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Anatomical sector analysis of load-bearing tibial bone structure during 90-day bed rest and 1-year recovery

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Summary

The aim of this study was to investigate whether the bone response to long bed rest–related immobility and during subsequent recovery differed at anatomically different sectors of tibial epiphysis and diaphysis. For this study, peripheral quantitative tomographic (pQCT) scans obtained from a previous 90-day 'Long Term Bed Rest' intervention were preprocessed with a new method based on statistical approach and re-analysed sector-wise. The pQCT was performed on 25 young healthy males twice before the bed rest, after the bed rest and after 1-year follow-up. All men underwent a strict bed rest intervention, and in addition, seven of them received pamidronate treatment and nine did flywheel exercises as countermeasures against disuse-related bone loss. Clearly, 3–9% sector-specific losses in trabecular density were observed at the tibial epiphysis on average. Similarly, cortical density decreased in a sector-specific way being the largest at the anterior sector of tibial diaphysis. During recovery, the bed rest–induced bone losses were practically restored and no consistent sector-specific modulation was observed in any subgroup. It is concluded that the sector-specific analysis of bone cross-sections has potential to reveal skeletal responses to various interventions that cannot be inferred from the average analysis of the whole bone cross-section. This approach is considered also useful for evaluating the bone responses from the biomechanical point of view.

Introduction

Bone structure can deteriorate in response to several factors such as disuse, immobilization, ageing, diseases and hormonal disturbances. Because the lower limb skeleton is primarily locomotive organ and capable to adapt to varying loading conditions (Frost, 2003; Ruff *et al.*, 2006), disuse irrespective of its primary cause provides a useful model to investigate responses of bone structure to reduced loading. Experimental bed rest with −6° head down tilt is an established ground-based model to simulate the physiological effects of spaceflight (Pavy-Le Traon *et al.*, 2007). Earlier bed rest studies have mostly relied on dual-energy X-ray absorptiometry (DXA) (LeBlanc *et al.*, 1990; Zerwekh *et al.*, 1998; Shackelford *et al.*, 2004; Armbrecht *et al.*, 2010) but with regard to bone structure these studies are limited by the inherent inability of DXA to yield tangible information on actual cross-sectional bone geometry, let alone separating the measured bone into trabecular and cortical compartments (Sievänen, 2000). Peripheral quantitative computed tomography (pQCT) offers a reasonable option to assess bone geometry and density without evident limitations of planar DXA (Sievänen *et al.*, 1998; Sievänen, 2000). The few studies which have used pQCT or QCT technology indicated that bone loss is most prominent at endocortical bone regions both after bed rest (Rittweger *et al.*, 2005, 2009, 2010) and space flight (Lang *et al.*, 2006).

It is obvious that different bone sectors experience specific loading environment in terms of biomechanics of the given site. A good example of sector-specific bone adaptation comes from patients with spinal cord injury, in whom electrical stimulation of the soleus muscle led to site-specific bone accrual in the posterior aspect of the distal tibia (Dudley-Javoroski & Shields, 2008). Apparently, normal locomotive muscle contractions mostly affect the anterior and posterior sectors of distal tibia via the ground reaction forces imposed on the feet in the front and the pulling forces mediated by the Achilles tendon in the back. Therefore, we hypothesized that the most substantial bone losses would occur at those sectors where the lack of locomotive loading is most evident because of immobility during bed rest and accordingly a faster recovery at the same sectors when the

locomotive loading was resumed. To our knowledge, disuse-related changes in anatomical sectors of bone structure have not been studied before.

To elaborate our hypothesis, we re-analysed the pQCT data from the 'Long Term Bed Rest' (LTBR) study that was carried out in 2001 and 2002 in Toulouse/France. The LTBR results indicated that administered countermeasures, pamidronate and flywheel exercise could only partly prevent the mean loss in tibial bone mineral content during bed rest (Rittweger *et al.*, 2005). However, after the resumption of locomotion, the bone loss fully recovered at the tibial shaft and also to a large extent at the distal epiphysis (Rittweger & Felsenberg, 2009). In this study, we assessed specifically whether (i) the bone responses to bed rest were different at anatomically different sectors of the distal tibia and tibial shaft in different subgroups and (ii) whether the sector-specific responses were different during the recovery. The latter research question can give valuable information as to whether the recovery is achieved not only in quantitative terms but also with anatomical specificity in line with anticipated biomechanics. We also expected different responses between the groups treated with countermeasures and the control group. While the flywheel exercise could halve the bone loss in the LTBR study (Watanabe *et al.*, 2004; Rittweger *et al.*, 2005), it is unclear whether this benefit is evenly distributed within the bone cross-section. Likewise, it is not known whether bisphosphonate treatment could modulate the sector-specific maintenance of bone mass during bed rest, while the general benefits of such treatments on DXA-measured bone mineral density have been consistently observed (Chappard *et al.*, 1989; Grigoriev *et al.*, 1992; Ruml *et al.*, 1995; LeBlanc *et al.*, 2002; Watanabe *et al.*, 2004; Rittweger *et al.*, 2005).

Material and methods

The details of the LTBR study as well as the countermeasures and scanning procedures have been described in detail elsewhere (Rittweger *et al.*, 2005). In short, 25 healthy young male volunteers were recruited to undergo 90 days of strict bed rest with −6° head down tilt. These men were randomly assigned into three groups: nine to the control group (Ctrl, bed rest only), seven received a single infusion of 60 mg pamidronate i.v. prior to bed rest (Pam) and the other nine participants practiced resistive flywheel exercises every 2–3 days (FW). The age range of the subjects was 23–41 years; weight range 60–80 kg and height range 167–185 cm. The study was approved by the local Ethics Committee and all participants gave their written informed consent before they were included into the study.

Peripheral quantitative computed tomography (XCT 2000; Stratec Medizintechnik GmbH, Pforzheim, Germany) was performed at distal 4% (epiphysis) and at 66% (diaphysis) sites of the tibia (Rittweger *et al.*, 2005). For the present study, pQCT data measured 2 weeks before the bed rest (BDC-14), 1 week before the bed rest (BDC-7), 2 weeks after cessation of the bed rest (R + 14) and 1 year after the bed rest (R + 360) were used. The reason to select R + 14 rather than the penultimate day of bed rest (HDT89) was that the bone changes continued and were largest around R + 14 (Rittweger *et al.*, 2010). Three subjects (one from Pam group and two from FW group) were excluded because they did not attend the R360 follow-up measurement. Also, one subject from FW group was moved into Ctrl group for second half of the study because of knee pain that was partly related to the flywheel training (Rittweger *et al.*, 2007). Repositioning for the pQCT scans included in this study was considered acceptable because the differences in total cross-sectional bone areas were within 25 mm^2 (∼1%) in all consecutive four scans of distal tibia. The mean total area and mean intra-individual standard deviation (in parentheses) between BDC-14 and BDC-7 were 2122 (10) mm^2 for epiphysis and 662 (0·1) mm^2 for diaphysis.

Peripheral quantitative computed tomography image analysis

A new preprocessing method for pQCT data, proposed recently by Cervinka *et al.* (2010), was applied to the raw pQCT data that were produced by the back projection algorithm of the pQCT system without preprocessing. The new method was chosen because it permitted more consistent detection of outer and inner cortical boundaries compared to common image-preprocessing techniques, such as median filtering. In brief, preprocessing of the image data was composed in two steps. First, a gray-level transformation was carried out by implementing a common, iteratively employed, piecewise linear conversion function. This transformation represents a non-equidistant re-quantization of the gray scale. Second, the transformed data were corrected for inaccuracies caused by the re-quantization procedure by using the Bayesian approach and Markov random fields with 3 × 3 linear neighbourhoods and redundant wavelet transform. The method is described in detail elsewhere (Cervinka *et al.*, 2010).

After preprocessing of the pQCT images, a modification of simple and fast edge tracing algorithm (described in Seits & Ruegsegger, 1983) was used for segmenting the bone cross-section into cortical and trabecular bone regions (Cervinka *et al.*, 2010). After detecting the outer cortical boundary according to the maximum values of the first derivative of the preprocessed image data, the region inside the traced bone contour was radially shrunk until the inner cortical boundary was found. The next local maximal points along the radii were considered to coincide with the inner boundary of the cortical shell (Cervinka *et al.*, 2010). The shrunken region corresponded to the trabecular bone area and subtraction of the original and shrunk bone regions provided the cortical bone area. The procedure for cortical contour detection is illustrated in Fig. 1.

Then, the conventional pQCT traits, cortical cross-sectional area (CoA) and trabecular density (TrD) for epiphysis, and CoA and cortical density (CoD) for diaphysis, were determined as descriptors of bone structure. Besides analysing the total bone cross-section, the cross-section was divided into four anatomical

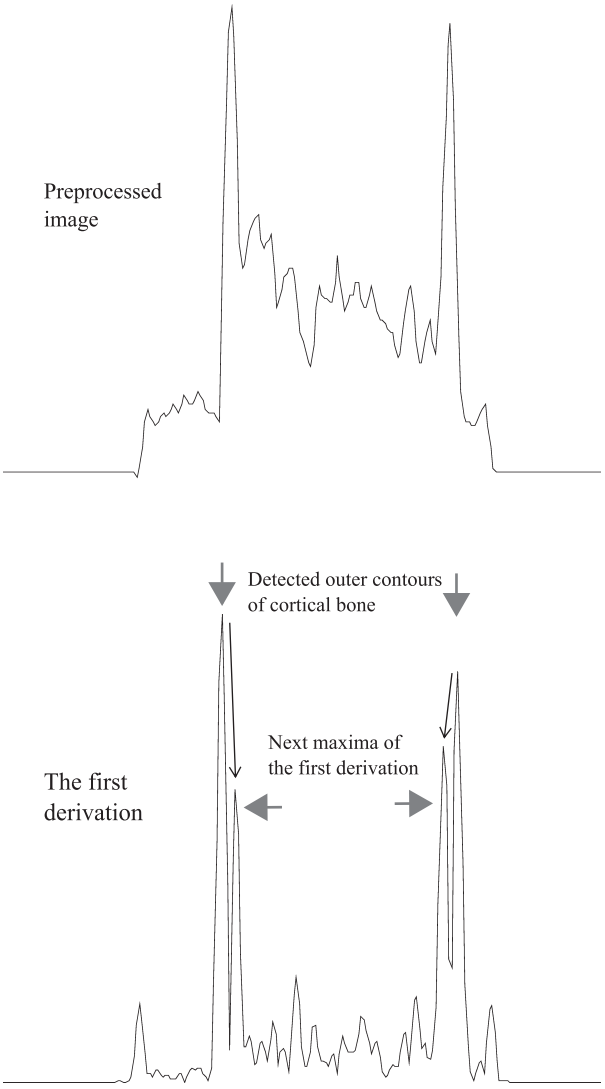

Figure 1 Cortical bone contour detection. The upper curve shows one radial X-ray attenuation profile along a radial line through the preprocessed bone image and the lower curve represents its first derivate. The greater attenuation of cortical bone is distinct. The maximum of the first derivative denotes the outer cortical boundary and the next local maximum denotes the inner cortical boundary. This algorithm is repeated across the whole bone cross-section and the area remaining between the maxima and next local maxima of the first derivative represented the cortical area.

sectors (lateral, posterior, medial and anterior) so that direction-specific features of the cortical and trabecular bone could be assessed (Fig. 2). The CoA was expressed as a percentage of total bone cross-sectional area or as a percentage of total bone area within the given anatomical sector, as appropriate. The TrD and CoD values represented the volumetric bone mineral apparent density of the given bone region. The conversion from gray scale values to bone mineral density was based on previously determined relationship between the pQCT-measured densities, known K_2HPO_4 concentrations and gray scale values (Sievänen *et al.*, 1998; Cervinka *et al.*, 2010).

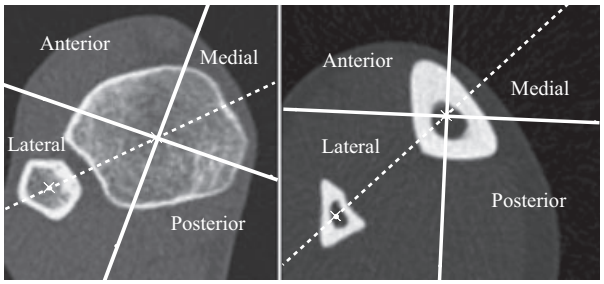

Figure 2 Definition of the anatomical sectors of interest for the distal tibia (4%) and tibia diaphysis (66%). First, a line was drawn through the centres-of-mass of the tibia and fibula. Then, two lines were drawn through the centre-of-mass of the tibia at angles of 45° and −45°. The sectors between these lines denote the four anatomical (posterior, medial, anterior and lateral) regions of interest.

Statistical analysis

Group mean values and 95% confidence intervals (95% CI) of bone traits are given as descriptive statistics. Changes in bone traits after bed rest (R + 14) and after recovery periods (R + 360) were expressed as percentage changes from baseline value, which was defined as the mean of BDC-14 and BDC-7 data measured before bed rest. These two pQCT assessments prior to bed rest provided also relevant information on short-term precision of bone traits and facilitated appropriate interpretation of changes in bone traits. Given the small number of subjects in study groups, the 95% CIs of percentage changes were considered adequate for indicating within-group changes during bed rest and subsequent recovery period or substantial between-group differences at different time points. In addition to mean changes over time, potential group differences in the variance of responses to bed rest and accompanying counter-measures at R + 14 and R + 360 were evaluated with the F-test.

Results

Epiphysis

Changes in CoA and TrD at the distal tibia during bed rest and 1-year recovery are shown in Fig. 3. Mean changes in CoA generally remained within ∼±2% in all groups without apparent trends or indication of statistical significance. However, the Pam group showed lower variance in the anterior and posterior changes in CoA during the bed rest compared to FW and Ctrl groups, respectively, ($P<0{\cdot}05$ for both) but higher variance in medial and anterior changes in CoA during recovery compared to Ctrl group ($P<0{\cdot}05$ for both sectors).

Mean trabecular loss during bed rest was systematically greater (∼3–8%) than the cortical loss, and it was most pronounced in Ctrl group and smallest in FW group. Besides the general decline within the whole bone cross-section, some anatomical sectors appeared to be more affected. During bed rest, the greatest (∼6–8%) mean losses in trabecular density occurred at the medial sector in Pam and FW groups, whereas in

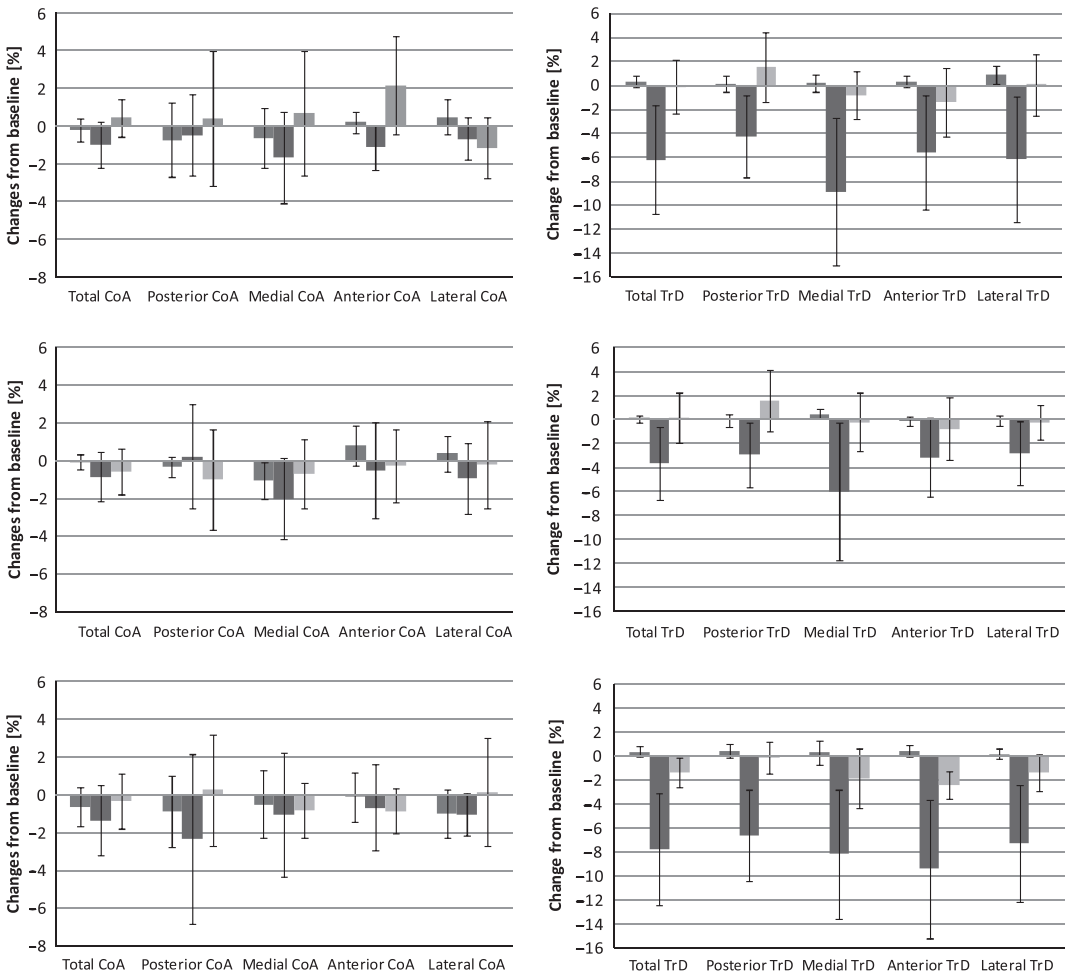

Figure 3 Distal tibia (4%): mean percentage changes (95% CI) of CoA (left panel) and TrD (right panel) in Pam (upper row), FW (middle row) and Ctrl (lower row) groups at baseline (mean of BDC-14 and BDC-7; left bars), from baseline to 2 weeks after 90 days bed rest (R + 14; middle bars) and from baseline to the end of 1-year recovery period (R + 360; right bars) in total and at four anatomical sectors of distal tibia.

Ctrl group, the greatest mean loss (∼10%) took place at the anterior sector. Compared to FW group, Ctrl group showed higher variance in density changes at anterior and lateral sectors during bed rest. During recovery, virtually all mean density levels returned back to baseline, the quite consistent ∼2% anterior loss in Ctrl group excluded. The variance at the anterior density in Ctrl group was lower compared to Pam group ($P<0{\cdot}05$). Individual changes in TrD during the bed rest and recovery periods are shown in Fig. 4.

Diaphysis

Mean changes in CoA and CoD at the tibial diaphysis during bed rest and 1-year recovery are shown in Fig. 5. In general, mean changes in CoA during the whole study period were marginal (mainly within ±1%) without apparent trends or indication of statistical significance. However, the variance in individual responses appeared to be great and some between-group differences were indicated. Compared to Ctrl group, changes in total CoA were less variant in Pam and FW groups during bed rest ($P<0{\cdot}05$ for both). In FW group, in turn, the variance in bed rest–related changes in medial CoA was higher compared to those in Ctrl and Pam groups ($P<0{\cdot}05$ for both). During recovery, changes in lateral CoA were more variant in Pam group than in Ctrl group.

The mean losses in CoD remained marginal (mostly clearly within ±1%) in Pam and FW groups but were somewhat larger at posterior (∼1·5%) and anterior (∼2%) cortical regions in Ctrl group. During bed rest, changes in medial and anterior CoD were more variant in FW group than in Pam or Ctrl groups and in Pam group, respectively ($P<0{\cdot}05$). In addition, changes in total, posterior and anterior CoD were more variant in Ctrl group compared to Pam group ($P<0{\cdot}05$). During recovery, there was virtually no systematic sector-wise effect on R + 360 and all mean density levels returned back to baseline. The variances were also similar in all groups. Individual changes in CoD during the bed rest and recovery are shown in Fig. 6.

Discussion

The main purpose of this study was to investigate whether the bone responses to bed rest and subsequent recovery differ

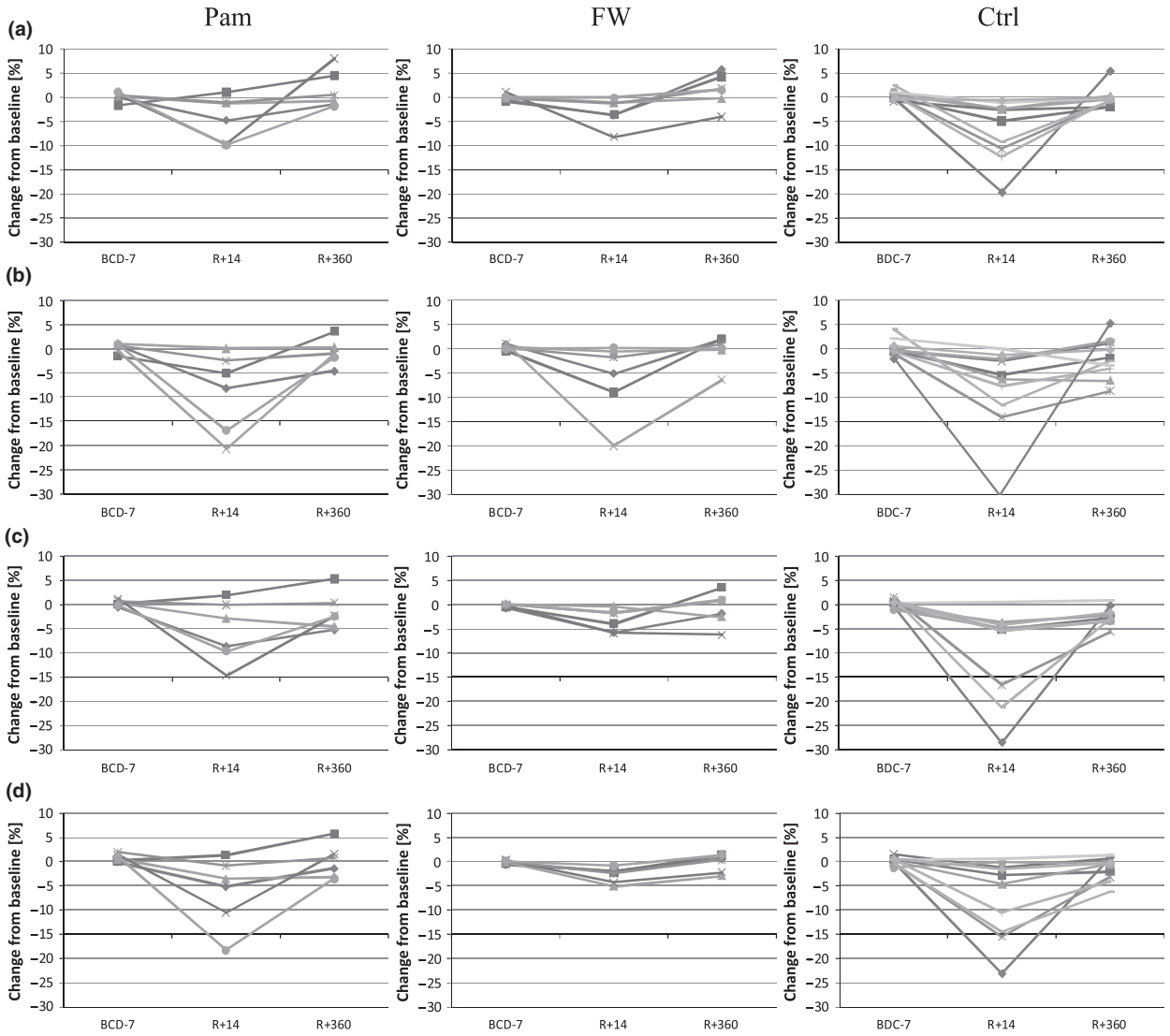

Figure 4 Individual percentage changes in TrD at distal tibia (4%) in Pam (left column), FW (middle column) and Ctrl (right column) at four anatomical sectors; posterior (a), medial (b), anterior (c) and lateral (d) at base line (mean of BDC-14 and BDC-7), R + 14 and R + 360.

between anatomically different sectors of the tibial epiphysis and diaphysis and whether they differ between different subgroups. Sector-specific modulation was evident and the bone losses were most pronounced in the trabecular compartment (reaching ~9% on average) of the distal tibia. This is in line with the results from long-term immobilization caused by spinal cord injury (Sievänen, 2010) but in apparent contrast to recent findings from bed rest studies (Rittweger & Felsenberg, 2009; Rittweger *et al.*, 2009, 2010; Armbrecht *et al.*, 2010). The latter have suggested that the most prominent bone loss occurred at the endocortical surface of cortical bone. During 1-year recovery, no consistent sector-specific modulation was observed in any subgroup and the bed rest–induced bone losses were practically restored, albeit with a small deficit was seen in trabecular density at the anterior sector of the control group. The general recovery of bone mass is perfectly in line with recent results of Rittweger & Felsenberg (2009).

Obviously, the structure and geometry of lower limb bones have developed under the regular influence of mechanical loading comprising largely the ground impacts and accompanying muscle forces during bipedal locomotion in different physical activities. Therefore, bed rest induces a drastic change to the loading environment of lower limb skeleton. Reportedly, significant (2–6%) mean losses occur in bone in response to bed rest and the magnitude of mean loss is related to the total duration of intervention (Rittweger & Felsenberg, 2009; Rittweger *et al.*, 2009, 2010; Armbrecht *et al.*, 2010; Sievänen, 2010). In the present study, the emphasis was uniquely placed on the sector-specific evaluation of cortical and trabecular bone responses to bed rest and consequent recovery in three different groups, one without countermeasure and two with countermeasures against bed rest–related bone loss.

Sector-specific analysis of bone structure has been previously applied in assessing the association of physical activity or

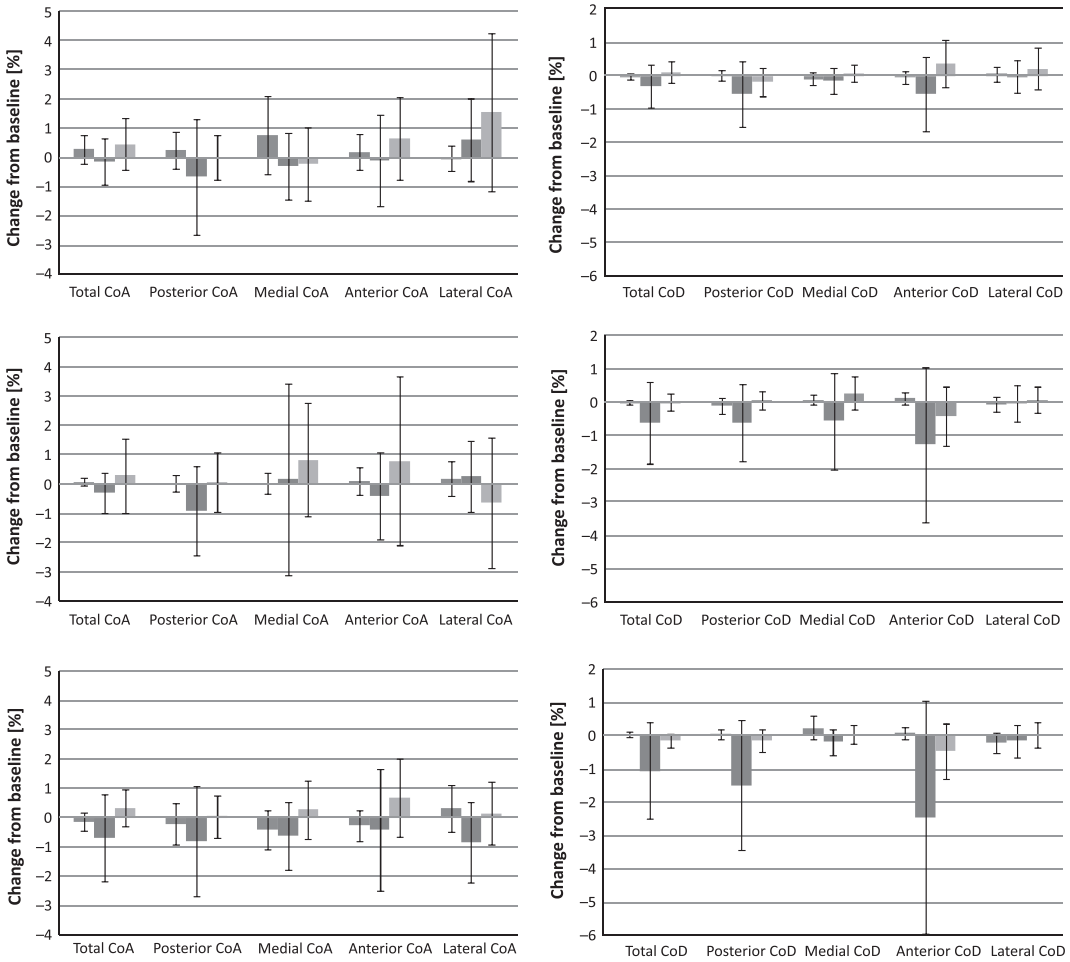

Figure 5 Tibia diaphysis (66%): mean percentage changes (95% CI) of CoA (left panel) and CoD (right panel) in Pam (upper row), FW (middle row) and Ctrl (lower row) groups at baseline (mean of BDC-14 and BDC-7; left bars), from baseline to 2 weeks after 90 days bed rest (R + 14; middle bars) and from baseline to the end of 1 year recovery period (R + 360; right bars) in total and at four anatomical sectors of the tibia shaft.

exercise loading with tibial geometry (Ma *et al.*, 2009; Macdonald *et al.*, 2009; Shaw & Stock, 2009; Rantalainen *et al.*, 2010). With regard to the present bed rest data, this approach allowed a good opportunity to test, at least tentatively, three following hypotheses all pertinent to bone physiology. First, a general bone loss should occur as a consequence of the lack of locomotive loading; second, the flywheel exercise should reduce this bone loss especially at sectors that are loaded most by pedalling (i.e. mainly along the antero-posterior axis because of muscle forces delivered via Achilles tendon); third, the pamidronate treatment should show a general preventive effect because of its apparently systemic influence on bone metabolism. Quite surprisingly, all three hypotheses were virtually confirmed for the cortical density only (Fig. 5), whereas the responses in trabecular density were not so distinct between groups (Fig. 3), let alone the marginal responses in cortical area (Figs 3 and 5).

As expected, the variance in individual skeletal responses was impressively great during the bed rest phase. In the light of such substantial inter-individual variability, the inter-subject variation during recovery has to be described as astoundingly small. It therefore seems that bone adaptation is more accurately governed when bone is accrued than when it is lost (Figs 4 and 6). Increased bone resorption, in particular, has been shown in a recent bed rest study on bone biomarkers (Armbrecht *et al.*, 2010).

Long bones, such as tibia, allow efficient movement of the body and provide stiffness against muscle contractions. The apparent goal of skeletal adaptation is to keep the loading-induced deformations (strains) within a specific physiological range (Frost, 2003), and when the regular loading is essentially reduced, the bone loses some of its rigidity. The contribution of cortical bone (geometry in conjunction with material elasticity) to the whole bone rigidity and strength is evident (Currey, 2001). Because the elasticity of cortical bone as a material is proportional to the third power of cortical density (Martin, 1991), the reduction in cortical density may be the first adaptive process to increase the flexibility of the given bone to meet the new loading environment – provided that the bone normal metabolism is not affected by antiresorptive medication. Indeed,

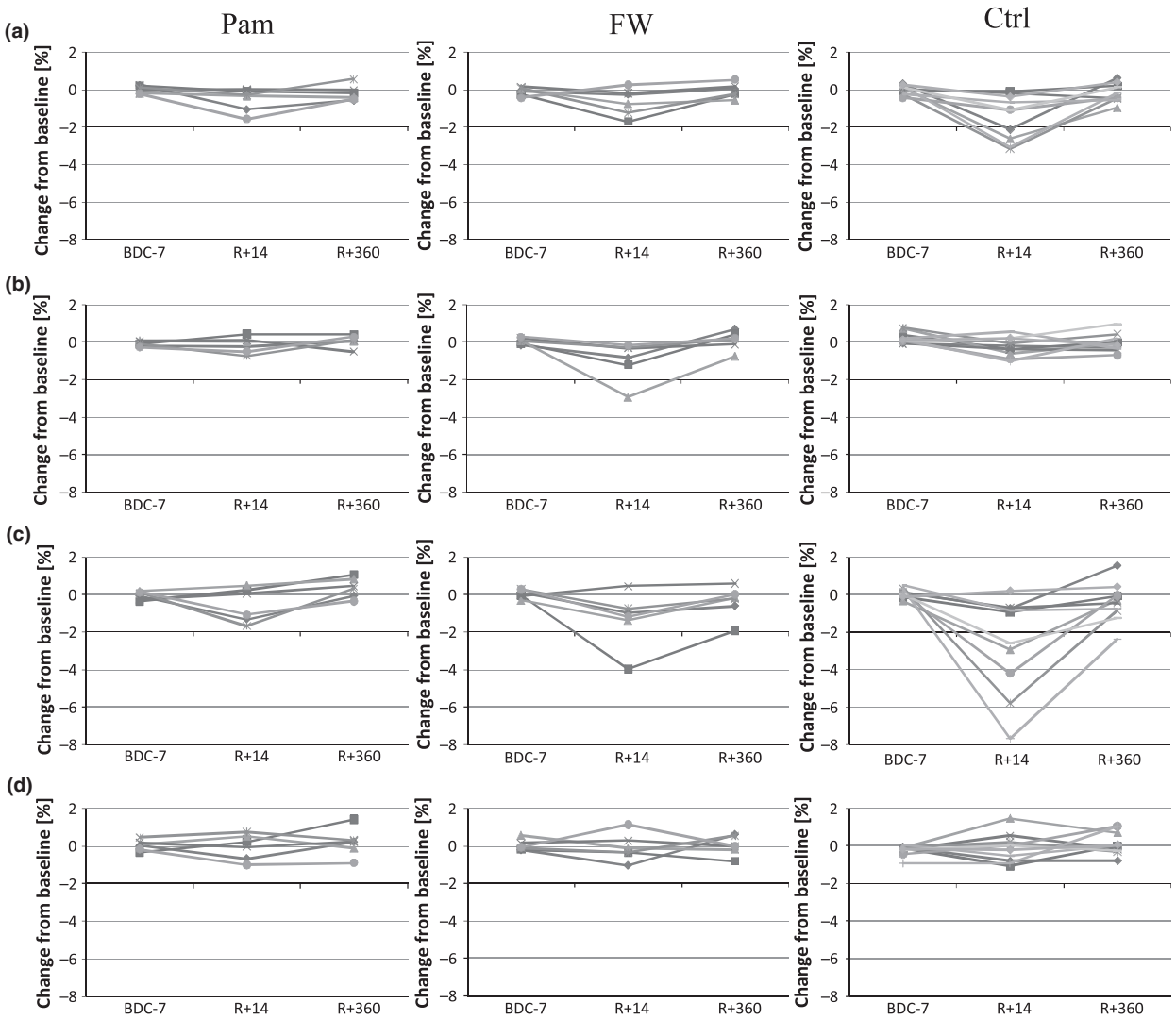

Figure 6 Individual percentage changes in CoD at tibia diaphysis (66%) in Pam (left column), FW (middle column) and Ctrl (right column) at four anatomical sectors: posterior (a), medial (b), anterior (c) and lateral (d) at base line (mean of BDC-14 and BDC-7), R + 14 and R + 360.

cortical density is shown to decline by 7–8% during the first year after spinal cord injury (Frey-Rindova *et al.*, 2000). Later, this density deficit may reduce back to some percents only while substantial (>30%) cortical thinning ensues (Eser *et al.*, 2004). This initial reduction in cortical density among paralysed persons is basically concordant what was seen in the present study. Apparently, the 3-month duration of the present bed rest intervention was too short to result in substantial reduction in cortical area, at least to the extent that could be reliably captured by low resolution pQCT.

Trabecular loss was much more substantial in magnitude and rather uniform without a distinct indication of direction-specific loss. The largest (∼9%) bone loss in the anterior sector and relative insensitivity of the medial loss to any countermeasure may be noted, however. In general, substantial trabecular bone loss is a well-known consequence of bone disuse (Sievänen, 2010). However, the specific nature of disuse, e.g. in bed rest studies, the body position and the total duration (0° and 35 days in (Rittweger *et al.*, 2009), 0° and 56 days in (Armbrecht *et al.*, 2010), and −6° head tilt down and 90 days in LTBR study (Rittweger *et al.*, 2005)) may account for the results.

Somewhat surprisingly, the most effective countermeasure against bed rest–induced trabecular loss appeared to be the flywheel exercise, not antiresorptive medication. This observation could stipulate the speculation whether incident mechanical loading is necessary for such a medication to be effective. Among ambulatory postmenopausal women, however, there is no indication for interaction between bisphosphonate treatment and exercise intervention; both interventions showed anticipated effects independently without interaction (Uusi-Rasi *et al.*, 2003). The time course of the bone losses in the present pamidronate group suggests that there was good efficacy in the first 4 weeks and that further injections might have led to better maintenance of bone mass (Rittweger *et al.*, 2005).

The strengths of the present study are the randomized prospective design with three interventional arms and a long follow-up period: the knowledge on the baseline variance which helped to put the observed skeletal responses in proper context; the sector-specific analysis of bone structure based on apparent ankle biomechanics; and the application of a new image processing method known to reduce noise in pQCT images (Cervinka *et al.*, 2010). It is known that the use of different thresholds and analysis methods can affect the results of pQCT studies (Kontulainen *et al.*, 2007) that may also account for the above-mentioned discrepant results observed in the present study compared to other bed rest studies (Rittweger & Felsenberg, 2009; Rittweger *et al.*, 2009, 2010; Armbrecht *et al.*, 2010).

The bed rest studies undoubtedly provide a useful model for bone loss (Pavy-Le *et al.*, 2007), but some inherent limitations of these demanding studies need to be recognized. First, the group sizes are deemed quite limited because of obvious challenges in financial, technical and practical execution of the intervention. Second, besides the small number of subjects in each study group, subject background characteristics within and between the groups can be heterogeneous, which may increase the variance in responses, confound their interpretation and further compromise the power of the study to detect between-group differences with statistical confidence. Therefore, many results obtained from bed rest studies remain descriptive and indicative only rather than conclusive in the statistical sense. Third, the bed rest intervention was too short to result in measurable changes in cortical area, but on the other hand, 3 months being bound to bed is close to maximum among healthy volunteers. Fourth, during the recovery, the assessment of biomechanical environment was based on coarse qualitative description because of lack of specific quantitative data on bone loading (the type, amount and intensity of physical activity of particular subjects were not known). Notwithstanding these limitations, observations from bed rest interventions because of their specific and well-controlled design are of utmost importance in unravelling not only the skeletal responses to disuse but also the effects of accompanying countermeasures on disuse-induced bone loss.

Keeping the inherent statistical limitations of bed rest interventions in mind, it is concluded that the sector-specific analysis of bone cross-sections has potential to reveal skeletal responses to various interventions that cannot be inferred from the average analysis of the whole bone cross-section. This approach is considered particularly useful for evaluating the responses from the biomechanical point of view and thus strengthening the interpretation of bone data from this relevant aspect of bone physiology.

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