

# Bone strength and ultrastructure

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Published online: 2 April 2009

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## Introduction

Bone is programmed to withstand low-energy trauma and repeated stimuli such as walking, running, and jumping to an extent. The ability of bone to withstand physiological stress is governed by not only its determinants including mass, geometry, and microarchitecture but also intrinsic bone tissue quality. Intrinsic bone tissue quality is governed by the degree of mineralization and matrix characteristics such as collagen fiber orientation and chemical structure, both of which help to characterize the bone ultrastructure, and while the exact roles these determinants play are poorly understood, they appear to be crucial for the overall understanding of bone mechanical properties. These determinants are affected by bone remodeling and may therefore be considered as targets of nutritional, hormonal, and therapeutic intervention and activity. If we are to understand how bone adapts to a loss of mass and responds to pathophysiological alteration or to treatment, all such affected constituents need to be considered systematically, including intrinsic bone tissue quality.

## Measurement of bone mechanical properties

Biomechanical tests of resistance to fracture provide an objective measure of overall bone quality. They are invasive

and performed only in animal models but are of major importance in the understanding of pathophysiological alteration of bone strength. In the animal model, biomechanical properties of both intact cortical and trabecular bone are investigated by axial compression of vertebral bodies and proximal tibiae. Purely cortical bone is tested by flexion, whereby force is applied at either three or four points. The load/deflection curve is then used to measure or extrapolate stiffness (the slope of the linear portion of the curve) and maximal load (load at point of fracture). The departure from linearity representing the separation between elastic (linear) deformation and plastic (nonlinear) deformation is defined as the yield point. The areas under these curves represent the energies absorbed during elastic and plastic deformation.

## Measurement of intrinsic bone tissue quality

Various techniques are available for assessing and quantifying intrinsic bone tissue quality at both the level of the bone structural units (BSU) by microindentation and at lamella level by nanoindentation. They both give an overall reading of intrinsic quality as influenced by the mineral and organic components, but only nanoindentation selectively evaluates the influence of each. Microindentation is used to measure microhardness at the BSU or tissue level in terms of resistance to indentation at a defined load over a defined time period. Nanoindentation measures hardness at the level of the individual lamella. The nanoindentation method involves fixing the loading and unloading rates in order to obtain a force displacement curve, which can then be employed to derive biomechanical parameters such as microhardness and Young's elastic modulus. Microindentation uses a set load for a set time, with only the depth,

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Data presented at the Third Meeting on Bone Quality, France, 24–25 June 2008: Bone Ultrastructure.

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imprint, and hence the diameter of the residual imprint varying with the test material. The dimensions of this residual imprint are used to calculate microhardness ( $\text{kg}/\text{mm}^2$ ), without the ability to distinguish between the elastic and plastic components. In the following discussion, results were obtained using nanoindentation.

### Relation between bone strength parameters and parameters determined using nanoindentation

In the absence of treatment, *ex vivo* studies show an excellent correlation between proximal femur bone mineral density (BMD) and the maximal load obtained using flexion tests of femoral neck and vertebral compression tests. BMD predicts 66% to 74% of maximal load variance. As a ratio between hydroxyapatite mineral content and scan surface, BMD incorporates bone dimensions in addition to mineral quantity. Indeed, the proficiency of BMD in predicting bone strength is due, at least in part, to its incorporation of bone size. In rats, vertebral BMD predicts 60% of the variance of maximal load. Including the modulus (elastic tissue properties) improves the prediction to 71.5% and hardness (plastic tissue properties) to 95%. This indicates that combining BMD and tissue quality allows for a reliable prediction of bone strength. There is a significant relation between energy measured by compression testing of the rat vertebra and dissipated energy measured at the tissue level by nanoindentation. This demonstrates that the determination of energy is dominated by the tissue quality, characterizing its plasticity. In contrast, there is no relation between stiffness measured by compression testing of the rat vertebra and modulus measured at the tissue level by nanoindentation. This demonstrates that the determination of stiffness is dominated by the geometry of the bone rather than by tissue quality. These different studies clearly demonstrate the crucial role of intrinsic bone tissue quality in the determination of bone strength alongside geometry and microarchitecture.

### The alteration of bone strength and the respective role of intrinsic bone tissue quality

Bone is a heterogeneous tissue made up of a mineral component (hydroxyapatite) and an organic (mostly collagenous) component. Each component is theoretically capable of influencing the intrinsic quality of bone tissue. The degree of mineralization remains the more studied aspect of bone tissue to date. Studies of the organic component have focused on collagen fiber orientation and maturity. The involvement of these parameters in tissue quality is especially evident in osteogenesis imperfecta where fiber

disorganization leads to the high fracture risk. Diseases resulting in the formation of woven bone (disorganization of the collagen matrix), such as Paget's, are also associated with decreased strength. Preliminary studies are revealing the importance of the intrinsic bone tissue quality. They have shown tissue to be heterogeneous throughout the different phases of bone remodeling. They also have found evidence of changes in response to protein intake, hormone impregnation, and osteoporosis treatments.

#### Intrinsic bone tissue quality: effect of estrogen deficiency

At the level of the femoral neck, ovariectomy resulted in an early decrease of the mechanical properties, followed by a recovery. The initial decrease was explained by a decreased bone mass, an alteration of microarchitecture and of intrinsic bone tissue quality. This was followed by a compensatory increase of the external diameter of the femoral neck, which alone explains the recovery of maximal load in ovariectomized rats. This effect on geometry is related to an increase in circulating IGF-I observed following ovariectomy. These observations highlight the importance of the geometry as a determinant of bone strength.

#### Intrinsic bone tissue quality: effect of protein intake

An isocaloric reduction of protein intake in rats impairs both cortical and trabecular intrinsic bone tissue quality without necessarily needing remodeling of the bone tissue involved. A recovery of material quality was also observed following protein supplementation in rats fed a low casein diet. A Fourier transform infrared imaging analysis shows an increased maturation of bone collagen cross-links as indicated by an improvement of Pyr/deH-DHLNL collagen cross-link ratio in rats given protein supplements. This was observed in areas of the bone not subject to bone remodeling. Altogether, the supplementation of protein restores bone mechanical properties by preventing further BMD losses, increasing cortical thickness, and restoring intrinsic bone tissue quality. Thus, protein intake can directly influence the bone matrix quality and be crucial for the determination of overall bone mechanical properties.

#### Intrinsic bone tissue quality: effects of osteoporosis treatments

In OVX rats, bone mechanical properties are improved by bisphosphonates, selective estrogen receptor modulator (SERM), and parathyroid hormone (PTH) treatment. Inhibitors of bone resorption prevent bone loss and the degradation of microarchitecture; in contrast, PTH increases bone mass. Intrinsic bone tissue quality was improved

following treatments with inhibitors of bone resorption: SERM>bisphosphonates, but not in PTH-treated rats. This positive effect was in relation to the level of mineralization achieved in bisphosphonate-treated rats, while in SERM-treated rats the origin of the improvement is yet to be elucidated. This underlines the selective effect of osteoporosis treatments on the different determinants of bone strength. Other works have shown the response of intrinsic bone tissue quality to strontium ranelate treatment, with significant increases in both elastic and plastic parameters to even higher values than those observed in sham controls. Strontium ranelate treatment improves trabecular and cortical intrinsic bone tissue quality. This novel property, namely, the improvement of the intrinsic quality of bone tissue that is newly formed in response to strontium ranelate, may lead to a decrease in the development and/or propagation of microcracks. Such an effect on intrinsic bone tissue quality may signify a new target for developers of treatments for osteoporosis.

## Conclusion

The direct measurement of bone strength provides an objective measurement of bone quality and integrates all the effects of the different determinants. Mechanical property determinants, such as geometry, microarchitecture, and intrinsic bone tissue quality, are influenced selectively by the different available treatments and nutritional and hormonal status. A recent study clearly indicated that intrinsic bone tissue quality plays a major role. It is altered by estrogen deficiency and low protein intake and partially restored by protein renutrition, bisphosphonates, and SERMs but not by PTH. The most effective treatment, however, was strontium ranelate which increased intrinsic bone tissue quality significantly, even compared to the control animals. This highlights the prominent role played by intrinsic bone tissue quality in determining bone

strength, which may thus become a new target for developers of anti-osteoporotic therapies.

## Further Reading

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