PERIPHERAL QUANTITATIVE COMPUTED TOMOGRAPHY (pQCT) FOR EVALUATING STRUCTURAL AND MECHANICAL PROPERTIES OF SMALL BONE.

Chapter 25 in "Practical Guide for Mechanical Testing of Bone"

Edited by
Yuehuet H. An, M.D.
Robert A. Draughn, D.Sc.

CRC Press, Boca Raton, FL, USA, 1999)

Author: José Luis Ferretti, M.D., Ph.D.

Member of the Research Career, Research Council, Natl. Univ. Of Rosario (CIUNR) and National Research Council (CONICET).

Director, Centre for Phosphocalcic Metabolism Studies (CEMFoC), Natl. Univ. of Rosario.

Basic Research Director, Metabolic Research Institute / Foundation (IDIM/FIM), Buenos Aires, Argentina.

CONTENTS

INTRODUCTION
.-1 WHAT IS A pQCT MACHINE
.-2 WHAT DOES pQCT ACTUALLY MEASURE
.-3 HOW DOES A pQCT MACHINE OPERATE
  -3.A ContMode
  -3.B PeelMode
  -3.C CortMode
.-4 WHAT KIND OF VARIABLES AND BONE PROPERTIES ARE MEASURED BY pQCT
  -4.A Indicators of bone "mass"
  -4.B Indicators of bone "apparent density"
  -4.C Indicators of bone architectural quality
  -4.D Indicators of the whole-bone quality
.-5 HOW CAN THE RAW INFORMATION PROVIDED BY pQCT BE APPLIED
  -5.A Analysis of the pathogenesis of some experimental effects on bone biomechanics.
  -5.B Analysis of the mechanostat status by means of "distribution / quality" curves.
  -5.C Analysis of the architectural efficiency of bone material distribution within bone diaphyses.
  -5.D Analysis of threshold-defined ROI's when cortical and trabecular bone are indistinguishable.
  -5.E Analysis of muscle / bone interrelationships.
CONCLUDING REMARKS
INTRODUCTION

Quantitative Computed Tomography (QCT) technology offers many opportunities for the investigation of bone biomechanics because it determines some indicators of bone properties that are relevant to bone strength, namely, the mass, the mechanical quality, and the spatial distribution of bone material [1-11].

Peripheral QCT was developed as a small-field, high-resolution extension of the existing QCT systems [12-15], to measure the peripheral skeleton with a substantial improvement of the image definition. Currently available pQCT machines perform transverse scans of a wide size-range of regions of interest, from large body segments such as the whole human head, neck, or thigh, to tiny excised bones such as mouse femurs or vertebrae. The gantry size of the machines limits the size of the specimen or body segment to be studied.

Stratec/Norland (Pforzheim, Germany) produces the widest range of pQCT machines in the market. These were developed from the original, XCT-960 model (the only pQCT device with FDA approval by 1993 [15,16]), which allowed a "research option" for studying relatively small bones. Some of these models are especially adapted for that purpose. The XCT Research SA ("SA" refers to "small animals") is designed to study bones from sheep or primates to rats in vivo or in vitro, and the high-resolution XCT Research M ("M" refers to "mouse") is used to study specimens ranging from mice to ferrets, even in vivo. A special achievement, the XCT Microscope, was also developed for quasi-histomorphometric analysis of small excised bones at a very high resolution, up to allowing the measurement of individual trabeculae. Scanco Medical AG (Bassersdorf, Switzerland) produces ultra-high resolution (microtomographic) machines [17,18] as the μCT-20, which is able to analyze 2-D and 3-D histomorphometrical images of trabecular bone specimens but can not determine bone density values. Some disadvantages of the high-resolution equipments are the high cost and the long determination time. Table 1 shows some distinctive characteristics of the equipment described.

25.1 What is a pQCT machine?

A basic pQCT machine consists of two major components: a scanner unit and a control/analysis computer system. The scanner unit contains a) a source that emits a very narrow X-ray beam, b) a detector of the emitted radiation fixed at a short distance from the source, which can measure the intensity of the radiation and the attenuation produced by the tissues studied, and
c) a mechanical system allowing radial, transverse and axial displacements of the source-detector couple in order to achieve different scanning positions of the bone (Fig.1-a). The bone sample is centrally located between the source and the detector with the aid of special supports adapted to in-vivo or in-vitro conditions (Fig.1-b). As a first step when the measurement is started, successive transverse displacements of the source-detector couple, repeated after small axial displacements, produce a computed radiograph or scout-view of the bone piece along its longitudinal axis, resembling a standard densitometric (DEXA) picture (Fig.1-c). Reference points on the screen allow selection of any convenient position along the bone axis. At that point, a series of transverse measurements are then performed after successive, partial rotation displacements of the couple until completion of a 180° excursion (translate/rotate mechanism).

The computer system a) controls the complete scanning procedure, b) integrates the information obtained into a composed image of the bone "slice", the tomographic scan that is shown on the screen (Fig.2), and c) analyses that scan as described below. The scan is integrated by the computer system by a complex procedure known as "backprojection with filtration". The complete field scanned is divided into a number of tiny square areal units (pixels). As the tomographic bone slice has a pre-determined, constant thickness, the pixels actually represent the bases of volume units which are more properly referred to as "voxels". The number of voxels per field is a fixed characteristic of each type of machine. The operator can change the size of the field, but not the number of voxels in the field. Therefore, the smaller the field selected, the smaller the voxels and the greater the information contained per unit of field area (i.e., the resolution of the machine), and vice-versa. For the earlier Stratec machines the voxel resolution was 1/128 of the variable scanning diameter [15]. The new machines provide 1024 x 1024 voxels. Some improvements such as automatic contextual segmentation algorithms to identify bone compartments [19] or post-processing of the images in a separate workstation with a region grow and skeletonization step [20] may approach some histomorphometric assessments of bone structure.

25.2 What does pQCT actually measure?

As an essentially absorptiometric technique, the pQCT measures only the attenuation of the radiation passing through the whole tomographic slice. This is the information from which the scan is performed. The magnitude of the attenuation depends on both the mass and the number of electrons in the atoms of the elements present in the tomographic slice, so that bone minerals are
most relevant for such measurement. The attenuation is expressed in cm\(^{-1}\) units as the linear attenuation coefficient (\(\mu\), a variable depending on the absorbing material) of the relationship between the intensities (\(I_0\), \(I\)) of the emitted radiation before and after absorption, according to the equation

\[
I = I_0 \cdot e^{-\mu d},
\]

(1)

where \(d\) is the layer thickness of the absorbing substance in cm.

The integration algorithm calculates the attenuation coefficient value corresponding to every particular voxel. This is a correlate of the amount (content) of absorbing matter in the voxel. However, the machine is unable to assess the absolute "mineral density" value of each voxel as a direct measurement. A special hydroxyapatite phantom is provided for this purpose (a particularly stable reference that does not require calibration too frequently [21]). The phantom allows the machine to transform the attenuation coefficients of every voxel into the "volumetric mineral content" (vBMC) and "volumetric mineral density" (vBMD = vBMC / voxel volume) data that are provided as the outcome. The machine is also able to assign different, default (rainbow scheme) or customizable voxel colors to successive ranges of attenuation/density values. Thus, the integrated image of the bone slice can be given a "semiquantitative" densitometric aspect (Fig.2).

The machines are arranged for assigning automatically a zero attenuation/density value to every voxel in the image corresponding to a measurement of pure fat [22]. Therefore, any above-zero attenuation measured for a given voxel should represent a certain content of matter denser than the fat it contains. Regardless of whether that corresponds to a soft or a hard (mineral-containing) tissue, or a mixture of both, the automatic reference to the phantom scale forces the machine to take and express that value as a "mineral content". The energy of the radiation used is specifically selected in order to minimize the error involved in such estimations of "mineral", as well as to optimize the "separation" of bone from soft tissues. On summing up the individual values assigned to each voxel, the machine can determine the mineral mass or volumetric bone mineral content (vBMC) and the volumetric bone mineral density (vBMD) of the whole bone slice or of a selected region of it. The vBMD represents the vBMC/bone volume relationship of the region and is usually expressed in mg/cm\(^3\).
It is critical to interpret correctly what kind of "bone density" the machine assesses. No available absorptiometric device can measure the "true" density of an ideal piece of pure "solid", absolutely pore-free bone substance (a hardly available specimen, if ever!). The established value of about 1.9 g of matter per cm$^3$ of solid tissue volume for such a material could only be estimated by calculation from what we know about its fractional composition. Practically, assuming it to be 58% matrix + 42% mineral, we can derive the following, approximate estimation.

\[
\begin{align*}
\text{Matrix density} & = 1.0 \text{ g/cm}^3 \times 0.58 = 0.58 \text{ g/cm}^3 \\
\text{Mineral density} & = 3.2 \text{ g/cm}^3 \times 0.42 = 1.34 \text{ g/cm}^3 \\
\text{Specific density of the solid bone matter} & = 1.92 \text{ g/cm}^3 \\
\end{align*}
\]

(matrix + mineral composite)

What is actually measurable in practice is the density of the whole bone including all hard substance, cells, vessels, marrow, etc. This is known as the "apparent" or Archimedean bone density. This concept is valid for the full range of bone porosity of either woven or lamellar, cortical or trabecular bone tissue, and also applies to bones as organs or to whole skeletons. The pQCT machines assess only this aspect of bone density in the bone slice. Moreover, they merely compare the absorptiometric data to a phantom scale of volumetric mineral densities. For that reason their outcome (vBMD) is expressed as a mineral mass per volume unit of whole-bone tissue.

Despite that limitation, the vBMD value could be regarded as a noninvasive indicator of the mechanical quality (specific stiffness, or elastic modulus) of the "solid" bone tissue. In fact, it represents at least one (the mineral amount per unit volume) of the most relevant determinants of that property. In support of that assumption, a close correlation between the chemically-assessed "vBMD" (ash content per unit of bone volume) and the mechanically-determined elastic modulus of the "solid" (cortical) bone tissue has been verified repeatedly [23-25].

It has also been demonstrated that the pQCT-assessed vBMD is more representative of the actual bone material quality than the "areal" bone mineral density (BMD) expressed as a mass of mineral per (square) unit of projected bone area provided by DEXA [1-9,26,27]. A further advantage of the pQCT-assessed vBMD values is that they are independent of the rotation of the
bone slice during the measurement, as well as (to some extent) of the bone size or shape. In addition, the information provided by the pQCT determinations can be processed by the machine in order to (1) distinguish between trabecular and cortical bone in many instances [3,22,28,29], and (2) calculate a number of variables which describe many aspects of bone architecture [4-11,22,29-40], as commented below.

The machine is "blind" concerning the type of tissue under measurement in each voxel because the vBMD is calculated from the attenuation coefficient value of the whole voxel, which reflects its bulk mineral mass content. In other words, voxels with trabecular bone of relatively low porosity and high "true" mineral density could not be distinguished from those containing cortical bone with relatively high microporosity if both of them show a similar attenuation coefficient. This pitfall gives raise to the so-called "partial volume" effect which is discussed below.

However, both the distinction between bone- and no-bone-containing voxels and the histological limitation between trabecular and cortical tissue are usually clear enough in practice to overcome the above inconveniences. The menu of operator-defined modes provided by the "Special Analysis Software" in the Norland/Stratec machines deals generally quite well with that purpose, as described below.

25.3 How does a pQCT machine operate?

Before starting the measurement, the operator must check the apparatus through the quality assurance steps, and then adjust the measurement parameters such as bone length, voxel size, number of steps in the scout-view scan, position of the reference line, number of sites to measure, and their distances to the reference line. These procedures vary for the different machines.

Some recommendations have to be followed concerning the management of the samples. As attenuation values for air are lower than for fat, it is convenient to avoid any interference from the air that may surround or be inside the bone sample, which would produce "negative density" values. Although this may not be relevant for in-vivo or whole-limb studies, it is recommended that operators (1) avoid measuring dried bones containing some air within the pores, as well as (2) immerse the fresh bones into water in tubes (Fig.1-b) during any in-vitro determination. Freezing the samples should not interfere the absorptiometric determinations, provided that air was not allowed to go into the bone cavity. However, it is not advisable to freeze the bones
during long periods of time (say, more than one month) if investigators also aim to perform mechanical testing on the same samples.

Positioning the sample is critical for the measurement. Some special devices as limb clamps for in-vivo studies (Fig.1-b) can be provided on request, but special supports or cradles usually would have to be prepared by each laboratory according to the particular aim of the study. The software helps to improve the reproducibility by detecting the same region of interest from one measurement to the next by testing the number of voxels [15,16]. The reproducibility of the determinations concerning repositioning should be assessed by calculating the corresponding coefficients of variation, for which quite low values have been reported [26,27,41,42].

Different field/voxel sizes can be conveniently selected according to the bone studied in order to optimize the resolution of the measurement. Ideally the bone slice should fit within the scanned field allowing just a minimal margin all around. Care should be taken to prevent the bone image from reaching the margins of the field, otherwise important sources of errors in the measurement could be introduced.

The first step of the determination is to obtain the scout-view, which is automatically made by the machine, and to define the scan sites on the screen. This can be arranged either symmetrically or asymmetrically concerning the starting reference line. However, there is a length limitation for the scout analysis (Table 1) that restricts the performance of multiple slices in a single measurement. Following the scout-view, a single command initiates the whole CT measurement automatically, under screen control to detect the misplacement of the sample within the field. The image obtained is then displayed, ready for analysis (Fig.2). Special tools ("Loop" functions) help when performing the standardized or customized analysis of the images. The most critical requisite for performing a good analysis (and hence that most subjected to discussion and further elaboration in the future) is the image definition concerning both the boundary between the bone section and the surrounding tissues and the one between the cortical and trabecular bone regions.

The Special Analysis Software in the Norland/Stratec machines (reference is made here to the newest available versions, 5.20 for XCT 960 A and 5.40 for XCT SA) allows customizing the methods (Modes) for separating (a) the soft tissue from the outer edge of the bone (ContMode), (b) the trabecular region from the cortical shell within the ContMode-defined bone to obtain a trabecular and a "cortical-subcortical" value (PeelMode), and (c) the cortical bone from the trabecular bone to obtain a cortical bone value (CortMode), as follows.
25.3.A ContMode

A pre-selected or user-defined attenuation threshold (the "THBD" threshold) causes the machine to automatically eliminate any voxel with an attenuation value below that level. A 0.5 cm\(^{-1}\) THBD value is recommended for that purpose (now it is possible to set the attenuation thresholds directly in mg/cm\(^3\) as a resource to standardize the procedure for the different machines). As the machine automatically works centripetally in this Mode until a complete bone perimeter is defined from the outside, every soft tissue surrounding the bone is removed that way (ContMode 1). For relatively large, regularly-shaped bones, a couple of iterative contour-detection procedures can also test the neighboring voxels with respect to either default or user-defined thresholds. This algorithm can be additionally activated in order to optimise the selection of a particular voxel as a boundary one (ContModes 2 & 3).

25.3.B. Peelmode

Working now from the outside bone edge in, this algorithm concentrically peels away an operator-defined percent of the outside area of the bone (usually 55% for the human radius; different values should be tried for animal bones). This default procedure (PeelMode 1) peels away (1) the outside cortical shell, (2) an inner area that is part cortical and part trabecular, and (3) a small portion of the inner area that is purely trabecular. It works well when the trabecular region is regularly shaped and hence the remaining, inner region can be considered to be purely trabecular.

PeelMode 1 may be unsatisfactory otherwise, including when the trabecular bone area must be analyzed as completely as possible. In these cases an operator-defined, inner attenuation threshold (the "THBD2" threshold) should be selected (proposedly, 0.63 cm\(^{-1}\) for rat bones) to separate a "trabecular" (attenuation values lower than threshold) and a "cortical-subcortical" region (values higher than threshold). A filtering process is used to ignore any high-attenuation voxel isolated within the trabecular area as well as in areas that are not continuous (PeelMode 2). If the operator wants also to eliminate the "subcortical" region as completely as possible, an additional peeling of the remaining area down to an indicated percentage can be ordered (PeelMode 3). An additional percentage of peeling can also be performed as an attempt to completely eliminate any high-density voxel inside the trabecular area which could influence the trabecular density (PeelMode 4).
When the cortical shell is well defined in the scan, the cortical/trabecular boundary can also be iteratively defined by testing the maximal attenuation gradient between the successive voxels working from the outside in, perpendicularly to the tangents to every voxel on the outer bone edge. This way the trabecular/cortical boundary is defined as a continuous chain of selected, high-slope voxels around a complete circle (PeelMode 5). This procedure works well for in-vivo rat studies. Additional peeling as in PeelModes 3 & 4 can also be commanded (PeelModes 6 & 7). These modes may be useful when PeelMode 5 worked well, for performing serial studies or if a low resolution had to be employed, respectively.

Abnormalities such as a coupling of a high attenuation value and a low trabecular density, or a high-density pocket within a low-trabecular-density region, can be identified by displaying the highest attenuation coefficient in trabecular bone, which allows one to see the density shifts (PeelMode 20).

25.3.C CortMode.

The cortical shell can be separated by selecting an adequate threshold (the "THCRT2" threshold; a 0.93 cm\(^{-1}\) value is recommended for rat bones, but in skeletally immature animals - younger than 9 months- it should be lowered to around 0.76 cm\(^{-1}\)). The machine will automatically peel-away every voxel showing an attenuation value below the selected one; i.e., the "trabecular" bone region (CortMode 1). When working at low resolutions, the remaining voxels showing a comparatively low attenuation with respect to their neighbors can also be additionally peeled away in order to ignore any low-density point within the cortical area (CortMode 2). An iterative contour detection algorithm, analogous to that employed in PeelMode 5 for the trabecular region, can also be employed to define the cortical shell from both the outer and inner sides (CortMode 3). This procedure may work well for lower resolutions such as those employed in in-vivo studies. The cortical region can also be defined after setting the outside bone edge by CortMode 1, working in, selecting a maximal attenuation for the inner threshold first and then lowering it until the whole cortex is displayed (CortMode 4). In many instances, the selected Modes succeed in defining the trabecular and cortical regions of interest (ROIs), which are shown on the screen. Then, special functions as "CalcBD" and "CortBD" yield the results of total, trabecular, cortical-subcortical, and cortical vBMD (in mg/cm\(^3\)) and the cross-sectional areas of the corresponding bone portions (in mm\(^2\)) as determined.
A two-color histogram represents the proportional distribution for total and "trabecular" voxels (the latter are characterized as those featuring within the "trabecular" region defined by the PeelMode) according to their attenuation values. The greater the accumulation of trabecular voxels in the "low-attenuation" region of the histogram, the better the boundary definition achieved between the "trabecular" and "cortical" ROIs. By modifying the selected thresholds the operator can improve that definition up to a certain limit. Bone images could hence be classified as apparently "well" or "poorly" defined concerning the sharpness (or the reliability) of the trabecular/cortical boundary. The standard analyses of "cortical" and "trabecular" bone provided by the machines obviously refer to the first case.

Nevertheless, whatever the method employed for defining the cortical and trabecular regions, the outcome will always be affected by the "partial volume effect" (PVE) [22,43]. The PVE is a source of error in the determination of the bone areas derived from the unavoidable inclusion of voxels that are not filled with mineralized tissue. It derives from the "blindness" of the machine as commented above and may lead to underestimation of the regional vBMD measurements that may exceed 15%. By optimizing the above Modes, as well as by selecting the smallest voxel sizes available, the PVE can be minimized, although it raises a severe limitation for the analyses of relatively thin cortical shells unless the definition achieved was actually very high.

The only way to test the PVE interference in the interpretation of the pQCT data is by measuring bones of the same kind but of different size and cortical thickness. On plotting the assessed cortical vBMD data (y) against the cortical thickness (x), a constant (horizontal) relationship for the greater values of the latter will be observed; however, toward the lowest end of the range the points should tend to shift to the lower-left region of the graph. This would point out the existence of the PVE and allow measurement of its relative significance.

25.4 What kind of variables and bone properties are measured by pQCT?

Many bone variables can be measured by pQCT in different long bones from small animals, principally in the midshafts (in order to correlate the data with those of bending tests, [37]), in the metaphyseal regions (where trabecular bone and remodeling are present in most species [44]), in femoral necks, and tentatively in vertebral bodies and hemimandibles, too. The available data can be classified according to their relevance as indicators of the different aspects of bone mechanical quality, namely, "mass", "apparent density", architectural design, and structural stiffness and strength, as follows.
25.4.A Indicators of bone "mass".

The vBMC of the trabecular, cortical, and total bone regions (0.6-1.9% in vitro and in vivo precision, 1.7-3.2% with repositioning with the XCT 960 A), as well as the cortical bone area (1.3-2.4 and average 3.8%, respectively), reflect the amount of mineralized tissue in the corresponding parts of the bone section [17,45]. Close correlations have been found between the pQCT- and histomorphometrically-assessed values of cortical area and other variables in rat tibiae and femora [40,42]. These indicators, especially those for cortical bone, should be regarded as relevant to bone strain and structural stiffness and strength in longitudinal compression [46-52]. The trabecular bone area (as arbitrarily defined by the above Modes) is not directly suitable for a proper biomechanical estimation of bone quality. The total bone area (calculated as the whole "solid" section area within the outer edge of the bone, regardless of the inner tissue structure) should only provide information on bone size.

25.4-B Indicators of bone "apparent density".

The vBMD of the trabecular region can be measured very precisely (0.4-0.6% in rats in vitro and in vivo, 2.9% with repositioning [8] and with a high statistical power, especially at the distal-femoral and proximal-tibial metaphyses. Changes can be detected within a few weeks after gonadectomy or treatment with raloxifene or PTH in small groups of rodents, much sooner than employing DEXA [8,9,27,28,41,53-55]. Rather than to represent a true "material property", the trabecular vBMD should be regarded as a correlate of the structural stiffness and strength of the trabecular network, i.e., a mechanical quality indicator expressed at the tissue level of complexity [56,57].

The vBMD of the cortical region (0.6-1.9% precision in vitro and in vivo, 3.3% with repositioning [8,10,53]), represents the "true" vBMD of the solid bone substance. Therefore, it may be regarded as indicative of one of the chief determinants of the intrinsic stiffness (elastic modulus) of the "solid" bone tissue [7,23-25]. Moreover, it has been shown to correlate well with the bone breaking force in femoral diaphyses and necks of rats and mice and in the human radius [11,29,51]. However, especially when measuring thin cortices at low resolutions, PVE-derived errors in the cortical area measurements (not in the vBMC determinant) may lead to underestimation of the vBMD [22,41,58,51]. High-resolution techniques should overcome this
problem [17]. Changes in the cortical vBMD are much slower than those in the trabecular density [8].

No directionality could be ascribed to these "qualitative" indicators because bone material or structural anisotropy is disregarded by the absorptiometric determinations. The total vBMD of the slice (a combination of the trabecular and cortical vBMD's) should not be an indicator of any particular bone mechanical property, analogously to the areal BMD provided by DEXA [20].

25.4.C Indicators of bone architectural quality.

The equatorial and polar second moments of inertia of the cross-sectional bone area (xCISMI, pCISMI; CV = 6.4% with the XCT-960 A in mice) are relevant to long-bone stiffness and strength in bending and torsion, respectively [1-7,29-37,39,47,52,59,60]. The Norland/Stratec machines calculate the CISMI's of the total or cortical bone areas as

\[ \text{CISMI} = \Sigma (A_i \cdot d_i^2) \]  \hspace{1cm} (2)

where \( A_i \) is the area of an individual voxel within the bone section and \( d_i \) is the distance from the center of that area to the reference, bending (x, y) or torsion (z) axis (Fig.1-d). As seen from the formula, the CISMI values (given in \( \text{mm}^4 \)) increase linearly with bone mass (A), but are also exponentially proportional to the distance (\( d^2 \)) of the bone material from the reference axis. Therefore, the equatorial and polar CISMI's are true indicators of the bending or torsional stiffness/strength of the assayed long bone, respectively, regardless of the bone material quality [7,10,11].

The machines can also measure the cross-sectional diameters, endosteal and periosteal perimeters, and average cortical thickness. These variables may help to evaluate the modeling-derived changes provoked by growth or by anabolic or anti-catabolic treatments [8]. However, they do not affect the quality of the architectural design or the mechanical competence of long bones as much as the CISMI's do [1-3,7].

25.4.D Indicators of the whole-bone quality.

The mechanical properties of the whole bones as measured by pQCT exceed the possibilities of standard densitometry [5-8,48]. The structural stiffness and strength of long hollow tubular structures are generally proportional to the product \( \text{CISMI} \times E \) (E being the elastic
modulus of the material of which the structure is made [61]). The elastic modulus can only be assessed mechanically, but it could be reasonably estimated by the apparent mineral density of the "solid" bone [23-25]. Hence, on replacing E by the pQCT-assessed vBMD of the cortical bone in formula (2), we developed the so-called Bone Strength Index (BSI) as the product

$$\text{BSI} = xCSMI \cdot \text{cortical vBMD} \quad (3)$$

We have shown that this BSI correlates strongly with the actual 3-point bending strength (breaking force) in a large sample of rat femur shafts, regardless of bone size and experimental conditions (Fig.3) [5]. Conversely, weak correlations were observed between the breaking force and the DEXA-assessed, areal BMD of the central diaphyseal region of the same bones. This is evidence of the greater ability of the tomographic BSI to describe the bone strength in the assayed conditions. Besides this BSI (xBSI), the pQCT machines also calculate its bending "y" and "torsion" versions (yBSI, pBSI, for which a mechanical validation is still needed), by using the yCSMI or the pCSMI (Fig.1-d) instead of the xCSMI in formula (3), respectively.

The BSI concept should not be freely generalized to the analysis of any bone region or method of deformation. Specially adapted formulae should be derived in order to achieve further, suitable BSI's for every method of bone deformation applied to every skeletal region of interest [5,11]. The Norland/Stratec machines provide also another kind of BSI for long bones, the "Stress/Strain Index" (SSI) [62,63], calculated as

$$\text{SSI} = \frac{\text{pCSMI} \cdot \text{cortical vBMD}_i}{d_{Mx} \cdot \text{vBMD}_{Mx}}, \quad (4)$$

where $d_{Mx}$ is the maximal distance from a voxel to the polar (z) axis in the image, and vBMD$_{Mx}$ is the maximal value the cortical vBMD could theoretically assume (i.e., 1200 mg/cm$^3$). This SSI was proposed to reflect the long-bone strength more generally than the above BSI's and as such it should be validated in future investigations. These BSI's or SSI's do not take into account any other relevant factors to bone material quality. They ignore the many microstructural determinants, including fatigue damage, that may also affect the mechanical ability of the bone.
tissue. Those indices may be useful, however, provided that these factors can be assumed to remain unaffected by the assayed treatments.

25.5 How can the raw information provided by pQCT be applied?

The standard information provided by pQCT studies in animals may be used for descriptive purposes [64]. However, it can also be used for other purposes [4,6,7,45,60], as the following examples suggest.

25.5.A Analysis of the pathogenesis of some experimental effects on bone biomechanics.

Data from separate pQCT evaluations of trabecular and cortical bone and the material and architectural properties of bones can be correlated with those from biomechanical studies in order to investigate the pathogenesis of any change in the whole-bone quality. Figure 4, upper, shows the ovariectomy (OX)-induced impairment and the alendronate-induced protection of the bending strength in rat femurs [65,66]. This effect must have resulted from changes in cortical material quality or in the diaphyseal architecture, or both. The lack of changes in the pQCT-assessed diaphyseal CSMI's in any group ruled out bone architecture as a source of such an effect. Parallel changes in the elastic modulus of the cortical tissue pointed to bone material quality as the cause of the observed variation in the whole-bone quality. However, the cortical vBMD did not vary between groups. Therefore, the changes in bone material quality must be ascribed to effects on some of the mineralization-unrelated, microstructural components of bone tissue that are known to affect bone material quality.

In support of that conclusion, the correlation between the measured breaking force and the calculated BSI of the same bones showed that bones from both the OX and risedronate-protected rats varied in opposite directions from the natural association between these variables (Fig.4, lower). The reason for that behavior should be a change in some factor(s) relevant to bone material quality that the BSI calculation disregarded (i.e., unrelated to bone mineralization). Findings like this may help to explain some differences between drug effects on fracture incidence and on the DEXA-assessed areal BMD in human studies.

25.5.B Analysis of the mechanostat status by means of "distribution/quality" curves.

We have described an opposite behavior of parameters of material quality (E) and architectural cross-sectional design (CSMI's) of femur shafts from growing rats of two different
lines [1,3]. This reflected a normalization of the bone strength to the body weight of the animals, due to a feed-back regulation of diaphyseal modeling by a function of the bone strain history (Frost's "mechanostat" theory [7,45,67-73]). The negative hyperbolic functions describing that relationship were called "distribution/quality" curves [4,6,7]. We also found that the same interrelationships could be shown by plotting the CSMI's vs the vBMD of the cortical bone as a correlate of the mechanical indicator, E, in many instances [1-3,29-36,65,66,74,75].

The effects of some treatments on the control of bone quality by the mechanostat can be described by such graphs in a useful way. On one hand, displacements of the points along the curve showing no departure from the normal relationship would reflect an indemnity of the mechanostatic control. On the other, any shift of the data to the upper-right or to the lower-left of the graph should indicate an anabolic (or anti-catabolic) or a catabolic (or anti-anabolic) shift of the mechanostat set-point [4,6,7], respectively. We have described in this way the effects of many treatments on rat bones [29-36,39,65,66,74,75]. As examples, a negative interaction of dexamethasone and a positive influence of anabolic PTH alone or combined with risedronate on bone mechanostat are summarized.

Dexamethasone administration to growing rats reduced all material (vCtBMD), architectural (CSMI's), and mechanical properties (breaking force) of femur shafts in a dose-dependent fashion [36,39]. Figure 5-a shows the anti-anabolic shift induced to the bone mechanostat setpoint. A 3-D representation (Fig.5-b) shows the combined, dose-dependent, negative influence of changes in both vCtBMD and CSMI on bone strength.

Low, intermittent doses of hPTH(1-38) given for 75 days to rats with a right hind limb immobilization and a mechanical overloading of the other leg enhanced all femur CSMI, vCtBMD, and bending breaking strength [30,31,40]. The distribution/quality curves (Fig.5-c) showed that these effects (1) reflected an anabolic interaction with the mechanostat setpoint, (2) were of a transient nature, (3) were maintained by a sequential administration of a remodeling inhibitor, the bisphosphonate olpadronate (an anti-catabolic interaction), and (4) were potentiated by the mechanical overload.

25.5.C  Analysis of the architectural efficiency of bone material distribution within bone diaphyses.

This interesting feature of bone modeling can be assessed by plotting any of the CSMI's (y) vs the cortical area of the same scan (x; Fig.6-a). The higher the slope of the correlation, the
better the architectural design that the same bone mass achieved. This method allowed a
description of gender-related differences in the design of human bones [38] and is most suitable
for analyzing the interactions of the mechanical usage of the limb with the effects of treatments
on bone architecture and strength in animal models.

25.5.D Analysis of threshold-defined ROI’s when cortical and trabecular bone are
indistinguishable.

When the distinction between cortical and trabecular bone is difficult but still possible,
one should use the function "Loop", working first at a fixed THBD, ContMode and CortMode
and varying the THBD2 to measure the "trabecular" bone. A second Loop should then be made at
a fixed THBD, ContMode, THBD2 and Cortmode and varying the THCRT2 to measure the
"cortical" bone. The software allows changing the ROI and the methods of analysis of the images
as desired while working with a given image. However, the parameters selected to perform the
image itself can not be changed a-posteriori, so one has to be careful when setting the conditions
at which the image is made. On following the above procedure, when a treatment blunts any
distinction between cortical and trabecular bone, its effects can be properly analyzed by
displaying the changes in the bone variables (y) along the whole range of attenuation thresholds
(x) at which the machine is able to work. So, the effects are described as differences between the
distribution curves of the assessed variables in the "threshold-defined" ROI’s. The resulting "type
of bone" is thus defined by the vBMD of the corresponding ROI, and the effects can be described
according to the THBD2 threshold range at which they were most evident in each group.

As an example, we failed to define the olpadronate-induced protection of metaphyseal bone of
tumor-implanted, hypercalcemic mice as related to pQCT-assessed changes in trabecular tissue
by performing the traditional pQCT measurements in PeelMode 1 (Fig.6-b). However, on varying
the THBD2 threshold (PeelMode 2, Fig.6-c) we were able to show that the differences between
the tumor- and olpadronate-induced effects varied widely [76]. So, we could conclude that a) the
tumor reduced the bone mass and made the "cortical bone" undetectable, and b) olpadronate
increased bone mass above normal values by protecting that "trabecular bone" from remodeling.
Those conclusions could not have been derived from standard pQCT determinations at a fixed
THBD2 threshold. This novel pQCT application is proposed as a useful tool in skeletal research,
avoiding many false negative results.
25.5.E Analysis of muscle/bone interrelationships.

The ability of pQCT machines to measure also the cross-sectional muscle areas allows evaluation of some muscle/bone interrelationships that are essential for assessing the state of bone mechanostat in different experimental conditions. In human studies, a close, linear relationship has been shown between bone CSMI's or BSI's (y) and the force or the pQCT-assessed, cross-sectional area of the regional muscles (x) in normal men and women. The slope of that relationship changed significantly after menopause [45,60,62,63,77] (Fig. 7, left). This offers the basis for distinguishing between disuse osteopenias (in which the mechanostat is still working properly) and true osteoporoses (in which the mechanostat setpoint is offset, most commonly because of an endocrine disorder) [60,67,68] (Fig. 7, right). The field is open for a wide variety of animal studies concerning this attractive proposal.

CONCLUDING REMARKS

Many new opportunities are open to future research that uses pQCT in animal models. pQCT can provide exclusive information concerning many biomechanical aspects of bone that are highly specific to the skeletal region studied. For that reason, special recommendations should be followed in future studies. They should avoid the understandable temptation to 1) extrapolate the pQCT data of a given skeletal region to a different site, and 2) compare the pQCT performance with those of other techniques that measure different aspects of bone mass - as the standard densitometry does - especially if these are inadequately taken as "gold standards".

REFERENCES

5. Ferretti, J. L., Capozza, R. and Zanchetta, J., Mechanical validation of a tomographic (pQCT) index for the noninvasive assessment of rat femur bending strength, Bone, 18, 97, 1996.


29. Ferretti, J. L., Gaffuri, O., Capozza, R., Cointry, G., Bozzini, C., Olivera, M., Zanchetta, J. and Bozzini, C.E., Dexamethasone effects on structural, geometric and material properties of rat femur diaphyses as described by peripheral quantitative computerized tomography (pQCT) and bending tests, *Bone*, 16, 119, 1995.
30. Capozza, R. F., Ferretti, J. L., Ma, Y. F., Meta, M., Alippi, R., Zanchetta, J. and Lee, W. S. S., Tomographic (pQCT) and biomechanical effects of hPTH(1-38) on chronically immobilized or overloaded rat femurs, *Bone*, 17(4S), S233, 1995.
35. Ferretti, J. L., Mondelo, N., Peluffò, V., Capozza, R., Cointry, G., Morillo, S., Zanchetta, J. and Montuori, E., Sub-chronic effects of high doses of miltorone on femur densitometric (DEXA), tomographic (pQCT) and mechanical properties in young rats, *Bone Miner.*, 25(S2), S12, 1994.
40. Ma, Y. F., Ferretti, J. L., Capozza, R., Cointry, G., Alippi, R., Zanchetta, J. and Jee, W. S. S., Effects of on/off anabolic hPTH and remodeling inhibitors on metaphyseal bone of immobilized rat femurs. Tomographical (pQCT) description and correlation with histomorphometric changes in tibial cancellous bone, Bone, 17(4S), S321, 1995.


57. Mosekilde, Li., Danielsen, C. and Gasser, J., The effect on vertebral bone mass and strength of longterm treatment with antiresorptive agents (estrogen and calcitonin), human parathyroid
hormone (1-38), and combination therapy, assessed in aged ovariectomized rats, *Endocrinology*, 134, 2126, 1994.


LEGENDS FOR FIGURES

Figure 1. (a and c were reproduced by permission of Stratec Medizintechnik GmbH, Germany)
   b. Standard support and tube provided for positioning rodent limbs and excised long bones for a pQCT measurement in the XCT-960 A.
   c. Example of a pQCT scout-view (human radius).
   d. Didactical representation of the meaning and calculation of the rectangular (related to x and y axes, relevant to bending analyses) and polar versions (related to z axis, relevant to torsional analyses) of the cross-sectional moments of inertia of a bone diaphysis (Cf. equation 2).

Figure 2.
   Examples of pQCT scans of the femoral distal metaphyses from the immobilized (IM) or overloaded legs (OL) of rats otherwise untreated or given anabolic doses of hPTH(1-38) for 75 days (P), withdrawn with no other treatment (Pw) or sequenced by risedronate (PR,R) during further 90 days [30,31,40] obtained with an adapted XCT-960 machine.

Figure 3. (Reproduced by permission of Elsevier Science, Inc., USA)
   Upper: Close, linear correlation between a BSI (x) assessed from pQCT scans of the midshafts and the actual fracture load in bending (y) of 206 femurs from rats of different ages and sizes [5], treated with dexamethasone [8,36] or aluminum hydroxyde [65] or studied as controls.
   Lower: Lack of correlation of the DEXA-assessed BMD of the central diaphyses with the same indicator of bone strength of the same bones.

Figure 4.
   Upper: Effects of peripubertal ovariectomy alone (OX) or immediately followed by 5 or 25 ug/kg sc 2/wk of alendronate for 6 months on the diaphyseal strength (fracture load) and architecture (body-weight adjusted CSMI, f) and the material quality (calculated elastic modulus, E) and vBMD of cortical bone of rat femurs [65,66]. Asterisks express the statistical significance of the inter-group differences.
   Lower: Correlation between the breaking force and the pQCT-assessed BSI of the bones from the OX- and 25-ug-alendronate-treated animals of the same experiment. Values for sham controls are represented by their 95% C.I.

Figure 5. (graph b is reproduced by permission of Elsevier Science Inc., USA)
   a. Distribution/quality graph showing the anti-anabolic effect of dexamethasone on rat femurs [29,39]. Control animals are represented by the 95% C.I. of the data.
   b. A 3-D representation of the combined impact of changes in bone architecture (xCSMI) and cortical material quality (as assessed by the vBMD) in the same bones.
   c. Distribution/quality curves showing the transient, anabolic interaction of hPTH with the mechanostatic control of bone architecture (upper) and the maintainence of that effect by the sequential administration of risedronate (lower) as described in Fig.2 [30,31,40]. Differences between OL and IM legs reflect the positive interaction of the mechanical usage with those effects.
Figure 6.
   a. Schematic representation of the assessment of differences in the efficiency of the diaphyseal architectural design (CSMI) per unit of available cortical material [38]. The higher the slope of the correlation, the greater the efficiency of bone mechanostat to stimulate and orient bone modeling.
   b. Distal-metaphyseal scans of the femurs from hypercalcemic, tumor-transplanted mice untreated (left) or treated with olpadronate (right) obtained with an XCT-960 A showing the impossible distinction between "trabecular" and "cortical" bone [76].
   c. Distribution curves of the counted voxels of cross-sectional area (CSA) and the vBMC of the animals from the same study, throughout the available range of THBD2 thresholds.

Figure 7.
   Left. Muscle/bone interrelationships as described in humans by the correlation between the tibial SSI and the cross-sectional muscle area of the calf of normal males and pre-menopausal females (upper) [77]. The same data from postmenopausal females depart significantly from the above correlation (lower).
   Right. Schematic representation of zones of normal "mechanostasis" (i.e., muscle/bone interrelationships under normal control by bone mechanostat) and "biomechanical incompetence" (because of a shift in the bone mechanostat setpoint), as derived from the evidence shown in the analogous graphs at the left. According to Frost's principles [78], this proposal (that can be tested employing animal models) should help to achieve a tomographic differentiation between osteopenias and osteoporoses.
<table>
<thead>
<tr>
<th>Machine and characteristics</th>
<th>Sample diam, Max</th>
<th>Voxel size</th>
<th>Max SV path</th>
<th>Min slice thickness</th>
</tr>
</thead>
<tbody>
<tr>
<td>XCT 960, research option, good for large bones</td>
<td>88 mm</td>
<td>90-690 μm</td>
<td>120 mm</td>
<td>2.50 mm</td>
</tr>
<tr>
<td>XCT Research SA, formerly 960 A, wide size-thickness range, variable slice capacity, avoids repositioning</td>
<td>90 mm</td>
<td>90-500 μm</td>
<td>180 mm</td>
<td>0.75 mm</td>
</tr>
<tr>
<td>XCT Research M, narrower, softer X-ray beam, higher and controlled resolution</td>
<td>50 mm</td>
<td>70-500 μm</td>
<td>180 mm</td>
<td>0.55 mm</td>
</tr>
<tr>
<td>XCT Microscope, higher mechanical precision, smaller source-center distance, special for thin cortical shells, quasi-histomorphometric scan, 3-D network reconstruction</td>
<td>50 mm</td>
<td>30-300 μm</td>
<td>180 mm</td>
<td>0.13 to 0.50 mm</td>
</tr>
<tr>
<td>Scanco uCT 20, ultra-high definition, static histomorphometry allowed, 3-D network reconstruction, no bone densitometry allowed.</td>
<td>17 mm</td>
<td>8-14 μm</td>
<td>50 mm</td>
<td>30 μm</td>
</tr>
</tbody>
</table>

b. Standard support and tube provided for positioning rodent limbs and excised long bones for a pQCT measurement in the XCT-960 A.

c. Example of a pQCT scout-view.

d. Didactical representation of the meaning and calculation of the rectangular (related to x and y axes, relevant to bending analyses) and polar versions (related to z axis, relevant to torsional analyses) of the cross-sectional moments of inertia (see equation 2).

Figure 1.
Figure 2.

Examples of pQCT scans of the femoral distal metaphyses from the immobilized (IM) or overloaded legs (OL) of rats otherwise untreated or given anabolic doses of hPTH(1-38) for 75 days (P), suspended with no other treatment (Pw) or sequenced by risedronate (PR,R) during further 90 days obtained with an adapted XCT-960 machine.
Figure 3.

Upper: Close, linear correlation between a BSI (x) assessed from pQCT scans of the midshafts and the actual fracture load in bending (y) of 206 femurs from rats of different ages and sizes, treated with doxamethasone or aluminum hydroxyde or studied as controls.

Lower: Lack of correlation of the DEXA-assessed BMD of the central diaphyses with the same indicator of bone strength of the same bones.
Upper: Effects of peripubertal ovariectomy alone (OX) or immediately followed by 5 or 25 mg/kg sc 2/wk of alendronate for 6 months on the diaphyseal strength (fracture load) and architecture (vCSM) and the material quality (elastic modulus, E) and vBMD of cortical bone of rat femur. Asterisks express statistical significance of the inter-group differences.

Lower: Correlation between the breaking force and the pQCT-assessed BSI of bones from the OX- and 25-ug alendronate-treated animals from the same experiment. Values for sham controls are indicated by their 95% C.I.

\[ \text{BSI} = \text{cortical vBMD (g/cm}^3\text{)} \times \text{lx (mm}^4\text{)} \]

Figure 4
Figure 5

- a. Distribution-quality graph showing the anti-anabolic effect of dexamethasone on rat femurs. Control animals are represented by the 95% C.I. of the data.
- b. A 3-D representation of the combined impact of changes in bone architecture (CSMI) and cortical material quality (as assessed by the vBMD) in the same bones.
- c. Distribution-quality curves showing the transient, anabolic interaction of hPTH with the mechanostatic control of bone architecture (upper) and the maintenance of that effect by the sequential administration of risedronate (lower) as described by the study shown in Fig. 2.

Differences between OL and IM legs reflect the positive interaction of the mechanical usage of the limb with those effects.
Figure 6.

a. Schematic representation of the assessment of differences in the efficiency of the diaphyseal architectural design (CSMI) per unit of available cortical material. The higher the slope of the correlation, the greater the efficiency of bone mechanostat to stimulate and orient bone modeling.

b. Distal-metaphyseal scans of the femurs from hypercalcemic, tumor-transplanted mice untreated (left) or treated with olpadronate (right) obtained with an XCT-960 A showing the impossible distinction between "trabecular" and "cortical" bone.

c. Distribution curves of the counted voxels of cross-sectional area (CSA) and the volumetric BMC of femurs from the same study, throughout the available range of THBDZ thresholds.
Figure 7.

Left. Muscle/bone interrelationships as described in humans by the correlation between the tibial SSI and the cross-sectional muscle area of the calf of normal males and pre-menopausal females (upper).

The same data from post-menopausal females significantly depart from the above correlation (lower).

Right. Schematic representation of zones of normal “mechanostasis” (i.e., muscle/bone interrelationships under normal control by bone mechanostat) and “biomechanical incompetence” (because of a shift in the bone mechanostat setpoint), as derived from the evidence shown in the analogous graph at the left. According to Frost’s principles, this proposal (that can be tested in many ways employing animal models) should help to achieve a tomographic differentiation between osteopenias and osteoporoses.