.

Conclusions

In conclusion, fibula diaphysis BMC is not substantially affected by disuse, although some epiphyseal loss appears to occur. Whilst the proximal diaphysis changes little in disuse, there is evidence that normal widening and thinning of the distal diaphysis through adulthood is diminished in disuse. Together, these results suggest that compressive loading does not substantially influence the structure of the fibula diaphysis, whilst the proximal diaphysis' structure also appears to be independent of bending and torsional loading. Observed effects of disuse on fibula are much less pronounced in absolute and relative terms than in the tibia throughout its length; these differences are not satisfactorily explained by differences in habitual loading or bone geometry. In addition, contrasting effects of long-term disuse on distal fibula diaphysis and tibia diaphysis occur. Further study of the mechanical environment of the shank could reveal important information pertaining to factors influencing bone mechanoadaptation. This includes the influence of components of deformation stimuli (magnitude, rate, mode, *etc*.), and the structure of mechanosensory apparatus such as osteocytes (shown to vary between the fibula and calvaria according to habitual loading patterns [31]).

Conflict of Interest: Alex Ireland, Ricardo Capozza, Gustavo Cointry, Laura Nocciolino, José Ferretti and Jörn Rittweger declare that they have no conflict of interest.

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pQCT Measurement	Main Effects		Interaction
	Site	Group	Site*Group
Total BMC ($mg.mm^{-1}$)	<0.001	0.22	>0.001
Cortical BMC ($mg.mm^{-1}$)	<0.001	0.67	0.29
Total Bone CSA (mm^2)	<0.001	0.26	0.95
Cortical Bone CSA (mm^2)	<0.001	0.67	0.29
Cortical BMD ($mg.mm^{-3}$)[a]	<0.001	0.69	0.97
Endoscortical Circumference (mm)	<0.001	0.08	0.002
Cortical Thickness (mm)	<0.001	0.9	0.001
Polar Moment of Inertia (MI_p, mm^4)	<0.001	0.91	0.98

Table 1. Main effects of group (SCI *vs* controls), site and group*site interaction for pQCT measures in 10-85% sites selected for cortical analysis. In all cases significant main effect of group indicated greater values in control than SCI. Site*Group interactions indicated where group differences varied by site – locations of these interactions are indicated on Figures 1a (total BMC), 2b (endocortical circumference) and 2c (cortical thickness). [a]Analysis restricted to 15-60% sites.

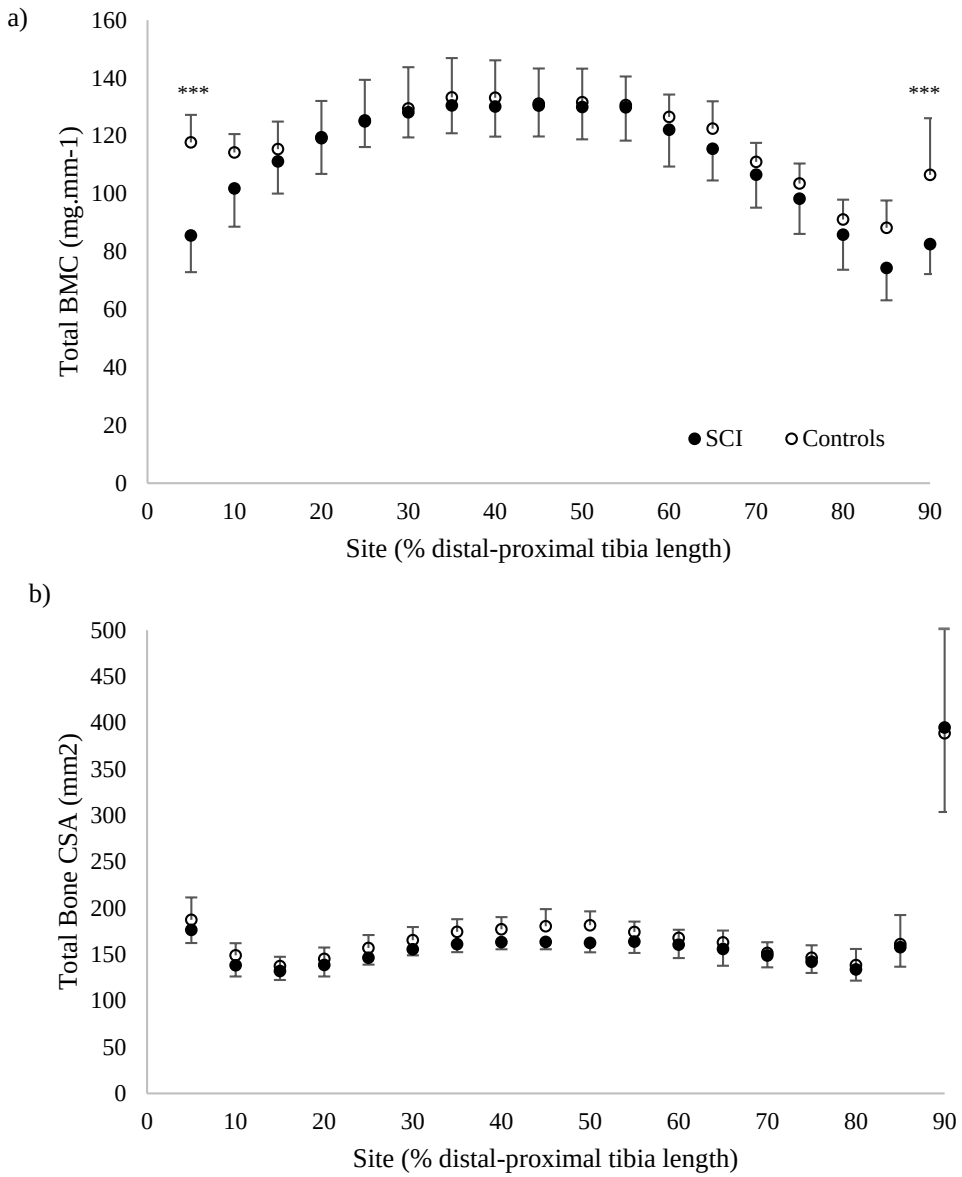

Figure 1. a) Total BMC and b) total bone CSA in SCI and controls throughout fibula length, as mean ± 95%CI. Asterisks indicate location of significant site-specific differences with respect to 80% site as identified by site*group interactions detailed in Table 1, *** - $P < 0.001$.

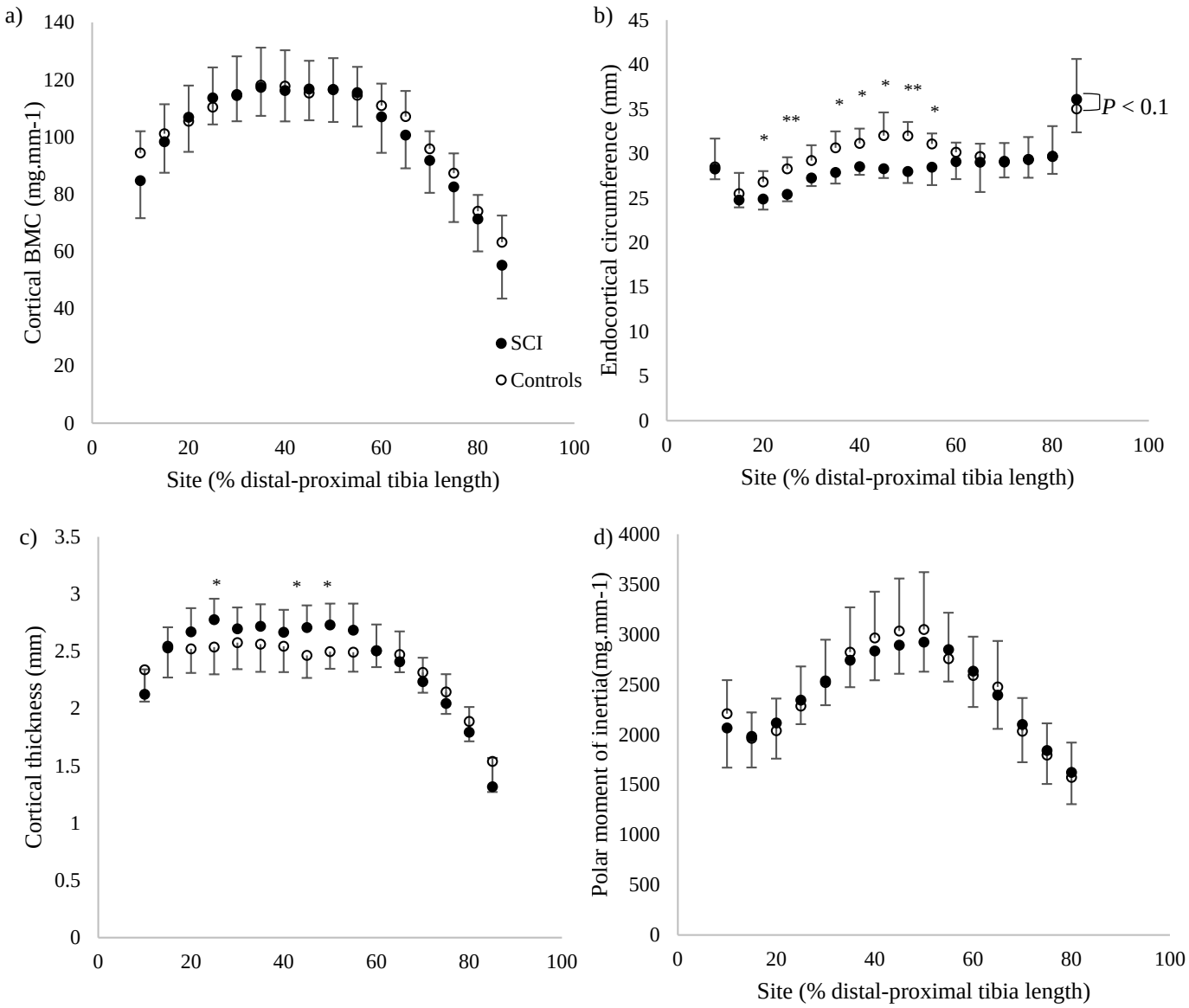

Figure 2. Cortical geometry in SCI and controls , as mean ± 95%CI. Asterisks indicate location of significant site-specific group differences with respect to 80% site as identified by site*group interactions detailed in Table 1, * - $P < 0.05$, ** - $P < 0.01$, *** - $P < 0.001$.

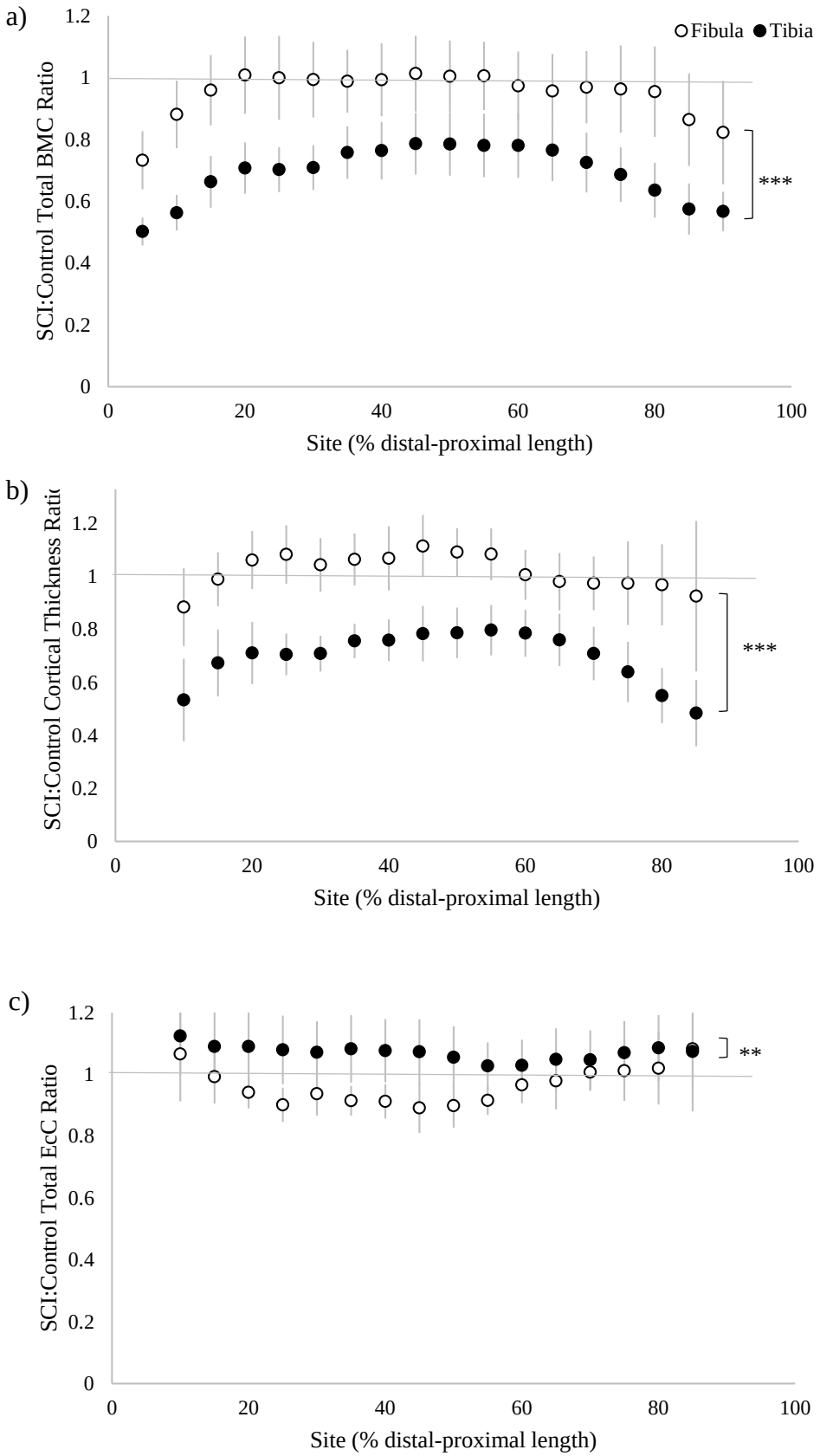

Figure 3. Comparison of tibia and fibula a) total BMC, b) cortical thickness and c) endocortical circumference group differences, as ratio of paired values ± 95%CI. Asterisks indicate main effect of bone (tibia/fibula); ** - $P < 0.01$, *** - $P < 0.001$.

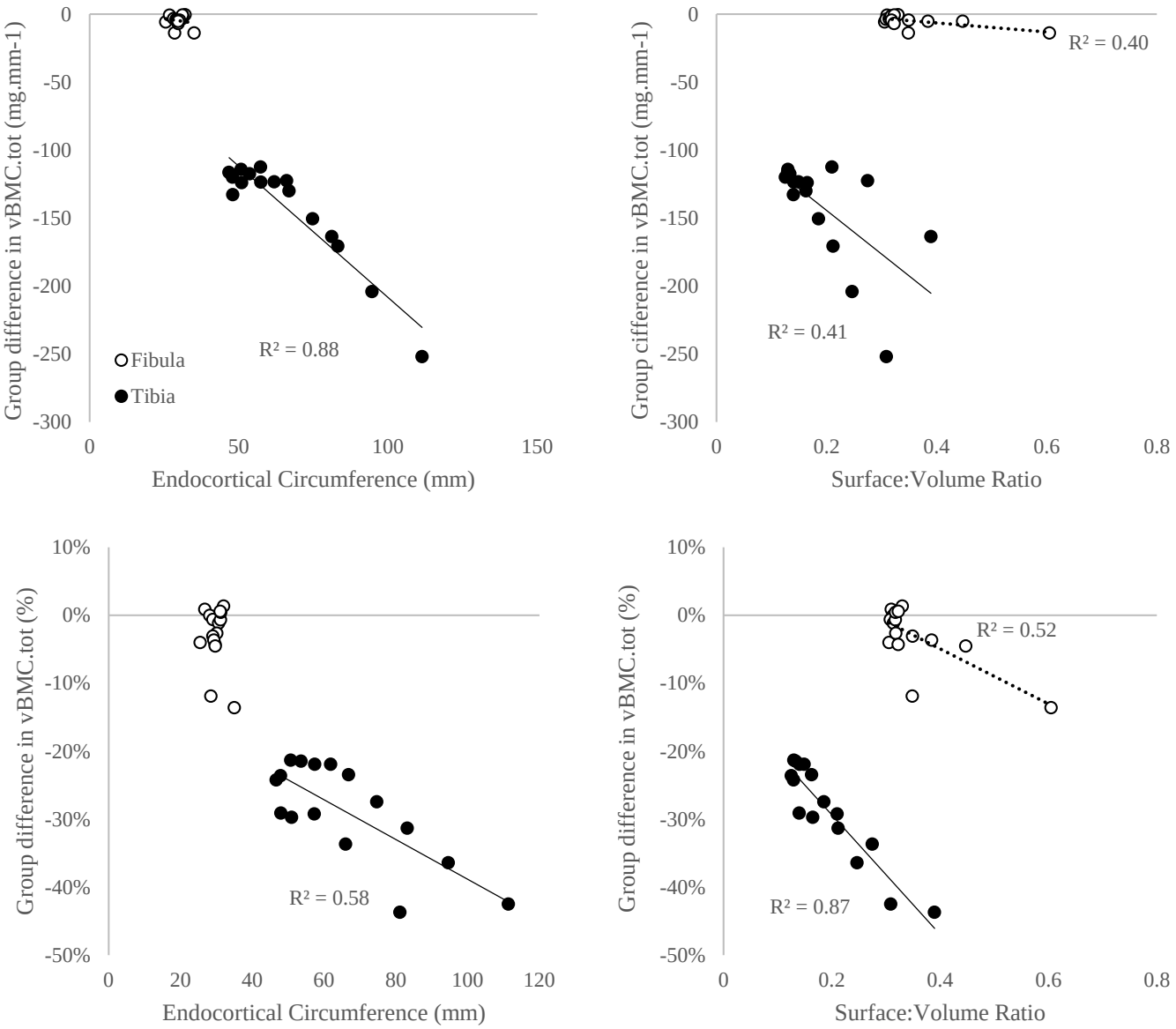

Figure 4. Associations between absolute and relative (%) paired differences in total BMC (vBMC.tot), and endocortical circumference and surface:volume ratio at different sites throughout tibia and fibula. Association significant at $P < 0.001$.