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Research Paper

Determining 'age at death' for forensic purposes using human bone by a laboratory-based biomechanical analytical method



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ABSTRACT

Determination of *age-at-death* (AAD) is an important and frequent requirement in contemporary forensic science and in the reconstruction of past populations and societies from their remains. Its estimation is relatively straightforward and accurate (± 3 yr) for immature skeletons by using morphological features and reference tables within the context of forensic anthropology. However, after skeletal maturity (>35 yr) estimates become inaccurate, particularly in the legal context. In line with the general migration of all the forensic sciences from reliance upon empirical criteria to those which are more evidence-based, AAD determination should rely more-and-more upon more quantitative methods. We explore here whether well-known changes in the biomechanical properties of bone and the properties of bone matrix, which have been seen to change with age even after skeletal maturity in a traceable manner, can be used to provide a reliable estimate of AAD. This method charts a combination of physical characteristics some of which are measured at a macroscopic level (wet & dry apparent density, porosity, organic/mineral/water fractions, collagen thermal degradation properties, ash content) and others at the microscopic level (Ca/P ratios, osteonal and matrix microhardness, image analysis of sections). This method produced successful age estimates on a cohort of 12 donors of age 53–85 yr (7 male, 5 female), where the age of the individual could be approximated within less than ± 1 yr. This represents a vastly improved level of accuracy than currently extant age estimation techniques. It also presents: (1) a greater level of reliability and objectivity as the results are not dependent on the experience and expertise of the observer, as is so often the case in forensic skeletal age estimation methods; (2) it is purely laboratory-based analytical technique which can be carried out by someone with technical skills and not the specialised forensic anthropology experience; (3) it can be applied worldwide following stringent laboratory protocols. As such, this technique contributes significantly to improving age estimation and therefore identification methods for forensic and other purposes.

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1. Introduction

Age at death is one of the main four physical attributes that a Forensic Anthropologist may be called to estimate when attempting to identify unknown skeletal or decomposed human remains, along with an estimation of sex, stature during life and ethnic ancestry. The accurate estimation of age at death in human remains is reliant on recognition of several age-related changes that happen in the skeleton at predictable times during an individual's development. In infants, juveniles and adolescents, these skeletal changes are governed mostly by intrinsic genetic and hormonal factors, which mean that there is little variation between the chronological age at which certain recognisable milestones, such as epiphyseal fusion of long bones, are reached. This makes age at death estimation of sub-adults much easier and more likely to be accurate than in skeletally mature individuals (Scheuer and Black, 2004).

Several qualitative methods for age at death (AAD) estimation exist, such as the assessment of the eruption of specific deciduous and permanent teeth, or the observation of epiphyseal fusion at different anatomical sites on the skeleton. In juveniles, dental eruption occurs at regular intervals to allow quite accurate (± 3 yr) age estimation. However, the reliability of age estimation declines with increasing age. Once an individual has reached skeletal maturity (in biomechanical terms this after about 35 yr) the age-related changes that are visible on the skeleton are mainly degenerative and are influenced by a combination of intrinsic and extrinsic factors such as genetics, diet, exercise and activity. This means that it is unlikely for two individuals of the same chronological age to show exactly the same age related skeletal changes. This variation between individuals means that age estimation of adults is notoriously unreliable (Scheuer, 2002).

After adulthood, age estimation is based on the assessment of degenerative changes to the symphyseal faces of the pubis and the auricular surface of the ilium; as well as progressive fusion and obliteration of the cranial sutures, the ossification of cartilage at the sternal end of the fourth rib, and the degree of femoral cortical remodelling (Baccino et al., 1999). AAD estimates based on these methods are not usually more accurate than ± 10 yr, and lead at best to an approximate age range and not an actual age.

There have been several attempts in the past twenty years to improve quantitative methods of AAD estimation, and to introduce methods that do not rely as heavily on the expertise and experience of the investigator, as is often the case with the observational morphometric techniques. These have focussed on the premise that intracortical porosity of bone and bone remodelling increases with age, and assessment of correlation between known chronological age and characteristics of histomorphological bone features, such as primary and secondary osteon numbers/size/maturity level, trabecular volume and cortical width (Bertelsen et al., 1995; Liu et al., 1996; Feik et al., 1996, 1997; Stein et al., 1999; Thomas et al., 2000; Castillo et al., 2012; Thompson and Galvin, 1983; Fernandez-Castillo et al., 2012). These methods have been found to be of only limited value as errors for age estimation exceeded ± 8 yr in over half of the cases in these studies.

The aspartic acid racemisation technique for age estimation (Helfman and Bada, 1976), first developed in 1976, has been tried and tested in the archaeological and forensic context (Csapo and Csapo-Kiss, 1998; Ohtani et al., 2007; Ritz et al., 1996). This method is very laboratory and protocol-dependent (Waite et al., 1999), and achieves an average accuracy of ± 5 yr in bone tissue and ± 3 yr at best in perfectly preserved teeth. An age-dependent accumulation of D-aspartic acid has also been demonstrated in bone osteocalcin (Ritz et al., 1996), which has led to age estimations within ± 5 yr (at a 95% prediction interval for the data). However, as a forensic technique, aspartic acid racemisation is complex, slow and inherently inaccurate for mature female remains (Ohtani et al., 2007).

1.1. Bone tissue and the chronological donor age

In contrast to previous histomorphometric studies of bone, which are only based in phenomenological changes in the bone cortex, Zioupos and Currey (1998), Zioupos et al. (1999), Zioupos (2001b), (2001a), Rho et al. (2002) have engaged in a material characterisation of the various bone phases at the macroscale and the microscale. In an attempt to evaluate the factors affecting the biomechanical properties of bone as function of age (for the benefit of orthopaedic and clinical biomechanics (Zioupos and Currey, 1998)) a number of physical characteristics were measured in situ, or in homogenised (bone powder) form. These physical measures were the bone stiffness and strength in relation to its porosity, mineral content, calcium to phosphorus ratios, the dry density (Zioupos, 2001a), the condition of collagen (thermal shrinkage and content in mature x-links) (Zioupos et al., 1999), the elasticity of osteonal and interstitial lamellae (Rho et al., 2002), the numerical and surface-density of the in vivo fatigue microcracks (Zioupos, 2001b) and other similar microstructural features. It was observed that meaningful relationships could be established that can predict some of these age related biomechanical bone characteristics as a function of others. The macromechanical Young's modulus can be predicted (Zioupos, 2001a) from the chronological age, the dry density and the mineral content with an $R^2=0.98$ as shown in Fig. 1.

In a similar study (Rho et al., 2002) the elastic modulus and hardness of secondary osteonal and interstitial bone (Fig. 2), was examined throughout the thickness of the cortex of human femoral bone from 9 male subjects (same cohort 35–95 yr of age) by nano-indentation, which provides both modulus of elasticity and hardness estimates for a material.

By combining results on the area fraction occupied by secondary osteons, the nanoproperties of these osteons and the intracortical porosity in a 'rule of mixtures' approach, the bending modulus of the whole bone could be predicted with an $R^2=0.88$ as shown in Fig. 3. If the chronological age of the donor is known and is added in as an extra independent variable, the R^2 increased to 0.94. This indicated that 'age' still carried extra non-quantifiable information about the quality of the bone of the

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