Bone 91 (2016) 11–19

Contents lists available at ScienceDirect

Bone

journal homepage: www.elsevier.com/locate/bone

Full Length Article

Osteocyte lacunar properties and cortical microstructure in human iliac crest as a function of age and sex

Fiona Linnea Bach-Gansmo [a], Annemarie Brüel [b], Michael Vinkel Jensen [b], Ebbe Nils Ebbesen [b], Henrik Birkedal [a,*,1], Jesper Skovhus Thomsen [b,**,1]

[a] Department of Chemistry and iNANO, Aarhus University, Aarhus, Denmark
[b] Department of Biomedicine, Aarhus University, Aarhus, Denmark

ARTICLE INFO

Article history:
Received 22 February 2016
Revised 27 June 2016
Accepted 6 July 2016
Available online 07 July 2016

Keywords:
Osteocytes
Osteocyte lacunae
Iliac crest
Cortical bone
Age
Sex
Nano-CT

ABSTRACT

Osteocytes are suggested to play a central role in bone remodeling. Evaluation of iliac crest biopsies is a standard procedure for evaluating bone conditions in the clinical setting. Despite the widespread use of such biopsies, little is known about the population of osteocytes in the iliac crest from normal individuals. Contradicting results have been reported on osteocyte lacunar properties in human bone. Hence, a solid understanding of the osteocyte population in healthy bone and the effect of age and sex is needed as good reference data are lacking. Furthermore, the role of cortical bone in bone quality has recently been suggested to be more important than previously realized. Therefore, the present study assesses osteocyte lacunar properties and cortical microstructure of the iliac crest as a function of age and sex. A total of 88 iliac crest bone samples from healthy individuals (46 women, aged 18.5–96.4 years and 42 men, aged 22.6–94.6 years) with an even age-distribution were examined using synchrotron radiation μCT and in house μCT, with $>5 \times 10^6$ osteocyte lacunae measured and analyzed. The study revealed that osteocyte lacunar volumes were unaffected by both age and sex. Osteocyte lacunar density did not differ between women and men, and only showed a significant decrease with age when pooling data from both sexes. Cortical porosity and Haversian canal density increased while cortical thickness decreased with age, with cortical thinning dominating the age-related cortical bone loss. None of the cortical microstructural parameters showed any sex dependency. Only weak links between osteocyte lacunar properties and cortical microstructural properties in iliac crest bone were found. Interestingly, the Haversian canal diameters were significantly but weakly negatively correlated with osteocyte lacunar volumes.

1. Introduction

The processes behind the age-related effects on bone are not fully understood, and with the increasing lifespan of the human population, this question is becoming increasingly relevant. Traditionally, age-related changes in bone have been considered more pronounced in women than in men, and therefore many studies have been conducted in women only. However, it remains unclear whether the age-related changes are dependent on sex or not, as there are several studies showing little or no difference between the sexes [1–4]. Most studies concerning aging of the normal skeleton have been performed in trabecular bone, whereas aging of cortical bone has been much less explored. This is surprising since cortical bone comprise approximately 80% of the total bone mass in the skeleton [5]. Moreover, cortical geometry and porosity has been linked to stiffness and strength of human bone [6,7], and loss of cortical bone has been related to proximal femur fractures [8]. Increasing the understanding of the cortical bone compartment therefore seems necessary in order to reveal its role in bone quality, as also pointed out by other researchers [9,10].

Recently, osteocytes have gained increasing attention, as it is becoming clear that they play a central role in the control of bone remodeling [11]. Osteocytes are by far the most abundant cells in bone, the number of osteocytes is estimated as ten times that of osteoblasts and thousand times that of osteoclasts [12]. Osteocytes are located in osteocyte lacunae throughout the bone matrix and are connected to other osteocytes via cellular processes running through canaliculi in the bone matrix. Multiple different roles have been suggested for the osteocytes including acting as mechanosensors [11,13], removing perilacunar bone

* Correspondence to: H. Birkedal, Department of Chemistry and iNANO, Aarhus University, 140 Langelandsgade, DK-8000 Aarhus C, Denmark.
** Correspondence to: J. Skovhus Thomsen, Department of Biomedicine, Aarhus University, Wilhelm Meyers Allé 3, DK-8000 Aarhus C, Denmark.
E-mail addresses: flb06@inano.au.dk (F.L. Bach-Gansmo), mb@biomed.au.dk (A. Brüel), mvinkel@gmail.com (M.V. Jensen), ene@dadlnet.dk (E.N. Ebbesen), hbirkedal@chem.au.dk (H. Birkedal), jst@biomed.au.dk (J.S. Thomsen).
[1] Joint senior author.

http://dx.doi.org/10.1016/j.bone.2016.07.003
8756-3282/

matrix (osteocytic osteolysis) [11,14,15], contributing to the regulation of osteoblasts through production of sclerostin [16], and contributing to recruiting osteoclasts through production of RANKL [17–19].

Different studies of human bone have shown varying results for osteocyte lacunar properties such as osteocyte lacunar volume, and it remains unknown whether osteocyte lacunar volume differs between women and men [20–25]. Additionally, it is becoming increasingly clear that large ranges and variations in osteocyte lacunar properties exist in normal individuals, even within the same anatomical site, hence making studies of large sample sizes necessary [20–23,26]. As a consequence, it is essential to determine the osteocyte lacunar properties in normal bone, and to investigate whether these properties change with age and whether they differ between women and men so that reference values can be ascertained.

The osteocyte lacunar geometric properties have previously been assessed using 2D techniques [27–29]. Since osteocyte lacunae are strongly anisotropic in shape, 2D methods are afflicted by potential sample sectioning bias. Therefore, 3D methods such as synchrotron based CT or confocal microscopy [30] are more appropriate [31]. Yet even with these tools, practical limitations arise from the compromise that must be made between resolution, investigated volume, and the number of individuals investigated. CT methods using synchrotrons have advanced tremendously and can now deliver sub-100 nm resolution [32–34], but studies in the literature continue to remain affected by either restricted volumes investigated, limited resolution, or a limited number of subjects. Indeed, only a few studies containing many subjects have been conducted on osteocyte lacunae in human bone. Thus, osteocyte lacunae in cortical bone from the proximal femoral shaft of women aged 20–86 years were studied using synchrotron radiation (SR) μCT [23]. When dividing the subjects into groups of women younger or older than 50 years, the osteocyte lacunar volumes were approximately 30% larger in the younger than in the older women, but no significant change in osteocyte lacunar density were found with age. In contrast, osteocyte lacunar density was reported to decrease with age in trabecular bone of transiliac crest biopsies [28]. No significant differences were found in size or shape of osteocyte lacunae in women with and without osteoporotic fractures in trabecular bone from femoral heads using confocal microscopy, but a large range of size and shapes were noted for each group [22]. Vashishth et al. found different effects of aging on osteocyte lacunar density in human vertebral cancellous bone form women and men using histomorphometry [27]. They also reported larger coefficient of variation in men than in women with age, and explained this with decreased bone turnover in men with age, hence increasing heterogeneity. The lack of a clear picture of the sex-dependence and age development of osteocyte lacunar properties therefore calls for a comprehensive study covering both sexes and a large age-span.

The mechanical properties of cortical bone is related to its porosity [35–37] and thickness [38]. Likewise, increased cortical porosity at the iliac crest [39] as well as other skeletal sites has been linked to increased fracture risk with age [40,41]. Furthermore, the cortical thickness is reduced with age [42–44]. Most previous studies of the age-related changes in cortical morphology in normal individuals has been conducted using conventional 2D histomorphometry, whereas studies of the age-related changes of the cortical morphology in normal individuals using μCT based 3D methods has been almost absent.

In contrast to e.g. the vertebral body, a substantial cortical shell with Haversian remodeling is found at the iliac crest. Moreover, iliac crest bone biopsies remains a valuable, widely used, and well-established clinical and research tool for studying the etiology, pathogenesis, and treatment of metabolic bone disorders [45]. Therefore, a solid understanding of the properties and aging in healthy cortical bone at this skeletal site using 3D methods is paramount. Consequently, the aim of the present study was to investigate age-related changes of human iliac crest cortical bone morphology in normal women and men on different length scales and to determine whether the changes in microstructural parameters are accompanied by changes in osteocyte lacunar properties.

2. Materials and methods

2.1. Bone samples

Iliac crest bone samples from 46 women aged 18.5–96.4 years and 42 men aged 22.6–94.6 years with an even age-distribution were included in the study. Individuals with known cancer, metabolic diseases, severe liver or kidney diseases, medication affecting bone metabolism, or periods of >2 weeks of immobilization prior to death were excluded from the study. The present study is a subset of a larger study called the "Danish in Vitro Bone Study" (DAVIBO) [2,4,46–48]. The collection of the material was approved by the local ethical committee.

At autopsy, approximately 5 cm × 5 cm iliac crest specimens were extracted from the left anterosuperior iliac spine, including the standard region for iliac crest biopsy [2]. The bone specimens were immediately frozen at −20 °C until further used. Subsequently, trans iliac crest bone samples were drilled with a trephine with an internal diameter of 7 mm from the frozen bone at the position normally used for iliac crest bone biopsies, i.e. 2 cm below and 2–3 cm behind the anterosuperior iliac spine (Fig. 1) and placed in 70% ethanol [49].

2.2. Conventional μCT

The bone samples were scanned in ethanol in a μCT scanner (μCT35, Scanco Medical AG, Brüttisellen, Switzerland) in high-resolution mode (1000 projections per 180°) with an isotropic voxel size of 6 μm, an X-ray tube voltage of 55 kVp, an X-ray tube current of 145 μA, and an integration time of 800 ms.

The cortical bone situated at either end of the iliac crest bone samples were delineated interactively using the standard software supplied with the μCT scanner. The 3D data sets were low-pass filtered with a Gaussian filter ($\sigma = 1.3$, support $= 2$) and segmented with a fixed threshold filter (480.3 mg HA/cm^3), which was the same as previously established when analyzing the trabecular bone from a subset of the bone samples [2].

The cortical thickness (Ct.Th) was calculated as the delineated cortex volume divided by the cross sectional area of the bone samples $\text{Ct.Th} = \text{TV}/(d^2 \times \pi/4)$, where the diameter d of the cylindrical bone samples were measured using a digital caliper [50]. By digitally swapping the bone and the marrow phase and by applying the standard μCT scanner software to this inverted image set the cortical porosity (Ct.Po), Haversian canal diameter Ha.Ca.Dm, Haversian canal separation Ha.Ca.Sp, and Haversian canal number Ha.Ca.N were determined [51] using the direct method without parallel plate assumptions [52]. Assuming that the Haversian canals can be represented by cylinders, the number of Haversian canals per area Ha.Ca.Dn can be estimated as: $\text{Ha.Ca.Dn} = \text{Ct.Po}/(\text{Ha.Ca.Dm}^2 \times \pi/4)$ [51]. Quality assurance was performed by weekly (density) and monthly (geometry) scans of the solid-state calibration phantom provided with the scanner.

2.3. SR μCT

After conventional μCT the iliac bone samples were investigated using SR μCT at the beamline for TOmographic Microscopy and Coherent rAdiology experimenTs (TOMCAT) at the Swiss Light Source (Paul Scherrer Institute, Villigen, Switzerland) [53] with an X-ray beam energy of 35 keV and an isotropic voxel size of 0.65 μm using local tomography. Before SR μCT measurements, the samples were removed from the ethanol, left to dry overnight in the hutch, and mounted using hot wax, with the long axis of the bone samples perpendicular to the beam. Sample exchange was facilitated by a robot sample exchange system installed at the beamline [54]. The cortical bone of the iliac crest samples was measured in air with one scan of each cortical plate. The in-plane field-of-view was 1.7 mm in diameter per scan. For each individual volume, 1201 projections were measured with an exposure time of 500 ms. The detector system consisted of a 4.3 megapixel CCD camera (PCO