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A non-destructive technique for 3-D microstructural phenotypic characterisation of bones in genetically altered mice: preliminary data in growth hormone transgenic animals and normal controls

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Abstract A non-destructive, three-dimensional technique for microstructural phenotypic characterisation of skeletal elements in genetically altered mice is presented. Preliminary data in bovine growth-hormone transgenic animals and control littermates are shown. The technique is based on microcomputed tomography (μCT) and digital postprocessing and allows for a differential quantitative analysis of the cortical and trabecular bone compartments in the axial and peripheral skeleton. The distal femora and the first lumbar vertebral bodies of six animals were CT scanned in the axial plane with an isotropic resolution of 20 μm. The periostal surface and the marrow spaces were segmented fully automatically, and the trabecular and cortical compartments were separated interactively. After 3-D reconstruction, various regions of interest (diaphyseal, metaphyseal and epiphyseal) were selected for the analysis. The femora and vertebrae of the transgenic animals showed obvious differences in size, shape, and trabecular arrangement compared with the control animals. The total bone mass was increased by a factor of two to three, but the trabecular bone was increased much more (up to 12 times) than the cortical bone. The transgenic animals showed an increased ratio of trabecular vs cortical bone (0.90 to 1.27 vs 0.14 to 0.36 in the femoral diaphysis) and an elevated trabecular bone volume fraction (49% to 73% vs 18% to 43% in the femoral metaphysis). The mean 3-D cortical thickness was similar in the normal and transgenic animals (values between 93 μm and 232 μm in the dia- and metaphyses), but the minimal cortical thickness was lower in the transgenic animals (22 to 31 μm vs 54 μm to 110 μm in the diaphysis). The technique presented is suitable for phenotypic characterisation of bone structure in genetically altered mice.

Key words Bone · Femur · Vertebra · Microcomputed tomography · 3-D reconstruction

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Introduction

Transgenic animal models have become a powerful research tool to elucidate the molecular and cellular role of different genes, in particular of those that play a role in the human disease process (reviewed Rossant and Nagy 1995; Wynshaw-Boris 1996). The ability to analyse the function of genes and their specific influence on various organ systems, such as bone, does, however, not only depend on the ability to alter the genotype, but also on being able to appropriately characterise the phenotypic changes in genetically manipulated animals. Because of the small size of murine bones, particular considerations and techniques are required.

The objective of the present study was therefore to present a non-destructive, three-dimensional (3-D) technique for microstructural phenotypic characterisation of skeletal elements in normal and genetically altered mice,

specifically focused on a differential quantitative analysis of the cortical and trabecular bone compartments in the axial and peripheral skeleton. We will apply the technique to mice carrying bovine growth hormone (bGH) fusion genes, expressing high serum levels of bGH (McGrane et al. 1988; Wolf et al. 1993; Blackburn et al. 1997), and to control littermates. A growth-hormone transgenic mouse model was selected, because overproduction of growth hormone has been shown to cause striking skeletal changes, including disproportionate gigantism (Wolf et al. 1991a; Wolf et al. 1991b; Oberbauer et al. 1992; reviewed by Wolf and Wanke 1996), but previous analyses have been confined to the measurement of external bone dimensions, rather than microstructural changes. We will report preliminary data on cortical and trabecular bone mass, trabecular bone volume fraction, and 3-D cortical thickness, measured in the diaphyseal, metaphyseal, and epiphyseal regions of the distal femur and in the first lumbar vertebra.

Materials and methods

Animals

We studied three female transgenic mice carrying fusion genes, in which the structural gene for bovine growth hormone (bGH) was placed under the transcriptional control of the rat phosphoenolpyruvate carboxykinase promoter (PEPCK). The mice were originally generated on a C57BL/6 x SJL genetic background (McGrane et al. 1988) and kindly provided by Dr. T.E. Wagner, Edison Biotechnology Center, Ohio University, Athens, Ohio, USA. The hemizygous PEPCK-bGH transgenic mice used in this study were derived from the 14th generation of sequential crossing with NMRI outbred mice (Charles River-Wiga). The animals were kept under conventional (nonbarrier) conditions and had free access to a standard rodent diet (Altromin 1324; Altromin, Lage, Germany) and tap water. Transgenic mice and controls were killed by bleeding from the retroorbital sinus while under ether anesthesia. All experiments were done according to the German Animal Protection Law. The right femur and thoraco-lumbar spine were removed and the bones stored at –20°C until further examination.

Previous investigations have shown that these mice express bGH specific mRNA in the liver and kidney (Wolf et al. 1995b), resulting in serum bGH concentrations in the range of 1–2 µg/ml (Wolf et al. 1993; Blackburn et al. 1997). For comparison, three non-transgenic sex- and age-matched littermates were investigated, the animals and their controls being 7.5, 10, and 24 weeks old, respectively. The body mass of the controls was 27, 32 and 44 g, and that of the transgenic mice 44, 50 and 56 g. The height of the first vertebral bodies was 2.00, 2.34 and 2.98 mm in the controls, and 2.44, 2.52 and 4.36 mm in the transgenic animals.

Micro-CT analysis, using a prototype fan beam microscope

The prototype measurement system employed was constructed and provided by Stratec Medizintechnik (Pforzheim, Germany). The system uses a fanbeam geometry for obtaining high resolution computed tomography (CT) images. Because of the small size of the bone samples, the X-ray tube and the detector are fixed and – unlike in normal CT systems – the sample itself is rotated (Fig. 1). This configuration allows for high precision and stability, the geometrical magnification of the system being about 1:40. The X-ray tube is a microfocus type with a square spot of 8 µm (high voltage = 36 kV, anode current = 100 µA, filtration = 18 µm copper [≅0.5 mm alloy]), the small samples making it possible to use a low X-ray energy (about 30 keV) to achieve high contrast in the CT-images. The system uses of a 256 pixel line detector with a length of 200 mm and a pixel size of 0.8 by 0.6 mm^2, its efficiency being about 90% of the used energy.

The acquisition of the detector signals was performed with a PC data acquisition card. For better stability, the detector was re-

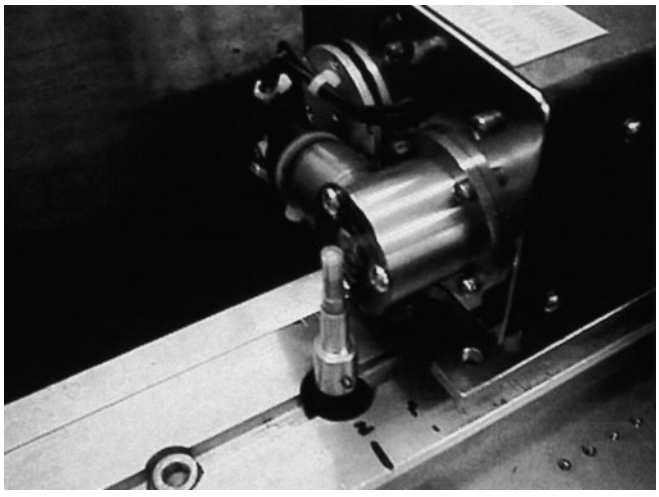


Fig. 1 Prototype measurement system (Stratec Medizintechnik, Pforzheim, Germany) working with a fanbeam geometry for obtaining high resolution CT images. The bone samples are placed in a tube. Because of the small size of the bone samples, the X-ray tube and the detector are fixed and – unlike in normal CT systems – the sample itself is rotated

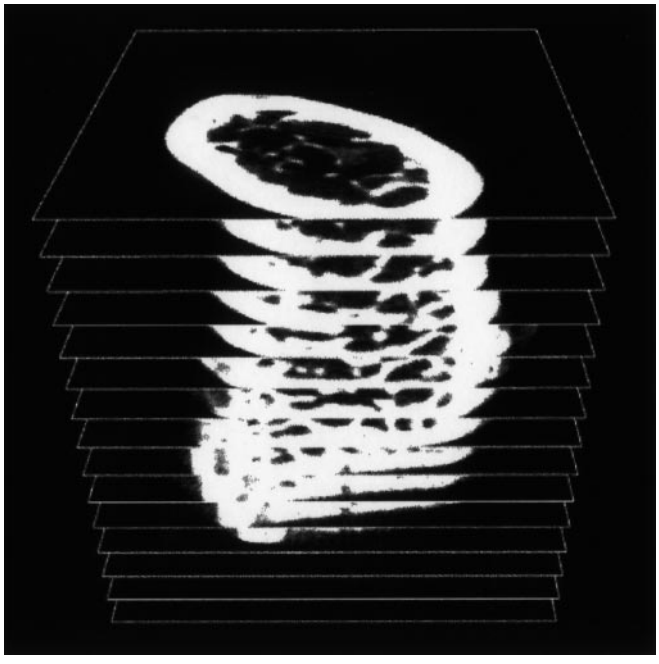
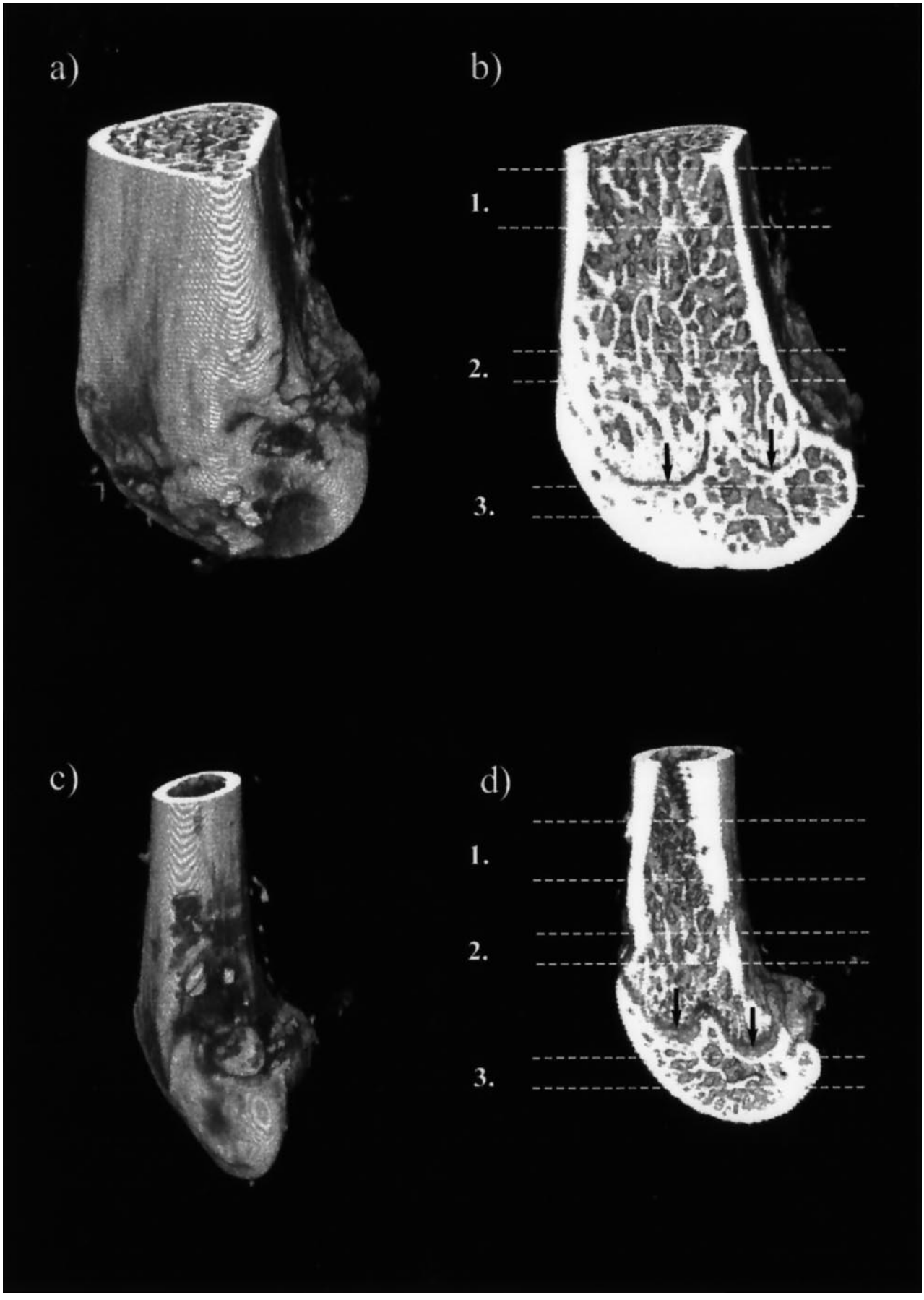


Fig. 2 Stack of high-resolution CT images of a distal femur of a normal mouse obtained with the prototype µCT system

Fig. 3 3-D reconstruction of the complete distal femur of a bGH transgenic mouse (**a**) and a control (**c**), aged 10 weeks. Sagittal section of the femur showing the larger amount of trabecular bone in the bGH transgenic mouse (**b**) and the normal trabecular bone structure of an age-matched littermate (**d**). Three regions of interest are defined: *1* diaphysis; *2* metaphysis; *3* epiphysis. The epiphyseal plate of the distal femur has been marked by *arrows*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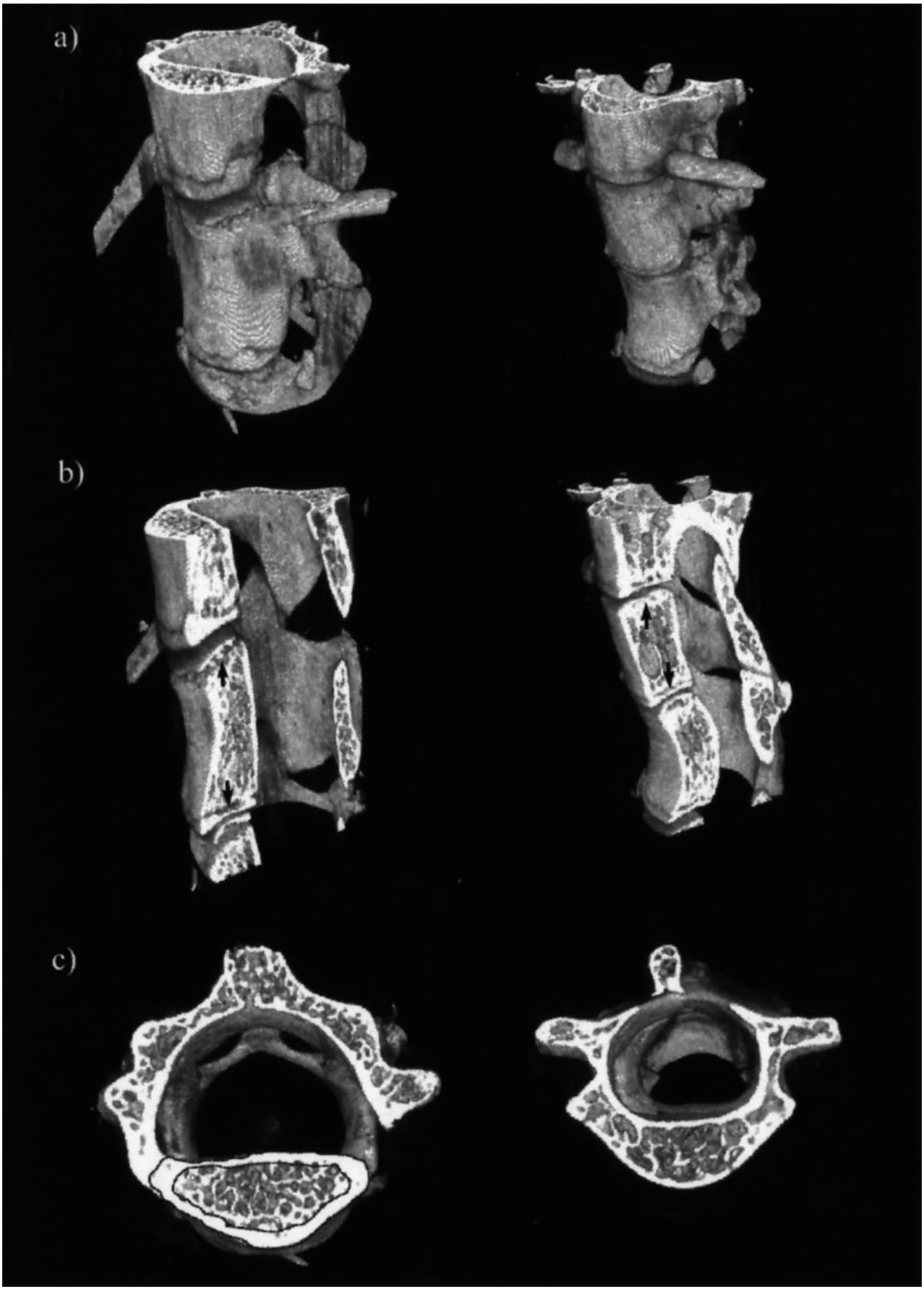
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calibrated between scans during measurements of multiple slices. In this configuration, the system has a measurement range of about 5 mm in diameter and an isotropic resolution of about 20 µm. A modified mechanical and electronic system of the Stratec XCT pQCT (peripheral quantitative CT) 2000 series was employed for positioning the sample in the z-direction of the CT co-ordinate system (perpendicular to the sectional images) and for rotating the probe during image acquisition. The software for the system was written with LabView (National Instruments); it controls the mechanics, the data acquisition, and the required steps for measuring multiple slices, which make it possible to obtain complete 3-D data sets. The reconstruction of the image data was made with a modified backprojection algorithm for fanbeam geometries. The measurement time for 1 slice (250 projections) was about 1.5 min.

The shaft of the femur and the thoraco-lumbar spine of the mice were orientated in the z-direction of the µCT co-ordinate system, and about 200 transverse images with a section thickness of 20 µm were obtained in each of the six distal femora (Fig. 2) and six first lumbar vertebrae. The total imaging time for each bone was about 5 h.

Three-dimensional digital image analysis

The µCT image data were transferred digitally to a symmetrical multiprocessing computer (ONYX, Silicon Graphics, Mountain View, Calif.) with two high performance graphic systems (Infinite Reality; Fig. 2). The segmentation of the external (periostal) contours of the bones and the marrow spaces was performed fully automatically, using a grey-value orientated, region-growing algorithm developed in our laboratory (Englmeier et al. 1997) and a constant threshold value for all bones. A 3-D reconstruction of the bones was then performed (Fig. 3), applying a contour based interpolation- and an optimised surface-constructing algorithm (Raya and Udupa 1990; Hee-Jong and Park 1992; Englmeier et al. 1997). In a next step, the reconstructed data sets were loaded in a virtual reality (VR) environment that allows one to visualise cross-sectional images in any direction and, more importantly, to define and analyse various regions of interest within the 3-D object representation (Fig. 3b, d).

In the distal femur, three regions of interest were selected: (1) the diaphysis – 26 slices, distant to the epiphyseal plate; (2) the metaphysis – 11 slices, directly proximal to the epiphyseal plate; (3) the epiphysis – 16 slices, between the subchondral area and the epiphyseal plate (Fig. 3b, d). In the spine, the complete first lumbar and its adjacent vertebrae were reconstructed (Fig. 4a), and the vertebral body (including the upper and lower end plates) was selected as a region of interest for further analysis (Fig. 4b, c). In these regions of interest, the total bone mass was quantified in terms of the number of volume elements (voxels) depicted as mineralised tissue.

For a quantitative evaluation of the cortical and trabecular bone mass, the trabecular compartment was separated from the cortical bone by means of an interactive B-spline Snake algorithm (Stammberger et al. 1998a) that delineates the endostal bone surface and cuts off the connecting trabeculae (Figs. 4c, 5a). In a next step, the trabecular bone volume fraction was calculated – that is the volume taken up by mineralised tissue, divided by the total volume of the trabecular compartment including the marrow spaces.

Fig. 4a–c Thoracolumbar spine (first lumbar vertebra in the centre), bGH transgenic mouse on the left and age-matched littermate on the right, both aged 10 weeks. **a** 3-D reconstruction seen from antero-lateral. **b** Sagittal section through the vertebral bodies; the epiphyseal plate of the first vertebral body has been marked by *arrows*. **c** Transverse section through the first lumbar vertebral body, showing the contours of the endostal and periostal surfaces after segmentation

The cortical bone thickness was analysed with a 3-D Euclidean distance transformation (Stammberger et al. 1998b). This algorithm calculates, independent of the specific section position and orientation, the true minimal 3-D distance between the periostal and endostal surfaces at each voxel (every 20 µm) in the 3-D object representation (Fig. 5a). The algorithm allows for the determination of the maximal, minimal and mean cortical bone thickness throughout the selected region of interest as well as for the variability of the cortical thickness values. The 3-D cortical thickness was determined for the diaphyseal and metaphyseal region of the distal femur, but not for the epiphysis and the vertebral body.

Results

Distal femur

Figure 3a, c shows 3-D reconstructions of the distal femora of one growth hormone (bGH) transgenic mouse and its age-matched littermate. For visualising the microstructural architecture, the bones were sectioned longitudinally (Fig. 3b, d), and in the transverse plane at the levels of the diaphysis (Fig. 5a), the metaphysis (Fig. 5b), the growth plate (Fig. 5c), and the epiphysis (Fig. 5d). It can be easily appreciated that the bones of the transgenic animals are substantially bigger and exhibit a triangular, rather than a rounded cross-sectional shape. The trabecular network is more regular in the bGH transgenic animals and is more prominent, in particular in the diaphyseal region.

The total bone mass (cortical plus trabecular mineralised tissue) was found to be between twice to three times higher in the distal femora of the transgenic animals. This difference was most marked in the diaphyses (Table 1), but it could also be seen in the meta- and epiphyseal regions (Tables 2, 3). The trabecular bone tissue was much more drastically elevated (up to 12-fold in the diaphysis) than in the cortical bone (less than 2-fold; Tables 1–3). In the diaphysis, the ratio of trabecular vs cortical bone mass ranged from 0.9 to 1.27 in the transgenic mice, whereas it was only between 0.14 and 0.36 in the normal controls (Table 1). The ratio between trabecular and cortical bone was also higher in the metaphysis (Table 2), but in the epiphysis the difference was less prominent (Table 3).

The trabecular bone volume fraction (BVF = percentage of the total volume of the trabecular compartment taken up by mineralised tissue), was substantially elevated in the femoral diaphysis of the bGH transgenic animals (34 to 55% vs 13 to 22% in controls; Table 1), and such differences were also observed in the metaphysis (49 to 73% vs 18% to 43%; Table 2), and in the epiphysis (57 to 76% vs 36 to 50%; Table 3).

The mean 3-D cortical thickness was relatively similar in the normal and bGH transgenic mice (93 to 227 µm in the diaphysis and 117 to 232 µm in the metaphysis), the values being sometimes higher in the growth-hormone-transgenic and sometimes in the control animals (Tables 1, 2). The same applied to the maximal cortical thickness (183 to 449) µm in the diaphysis and 220 to 568 µm in the metaphysis), indicating that the rel-

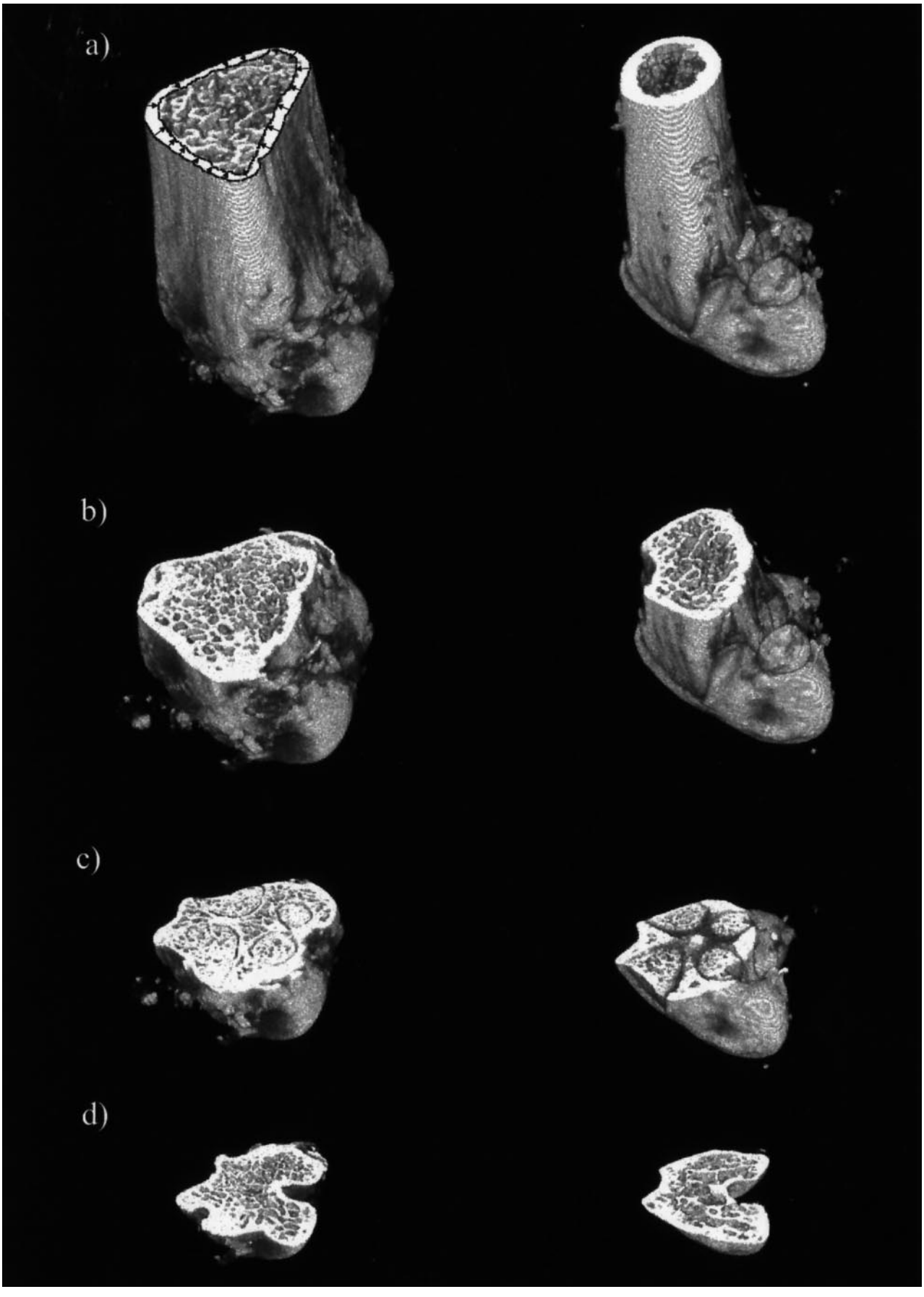
a)
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Table 1 Quantitative analysis of the trabecular and cortical bone tissue in the diaphysis of the distal femur: result for three transgenic animals and control littermates [(*Con* control littermate, *Tg* transgenic mouse, *vol*. volume, * unit 1000 voxels of 20 µm³, *Cort*. cortical, *Trab*. trabecular, *BVF* bone volume fraction (% of the trabecular compartment taken up by mineralized tissue), *CV%* coefficient of variation (SD/mean) of the thickness values]

	7.5-Weeks old		10-Weeks old		24-Weeks old	
	Con	Tg	Con	Tg	Con	Tg
Total vol.*	97.8	220.5	128.5	247.4	138.9	333.1
Total bone*	52.2	108.1	66.8	163.8	75.6	238.9
Cort. bone*	42.8	49.7	49.0	72.1	66.4	125.5
Trab. bone*	9.3	58.4	17.8	91.7	9.2	113.5
Ratio Trab./Cort.	0.22	1.18	0.36	1.27	0.14	0.90
Trab. BVF (%)	17%	34%	22%	52%	13%	55%
Cort. thickness (µm)						
Mean	147	93	136	132	191	227
Max.	230	183	221	247	308	449
Min.	66	22	54	22	110	31
CV%	17%	27%	22%	29%	19%	32%

Table 2 Quantiative analysis of the trabecular and cortical bone tissue in the metaphysis of the distal femur: result for 3 transgenic animals and control littermates [*Con* control littermate, *Tg* transgenic mouse, *vol*. volume, * unit 1000 voxels of 20 µm³, *Cort*. cortical, *Trab*. trabecular, *BVF* bone volume fraction (% of the trabecular compartment taken up by mineralized tissue), *CV%* coefficient of variation (SD/mean) of the thickness values]

	7.5-Weeks old		10-Weeks old		24-Weeks old	
	Con	Tg	Con	Tg	Con	Tg
Total vol.*	60.2	128.0	80.6	118.5	73.5	146.9
Total bone*	37.2	77.7	47.6	87.1	37.3	122.0
Cort. bone*	20.1	30.3	23.4	29.8	29.5	54.2
Trab. bone*	17.1	47.4	24.2	57.3	7.8	67.8
Ratio Trab./Cort.	0.85	1.68	1.04	1.93	0.27	1.25
Trab. BVF (%)	43%	49%	42%	65%	18%	73%
Cort. thickness (µm)						
Mean	120	117	119	118	174	232
Max.	220	238	264	265	385	568
Min.	22	31	22	31	49	44
CV%	34%	30%	28%	31%	34%	43%

Table 3 Quantitative analysis of the trabecular and cortical bone tissue in the epiphysis of the distal femur: result for three transgenic animals and control littermates [*Con* control littermate, *Tg* transgenic mouse, *vol*. volume, * unit 1000 voxels of 20 µm³, *Cort*. cortical, *Trab*. trabecular, *BVF* bone volume fraction (% of the trabecular compartment taken up by mineralized tissue), *CV%* coefficient of variation (SD/mean) of the thickness values]

	7.5-Weeks old		10-Weeks old		24-Weeks old	
	Con	Tg	Con	Tg	Con	Tg
Total vol.*	95.1	164.7	118.2	177.9	137.7	194.1
Total bone*	70.8	127.7	82.9	153.1	97.7	172.3
Cort. bone*	46.5	79.3	47.3	76.6	65.1	117.3
Trab. bone*	24.3	48.4	35.6	76.6	32.6	55.0
Ratio Trab./Cort.	0.53	0.61	0.75	1.00	0.50	0.47
Trab. BVF (%)	36%	57%	50%	76%	45%	72%

Fig. 5a–d Transversal sections through the femora of a bGH transgenic mouse (*left*) and a control (*right*), both aged 10 weeks. **a** Superior view of the diaphysis, showing the contours of the endostal and periostal surfaces. The thickness measurements of the cortical bone (*arrows*) were made from every pixel at the periostal surface (intervals = 20 µm) throughout the entire region of interest, independent of the specific section orientation, using a 3-D Euclidian distance transformation. **b** Oblique view onto the metaphysis, **c** the growth plate, and **d** the epiphysis. In all regions, the amount of trabecular bone was higher in the transgenic mice

atively higher cortical bone mass was not accompanied by an increase in true (3-D) cortical thickness. Whereas in the control animals the cortical thickness was always higher in the diaphysis than in the metaphysis, this was not always the case in the bGH transgenic mice (Tables 1, 2). Interestingly, the minimal cortical thickness yielded considerably lower values in the diaphysis of the transgenic animals (22 to 31 µm vs 54 to 110 µm), the variation of the cortical thickness being higher (27 to 32% vs 17 to 22%; Table 1). In the metaphysis, this difference was not as pronounced (Table 2).

Table 4 Quantitative analysis of the trabecular and cortical bone tissue in the 1st vertebral body: results for three transgenic animals and control littermates [*Con* control littermate, *Tg* transgenic mouse, *vol*. volume, * unit 1000 voxels of 20 µm³, *Cort*. cortical, *Trab*. trabecular, *BVF* bone volume fraction (% of the trabecular compartment taken up by mineralized tissue), *CV%* coefficient of variation (SD/mean) of the thickness values]

	7.5-Weeks		10-Weeks		24-Weeks	
	Con	Tg	Con	Tg	Con	Tg
Total vol.*	216.3	420.2	317.1	488.2	413.3	1020.0
Total bone*	151.6	283.3	234.8	421.8	303.7	932.2
Cort. bone*	91.1	137.1	136.5	219.0	161.5	502.5
Trab. bone*	60.6	146.2	98.3	202.8	142.3	429.7
Ratio Trab./Cort.	0.67	1.07	0.72	0.93	0.88	0.86
Trab. BVF (%)	48%	52%	54%	75%	57%	83%

First lumbar vertebral body

Figure 4a demonstrates a 3-D reconstruction of the first vertebral body of a bGH transgenic mouse and its age-matched littermate. An axial and a sagittal section through the vertebral body are shown in Fig. 4b, c. Again, differences in size and shape of the bone as well as differences in the trabecular pattern are obvious in the bGH transgenic animals, the vertebra of the transgenic mice being more elongated.

The total volume of the vertebral body was elevated by a factor of 1.5 to 2.5, and the total bone mass by about two to three in the bGH-expressing mice (Table 4). Again, the differences were generally more pronounced in the trabecular than in the cortical bone compartment. The ratio of trabecular vs cortical bone ranged from 0.86 to 1.07 in the bGH transgenic animals, and from 0.67 to 0.88 in the controls (Table 4). The trabecular bone volume fraction was also higher in the transgenic mice (52 to 83% vs 48% to 57%; Table 4).

Discussion

A non-destructive technique for 3-D microstructural phenotypic characterisation of skeletal elements in genetically altered mice has been presented. Our data in bovine growth-hormone transgenic animals show that transgenic mice have an altered ratio between trabecular and cortical bone mass. Whereas cortical bone mass is increased proportional to bone size (the mean cortical thickness being identical, and the minimal cortical thickness being even reduced), the trabecular bone mass is increased considerably in transgenic animals, with a marked increase in trabecular bone volume fraction.

Technical considerations

So far analyses of the skeletal system in bGH transgenic mice have been focused on the measurements of external bone dimensions (Wolf et al. 1991a; Wolf et al. 1991b). These can provide valuable data on total bone length and diameter, but not on the microstructural arrangement of the bone tissue and the relationship between cortical and trabecular bone.

Histologic sections make it possible to delineate single trabeculae and the cortex; however, quantitative analyses are difficult, because the 3-D context is lost (making it necessary to appropriately realign the sections) and because the slices may be geometrically distorted during the sectioning process. The realignment and "linear" geometric distortion may to be corrected based on external markers (e.g., Sundsten and Prothero 1983; Streicher et al. 1997), but these procedures are extremely time-consuming. Moreover, when sectioning tissues of different mechanical stiffness, such as bone and soft tissues, non-linear distortions can occur in the sections, and these are extremely difficult to deal with (Schormann et al. 1995). The problem of non-linear distortion may be overcome by so-called "episcopic" imaging techniques, using "on-block" staining and digitisation before 3-D reconstruction (Odgaard et al. 1990; Odgaard 1997; Weninger et al. 1998). However, the great advantage of the CT-based technique is not only that it is geometrically highly accurate, but that it is entirely non-destructive, making it possible to examine the bones repeatedly or subjecting them to further complementary examinations, such as biochemical analyses, histology, or mechanical testing.

To be able to identify the cortical shell and single trabeculae and to make quantitative measurements on a microstructural level, a high spatial resolution is required in all three dimensions, and this resolution is not available on standard CT scanners. Among the first to use high-resolution CT for the study of 3-D bone structure were Feldkamp et al. (1989) and Kuhn et al. (1990), and the spatial resolution of this technique has been improved rapidly over recent years (e.g. Durand and Rüegsegger 1991; Engelke et al. 1993; Müller et al. 1994; Rüegsegger et al. 1996; Müller et al. 1996; for a recent review, Odgaard 1997). Acquiring data at an isotropic resolution (here 20 µm³) has the advantage that secondary slices can be obtained in various planes, without the need of interpolation and/or loss of detail. To identify and reconstruct the object of interest within serial images, a high contrast with the surrounding media is required. As the hydroxy-apatite in the bone yields a high degree of X-ray attenuation, but not the tissue within the bone (marrow and fat) and around it (muscles, tendons and other soft-tissues), the mineralised tissue is delineated with very high contrast in the CT images. Therefore, 3-D reconstruction can be performed fully automatically, provided appropriate algorithms are at hand.

Three-dimensional reconstruction is not only advantageous for better visualisation of the bones and their 3-D shape, but also for accurate quantitative analysis of apparent trabecular bone volume fraction and cortical thickness. If, for instance, the same analysis was based on a single section, the cortical thickness might appear distorted, if the image was not acquired perpendicular relative to the long axes of the bone. These problems could be overcome in the present study, by applying a 3-D Euclidean distance transformation algorithm (Stammberger et al. 1998b) that computes the minimal spatial distance between the periostal to the endostal surface, independent of the original section orientation. Moreover, the 3-D reconstruction technique renders the measurement less dependent on the selection of a specific section level within the bone. Another advantage of the technique presented here is that the definition of various regions of interest (diaphysis, metaphysis, epiphysis) can be carried out after the 3-D reconstruction in a virtual-reality (VR) environment. This allows one to select regions of interest in the bone "a posteriori", rather than having to choose them before the CT scanning. Because the digital analysis is based on the whole region (rather than on only one supposedly standardised section) the measurements are more reliable, and can be more readily compared between animals. Moreover, the acquisition of real 3-D geometric data is required, if microstructural finite element models of bones are to be created. Such computer models make it possible to calculate bone stress and bone failure at the level of the mineralised tissue, and to assess the relationship between microstructure and whole-bone strength (van Rietbergen et al. 1997).

The only interactive segmentation process required in our study was that of separating the trabecular and cortical bone at the endostal surface. Here, the cortical bone is continuous with the trabeculae, and thus no fully automated segmentation is possible based on thresholding or edge detection alone. However, by employing the interactive Snake algorithm (Stammberger et al. 1998a) the amount of user interaction can be reduced to a minimum, the time required for the segmentation amounting to between 15 and 45 min for each region of interest. The algorithm exploits the grey-value gradients inherent in the image and the information from the segmentation of previous sections. Its performance is controlled visually on a section-by-section basis and can be corrected by changing the specific settings or manually, if required.

Preliminary results in growth-hormone transgenic mice

Owing to the small sample size, this study can only provide preliminary but not conclusive data on differences in the skeletal elements of bGH transgenic mice. However, we find very pronounced differences between the transgenic and control animals. Apart from the much greater bone size, which has also been described in previous studies (Wolf et al. 1991a, b), we find a strikingly altered relationship between the trabecular and cortical bone. Whereas the cortical bone mass is found to be increased proportional to the bone size (resulting in a very similar cortical thickness in transgenic and control animals), the trabecular volume fraction and the ratio of trabecular vs cortical bone mass are substantially increased in the mice expressing the bGH. The relative increase of trabecular vs cortical bone is more pronounced in the diaphysis and metaphysis of the distal femur than in the epiphysis or the first lumbar vertebra.

Tseng et al. (1996) investigated the cortical bone in another strain of transgenic mice expressing human growth hormone that was directed specifically to osteoblasts with minimal systemic overproduction. They investigated the femoral mid-diaphysis with μCT, quantifying the cortical thickness in a sequence of 2-D images and reported a small increase in cortical thickness in the transgenic animals. The authors also found a greater porosity and a reduced apparent elastic modulus of the cortical bone (determined by histomorphometry and mechanical testing). However, they did not analyse skeletal sites that included trabecular bone and thus did not observe the altered quantitative relationship between both bone compartments.

Growth hormone has been shown to be important in bone formation as well as bone resorption, the anabolic response being conveyed via a direct interaction with growth hormone receptors on osteoblasts (and possibly also on osteoclasts) and via the induction of endocrine and autocrine/paracrine IGF I (insulin-like growth factor; recent review by Ohlsson et al. 1998). It is interesting to note that – in the bGH transgenic mice – the net bone resorption is decreased at places where the control mice have already formed a marrow cavity. Although resorption has been shown to be increased with GH administration (Ohlsson et al. 1998), a much more regular and dense network of trabecular bone appears to be maintained in the bGH transgenic mice compared with normal controls. This may imply that, mechanically, the whole bone remains relatively stiff and that therefore the thickness of the cortex may not need to be adapted in proportion to the gain in total bone size.

Conclusions

The non-destructive CT based method presented in this study is capable of differentiating between changes in the trabecular and the cortical bone compartments of the peripheral and axial skeleton of normal and genetically altered mice. The technique has been applied to mice carrying bovine growth hormone fusion genes and normal controls, and the data indicate that trabecular bone is increased substantially in transgenic animals. The cortical bone on the other hand is increased proportional to the total bone size, the cortical thickness being similar to that of the control animals and the minimal thickness being even reduced.

The technique can be readily applied to other bones or other mutants, transgenic or knock-out mouse models

(e.g., IGF I and II transgenic mice: Mathews et al. 1988; Wolf et al. 1995a). In this way, the specific effect of certain genes and molecules on the skeletal phenotype can be identified and their roles in differentiation, growth, maintenance, and degeneration may be characterised with high accuracy. In addition, techniques for reliable quantification of bone parameters are required in mutagenesis screen programs, aiming at the systematic production of mouse models for human diseases, e.g., osteoporosis (Balling et al. 1998). With improved scanning times, the technique presented may potentially be extended to studying mouse limbs in vivo, and thus may become a powerful tool in these screening programs.

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