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Estimation of chronologic age using the aspartic acid racemization method. II. On human cortical bone

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Abstract Over the last 20 years a new chemical method, base on the racemization of aspartic acid, has been developed to be used for the estimation of chronologic age in adult individuals. The method has a good accuracy when used on dental enamel, dentine and cartilage. However, in forensic and archeological cases teeth and cartilage are not always available. Since preliminary studies have shown that there are some age-related changes of the D/L aspartic acid ratio also in bone, this study was carried out to further explore if the method could be used for age estimations of bone. Bone samples from 24 individuals, aged 0.2 to 95.6 years were analysed for the D/L ratios with HPLC-technique. Two different fractions of the bone were examined, an acid-soluble peptide fraction and an acid-insoluble collagen-rich fraction. The analyses showed age-related racemizations in both fractions, although of different rates. The correlation coefficients with age were 0.72 in the peptide fraction, and 0.84 in the collagen-rich fraction. It thus seems as if bone may be used for age estimations when more stable tissues like dentine and cartilage are not available.

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Introduction

Several studies have shown the possibility to estimate the chronologic age with aid of the aspartic acid racemization method in dental enamel [1], dentine [e.g. 2–6], the gray matter of the brain [7], the lens of the eye [8], and lately cartilage [9, 10], elastic fibres of the lung parenchyma [11] and bone [12, 13].

At present, dentine seems to be the most suitable tissue for this kind of analysis. However, in practice there are often cases where teeth are not available, either because of extractions or because the head of a corpse is missing. In these cases cartilage may be used, although with less accuracy than with dentine. There may also be cases where cartilage is no longer present owing to advanced tissue degradation, and where the bone is the only remaining tissue. Bone is generally considered to be subjected to a lifelong renewal process with continuous resorptions and appositions, and it is therefore theoretically questionable if bone tissue can be used for age estimations. However, some preliminary studies [12, 13] and a pilot study by ourselves have indicated that there is also a correlation between the ratio of D/L aspartic acid and chronologic age in bone.

The aim of this study was to further explore the accuracy with which bone can be used for estimation of chronologic age with the "aspartic acid racemization method". Since earlier studies on both dentine and cartilage have shown that the racemization rate, and the correlation between age and the ratio of D/L aspartic acid, varies in different fractions of the tissues, we studied both an acid-soluble peptide fraction and an acid-insoluble collagen-rich fraction of bone.

Materials and methods

Material. Samples of bone were taken from 24 individuals aged 0.2–95.6 years (mean 48.9 ± 26.2 years) of both sexes (12 males and 12 females). The bone samples were collected at autopsies from the central part of the frontal bone. After fixation overnight in 10% neutral formalin at 4° C, the outer cortical bone was prepared to remove as much bone marrow as possible. The bone samples were washed in distilled water, briefly dried in air and frozen at –18° C until used. The further handling of the tissue has been described previously [10].

Analytical procedures. The analytical procedures have been described in detail in a previous paper [10].

Regression model. The ratio of D- and L-aspartic acid was calculated from the areas under the eluted peaks (mV*sec). A linear regression model was then constructed using the expression: $\ln[(1 + D/L)/(1 - D/L)]_t - \ln[(1 + D/L)/(1 - D/L)]_{t=0} = 2k_t * t$, where D/L is the ratio of D- and L-aspartic acids, t is any given time during racemization and the logarithmic term at t = 0 describes the amount of D-aspartic acid formed during hydrolysis.

Results

Plots of ln[(1 + D/L)/(1 – D/L)] of aspartic acid against ages of the individuals are shown in Figs. 1 and 2. The expressions obtained by linear regression with the ratio of D- and L-forms as the dependent variable were in the acid-soluble peptide fraction: ln[(1 + D/L)/(1 – D/L)] = 0.028373 + 0.001819 * t (r = 0.72, std err est = 0.0478399, $n = 24$) and in the acid-insoluble collagen-rich fraction: ln[(1 + D/L)/(1 – D/L)] = 0.026354 + 0.000474 * t (r = 0.84, std err est = 0.008326, $n = 24$).

To calculate the age in years of an individual form the insoluble collagen-rich fraction of the bone from the D/L ratio, the following equation was derived: AGE = ln[(1 + D/L)/(1 – D/L)] * 1494.64 – 25.12 (std err est = 14.79).

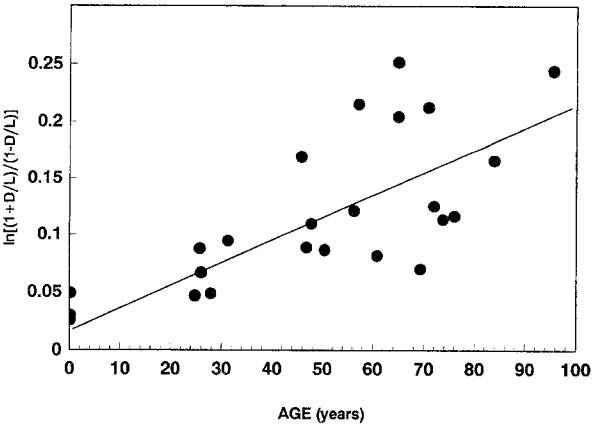

Fig. 1 Plot of ln[(1 + D/L)/(1 – D/L)] of aspartic acid from the acid-soluble fraction of bone against age. The slope defines the rate constant (2k) of racemization

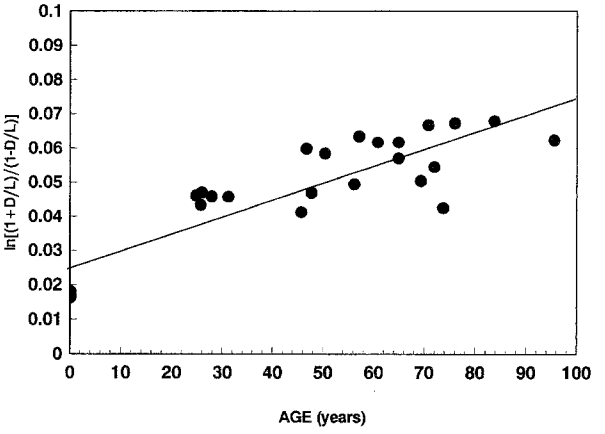

Fig. 2 Plot of ln[(1 + D/L)/(1 – D/L)] of aspartic acid from the acid-insoluble collagen-rich fraction of bone against age. The slope defines the rate constant (2k) of racemization

Discussion

An age-related correlation with the ratio of D/L aspartic acid in bone was not to be expected, since this tissue is generally considered to be subjected to constant, life-long remodelling, in which old tissue with accumulated D-aspartic acid is removed and substituted with new constituents high in the L-form. Preliminary pilot studies on bone from the maxilla and the mandible, however, showed an unequivocal, although somewhat irregular, relationship between the relative amount of accumulated D-aspartic acid and age. This finding encouraged us to explore the relationship in more detail. In order to avoid the influence of physiological remodelling as much as possible we tested samples from the frontal bone, which could be expected to be subjected to only minor functional load and stress. We also aimed at getting a bone tissue which was contaminated as little as possible with connective tissue and bone marrow, and which was easy to access and prepare in a reproducible way.

The relative amount of accumulated D-aspartic acid was lower than in other tissues studied. The racemization rate in the acid-soluble peptide fraction of dentine is 5.3 * 10^{-3} [6] and of cartilage about 3.1 * 10^{-3} [10]. In the corresponding fraction of bone it was only 1.8 * 10^{-3}. The racemization rate in the acid insoluble collagen fraction was about 1.0 * 10^{-3} in dentine [14], and 3.3 * 10^{-3} in cartilage [10]. In the corresponding fraction of bone it was only 0.47 * 10^{-3}. The racemization slope of the same tissue also seems to differ between the various laboratories and methods used, but it seems obvious that the racemization rate is slower in bone than in dentine and cartilage, and that this difference is largest in the collagen-rich fraction. It thus seems as if the tissue-turnover rate is faster in bone than in dentine and cartilage, and that the collagenous fraction has the highest turnover rate.

It is unclear why the racemization rates differ between the 2 different fractions of bone but not of cartilage, where they are of about the same order of size. The difference between the 2 compartments in dentine is easier to explain, since collagen in this tissue is not subjected to any remodelling whilst the acid-soluble peptide fraction may have some nutritional exchange with the circulation via

the dentinal tubules and the peritubular dentine. In cartilage there seems to be a higher turnover rate of proteoglycans in the ground substance than of the collagen, which is almost completely stable [15]. It would be expected that the collagen fraction and the surrounding ground substance of bone should be exchanged simultaneously at remodelling. The current results suggest that the collagen is exchanged, but the ground substance is not. This is corroborated by Turzynski and Ritz [13] who suggested that osteocalcin extracted from bone had a high longevity.

The correlation between the D/L ratio of aspartic acid and age is much higher in both dentine and cartilage than in bone. This may be an expression of the generally accepted remodelling of bone, and that the degree of racemization is the average of active and passive areas within the sample. The individual variation of remodelling probably accounts for a large part of the dispersion around the common regression line. Another explanation is the fact that the rate of racemization of amino acids in the living body is mainly determined by temperature. Normal differences in the body temperature (36.2–37.6) may influence the rate of racemization to such a degree that age estimations of old individuals may differ by up to 20 years [2].

It is further accepted that remodelling is higher in bone tissue that is subjected to physical load than in bone without weight-bearing function. It seems as if the bone used in this study had been subjected to remodelling, since both fractions studied had a significantly lower racemization rate than the corresponding fractions of dentine and cartilage.

Other possibilities for the lower correlation in bone than in dentine and cartilage may be that when small samples are analysed contamination with proteins from other sources, such as connective tissue and blood, may significantly increase the relative amount of the L-form, giving a too low estimate of the age. Similarly, contamination with bacteria with D-form aspartic acid in their cell walls may cause an apparent too high age. We were cautious to handle the tissue samples as cleanly as possible to avoid any kind of contamination.

In the calculations of the regression models it was assumed that the correlation between the D/L ratio and age was linear over the whole life span. This is probably a simplification, since it may be suggested that the correlation is divided into two parts, one over the first 16–18 years, when bone is formed, and one over the remaining years when ageing constitutes the largest part.

In conclusion, it seems as if the "aspartic acid racemization method" used on bone can be used for age determination of adult individuals when teeth or cartilage are not available. However, the precision seems, logically, to be less when bone is used than when dentine and cartilage is used.

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