Analysis of biomechanical effects on bone and on the muscle-bone interactions in small animal models

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Abstract

Animal models are suitable to study many aspects of bone structure and strength. This article reviews some general principles of current bone biomechanics and describes the scope of the available methodology for biomechanical studies of the musculoskeletal system employing those models. The analysis comprises bone and muscle "mass" indicators provided by standard densitometry (DEXA); bone "mass", "apparent density", geometry or architectural design and strength and muscle strength indicators that can be determined by peripheral quantitative computed tomography (pQCT), and bone material and structural (whole-bone) properties than can be directly assessed by destructive mechanical tests. Some novel interrelationships that can be investigated that way are discussed, namely, 1. the pathogenetic analysis of the effects on whole-bone strength, 2. the discrimination between mineralization and microstructural factors as determinants of changes in the bone material or structural properties, 3. the evaluation of the interaction of a treatment with the ability of bone "mechanostat" to optimize the bone architectural design by "distribution / mass" and "distribution / quality" curves, and 4. the analysis of effects on the muscle-bone interactions for a differential diagnosis between "physiological" or "disuse" and "true" osteopenias and osteoporoses.

Keywords: Bone Biomechanics, Bone Architecture, Bone Strength, pQCT, Animal Models

Mechanical properties of bones and bone tissue

The mechanical properties of bones include their stiffness and strength and their ability to absorb energy elastically (i.e., *reversibly*). These properties are determined at two different levels of biological complexity, namely, the tissue level (bone *"material" properties*) and the organ level (bone *"structural" properties*)[1,2]. The link between these two levels is provided by bone architecture (bone *"geometric" properties*).

Bone's material properties are determined by two different factors, namely, 1. the calcification of the "solid" bone matrix (*mineralization-related* factor) and 2. the composition and spatial arrangement of crystals, collagen fibers, lamellae, osteons and cement lines, and the density of microdamage inside the calcified tissue (mineralization-unrelated, *"microstructural"* factors)[3].

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The bone geometric properties concern not only the amount (mass, size) but also the spatial distribution of the calcified material that confers on bones their architectural design as supporting organs (shape, macrostructure of the cortex and the trabecular network).

The bone structural properties are determined by a combination of the material and geometric properties[1,4]. They can be viewed as a function of the product of a bone material indicator and a geometric indicator related to the bone mass and architectural design[3,4] (Table 1).

Therefore, a proper analysis of whole-bone strength should involve the determination of reliable indicators of both the bone material and geometric properties[1,4].

Furthermore, bones are self-controlled structures because they regulate their structural stiffness (resistance of the whole bone to deformation by a load). This regulation is achieved through a response of bone cells to the strains produced by customary mechanical usage (bone "mechanostat" theory)[5,6]. As a result, bone modeling and remodeling are directionally modulated (Fig. 1) so that the strain environment is controlled and bones tend to show a fairly constant structural stiffness relative to the loads on them.
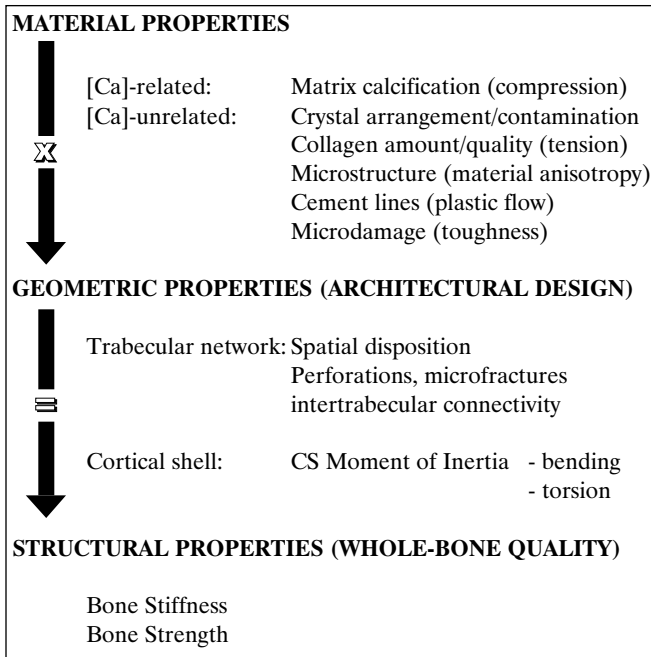
MATERIAL PROPERTIES

    [Ca]-related: Matrix calcification (compression)
    [Ca]-unrelated: Crystal arrangement/contamination
                           Collagen amount/quality (tension)
                           Microstructure (material anisotropy)
                           Cement lines (plastic flow)
                           Microdamage (toughness)

GEOMETRIC PROPERTIES (ARCHITECTURAL DESIGN)

    Trabecular network: Spatial disposition
                             Perforations, microfractures
                             intertrabecular connectivity

    Cortical shell: CS Moment of Inertia - bending
                                       - torsion

STRUCTURAL PROPERTIES (WHOLE-BONE QUALITY)

    Bone Stiffness
    Bone Strength

Table 1. Determination of the structural properties (whole - bone quality) by the bone material and geometric properties.
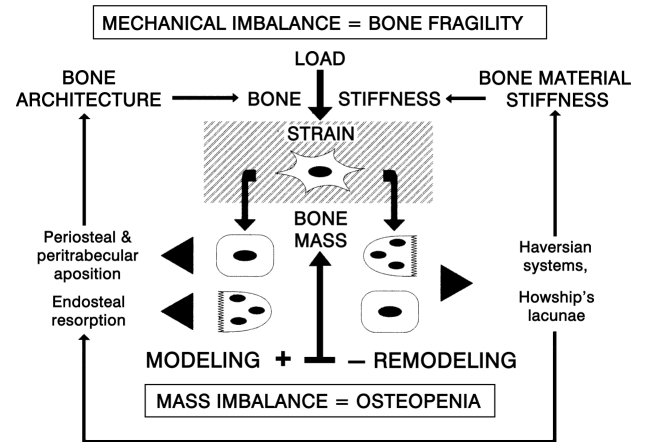

Figure 1. Schematic representation of the homeostatic regulation of the whole-bone stiffness by the bone "mechanostat" showing the sensors (osteocytes) and effector cells (osteoblasts, osteoclasts) of the system, the directional modulation of bone modeling and remodeling, and the independence between the resulting mass and mechanical balances. control, possible.

Within physiological limits, bone strength is fairly proportional to bone stiffness. Therefore, the strength balance resulting from that control can be independent of the correlative mass balance. Hence, the metabolic and anthropometric concept of bone mass should not be related to the biomechanical concept of bone strength.

The contractions of the regional muscles produce most of the strain the skeleton undergoes in daily life. Therefore, no structural/biomechanical study of the skeleton would be complete without a comprehensive analysis of the muscle-bone interactions.

It is possible to analyze some bone material, geometric and structural properties, as well as the necessary muscle-bone interactions that allow interpretation of the bio-mechanical condition of bones and its evolution during a given treatment. This can be done by using some modern technologies for studying the structural and biomechanical features of the musculoskeletal system in small laboratory animals.

• High-resolution QCT (peripheral QCT or pQCT, micro-QCT) can describe the mass, volumetric density and distribution of the bone mineralized tissue non-invasively. This reveals some aspects of bone's material and geometric properties and can predict the actual bone strength both in vitro and in vivo. It is also able to measure the cross-sectional areas of the regional muscles that are indicators of their strength[1,3,4].

• Mechanical tests of bone performed in vitro provide direct measurements of the whole-bone structural proper-ties[1,4].

• Combinations of mechanical and geometrical data allow indirect calculation of some bone material properties, too[1].

That information describes the bone material, geometric and structural properties, their interrelationships, and their natural interaction with mechanical forces more completely than data provided by technologies such as dual-energy X-ray absorptiometry (DEXA) or ultrasonometry do (Fig. 2). Ultrasonometry can provide some data related to the bone material properties as a whole (i.e. considering all mineralization and microstructural factors) but it is not suitable for studying small bones.

A general description of the variables that can be determined employing both mechanical and absorptiometric (DEXA, pQCT) techniques in small animals, and the way that information can be analyzed and interpreted is given below.

Scope of the available methodology for bio-mechanical studies of the skeleton

A. What kind of bone and muscle mechanical properties can be measured by standard densi-tometry (DEXA) ?

1. Bone mineral content (BMC)

Standard densitometry determines the mass of mineral present in the whole body or in the selected skeletal region, either in vivo or in vitro. This does not represent the mass of calcified tissue, because the mineral concentration in the bone tissue may vary. It does not represent the concentration of mineral within any specific type of bone tissue or structure, because the measurement includes a mixture of different types of bone tissue and bone-free pores, as well as the marrow cavity.
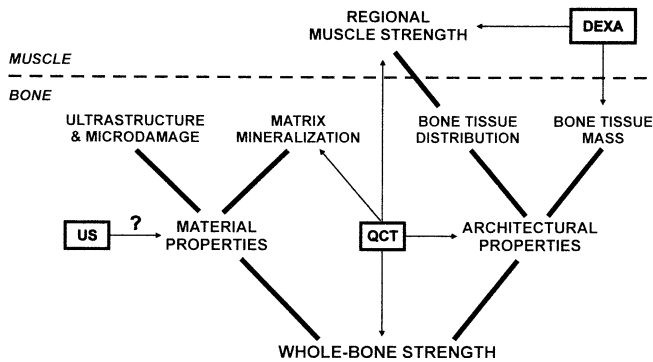
Figure 2. Schematic representation of other factors that determine whole-bone strength and the muscle-bone interactions, showing the properties that can be determined by dual-energy X-ray absorptiometry (DEXA), quantitative computed tomography (QCT) and ultrasonometry (US).

Changes in the BMC indicate just the result of the metabolic, "mass" balance between bone formation and destruction. They should not be directly ascribed to changes in the bone structure because they may reflect the variation of the amount and/or the mineral concentration of one or more types of mineralized tissues in the studied region, disregarding the spatial distribution of that material and the size of the pores or the marrow cavity.

2. "Areal" bone mineral density (BMD)

The BMC can be expressed in crude mass values (g of mineral) and/or related to the projected bone area, as the "areal bone mineral density" (BMD, in g/cm^2). The DEXA-BMD is not a volumetric density. It represents the whole mass of mineral present in the bone region studied (regardless of the bone structure in that region) expressed per unit of projected bone area. Expression per bone area provides just a partial anthropometric correction for bone size. The allometric relationships are not completely neutralized that way, because only 2 of the 3 spatial axes are captured by that adjustment. Thus, the BMD is still a size-dependent indicator of the bone mineral content.

The BMD can be considered an indicator of the degree of concentration of mineral within the whole bone in the region studied, but not as an indicator of the volumetric mineral density of the ideally "solid" bone material (bone "true" mineral density) or that including the pores (bone "apparent" mineral density). Therefore, changes in the BMD are in many ways analogous to those in the BMC, and for this reason they should not be directly ascribed to changes in the bone structure. No information is provided by DEXA on the bone material or geometric properties that are the true determinants of the bone structural properties.

Therefore, no DEXA data should be regarded as indicating the state of or changes in bone strength or fragility. This implies that, although DEXA is useful for diagnosing osteopenia (defined as a low value of bone mass),

it is unsuitable for diagnosing osteoporosis (defined as an osteopenic condition leading to a demonstrable reduction of bone strength)[7].

3. Lean mass (LM)

Lean mass, regarded as proportional to muscle mass, can be measured by DEXA in the whole body and also in selected body regions. The LM data allow assessing the anthropometrical relationships between muscle and bone masses that are suitable for the differential diagnosis of osteopenia. Once a low value of bone mass has been established, the determination of a proportionate or a disproportionate muscle/bone mass ratio would indicate the merely allometric ("physiological") or a "true" nature of that osteopenia, respectively[8,9].

B. What kind of bone and muscle mechanical properties can be measured by peripheral QCT (pQCT) ?

Peripheral QCT analyzes cross-sectional slices of bones and muscles that have a constant thickness and hence a known volume. In this way both bone volumetric densitometry and bone and muscle geometric measurements can be made[10-12].

Many bone variables can be measured in different long bones from small animals, principally in the midshafts (in order to correlate the data with those of bending tests[13]), in the metaphyseal regions (where trabecular bone and remodeling are present in most species[14]), in femoral necks, and tentatively in vertebral bodies and hemimandibles, too. Measurements can be made either in the whole bone scan or in selected regions of it (such as separate determinations in cortical and trabecular bone, etc.). 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.

1. Indicators of bone "mass"

Volumetric bone mineral content (vBMC)

The vBMC can be measured separately in the trabecular, cortical, and total bone regions of the scan. It reflects the amount of mineral (not that of the mineralized tissue, because of the same limitations that affect DEXA determinations) in the selected bone region[5,15] and hence it should be interpreted analogously to the DEXA-BMC.

Cross-sectional bone areas (CSA's)

Separate measurements of total, cortical and trabecular bone CSA's can be performed[16,17]. The cortical bone area

should be regarded as relevant to bone structural stiffness/strength chiefly in uniaxial compression[4,18-23].

The trabecular bone area (as defined by the apparatus) is not directly suitable for a proper biomechanical estimation of bone quality[12].

The total bone area (calculated as the whole "solid" section area within the outer edge of the bone, regardless of the inner tissue structure) provides information on only bone size[12].

2. Indicators of bone "apparent density" (volumetric BMD, vBMD)

Volumetric BMD of the trabecular region

It can be measured in vitro and in vivo, especially at the distal-femoral and proximal-tibial metaphyses. Changes can be rapidly detected after gonadectomy or treatment with raloxifene or PTH in rodents[2,24-31]. The trabecular vBMD should not be regarded as a true indicator of a "material property". It is rather an indicator of the structural stiffness and strength of the trabecular network i.e., a structure arranged at a higher level of biological complexity than that of the "solid" bone material[31,32].

Volumetric BMD of the cortical region

Measurable in vitro and in vivo[2,24,33], it represents the "apparent" mineral density (i.e., including the pores) of the cortical tissue. It could also approach the "true" vBMD of the "solid" bone substance provided that the intracortical porosity is not too high and the cortex is not thin enough to induce significant "partial-volume" errors[23,28,34,35]. If those conditions can be certified somehow, the cortical vBMD could be regarded as one of the significant determinants of the intrinsic stiffness (elastic modulus) of the "solid" bone tissue (the most relevant bone material property)[1,36-38]. It correlated well with the ultimate strength in femoral diaphyses and necks of rats and mice and in the human radius[23,39,40].

The vBMD of the whole bone slice

As a combination of the trabecular and cortical vBMDs, it expresses the concentration of bone tissue within the intact bone in the region studied. Hence, it should not be an indicator of any particular bone mechanical property, analogously to the areal BMD provided by DEXA[41].

3. Indicators of bone architectural quality (bone geometric properties)

Equatorial and polar moments of inertia

Equatorial and polar moments of inertia of the cortical bone CSA (xCSMI, pCSMI) are relevant to long-bone

strength in bending and torsion, respectively[1,4,10,11,13,19,40,46-55]. They can be calculated as:

$$CSMI = S\ (A_i \cdot d_i^2)$$

where A_i is the area of an individual voxel within the bone section and d_i is the distance from that voxel to the reference, bending (x, y) or torsion (z) axis (Fig. 3). The CSMI values (given in mm^4) increase linearly with bone mass (A), but are also proportional to the squared distance (d^2) from the bone cortex to the reference axis. The more peripheral the disposition of the cortical tissue with respect to the reference axis, the higher the corresponding moments of inertia and the bending or torsion stiffness or strength of the bone in the assayed conditions, independently of the bone mass and material properties[1,33,39].

Cross-sectional diameters, endosteal and periosteal perimeters, average cortical thickness

These variables may help to evaluate the modeling-derived changes provoked by growth or by anabolic, catabolic, or anti-catabolic treatments. However, they do not reflect the architectural design or the structural properties of long bones as well as the CSMI's do[1,42-44].

4. Indicators of the whole-bone quality (bone structural properties)

Bone Strength Indices (BSI's)

The structural stiffness and strength of hollow tubular structures are generally proportional to the product $CSMI \cdot E$ (E being the elastic modulus of the material of which the structure is made[56]). The elastic modulus can only be assessed mechanically, but, as commented above, it could be reasonably estimated by the "apparent" mineral density of the "solid" (cortical) bone[36-38]. We have shown that the product:

$$xCSMI \cdot cortical\ vBMD$$

is a reliable predictor of the actual bending strength (xBSI)

xCSMI or Ix = Sum (Ai d²x)
yCSMI or Iy = Sum (Ai d²y)
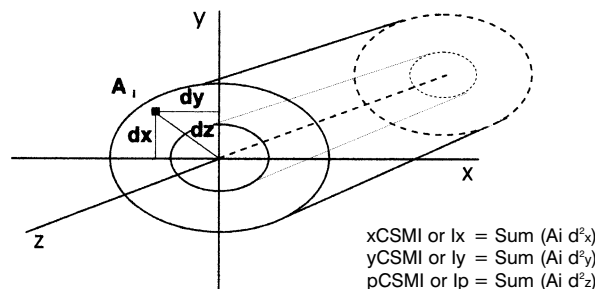pCSMI or Ip = Sum (Ai d²z)

Figure 3. Calculation of the rectangular (related to x and y axes, relevant to bending analyses) and polar versions (related to z axis, relevant to torsional analysis) of the cross-sectional moments of inertia.

of rat femur diaphyses regardless of bone size and experimental conditions[45] (Fig. 4). Conversely, weak correlations were observed between the breaking force and the DEXA-assessed, areal BMD of the central diaphyseal region of the same bones. A "torsion" version of the BSI (pBSI, for which a mechanical validation is still needed) can also be calculated by using the pCSMI instead of the xCSMI.

Stress-Strain Index (SSI)

Another kind of BSI for long bones[57,58] can also be calculated as:

$$SSI = \frac{pCSMI \cdot cortical\ vBMD_i}{d_{Mx} \cdot vBMD_{Mx}}$$

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., 1.2 g/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.

The extrapolation of BSI, SSI, etc. data to bone strength estimations has two important limitations.

1. The above BSI's or SSI's do not take into account any other factors relevant to bone's material properties than bone mineralization. They ignore the many microstructural determinants, including fatigue damage, that may also affect the mechanical properties of the bone tissue[45]. Those indices may be useful, however, provided that these factors can be assumed to remain unaffected by the assayed treatments.

2. Generally speaking, the BSI's are highly sensitive to the bone region and the type of deformation considered. Therefore, specially adapted formulae should be derived in order to achieve suitable BSI's for each method of bone deformation applied to each skeletal region of interest[39,45].

5. Indicators of muscle strength

Muscle cross-section area

The cross-section areas of muscles are significant (but not exclusive) determinants of their strength. The pQCT technology allows determination of both crude and fat-deprived cross-section areas of muscles, in vivo and in vitro.

When muscle and bone determinations are performed in the same region of the body it is possible to analyze the muscle-bone relationships and draw interesting biomechanical conclusions, as described below.

C. What kind of bone properties can be assayed directly or indirectly by destructive mechanical tests ?

1. Direct determination of the mechanical properties of the whole bones (bone structural properties)

Bones are usually tested mechanically by letting a load act on some part of their structure in such a way that some kind of strain (compression, bending, torsion, or shear strain) is induced, until a fracture is produced. The main bone structural properties usually assessed this way are the resistance to deformation (stiffness) and fracture (strength), and the ability of the structure to absorb energy[1,4,56].

The strain rate is an important feature of the mechanical tests. High strain rates are suitable for analyzing the bone behavior under the usual, fracture-inducing traumas. The more widely employed, low-strain rates ("passive load" tests) are useful for describing the "static" properties of the bone structure. This way the load (W) / deformation (d) curves obtained allow analyzing the successive "elastic" and "plastic" periods of bone behavior, separated by the "yield point" (Fig. 5), with minimal variance. "Elastic" here means linear proportionality between the load and the reversible deformation, a condition that is accomplished provided that no microcracks have been produced in the structure. "Plastic" refers to nonlinear relationship between the load
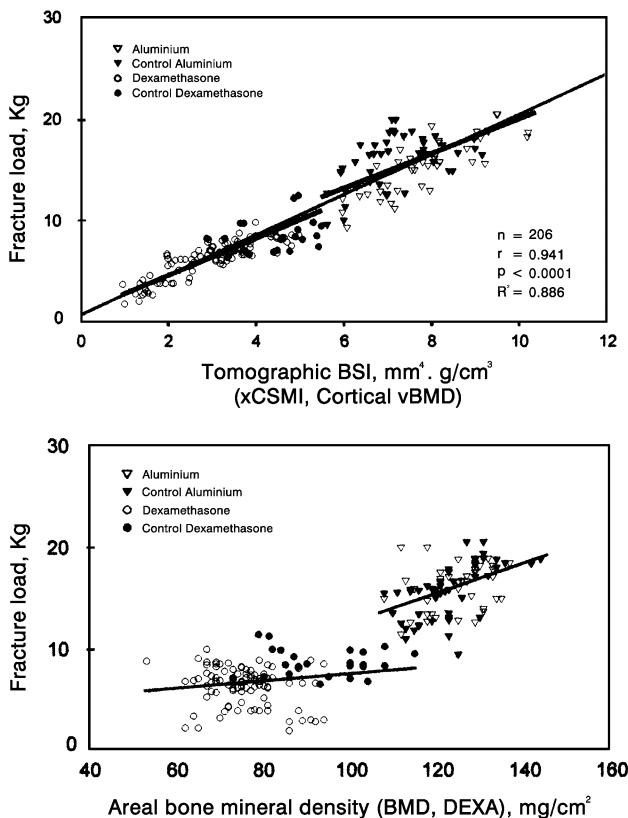
Figure 4. Upper: Close, linear correlation between the xBSI (x) assessed from pQCT scans of the midshafts and the actual fracture load in bending (y) of 206 femurs from rats of different ages and sizes, treated with dexamethasone[40,53] or aluminum hydroxide[61] or studied as controls. Lower: Lack of correlation between the DEXA-assessed BMD of the central diaphyses and the fracture load of the same bones[45].
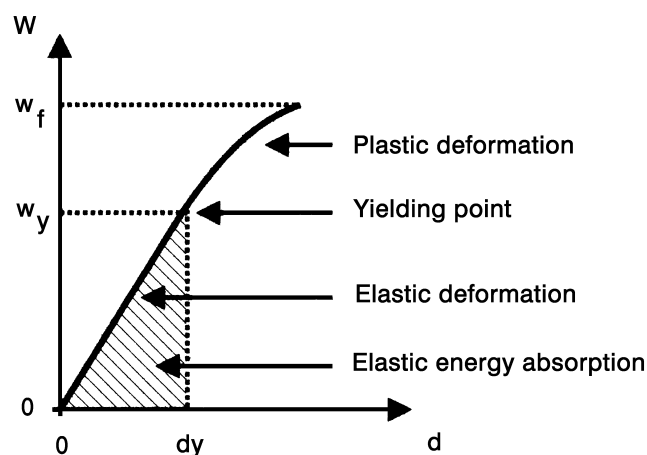
Figure 5. Typical load (W) / deformation (d) curve showing the first, elastic behavior and the final, plastic deformation of the assayed bone, separated by the yielding point (y).

and the irreversible deformation that occurs as a natural consequence of the presence of fatigue damage. Geometric properties aside, the elastic behavior of bones is chiefly affected by the collagen quality and mineralization, while bone properties under plastic conditions rather depend on microstructural factors and microdamage that affect the ability of the tissue to resist fracture. Regardless of the method of deformation employed, the tests usually allow direct determination of the following bone structural properties:

Maximum elastic load (yield load, Wy, in N)

It indicates the load needed to reach the yield point, representing the maximal whole-bone strength in elastic conditions as determined by the test. This is the main and the best studied component of the structural bone strength, much affected by calcification and collagen quality.

Maximum elastic deformation (yield deformation, dy, in mm)

It indicates the bone deformability in elastic conditions as determined by the test. This is not a useful indicator per se.

Load-to-deformation ratio (bone stiffness, Wy/dy, in N/mm)

It indicates the whole-bone stiffness in elastic conditions as determined by the test. Generally proportional to the bone structural strength, it may also vary independently under certain treatments.

Energy absorption by the whole bone during elastic behavior (Wy·dy/2, in N·mm)

It indicates the amount of energy the bone can absorb during the whole elastic period as determined by the test. It can be affected independently from the bone strength. High values of this property may be associated with the production of comminuted fractures.

Fracture or ultimate load (bone strength, Wf, in N)

It indicates the minimum necessary load to fracture the bone in the assayed conditions, and estimates the structural bone strength as determined by the test. This is the most important variable determined as long as it expresses directly the resistance of the whole bone to fracture, incorporating both the elastic and plastic behaviors.

Plastic / elastic ratio ([Wf-Wy] / Wy · 100 percent fraction of the fracture load that is supported in plastic conditions)

It describes the amount of bone resistance to load while undergoing microcracks, and reflects the state of the microstructural determinants of the bone "material" properties, including crystal abnormalities, as determined by the test. This aspect of bone strength, not much influenced by the degree of mineralization per se, can be affected by some modern treatments and deserves careful investigation.

2. Direct and indirect determination of the mechanical properties of the "solid" (cortical) bone tissue (approach to the bone material properties)

The bone material properties can be assayed directly by testing machined pieces of bone tissue (a little employed resource). They are more usually approached by indirect calculation employing a combination of the bone structural properties determined mechanically and the bone geometric properties assessed by any suitable procedure. The chief bone material properties that are usually determined are the following.

Young's elastic modulus (intrinsic bone material stiffness as expressed during the elastic behavior, much influenced by bone calcification and collagen quality)

When machined pieces of bone are directly assayed, the elastic modulus can be directly calculated as $E = f (Wy / dy)$, in MPa or GPa. It represents the most useful determinant among the bone material properties, and provides a way to express the stiffness of the ideally "solid" bone tissue avoiding the influence of bone size. It usually varies relatively little in normal conditions but may be affected by many treatments and is an essential determinant of the structural bone strength. When determined in highly-porous structures as in trabecular bone samples, E represents the structural rather than the material stiffness and should not be regarded as an indicator of any bone material property.

The pQCT-assessed cortical vBMD can directly estimate the mineralization of the "solid" bone tissue, that varies linearly with its elastic modulus[37,38]. Hence, the cortical vBMD can be regarded as proportional to E provided that the remaining (microstructural) determinants do not vary significantly from normal. In long bones assayed in bending or torsion, E can also be indirectly calculated as a function of Wy/(dy·CSMI).

Maximum elastic stress

Bone stress is the reacting force opposed by the deformed bone tissue to the deforming load. The maximum elastic stress is the value that force assumes at the yield point, either mechanically assessed or indirectly calculated in force/area units (MPa) as a size-independent indicator of the intrinsic strength of the bone tissue.

Energy absorption by bone tissue during the elastic behavior (calculated as Wy·dy / 2·vol, in N/mm)

It is a little investigated, size-unrelated estimator of the amount of energy elastically absorbed per unit of volume of bone tissue.

Figure 6. 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 (xCSMI) and the material quality (elastic modulus) and vBMD of cortical bone of rat femurs. 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 controls and the OX + 25-ug-alendronate-treated animals from the same experiment. Values for sham controls are indicated by their 95% C.I.

Novel functional interrelationships that can be investigated

The experimentally-induced changes in bone material, geometric and structural properties can be interpreted differently, according to the way they are analyzed. This is a most interesting field in skeletal physiology that is under constant development. Standard inter-group comparisons can describe the changes induced in those bone properties, but they may not provide a true interpretation of the underlying, pathophysiological interrelationships. From our point of view, the following, more elaborate approaches can further investigate many functional associations with potential pharmacological interest.

1. Pathogenetic analysis of the effects on whole-bone strength

The effects of any treatment on the bone structural properties should involve changes in the material and/or the geometric properties. Tomographic data can be correlated with those from mechanical tests in order to investigate the pathogenesis of any change in whole-bone quality. Figure 6, upper shows the ovariectomy (OX)-induced impairment and the alendronate-induced protection of the bending strength in rat femurs[59,60]. This effect must have resulted from changes in cortical material quality or in the diaphyseal architecture, or both. The lack of changes in the diaphyseal CSMI's in any group ruled out bone architecture as a source of that effect. Parallel changes in the elastic modulus of the cortical tissue indicated that the observed variation in whole-bone strength should have been caused by changes in the bone material properties. However, the cortical vBMD did not vary between groups. Therefore, the changes in bone material properties must be ascribed to effects on the mineralization-unrelated, microstructural components of the bone tissue quality.

Bone material and geometric properties are normally interrelated by the bone mechanostat (Fig. 1), a region-and gender-specific mechanism that controls whole-bone stiffness. Therefore, it may be difficult to assess which of them has actually been affected, and if so, how much. These combined influences may be described by multiple regression analyses between bone structural properties (yield stiffness, yield or fracture load of the whole bones, etc., y) and indicators of both bone material properties (calculated elastic modulus, pQCT-assessed cortical vBMD, etc., x_1) and bone geometric properties either related to mass (BMC in vertebral bodies working in compression, etc.) or distribution of the tissue (CSMI's in long bones working in bending or torsion, etc.) (x_2).

2. Discrimination between mineralization and microstructural factors as determinants of changes in the bone material or structural properties

When a correlation analysis demonstrates an effect of a treatment on the bone material properties, it is essential to
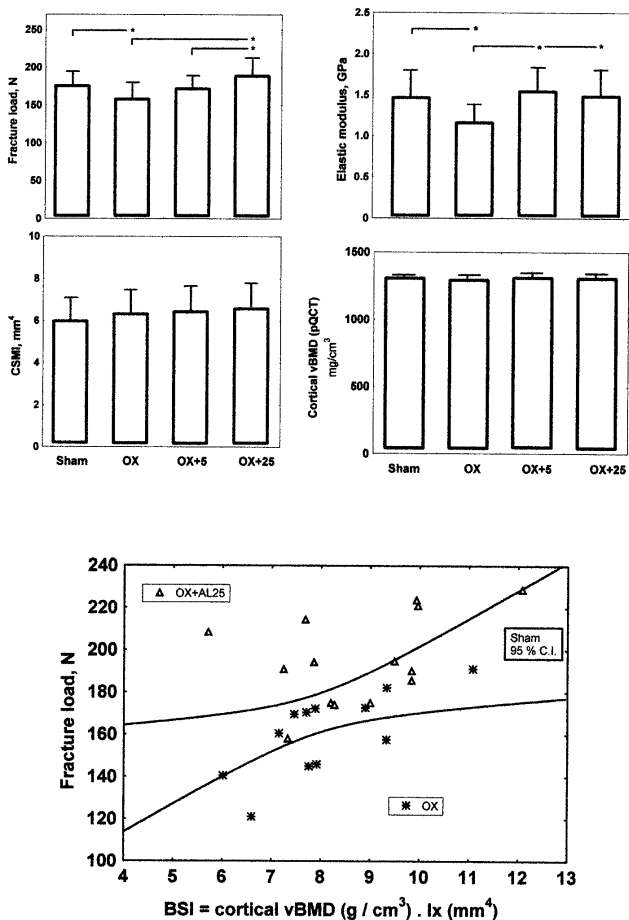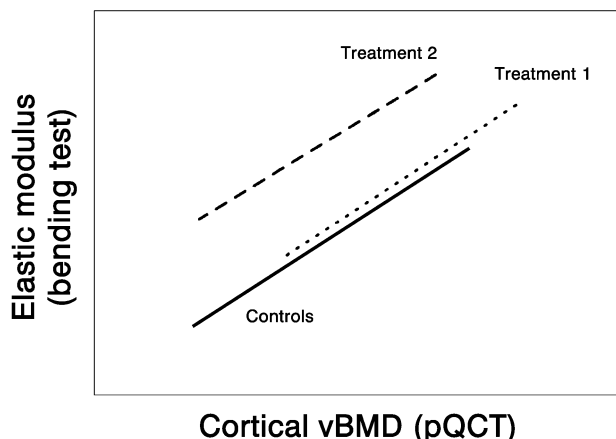
Figure 7. Sketch of a correlation graph between the elastic modulus and the volumetric mineral density of the cortical bone tissue as referred to in the text.

Figure 8. 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.

assess whether it was exerted on factors related to tissue mineralization or to microstructure. This is an important point because effects on microstructural factors can not be monitored by standard absorptiometric techniques in clinical practice. Current techniques only assess bone mineralization, disregarding the other factors. The simple finding of a positive, significant correlation between the elastic modulus (y) and the vBMD of the cortical tissue (x) in the treated group(s) may be a mere expression of the natural relationship between these two variables that may also be shown by the control animals. More elaborate analyses are needed to answer that question.

One such test is the ANCOVA of the regression curves between the elastic modulus (y) and the pQCT-assessed vBMD of the cortical bone (x), for different experimental groups (Fig. 7). No inter-group differences in slopes or intercepts would suggest a pure effect on bone mineralization (Treatment 1 in Figure 7). Conversely, any difference would suggest some independent effect(s) occurred on the microstructural factors (Treatment 2).

Another simple tool for that purpose is based on the facts that the BSI's estimate the actual bending or torsion strength of long bones as the product of a geometric indicator (one of the CSMI's) and the vBMD of the cortical bone as just a partial estimator of the elastic modulus of bone tissue. In fact the vBMD and the elastic modulus of cortical bone correlate closely only if the microstructural determinants of the elastic modulus do not vary[37,38]. Therefore, the BSI's can only predict the actual strength of long bones in that specific instance. If so, then the regression curves between the actual long-bone strength (y) and a BSI (x,) for different experimental groups can be compared with control data or suitable reference curves (Fig. 6, lower) by ANCOVA tests. If the experimental conditions do not affect the microstructural determinants the BSI should accurately estimate the actual bone strength according to the reference curves. Failure of the data to fit the reference curve (differences between slopes or intercepts) would suggest that the treatment affected the
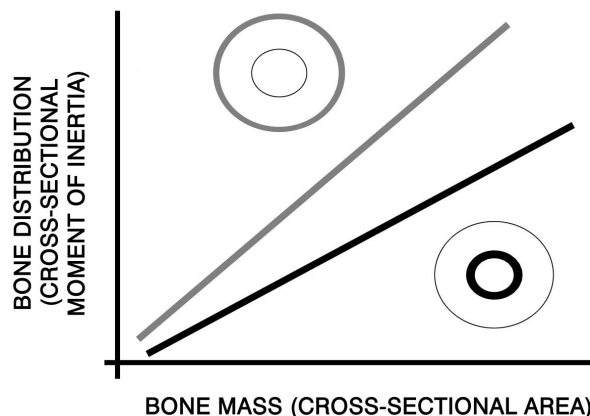
microstructural factors of bone tissue quality, that are disregarded by the index calculation.

3. Evaluation of the interaction of a treatment with the ability of bone "mechanostat" to optimize the bone architectural design

Perhaps the most important goal to be achieved by any treatment for a bone-weakening disease should be to improve, normalize, or at least not disturb the regional regulation by the bone mechanostat of the mechanical efficiency of the bone structure as it relates to the customary mechanical usage. We have developed two kinds of analyses for describing such interactions, based on what could be called "distribution/mass" and "distribution/quality" curves.

a. "Distribution/mass" curves

When a treatment improved the bone architectural design, it is important to establish whether this improvement has been achieved by following the natural relationships between bone tissue distribution (y) and availability (x). Bone tissue distribution and availability can be estimated by pQCT-assessed indicators of bone architecture (e.g., the CSMI's) and mass (e.g., the cortical CSA), respectively.

An ANCOVA test of the differences in slopes and intercepts of the positive, linear correlations between these variables (Fig. 8) will answer that question. Coincidence of the curves for control and treated animals would imply that treatment did not interfere with the control of bone modeling/remodeling by the bone mechanostat in the studied region. Positive or negative differences between slopes or intercepts would indicate a positive or negative interaction of treatment, respectively, with the sensor (osteocytes) or effector cells of that system (osteoblasts, osteoclasts) that should have influenced the peripheral distribution of the

cortical bone tissue as indicated in the Figure. Muscle strength and physical activity should enhance that interaction. Reference curves are needed in order to avoid the misleading influence of gender (the slopes and/or the intercepts of the curves are generally higher in males than females) on those relationships.

b. "Distribution / quality" curves

The indicators of bone geometric and material properties usually vary reciprocally, reflecting the feedback regulation
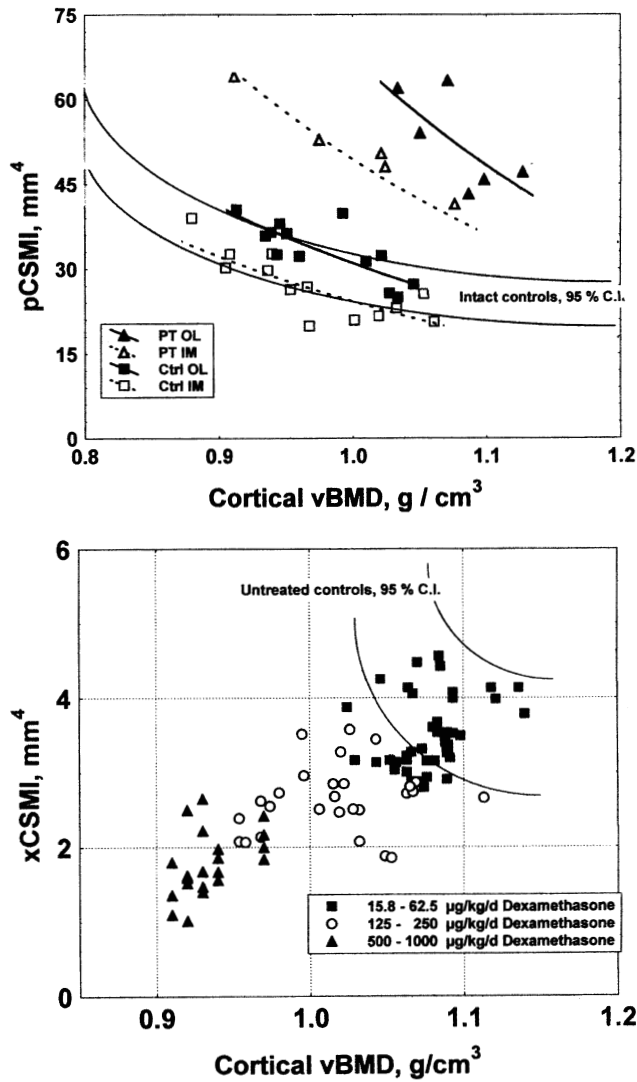
of bone modeling as a function of the bone strain history by the bone mechanostat. The negative, hyperbolic functions describing that relationship[1,10,11] (Fig. 9) can be shown by plotting the CSMI's as geometric indicators (y) vs the elastic modulus or the cortical vBMD as a material quality (stiffness) indicator (x)[40,42-44,46-52,59-62].

The effects of many treatments on the control of bone quality by the mechanostat can be described by such graphs. Displacements of the points along a normal reference curve showing no departure from the natural relationship would indicate that treatment did not affect the homeostasis. 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 analyzed in this way some natural changes and the effects of many treatments on rat bones[10,11,16,40,42-44,46-53,62] as described by the following two examples.

1. Low, intermittent doses of hPTH(1-38) chronically given to rats with a right hind limb immobilization and a mechanical overloading of the other leg enhanced all femur CSMI, cortical vBMD, and bending breaking strength[16,46,47]. The distribution/quality curves (Fig. 9, upper) showed that these effects: a) reflected an anabolic interaction with the mechanostat setpoint and b) were enhanced by the mechanical overload.

2. Dexamethasone administration to growing rats reduced all material (cortical vBMD), geometric (CSMI's), and structural properties (breaking force) of femur shafts in a
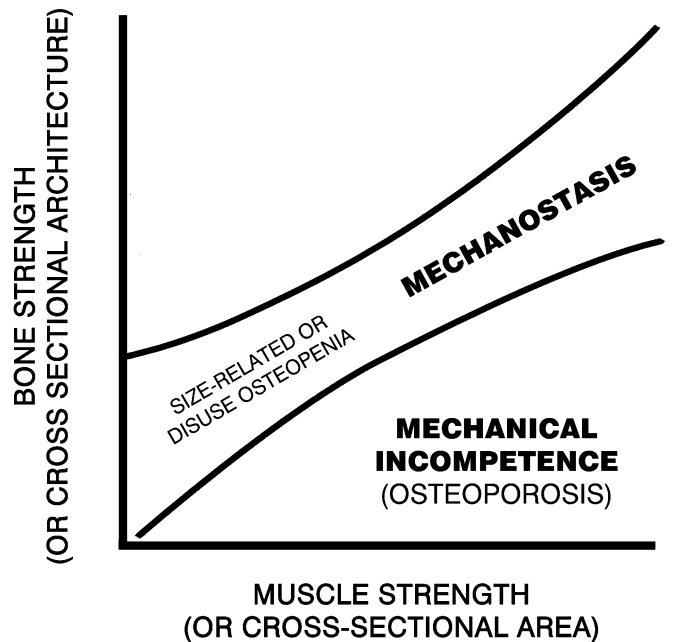

Figure 9. Upper: Distribution / quality curves showing the anabolic interaction of hPTH (PT) with the control of the diaphyseal architecture of the femur shafts (pCSMI) by the bone "mechanostat" as a function of the bone material properties (cortical vBMD) in rats with one rear leg immobilized (IM) and the contralateral leg overloaded (OL)[46,47]. Differences between OL and IM legs reflect the positive interaction of the mechanical usage with those effects. Lower: Distribution / quality graph showing analogously the anti-anabolic effect of dexamethasone on rat femurs[40,53]. Control animals are represented by the 95% C.I. of the data in both graphs.

Figure 10. Schematic representation of zones of normal "mechanostasis" (i.e., muscle / bone interrelationships under normal control by the bone "mechanostat") and "biomechanical incompetence" (because of a shift in the bone mechanostat setpoint). This interpretation should help to achieve a tomographic differentiation between osteopenias and osteoporoses[8].

dose-dependent fashion[40,53]. Figure 9, lower shows the anti-anabolic shift induced in the bone mechanostat setpoint.

4. Effects on the muscle-bone interactions

Muscles and bones are associated both anthropometrically (as defined by mass/mass relationships that are testable by DEXA and concern the diagnosis of osteopenias) and functionally, through strength/strength relationships that have to be tested and could help to define the diagnosis of osteoporoses[8] (Fig. 10).

In rat studies, these relationships can be investigated by correlating the bone geometric (pQCT) and mechanical properties with indicators of the status of the regional muscles. Muscle mass can be assessed by weighing the dissected muscles. Muscle strength could only be estimated in vivo by measuring the muscle cross-sections tomographically, ideally from filtered, fat-free images.

The ANCOVA of inter-group differences between the slopes and/or intercepts of the correlation curves between indicators of bone geometry or strength (y) and muscle strength (x) will elucidate whether the effects of the treatment impaired or improved the normal relationships between muscle strength and bone properties.

This question is important concerning the recent development of anabolic agents that are thought to interact positively with the bone mechanostat; and whenever it is desired to define the type of interaction a treatment may have with that homeostatic system (Fig. 9).

Concluding remarks

This article aimed at reviewing the more widely used methods for assessing the effects of modern treatments on bone structure and strength in small animals. The analysis concerned the separate assessment of the biomechanical determinants of bone stiffness and strength, the effects of the cybernetic regulation of bone stiffness, and the evaluation of the influences of the contractions of the regional muscles on bone development and strength. Those analyses could help to tell if a given treatment can improve bone strength by:

a. Acting only on clinically relevant sites prone to fracture.
b. Improving the mechanical properties of bone.
c. Normalizing, or at least not disturbing, the functional relationships involved in the proposed mechanism for the bone mechanostat.
d. Optimizing the stiffness of newly-formed bone, either through effects on mineralization or on any other determinant of its mechanical quality.
e. Ensuring an adequate mechanical stimulation of bone in the affected region, by an adequate regimen of physical activity, in order to help in dealing with a and c.

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