DIETARY HABITS AND FRESHWATER RESERVOIR EFFECTS IN BONES FROM A NEOLITHIC NE GERMAN CEMETERY

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ABSTRACT. Within a project on Stone Age sites of NE Germany, 26 burials from the Ostorf cemetery and some further Neolithic sites have been analyzed by more than 40 accelerator mass spectrometry (AMS) dates. We here present the results of stable isotope and radiocarbon measurements together with reference ^{14}C dates on grave goods from terrestrial animals such as tooth pendants found in 10 of the graves. Age differences between human individuals and their associated grave goods are used to calculate ^{14}C reservoir effects. The resulting substantial reservoir effects have revealed misleadingly high ^{14}C ages of their remains, which originally indicated a surprisingly early occurrence of graves and long-term use of this Neolithic burial site. We demonstrate that in order to ^{14}C date the human bones from Ostorf cemetery, it is of utmost importance to distinguish between terrestrial- and freshwater-influenced diet. The latter may result in significantly higher than marine reservoir ages with apparent ^{14}C ages up to ~800 yr too old. The carbon and nitrogen isotopic composition may provide a basis for or an indicator of necessary corrections of dates on humans where no datable grave goods of terrestrial origin such as tooth pendants or tusks are available. Based on the associated age control animals, there is no evidence that the dated earliest burials occurred any earlier than 3300 BC, in contrast to the original first impression of the grave site (~3800 BC).

INTRODUCTION

Marine or freshwater diets result in radiocarbon ages apparently too old due to the so-called reservoir effect. Marine and freshwater organisms are depleted in ^{14}C relative to the atmosphere, resulting in apparent ^{14}C ages that are too old compared to contemporaneous terrestrial organisms. This difference is called the reservoir age (R). For marine organisms living in the upper, mixed layer of the open ocean, the reservoir age is generally about 400 ^{14}C yr (Stuiver and Braziunas 1993; Hughen et al. 2004). In shallow marine waters, the reservoir age can be higher or lower, depending on atmospheric CO_2 exchange, influx of hard freshwater, and upwelling of deep ocean waters (Heier-Nielsen et al. 1995). Freshwater may contain dissolved $CaCO_3$ from fossil carbonate in soils. The freshwater reservoir age is known to vary considerably with time and location (Heier-Nielsen et al. 1995; Lanting and van der Plicht 1998; Olsen et al. 2009). Hence individuals who subsisted or partly subsisted on marine or freshwater diets are often very difficult to date directly by their bone remains (Lanting and van der Plicht 1998; Arneborg et al. 1999; Bonsall et al. 2004; Fischer et al. 2007).

Since the early 1980s, measurements of stable isotopes of carbon and nitrogen in bone collagen have proven a useful way of obtaining information on the protein part of diets (Wada et al. 1991; Ambrose 1993; Richards and Hedges 1999; Ambrose and Krigbaum 2003; Erikson 2003; Richards et al. 2003). The stable isotope ratio of carbon (δ^{13}C) is especially useful for estimating the proportion of marine relative to terrestrial diets. This is because the carbon reservoirs of the marine and terrestrial environment have different isotopic compositions, and this difference is passed on to the consumer from either of these reservoirs. Carbon isotopes are, however, much less suited to distinguish between a terrestrial and freshwater diet as the carbon isotopic compositions of these reservoirs often have similar values.

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The nitrogen stable isotope composition ($\delta^{15}N$) reflects the trophic level of a consumer. Thus, herbivores have significantly lower $\delta^{15}N$ values than carnivores (Schoeninger and DeNiro 1984). Because aquatic (i.e. marine and freshwater) food webs are longer than their terrestrial counterparts, diets dominated by food from fresh and/or marine waters can usually be distinguished from more terrestrial diets through their elevated $\delta^{15}N$ values.

Although lipids and carbohydrates in diets may constitute significant parts of the energy intake, they have little influence on $\delta^{13}C$ of the protein in bone collagen—at least in protein-rich diets (Hedges 2004). Since they contain no nitrogen, they have no effect on $\delta^{15}N$ either. The isotopic composition of collagen from the most compact bones of adult humans represents the average diet over a considerable time (of the order of 20 yr) prior to death (Geyh 2001; Wild et al. 2000). By contrast, collagen from non-compact (trabecular) bones from adult humans represents the average diet over a much shorter period, ~4 yr (Martin et al. 1998).

The effect of possible reservoir ^{14}C age offsets may be revealed by stable isotope analysis on bone remains for dietary reconstruction or if the individuals can be associated with ^{14}C datable material reflecting their true age. The latter is the case for the humans excavated from the Ostorf cemetery in Schwerin, NE Germany (Figure 1), who in many cases were buried with grave goods such as animal tooth pendants from badgers, beavers, roe deer, and wild boars. Terrestrial tooth pendants provide an opportunity to 1) obtain an absolute age of the humans despite their possible aquatic diet, 2) estimate the reservoir effect, and 3) obtain independent information on their diet that can confirm or supplement isotopic dietary data. The Ostorf cemetery is part of a larger Neolithic research project in NE Germany covering 5 sites and 29 burials and includes more than 30 accelerator mass spectrometry (AMS) ^{14}C dates (Figure 1). Lakes, rivers, and groundwater of the surrounding area of the 5 localities are characterized by a high content of dissolved carbonates, i.e. by hard waters. These carbonate ions originate from dissolution of fossil carbonate minerals having no ^{14}C activity. A high content of ^{14}C-free carbon may significantly increase the reservoir age of freshwater systems, i.e. the so-called hardwater effect (Boaretto et al. 1998; Dye 1994). Thus, the hardwater effect results in increased apparent ^{14}C ages of humans with a subsistence based on freshwater environments. Here, the human remains from the Ostorf cemetery are used as case study on the ^{14}C hardwater effect on humans with a freshwater diet. This study also illustrates further implications that the ^{14}C hardwater effect may have on dating, for example, food residue on pottery (Fischer and Heinemeier 2003; Hart and Lovis 2007). The results presented here contrast the original first impression of the Ostorf grave site, which dated the first occurrence to around 3800 BC. For a further presentation and discussion of the archaeological context, see Lübke et al. (2009) and Olsen and Heinemeier (2009).

METHODS

For samples prepared and measured at the AMS ^{14}C Dating Centre at the University of Aarhus (denoted AAR- in Table 1), collagen was extracted from human and animal bones following standard protocol (DeNiro and Epstein 1981; Jørkov et al. 2007). Bone samples were demineralized in 1M HCl at 4 °C for 1 hr, being stirred every 5 min. They were then rinsed to neutral pH with deionized water. Then, 0.2M NaOH was added to remove contaminating humic acids. Samples were rinsed again to neutral pH with deionized water. HCl was added to the sample tubes to reach a pH of 2.5. The samples were then covered and gelatinized in this weak acid solution at 70 °C for 16 hr in order to dissolve the protein components. After removing insoluble residues by centrifuging the samples at 2500 rpm for 10 min, the remaining supernatant solution was concentrated to ~3 mL by evaporation at 100 °C for 6 hr. The solution was then freeze-dried for 24 hr. The extracted collagen material has been quality-checked in terms of collagen percentage and C:N values (DeNiro 1985;

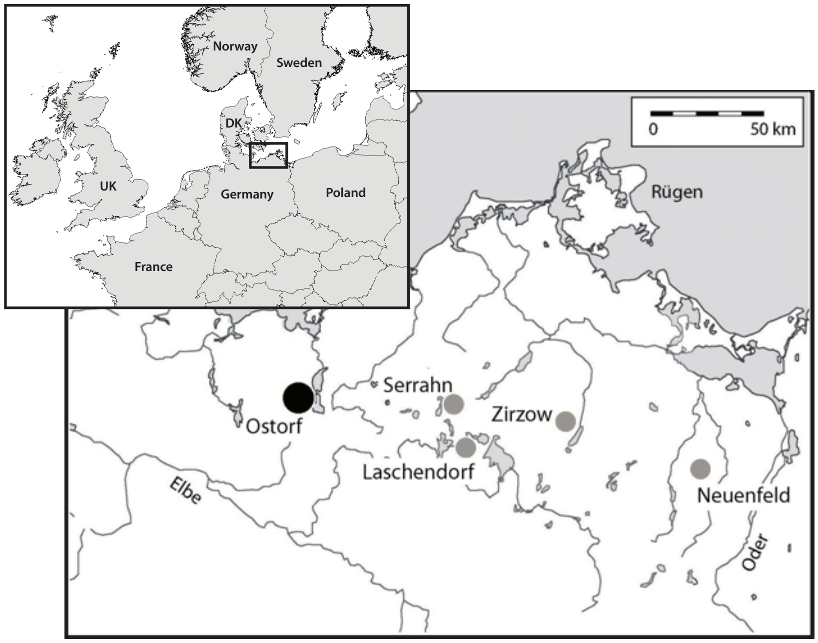

Figure 1 Map showing the location of the Ostorf cemetery, excavated in Schwerin, Germany

van Klinken 1999). The collagen was then converted to CO_2 by combustion in sealed evacuated ampoules with CuO. Part of the resulting CO_2 gas was used for $\delta^{13}C$ analysis on a GV Instruments IsoPrime stable isotope mass spectrometer to a precision of 0.2‰, while the rest was converted to graphite for AMS ^{14}C measurements by reduction with H_2 using cobalt as a catalyst (Vogel et al. 1984). The results are reported according to international convention (Stuiver and Polach 1977) as conventional ^{14}C dates in ^{14}C yr BP (before AD 1950) based on the measured $^{14}C/^{13}C$ ratio corrected for the natural isotopic fractionation by normalizing the result to the standard $\delta^{13}C$ value of –25‰ VPDB (Andersen et al. 1989). The $\delta^{15}N$ values were measured on collagen powder on a GV Instruments IsoPrime stable isotope mass spectrometer to a precision of 0.2‰.

RESULTS AND DISCUSSION

The results of 25 carbon and nitrogen stable isotope measurement together with 26 ^{14}C measurements on human and animal bone remains from the Ostorf cemetery are presented in Table 1. Sixteen ^{14}C dates on human bones had been previously measured by the Utrecht AMS laboratory (UtC-lab nr, Table 1). Laboratory numbers AAR-#-I indicate isotope measurements from Aarhus.

Figure 2 illustrates the carbon and nitrogen stable isotope data of the animal tooth pendants associated with human remains from the Ostorf cemetery. Also shown are values for prehistoric animals from a Danish study (Fischer et al. 2007). The 5 boxes in Figure 2 indicate isotopic ranges from minimum to maximum values of $\delta^{13}C$ and $\delta^{15}N$. Hence, these 5 boxes distinguish between 5 main dietary protein sources: terrestrial herbivores (roe deer, red deer, and domestic cattle, all in one box), 2 freshwater species (pike and tench, in 2 separate boxes), and 2 marine food sources (fish and seal, each in a separate box).

Though the Ostorf animal samples may not be directly comparable to the Danish material, it is clear that the carbon and nitrogen isotopic values of the low trophic herbivores roe deer and beaver fall

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Table 1 Radiocarbon and stable isotope results for the Ostorf humans and associated animal tooth pendants. AAR-10733-I and AAR-10734-I represent the pair of a mother and baby, respectively.

	Animal pendants							Humans						
Grave	Lab nr	Species	Collagen yield %	$\delta^{13}C$ ‰	$\delta^{15}N$ ‰	C:N	^{14}C age BP	Lab nr	Collagen yield %	$\delta^{13}C$ VPDB ‰	$\delta^{15}N$ AIR ‰	C:N	^{14}C age BP	Age offset yr
1904-01	AAR-10590	Wild boar, tooth	14.8	–22.4	6.9	3.3	4400 ± 34	UtC-8173					4568 ± 40	168 ± 52
1961-01	AAR-10589	Wild boar, tooth	15.1	–21.2	7.2	3.4	4435 ± 36	AAR-9758-I / UtC-7447	14.8	–20.6	12.8	3.4	4699 ± 49	264 ± 61
1904-02	AAR-10591	Wild boar, tooth	19.1	–22.1	7.2	3.2	4449 ± 34	AAR-9757-I / UtC-7440	13.1	–22.0	13.9	3.3	4737 ± 41	288 ± 53
1935-03	AAR-10595	Wild boar, tooth	14.0	–22.1	6.7	2.9	4387 ± 35	AAR-9763-I / UtC-7445	12.3	–20.8	13.4	3.5	4356 ± 44	–31 ± 56
1935-04	AAR-10596	Badger, tooth	4.7	–18.9	10.5	3.3	4402 ± 31	AAR-9764-I / UtC-7446	10.5	–20.8	13.7	3.3	4299 ± 50	–103 ± 59
1961-04	AAR-10592	Beaver, tooth	2.3	–22.3	4.0	3.2	4500 ± 65	AAR-9756-I / UtC-7448	11.2	–20.9	14.2	3.0	4827 ± 47	327 ± 80
1961-05	AAR-10594	Wild boar	12.3	–20.6	5.6	3.1	4398 ± 34	AAR-10733-I / UtC-8180	10.0	–20.0	13.0	3.4	4833 ± 38	435 ± 51
								AAR-10734-I / UtC-8181	11.0	–19.9	13.3	3.3	4795 ± 38	397 ± 51
1961-07	AAR-10593	Roe deer	10.7	–22.9	4.0	3.2	4429 ± 32	AAR-9752	15.4	–20.6	13.2	3.3	4830 ± 47	401 ± 57
1961-08	AAR-10597	Badger, tooth	1.7	–19.5	11.9	3.4	4135 ± 41	AAR-9754-I / UtC-7449	15.2	–19.8	15.2	3.3	4970 ± 50	835 ± 65
1961-09	AAR-10598	Roe deer	5.7	–21.0	4.6	3.4	4303 ± 34	AAR-9755-I / UtC-8179	9.4	–19.4	15.3	3.4	4855 ± 49	552 ± 60
1904-16								AAR-9753-I / UtC-7443	15.0	–20.3	14.8	3.4	5023 ± 45	
1935-05								AAR-9759-I / UtC-8177	9.8	–19.2	14.9	3.3	4591 ± 49	
1904-05								AAR-9760-I / UtC-7441	15.4	–21.0	13.1	3.3	4659 ± 43	
1904-06								AAR-9761-I / UtC-8106	14.6	–21.5	13.4	3.1	4486 ± 44	
1935-02								AAR-9762-I / UtC-7444	13.3	–20.7	13.0	3.3	4383 ± 42	

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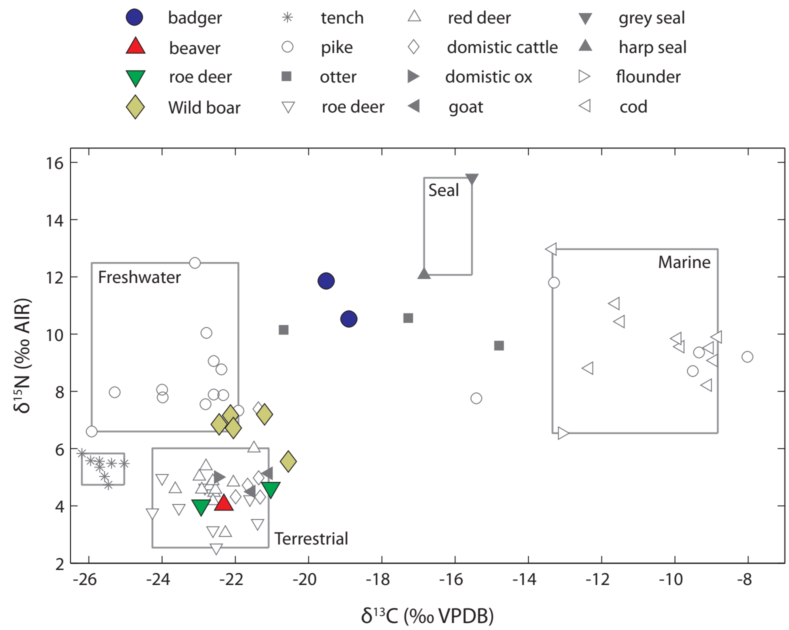

Figure 2 Measured $\delta^{13}C$ and $\delta^{15}N$ bone collagen values of animal pendants from the Ostorf cemetery (large symbols) shown together with isotopic values for animals from Denmark (small symbols) (Fischer et al. 2007). The boxes represent the different isotopic ranges of terrestrial, marine, and freshwater animals.

within the terrestrial food item box. The wild boar samples have on average a $\delta^{15}N$ value that is 2.7‰ higher than the average $\delta^{15}N$ value of the herbivores and show very similar carbon isotope values (Table 1, Figure 2). This suggests the wild boar to be about 1 trophic level above the herbivores. In contrast, the badger samples display very elevated values in both $\delta^{15}N$ and $\delta^{13}C$. Surprisingly, the $\delta^{15}N$ values are comparable with high trophic marine and freshwater fish.

Figure 3 illustrates the 15 human carbon and nitrogen isotope measurements together with the associated animal samples. In addition, the 5 boxes are taken from Figure 2 (dashed) and displaced by 1‰ for $\delta^{13}C$ and 3.5‰ for $\delta^{15}N$ (solid line) to account for the diet-to-consumer shift (trophic level) (Schoeninger and DeNiro 1984; Lidén 1995; Richards and Hedges 1999). Thus, the solid-line boxes define the isotopic range in which a human would end up if consuming only food from one of these food categories. Consumer isotopic values outside the boxes indicate a mixture of 2 or more different food sources. The Ostorf humans all display elevated $\delta^{15}N$ values, but similar $\delta^{13}C$ values compared to the herbivores. Therefore, based on the isotopic evidence, it seems very probable that the Ostorf humans have subsisted on a significant component of freshwater resources. Further, their $\delta^{13}C$ and $\delta^{15}N$ isotopic values are weakly correlated ($\rho = 0.50$), suggesting that their diets may stem from a few resources only with distinct carbon and nitrogen isotopic end-member values. The $\delta^{13}C$ values range from –19.2‰ to –22.0‰ VPDB and the $\delta^{15}N$ values range from 12.8‰ to 15.3‰ AIR. The relatively small span of $\delta^{13}C$ and $\delta^{15}N$ values indicates that this population must have had rather uniform dietary choice. The $\delta^{15}N$ span indicates about 1 trophic level difference between the most extreme values. Contrary to the expected $\delta^{15}N$ difference between parent and offspring of 3‰, the mother and child presented here give almost similar $\delta^{15}N$ values (Table 1, Figure 3). A more thorough analysis of the dietary habits of the Ostorf humans requires a suite of reference animals and cannot be carried out on the data presented here.

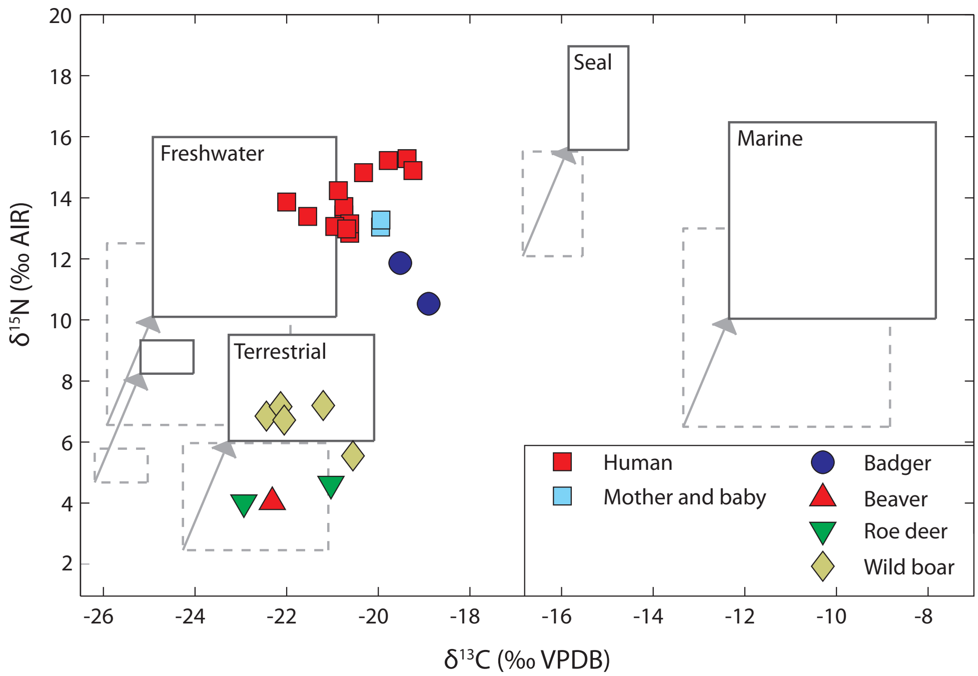

Figure 3 Measured $\delta^{13}C$ and $\delta^{15}N$ bone collagen values for the Ostorf cemetery humans shown together with the isotopic values of animal tooth pendants from associated grave goods. The solid boxes represent food items displaced to the equivalent consumer values by 1‰ for $\delta^{13}C$ and 3.5‰ for $\delta^{15}N$ to account for trophic level shift relative to the reference animal values (dotted boxes). This shift is indicated by the arrows. See also Figure 2.

Due to their elevated $\delta^{15}N$ values indicating a fish diet, the Ostorf humans may be expected to exhibit apparent ^{14}C ages that are too old, depending on the reservoir age of the local freshwater environment. However, their associated tooth pendants from terrestrial animals are not expected to be influenced by reservoir effects and should therefore provide the correct age for each of the Ostorf individuals buried with such grave goods. The reservoir age effect defined as the ^{14}C differences between ^{14}C age of the human and associated animal pendant can then be calculated (Table 1). As illustrated by Figures 4, 5A and B, about 80% of the dated Ostorf humans have apparent ^{14}C ages older than their associated terrestrial animal pendants. Furthermore, it is striking that the human apparent ^{14}C dates indicate a very extended inhumation period of the Ostorf cemetery ranging from 3800 to 2900 BC, whereas the animals reveal a shorter and later inhumation phase from 3350 to 2600 BC (Figure 4). Also, it is remarkable that apart from the 2 youngest animals (08-1961 and 09-1961, Table 1, Figure 4), the animal ^{14}C dates show no statistical difference (χ_{meas}: $4.7 \leq \chi$: 16.5). This suggests that these 9 individuals may reflect a single burial event or a very short inhumation phase of the cemetery with the 2 humans from grave 08-1961 and 09-1961 being buried later.

The calculated reservoir age (Table 1, Figure 5) shows considerable variability, ranging from −103 to 835 yr, which may reflect individual dietary preferences, i.e. amounts of freshwater fish, or subsistence from multiple lakes with different freshwater reservoir ages. The low collagen yield for the terrestrial reference dates AAR-10596, -10592, and -10597 may question the validity of their ^{14}C age and in turn the calculated reservoir age. However, the C:N ratios are in the acceptable range of 2.7–3.6 (Schoeninger and DeNiro 1984), and only AAR-10597 deviates appreciably from the general age pattern (Table 1). Notably, the negative reservoir ages obtained for 2 individuals (03-1935

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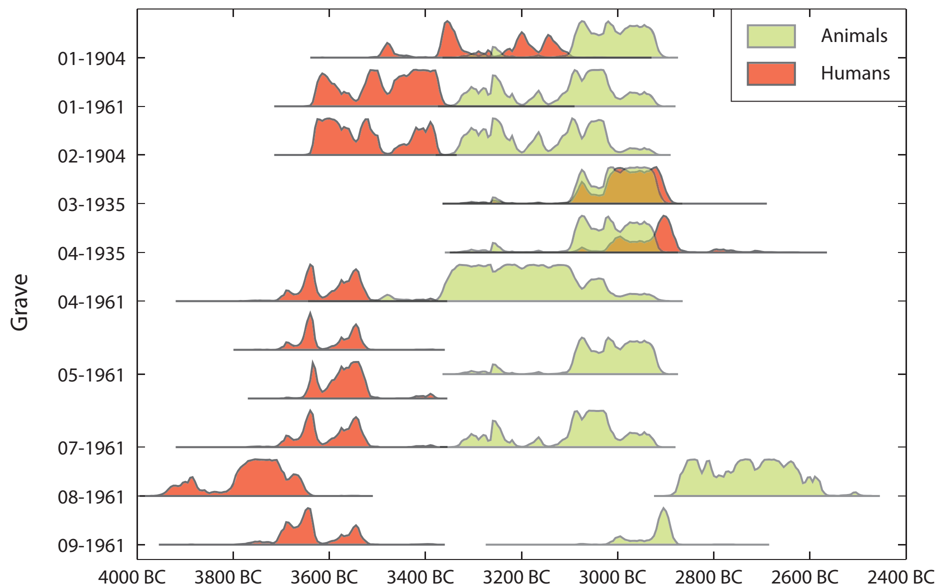

Figure 4 Calibrated age probability distributions of humans shown together with associated animal tooth pendants for 10 of the Ostorf graves using OxCal 4.1 (Bronk Ramsey 2009) and the IntCal04 calibration curve (Reimer et al. 2004). The generally much higher ages for the human samples are due to (freshwater) reservoir effects.

and 04-1935) are not significantly different from a 0 yr reservoir age. They may thus indicate no consumption of freshwater fish or at least a diet, perhaps consisting of freshwater fish, in ^{14}C equilibrium with the ^{14}C content of the atmosphere. The latter can be true for freshwater lakes containing no dissolved fossil carbonate, though these are very rare if not absent in the study area.

Obviously, the most suitable reference material for calculating the reservoir ages are herbivores, whereas animals like wild boar and in particular badger may obtain their food from sources depleted in ^{14}C. The badger, known to digest amphibians, may therefore reflect apparent ^{14}C ages being too old. If the associated animal control samples turn out to be too old, this would only underestimate the calculated reservoir age of the humans. Therefore, the negative reservoir ages may be explained by associated control animals having too old ages. In support of this hypothesis, most of the reservoir age variability is exhibited by associated control animals like the badger and wild boar (see Table 1 and Figure 5C, D). On the other hand, the consistent ages of all associated control animals speak against this possibility.

The Ostorf cemetery data set offers a unique opportunity to investigate the relationship between the stable isotopes dietary evidence and the resulting reservoir age (Figure 5C, D). Both the human δ^{13}C and δ^{15}N values are only weakly correlated with the reservoir age ($\rho = 0.54$ and $\rho = 0.53$). However, removing the 2 samples with negative reservoir ages increases the correlation to 0.64 and 0.71 for δ^{13}C and δ^{15}N, respectively. This is a clear indication of a correlation between diet and reservoir age of an individual. Thus, the human carbon and nitrogen isotopic values may be applied for estimating the reservoir age and thus to yield crudely corrected dates for the humans found in a similar environment with no associated animal grave goods. The lack of a better correlation is probably because humans may consume both smaller and larger fish exhibiting different δ^{15}N values but similar reservoir age. Also, different lakes may have different reservoir ages, contributing further to the observed scatter (Figure 5C and D). However, the removal of 2 samples in order to obtain an accept-

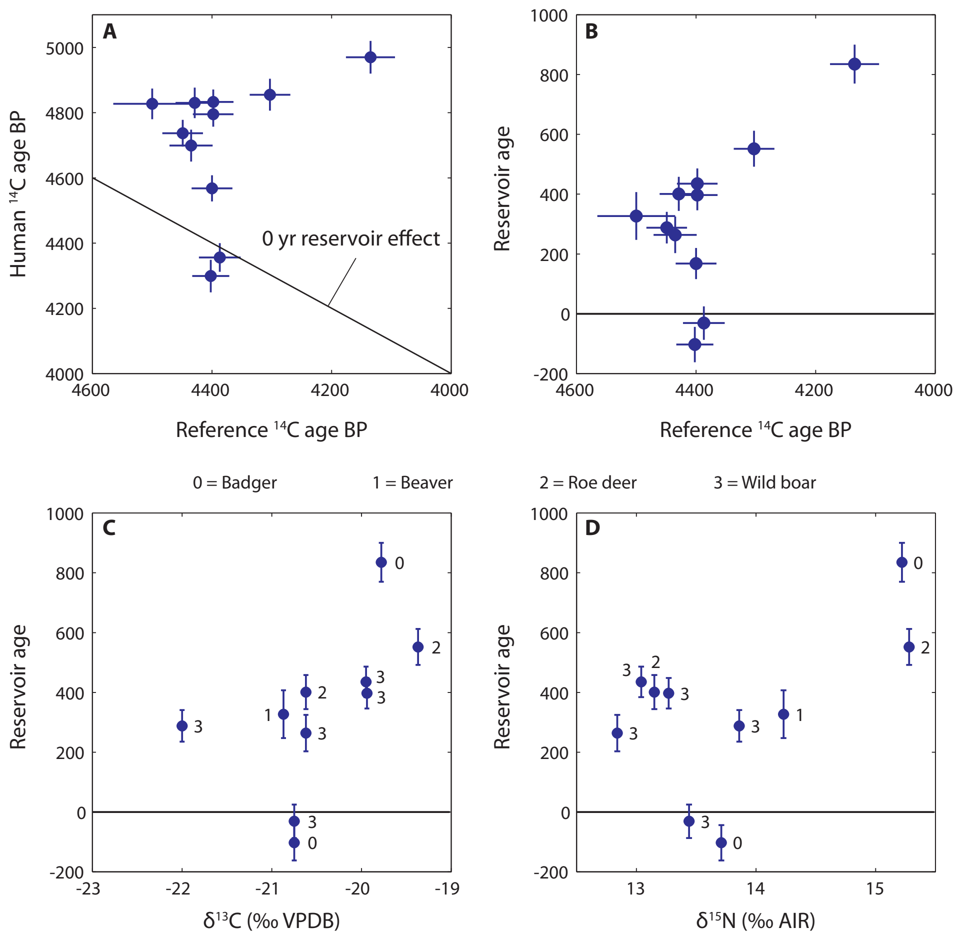

Figure 5 A) Measured human ^{14}C ages versus the associated terrestrial animal tooth pendant ^{14}C age (reference age). The solid black line indicates no reservoir effect, i.e. similar animal tooth pendant and human ^{14}C age (x = y line). Note that the reservoir age is calculated as the vertical distance from the no reservoir effect line to the human ^{14}C age. B) Associated terrestrial animal tooth pendant ^{14}C age (reference age) versus calculated reservoir age R. C) The human δ^{13}C values versus the calculated reservoir age. D) The human δ^{15}N values versus the calculated reservoir age R. For each data point in C and D the species of the reference animal is indicated by a number.

able correlation between the calculated reservoir age, δ^{13}C and δ^{15}N values fundamentally show that estimation of reservoir ages based on stable isotope models may in general yield considerably misleading results and therefore should be carried out with great caution (e.g. Lanting and van der Plicht 1998).

CONCLUSION

We have demonstrated that in order to ^{14}C date the human bones from Ostorf cemetery, it is of utmost importance to obtain dietary knowledge to distinguish between terrestrial- and freshwater-influenced diet. The latter may result in significant reservoir ages with apparent ^{14}C ages up to ~800 yr too old. The carbon and nitrogen isotopic composition may provide a basis for, or at least a warning against, necessary reservoir corrections of dates on humans where no datable grave goods of terrestrial origin such as tooth pendants or tusks are available. Based on the associated control animals, there is no evidence that the first burials occurred any earlier than 3300 BC, a result that contradicts the original first impression of the grave site (3800 BC) as discussed by Lübke et al. (2009).

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