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Bone histomorphometry in transiliac biopsies from 48 normal, healthy men

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ABSTRACT

Investigators and clinicians use bone histomorphometry data from iliac bone biopsies to study bone abnormalities in diseased patients, and to understand the safety and effectiveness of pharmaceutical interventions. This requires access to a high quality normal data-set to be used for comparisons, a resource that has not been adequate to date. The objective of this work is to present static and dynamic bone histomorphometry data from transilial bone biopsies performed on 48 healthy males, evenly distributed between ages 45 and 75. In addition, we compared these results with results from our earlier study in normal postmenopausal women (Recker et al., 1988 [1]). The data include bone density and anthropometric measurements, micro-CT, and a collection of serum biochemical measurements. We found that several of the histomorphometry variables were correlated with serum measurements, i.e. serum testosterone and sex hormone-binding globulin (SHBG). Micro-CT variables were correlated with the static histomorphometry variables, and were very similar. Age-related changes were observed for both histomorphometry and Micro-CT, but were surprisingly small in most cases. Comparisons with our previously reported histomorphometry data from normal women were surprisingly similar, but there was a significant age by gender interaction in the wall thickness (W.Th) measurements, i.e. there was a small increase in this variable with age in men, and a significant decline with age in women. The population selected for this study, and the prior study in normal women, were carefully chosen so as to rule out the presence of clinical, life-style or other confounding factors. While the cohort chosen herein was a convenience sample, and not a population-based sample, we believe it can be used as a reference standard with proper precautions in its interpretation and in its comparisons with diseased populations.

1. Introduction

The purpose of this study is to provide normal reference data for quantitative bone histomorphometry and micro-CT measurements from transiliac bone biopsies from healthy adult Caucasian men. This information is provided to understand and interpret data from transilial biopsies taken from men with bone diseases such as osteoporosis, and to interpret treatment effects and safety of pharmaceutical agents used in men for such diseases. Data we have published on normal women of the same age range will be compared with these data from normal men. Normal histomorphometry data have been available for women [1]; however, due to known differences in bone histomorphometry values between women and men, and the increasing importance and incidence of osteoporosis and other bone diseases in men, it has become necessary to obtain similar data from a population of normal men to use as a standard reference data-base.

2. Methods

2.1. Recruitment

We recruited 48 healthy Caucasian males who were evenly distributed between ages 45 and 75. The project was approved by the Creighton University Institutional Review Board. The subjects were contacted by phone and asked to volunteer for the study. They were identified by two methods: 1) by query of our clinic outpatient archive which is maintained for recruitments such as this, and 2) by response to local advertising. All subjects in our archive have indicated a willingness to be contacted for future research studies, and each has signed consent to be contacted for recruitment for studies such as this. After signing a consent form for this study, each underwent clinical and laboratory examination to establish good health, and DXA measurements of bone mineral density (BMD) of the total hip, lumbar spine (L1–L4) and total body. The age-range of those enrolled was identical to the age-range

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of the healthy postmenopausal women recruited for the previous study [1]. We recruited 8 subjects from each 5-year age interval, 45–49, 50–54, 55–59, 60–64, 65–69 and 70–75 for a total enrollment of 48. To minimize potential confounding effects of known conditions (e.g., physiological and pharmacological) on bone status, and to enhance the power of the study by recruiting a homogenous group of subjects, we established an extensive list of exclusion criteria for candidate subjects that are listed in Appendix 1. The purpose of these exclusion criteria was to eliminate any person with a diagnosis or treatment that might affect bone health.

2.2. Biopsies

Each volunteer underwent transiliac bone biopsy according to a long- and well-established protocol. The procedure was performed in the outpatient surgery suite of Creighton University Medical Center under local anesthesia with proper monitoring (blood pressure, pulse oximetry and electrocardiogram), and conscious sedation. A 7.5 mm diameter cylindrical specimen of bone was taken from a location about 2 cm posterior and inferior to the anterior-superior spine of the pelvis through a 2 cm skin incision. Each specimen, including both cortices and the intervening trabecular bone, was placed in 70% ethanol fixative and transported to the histomorphometry laboratory. The wound was closed, and a dressing applied. The subjects were given instructions on physical activity and wound care, and returned for removal of suture seven days after the procedure. Prior to biopsy, each subject received in vivo double tetracycline labeling as follows: oral tetracycline hydrochloride (250 mg qid) for three days (Label 1) followed by a 14-day label-free interval, and then three days of oral tetracycline hydrochloride (250 mg qid) (Label 2) [1–4]. Five to 14 days after the end of Label 2 administration, the transiliac biopsy was performed.

2.3. Processing

The transilial bone biopsies were processed using standard histomorphometry techniques [1]. Each specimen was placed in 70% ethanol for 48 h and then dehydrated, defatted and embedded in methylmethacrylate. Near-serial sections were obtained with a motorized microtome (Reichert-Jung SuperCut 2050) with knife blades sharpened at 55° angulation. Sections from two areas of the specimen, each area separated by >250 μm, were prepared, stained and mounted on glass slides for analyses. All the microscope work was performed on an Olympus Microscope (BX 60, Hitschfels Instruments, St. Louis). The stained sections were read at 125× magnification using an operator interactive, semi-automatic image analysis system (Bioquant, Nashville TN). The intact biopsy specimens were subjected to microCT analysis after embedding in methylmethacrylate, prior to histomorphometric analysis. The microCT measurements were performed on a SCANCO-40 instrument (micro-CT-40, Scanco Medical AG, Bassersdorf, Switzerland). Embedded specimens were scanned at 30-μm resolution so that the region of interest was similar in resolution to that in the two dimensional (2-D) histomorphometric analyses. The micro-CT outputs included measurements of trabecular bone volume, trabecular thickness, trabecular number, trabecular separation and connectivity, all expressed in 2D and 3D units. Only the 3D units are reported here.

The total number of sections combined from the two areas of the specimen, separated by >250 μm, were prepared, mounted and stained, as follows:

1) ten 5 μm sections with Goldner's modification of Masson's trichrome stain;
2) four 5 μm sections with toluidine blue;
3) four 8 μm sections remained unstained (for tetracycline label analysis).

The section areas examined for histomorphometry measurements ranged from 36 to 58 mm^2 from each section from each site. We performed microscopic measurements with a user-interactive, automated image analysis system (Bioquant Osteo v7.10.10; Bioquant Image Analysis Corporation Nashville Tenn.). The drawing tablet was calibrated for each magnification with a stage micrometer. The section fields that were read were chosen from the area beginning one trabecular thickness from each cortico-endosteal surface. The following surfaces were identified and traced with a cursor containing a pin point light which was superimposed on the microscopic image through the light microscope and camera lucida:

a- osteoid without osteoblasts
b- osteoid with osteoblasts
c- resorptive surface without osteoclasts
d- resorptive surface with osteoclasts
e- resting surface.

Osteoid seam thickness was measured with the micrometer at 400× magnification on the Goldner-stained sections, using a randomizing process previously described [2]. Wall thickness was measured on toluidine blue sections at 250× magnification. The measurement was reported as the average distance between cement lines and trabecular surfaces on completed, inactive trabecular osteons, and was measured with the drawing tablet. Tetracycline labeling on bone surfaces bearing single, double or no label was read under ultraviolet light at 125× magnification on the unstained, ten micron sections. Interlabel width measurements were made with the eyepiece micrometer at 450× magnification. Both selection of the measurement sites and actual measurements were done by a randomizing procedure [2]. All width measurements were converted to thickness measurements by correcting for section obliquity by the following method: thickness = width × π / 4. Calculation of the static and dynamic variables has been described [3]. All raw data were entered and stored from the drawing tablet electronically on the lab file server which has daily duplicate back-up. The data are reported as 31 standard bone histomorphometric values. The variables conform to the Report of Standard Nomenclature [4]. It is important to note that when comparing data from these subjects with published data from normal white males (5–10) technical issues need clarification; 1) in this report all width measurements performed at the microscope were adjusted by an obliquity correction of π/4 in order to express the derived variables in three dimensions [9,10], 2) the cortical-endosteal surface and the transitional zone [11] were not included in the measurements, and 3) the Tt.Ar of microscopic sections that were examined ranged between 37 and 59 mm^2 and the Tt.Pm ranged between 86 and 237 mm.

2.4. Micro-CT

The intact bone samples were measured with compact cone-beam type tomography using a desktop micro-CT (micro-CT-40, Scanco Medical AG, Bassersdorf, Switzerland). A microfocus X-ray tube with a focal spot of 10 μm was used as a photon source. To perform a measurement, each specimen was placed in a plastic tube that was mounted on a turntable that can be shifted automatically in the axial direction. Using 30 μm resolution, 3 dimensional isotropic images of the iliac bone biopsies were collected with an integration of 250 ms [12,13]. An average of 110 slices was used for the trabecular micro-architectural analyses. A standard convolution-back-projection procedure with a Shepp and Logan filter was used to reconstruct the CT images in 1024 × 1024 pixel matrices. A Digital AlphaStation 500/333 workstation (Digital Equipment Corporation, Maynard, MA, USA) was used to steer the stepping motors, to synchronize rotation, axial shift, and data recording, as well as for the image reconstruction. We used a customized threshold technique (Scanco) that provided the best segmentation of the bone tissue. We