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AUTHOR(S)

Daniel Belavy, G Beller, Z Ritter, D Felsenberg

PUBLICATION DATE

01-09-2011

HANDLE

10536/DRO/DU:30071015

J Musculoskelet Neuronal Interact 2011; 11(3):215-226

Original Article

Hylonome

Bone structure and density via HR-pQCT in 60d bed-rest, 2-years recovery with and without countermeasures

D.L. Belavý, G. Beller, Z. Ritter, D. Felsenberg

Charité Universitätsmedizin Berlin, Center of Muscle and Bone Research, Hindenburgdamm 30, 12203 Berlin, Germany

Abstract

We examined the effects of bed-rest, recovery and exercise countermeasures on bone density and structure at the distal tibia and radius as measured via high-resolution peripheral computed tomography. 24 subjects underwent 60-days of head-down tilt bed-rest and performed either resistive vibration exercise (RVE; *n*=7), resistive exercise only (RE; *n*=8) or no exercise (*n*=9; 2nd Berlin BedRest Study; BBR2-2). Measurements were performed regularly during and up to 2-years after 60d bed-rest. At the distal tibia marked reductions in cortical area, cortical thickness and bone density but increases in periosteal perimeter and trabecular area were seen (p all<0.001). Recovery of most parameters occurred within 180d after bed-rest. At the distal radius, persistent increases in cortical area, cortical thickness, cortical density and total density and decreases in trabecular area were seen (p all≤0.005). A significant effect of RVE (p=0.003), but not RE, was seen on cortical area at the distal tibia, with few effects of the countermeasures observed on the remaining parameters. The current study represents the first implementation of high-resolution peripheral computed tomography in bed-rest in male subjects and helps to understand the patterns of bone remodeling due to bed-rest and recovery.

Keywords: micro-CT, 3D-pQCT, Spaceflight, Immobilization, Training

Introduction

Prolonged bed-rest is a methodology used by space agencies to simulate the physiological effects of spaceflight on the human body[1]. Whilst an underlying aim of such studies is to assess potential countermeasures against the changes in the human body seen in spaceflight, astronauts and cosmonauts are a relatively small and specialised clientele. Bed-rest represents, however, extreme inactivity[2]. With many patients subject to immobilization, inactivity or bed-rest due to medical conditions or medical interventions, an overarching aim of bed-rest studies is to understand the effects of "inactivity" on the human body.

One of the main organ systems affected by bed-rest is bone. Bone loss in bed-rest is typically characterised by reduction in bone mass and density in the "load-bearing" regions (legs, hip, and pelvis), with comparatively less bone loss in body regions where the loading patterns in bed-rest or spaceflight are less affected (such as the wrist and skull)[3,4]. Investigations in bed-rest and spaceflight on bone to date have largely been restricted to assessment of its mass and density. Whilst adequate bone density is considered critical for resistance to bone fracture, other factors, such as the structure of bone are also important. The organisation of bone tissue has an important impact on its mechanical properties[5]. With recent advances in scanning technology, assessment of bone structure has become possible.

High-resolution peripheral computed tomography (HR-pQCT) has been implemented in recent years to provide high resolution (<100 μm thickness) scans of bone at the distal tibia and distal radius. In addition to more mundane parameters of bone density, HR-pQCT can provide information on a number of bone structure parameters, such as cortical thickness and trabecular number in different regions of bone, such as the inner-trabecular region, trabeculae closer to the endocortical surface and the bone cortex[6-12]. Some of these parameters, such as cortical thickness, have been shown by prior work to relate directly the mechanical properties of bone[13]. To date, HR-pQCT has been used to investigate the effects of pharmaceu-

Dieter Felsenberg has consultancies for European Space Agency and Novotec Medical GmbH (no financial interest in this consultancy). All other authors have no conflicts of interest.

Corresponding author: Daniel L. Belavý B.Phty, PhD, Zentrum für Muskel- und Knochenforschung, Charité Campus Benjamin Franklin, Hindenburgdamm 30, D-12203 Berlin, Germany
E-mail: belavy@gmail.com

Edited by: F. Rauch
Accepted 12 July 2011

tical intervention on bone structure[14-16], changes in bone structure with weight gain in anorexia[17], as well as the association of physical activity in youth[18] and age in the elderly[19] on bone structure in cross-sectional studies. To the best of our knowledge, however, there have been no published prospective studies on the effects of physical inactivity or exercise on bone structure in men, as measured by HR-pQCT.

The 2nd Berlin BedRest Study[20,21] was implemented to investigate the effects of inactivity and two different countermeasure exercise protocols on changes in male subjects during 60d bed-rest. The primary outcome measure of this study was bone mass at the distal tibia, as measured with conventional peripheral quantitative computed tomography[21]. However, an additional aim of our research group is to understand the effects of inactivity on bone as measured with HR-pQCT. In the current work, we address this goal using HR-pQCT measurements in prolonged (60d) bed-rest in male subjects with and without exercise interventions.

Materials and Methods

Bed-rest protocol and subjects

Details of the study protocol have been published elsewhere[20]. However, in brief: 24 medically and psychologically healthy males participated in the 2nd Berlin BedRest Study which was conducted at the Charité Campus Benjamin Franklin in Berlin, Germany, by the Centre for Muscle and Bone Research from 2007 to 2010. The subjects had no history of bone or joint disease and were not osteopenic or osteoporotic (i.e. lumbar spine and hip dual X-ray absorptiometry >-1.5 SD and lumbar spine trabecular bone density on quantitative computed tomography >120 mg/ml). Further inclusion and exclusion criteria are listed elsewhere[20]. Subjects attended the facility for nine days of baseline data collection (BDC-9 to BDC-1), underwent 60-days of six-degree head-down tilt bed-rest (HDT), remained in the facility for seven days after reambulation (R+1 to R+7) and returned to the facility 14, 30, 180, 360 and 720 days after reambulation (R+14, R+30, R+90, R+180, R+360 and R+720) for follow-up testing. The study was approved by the ethical committee of the Charité Universitätsmedizin Berlin and the radiological examinations were approved by the Bundesamt für Strahlenschutz. All subjects gave their informed written consent prior to participation in the study.

Subjects were randomized to three different groups: one that performed resistive exercises with whole-body vibration during bed-rest (RVE; n=7), one that performed resistive exercise only (RE; n=8) and one that performed no exercise and served as a control group (CTR; n=9)[20].

Countermeasure exercise during bed-rest

The countermeasure exercise protocol is discussed in detail elsewhere[20]. In brief, however, exercise maneuvers were chosen to target those load-bearing regions of the body that are most affected by bed-rest (i.e. lower-quadrant). Training was performed three days a week during the HDT-phase. After a short warm up, the following exercises were performed on the Galileo Space exercise device (Novotec Medical GmbH, Pforzheim, Germany; Figure 1): bilateral squats (~75-80% of pre bed-rest maximum voluntary contraction: in RVE-group vibration frequency 24 Hz, amplitude 3.5-4 mm, peak acceleration ~8.7 g where g=9.81 ms^{-2}), single leg heel raises (~1.3 times body-weight; in RVE-group vibration frequency 26Hz, amplitude 3.5-4 mm, peak acceleration ~10.2 g), double leg heel raises (~1.8 times body-weight; in RVE-group vibration frequency 26 Hz, amplitude 3.5-4 mm, peak acceleration ~10.2 g), back and heel raise (performing hip and lumbar spine extension against gravity with ankle dorsiflexion but with ~1.5 times body-weight applied at the shoulders; in RVE-group vibration frequency 16 Hz, amplitude 3.5-4 mm, acceleration ~3.9 g). The RVE-group performed the same exercises as the RE-group except that whole-body vibration was applied[20]. Note that the acceleration parameters stated refer to the acceleration of the platform itself, effective accelerations on the subject are much lower. The maximum resulting ground reaction forces transmitted to the feet of the subjects result in effective acceleration at the feet in the order of 0.7 g (unpublished observations).

High-resolution peripheral computed tomography

The left distal forearm and left distal tibia of the volunteers were measured with a high resolution HR-pQCT system (XtremeCT; Scanco Medical AG, Bassersdorf, Switzerland)[6-12] to assess density and micro-architecture of the bone tissue. If any conditions (e.g. prior fracture) were found which precluded a valid measurement, the right side was measured for the duration of the study. Measurements were performed by the same operator 3-days prior to the bed-rest (BDC), on day-30 (HDT30) and day-59 (HDT59) during the bed-rest phase and day-3, -15, -30, -90, -180, -360 and -720 in the post-bed-rest recovery phase (R+3, R+15, R+30, R+90, R+180, R+360 and R+720). A total scan length of 9.02 mm in axial direction divided into 110 slices was simultaneously measured with a nominal isotropic resolution of 82 μm over a scan time of 2.8 minutes. The standard patient settings were applied concerning effective energy, x-ray tube current and matrix size (60 kVp, 95 mAs and 1536x1536 pixels, respectively). Total effective dose was less than 3 μSv per scan. The standard control files for the tibia and radius were used.

In order to achieve a high reproducibility, the subjects were positioned carefully in a formed carbon fiber cast and fixated in the gantry. Measurements were performed in supine lying. An anteroposterior scout view was used to determine the start position of the measurement. A reference line was positioned at a certain landmark in the joint space and the measurement started 9.5 mm and 22.5 mm proximal to this line in the radius and tibia respectively. A number of criteria were used to determine whether a measurement was valid or if it had to be repeated directly. In some cases a certain measurement day could not be included in further analysis as no valid measurement was achieved. If a subject moved during the image acquisition the segmentation of bone and soft tissue can be affected. Therefore the measurements were validated and classified as follows:

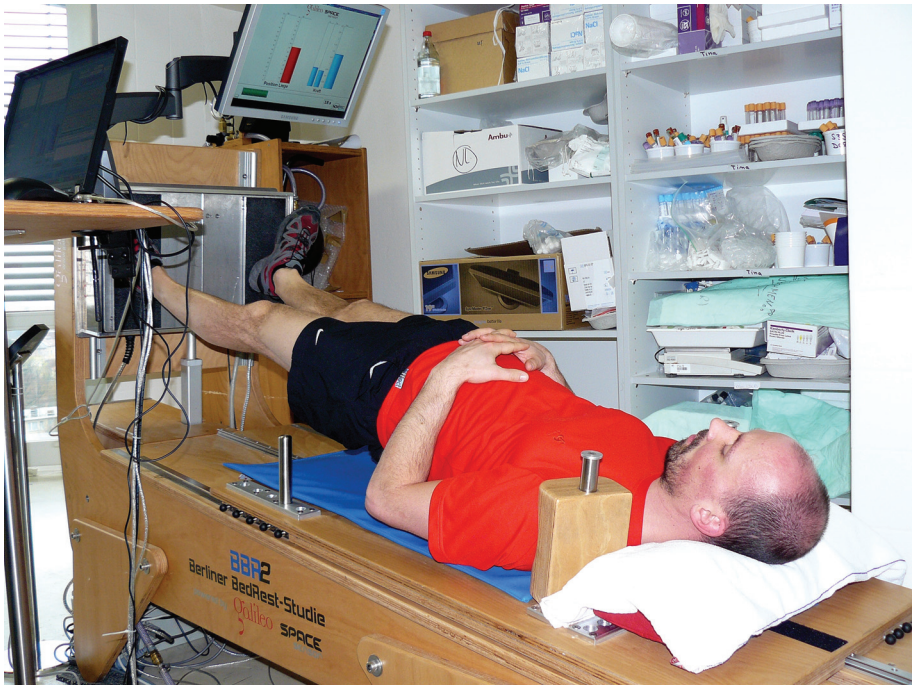

Figure 1. Countermeasure exercise training. Both the resistance exercise only (RE) and resistance exercise with whole-body vibration (RVE) groups performed their exercises on the specially designed Galileo Space exercise device. Subjects were positioned in head-down tilt on a moveable platform with shoulder pads and hand grips preventing downward movement and permitting application of force via the platform. A pneumatic system (not shown) generated the force, applied through the moveable platform, against which the subject needed to resist and move (via the shoulder pads and hand grips). The feet were positioned either side of a platform which could be set to vibrate in the RVE group. Subjects were given visual feedback of their actual and target position in the exercise via a monitor placed in the subjects' field of view. Here the subject is performing the combined back lift and ankle dorsiflexion exercise.

- The measurement shows no signs of motion artifacts (grade 1)
- A minimal non-sharpness of the contours can be indicated (grade 2)
- The measurement is significantly blurred (visible artificial lines inside the image; grade 3).
- The contours of the cortical structures are discontinued and broken. The results of the measurement are not valid (grade 4).

Only images with grades of three or less were considered valid and hence used in final analyses. Another criterion for an invalid measurement was an overlap of less than 80% in the length of the region scanned in follow-up measurements compared to that of the baseline measurement (common region).

For evaluation, the bone volume is separated from the surrounding soft tissue by a threshold based contour algorithm. The standard measurement protocol (software version number 6.0G) was used for image analysis. The following parameters were calculated using this protocol: density of the entire measurement volume, density of the cortical and trabecular regions, density of the outer 40% of the trabecular sub-region, density of the inner 60% of the trabecular sub-region and thickness of the cortical layer. Cortical area, trabecular area and the circumference of the outer cortical perimeter were calculated. The number of trabeculae, trabecular thickness, trabecular separation and the index of trabecular network inhomogenity were also calculated within the entire trabecular region.

The sample size estimates of the 2nd Berlin BedRest Study were based upon distal tibia bone mineral content as measured by conventional peripheral quantitative computed tomography (pQCT)[21] and was not powered to the HR-pQCT measurements considered in the current investigations. As there is limited data on the HR-pQCT data in bed-rest with and/or without countermeasures, it was difficult to conduct a sensitivity analysis for this study. Thus, we consider the current work to be an exploratory study for the comparison between CTR, RE, RVE.

Statistical analyses

To evaluate the effect of bed-rest, recovery and the impact of countermeasures, linear-mixed effects models[22] were used. For each of the outcome parameters, analysis was first conducted on absolute-values with 'group' (RVE, RE, CTR) and 'study-date' (BDC, HDT30, HDT59, R+3, R+15, R+30, R+90, R+180, R+360 and R+720) main effects and their interaction. Allowances were made for heterogeneity of variance for study-date and group with random effects for each subject. Where there the group×study-date interaction showed a p-value less than 0.05, further testing was done: (a) the models were repeated using data on percentage change compared to baseline to try to rule out potential effect of subtle differences in baseline data between groups

Group	Age (years)	Weight (kg)	Height (cm)
CTR	33.1(7.8)	80.6(5.2)	181.3(6.0)
RE	31.1(5.1)	75.0(12.8)	179.3(7.7)
RVE	32.2(10.4)	81.5(6.2)	179.6(5.8)

Values for age, weight and height are mean(SD). CTR: inactive control group; RE: resistive exercise only group; RVE: resistive exercise with whole-body vibration group. There were no differences between groups for any of these variables (F all<1.2, p>.33).

Table 1. Baseline subject characteristics.

Parameter			
Abbreviation and unit	**Description**	**Tibia**	**Radius**
CortArea [mm²]	Cortical area	158.3(23.2)	71.2(11.3)
Ct.Pm [mm]	Cortial periosteal perimeter	117.0(11.4)	81.1(6.5)
Ct.Th [μm]	Cortical thickness	1.4(0.2)	0.9(0.1)
D100 [mg/cm³]	Total density	327.3(53.3)	337.6(53.8)
Dcomp [mg/cm³]	Cortical density	886.1(32.0)	876.7(31.6)
Dinn [mg/cm³]	Density of inner 40% of trabecular region	156.5(37.1)	149.5(41.2)
Dmeta [mg/cm³]	Density of outer 60% of trabecular region	260.8(38.1)	237.2(36.0)
Dtrab [mg/cm³]	Trabecular density	198.7(37.1)	185.3(38.5)
TrabArea [mm²]	Trabecular area	722.8(169.7)	276.7(55.3)
Tb.1/N.SD [μm]	Trabecular network inhomogeneity	0.18(0.04)	0.19(0.03)
Tb.N [mm-1]	Trabecular number	2.04(0.23)	1.80(0.17)
Tb.Sp [μm]	Trabecular separation	0.42(0.07)	0.47(0.06)
Tb.Th [μm]	Trabecular thickness	0.08(0.01)	0.09(0.01)

Values are mean(SD). N=24. For changes over time for all subjects pooled, see Figures 3 and 4. For baseline data in each group and changes within each group, see Tables 3 and 4.

Table 2. Baseline HR-pQCT parameters for all subjects.

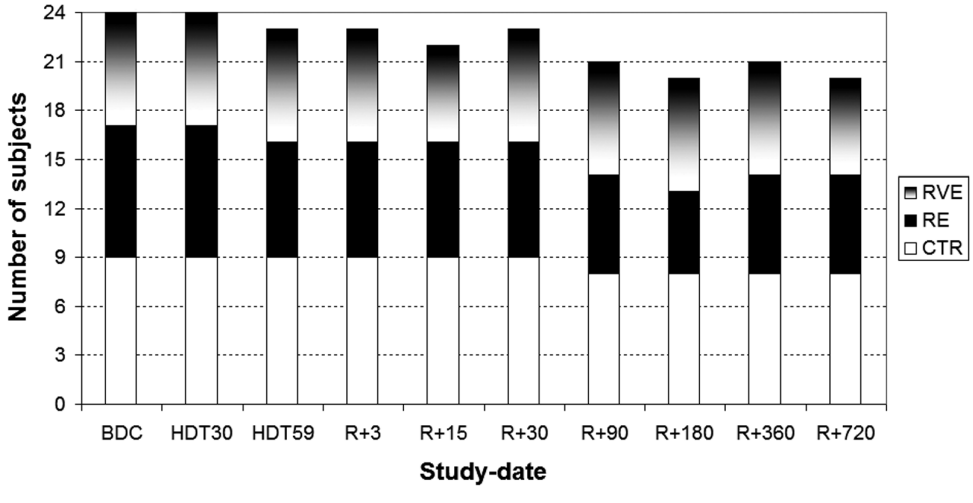

Figure 2. Number of HR-pQCT data sets available on each study-date. CTR: inactive control group, RE: resistive exercise only group, RVE: resistive exercise with whole-body vibration group. BDC: baseline data collection 3 days prior to bed-rest, HDT: day of head down tilt bed-rest, R+: day of recovery. One subject (RE group) dropped out after the 30th day of bed-rest for medical reasons unrelated to the investigations reported here. Note the values given are for the tibia measurements. For the radius, data of sufficient quality could not be obtained from one subject each on HDT30 (RVE group), R+3 (RVE group) and R+720 (CTR group). For more information on image quality, see text.

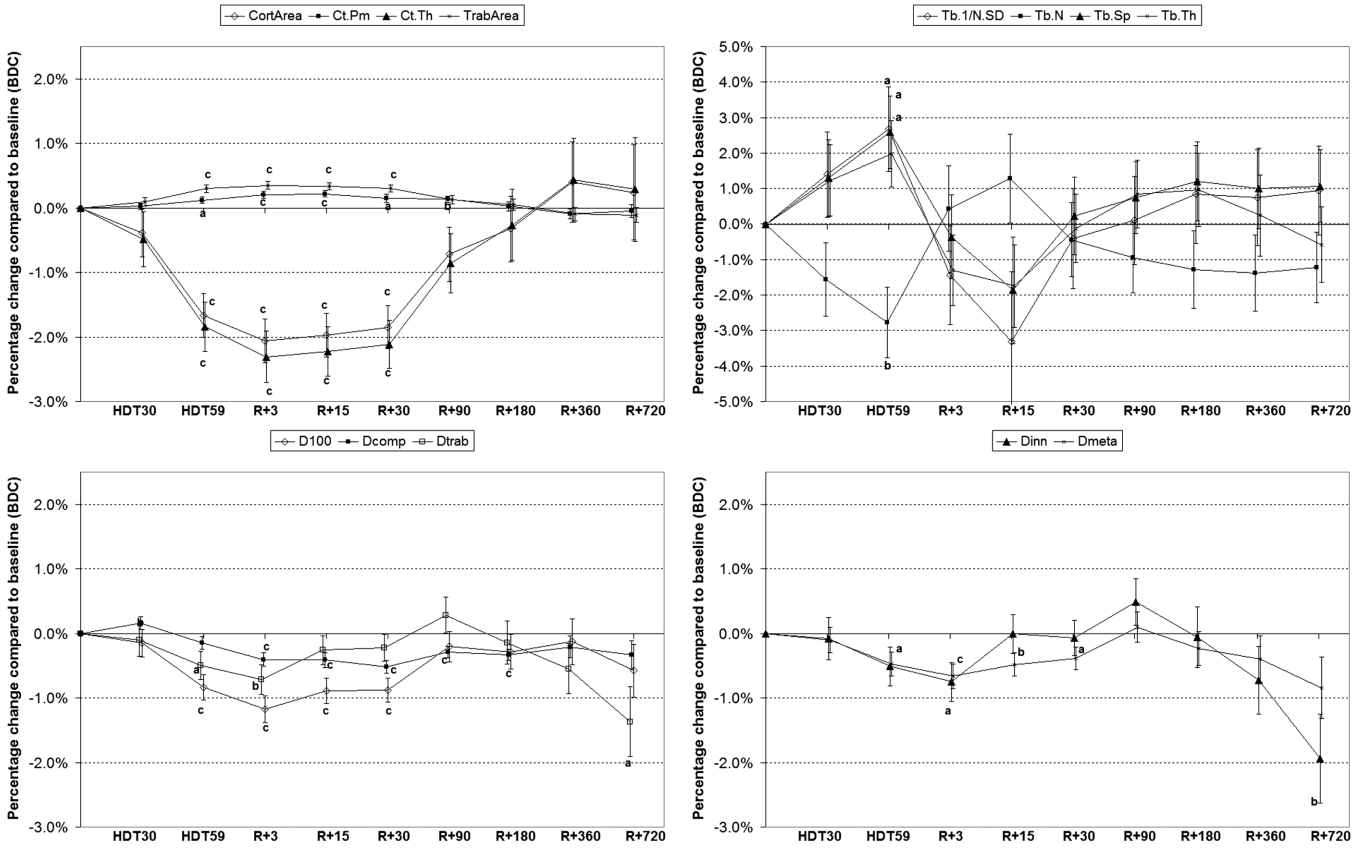

Figure 3. Distal tibia HR-pQCT parameters during and after bed-rest pooled across all subject groups. Values are mean(SEM) percentage change compared to baseline (significance indicated by: a: $p<0.05$; b: $p<0.01$; c: $p<0.001$). All parameters showed evidence on ANOVA ($p<0.01$) for changes over the course of the study in this pooled data set. All analyses performed on absolute values. Analysis of percentage change data yields same statistical results (data not shown). HDT: day of head down tilt bed-rest, R+: day of recovery. For CortArea, Ct.Th, Tb.1/N.SD, Tb.N, Tb.Sp and Tb.Th there was some statistical evidence for a different response in the three subject groups (see text and Table 3) for further details. See Table 2 for parameter name descriptions.

and, then, (b) if the effect still persisted, subsequent two-group models (i.e. CTR vs. RE, CTR vs. RVE and RE vs. RVE) using percentage change data were conducted to examine which group(s) could have been responsible for the effect. Furthermore, *apriori* contrasts comparing days HDT30 and beyond to baseline within each group and also for all groups pooled. An alpha level of 0.01 was used for statistical significance for the 'study-date' main-effect and 'group×study-date' interaction on ANOVA, and *p*-values for these model terms being less than 0.05 but greater than 0.01 were considered trends. For analysis of changes during and after bed-rest, as multiple measurement sessions were undertaken on the same subjects, a Bonferroni adjustment was not performed, rather we looked for consistent "significant" differences across time points. Subject age, height and weight had little influence on the findings if they were incorporated in the models as linear co-variates and were hence excluded from the analyses presented here. The statistical analyses were performed with the "R" environment for statistical computing and graphics version 2.10.1 (www.r-project.org).

Results

Subject baseline anthropometric data are given in Table 1. The numbers of subjects whose data were available for analyses are presented in Figure 2. The median(min-max) common-region at the tibia was 94(81-97)% and 91(87-95)% at the radius. It was typically easer to obtain images free of movement artefacts from the tibia: 215 images were graded as quality-1, six as quality-2, zero as quality-3 and one as quality-4, whereas at the radius: 115 images were graded as quality-1, 88 as quality-2, 15 as quality-3 and four as quality-4. For the tibia, inclusion of the six quality-2 images in the statistical analyses strongly influenced the results for trabecular separation and trabecular number, but not other parameters. These data points from these six quality-2 images were obvious outliers compared to the remaining data points from each subject (data not shown), were hence excluded from further statistical analysis and only data from images of quality-1 were included in the analyses presented here. For the radius, the quality of

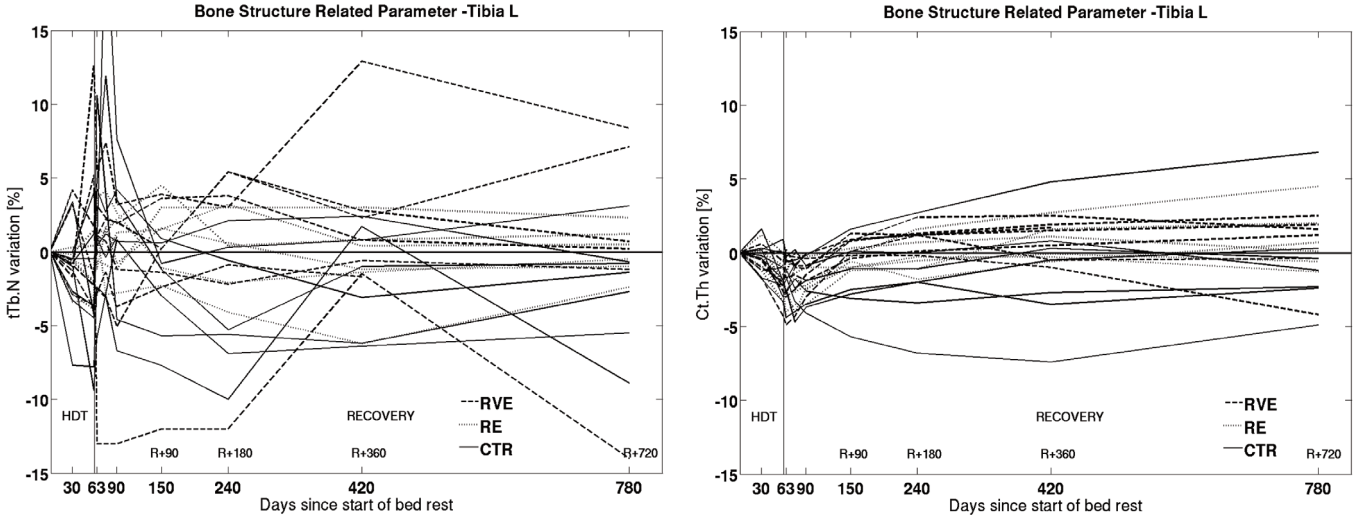

Figure 4. Raw data on cortical thickness (top) and trabecular number (bottom). Values are changes in each subject in each group expressed as percentage change compared to baseline. Vertical line indicates end of bed-rest phase. CTR: inactive control group; RE: resistive exercise only group; RVE: resistive exercise with whole-body vibration group. We consider it unlikely that the much higher variability of the trabecular number (Tb.N) parameter was due to actual physical processes, rather due to measurement error.

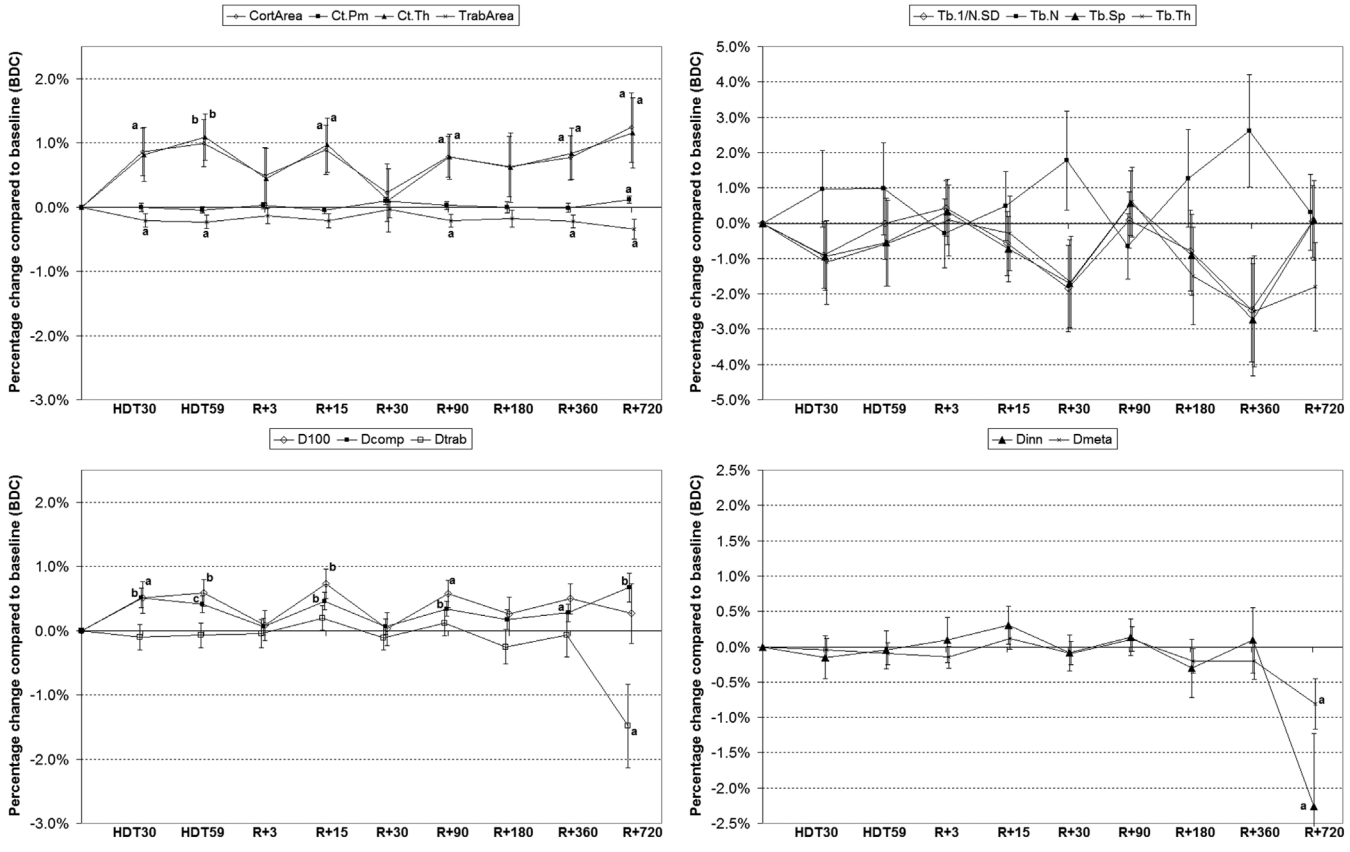

Figure 5. Distal radius HR-pQCT parameters during and after bed-rest pooled across all subject groups. Values are mean(SEM) percentage change compared to baseline (significance indicated by: a: $p<0.05$; b: $p<0.01$; c: $p<0.001$). All analyses performed on absolute values. Analysis of percentage change data yields same statistical results (data not shown). HDT: day of head down tilt bed-rest, R+: day of recovery. There was no evidence for differences in the response of the different subject-groups; see text and Table 4 for further details. See Table 2 for parameter name descriptions.

images had limited effect on the outcome of statistical analysis (data not shown) and hence images of quality 1, 2 and 3 were included in the analyses presented here.

Effect of bed-rest and recovery

At the distal tibia all parameters showed evidence on ANOVA ($p<0.01$; Figure 3) for changes over the course of the study. Cortical area and thickness decreased during bed-rest (HDT59: $p<0.001$) and remained so up until R+30 ($p<0.001$) with the reductions at R+90 and R+180 no longer being significant. Cortical perimeter ($p=0.017$) and trabecular area ($p<0.001$) increased significantly by HDT59 and remained so until R+180 and the sustained increase at R+180 was significant in cortical perimeter ($p=0.006$) but not trabecular area ($p=0.066$). Total density decreased significantly by HDT59 ($p<0.001$) with this reduction remaining significant up to and including R+30 ($p<0.001$). Cortical density first showed significant reductions 3d after bed-rest (R+3; $p<0.001$) with this significant ($p<0.001$) effect persisting up until R+180. At R+360 ($p=0.26$) and R+720 ($p=0.14$) the reduction of cortical density was no longer significant. Reductions of trabecular density reached significance at HDT59 ($p=0.028$) with this reduction persisting significantly only up until R+3 ($p=0.002$). The two sub-components of trabecular density, density of the inner 40% (Dinn) and outer 60% (Dmeta) of the trabecular network also showed reductions with Dinn being significantly reduced at R+3 only ($p=0.013$). Whilst Dmeta showed a similar pattern of loss as Dinn, reductions in this former parameter were significant from HDT59 to R+30 ($0.0007\leq p\leq 0.027$). At R+720, both Dtrab ($p=0.013$) and Dinn ($p=0.005$) were decreased significantly, and these effects persisted if subjects with incomplete data sets were excluded from analyses (data not shown). Whilst trabecular number decreased significantly ($p=0.006$) at HDT59 whilst trabecular network inhomogeneity, trabecular separation and trabecular thickness were increased at the same time-point ($0.012\leq p\leq 0.034$), these parameters were typically associated with high variability and no clear pattern of recovery could be discerned as in the other HR-pQCT parameters (Figures 3 and 4).

At the distal radius, cortical area, cortical thickness, total density, and cortical density increased and trabecular area decreased at a number of time points from HDT30 until the end of the study (Figure 5). Whilst the study-date main effect on ANOVA of the absolute values for these effects was significant for cortical density only ($p=0.002$) and not for the remaining parameters ($p\geq 0.070$), ANOVA of the data as percentage change compared to baseline showed that the changes in these parameters were indeed significant ($p\leq 0.005$). For cortical perimeter, ANOVA indicated a trend for a change at some stage over the course of the study ($p=0.031$), but this effect was restricted to the final measurement date. No statistically significant changes ($p\geq 0.23$) were observed on ANOVA for trabecular density, its sub-components (Dinn and Dmeta) or on the trabecular network structure parameters at the distal radius. The "significant" effects seen at R+720 on distal radius cortical perimeter, trabecular density and its subcomponents (Dinn and Dmeta) did not persist if subjects with incomplete data sets were excluded from analyses (data not shown).

Effect of countermeasures

At the distal tibia, evidence was apparent from ANOVA for a different response between the three subject groups for cortical area ($p=0.003$), cortical thickness ($p=0.023$), trabecular network inhomogeneity ($p<0.001$), trabecular number ($p<0.001$), trabecular separation ($p<0.001$) and trabecular thickness ($p=0.004$; Table 3). For cortical area, the RVE group showed less loss than the CTR ($p=0.034$) and RE ($p=0.003$) groups. For the trend observed for cortical thickness, the RVE group showed less loss than the CTR group ($p=0.024$), whereas this effect did not reach significance in the RE-group (vs. CTR $p=0.557$). For the parameters of trabecular network inhomogeneity, trabecular number, trabecular separation and trabecular thickness, significant differences on two-group ANOVAs comparing the RVE to CTR ($p\leq 0.005$) as well as RE to CTR ($p\leq 0.018$), but not RVE and RE ($p\geq 0.13$) were seen. Where significant changes were found within the CTR group, increases in network inhomogeneity, decreases in trabecular number, increases in trabecular separation, and increases in trabecular thickness were observed (Table 3). The RVE and RE groups typically did not show these changes, or show them to the same extent.

At the distal radius, no significant differences between groups were seen in the response during bed-rest and recovery ($p\geq 0.052$; Table 4).

Discussion

To our knowledge, the current study is the first to prospectively evaluate changes in bone using HR-pQCT associated with physical activity in men: during and after prolonged bed-rest with and without exercise countermeasures. In addition to the evaluation of bone density in various regions of the bone in the distal tibia and distal radius, HR-pQCT permits the evaluation of bone structure parameters such as cortical thickness and area, periosteal perimeter length and also trabecular number, area and separation.

In terms of the effect of bed-rest, the HR-pQCT parameters at the distal tibia showed greater changes than the distal radius. This is a finding consistent with data from other measurement modalities such as dual X-ray absorptiometry[3,4] and peripheral quantitative computed tomography (pQCT)[23,24] which consistently show that bones in load-bearing regions (such as the tibia) show a greater extent of changes during bed-rest than non-load bearing regions (such as the forearm). At the tibia, reductions in cortical area and cortical thickness were seen during bed-rest whilst trabecular area and the cortical periosteal perimeter increased concurrently. This finding of increased trabecular area is consistent with findings during bed-rest using conventional pQCT at the distal tibia[23], although in this prior work, no reductions in cortical area were seen. Also, as could be expected from prior work, reductions in bone density were seen at the distal

Table 3. Distal tibia HR-pQCT parameters during and after bed-rest in each group.

Group	Study-date									
	BDC	**HDT30**	**HDT59**	**R+3**	**R+15**	**R+30**	**R+90**	**R+180**	**R+360**	**R+720**
					CortArea [mm²] #; †; e ƒ					
CTR	167.3(22.6)	-0.6(2.4)%	-1.9(2.1)%b	-2.4(2.1)%b	-2.5(2.1)%c	-2.5(2.1)%c	-2.1(2.4)%a	-1.6(2.9)%	-0.6(4.1)%	-0.5(4.0)%
RE	160.1(22.4)	-0.1(0.8)%	-1.6(0.9)%c	-2.0(0.9)%c	-1.7(1.2)%c	-1.5(1.0)%c	-0.3(0.7)%	0.2(1.2)%	0.9(1.4)%	1.2(2.5)%
RVE	144.9(22.4)	-0.7(0.8)%a	-1.8(1.7)%b	-1.7(1.7)%a	-1.8(1.2)%c	-1.2(1.0)%b	0.5(0.6)%a	0.8(0.7)%b	0.9(1.3)%	0.4(2.1)%
					Ct.Pm [mm]					
CTR	118.5(11.9)	0.0(0.2)%	0.1(0.2)%a	0.2(0.2)%b	0.3(0.2)%c	0.2(0.4)%	0.2(0.2)%b	0.2(0.2)%	0.0(0.3)%	-0.2(0.4)%
RE	116.2(11.9)	0.0(0.4)%	0.1(0.3)%	0.3(0.3)%a	0.2(0.3)%a	0.1(0.4)%	0.1(0.3)%	0.1(0.4)%	-0.1(0.4)%	-0.1(0.4)%
RVE	116.0(11.9)	0.0(0.2)%	0.1(0.3)%	0.1(0.3)%	0.1(0.1)%a	0.1(0.2)%	0.1(0.2)%	-0.1(0.3)%	-0.2(0.4)%	0.2(0.4)%
					*Ct.Th [µm] *; e ƒ*					
CTR	1.4(0.2)	-0.7(2.8)%	-2.2(2.5)%b	-2.7(2.5)%b	-2.9(2.5)%c	-2.8(2.6)%b	-2.3(2.8)%a	-1.7(3.3)%	-0.6(4.3)%	-0.4(4.4)%
RE	1.4(0.2)	-0.1(0.9)%	-1.6(0.9)%c	-2.1(0.8)%c	-1.8(1.2)%c	-1.7(0.8)%c	-0.5(0.8)%	0.1(1.4)%	1.0(1.6)%	1.3(2.8)%
RVE	1.3(0.2)	-0.9(0.9)%b	-1.8(1.3)%c	-1.9(1.7)%b	-1.8(1.0)%c	-1.6(0.8)%c	0.5(1.1)%	1.1(1.4)%a	1.1(1.8)%	0.1(3.0)%
					D100 [mg/cm³]					
CTR	330.3(52.2)	-0.2(1.1)%	-1.0(1.0)%b	-1.2(1.0)%c	-1.2(1.1)%b	-1.2(1.1)%c	-0.7(1.2)%	-1.1(1.3)%a	-0.7(2.2)%	-1.3(2.4)%
RE	347.2(52.2)	0.1(0.5)%	-0.8(0.5)%c	-1.1(0.6)%c	-0.7(0.6)%b	-0.7(0.6)%b	-0.3(0.6)%	0.1(0.7)%	0.0(1.0)%	0.0(1.5)%
RVE	300.8(52.2)	-0.4(1.0)%	-0.7(1.2)%	-1.2(1.2)%b	-0.7(1.0)%	-0.6(0.8)%	0.5(1.1)%	0.4(1.3)%	0.4(1.5)%	-0.3(1.3)%
					Dcomp [mg/cm³]					
CTR	891.8(33.0)	0.1(0.4)%	-0.2(0.4)%	-0.4(0.5)%a	-0.5(0.6)%a	-0.6(0.6)%b	-0.4(0.3)%c	-0.5(0.2)%c	-0.1(0.9)%	-0.3(1.0)%
RE	886.5(33.1)	0.3(0.4)%a	-0.2(0.4)%	-0.4(0.5)%a	-0.4(0.4)%a	-0.5(0.4)%b	-0.3(0.4)%	-0.2(0.4)%	-0.3(0.5)%	-0.4(0.8)%
RVE	878.3(33.2)	0.0(0.5)%	-0.1(0.8)%	-0.4(0.6)%	-0.3(0.6)%	-0.5(0.5)%a	-0.1(0.4)%	-0.2(0.4)%	-0.2(0.6)%	-0.4(1.0)%
					Dinn [mg/cm³]					
CTR	153.0(34.9)	0.0(1.0)%	-0.7(1.1)%a	-0.5(0.9)%	0.0(1.0)%	0.0(0.7)%	0.7(1.8)%	-0.6(1.6)%	-1.1(2.3)%	-3.0(3.5)%a
RE	176.9(35.0)	0.1(1.3)%	-0.6(1.1)%	-0.9(1.2)%	-0.1(1.2)%	-0.2(1.1)%	-0.1(1.3)%	0.3(1.1)%	-0.5(1.7)%	-0.9(2.3)%
RVE	137.7(35.0)	-0.6(2.6)%	-0.1(2.3)%	-1.0(2.3)%	0.1(2.2)%	-0.1(2.1)%	0.9(2.3)%	0.3(3.5)%	-0.5(3.9)%	-1.7(3.6)%
					Dmeta [mg/cm³] #					
CTR	262.2(34.1)	-0.1(0.9)%	-0.6(0.8)%a	-0.7(0.8)%a	-0.7(0.8)%b	-0.6(0.8)%a	-0.2(1.3)%	-0.8(1.4)%	-1.2(2.2)%	-2.1(2.6)%a
RE	283.1(34.1)	0.0(0.6)%	-0.5(0.6)%a	-0.6(0.7)%a	-0.5(0.5)%a	-0.4(0.6)%	-0.2(0.6)%	-0.1(0.7)%	-0.3(1.0)%	-0.6(1.2)%
RVE	233.6(34.1)	-0.3(1.3)%	-0.3(1.4)%	-0.7(1.4)%	-0.1(1.2)%	-0.1(1.2)%	0.8(1.4)%	0.4(1.4)%	0.5(1.5)%	0.8(1.6)%
					Dtrab [mg/cm³] #					
CTR	197.2(34.2)	-0.1(0.8)%	-0.7(0.8)%b	-0.6(0.7)%a	-0.4(0.8)%	-0.3(0.7)%	0.3(1.5)%	-0.7(1.3)%	-1.2(2.1)%	-2.6(3.0)%a
RE	219.9(34.2)	0.0(0.8)%	-0.5(0.6)%a	-0.7(0.8)%b	-0.3(0.7)%	-0.3(0.8)%	-0.1(0.8)%	0.1(0.9)%	-0.4(1.3)%	-0.7(1.6)%
RVE	176.4(34.3)	-0.4(1.9)%	-0.1(1.7)%	-0.9(1.8)%	0.0(1.6)%	0.0(1.7)%	0.8(1.8)%	0.4(2.3)%	0.1(2.3)%	-0.4(2.3)%
					TrabArea [mm²]					
CTR	735.8(177.1)	0.1(0.5)%	0.4(0.4)%b	0.4(0.4)%b	0.4(0.4)%c	0.4(0.4)%c	0.4(0.4)%b	0.3(0.5)%	0.1(0.7)%	0.0(0.6)%
RE	714.2(177.1)	0.1(0.2)%	0.3(0.2)%c	0.3(0.2)%c	0.3(0.2)%b	0.2(0.2)%b	0.0(0.1)%	-0.1(0.2)%	-0.2(0.3)%a	-0.3(0.4)%a
RVE	716.0(177.1)	0.1(0.1)%a	0.3(0.3)%b	0.3(0.2)%b	0.3(0.1)%c	0.2(0.1)%c	-0.1(0.2)%	-0.2(0.2)%a	-0.2(0.3)%	0.0(0.2)%
					Tb.1/N.SD [µm] ‡; d ,e					
CTR	0.17(0.03)	2.4(6.1)%	4.1(6.3)%	-2.4(7.4)%	-6.2(12.1)%	-1.9(6.9)%	3.1(6.3)%	4.9(6.4)%a	4.5(6.1)%a	4.0(5.2)%a
RE	0.17(0.03)	-0.1(4.1)%	0.8(4.4)%	-2.5(4.9)%	-2.2(4.4)%	-2.0(4.2)%	-3.7(3.7)%a	-1.4(4.3)%	-0.8(4.2)%	-1.3(3.4)%
RVE	0.20(0.03)	1.7(8.0)%	2.3(6.7)%	0.6(6.6)%	-0.6(6.4)%	2.7(7.0)%	0.4(7.0)%	-1.5(8.0)%	-1.5(7.7)%	-0.6(7.3)%
					Tb.N [mm-1] ‡; d ,e					
CTR	2.08(0.21)	-2.5(5.0)%	-4.2(4.8)%b	0.7(5.7)%	2.4(5.5)%	-0.4(4.8)%	-3.4(4.5)%a	-4.4(4.8)%a	-4.2(4.8)%a	-3.7(4.4)%a
RE	2.11(0.21)	-0.1(4.9)%	-0.5(4.5)%	1.7(5.4)%	1.6(5.2)%	1.4(4.5)%	2.4(4.1)%	1.0(4.2)%	0.4(4.4)%	1.1(4.1)%
RVE	1.90(0.21)	-2.2(5.6)%	-3.5(5.4)%	-1.6(6.2)%	-1.1(5.7)%	-2.7(5.3)%	-1.5(5.1)%	0.2(5.4)%	0.2(5.5)%	-0.5(4.8)%
					Tb.Sp [µm] ‡; d ,e					
CTR	0.41(0.06)	2.2(5.3)%	3.9(4.9)%a	-0.5(6.6)%	-3.6(8.0)%	-0.2(4.9)%	3.3(5.2)%	4.6(5.0)%a	4.4(4.8)%a	3.7(4.0)%a
RE	0.39(0.06)	-0.3(4.4)%	0.2(4.3)%	-2.0(5.0)%	-2.1(4.8)%	-1.8(4.6)%	-2.9(4.1)%	-1.5(3.9)%	-1.0(4.4)%	-1.8(4.0)%
RVE	0.45(0.06)	2.0(6.8)%	2.9(5.7)%	1.0(5.7)%	0.5(5.4)%	2.3(5.8)%	0.9(5.8)%	-0.4(6.7)%	-1.0(7.0)%	0.2(6.2)%
					Tb.Th [µm] †; d ,e					
CTR	0.08(0.01)	2.4(4.7)%	3.3(4.2)%a	-1.3(4.8)%	-3.0(6.1)%	-0.1(4.4)%	3.3(3.7)%a	3.6(4.1)%a	2.6(4.4)%	0.7(4.4)%
RE	0.09(0.01)	-0.1(3.8)%	-0.3(3.7)%	-2.6(4.3)%	-2.2(4.0)%	-2.2(4.0)%	-3.2(3.7)%a	-0.9(3.3)%	-1.5(4.7)%	-2.4(4.6)%
RVE	0.08(0.01)	1.4(7.4)%	2.5(6.4)%	-0.2(6.1)%	0.4(5.9)%	2.0(6.1)%	2.0(6.3)%	-0.2(6.9)%	-0.9(7.5)%	-0.5(6.8)%

*Values at baseline (BDC) are mean(SD) in absolute values. Beyond this, values are mean(SD) percentage change compared to baseline (significance indicated by: a: p<0.05; b: p<0.01; c: p<0.001). "#" indicates evidence (p<0.05) for differences between groups in baseline values. Significance of group×study-date interaction from ANOVA indicated by *: p<0.05; †: p<0.01; ‡: p<0.001. "d", "e", "f" indicate, respectively, significant (p<0.05) group×study-date interactions for two-group CTR vs. RE, RVE vs. CTR and RVE vs. RE comparisons. All analyses performed on absolute values. Analysis of percentage change data yields same statistical results (data not shown). CTR: inactive control group; RE: resistive exercise only group; RVE: resistive exercise with whole-body vibration group. HDT: day of head down tilt bed-rest, R+: day of recovery. See Table 2 for parameter name descriptions.*

Table 4. Distal radius HR-pQCT parameters during and after bed-rest in each group.

Group	Study-date									
	BDC	**HDT30**	**HDT59**	**R+3**	**R+15**	**R+30**	**R+90**	**R+180**	**R+360**	**R+720**
					CortArea [mm²] #					
CTR	76.1(10.1)	0.7(2.0)%	0.9(2.0)%	0.5(2.2)%	0.6(1.8)%	0.2(2.6)%	0.4(1.7)%	0.5(3.0)%	0.0(1.6)%	1.4(1.7)%a
RE	73.5(10.1)	1.1(1.8)%	0.8(1.9)%	0.9(2.4)%	1.2(1.4)%a	0.5(2.0)%	1.2(0.4)%c	0.3(1.0)%	1.6(2.0)%a	2.4(2.6)%a
RVE	62.3(10.1)	0.9(1.5)%	1.3(1.3)%b	0.0(1.3)%	1.1(2.6)%	-0.1(1.0)%	0.9(1.5)%	1.0(1.2)%a	1.1(1.0)%b	-0.3(2.5)%
					Ct.Pm [mm]					
CTR	82.3(6.4)	0.1(0.4)%	0.0(0.2)%	0.1(0.2)%	0.1(0.2)%	0.2(0.3)%	0.1(0.3)%	0.1(0.5)%	0.0(0.4)%	0.2(0.2)%a
RE	82.5(6.4)	-0.1(0.3)%	-0.1(0.3)%	0.0(0.2)%	-0.2(0.3)%	0.1(0.2)%	-0.1(0.2)%	-0.2(0.2)%	0.0(0.3)%	0.0(0.3)%
RVE	78.0(6.4)	0.0(0.3)%	0.0(0.3)%	-0.1(0.2)%	-0.1(0.2)%	0.0(0.2)%	0.1(0.3)%	0.0(0.3)%	-0.1(0.3)%	0.1(0.1)%a
					Ct.Th [μm]					
CTR	0.9(0.1)	0.5(2.0)%	1.0(1.7)%	0.2(2.2)%	0.5(2.0)%	0.0(2.3)%	0.3(1.6)%	0.3(2.4)%	0.0(1.7)%	1.2(2.1)%
RE	0.9(0.1)	1.3(2.1)%	1.1(1.7)%	0.9(2.3)%	1.4(2.0)%	0.3(2.4)%	1.5(1.6)%a	0.4(2.4)%	1.7(1.8)%a	2.4(2.2)%b
RVE	0.8(0.1)	0.8(2.2)%	1.3(2.0)%	0.1(2.5)%	1.2(2.2)%	0.0(2.7)%	0.7(1.9)%	1.1(2.8)%	1.1(2.1)%	-0.3(2.5)%
					D100 [mg/cm³]					
CTR	340.6(55.0)	0.3(0.9)%	0.6(1.1)%	0.2(1.4)%	0.6(1.0)%	-0.1(1.5)%	0.4(0.8)%	0.1(1.4)%	-0.2(1.5)%	0.3(2.0)%
RE	348.7(55.1)	0.6(1.1)%	0.2(1.2)%	0.2(1.6)%	0.6(1.1)%	0.0(1.3)%	0.6(1.1)%	-0.2(0.8)%	0.7(1.0)%	0.8(1.7)%
RVE	321.2(55.0)	0.6(1.2)%	0.8(0.8)%a	0.5(0.7)%	0.7(1.4)%	0.1(0.5)%	0.7(1.0)%	0.6(0.6)%b	0.6(0.9)%	-0.7(2.1)%
					Dcomp [mg/cm³]					
CTR	886.4(31.9)	0.3(0.8)%	0.4(0.7)%	0.0(0.8)%	0.3(0.6)%	0.1(0.7)%	0.2(0.5)%	0.1(0.9)%	0.1(0.6)%	0.9(0.8)%b
RE	873.9(31.9)	0.4(0.7)%	0.4(0.7)%	0.2(0.9)%	0.5(0.7)%	0.0(0.6)%	0.3(0.5)%	-0.1(0.6)%	0.3(0.7)%	0.8(0.9)%a
RVE	867.0(31.7)	0.7(0.8)%a	0.5(0.6)%a	0.2(0.4)%	0.4(0.7)%	0.1(0.4)%	0.5(0.6)%a	0.4(0.2)%c	0.6(0.5)%b	0.2(0.9)%
					Dinn [mg/cm³]					
CTR	144.2(41.4)	-0.3(1.2)%	0.4(1.4)%	0.2(1.2)%	0.6(1.3)%	-0.2(1.1)%	0.6(1.0)%	-0.6(2.0)%	-0.6(4.4)%	-2.8(6.1)%
RE	166.3(41.4)	0.2(1.5)%	-0.8(1.3)%	-0.8(1.6)%	-0.4(1.3)%	-0.3(1.3)%	-0.3(1.4)%	-0.6(1.5)%	-0.5(1.7)%	-1.7(3.2)%
RVE	137.5(41.4)	-0.6(1.4)%	-0.1(1.6)%	1.1(1.3)%a	0.2(1.6)%	0.0(1.6)%	-0.1(1.4)%	0.0(2.0)%	-0.6(2.1)%	-2.7(3.8)%
					Dmeta [mg/cm³]					
CTR	232.6(36.8)	0.0(0.9)%	-0.2(0.6)%	-0.2(0.7)%	0.0(0.8)%	-0.4(0.6)%a	0.1(0.6)%	-0.3(0.9)%	-0.6(1.4)%	-1.5(1.7)%a
RE	247.9(36.9)	0.0(0.9)%	-0.2(0.9)%	-0.2(1.3)%	0.0(0.9)%	-0.2(0.9)%	0.1(1.0)%	-0.4(0.8)%	0.1(0.7)%	-0.4(1.2)%
RVE	231.3(36.8)	0.1(0.8)%	-0.1(0.9)%	0.3(0.5)%	0.0(0.7)%	0.1(1.0)%	0.0(1.0)%	-0.2(0.7)%	-0.3(1.4)%	-0.6(1.6)%
					Dtrab [mg/cm³]					
CTR	180.3(38.9)	-0.2(1.0)%	0.1(1.0)%	0.0(0.9)%	0.3(1.0)%	-0.3(0.8)%	0.3(0.8)%	-0.4(1.4)%	-0.6(2.8)%	-2.1(3.7)%
RE	199.6(39.0)	0.1(1.1)%	-0.6(1.1)%	-0.6(1.5)%	-0.3(1.1)%	-0.3(1.2)%	-0.2(1.2)%	-0.6(1.1)%	-0.2(1.2)%	-1.2(2.2)%
RVE	175.8(38.9)	-0.2(0.9)%	0.0(0.9)%	0.6(0.6)%a	0.1(0.8)%	0.1(1.0)%	0.0(0.9)%	-0.1(1.1)%	-0.4(1.5)%	-1.6(2.5)%
					TrabArea [mm²]					
CTR	284.9(56.3)	-0.2(0.6)%	-0.2(0.6)%	-0.1(0.7)%	-0.1(0.6)%	0.0(0.8)%	-0.1(0.5)%	-0.2(0.8)%	0.0(0.5)%	-0.4(0.5)%a
RE	285.3(56.3)	-0.3(0.5)%	-0.2(0.6)%	-0.3(0.7)%	-0.3(0.4)%	-0.1(0.5)%	-0.4(0.1)%c	-0.1(0.2)%	-0.5(0.5)%a	-0.7(0.6)%b
RVE	256.4(56.3)	-0.2(0.4)%	-0.3(0.3)%a	0.0(0.4)%	-0.2(0.6)%	0.0(0.4)%	-0.3(0.4)%	-0.2(0.3)%	-0.3(0.3)%a	0.1(0.8)%
					Tb.1/N.SD [μm]					
CTR	0.20(0.03)	-1.8(6.0)%	1.3(4.6)%	0.8(3.8)%	0.7(2.8)%	-1.3(5.8)%	0.7(4.5)%	-2.1(7.2)%	-1.4(7.2)%	1.6(6.6)%
RE	0.20(0.03)	-1.5(5.3)%	0.4(4.2)%	-3.7(9.0)%	-1.5(5.3)%	-1.0(4.9)%	-0.9(4.1)%	1.1(4.1)%	-2.6(4.8)%	-1.8(3.9)%
RVE	0.19(0.03)	0.2(4.5)%	-1.0(5.2)%	-0.5(3.4)%	-1.4(4.7)%	-2.3(7.9)%	0.9(4.2)%	-0.8(4.2)%	-2.2(6.0)%	0.9(4.3)%
					Tb.N [mm⁻¹]					
CTR	1.80(0.17)	2.0(6.4)%	-0.7(6.1)%	-0.7(5.5)%	-1.0(4.1)%	0.7(6.0)%	-0.8(6.0)%	3.0(9.1)%	1.3(6.4)%	-0.8(6.3)%
RE	1.79(0.18)	2.4(6.4)%	0.8(4.6)%	4.4(9.6)%	1.4(5.4)%	1.5(5.4)%	1.4(4.6)%	-1.5(4.7)%	3.3(5.9)%	1.7(4.2)%
RVE	1.84(0.18)	-0.3(5.2)%	2.0(7.1)%	0.9(4.2)%	1.9(5.4)%	2.1(9.4)%	-2.2(4.6)%	1.7(4.5)%	3.2(7.5)%	-0.6(5.4)%
					Tb.Sp [μm]					
CTR	0.48(0.06)	-1.8(5.6)%	1.7(6.0)%	1.1(4.9)%	0.9(4.1)%	-0.8(6.0)%	1.3(6.3)%	-1.5(7.9)%	-1.5(7.5)%	2.0(7.2)%
RE	0.47(0.06)	-2.5(5.5)%	-0.8(4.1)%	-4.0(8.8)%	-1.8(5.2)%	-1.4(4.6)%	-1.7(3.8)%	1.1(4.1)%	-3.3(4.8)%	-2.1(3.8)%
RVE	0.47(0.06)	0.2(4.9)%	-1.8(6.7)%	-1.2(4.1)%	-1.7(5.2)%	-1.8(8.4)%	2.0(4.6)%	-1.7(4.4)%	-2.9(6.7)%	0.9(5.0)%
					Tb.Th [μm]					
CTR	0.08(0.01)	-1.9(4.3)%	1.3(6.0)%	1.2(5.2)%	0.9(3.9)%	-1.3(5.9)%	0.9(5.3)%	-3.3(7.6)%	-1.6(6.9)%	-1.5(6.2)%
RE	0.09(0.01)	-1.9(6.5)%	-0.9(4.5)%	-4.3(7.5)%	-1.0(5.6)%	-1.5(4.9)%	-1.5(4.6)%	0.9(4.8)%	-3.1(6.4)%	-2.8(4.8)%
RVE	0.08(0.01)	-0.3(5.6)%	-2.0(7.1)%	-0.7(5.0)%	-1.9(6.2)%	-1.6(8.8)%	1.8(5.4)%	-2.1(5.5)%	-3.4(7.0)%	-1.2(6.3)%

*No differences in the response of the three groups were seen at the distal radius. Values at baseline (BDC) are mean(SD) in absolute values. Beyond this, values are mean(SD) percentage change compared to baseline (significance indicated by: a: p<0.05; b: p<0.01; c: p<0.001). "#" indicates evidence (p<0.05) for differences between groups in baseline values. Significance of group×study-date interaction from ANOVA indicated by *: p<0.05; †: p<0.01; ‡: p<0.001. "d", "e", "f" indicate, respectively, significant (p<0.05) group×study-date interactions for two-group CTR vs. RE, RVE vs. CTR and RVE vs. RE comparisons. All analyses performed on absolute values. Analysis of percentage change data yields same statistical results (data not shown). CTR: inactive control group; RE: resistive exercise only group; RVE: resistive exercise with whole-body vibration group. HDT: day of head down tilt bed-rest, R+: day of recovery. See Table 2 for parameter name descriptions.*

tibia. Separation of the trabecular compartment into the inner 40% of the trabecular compartment and outer 60% did not appear to yield differential density changes during the bed-rest phase. In the bed-rest phase, reductions in trabecular number but increases in trabecular thickness and trabecular separation were seen at the tibia. At the distal radius cortical area, cortical thickness, cortical density and total density *increased* significantly with trabecular area *decreasing* significantly during bed-rest. These effects persisted up to the end of the 2-year follow-up recovery phase. Other works using lower-resolution conventional pQCT have not observed such changes[23,24]. Whilst further work would be necessary to investigate whether this is a consistent effect of bed-rest, as measured with HR-pQCT, it is possible that altered activity patterns (i.e. greater use of hands) during bed-rest and hence altered bone loading patterns could be responsible. This, however, cannot wholly explain the persistence of the changes long after bed-rest.

The pattern of bone recovery after bed-rest was seen most clearly at the distal tibia. Recovery of distal tibia cortical area, cortical thickness, trabecular area and cortical periosteal perimeter typically began first between 3d and 15d after bed-rest with this recovery process apparently complete approximately 6-months after bed-rest. Similarly, of the density parameters at the distal tibia, total density and trabecular density began to show recovery between 3d and 15d after bed-rest with this process being complete within 90d after bed-rest. Cortical density (at the distal tibia remained consistently below baseline level after bed-rest. Prior work has shown that bone recovery can take quite some time after bed-rest, estimated up to two-years[25], depending upon bone region. More specifically, data on cortical bone density measured with conventional pQCT[23], showed recovery within one-year after 56d bed-rest at the distal tibia (epiphysis) but not at the tibia diaphysis, although this effect was not significant statistically in this prior work. These data help to underscore recent findings of the cortical compartment of bone being the most susceptible to interventions, such as bisphosphonate treatment[26,27] and immobilization[28]. Since trabecular bone was assumed to have higher surface area to volume ratio than cortical bone and since bone remodelling occurs on existing bone surfaces, conventional wisdom dictated that bone turnover, and hence bone loss, should be most in the trabecular region. The mounting evidence, including that of the current study, suggests however, that these assumptions are not true and that cortical bone is the region of strongest bone loss in bed-rest. Further work should consider the recovery of the cortical compartment of the tibia in more detail, to better understand to what extent complete recovery does indeed occur long-term and if not, what processes could be underlying it. Finally, whilst in the parameters of trabecular network inhomogeneity, trabecular number, trabecular separation and trabecular thickness some changes were seen during bed-rest no consistent pattern during recovery was seen, likely due to high levels of measurement variability in these parameters.

An additional goal of the current study was to evaluate the effect of the exercise countermeasures on the HR-pQCT parameters considered. At the distal radius, just as there was a limited effect of bed-rest observed on the HR-pQCT parameters in this region of bone, there was similarly little impact of the exercise countermeasures, which themselves were targeted at the load-bearing regions of the body (i.e. lower quadrant). At the distal tibia, there were indeed some effects of the countermeasures seen with less loss of cortical area in the RVE-group than in the CTR- and RE-groups whereas the differences between the RE- and CTR-groups did not reach significance. A similar effect was seen for cortical thickness, though this effect was statistically weaker. Whilst the trabecular parameters of number, separation, thickness and network inhomogeneity generally showed less extreme changes in the RE- and RVE-groups than in the CTR-group, this finding should be treated with some caution as these variables in particular were subject to higher measurement variability and the within group changes were typically less consistent. Of note is that the density parameters at the distal tibia showed no significant differences between groups, whereas bone mineral content data from lower-resolution conventional pQCT in the same subjects[21] did show an impact of the exercise countermeasures, particularly RVE. Data from our own group show that measurement reproducibility at the distal tibia for bone mineral density as measured HR-pQCT is similar to than for bone mineral density and content as measured by conventional pQCT (*unpublished observations*). Hence, "greater" measurement error cannot explain the differences in findings on the same subjects with the two different methodologies, and further work comparing HR-pQCT and conventional pQCT needs to be undertaken.

It is appropriate to consider some of the limitations of the current study. Firstly, there were a limited number of subjects in the current study. The sample size of the 2nd Berlin BedRest Study was based upon distal tibia bone mineral content as measured by conventional pQCT[21]. It is likely that the current sample size may very well have played a role in the non-significance of some effects, such as that of the countermeasures or changes in some parameters compared to baseline. At the distal radius, it was more difficult to obtain images free of movement artefacts, despite measuring a young, healthy, male collective. Consequently, it is to be expected that the measurement error of the HR-pQCT parameters at the radius is higher than at the tibia (*unpublished observations*). This could indeed be part of the reason why we did not find many significant statistical effects at the distal radius. It may well be that HR-pQCT is not the best methodology to capture the effects of an intervention on bone in small sample sizes, and that lower resolution pQCT may be more appropriate. This issue would, however, need to be examined more in future work. Another consideration is that whilst we did find significant changes in some parameters, particularly at the tibia, the magnitude of these changes reached at most 2.5%. One may question whether such "small" changes would be associated with any changes in the mechanical properties of bone. In this regard, it is important to remember that prior work has shown that relatively modest (~5%) increases in bone mineral density result in much greater (>60%) increases in the ultimate force and energy to failure of bone[29]. Hence it is possible that the changes

observed in the current study are well associated with changes in the response of bone to loading. Nonetheless, whilst changes in some parameters, such as cortical thickness (Ct.Th), have been associated with changes in mechanical properties of bone[13], for a number of other HR-pQCT parameters, work still needs to be performed to see if changes in these HR-pQCT parameters actually relate to changes in the response of bone to loading, and if so, to what extent.

It is also appropriate to consider and some issues specific to HR-pQCT measurement methodology. The grading of the images performed accounts only for movement artefacts and currently the orientation of the image relative to the bone is not accounted for. Subtle changes in subject positioning during measurement from one day to the next, such that the angle at which the images transect the bone may vary one day to the next. This no doubt contributes to decreased reproducibility. Future work should examine to what extent changes in orientation affect the results and develop a system of either controlling for these orientation changes or at least alerting operators when it occurs. The grading conducted was also implemented by only one individual and future work needs to examine inter- and intra-rater reliability of such a grading system. It should be remembered also that HR-pQCT measurements rely on positioning of a scan region of fixed length at an arbitrary distance from the joint line. This does mean that within an individual one can ensure a similar anatomical region is measured one day to the next. However, due to differences in bone length from one individual to the next, we can be certain that between individuals we do not consistently capture the same anatomical regions. This is an issue is difficult to resolve, and a challenge for the HR-pQCT research community, particularly in cross-sectional studies, but is not a major concern for the current study as we were interested in the changes within individuals over time. Finally, a number of parameters (e.g. trabecular number) are not measured directly, but are calculated from other parameters that are indeed directly measured from the images taken (trabecular number is calculated from trabecular separation). For this reason, for example, we did not include data on bone volume to tissue volume because this parameter correlates perfectly with trabecular density and hence gives little additional insight into changes in the bone. Also the so-called "structural parameters" depend on density-based threshold detection, such that decreases in bone density may result in, for example, the detection of a "thinner" cortex. Nonetheless, changes in other parameters do correlate highly with one another (such as cortical area and thickness) and further work should examine which HR-pQCT parameters are most informative and which parameters in comparison deliver little additional information.

In conclusion, the main value of the current study is the description of changes in bone, as measured with HR-pQCT, during inactivity. Such information could also help to understand what occurs due to immobilization or inactivity in a clinical population. In terms of the effect of bed-rest at the distal radius, increases in cortical area, cortical thickness, cortical density and total density but decreases in trabecular area were seen. These changes at the distal radius persisted up to 2-years after bed-rest. The strongest effects were seen at the distal tibia, however, with expected losses in bone density, but also reductions in cortical area, cortical thickness, trabecular number but increases in trabecular area, cortical periosteal perimeter, trabecular thickness and trabecular separation. Most parameters at the distal tibia recovered within 180d after bed-rest. In terms of the countermeasures, although prior work using pQCT in the same subjects showed a significant effect of RVE at the tibia, only a significant effect of RVE, and not RE, was seen on the cortical area variable at the distal tibia was seen.

Acknowledgements

Martina Kratzsch is thanked for her assistance with the HR-pQCT measures. The 2nd Berlin Bed-Rest Study (BBR2-2) was supported by grant 14431/02/NL/SH2 from the European Space Agency and grant 50WB0720 from the German Aerospace Center (DLR). The 2nd Berlin Bed-Rest Study was also sponsored by Novotec Medical, Charité Universitätsmedizin Berlin, Siemens, Osteomedical Group, Wyeth Pharma, Servier Deutschland, P&G, Kubivent, Seca, Astra-Zeneka and General Electric. Daniel L. Belavý was supported by a post-doctoral fellowship from the Alexander von Humboldt Foundation.

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