Original Paper

Evaluation of the 3D spatial distribution of the Calcium/Phosphorus ratio in bone using computed-tomography dual-energy analysis

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ABSTRACT

Purpose: The Calcium/Phosphorus (Ca/P) ratio was shown to vary between healthy bones and bones with osteoporotic symptoms. The relation of the Ca/P ratio to bone quality remains under investigation. To study this relation and determine if the ratio can be used to predict bone fractures, a non-invasive 3D imaging technique is required. The first aim of this study was to test the effectiveness of a computed-tomography dual-energy analysis (CT-DEA) technique developed to assess the Ca/P ratio in bone apatite (collagen-free bone) in identifying differences between healthy and inflammation-mediated osteoporotic (IMO) bones. The second aim was to extend the above technique for its application to a more complex structure, intact bone, that could potentially lead to clinical use.
Methods: For the first aim, healthy and IMO rabbit cortical bone apatite samples were assessed. For the second aim, some changes were made to the technique, which was applied to healthy and IMO intact bone samples.
Results: Statistically significant differences between healthy and IMO bone apatite were found for the bulk Ca/P ratio, low Ca/P ratio proportion and interconnected low Ca/P ratio proportion. For the intact bone samples, the bulk Ca/P ratio was found to be significantly different between healthy and IMO.
Conclusions: Results show that the CT-DEA technique can be used to identify differences in the Ca/P ratio between healthy and osteoporotic, in both bone apatite and intact bone. With quantitative imaging becoming an increasingly important advancement in medical imaging, CT-DEA for bone decomposition could potentially have several applications.

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Introduction

Dual energy X-ray absorptiometry (DEXA) is the conventional method of assessing osteoporosis. However, it has a number of limitations when used to classify bone [1,2]. One of the major problems that have been reported is that the World Health Organization (WHO) classification of osteoporosis is based on DEXA data collected from epidemiological studies on white, postmenopausal women and does not necessarily apply at other cohorts of population, e.g. men, younger or older people. Moreover, DEXA calculates bone mineral density (BMD) using area (aBMD) and it is not an accurate measurement of true bone mineral density due to the missing depth value in the calculation of bone mineral density. Furthermore, planar imaging cannot differentiate between trabecular and cortical bones. Thus BMD measurement is independent of the ratio of trabecular to cortical bone and therefore does not give a complete picture of the strength of bone. There is a need for better understanding of bone pathogenesis and for an enhanced/earlier fracture prediction method.

One possible indicator to predict bone fracture is the ratio of the two main constituents of bone, Calcium (Ca) and Phosphorus (P). Increased Ca intake increases bone density [3,4], while increased P intake combined with a low Ca intake can cause secondary hyperparathyroidism and decrease in bone mineral content [5]. Using 2D or 3D synchrotron-based techniques, it has been observed that the Ca/P ratio and its distribution in animal and human bones vary significantly between healthy and inflammation-mediated osteoporotic (IMO) and aged human bones [6–10]. However, the exact reason for the lowered Ca/P ratio in IMO and aged human bones is still under investigation. As such, the 3D spatial distribution of the Ca/P ratio could be correlated to bone quality indicators. For this, a laboratory based technique is initially required that can assess in 3D the Ca/P ratio in bone.

Interest in decomposing the main bone components using dual-energy analysis (DEA) has existed since the early 1960s and still exists

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today [7]. In 1963, Camerson and Sorenson proposed a 2D DEA model for finding the concentrations of the elements present in bone using projection radiography [11]. CT-DEA was first conceived in 1976 [12] and different techniques for material decomposition were developed. A DEA system and model were developed in 1997 to assess quantitatively the amount of Ca/P ratio in animal and human bones, in 2D, using radioactive sources [6] and a commercial X-ray source [8]. Limitations in generator powers, tube heat capacity, tube cooling, spatial and temporal resolutions of earlier X-ray imaging systems together with the challenge of decomposing the materials of similar X-ray attenuation put a limit to the effectiveness and potential of DEA. The differentiation of body tissues without the application of contrast media is considered an ambitious aim. In bone specifically, the decomposition of bone materials using two X-ray beams is challenging due to the complexity of bone composition [13] and the similar attenuation of its components to X-rays, which can effectively decrease the accuracy of the assessed quantity/ties. To date, no techniques for the assessment of the 3D spatial distribution of the Ca/P ratio in intact bone using CT-DEA and a polychromatic X-ray source have been published.

In our previous studies [14,15] a CT-DEA technique for the 3D assessment of Ca/P ratio was developed and validated for measurements on cortical bone apatite (this technique will be referred to as CT-DEA$_{BA}$). In these samples bone collagen had been removed, thus reducing the effect of bone complexity on assessed Ca/P ratio accuracy. CT-DEA$_{BA}$ was optimised for a micro-CT tomography system with a polychromatic X-ray beam, demonstrating that the technique could be applied in a clinical system. The first aim of this study was to apply CT-DEA$_{BA}$ to an extended population, thus allowing statistical tests to be used to conclude on the efficacy of the technique. The CT-DEA$_{BA}$ technique was firstly applied to 18 collagen-free rabbit cortical bone apatite samples (9 healthy and 9 IMO) to statistically verify that it can identify differences between healthy (high Ca/P ratio) and IMO (low Ca/P ratio). The second aim was to extend the above technique to bones in which the composition and quality had not been altered in any way. These samples have a more complex structure as they contain collagen, and are referred to as 'intact bone'. This technique will be referred to as CT-DEA$_{IB}$. As in CT-DEA$_{BA}$, both healthy and IMO samples, but this time intact bone samples, were assessed with CT-DEA$_{IB}$. In this contribution the necessary amendments to the technique for the quantification of the Ca/P ratio in intact bone are described. The feasibility of CT-DEA$_{IB}$ for intact bone was tested by applying it to 18 intact rabbit cortical bone samples (9 healthy and 9 IMO). This manuscript presents an attempt at measuring the Ca/P ratio non-invasively in bone, verifies CT-DEA$_{BA}$ and CT-DEA$_{IB}$ and discusses possible applications of them as well as potential improvements.

Method

CT-DEA

In principle, CT-DEA cannot decompose more than two constituent materials in a mixture as it only provides two independent measurements. Bone though is a complex structure consisting of a large number of components including calcium phosphates, water, carbonates, citrates, sodium, magnesium, collagen and non-collagenous proteins [13]. Four of the main components in bone are Ca, PO$_4$ (together ≥60% by weight), collagen (≥15% by weight) and water (water ≤10% by weight). The rest of the elements in bone are of reduced quantity and their effect in X-ray attenuation is low. In previous work collagen was removed by heating [14], producing 'bone apatite', and the contributions of Ca, PO$_4$ and water were decomposed (the CT-DEA$_{BA}$ technique). For a non-invasive technique such heating would not be possible and in a better approximation bone can be decomposed into Ca, PO$_4$ and collagen.

For a three-material decomposition using two different spectral measurements, one additional condition must be provided to solve for three unknowns. Mass conservation can be assumed, i.e. the sum of the masses of three constituent materials is equivalent to the total mass of the mixture. Two spectral measurements and a mass-conservation based, 3-material decomposition CT-DEA algorithm were thus used to determine the proportions of the three main components in bone: Ca, PO$_4$ and collagen. The necessary amendments from CT-DEA$_{BA}$ to CT-DEA$_{IB}$ are described below.

To calculate the mass fraction of the three unknown components in bone the three equations below need to be solved simultaneously for f$_{Ca}$, f$_{PO_4}$ and f$_{collagen}$.

$$\frac{\bar{\mu}_L}{\rho_{\text{eff}}} = \bar{m}_{Ca,L}f_{Ca} + \bar{m}_{PO_4,L}f_{PO_4} + \bar{m}_{collagen,L}f_{collagen} \tag{1}$$

$$\frac{\bar{\mu}_H}{\rho_{\text{eff}}} = \bar{m}_{Ca,H}f_{Ca} + \bar{m}_{PO_4,H}f_{PO_4} + \bar{m}_{collagen,H}f_{collagen} \tag{2}$$

$$1 = f_{Ca} + f_{PO_4} + f_{collagen} \tag{3}$$

In these equations, L and H are the low and high energies respectively, ρ_{eff}, $\bar{\mu}$ and $\bar{m}$ are the effective density, average linear attenuation coefficient (given by CT) and average mass attenuation coefficient respectively. If the X-ray spectrum is known, $\bar{m}$ can be calculated as:

$$\bar{m} = \frac{\int P(E) \cdot E \cdot m(E) \cdot dE}{\int P(E) \cdot E \cdot dE} \tag{4}$$

where P is the number of photons.

Density, ρ_{eff}, is determined using two predefined mathematical relations of: (i) the fraction of the average linear attenuation coefficients, at the low and high energies, $F(Z_{\text{eff}})$, to the atomic number of the sample, Z_{eff} and (ii) Z_{eff} to $\bar{m}_L$, and by dividing the linear attenuation coefficient by $\bar{m}_L$. In this study these relations were approximated by second order polynomials (with $R^2 = 0.99$ each) using the chemical formulae of four calibrating bone phantoms of different Ca/P ratio (Ca(H$_2$PO$_4$)$_2$(H$_2$O), CaHPO$_4$, Ca$_3$(PO$_4$)$_2$, Ca$_5$OH(PO$_4$)$_3$). The technique was calibrated for collagen being present in the bone samples in a weight proportion of 15%.

After determining ρ_{eff} the fractions of Ca, PO$_4$ and collagen can be determined by solving Eqs. (1)–(3) above. Finally, knowing f_{Ca}, f_{PO_4} and $f_{collagen}$ allows the Ca/P ratio to be calculated. The molecular weight ratio (PO$_4$/P) is 3.0679, and the ratio of the molar masses of Ca and P is 1.2940, thus the atomic Ca/P ratio is given by:

$$\text{Ca/P ratio} = \frac{f_{Ca}}{f_{PO_4}} \frac{3.0679}{1.2940}. \tag{5}$$

It is worth noting here that the above technique is not sensitive enough to observe chemical bonding energies and is only likely to show the total number of any given atomic species. Therefore, it does not indicate the chemical composition of the bone.

Samples

Bone samples were used to investigate the performance of the technique in assessing Ca/P ratio in bone apatite and intact cortical bone. Bone samples were obtained from six female New Zealand white rabbits. All study protocols were approved by the Ioannina University Institutional Animal Care and Use Committee.

In three of the animals, at eight months of age, inflammation-mediated osteoporosis (IMOs) was induced by injections of talkum (Sigma-Aldrich Ltd) on the back of the rabbit [16]. IMOs is one of