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Interspecies Differences in Bone Composition, Density, and Quality: Potential Implications for *in Vivo* Bone Research*

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ABSTRACT

This study compares bone composition, density, and quality in bone samples derived from seven vertebrates that are commonly used in bone research: human, dog, pig, cow, sheep, chicken, and rat. Cortical femoral bone samples were analyzed for their content of ash, collagen, extractable proteins, and insulin-like growth factor-I. These parameters were also measured in bone powder fractions that were obtained after separation of bone particles according to their density. Large interspecies differences were observed in all analyses. Of all species included in the biochemical analyses, rat bone was most different, whereas canine bone best resembled human bone. In addition, bone density and mechanical testing analyses were performed on cylindrical trabecular bone cores. Both analyses demonstrated large interspecies variations. The lowest bone density and fracture stress values were found in the human samples; porcine and canine bone best resembled these samples. The relative contribution of bone density to bone mechanical competence was largely species-dependent. Together, the data reported here suggest that interspecies differences are likely to be found in other clinical and experimental bone parameters and should therefore be considered when choosing an appropriate animal model for bone research. (*Endocrinology* **139:** 663–670, 1998)

All animals are equal, but some animals are more equal than others (G. Orwell, Animal Farm, 1945).

Although over the past 10 yr significant advances have been made in the biology of bone cells, especially because of the technical improvements of *in vitro* systems of bone cell cultures, it has also become clear that such *in vitro* systems oversimplify the *in vivo* situation. The study of animal models will therefore remain an important part of research to address the pathogenesis of bone disorders and/or the effects of newly developed drugs on bone metabolism. Moreover, before accepting any agent for clinical osteoporosis trials, the Food and Drug Administration (FDA) now requires its efficacy in at least one small (rodent) animal model and one larger animal with known intracortical bone remodeling (1).

The rat is by far the most commonly used animal in bone research because of a number of practical advantages: its low cost and ease of accomodation and care, its short lifespan that enables studies of age-related changes, its well defined genetic background, and its thoroughly documented mineral metabolism (1–5). Several rat models have been used, including the growing rat (6, 7), the aged rat (8, 9), the mature ovariectomized rat (2, 3), and the aged ovariectomized rat (10). As an alternative to the rat, other small animals, such as the guinea pig (11) or the mouse (12), have been proposed. The choice of an appropriate larger animal model is usually much more difficult because the suitability of each model depends largely upon the objectives of the study. There is no ideal model for the study of all aspects of a metabolic bone disorder such as osteoporosis; all have advantages and disadvantages that should be taken into account when selecting animal models for the study of a specific metabolic bone disorder (13–15). A nonlimited list of larger animals that are being used in bone research includes dogs (16, 17), sheep (15), (mini)pigs (18), poultry (19), dairy cows (20), and nonhuman primates (21, 22).

The suitability of an animal model in bone research is closely related to the degree of similarity between the results obtained in the model compared with the human situation. After all, the ultimate goal of the use of an animal model is to transfer the obtained results to clinical practice. Differential responses in animals compared with humans can be caused by many different factors, acting directly or indirectly on the bone metabolism. Usually, animal studies aim to evaluate the impact of a specific treatment or therapy during an experimental period on a predefined set of parameters relevant in bone research. However, little attention has focused on how the baseline values of these parameters in animal models compare with humans; yet this may largely influence whether or not the results of a study may also apply to the clinical situation. In this regard, the objective of the present study was to document differences in bone mineral density, mechanical competence, and biochemical composition in bone specimens from different species, including human samples.

Materials and Methods

Bone specimen collection

Femoral shaft bone samples were obtained immediately after death from three physiologically mature and healthy female humans (age

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* Partial and preliminary data of this study have been presented as a selected poster at the XXIVth European Symposium on Calcified Tissues (Aarhus, Denmark, May, 1995).

30–60 yr), dogs (age 8–15 months), cows (age 2–4 yr), sheep (age 1–2 yr), pigs (age 18 months), rats (age 12 months), and chickens (age 12 months), respectively. From the larger species (human, dog, cow, sheep, and pig), a lumbar spine (L2) specimen was also excised. According to the protocol of our study, the preparation of a cylindrical core with fixed size (10 mm height, 8.2 mm diameter) was required to perform all the foreseen analyses (see below). Because the preparation of a core sample with these dimensions was not possible, no lumbar spine specimens were excised from the rat and chicken.

Bone sample preparation

Cortical bone powder samples were prepared from femoral bone specimens, according to previously established methods (7, 23), before analysis of their biochemical composition. Briefly, the bones were thoroughly cleaned from periosteum, associated soft tissue, and bone marrow. The femoral shafts were defatted for 2 days in trichloroethylene (renewed once daily), washed in distilled water, and dried. Bone was pulverized with a beater mill cooled with liquid nitrogen until bone powder with particle size between 40 and 160 μm was retained. For density fractionation analysis (see below), bone powder with a particle size smaller than 20 μm was prepared.

From the lumbar spine specimens, one (or two, if possible) cylindrical core(s) of trabecular bone (8.2 mm diameter) was taken in the axial direction of the specimens, using a core drill. From these samples, the end plates were cut plane-parallel, using a low-speed diamond saw, such that the height of the cylinders was exactly 10 mm. The obtained cylindrical cores with fixed dimensions were stored in PBS (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, 1.4 mM KH_2PO_4, pH 7.3) at 4 C until bone mineral density measurements and mechanical testing were done. The remaining trabecular bone parts of the lumbar spine samples were collected immediately after the isolation of the cylindrical core(s) and treated as described above for the cortical femoral bone samples, and chemical composition was analyzed.

Bone mass measurement

Bone mineral content (BMC) and projected area (area) of the trabecular fixed-size cylinders were measured by dual-energy x-ray absorptiometry, using a quantitative digital radiography device (QDR-1000/W, Hologic, Waltham, MA) that was calibrated with a Hologic hydroxyapatite anthropometric spine phantom. The specimens were scanned, with standard collimation of the x-ray beam, in ultrahigh resolution mode and manufacturer-supplied software (Hologic, V4.47), using 140/70 keV, average 3 mA, a scan step of 0.5 mm, and point resolution of 0.5 mm. All specimens were scanned in two positions. First, they were positioned with the cylinder axis perpendicular to the scanning table (axial direction); second, their axis was parallel to the table (lateral direction). Reproducibility, as coefficient of variation (CV) from five measurements of the same bone specimen after repositioning, was 0.38% (BMC) and 0.8% (area) for lateral measurements, respectively, and 0.58% (BMC) and 0.45% (area) for measurements in the axial direction through the cylinder. The BMC was taken as the average value obtained for these two scanning positions. The exact height of the core samples was recorded using a micrometer with a readout up to 0.01 mm. From these data, the volumetric bone mineral density (vBMD) was then calculated by dividing the BMC (mg) through the volume of the core (calculated by multiplication of the circular area measured in axial direction with the measured core height).

Mechanical analysis

A compression test was made between the two parallel surfaces of the wet cylindrical cores, as described earlier (24). Briefly, the specimens were placed between flat platens, and a downward displacement of the upper platen produced compression loading. The force was applied by means of a hydraulic piston, moving downward at high speed (about 0.12 meters per second). The force was measured using a load cell (Lebow model 3157, Philadelphia, PA) and the deformation of the sample (displacement of the piston) with a linear extensometer (Philips LO1314, Eindhoven, The Netherlands). The maximum force was determined as the point at which the derivative of the curve force *vs.* displacement is equal to zero. The maximum force was divided by the area of cross-section of the samples, to yield the fracture stress.

Density fractionation of bone powder

The density fractionation by stepwise centrifugation method was performed as described by Grynpas *et al.* (21), using the cortical bone powder samples. Sieved bone powder, 200–300 mg (particle size <20 μm), was added to a tube containing 35 ml of a 2.0 g/ml density solution made up by a bromoform-toluene mixture and calibrated with sink floats (Cargille Laboratories Inc, Cedar Grove, NJ). After centrifugation of the solution for 30 min at 10,000 rpm (JA20, Beckman Instruments, Fullerton, CA), the supernatant was separated from the pellet and transferred to a new tube. The density of the supernatant was modified to 1.9 g/ml by the addition of toluene and recentrifuged. Under the same conditions, each precipitate obtained from solutions of progressively decreasing density (at steps of 0.1 g/ml) was collected. To obtain a range of mineral density greater than 2 g/ml, the precipitate obtained from the initial 2 g/ml density solution was resuspended in a solution of 2.3 g/ml. Successive centrifugation of precipitates at progressively decreasing densities (steps of 0.1 g/ml) provided the higher density fractions. The finally obtained series of specific gravity fractions were centrifuged in 100% ethanol to remove bromophenol and toluene residues, dried, and weighed. From weight determinations of each fraction, the percent distribution of the various bone density fractions in a sample of unfractionated bone powder was calculated to generate mineralization profiles.

Ash weight and collagen analysis

Small amounts (~10 mg) of each bone powder sample were ashed at 700 C for 6 h in a muffled furnace. The ashed segment was weighed and expressed as percentage of dry bone weight. Hydroxyproline concentration, as a marker for collagen content, was measured by the method of Kivirikko *et al.* (25) and was used previously in our laboratory for the analysis of bone samples (7, 26).

Analysis of the total amount of extractable proteins and insulin-like growth factor-I (IGF-I)

Based on previous experience (7, 23, 26), the noncollagenous proteins were extracted from the bone samples as described below. Approximately 15 mg of each dry bone powder sample were extracted subsequently with 1.5 ml of 0.5 M ammonium-EDTA (pH 8.0) and 4 M guanidinium-HCl (pH 7.4). Each of the extraction solutions contained a mixture of protease inhibitors (5 mM benzamidine, 10 mM 6-aminocaproic acid, 100 μM *p*-hydroxymercuribenzoic acid, pH 6.2) at 4 C. The extractions were carried out overnight in microcentrifuge tubes by end-over-end rotation. After 18 h the solution was centrifuged (12,000 rpm for 30 min), the supernatant containing noncollagenous proteins was separated from the collagenous residue and desalted on a Sephadex PD-10 column (Pharmacia, Uppsala, Sweden). Both extracts (with EDTA and guanidinium-HCl, respectively) were analyzed separately; the presented data represent the sum of the values found in both extracts. The total protein concentration in the extracts was determined by spectrophotometry (λ = 280 nm) using BSA as standard. Before IGF-I determination the desalted extracts were lyophilized in a Speed Vac Concentrator (Heto, Allerod, Denmark) and dissolved in assay buffer. IGF-I was determined by a RIA as previously outlined in detail (27). Briefly, recombinant human IGF-I was used as standard, and a polyclonal antiserum was raised in a guinea pig. The antiserum displayed less than 0.01% cross-reaction with insulin and less than 1% cross-reaction with IGF-II. Inter- and intraassay coefficients of variation of the RIA were 7.7 and 7.4%, respectively, with a detection limit of 1.3 ng IGF-I/tube. The analytical recovery was 100 $\pm$ 2% (mean $\pm$ SEM, n = 6). Interference with IGF-binding proteins was evaluated by column chromatography (7). Different bone powder extracts were spiked with radioactive labeled IGF-I and eluted on a superose 12 column (Pharmacia). A single peak was observed, indicating the lack of interference between IGF-I and its binding proteins in these samples.

Results

Femoral samples (cortical bone)

The results of the biochemical analyses of the cortical bone powder samples are presented in Fig. 1. Large variations were observed between the different species for all examined parameters. Ash content was similar in human, dog, and pig cortical bone, intermediate in cow, sheep, and chicken, and highest in the rat. In five of the seven species, the collagen content, measured by hydroxyproline, was inversely related to the degree of mineralization. The femoral samples from pig and sheep, however, showed lower collagen concentrations than expected, but this was compensated for by a larger amount of extractable noncollagenous proteins compared with the other species. Rat bone also showed a high concentration of extractable proteins, whereas human and cow samples had the lowest concentration. These extracts of the bone powder samples were analyzed for IGF-I, a growth factor known to be involved in bone metabolism and produced by osteoblasts, and showing a well conserved sequence across species. IGF-I concentrations were highest in rat bone, intermediate in human and dog bone, and very low in the other examined species.

To determine whether these interspecies differences of the bone powder samples are found in each of the microscopic bone particles that constitute the whole bone samples or, alternatively, are due to the altered composition of a part of the microscopic bone particles, resulting in the differences observed in the whole bone samples, the bone powder samples were further analyzed by the density fractionation technique. This technique separates the small bone particles (<20 μm) that are present in a bone powder sample according to their density, ultimately leading to the construction of so-called mineralization profiles (Fig. 2). A shift from the bone particles to higher density fractions was observed in the species with a higher ash content in the unfractionated bone powder samples. The order of the species from lowest to highest proportion of high density bone particles was as follows: human, dog, pig, chicken, cow, sheep, and rat. The chemical composition of the two main bone particle fractions (density 2.0–2.1 and 2.1–2.2 g/ml, respectively) was further analyzed, and the results are presented in Table 1. An increase of ash and a decrease of collagen concentration were observed with increasing density in all species. Within a specific density range, significant variations of ash and collagen were observed between the species, similar to the variations in the whole bone samples (Fig. 1). This indicates that species differences of bone composition in the whole bone powder samples are found in all the bone particles of the

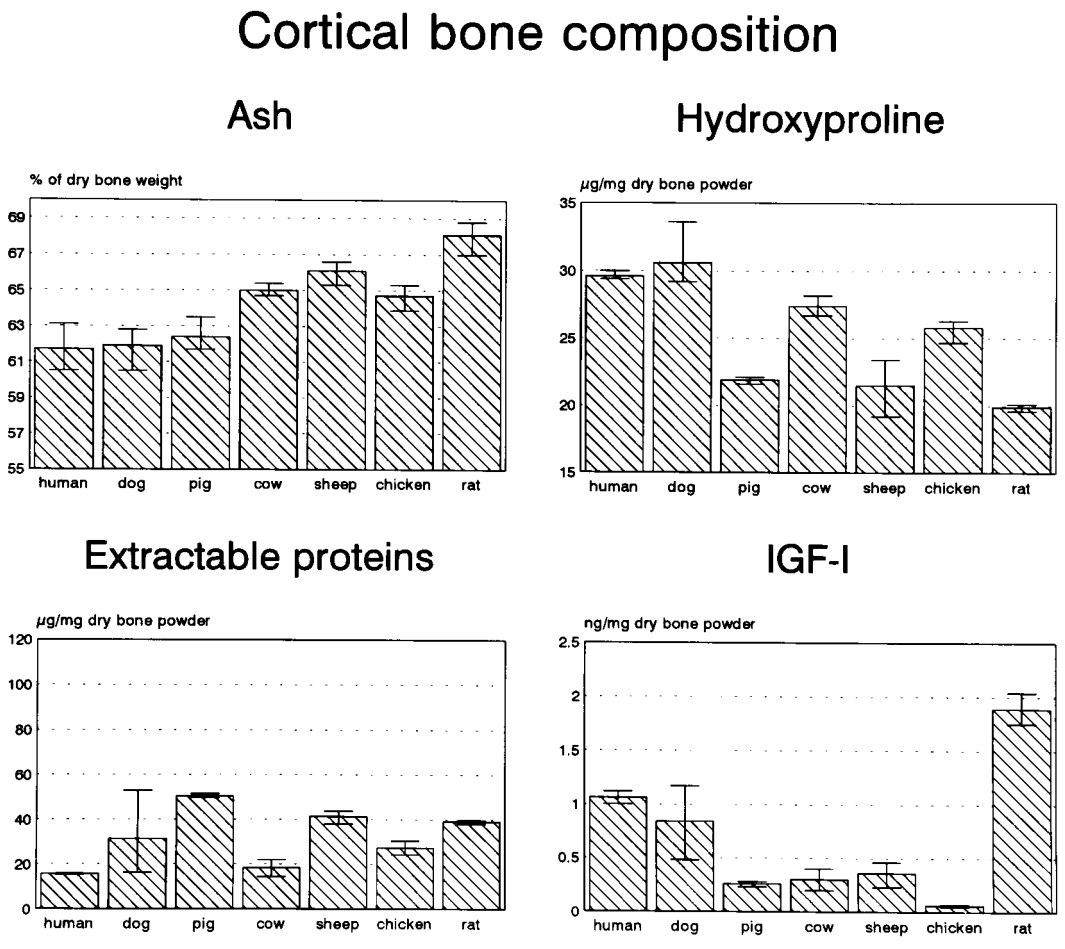

FIG. 1. Bone composition of cortical femoral bone samples of seven different species: human, dog, pig, cow, sheep, chicken, and rat. *Bars* indicate the mean value of three examined cases per species; *error bars* indicate the range.

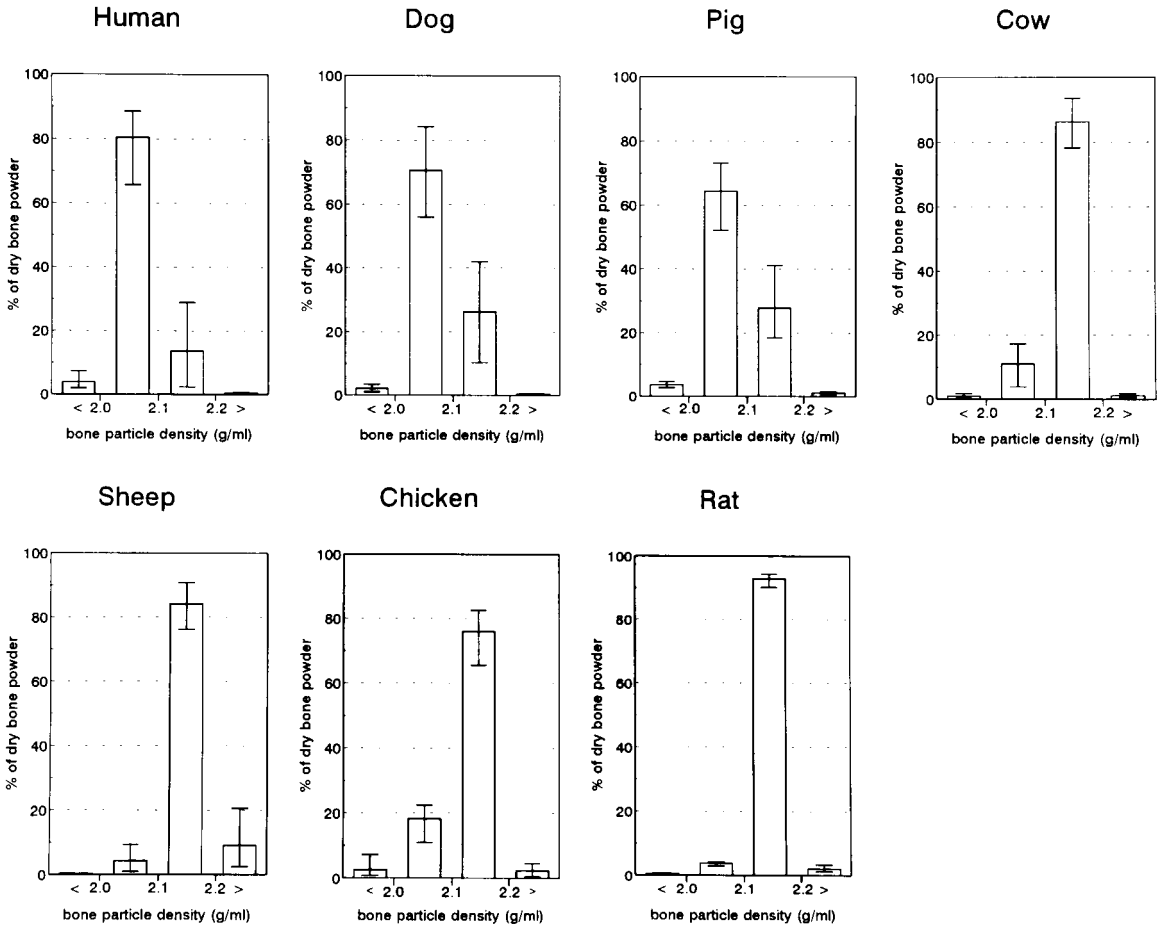

FIG. 2. Density distribution profiles of cortical femoral bone powder of seven different species: human, dog, pig, cow, sheep, chicken, and rat. Values are expressed for each density fraction as the percentage of the total bone sample weight (± SD).

sample and are not solely the result of a relative shift of a part of the bone particles to a higher/lower density. The total amount of extractable proteins was in general higher in the lower mineralized density fraction (2.0–2.1 g/ml), but the concentration varied significantly among species (Table 1). Bone powder extracts from rats, however, showed a higher concentration of extractable proteins in the higher mineralized density fraction (2.1–2.2 g/ml). The IGF-I did not show large variations among the different density fractions of a species; a similar interspecies variation was found as in the unfractionated bone powder samples.

Lumbar spine samples (trabecular bone)

The results of the biochemical analyses of the trabecular bone samples derived from the lumbar spine specimens are shown in Fig. 3. In general, much less variation was found among the examined species with regard to trabecular bone composition compared with the compact bone samples (Fig. 1). The ash concentrations in dog, pig, and sheep were very similar to the human samples; only the ash content of the samples derived from the cow was somewhat lower compared with the other samples. Inversely, collagen concentration was higher in the cow samples compared with the other species and was somewhat lower in pig and sheep. The cow showed the largest contrast between cortical and trabecular bone samples: a relative high ash content in cortical bone was combined with a relative low ash content in the trabecular bone samples. In all species, the amount of extractable proteins was significantly higher (on average, a doubling of the amount) in the lumbar spine bone powder samples compared with the cortical samples derived from the femur. The relative differences between the species was similar, however, in the trabecular and the cortical bone samples. Only the trabecular samples derived from the cow showed a higher-than-expected amount of extractable proteins based on the results from the cortical samples. Although IGF-I contributes to the large pool of extractable proteins, large differences between trabecular and cortical bone samples were observed with regard to the IGF-I concentration.

Fixed-size cylindrical cores, prepared from the lumbar spine specimens, were analyzed by bone mineral density measurements and mechanical testing. The results are summarized in Table 2. Both the BMC and vBMD values were approximately 50% lower in the human samples compared with the samples from the dog and pig. The BMC and vBMD in dog and pig were in turn much lower in comparison with cow and sheep. From the raw data of the mechanical tests, the fracture stress was derived. Fracture stress was lowest in the samples from human and pig, intermediate in dog and cow samples, and highest in sheep samples. The relation

TABLE 1. Concentration of biochemical bone components in cortical bone powder sample fractions with a density of 2.0–2.1 g/ml and 2.1–2.2 g/ml, respectively

Parameter	Density fraction (g/ml)	Human	Dog	Pig	Cow	Chicken	Sheep	Rat
Ash (% of dry bone)	2.0–2.1	61.3 (61.0–61.6)	62.5 (60.8–63.3)	64.3 (63.4–65.5)	61.5 (61.3–61.7)	61.9 (60.8–62.5)	63.4 (62.6–64.2)	62.2 (62.1–62.3)
	2.1–2.2	63.4 (62.6–64.2)	63.7 (63.3–64.4)	66.3 (65.1–67.9)	65.4 (65.2–65.8)	65.0 (63.7–66.5)	69.0 (68.2–69.7)	66.9 (66.5–67.6)
Hydroxyproline (μg/mg dry bone)	2.0–2.1	30.0 (28.7–30.7)	30.4 (30.1–30.8)	22.8 (22.4–23.4)	31.8 (30.8–32.8)	26.9 (26.6–27.3)	25.9 (23.9–27.9)	23.7 (23.6–23.7)
	2.1–2.2	27.8 (27.5–28.2)	27.1 (26.4–28.1)	20.4 (20.2–20.6)	27.0 (26.7–27.3)	24.8 (23.6–26.1)	20.4 (19.8–20.8)	18.4 (18.2–18.7)
Total protein (μ/mg dry bone)	2.0–2.1	64.1 (56.7–77.5)	74.1 (70.6–77.5)	124.6 (120.0–124.4)	79.7 (79.7–79.7)	128.8 (109.1–150.7)	120.1 (114.1–126.1)	60.5 (58.6–64.3)
	2.1–2.2	49.2 (40.1–58.3)	61.0 (57.9–62.9)	84.5 (75.6–96.6)	56.0 (49.7–61.5)	56.6 (52.1–70.0)	80.7 (68.1–90.9)	89.3 (74.3–101.1)
IGF-I (ng/mg dry bone)	2.0–2.1	1.12 (1.09–1.22)	1.1 (0.7–1.2)	0.26 (0.23–0.29)	0.49 (0.49–0.49)	0.09 (0.06–0.11)	0.34 (0.30–0.38)	2.1 (1.9–2.3)
	2.1–2.2	1.09 (0.94–1.24)	0.9 (0.6–1.2)	0.28 (0.23–0.34)	0.42 (0.30–0.49)	0.11 (0.08–0.15)	0.35 (0.25–0.43)	1.6 (1.5–1.7)

The samples were prepared by gradient density fractionation of bone powder from femoral bones of seven different species. Results are presented as mean and the range.

between fracture stress and mineral mass (BMC) and density (vBMD), respectively, in the trabecular bone samples derived from different species is visualized in Fig. 4. This figure illustrates that each of the examined species forms a separate entity with no or only limited overlaps with the data from other species. It suggests, furthermore, that the relative contribution of BMC and/or vBMD measurements to the bone quality, measured as fracture stress, is likely to be species-dependent.

Discussion

Animal models are being widely used to study all aspects of bone research, *e.g.* (postovariectomy) bone loss (2, 15, 16, 28), aging (12, 16, 22), fracture healing (29), calcium homeostasis (20), effects of drug treatment (6, 7, 23), etc. As the FDA currently requires the evaluation of new drugs in at least one small (rodent) and one large animal model, it is obvious that the choice of an appropriate animal model for these studies is extremely important. An ideal model that can be used for all studies in bone research does not exist; whether or not an animal model is useful depends largely on the specific objectives of the study. Numerous reviews, discussing the advantages and disadvantages of different animal models in specific bone research areas, have been published during the past few years (1, 4, 13–15, 28, 30). However, only a few studies have been undertaken to systematically and directly compare different species with regard to parameters relevant in bone research (31–33). It is obvious, however, that similarity or dissimilarity of the normal values of such parameters may significantly contribute to the usefulness of an animal model. The present study is, to our knowledge, the first to document interspecies differences in bone composition, bone density, and bone quality, based on a systematic comparison of a large set of different species that are frequently used in bone research.

Biochemical analysis of the trabecular and cortical bone of the included species reveals several interesting points. First, significant interspecies differences are present for each of the examined bone composition parameters, both in cortical and trabecular bone. An early study by Biltz and Pellegrino (31) reported on the biochemical analysis of a limited number of cortical bone samples, derived from a variety of different species. The data on ash and hydroxyproline content in these samples were comparable with the data of the present study. The composition of human and rat cortical bone has also been investigated by Mbuyi and Dequeker (32), who compared the collagen content and the content of some nonspecific markers for noncollagenous proteins, such as sialic acid, uronic acid, and hexoses. Higher collagen and lower EDTA-extractable protein contents were found in human compared with rat bone. This was confirmed by the results of the present study, which extends the biochemical analyses of the latter two studies to the analysis of several other species currently used in bone research, to the simultaneous analysis of trabecular bone, and to the analysis of more specific parameters. However, the physiological and phylogenetic implications of the species variations in bone composition remain to be determined. Second, the density fractionation analyses of the cortical bone samples indicate that both a shift

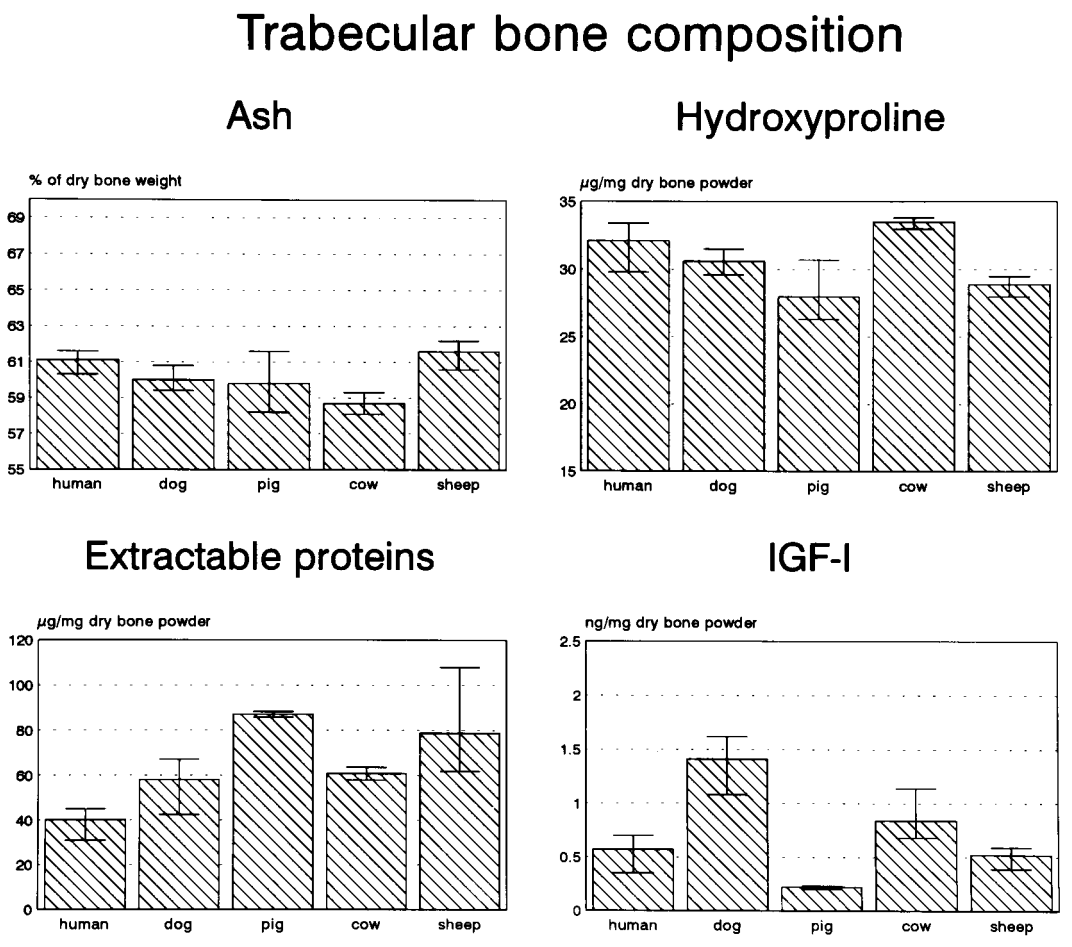

FIG. 3. Bone composition of trabecular samples from the lumbar spine of five different species: human, dog, pig, cow, and sheep. *Bars* indicate the mean value of three examined cases per species; *error bars* indicate the range.

of a portion of the microscopic bone particles toward another density and composition, as well as compositional differences within a specific density range, may contribute to the interspecies differences observed in the samples of whole bone. These results support the hypothesis that the material properties of the bone particles are highly species-dependent and might contribute to interspecies differences in bone quality characteristics. It is well recognized that, in addition to bone mass and bone architecture, bone composition contributes to bone quality properties (34). Third, it is clear that bone composition in some species more closely resembles human bone composition than others. In particular, it should be noted that, of all species examined, the rat differs most, whereas the bone composition of the dog most resembles that of human bone. This observation supports the requirement of the FDA that evaluation of drugs should be performed, in addition to the rat, in at least one larger animal. Our results support the recommendation by Rodgers *et al.* (28) to use the relatively inexpensive and well characterized rat model for preliminary screenings of new pharmacological agents or therapeutic modalities, followed by verification in other species, before undertaking clinical trials in human patients.

Results of bone density measurements and mechanical testing also show significant interspecies differences. Human bone specimens constitute a clearly separated entity compared with all other species, both with regard to BMC and vBMD analyses. It is, however, speculative to ascribe the higher fracture incidence in humans compared with other species to the fact that humans show the lowest BMC, vBMD, and fracture stress values. Based on these analyses, the pig and dog most resemble the human situation. A positive intraspecies correlation between bone density and fracture stress was observed. In general, this correlation was also found at the interspecies level. However, comparison of individual species does not necessarily follow this correlation. Fracture stress in dogs is higher compared with pigs, although similar BMC and vBMD values are observed. Similarly, sheep show higher fracture stress compared with cows, but bone density measurement data are comparable. This suggests that the relative contribution of BMC and/or vBMD data to the bone quality, measured as fracture stress, is species-dependent. Whereas BMC and BMD measurements in humans have been shown to predict up to 70% of bone strength (35), it is clear from our data that such a relationship cannot simply be transferred to the situation in animal models or vice versa.

The present analysis has several limitations. The number of animals per species was very limited; yet the intraspecies

TABLE 2. Summarized results of bone mass (BMC and BMD) measurements and mechanical testing of trabecular bone samples (cylindrical cores) obtained from the lumbar spine of five different species

Species	Human (n = 4)	Dog (n = 5)	Pig (n = 6)	Cow (n = 6)	Sheep (n = 6)
BMC (mg)					
Mean	76.3	173.1	173.0	232.7	236.1
Range	(45.8–110.6)	(138.1–221.3)	(162.7–192.9)	(193.1–294.6)	(212.4–273.1)
Axial area (circle) (mm^2)					
Mean	47.6	56.6	55.8	57.1	57.3
Range	(41.6–50.0)	(53.2–60.5)	(52.0–58.0)	(54.8–62.3)	(56.0–58.3)
Height (mm)					
Mean	8.97	8.98	8.46	9.07	9.41
Range	(8.21–9.61)	(8.57–9.34)	(7.43–9.11)	(8.62–9.33)	(9.02–9.73)
vBMD (mg/cm^3)					
Mean	178	340	373	449	437
Range	(98–270)	(297–390)	(348–441)	(342–536)	(400–477)
Fracture stress (N/mm^2)					
Mean	1.21	6.12	2.40	5.67	13.22
Range	(0.08–2.40)	(4.32–7.78)	(1.70–3.76)	(1.98–9.28)	(9.30–15.79)

n indicates the number of examined cylindrical cores per species (one or two per subject).

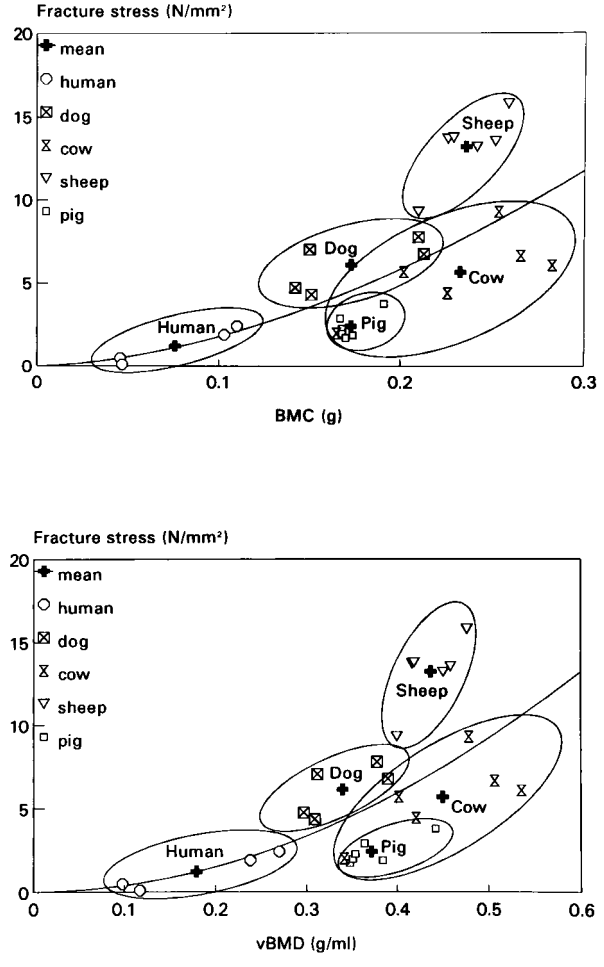

FIG. 4. Relationship between fracture stress and BMC and vBMD, respectively, in cylindrical cores obtained from the lumbar spine of five different species. Individual values are indicated with *open symbols* and grouped per species with *ovals*. The mean value per species is shown in *bold*. The *solid line* indicates the curve of best fit, based upon all the individual data points.

variation was much lower compared with the interspecies variation. The age and sex of the animals may also influence the results. To minimize the bias of these confounders, we used sexually mature female animals as is frequently done in bone studies. Other intraspecies differences, *e.g.* animal strain, are difficult to correct for, unless multiple breeds per species are included in the study. This was, however, beyond the objectives of this study. Finally, a limiting factor of our study might be found in the choice of the examined anatomical sites. Although bone samples from the same anatomical sites (femur and lumbar spine) were analyzed for all species examined, it cannot be excluded that at least part of the observed differences may be attributed to differences in load-bearing of the lumbar spine and the femur. From among these species, humans are the only ones to walk in an upright position, and it could be expected that, by evolution, this leads to differences in architectural and mechanical bone properties. Indeed, the human lumbar spine is clearly different from all other examined species, in that its bone density and fracture stress are much lower compared with the other species. It would be of interest to document whether similar characteristics of the lumbar spine are also observed in other upright walking species, such as nonhuman primates.

It seems unlikely that the large interspecies differences reported here can all be attributed to the above-mentioned confounding factors alone. The fact that all the analyses described in this study have been performed in a single laboratory using the same analytical procedures for all samples strengthens the present interspecies comparison. Nevertheless, it is impossible to exclude the impact of all these confounders on the results from the real genetic interspecies differences. Moreover, the small number of animals per species does not allow statistical analyses. The presented data should therefore be interpreted as relative indications of qualitative differences only, and not as absolute reference values for further studies.

In summary, this study shows marked interspecies dif-

ferences with regard to bone composition, bone density, and bone mechanical competence. None of these animal models is similar to the human situation for all examined parameters. Some animals, however, more closely resemble humans than others. In particular, with regard to bone density and quality parameters of the lumbar spine, humans appear to be very different from the other species examined. Based on a combination of all the parameters examined in the animals of our study, we conclude that the characteristics of human bone are best approximated by the properties of dog bone. The large biochemical differences in bone composition in the rat and human indicate that bone research data derived from this most frequently used animal model should be transferred to the clinical situation with utmost care. It would be speculative to predict whether the human bone response to a treatment will also be best resembled by the dog model. Moreover, other factors (*e.g.* physiology, endocrinology) that were not evaluated in this study also influence bone response. It is obvious that the interspecies variations that are demonstrated in this study may affect other clinical and experimental bone parameters and should therefore be taken into account when selecting an animal model for bone research.

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