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Variation of trabecular architecture in proximal femur of postmenopausal women

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ABSTRACT

This investigation of microstructure in the human proximal femur probes the relationship between the parameters of the FRAX index of fracture risk and the parameters of bone microstructure. The specificity of fracture sites at the proximal femur raises the question of whether trabecular parameters are site-specific during post-menopause, before occurrence of fragility fracture. The donated proximal femurs of sixteen post-menopausal women in the sixth and seventh decades of life, free of metabolic pathologies and therapeutic interventions that could have altered the bone tissue, constituted the material of the study. We assessed bone mineral density of the proximal femurs by dual energy X-ray absorptiometry and then sectioned the femurs through the center of the femoral head and along the femoral neck axis. For each proximal femur, morphometry of trabeculae was conducted on the plane of the section divided into conventional regions and sub-regions consistent with the previously identified trabecular families that provide regions of relatively homogeneous microstructure. Mean trabecular width and percent bone area were calculated at such sites. Our findings indicate that each of mean trabecular width and percent bone area vary within each proximal femur independently from each other, with dependence on site. Both trabecular parameters show significant differences between pairs of sites. We speculate that a high FRAX index at the hip corresponds to a reduced percent bone area among sites that gives a more homogeneous and less site-specific quality to the proximal femur. This phenomenon may render the local tissue less able to carry out the expected mechanical function.

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1. Introduction

Fragility fractures due to degradation of bone tissue during normal function are responsible for significant morbidity and mortality in elderly patients (Ulrich et al., 1999; Brenneman et al., 2006; Seeman and Delmas, 2006). Accordingly, accurate assessment of absolute fracture risk earlier in life is critical to establishing the threshold for clinical intervention (Cheng et al., 1997; Dequeker, 1994; Abrahamsen et al., 2006). After the vertebral bodies, the proximal femur is the anatomical site most likely to experience non-traumatic fractures (Wasnich, 1996; Patel and Murphy, 2006; Pulkkinen et al., 2006; Sornay-Rendu et al., 2007). A variety of factors contributes to fragility fractures at the proximal femur (Wasnich, 1996; De Laet et al., 2005; Bousson et al., 2006; Havill et al., 2007).

Bone mineral density (BMD) as initially assessed using dual energy X-ray absorptiometry (DXA) at the conventional regions (neck, Ward's triangle, greater trochanter and intermediate region between the epiphysis and diaphysis) is insufficient to explain fracture risk in patients. Additional technologies are currently employed to assess BMD as well as microstructural parameters (Tothill, 1989; Mazess, et al., 1992; Dequeker, 1994; Kothari et al., 1998; Kowalczyk, 2010; Krug et al., 2005; Krieg et al., 2006; Abrahamsen et al., 2006; Patel and Murphy, 2006; Hernandez et al., 2008; Premaor et al., 2010; Greenspan et al., 2010), with a view towards understanding the influence of the factors of fracture risk (age, sex, weight, height, previous fracture, parent fractured hip, current smoking, glucocorticoids, rheumatoid arthritis, secondary osteoporosis, alcohol consumption, femoral neck BMD) established through epidemiological clinical studies and incorporated within the FRAX index (Kanis, 2002; Premaor et al., 2010; http://www.sheffield.ac.uk/FRAX/), on bone microstructure. Such understanding would aid in formulating appropriate decisions about therapeutic interventions to avoid occurrence of fracture later in life.

The patterns of distribution of the cortical and trabecular parameters that yield a specific BMD at any given region of the proximal femur is unknown. For instance, fractures at the proximal

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femur, whose location depends on age and absence/presence of bone pathology, are yet to be explained in terms of ultra- and micro-structural parameters (Zuckerman, 1996; Tsangari et al., 2006). Likewise, a method of measuring the degree of organization of the trabecular network by characterizing trabecular anisotropy remains elusive (Chappard et al., 2005). The understanding of propagation of femoral fractures requires continued investigation of bone's ultra- and micro-structural parameters.

The microstructural specifications pertinent to healthy bone as well as those pertinent to bone affected by metabolic disease are the object of abundant inquiry. The current study was motivated by our qualitative observations on donor proximal femur that: (1) the width of trabeculae and the space between trabeculae depends on site; (2) specific sites show large space between trabeculae (e.g. upper portion of the greater trochanter in various older subjects) relative to other sites (e.g. neck). We hypothesized that the two trabecular parameters of trabecular width and percent bone area (nomenclature follows definitions in Parfitt (1987)) vary independently from each other, but with dependence on site. This study refers to the proximal femur of post-menopausal female donors aged 52–70 with a clinical history free of bone pathology and of medical therapies that could have altered bone metabolism. We assessed the organization of the trabecular network in terms of trabecular width and percent bone area and established microstructurally significant differences among proximal regions. We investigated the link between the two parameters of trabecular width and percent bone area, BMD assessed by DXA scan and fracture risk.

This work on the trabecular bone is developing along the conceptual lines of analogous research on the morphology of the compact bone microstructure (Ascenzi, 1988; Ascenzi et al., 2004, 2008). The studies of patterns of micro-structure within the macro-structure yield findings in both instances suitable for inclusion in, and verification of, finite element modeling for hierarchical simulation of biomechanical function.

2. Methods

2.1. Preparation and selection of specimens

The International Institute for the Advancement of Medicine, the Indiana Organ Procurement Organization, the Musculoskeletal Transplant Foundation, The National Disease Research Interchange and ScienceCare Anatomical provided the Caucasian female human femurs ranging in age from 52 to 70 used for this study. The clinical history of each donor was available and confirmed as free from medical therapies that could have altered the bone tissue and from metabolic pathologies. After removal and defleshing, histology of the trabecular tissue from the distal femur was assessed to establish either presence or absence of osteomalacia through thickness of osteoid border. The undecalcified bone was dehydrated in alcohol (70%; 100%), cleared with xylene and embedded in plastic resin methylmethacrylate. Static parameters of osteoid volume and thickness were observed in 5 μm sections treated with toluidine blue stain (Hernandez et al., 2008). Appearance of osteoid volume and thickness larger than normal was interpreted as potential osteomalacia. Osteomalacia was excluded in twelve out of sixteen donors.

Each femur was embedded in rice bags for standard simulation of soft tissue (Hologic Inc., 1996) and positioned under a DXA scanner Delphi A (Hologic Inc.) equivalently to the femur of a patient lying supine under the scanner. The DXA scan provided the assessment of BMD at specific regions of the proximal femur: neck, Ward's triangle, greater trochanter and intermediate region between the epiphysis and diaphysis. So-called total BMD is the BMD measure of the region that includes neck, greater trochanter, intermediate area between epyphisis and diaphisis with exclusion of Ward's triangle and femoral head. The *T*-score of total BMD was computed as the number of standard deviations from the mean total BMD of sex-matched young adults at their peak bone mass. The FRAX index at the hip was then computed, except for the few donors for whom height and weight were unavailable. Each proximal femur was then sectioned longitudinally through the center of the femoral head along the femoral neck axis (Fig. 1a) by means of a high-precision sectioning saw (Harrington Tool Co., Michigan). The bone marrow closer to the cut surface of each half proximal femur was removed by enzymatic digestion enhanced by a solution of water and Tergazyme (Alconox, Inc.) (Boyde, 1984).

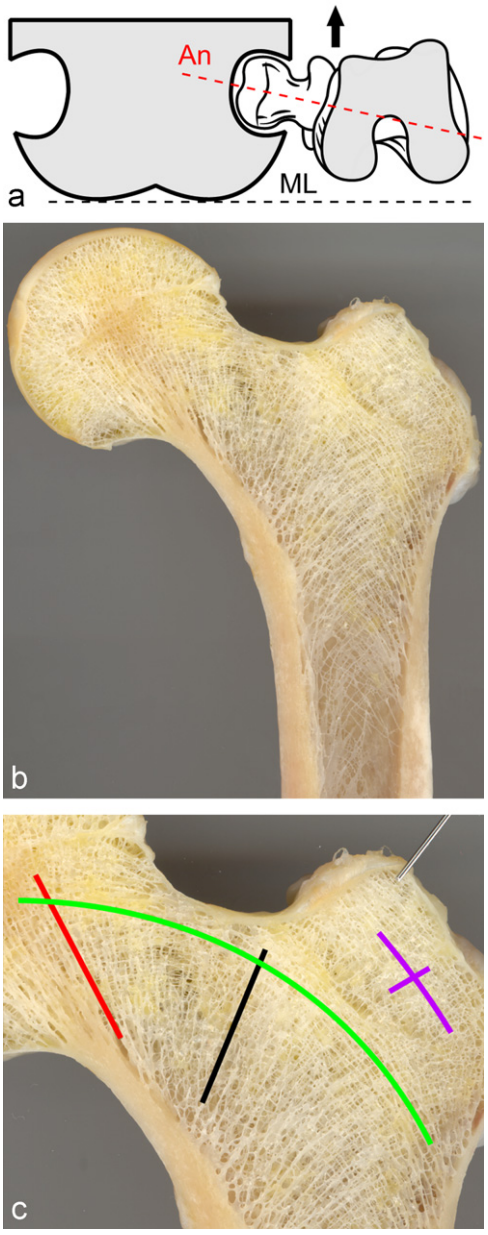

Fig. 1. The investigated proximal femur. (a) Schematic showing the position of the longitudinal cut along the axis (An) of the femoral neck through the center of the femoral head with respect to the medial-lateral (ML) axis. The arrow points to the anterior aspect. (b) The trabeculae are exposed for halved proximal femur of 66 year old donor #11 with lower than normal BMD, at 16% reduction. (c) The *right family* (general pattern in red) of trabeculae runs from the medial metaphysis through the neck and fans out in the head; the *bent family* (in green) runs from the lateral metaphysis through the neck and crosses with the right family in the central head; the *medial family* (in black) crosses the bent family at an acute angle to enter the medial metaphysis; and the *greater trochanter family* (in violet) spans the upper portion of the greater trochanter (Ward, 1838; Singh et al., 1970).

2.2. Imaging and morphometry

For each sectioned femur, an image of the complete section of the femur was obtained using an HP Scanjet 4890 desktop scanner set to a resolution of 1000 dpi (Fig. 1b). The images were imported in graphic software XaraX1 (XaraX Co) and analyzed at 40x. The morphometric analysis of each image referred to the trabecular structure (Fig. 1c) exposed by sectioning (Scolamacchia, 1999). Specifically, on each trabecula a line segment was manually drawn perpendicularly to the trabecular walls with a minimum of 200 μm between subsequent segments along a non-branching trabecula (Fig. 2a). Further, a 1 mm grid was superimposed electronically to each image to assess percent bone area. A green dot placed at a given intersection point of the grid denoted a hit, i.e. the presence of a trabecula, while a red dot denoted a miss, i.e. lack of trabecula at such intersection point (Fig. 2b). This method is described in Parfitt (1983) to compute