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Age Estimation of Archaeological Remains Using Amino Acid Racemization in Dental Enamel: A Comparison of Morphological, Biochemical, and Known Ages-At-Death

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ABSTRACT The poor accuracy of most current methods for estimating age-at-death in adult human skeletal remains is among the key problems facing palaeodemography. In forensic science, this problem has been solved for unburnt remains by the development of a chemical method for age estimation, using amino acid racemization in collagen extracted from dentine. Previous application of racemization methods to archaeological material has proven problematic. This study presents the application to archaeological human remains of a new age estimation method utilizing amino acid racemization in a potentially closed system—the dental enamel. The amino acid composition and extent of racemization in enamel from two Medieval cemeteries (Newcastle Blackgate and Grantham, England) and from a documented age-at-death sample from a 19th century cemetery (Spitalfriedhof St Johann, Switzerland) were determined. Alterations in the amino acid composition were detected in all populations, indicating that diagenetic change had taken place. However, in the Medieval populations, these changes did not appear to have substantially affected the relationship between racemization and age-at-death, with a strong relationship being retained between aspartic acid racemization and the morphological age estimates. In contrast, there was a poor relationship between racemization and age in the post-medieval documented age-at-death population from Switzerland. This appears to be due to leaching of amino acids post-mortem, indicating that enamel is not functioning as a perfectly closed system. Isolation of amino acids from a fraction of enamel which is less susceptible to leaching may improve the success of amino acid racemization for archaeological age estimation. Am J Phys Anthropol 140:244–252, 2009.

Accurate age estimation remains one of the central problems in palaeodemography. While numerous methods exist for estimating age-at-death from the skeleton, the majority of these techniques rely upon degenerative changes, which show substantial variations in the timing of their occurrence between individuals (Kemkes-Grottenthaler, 2002). As a result, age estimates produced using these techniques have large error margins and may show bias towards underestimation of the age of older individuals and overestimation of the age of younger individuals (Aykroyd et al., 1999). There is, therefore, a need for the further development of age-at-death estimation methods which are less susceptible to such problems.

In forensic human identification work, age estimation using amino acid racemization (AAR) of aspartic acid and asparagine (Asx) in tooth dentine has proven to be able to effectively overcome these difficulties (e.g. Ohtani et al., 1988; Ohtani, 1995; Ritz-Timme et al., 2000). This technique is based upon the fact that amino acids can exist in two different enantiomeric forms, L and D, which are non-superimposable mirror images. In the human body, all the amino acids are initially synthesized in the L form, but this situation is thermodynamically unstable. Where there is no tissue turnover, such as in teeth, over time a spontaneous racemization reaction will occur until an equilibrium of L and D enantiomers is formed. The racemization process is strongly temperature dependent. As a result of the relatively high temperature of the body (37.5°C), racemization occurs at an appreciable rate during life. If the post-mortem environment of an individual is relatively cold, for example if the individual is buried in a cool, temperate climate, then racemization will only occur very slowly after death. Therefore, in tissues which form early in life and which experience little biochemical turnover, the ratio of L to D enantiomers will reflect the age-at-death of a recently deceased individual.

Although AAR has proven effective in forensic identification, the application of AAR in dentine to archaeological samples has been less consistently successful. When the method has been applied to archaeological

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populations where only estimated ages-at-death are available, the age estimates produced using AAR showed a good correlation with the morphological age estimates (Masters and Zimmerman, 1978; Masters, 1984; Csapo et al., 2004). This has been interpreted as indicating that the method can be successfully applied to archaeological populations, in spite of the large errors in the morphological age estimates used for comparison. However, the application of AAR to populations with known ages-at-death has shown a poor correlation between the AAR age estimates and the known ages-at-death (Gillard et al., 1992; Carolan et al., 1997a). It seems likely that the better performance of AAR age estimation on material where the age-at-death is unknown is due to the low accuracy of age-at-death estimates on archaeological skeletal remains previously mentioned. If the ages-at-death are not known accurately then it is difficult to determine whether there is a correlation between AAR and the true age-at-death of the individual. Greater emphasis should therefore be placed upon the results obtained on skeletal remains with known ages-at-death, where poor correlations between AAR in tooth dentine and age are consistently observed.

The poor relationship between AAR and age-at-death observed in dentine from known age-at-death skeletal remains is probably due in part to diagenetic changes taking place in collagen, the main protein in dentine, in the burial environment. Van Duin and Collins (1998) and Collins et al. (1999) have argued that Asx residues in the triple helix of collagen are unable to racemize, a hypothesis that has been borne out by recent observations in bone collagen (Dobberstein, 2005). After a certain period of time in the burial environment, all non-helical Asx sites will have reached equilibrium, and racemization of the Asx amino acids in collagen will "equilibrate" at a D/L of 0.09 (equivalent to approximately 3400 years of burial at 10°C, or 110 years at the normal body temperature of 37°C), until diagenetic breakdown of the collagen molecules releases more Asx sites for racemization. Thus, for remains with a long post-mortem interval it will not be possible to retrieve meaningful age information.

One possible alternative approach to the application of AAR to archaeological remains is to use a different source of proteins. Collins and Riley (2000) have argued that in order to make meaningful estimates of age from racemization in biomineralized protein it is necessary to isolate a closed system, a suggestion strongly supported by research work on fossil mollusc shells (Penkman et al., 2008). A likely candidate tissue is dental enamel, being often well preserved in the burial environment, and in addition containing non-collagenous proteins. Although enamel has a low organic content, its proteins have already been degraded into small fragments during the formation of the enamel (Robinson and Kirkham, 1984), and some of these protein fragments may be protected from the burial environment by their intimate association with the crystal structure of the enamel mineral. Thus, these proteins may provide a potential new source of amino acids for AAR age estimation. Analysis of enamel has to date only seen limited application in age estimation studies, due to the low protein content of this tissue. However, recent developments in AAR analysis have allowed the use of reverse phase-high pressure liquid chromatography (RP-HPLC) to separate and measure much smaller amounts of amino acids than was previously possible (Kaufman and Manley, 1998; Benesova et al., 2004). The greater sensitivity of the RP-HPLC technique has already allowed the successful application of AAR age estimation to enamel from living individuals (Griffin et al., 2008a). However, this method has not yet been applied to archaeological enamel.

This study applied the new sample preparation method of Griffin et al. (2008a,b) to samples from three archaeological sites, in order to investigate its suitability for age estimation of archaeological skeletal remains. Dental samples from the Medieval English cemeteries of Newcastle Blackgate and Grantham were analyzed, along with samples from the post-medieval known age-at-death collection of Spitalfriedhof St Johann, from Basel, Switzerland. By comparing the results from populations with and without documented ages at death, it may be possible to determine whether discrepancies observed in the success of AAR in age estimation between these groups are due to the low accuracy of morphological age estimation techniques. Little information is available on the burial environments of the three archaeological populations; however, if enamel is acting as a closed system, the burial environment should not significantly influence the extent of racemization post-mortem. However, the varying post-mortem intervals of the three populations will have an impact on the AAR values obtained. Newcastle Blackgate has the longest post-mortem interval, followed by Grantham and Spitalfriedhof St Johann. It would therefore be expected that the AAR values for Newcastle Blackgate will be higher for any given age-at-death than those for Grantham, due to the longer time during which post-mortem racemization could have occurred. Likewise, the AAR values for any given age-at-death will be expected to be higher for an individual from Grantham than for an individual from Spitalfriedhof St Johann.

MATERIALS AND METHODS

Newcastle Blackgate (UK)

The first population chosen for study was represented by human remains from the early medieval cemetery of Newcastle Blackgate. The radiocarbon dates and artefactual evidence obtained from the cemetery showed that it was in use from approximately AD 750 to 1150 (Cummings and Rega, 2007). During excavation of the cemetery by the Newcastle City Archaeological Unit, in 1977–78 and 1990–92, the remains of 646 articulated skeletons and 175 disarticulated individuals were recovered (Cummings and Rega, 2007). Thirteen human teeth were selected from among the disarticulated remains from the cemetery, representing individuals whose age estimates were not open ended and whose teeth could be removed from the jaw without damage to the skeleton. Age-at-death was estimated by Professor Chamberlain and ranged from approximately 5 years to 30–40 years (Table 1).

The errors in the morphological age estimates for Newcastle Blackgate and Grantham vary according to the age of the individual. For individuals under 20 years of age, the age estimates were based upon dental development (Smith, 1991; Reid and Dean, 2000), which follows a fairly predictable pattern with age. Because of the relative consistency of the timing of dental development within and between populations, ages-at-death produced using this method are highly accurate. As a result, the errors associated with these age estimates are approximately ±2 years for children under the age

TABLE 1. Morphological age-at-death estimates for the individuals from the Newcastle Blackgate and Grantham cemeteries

Specimen	Teeth sampled	Estimation method	Estimated age
Newcastle Blackgate			
BG90 3171–A	m_1, m_2	4 (M_1 root ½ complete; M_2 crown ½ complete)	5 yr
BG90 3171–B	M_2	4 (M_2 root ¾ complete; M_3 crown ½ complete)	11 yr
BG90 3171–D	M^2	4 (M^2 root ½ complete)	10 yr
BG90 3208	P_2	2	20–25 yr
BG90 3208	C_1, M_2	2	30–40 yr
BG90 3231–A	P_1, M_1, M_2	4 (M_2 apex closed; M_3 unerupted)	15 yr
BG90 3268	M_1, M_2	2	20–25 yr
BG92 3693–A	M_2	4 (M_2 with apex ½ closed)	14 yr
Grantham			
GLR US Mand 1	m_1, m_2	3 (I_1 root initiated)	4 yr
GLR US 01	m^1, m^2	3 (I^1 root initiated)	5 yr
GLR US 02	M_1, M_3	2	25–35 yr
GLR US 03	M_1	2	40+ yr
GLR US 04	M_1, M_1	4 (P_1 root ¾ complete; M_2 root ¼ complete)	10 yr
GLR SK 019	M_1	4 (M_2 root apex closed, M_3 root ¼ complete)	15 yr
GLR SK 027	M_2, M_3	2	40+ yr
GLR SK 040	P_1, M_3	1, 2	30–40 yr
GLR SK 046	P_2, M_3	1, 2	30–40 yr

Estimation methods: 1 (Katz and Suchey 1986), 2 (Miles 1963), 3 (Reid and Dean 2000), 4 (Smith 1991).

of 10, and ±3 years for individuals between 10 and 20 years. For older individuals, the age estimates were determined from the extent of tooth wear (Miles, 1963), which is a less accurate method for age estimation, due to the high level of variation in the rate of tooth wear within and between populations. Therefore, the errors in the age estimates for the adults examined were ±5 years for individuals aged between 20 and 30 years and ±10 years for individuals over 30 years of age.

Grantham (UK)

The second population studied was from a medieval cemetery excavated at London Road, Grantham, which is believed to have belonged to "the hospital of St Leonard "by the Spittelgate" of Grantham" (Page, 1906). The cemetery was in use during the 12th to the 15th centuries (Trimble et al., 1991). Part of the cemetery was excavated in 1991 by Heritage Lincolnshire, and the remains of 53 individuals were recovered, most of which showed good bone cortical surface preservation. The sixteen human teeth analyzed here were selected from both articulated individual skeletons (four individuals) and commingled remains (five jaws). Age-at-death was estimated by Professor Chamberlain using the same methods as for the remains from Newcastle Blackgate, supplemented by evidence for age from the pubic symphysis where possible (Katz and Suchey, 1986) (Table 1).

Spitalfriedhof St Johann (Switzerland)

One hundred teeth were analyzed from the Spitalfriedhof St Johann known age reference collection. The cemetery of Spitalfriedhof St Johann was associated with the Bürgerspital hospital of Basel, and was in use from AD 1845 to 1868. More than two thirds of the patients who died in the hospital during this period were buried in the cemetery, making the collection reasonably representative of the hospital mortality patterns at this time. The cemetery was excavated in 1988–1989 prior to the development of the site.

Due to the incomplete excavation of the site, of the more than 2000 individuals buried in the cemetery, only 1061 were recovered. From the excavated skeletal remains, 220 skeletons are now curated by the Natural History Museum, Basel, as the Spitalfriedhof St Johann known age collection, representing those individuals who could be identified from the hospital and cemetery records. Individuals were identified by comparing the grave register with the death register of the hospital and the identifications verified by comparing the sequence of age and sex in the excavated individuals with the sequence in the burial register. In a number of cases, further verification was possible where the medical records and/or autopsy reports from the hospital provided details of pathological conditions which could then be corroborated by inspection of the skeleton.

One hundred human teeth from the Spitalfriedhof St Johann known age collection were analyzed in a blind test of the amino acid racemization age estimation method outlined below. The teeth were selected by Dr Gerhard Hotz to cover the full range of adult ages. Where possible, priority was given to teeth which were free of dental disease and showed good preservation.

Modern teeth

The data from the three archaeological populations will be compared with previously published data obtained using the same methodology on modern human teeth extracted from living individuals of known age (Griffin et al., 2008a).

METHODS

The amino acids analyzed were obtained by sequentially acid etching the enamel surface of the tooth crown using the method developed in Griffin et al. (2008a). The labial and lingual surfaces of each tooth were cleaned by applying a 0.2 ml PCR tube filled with 6 M HCl (total volume approximately 230 μl) to each surface for one minute, then rinsing the tooth surfaces with HPLC grade methanol. In order to minimize the amount of contaminating protein present on the tooth surface due to handling of the teeth, they were placed in 12% (wt/vol) sodium hypochlorite for two days and then rinsed in ultrapure water and HPLC grade methanol. Samples of the acid soluble fraction of enamel proteins were

collected by applying two 0.2 ml PCR tubes filled with 6 M HCl (total volume of each tube approximately 230 μl) to each of the labial and lingual surfaces for two consecutive time intervals of one minute. This depth of sampling was selected, as it represents the shallowest depth of etching at which relatively consistent Asx D/L values are obtained, whilst being shallow enough to avoid sampling of the dentine (Griffin et al., 2008b). The teeth from Newcastle Blackgate were sampled to a greater depth than the other teeth in this study, as these teeth had previously been used in the development of the sampling method. For four randomly-selected samples from each population, in order to measure the amount of free amino acids in each sample, a 30–50 μl subsample was taken prior to hydrolysis, which was dried on a centrifugal evaporator for 4–5 h.

The second sample obtained from each tooth surface was hydrolyzed at 110°C for 6 h in an N_2 enriched atmosphere in sterile glass vials. All the samples were either hydrolyzed shortly after collection or stored in a freezer until they were hydrolyzed in order to minimize the extent of induced racemization. This study used a hydrolysis temperature of 110°C, as recommended by Kaufman and Manley (1998), rather than the 100°C used in previous studies of enamel racemization (Helfman and Bada, 1976; Ohtani and Yamamoto, 1992). While this difference in hydrolysis temperature should result in a larger amount of induced racemization during the hydrolysis step than observed in previous research, the amount of induced racemization should be approximately the same for all the samples. The Asx D/L values obtained in the present study will thus be slightly greater than those seen in previous research, but should show a consistent relationship to these values.

After hydrolysis, the samples were dried on a centrifugal evaporator overnight. When dry, the samples were rehydrated in 50 μl of a solution of 0.003 M HCl, 0.01 mM L-homo-arginine (as an internal standard) and 0.77 mM sodium azide, with a pH of 2. The sample vials were vortexed to aid dissolution, centrifuged and the supernatant collected and analyzed on the RP-HPLC. Samples were separated on a C_{18} Hypersil BDS column by reverse phase HPLC using modified methods based on that of Kaufman and Manley (1998). During hydrolysis, both asparagine and glutamine undergo rapid irreversible deamination to aspartic acid and glutamic acid, respectively (Hill, 1965). Therefore it is not possible to distinguish these amino acids and they are reported as Asx and Glx respectively. Two analyses were conducted for each sample, one using a shortened version of the chromatographic method of Penkman (2005), enabling the separation of L-Asx, D-Asx, L-Glx, D-Glx, L-serine (L-Ser), D-Ser, glycine (Gly), L-alanine (L-Ala) and L-homo-arginine, and the second using an adaptation of this method, with part of the elution gradient halved in order to enhance separation of the L- and D- Asx peaks (Fig. 1). The Asx D/L values for each sample were calculated from the second analysis, while the D/L values for all the other amino acids were calculated from the first analysis. In some chromatographic runs it was also possible to separate L-threonine (L-Thr), L-histidine (L-His) and L-arginine (L-Arg). Absolute concentrations of L- and D-amino acids were calculated from the integrated area under each peak, normalized to the peak area of the internal standard, L-homo-arginine. The Asx or Ser D/L values obtained from the two sampled sides of each tooth were averaged to produce an overall Asx or Ser D/L value for that tooth.

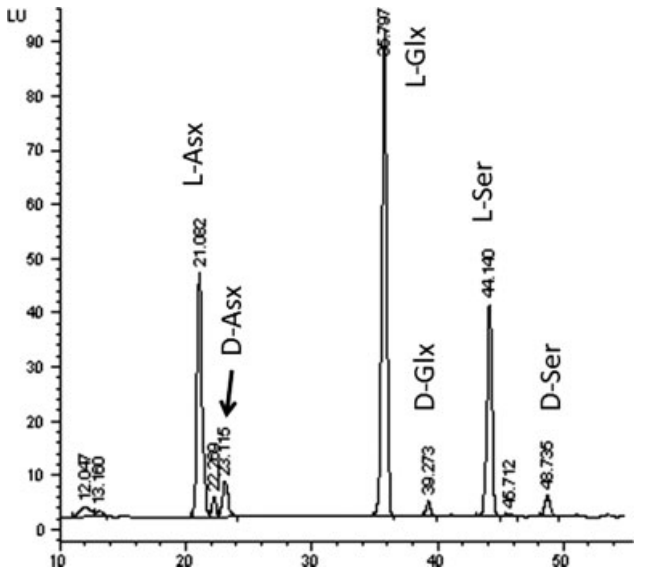

Fig. 1. Typical chromatogram of an archaeological tooth sample.

RESULTS

The amino acid compositions relative to glycine within the acid soluble fraction of enamel proteins from the teeth of all three archaeological populations were significantly different to those observed for modern teeth (Griffin et al., 2008a) (Table 2). The teeth from Newcastle Blackgate and Grantham were depleted in Glx, Ser, L-Thr and L-Arg, while the teeth from Spitalfriedhof St Johann also showed a reduction in L-Ala in comparison to the concentrations observed in modern healthy teeth. However, despite the similarity in the depletion of certain amino acids, the three archaeological populations displayed different behavior in terms of their total amino acid concentrations (calculated from the sum of Asx, Glx, Ser, Glycine and Ala concentrations for each sample). The average total amino acid concentration of the teeth from Newcastle Blackgate was significantly higher than that observed in modern teeth (Griffin et al., 2008a), probably due to the sampling of slightly deeper enamel in the teeth from this population, while that of the teeth from Grantham was significantly reduced, and that of Spitalfriedhof St Johann showed no significant change (Table 2). This suggests that the amino acid content of the enamel in the medieval samples has undergone diagenetic alteration post-mortem. Such changes in the amino acid composition of the enamel may alter the relationship between amino acid racemization and age in these populations.

In spite of the changes observed in their amino acid compositions, for the teeth from Newcastle Blackgate and Grantham the relationship between Asx D/L in the acid soluble fraction of the enamel and tooth age (determined by subtracting the age at which crown formation is completed for that tooth type from the age-at-death of the individual) showed a log linear increase with the age of the tooth, similar to that reported for modern teeth (Fig. 2). However, the extent of Asx racemization in these two archaeological populations was higher than had been observed for modern teeth at any given age, with the values for Grantham being generally slightly lower than those for Newcastle Blackgate.

TABLE 2. Means and standard deviations of amino acid concentrations [aa] relative to glycine of samples from the three archaeological populations (pmol/μl) for aspartic acid/asparagine (Asx), glutamic acid/glutamine (Glx), serine (Ser), L-threonine (L-Thr), L-histidine (L-His), L-arginine (L-Arg) and alanine (Ala)

		Asx	Glx	Ser	L-Thr	L-His	L-Arg	Ala	Total
Modern (n = 31)	[aa]	37	64	39	25	12	12	21	59
	S.D.	8	13	8	7	3	3	5	15
Newcastle Blackgate	[aa]	31	51	28	18	10	10	20	66
(n = 13)	S.D.	5	6	4	3	2	1	4	16
t-test P values		2.8×10^{-4}	6.2×10^{-8}	4.4×10^{-12}	1.6×10^{-7}	0.067	0.0013	0.069	0.024
Grantham (n = 16)	[aa]	35	58	31	19	13	10	24	49
	S.D.	9	10	7	4	4	2	8	14
t-test P values		0.36	0.038	5.7×10^{-7}	6.2×10^{-5}	0.15	0.00050	0.045	0.0033
Spitalfriedhof St Johann	[aa]	39	58	32	19	13	10	23	54
(n = 73)	S.D.	9	10	10	4	2	5	7	28
t-test P values		0.048	3.0×10^{-5}	3.3×10^{-9}	7.6×10^{-20}	0.21	1.3×10^{-6}	0.0063	0.24

Modern data are from Griffin et al. (2008a). P values are of 2-tailed t-tests between the archaeological samples and the modern population.

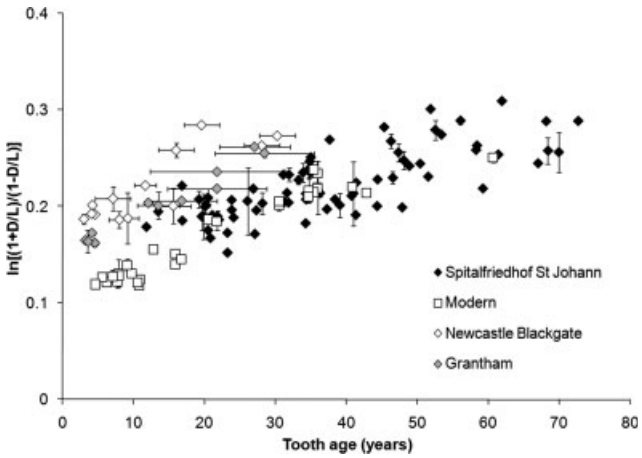

Fig. 2. Relationship between ln [(1 + Asx(D/L))/(1 − Asx(D/L))] and tooth age for all the populations in this study. Ages for modern teeth and for the Spitalfriedhof St Johann population are known, ages for the Newcastle Blackgate and Grantham populations are those determined from dental development and dental wear. *Y* error bars represent 1st standard deviation about the mean for multiple measurements from the same tooth. *X* error bars represent the errors in the morphological age estimates.

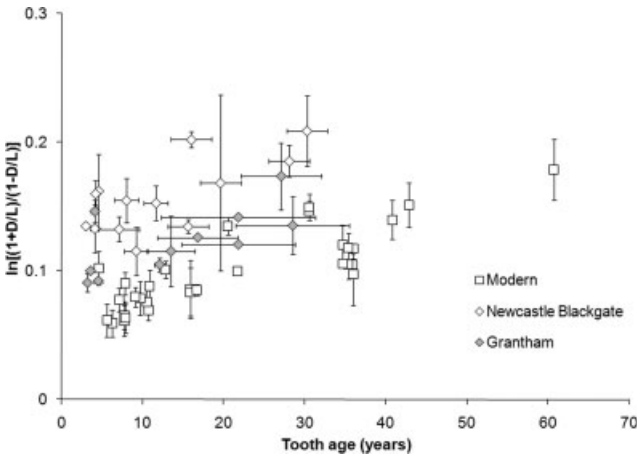

Fig. 3. ln [(1 + Ser(D/L))/(1 − Ser(D/L))] versus age for archaeological and modern teeth. Ages for modern teeth are known, ages for the Newcastle Blackgate and Grantham populations are those determined from dental development and dental wear. *Y* error bars represent 1st standard deviation about the mean for multiple measurements from the same tooth. *X* error bars represent the errors in the morphological age estimates.

In contrast, the correlation between Asx D/L and age for the Spitalfriedhof St Johann population (r^2 = 0.59) was not as strong as observed in modern teeth (r^2 = 0.92) (Griffin et al., 2008a) or in the two other archaeological populations (r^2 = 0.73 for Newcastle Blackgate, r^2 = 0.96 for Grantham) (Fig. 2), although in these latter two populations, age was estimated rather than known. Although a correlation between the extent of racemization and tooth age could still be identified at Spitalfriedhof St Johann, the relationship was not as close and the gradient of the relationship was markedly different to that observed in modern teeth.

For the archaeological populations of Newcastle Blackgate and Grantham, only a weak relationship could be detected between Ser D/L in the acid soluble fraction of enamel and tooth age (Fig. 3). As had been observed for Asx, the teeth from these populations showed a higher extent of Ser racemization than had been observed in modern teeth (Griffin et al., 2008a). Again, Ser D/L in the Spitalfriedhof St Johann population (r^2 = 0.58) was not as closely linked to tooth age as it was for the modern samples (r^2 = 0.71), reflected in the lower r^2 for the relationship between Ser D/L and age in this population, although the gradient of the relationship was still similar to that observed in modern teeth (Fig. 4).

The racemization values obtained were converted to ages at death using the calibration curve shown in Griffin et al. (2008a). Before the calibration curve could be applied, however, the extent of racemization occurring post-mortem needed to be estimated. This was achieved by comparing the ages estimated by Asx racemization for the five teeth from juvenile individuals at Grantham and the seven teeth from juvenile individuals at Newcastle Blackgate with the ages determined for these teeth using tooth development. Age-at-death estimates of juveniles using dental development are relatively accurate (Saunders, 2000), making it possible to determine the amount of racemization that should have taken place during life, and hence the amount occurring post-mortem. By determining this value for each of the juveniles analyzed, it was possible to estimate the average offset due to racemization in the burial environment at each site, by averaging the differences between the AAR and

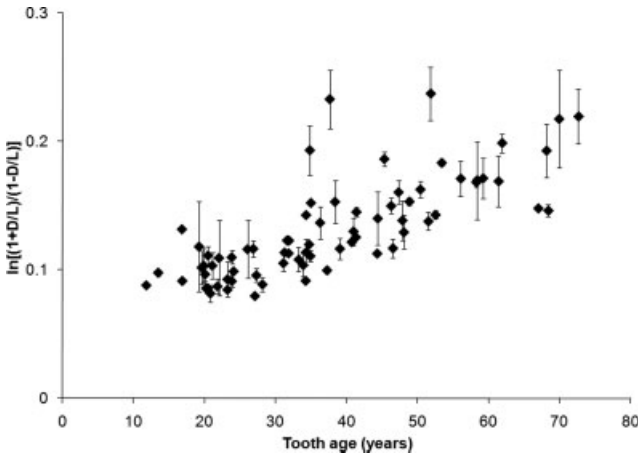

Fig. 4. ln [(1 + Ser(D/L))/(1 − Ser(D/L))] versus age for the Basel Spitalfriedhof St Johann population. Error bars represent 1st standard deviation about the mean for multiple measurements from the same tooth.

TABLE 3. Calculated offsets for the three archaeological populations from the modern calibration curve

Population	Offset (yr)	S.D. of offset (yr)
Grantham	17.9	2.5
Newcastle Blackgate	24.7	3.9

TABLE 4. 95% confidence intervals for the AAR age estimates for the archaeological and modern populations analyzed

Population	95% confidence interval (yr)
Modern	±8.7
Spitalfriedhof St Johann	±20.2
Grantham	±7.5
Newcastle Blackgate	±13

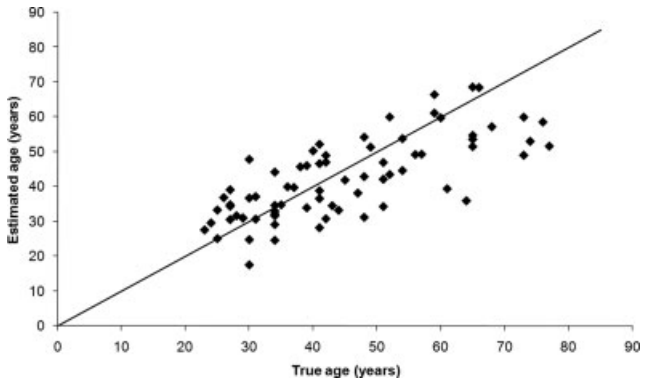

Fig. 5. Relationship between true and estimated ages-at-death for the Basel Spitalfriedhof St Johann individuals. Line shown is $x = y$.

morphological age estimates for the selected juvenile teeth. The teeth used for this analysis included both permanent and deciduous teeth, as no significant difference was observed in the relationship between AAR and age-at-death between the permanent and deciduous teeth analyzed. This offset could then be subtracted from the age estimated using the regression equation of (Griffin et al., 2008a) (Table 3). Although the application of regression to age-at-death estimation has received much criticism in recent years (Gowland and Chamberlain, 2002), it still remains the most frequently used statistical method in forensic and archaeological applications of AAR. Therefore, linear regression was used for age estimation in the present study, to enhance its comparability with previous work on AAR age estimation.

The method described above appeared to be able to produce age estimates for the populations from Newcastle Blackgate and Grantham that were similar to those determined using morphological methods. The age estimates produced using this approach were closer to the ages estimated from dental development and tooth wear for the Grantham population than for those of Newcastle Blackgate. This was reflected in the smaller 95% confidence interval for this population (Table 4), which was even lower than that observed in modern teeth (Griffin et al., 2008a). The better performance of AAR age estimation in the Grantham population was probably due to the smaller sample size and narrower age range of this population relative to the sample of modern teeth examined in Griffin et al. (2008a).

Age estimates for Spitalfriedhof St Johann were generated for the teeth prior to knowledge of their true ages, as described above. The extent of racemization occurring during the burial period was estimated from the temperature records for Basel (Baker et al., 1994), rather than by the method used for the Newcastle Blackgate and Grantham populations, due to the lack of juvenile individuals in the sample provided. The average temperature at Basel for each year since burial was determined from temperature records for the city (Baker et al., 1994), and the amount of racemization which would have taken place was determined from the equation $\ln k = \ln A - E_a/RT$, where k is the rate constant of the reaction, A is the "pre-exponential factor", a constant independent of temperature, E_a is the activation energy of the reaction [106 kJ mol^{-1} for Asx racemization in enamel (Griffin, 2006)], R is the gas constant (8.3143 J K^{-1} mol^{-1}), and T is the temperature in Kelvin. This figure was calculated for each individual from their known date of burial, and was subtracted from that individual's age-at-death, which had been calculated from the regression equation of Griffin (2006). The ability of this AAR age estimation methodology to produce accurate ages at death appeared to be poorer in the Spitalfriedhof St Johann population than in the previous archaeological populations (Figs. 5 and 6). While 68% of the age estimates were within ten years of the true age-at-death, for many teeth the age estimates were very inaccurate. Because of this, the 95% confidence interval for the age estimates produced for this population was approximately twice that of the other archaeological populations analyzed (Table 4). There was also evidence for bias in the age estimates produced, with individuals more frequently underaged than overaged, particularly those who were over 60 years of age.

The proportion of amino acids in the acid soluble portion of the enamel which are not protein bound, known as free amino acids, was also found to differ between the populations analyzed here (Fig. 7). In the modern teeth analyzed by Griffin et al. (2008a) there was an unexpectedly high level of free amino acids in the enamel, perhaps reflecting entrapped protein fragments resulting from the processing of the enamel matrix during miner-

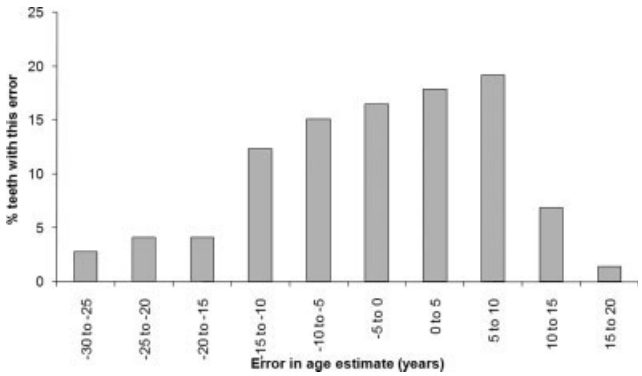

Fig. 6. Errors in age estimates for the Basel Spitalfriedhof St Johann population.

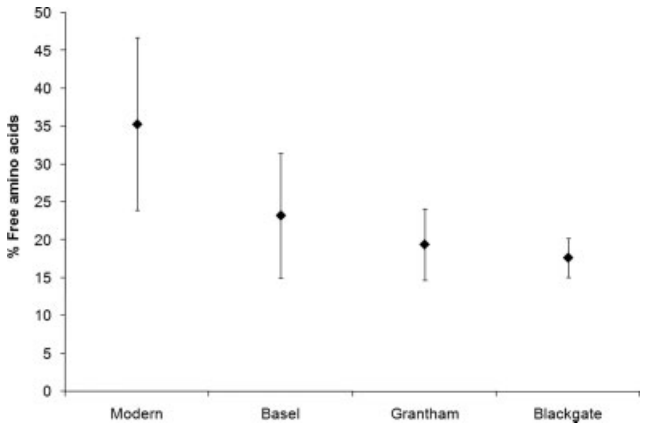

Fig. 7. Percentage of free amino acids for each population analyzed (values shown are the sum of those for Asx, Glx, Ser, Gly and Ala). Error bars represent 1st standard deviation about the mean for the population.

alization. However, the proportion of free amino acids decreased progressively in the three archaeological populations as the length of the burial period increased. These results may indicate the loss of amino acids from the enamel into the burial environment through leaching, a possibility supported by the changes in amino acid concentration observed in all three archaeological populations, in contrast to previous studies with bleached shells (Penkman et al., 2008).

DISCUSSION

Amino acid concentrations

The differences observed in the amino acid compositions relative to glycine of the enamel from all three archaeological populations from that of modern teeth indicate that post-mortem change is occurring in the amino acid content of the acid soluble fraction of enamel in the archaeological specimens. The pattern of changes in amino acid composition relative to glycine was similar in the three archaeological populations, suggesting that they may be due to common post-mortem diagenetic processes. Although in modern teeth caries can have a significant impact on the amino acid composition of the enamel proteins (Griffin et al., 2008a), and potentially also the relationship between Asx D/L and age, this was not observed in the teeth analyzed here. The observation of alterations in the amino acid composition of the enamel post-mortem provides a strong indication that the teeth from these populations are not acting as a closed system in the burial environment, which we believe to be essential for the application of amino acid degradation studies for age estimation (e.g., Penkman et al., 2008).

Amino acid racemization

Although racemization in the burial environment will be much slower than racemization during life, the results presented here show that it is occurring at a measurable rate over the burial period examined. If the enamel is acting as a closed system, then the extent of racemization post-mortem should be determined only by temperature and time since burial. If this was the case, it would be expected that the Asx D/L values for any archaeological population should be offset from the modern racemization calibration curve by approximately the same value, although differences in burial temperature and time of burial with a cemetery will increase variability. However, in populations where burials occurred over only one or two centuries, in particular when burial temperatures are low (e.g. burial temperatures in Northern Europe range between 8°C and 12°C), then the variation in the offset should be relatively small. Thus, it should be possible to take these changes into account when preparing age estimates using this method.

This idea is supported by the observed relationship between racemization and age-at-death for the Newcastle Blackgate and Grantham populations. The Asx D/L values for these two populations were consistently higher at any given age than those for modern teeth. The similarity of the slopes of Asx D/L against tooth age in the two archaeological populations and in modern teeth suggests that post-mortem changes are not greatly affecting the pattern of racemization in these two populations. Furthermore, the teeth from Grantham tended to show a lower extent of racemization than those from Newcastle Blackgate. Such a finding is consistent with the more recent deposition of the remains from Grantham (12th to 15th century AD) compared to Newcastle Blackgate (8th to 12th century AD).

In contrast, the results obtained from the Spitalfriedhof St Johann population showed a much poorer correlation with age than had been observed in the previous two archaeological populations. The rate of racemization observed for this population was much lower than that reported for modern individuals, and was also lower than that seen for the other archaeological populations presented in this study. The different Asx racemization behavior detected in this population implies that changes are occurring in the burial environment which are having a greater impact on the rate of Asx racemization than had been observed in the two other archaeological populations. Analysis of the data using Generalized Linear Modelling (GLM; using SPSS 15.0) confirmed that the relationship between Asx racemization and age-at-death was significantly different in the population from Spitalfriedhof St Johann to that in the modern population of Griffin et al. (2008a). The relationship between Asx D/L and age observed here is similar to that reported by researchers working on archaeological dentine of similar age to the Spitalfriedhof St Johann cemetery (Carolan et al., 1997a,b). This indicates that both tissues appear to be susceptible to diagenetic change

post-mortem, although the mechanisms by which diagenesis affects Asx D/L are likely to be different in enamel and dentine.

The behavior of Ser D/L in the remains from Spitalfriedhof St Johann supports this theory. As had been seen for Asx D/L, the Spitalfriedhof St Johann population showed more dispersal of Ser D/L values around the trendline than had been seen in modern teeth. However, this variability did not alter the slope of the relationship between D/L and tooth age, unlike that seen for Asx.

Comparison of Spitalfriedhof St Johann and the Medieval populations

The differing relationships between Asx D/L and age observed in the medieval populations and in the population of Spitalfriedhof St Johann may be affected by differences in the number of teeth analyzed from each site. The small size of the two samples from unknown age-at-death archaeological populations may have given a false impression of the level of variability in Asx D/L with age. The use of linear regression to produce age estimates from Asx D/L values may also have had an impact upon the age estimates produced, as has been seen when other age estimation methods have been applied to archaeological known-age-at death populations (e.g., Molleson and Cox, 1993). However, it seems most likely that the lower accuracy of the age estimates for the two Medieval populations is obscuring the true relationship between Asx D/L and age for these populations. As the relationship between Asx D/L and age-at-death is not strong when applied to known age-at-death archaeological skeletal remains, it is probable that this weaker relationship holds for other archaeological material as well.

One potential reason for this low correlation between AAR and age may lie in post-mortem changes occurring in the chemical composition of the tooth enamel. Analysis of the proportions of free amino acids in the teeth from each of the three populations and from the modern teeth presented in Griffin et al. (2008a) indicates that there is a tendency for free amino acids to be leached into the burial environment over time (Fig. 7). Such a finding contradicts the widely held view that enamel acts as a closed system post-mortem, and casts doubt upon the ability of amino acid racemization in enamel to be used for age estimation of archaeological remains, as the assumption that the enamel is acting as a closed system is critical for the reliability and meaningful application of this technique.

However, analysis of a number of Javanese pig teeth from burial contexts dated up to 44,000 BP has shown significant retention of amino acids over long burial periods (Griffin et al., in preparation). This suggests that there is a portion of the amino acids of the enamel that does act as a closed system post-mortem, while the remainder of the amino acids are readily leached to the burial environment. If this is indeed the case, then it would be expected that the portion of the amino acids which is being retained may be more useful in age estimation than the fraction of enamel proteins analyzed here. This portion of amino acids is likely to make up a significantly larger proportion of the amino acids extracted from the enamel of individuals from the two Medieval populations than from the individuals from Spitalfriedhof St Johann, as a greater amount of the free amino acids will have been leached into the burial environment due to their longer post-mortem interval. This may go some way towards explaining the closer relationship between Asx D/L and age-at-death in these two populations. Future work may allow the development of a better method for isolating this well-preserved portion of amino acids, enhancing the ability of AAR to estimate age-at-death in archaeological populations. Recent research on archaeological shell has produced new methods for extracting closed system amino acids (Penkman et al., 2008) and it is possible that application of similar methodologies to archaeological enamel may allow the isolation of well-preserved amino acids from this tissue as well.

CONCLUSIONS

Amino acid racemization in the acid soluble fraction of the enamel can be correlated with age-at-death in archaeological skeletal remains. However, the extent to which this relationship can be used to produce ages-at-death is highly variable. Although a good relationship was observed between AAR and age-at-death for teeth from two Medieval populations, when tested on more recently buried known age-at-death material, AAR in enamel appeared to be a poor age indicator, with a high level of variability in the relationship between AAR and age-at-death. It seems likely that this phenomenon is due to open system diagenesis in the burial environment, with free amino acids being leached into the burial environment post-mortem. Therefore, while AAR has shown some potential for use in archaeological age-at-death estimation, further work is needed to improve its accuracy and reliability when applied to archaeological skeletal remains.

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