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Bone 38 (2006) 265–272

BONE

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Regional distinctions in cortical bone mineral density measured by pQCT can predict alterations in material property at the tibial diaphysis of the Cynomolgus monkey

Kiichi Nonaka [a,b,c,*], Satoshi Fukuda [b], Kazuhiro Aoki [a], Takashi Yoshida [d], Keiichi Ohya [a]

[a] *Section of Pharmacology, Department of Hard Tissue Engineering, Division of Bio-Matrix, Graduate School, Tokyo Medical and Dental University, Japan*
[b] *International Space Radiation Laboratory, National Institute of Radiological Sciences, Japan*
[c] *Section of Health Science, ELK Corporation, Japan*
[d] *Tsukuba Primate Research Center, National Institute of Biomedical Innovation, Japan*

Received 17 December 2004; revised 14 July 2005; accepted 17 August 2005
Available online 5 October 2005

Abstract

We examined whether regional differences in cortical bone mineral density (Ct.BMD) measured by peripheral quantitative computed tomography is related to the heterogeneity of bone tissue and whether regional Ct.BMD is a better indicator of changes in bone material properties. Bilateral tibiae were obtained from 17 female adult Cynomolgus monkeys (*Macaca fascicularis*; mean age 16.8 years). After determining that Ct.BMD was similar between the right and left tibiae, the left tibiae were used for bone histomorphometry and the right for a three-point bending test. The Ct.BMD in the posterior quadrant was significantly higher than that in the anterior quadrant. In the bone histomorphometric analysis, all parameters (i.e., average osteonal area, average osteonal bone area, osteon population density, percent osteonal area [%On.Ar], percent osteonal bone area [%On.B.Ar], percent osteonal area of initial remodeling [%Il.On.Ar], percent osteonal area of secondary remodeling [%Sd.On.Ar], porosity, and percent osteoid area in the posterior region) were significantly lower than those in the anterior region. The results indicated that in the same cross-section, bone tissue structure was heterogeneous. Both total- and posterior-Ct.BMD were positively correlated with breaking stress and negatively correlated with toughness, whereas anterior-Ct.BMD was positively correlated with elastic modulus. Backward stepwise multiple regression analyses indicated that posterior-Ct.BMD and total-Ct.BMD were the best variables for predicting breaking stress and toughness, respectively, when age is taken into account. The %On.Ar, %On.B.Ar, and %Il.On.Ar in the posterior region were negatively correlated with elastic modulus. The %On.Ar, %On.B.Ar, and %Sd.On.Ar in the posterior region were positively correlated with toughness. These findings indicated that regional Ct.BMD measurement is useful to assess changes in the material properties of bone associated with the degree of mineralization. In particular, anterior-, posterior-, and total-Ct.BMD can be used separately to predict changes in the material properties of the tibial diaphysis.

Keywords: pQCT; Cortical bone; Mechanical testing; Mineralization; Cynomolgus monkey

Introduction

Knowledge of both geometric and material properties is necessary to assess whole-bone mechanical properties [1–5]. Peripheral quantitative computed tomography (pQCT), which is widely used for animal experiments and clinical diagnosis, can be used to analyze section modulus and cross-sectional moment of inertia as geometric properties under bending or torsional loading conditions [6–11]. It can also be used to analyze volumetric cortical bone mineral density (Ct.BMD; g/cm^3), which may in turn be used to predict the mechanical properties of bone material [12,13]. In contrast, the value of bone mineral density (BMD; g/cm^2) obtained by dual-energy X-ray absorptiometry, which is also extensively used clinically [14–16], is expressed as bone mineral content per projected bone area. Because the BMD determined by dual-energy X-ray absorptiometry derives from the sum of both trabecular and cortical bone mineral content and is dependent

* Corresponding author. Section of Health Science, ELK Corporation, 2-17-4, Yushima, Bunkyo-Ku, Tokyo 113-0034, Japan. Fax: +81 3 3818 9385.
E-mail address: kiichi_nonaka@elkc.co.jp (K. Nonaka).

8756-3282/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.bone.2005.08.012

on bone size, it is not a predictor of the tissue material properties and is fundamentally different from Ct.BMD determined by pQCT.

Previous studies indicate that toughness of human bone is negatively correlated with age and ash content [17,18], that is, older bone is less tough than younger bone due to the high degree of mineralization. Decreased toughness reduces the ability of bone to absorb the energy of impacts and makes it more brittle. Although increased mineralization increases bone strength in static tests, it reduces toughness and makes bone more likely to fracture on impact. Although Ct.BMD positively relates to breaking stress and elastic modulus [12,13], the relationship between Ct.BMD and toughness remains unclear [19–21]. Ct.BMD is calculated from the whole cortical bone area based on the assumption of homogeneity. There is, however, some heterogeneity in bone tissue, such as porosity, remodeling, microdamage accumulation, osteonal population density, and mineralization [22–27].

We hypothesized that, in the same cross-section, there are regional differences in Ct.BMD measured by pQCT. Our objective was to examine whether regional analysis of Ct. BMD, measured by pQCT, could improve the assessment of material properties by analyzing its relation to the values of intracortical bone histomorphometry and structural mechanical testing in the tibial diaphysis of Cynomolgus monkey.

Materials and methods

Experimental animals

Seventeen female Cynomolgus monkeys (*Macaca fascicularis*), aged 16.8 ± 2.6 y (mean ± SD) and weighing 4.6 ± 1.1 kg, were used. All animals were bred in a cage with standard diets at the Tsukuba Primate Research Center, National Institute of Biomedical Innovation, and had no diseases that affect bone metabolism. After the animals were euthanized, all extremities were fixed in 10% neutral buffered formalin, and the soft tissue was removed. Thereafter, the tibiae were excised and transferred to 70% ethyl alcohol. The lengths of the right tibiae were measured using calipers (120.4 ± 5.1 mm). All animals in the study were treated and/or handled according to the "Recommendations for the Handling of Laboratory Animals for Biomedical Research" at Tsukuba Primate Research Center.

Bone densitometry

The mid-diaphyses of the right and left tibiae were scanned using a pQCT device (XCT Research SA+, Stratec Medizintechnik GmbH, Pforzheim, Germany) with pixel dimensions of 0.3 mm × 0.3 mm and a slice thickness of 0.77 mm. Total-Ct.BMD was analyzed for the whole cortical compartment, which was determined using a threshold value of 690 mg/cm^3, using pQCT software, Rev. 5.40. We also analyzed cross-sectional moment of inertia about the x axis (I_x) and section modulus about the x axis (Z_x), which was defined as I_x divided by half the depth of the cross-section. The cross-sectional images were divided into quadrants to analyze regional Ct.BMD in the anterior and posterior regions (Fig. 1). The coefficients of variation (%CV; standard deviation/mean) in our laboratory were 0.35% for Ct.BMD, 1.38% for I_x, and 2.26% for Z_x. These values were averaged %CVs determined from five repeated measurements of each of three tibiae, with sample repositioning before each measurement.

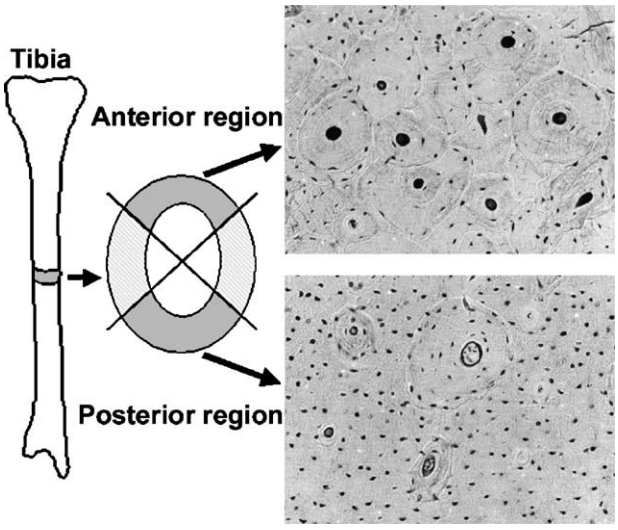

Fig. 1. Regions analyzed for pQCT and bone histomorphometry. Cross-sectional images measured by pQCT at the middle of the diaphyses were divided into quadrants. Cortical bone mineral density (Ct.BMD) was obtained from the total, anterior, and posterior cortex regions. The cross-sections of bone histomorphometry were also divided into quadrants to distinguish the anterior and posterior regions, corresponding to the same regions analyzed by pQCT.

Bone histomorphometry

Tissue preparation

Bone samples (7 mm thick) were taken from the middle of the left tibiae, immersed in Villanueva's bone stain solution, and later embedded in methylmethacrylate. Sections (25 μm thick) were prepared from the bone blocks using an auto-microtome (SP1600, Leica, Nussloch, Germany) and mounted on glass slides.

Intracortical histomorphometry

Segmented images of bone samples (1300 × 1030 pixels) were recorded using a digitizing system (Axiovision, Carl Zeiss, Germany) attached to a microscope (AxioPhoto, Carl Zeiss, Germany) at 50× magnification. The whole cross-sectional image was later reconstructed using image editing software (Adobe Photoshop Elements, Adobe Systems Inc., San Jose, CA). The entire cross-sectional image was divided into quadrants to distinguish the anterior and posterior regions, corresponding to the same area analyzed by pQCT (Fig. 1). Cortical area (Ct.Ar) of the anterior and posterior cortices was measured from the reconstructed image. Bone histomorphometry was performed using image analysis software (KS-400, Carl Zeiss, Germany) at 100× magnification. Primary and secondary parameters are presented in Table 1. The variables employed for this study were as follows; average osteonal area (av-On.Ar), average osteonal bone area (av-On.B.Ar), osteon population density (On.D), percent osteonal area (%On.Ar), percent osteonal bone area (%On.B.Ar), percent osteonal area of initial remodeling (%Il.On.Ar), percent osteonal area of secondary remodeling (%Sd.On.Ar), porosity (Po), and percent osteoid area (% O.Ar). The histomorphometric nomenclature used is that of the American Society for Bone and Mineral Research Committee [28].

Mechanical testing

The mechanical properties of the right tibia were measured by a three-point bending method using a mechanical testing machine (MZ-500S, Maruto Co. Ltd., Tokyo, Japan). The bending load was applied on the posterior surface of tibia at a speed of 10 mm/min until fracture, with the anterior surface of the tibia facing downwards on end supports separated by 60 mm. Breaking force, stiffness (slope of the linear portion of the load deformation curve), and work of fracture (area under the load deformation curve before fracture) were measured as whole-bone mechanical properties [29,30]. These values were normalized by cross-sectional moment of inertia and section modulus analyzed by pQCT to