Ca/P concentration ratio of bone tissues evaluated by energy dispersive x-ray spectroscopy and x-ray microtomography.

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Calcium (Ca) and Phosphorus (P) are the central high Z elements of the bone mineral matrix. Ca is the body’s most abundant cation, generally tied up in the mineral phase and partially released into the bloodstream via bone resorption. Similarly, 85% of the phosphate concentration is present in the mineral phase. The co-dependence of Ca and P are prerequisite for bone growth and development (Shapiro R. et al., Bone 32: 532-540, 2003) although their concentration does not vary mutually. Various hormonal and physicochemical factors affect their relative content which is critical for sustaining mineral homeostasis and bone metabolism. The Ca/P ratio is a promising biomarker for the assessment of bone health (Coats A.M. et al., Calc. Tissue Int 73: 66-71, 2003) which, in clinical practice, DXA is physically unable to measure from relative mass variations. Furthermore, Ca/P ratio has been also associated with rheumatoid arthritis and the generation of reactive oxygen species (Calc. Tissue Int 62: 193-198, 1998). Previous studies have also shown that the value and distribution of Ca/P ratio in bone varies between normal and osteoporotic bone (Kourkoumelis N. et al., J. Biol. Phys. 38: 279-291, 2012). Thus, it is of importance to carefully select the analytical techniques able to provide accurate relative Ca and P concentration measurements in bone tissue.

Energy dispersive x-ray spectroscopy (EDX) is a sensitive qualitative and semiquantitative technique for evaluating the mineral content in microscopic regions of bone with spatial resolution of cubic micrometers. The analytical resolution depends on the incident beam energy, the critical excitation energy for the x-rays of interest, the atomic weight, the atomic number and the density of the sample. Quantitative information is based on the relative elemental abundance. Micro-computed tomography (μ-CT) is an important tool for a wide range of research related to the biology of bone. High spatial resolution and high accuracy in the linear attenuation coefficients, allow different chemical components to be studied throughout the volume of either trabecular or cortical bones. A novel Dual Energy Analysis (DEA) algorithm was developed to differentiate calcium, phosphate and water which, as a first approximation, can be regarded as the major x-ray attenuators in bone.

Results show that Ca/P ratio values are positively related to induced bone loss while cortical and trabecular bone exhibit a different correlation between Ca and P. The novel application of the DEA technique has managed to (i) accurately determine mass fractions of Ca, PO₄ and H₂O in phantoms and (ii) trace Ca/P variations along the 3D bone structure.