

Oxygen Isotope Analysis of Human Bone Phosphate Evidences Weaning Age in Archaeological Populations

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ABSTRACT

Here we report bone phosphate oxygen ($\delta^{18}\text{O}_p$) values from perinates/neonates and infants (<3.5 yrs; $n=32$); children (4-12 yrs; $n=12$); unsexed juveniles (16-18 yrs; $n=2$) and adult bones ($n=17$) from Wharram Percy, North Yorkshire, England, in order to explore the potential of this method to investigate patterns of past breastfeeding and weaning. In prior studies, $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ analyses of bone collagen have been utilised to explore weaning age in this large and well-studied assemblage, rendering this material highly appropriate for the testing and development of this alternative method targeting the inorganic phase of bone. Data produced reveal ^{18}O -enrichment in the youngest perinatal/neonatal and infant samples, and an association between age and bone $\delta^{18}\text{O}_p$ (and previously-published $\delta^{15}\text{N}$ values), with high values in both these isotope systems likely due to breastfeeding. After the age of 2-3 years, $\delta^{18}\text{O}_p$ values are lower, and all children between the ages of 4 and 12, along with the vast majority of sub-adults and adults sampled (aged 16 to >50 years), have $\delta^{18}\text{O}_p$ values consistent with the consumption of local modern drinking water. The implications of this study for the reconstruction of weaning practices in archaeological populations are discussed, including variations observed with bone $\delta^{15}\text{N}_{\text{coll}}$ and $\delta^{18}\text{O}_p$ co-analysis and the influence of culturally-modified drinking water and seasonality. The use of this method to explore human mobility and palaeoclimatic conditions are also discussed with reference to the data presented.

Breastfeeding and weaning practices in past populations have become an increasingly important and studied area of archaeology and anthropology in recent years. It has been suggested that the natural age for the cessation of human breastfeeding lies between approximately 2.5 and 7 years (Dettwyler 1995; Dettwyler 2004), based on anthropological literature and various predictions concerning human physiology and reproduction (see review in Dettwyler 2004). However, incidences of breastfeeding beyond 4 years of age are unusual, with weaning most commonly occurring between 2-3 years amongst modern humans living in non-industrialised and traditional, natural fertility societies (Alvarez 2000; Sellen 2001, 2007; Kennedy 2005). Prior to the cessation of breastfeeding, most infant diets also include complementary liquid and solid foods, in many cases before the age of 6 months (Sellen 2001). However, practices are varied (Sellen 2001; Kennedy 2005), and cultural factors are known to strongly influence the duration and nature of breastfeeding (and complementary feeding) in past and present societies (Stuart-Macadam and Dettwyler 1995; Dettwyler 2004). Therefore, the investigation of breastfeeding (including the processes by which other foods are introduced to the infant and breastfeeding declines and ceases) can illuminate past cultural practices and lifeways in earlier societies, particularly aspects of life pertaining to children and women. These practices also have broader implications for our understanding of human ecology, health, population dynamics and demographics in the past. As well as providing total nutritional requirements in early life (Butte et al. 2002), breast milk provides immunological protection to infants, primarily against gut/diarrheal diseases but also against extra-intestinal diseases (e.g. Arifeen et al. 2001; Duijts et al. 2010; see review in Horta and Victora 2013a) and (possibly) even longer-term conditions (e.g. Rich-Edwards et al. 2004; see review in Horta and Victora 2013b), and may also have benefits for a child's

cognitive development (e.g. Quinn et al. 2001). Lactation can also determine fertility in breastfeeding females (through ovulation suppression) and therefore influence birth spacing, and through this, impact maternal health (Vitzhum 1994).

In archaeological case studies, nitrogen isotope analyses of tooth and bone collagen are commonly used to reconstruct breastfeeding and weaning practices (e.g. Fogel et al. 1989; Mays et al. 2002; Richards et al. 2002; Fuller et al. 2003, 2006a; Jay et al. 2008), given the established trophic level effect on $\delta^{15}\text{N}$ and subsequent drop in $\delta^{15}\text{N}$ during/following weaning. This effect has been evidenced in modern experimental studies on humans (hair and fingernails; Fogel et al. 1989; Fuller et al. 2006b) and controlled feeding experiments on domestic animals (dentinal collagen; Balasse et al. 2001), as well as studies of blood plasma and milk proteins in lactating females and nursing offspring of a range of wild species (e.g. Jenkins et al. 2001; Polischuk et al. 2001). In contrast, the application of oxygen ($\delta^{18}\text{O}$) isotope techniques to archaeological, anthropological and palaeoecological case studies have largely focused on reconstructions of palaeotemperature and palaeoclimate (e.g. Longinelli 1984; D'Angela and Longinelli 1990; Bryant et al. 1994; Sánchez Chillón et al. 1994; Delgado Huertas et al. 1995; Stuart-Williams and Schwarcz 1997; Genoni et al. 1998; Longinelli et al. 2003; Mannino et al. 2003; Hoppe et al. 2004; Hoppe 2006; Bernard et al. 2009) and also, through this, their use as a tool to determine geographical provenience and mobility in humans (e.g. White et al. 2000; Evans et al. 2006a,b; Eckardt et al. 2009; Chenery et al. 2010) and wild and domestic animals (e.g. Evans et al. 2007; Pellegrini et al. 2008; Britton et al. 2009). These approaches are all based on the premise that the oxygen isotope composition of tissues developed during life will be directly related to that of local drinking water, the latter being primarily controlled by local air

temperatures and, therefore, geographic setting (Dansgaard 1964; Darling and Talbot 2003).

A small number of studies have also employed oxygen isotope analysis to investigate breastfeeding practices and determine weaning age in past societies. These applications are based on the premise that – due to isotopic fractionation – the breast milk of a lactating mother will be isotopically-enriched in the heavier isotope (^{18}O) compared to the water she ingests, inducing elevated $\delta^{18}\text{O}$ values in infant tissues. Studies have targeted both the oxygen-bearing carbonate and phosphate components of tooth enamel (Wright and Schwarcz 1998, 1999) and, in a single study, the phosphate component of bone (White et al. 2004; $n=3$ infant bones). Generally considered to be less prone to diagenetic alteration than the oxygen-bearing carbonate fraction of the skeleton (Kolodny et al. 1983; Luz et al. 1984; Nelson et al. 1986; Kohn et al. 1999), analysis of oxygen isotope ratios in bone phosphate offers the potential for the reconstruction of breastfeeding and weaning practices where collagen is not preserved. Furthermore, analysis of bone phosphate in adult humans could permit the reconstruction of mean annual temperatures, as demonstrated in other mammalian species (Longinelli 1984; Luz et al. 1984; Ayliffe and Chivas 1990; Sánchez Chillón et al. 1994; Stuart-Williams and Schwarcz 1997). However, despite the strength of the P-O bonds within the PO_4 component, the recrystallization of the nano-crystalline bone bioapatite and isotope exchange with ambient pore fluids may render the material unsuitable for such studies due to chemical alteration in the burial environment (Tütken et al. 2008). Furthermore, recent studies have demonstrated that the composition of diet or even food culinary practices (such as stewing and brewing) can influence the $\delta^{18}\text{O}$ of ingested water, and therefore could have the potential to induce isotopic enrichment

or depletion in body water and, ultimately, in bodily tissues (Daux et al. 2008; Brettell et al. 2012).

Here we present the results of oxygen isotope analyses of bone and tooth phosphate from Medieval perinatal/neonatal, infant, juvenile and adult skeletons from the site of Wharram Percy, Yorkshire, UK (Mays 2007). The purpose of this study is to explore the application of oxygen phosphate studies of bioapatite to the investigation of breastfeeding and weaning in archaeological populations. This material offers the potential for methodological development as it allows the sampling of bone (and tooth) phosphate from a large number of individuals, including infants, sub-adults, and adults. Furthermore, studies utilising carbon and nitrogen isotope analysis of bone collagen to investigate weaning from the same materials have already been published (Mays et al. 2002; Richards et al. 2002; Fuller et al. 2003), allowing for direct data comparison.

THE APPLICATION OF OXYGEN ISOTOPES IN ARCHAEOLOGICAL AND ANTHROPOLOGICAL STUDIES

The ratios of ^{18}O to ^{16}O ($^{18}\text{O}/^{16}\text{O}$ or $\delta^{18}\text{O}$) in precipitation are altered by natural environmental process through fractionation occurring at various points in the hydrological cycle. The isotope composition of rainfall and other types of precipitation is depleted in the heavier isotope (^{18}O) compared to the ocean and other surface water. Evaporation from these water bodies serves to initially deplete the evaporating water, making it isotopically lighter than the source. Global and local meteoric processes then continue to bring about further depletions. These are the result of thermal and geographical parameters, related to local and regional climate, the biggest influence on the depletion of ^{18}O in precipitation being local temperature variations (Dansgaard 1964). Given the relationship between temperature and fractionation in precipitation,

there are also variations with altitude, latitude and season (Dansgaard 1964; Yurtsever 1975; Gat 1980; Clark and Fritz 1997).

Oxygen isotope studies of skeletal materials are based on the correlation between the oxygen isotope composition of animal tissues and that of water ingested during life (Longinelli 1965, 1966, 1984; Longinelli and Nuti 1968, 1973). As endothermic homeotherms, mammals have a metabolically-controlled, relatively constant body temperature ($\sim 37^{\circ}\text{C}$), and their bioapatite (a carbonated hydroxyapatite) precipitates in oxygen isotope equilibrium with body water (Longinelli 1984; Luz et al. 1984). Fractionation occurs between ingested water and body water, and this varies between different species. Fractionation factors have been established for a number of extant species, including humans (Luz et al. 1984; Levinson et al. 1987; Daux et al. 2008), and despite demonstrable inter-specific variations, the body water-drinking water relationship appears to be roughly linear. Therefore the $\delta^{18}\text{O}$ of bodily tissues directly reflects that of drinking water (Land et al. 1980; Longinelli 1984; Luz et al. 1984; Ayliffe et al. 1992; Bryant and Froelich 1995; Kohn 1996; Kohn et al. 1996), albeit with modifications due to admixture with respiratory oxygen and chemically-bound oxygen in ingested food, species-specific drinking strategies and metabolisms, and water turnover rates (e.g. Luz et al. 1984; Ayliffe and Chivas 1990; Ayliffe et al. 1992; Bryant and Froelich 1995; Kohn 1996; Levin et al. 2006; Podlesak et al. 2008).

Responses in body water and tissue values within an organism are not instantaneous. Where the daily turnover of total oxygen is 5-10%, a time-lag of 10-20 days can be predicted between body water and newly forming tissues (Kohn and Cerling 2002). The rate of body water turnover has been confirmed using tracers and through experimental studies using dietary switches (from depleted to enriched drinking waters; e.g. Sharp and Cerling 1998). Although there are intra- and inter-

specific variations, this period of time is universally fairly restricted, with full isotopic equilibrium in body water reached within two weeks, and that new equilibrium being reflected in newly-forming tissues – such as growing tooth enamel – in just over three weeks (Luz et al. 1984; Podlesak et al. 2008). This could be expected to vary between species, depending on their degree of water-dependence or drought-resistance – with more water dependent species having a shorter time-lag and recording isotopic input variations with a greater fidelity in co-forming tissues. However, the rate of turnover in tissues constantly undergoing remodeling (such as bone) is far slower. For example, the mean annual turnover rate for adult bone is between 5% and 25% (Martin et al. 1998); therefore, bone phosphate oxygen isotope values can be expected to represent averaged isotopic inputs spanning years. This turnover rate could be expected to be higher during infancy and childhood (a period rapid of skeletal growth), although perhaps less rapid than the synthesis of the organic matrix and therefore turnover of bone collagen (Tsutaya and Yoneda 2013: e72327[2]).

Where local drinking water sources largely originate from precipitation, the mean $\delta^{18}\text{O}$ of local drinking water should broadly reflect (and be characteristic of) local climatic conditions. Oxygen isotope ratios of modern drinking water and bodily tissues have been demonstrated to correlate with both the mean average and seasonally-varied geographic patterns in precipitation $\delta^{18}\text{O}$ (e.g. Ehleringer et al. 2008; Kennedy et al. 2011), leading to the application of oxygen isotope analysis to archaeological and fossil bone and teeth in order to reconstruct mean annual and seasonal palaeotemperatures (Koch et al. 1989; Bryant et al. 1994, 1996a; Fricke et al. 1998), and – through this – infer geographical provenance in humans (e.g. Evans et al. 2006a; Eckardt et al. 2009; Müldner et al. 2009; Chenery et al. 2010). Regression equations are required to convert bioapatite $\delta^{18}\text{O}$ values to predicted drinking water (i.e. environmental water) values

($\delta^{18}\text{O}_{\text{dw}}$), although there are errors associated with these. In a recent study, Pollard et al. determined that even a small margin of error (e.g. 95% confidence level) for predicted values $\delta^{18}\text{O}_{\text{dw}}$ is likely to be $\pm 1\text{‰}$ and may be as high as $\pm 3.5\text{‰}$ (Pollard et al. 2011). This is consistent with other research, for example, that by Daux et al (2008), who determined the 95% error range of predicted $\delta^{18}\text{O}_{\text{dw}}$ values (based on their calibration data) to be $\sim 1.5\text{‰}$ at best. While such predicted values can perhaps be cautiously determined in archaeological oxygen isotope studies, the use of direct measured values of $\delta^{18}\text{O}_{\text{p}}$ (in animals or humans) as a means of comparison (which are only subject to measurement error, typically $\pm 0.1\text{‰}$ to $\pm 0.4\text{‰}$) rather than the use of predicted values is increasingly being advocated (Müldner et al. 2011; Pollard et al. 2011). Furthermore, it should be noted that recent studies have also demonstrated that the composition of diet or even food preparation/culinary practices (such as stewing and brewing) can influence the $\delta^{18}\text{O}$ of drinking water, and therefore could have the potential to induce isotopic enrichment or depletion in body water and, ultimately, in bodily tissues (Daux et al. 2008; Brettell et al. 2012). These studies highlight some potential limitations for the usefulness of the oxygen isotope analysis of adult human remains in provenance/mobility or palaeoclimatic studies, although the prevalence of this issue in the study of the past and the archaeological implications have yet to be explored.

The application of oxygen isotope analyses to studies of breastfeeding and weaning relies on the principal that – due to isotopic fractionation – the breast milk of a lactating mother will be isotopically-enriched in the heavier isotope (^{18}O) compared to the water she ingests. This is because the oxygen in body water is enriched in ^{18}O relative to drinking water, largely due to the discrimination against ^{18}O during the expiration of water vapour (Bryant and Froelich 1995; Kohn et al. 1996; Wright and

Szwarcz 1998). Given that breast milk is formed from ^{18}O -enriched body water, it is isotopically heavier compared to the mother's drinking water. In a recent study comparing $\delta^{18}\text{O}$ in raw cows' milk to that of water from the same farms, milk $\delta^{18}\text{O}$ was on average ~4‰ elevated compared to water $\delta^{18}\text{O}$ (Lin et al. 2003: 2191). It is therefore anticipated that – given that breast milk would constitute the main source of ingested water for offspring prior to weaning – mineral tissues formed prior to weaning, either tooth enamel or bone bioapatite, will reflect this enrichment and exhibit elevated $\delta^{18}\text{O}$ values until after the complete cessation of breastfeeding. When weaning occurs, the oxygen isotope values in successively forming tissues (new enamel formation, or bone growth/remodelling) should decrease. Past studies have suggested $\delta^{18}\text{O}$ in archaeological teeth can be related to breastfeeding and weaning, with early-forming teeth demonstrating enrichment of between 0.5‰ and 1.2‰ in enamel carbonate and/or phosphate compared to adult averages (Wright and Szwarcz 1998, 1999). Another prior study also included a limited number of infant bones from Nubian mummies (<3 yrs; $n=3$) suggested more extensive ^{18}O -enrichment (+~2‰, compared to the adult average in the same study; White et al. 2004). However, metabolic differences associated with ill health, extensive intra-annual climatic variability, birth seasonality, and immigration could all potentially influence oxygen isotope values in infants and may limit the application of these techniques to archaeological case studies (White et al. 2004).

The purpose of this study was to further explore the potential application of oxygen phosphate studies of bone bioapatite to the investigation of breastfeeding and weaning in archaeological populations, through the analyses of bone (and a limited number of teeth) from perinatal/neonatal, infant, juvenile and adult skeletons from the Medieval site of Wharram Percy. This large and well-studied assemblage represents a

temporally and geographically-definable group – the inhabitants of the village and nearby parishes. We hypothesise that – provided local, ‘culturally-unmodified’ water was consumed by adult females in Medieval Wharram Percy, and bone phosphate is not subject to diagenetic alteration in this instance (see ‘Materials’) – bone phosphate oxygen ratios should be elevated in infants who died prior to the cessation of breastfeeding and lower in older infants who died post-weaning. Furthermore, with the same provisos, the older juvenile and adult bone phosphate at Medieval Wharram Percy should reflect local predicted environmental oxygen isotope values.

MATERIALS

Wharram Percy, United Kingdom

Wharram Percy is a deserted Medieval village in North Yorkshire in the North-East of England, close to the modern city of York (Figure 1). The site has been the subject of detailed investigation in recent decades, including long-running archaeological excavation (Beresford and Hurst 1990; Mays et al. 2007) and a number of associated research projects (e.g. Sofaer Derevenski 2000; Mays et al. 2002; Richards et al. 2002; Fuller et al. 2003). The human skeletal assemblage from Wharram Percy is extensive, with 688 articulated skeletons (including 328 infants, juveniles and sub-adults) excavated from St Martin’s church and churchyard. This large assemblage dates from the 10th to 19th centuries AD, with the bulk (including those utilised in this study) dating from the 11th-14th century AD (Mays et al., 2007). The skeletal remains represent the population of the village and local rural parish, a chronologically- and geographically-defined group.

Bone and tooth samples

Rib bone was selected from 63 individuals, including perinates/neonates and infants (<3.5 yrs; $n=32$); children (4-12 yrs; $n=12$); unsexed juveniles (16-18 yrs; $n=2$) and adult bones ($n=17$; females $n=10$, males $n=7$). Each of the human bones involved in this study has previously been the subject of bone collagen isotope studies, demonstrating good preservation of collagen (Mays et al. 2002; Richards et al. 2002; Fuller et al. 2003). In addition to the organic component, primarily collagen, bone is principally comprised of inorganic material (~70% by dry weight), largely a composite of calcium phosphate minerals. The mineral fraction of bone bears close similarities to naturally-occurring hydroxyapatite but, due to a lack of stoichiometry, small crystal size, structural disorganisation and a high incidence of substitutions in the apatite crystal lattice, it is often referred to as biological hydroxyapatite, or 'bioapatite'. Substitutions commonly occur at the phosphate (PO_4) and hydroxyl (OH) sites, often with carbonate (CO_3), therefore resulting in a range of potential oxygen-bearing sites in bioapatite (Martin et al. 1998).

Despite the relative ease with which oxygen can be extracted and analysed from the carbonate component, phosphate is normally preferentially selected for oxygen isotope analysis, due to the strong correlation between phosphate oxygen and the oxygen isotope composition of ingested water (Kolodny et al. 1983; Luz et al. 1984), and the strength of the P-O bonds within the PO_4 component of bioapatite, resulting in a greater resistance to diagenetic alteration than carbonate components from the same samples (Kolodny et al. 1983; Luz et al. 1984; Kohn et al. 1999). It has also been demonstrated that, although carbonate components are often diagenetically-altered through the more frequently-occurring chemical contamination in the burial environment, it is the less common biological and enzymatic attack that is more likely to affect the phosphate component (Zazzo et al. 2004a). Although currently there are

no comprehensive chemical pre-treatments to remove the effects of diagenesis or quality indicators to identify diagenetically-altered samples (akin to those for bone collagen, e.g. Ambrose 1990; van Klinken 1999), there are a range of techniques that are commonly used for evaluating diagenesis in ancient bones. Although scanning electron microscopy of selected material from Wharram Percy has previously suggested preservation of histological detail is poor (Nielsen-Marsh and Hedges 2000; Turner-Walker and Syversen 2002), which can be related to the activities of soil-dwelling microorganisms, this does not appear to have influenced mineral integrity of bone at Wharram Percy (measured as Ca: P ratios), which resemble that of modern bone (Mays 2003: 733-734).

In addition to bone, enamel was also analysed from a limited number of the individuals included in this study, comprising of deciduous second molars from three children, and permanent canines and third molars from three adults. The crowns of deciduous second molars begin forming at about 6 months *in utero* and are complete by the age of about 11 months post-natal; permanent canine tooth enamel begins mineralising around the age of 4-5 months, completing at around the age of 6 years (Gustafson and Koch 1974). The third molar crowns form between the ages of about 9 and 14 years (Garn et al. 1962; Anderson et al. 1976; Levesque et al. 1981). Ages and sexes were determined for a previous study on the same materials, and details of the methodologies used can be found in Richards et al. (2002: 206). It should be noted that, although crown formation times can provide brackets for the period of childhood represented by isotopic values obtained from specific teeth, the timing and periodicity of mineralisation (i.e. when the majority of oxygen in the tooth enamel is incorporated) are poorly understood. Although enamel formation may take a number of years in specific teeth, mineralisation may occur over a more restricted time within that

framework, and therefore isotopic data from enamel could represent isotopic contributions from a considerably shorter period (Montgomery 2002: 53).

Establishing local isotope ranges

In order to assess any nursing or weaning signal in the infant bones and teeth, it is first necessary to determine the likely oxygen isotope values of local waters. The oxygen isotope composition of modern surface water and groundwater has been well characterised for the UK by previous studies, and contour maps have been produced (e.g. Darling et al. 2003; Darling and Talbot 2003). These modern values can be applied to archaeological case studies as groundwater reflects averaged rainwater inputs over periods of tens and hundreds of years, and it has been suggested that (despite small-scale climatic variations) there has been little variation in ground and surface water values throughout the Holocene (Darling et al. 2003: 191-192). Groundwater in Britain has a measured range of around 4‰, from -9.0‰ and -4.5‰, with the least negative values being exhibited in areas of the extreme west (e.g. the Western Isles of Scotland). The area of North Yorkshire surrounding Wharram Percy is situated within the -8.0‰ to -7.5‰ contour for UK groundwater (Darling et al. 2003), but close to the <-8.0‰ region – a small area proximal to the site. Recently measured tapwater $\delta^{18}\text{O}$ compositions in the region surrounding the site include values of -8.1‰ and -8.3‰ (Darling et al. 2003: 193). Therefore, the total range of mean annual local water values in this area, and the surrounding parts of Britain, can be conservatively considered to be between -8.5‰ and -7.5‰.

Various regression equations have been proposed to convert skeletal phosphate values ($\delta^{18}\text{O}_p$) to approximate drinking water values ($\delta^{18}\text{O}_{dw}$) and vice-versa (Longinelli 1984; Luz et al. 1984; Levinson et al. 1987; Daux et al. 2008). Such

equations are required given that isotopic fractionation occurs in the body as water is metabolised, which enriches the bioapatite in the heavier isotope (^{18}O) relative to the drinking water. Using the likely total range of average local water values for this area (-8.5‰ to -7.5‰, see above) and on the recommendations of Daux et al. (2008: 1143, equation 6), local and regional bioapatite ($\delta^{18}\text{O}_p$) values for humans drinking local water might be expected to lie between 16.4‰ ($\delta^{18}\text{O}_{\text{dw}}=-8.5\text{‰}$) and 17.0‰ ($\delta^{18}\text{O}_{\text{dw}}=-7.5\text{‰}$). However, as discussed above, error ranges associated with such conversion equations are high (in this instance, $\pm\sim 2\text{‰}$), and can therefore encompass all predicted British variability (Pollard et al. 2011). Directly measured values of $\delta^{18}\text{O}_p$ from previous studies (local humans or animals) can provide a useful means for comparison and aid in the estimation of local bioapatite values (Müldner et al. 2011; Pollard et al. 2011). Previously measured archaeological enamel ($\delta^{18}\text{O}_p$) of ‘local’ individuals from the site of West Heslerton, North Yorkshire, which lies less than 10 miles north-west of Wharram Percy determined a mean value of $17.1\pm 0.4\text{‰}$ (2σ) (Budd et al. 2004, recalculated in Chenery et al. 2010: 161) are in line with the range calculated above. These values, and the predicted values, also correspond well with the broader bioapatite $\delta^{18}\text{O}_p$ British ‘baseline’ suggested in Chenery et al. (2010) of 16.8‰ to 18.6‰ (mean= $17.7\text{‰}\pm 0.9$ [2σ]), who calculated this range from previously-published data on archaeological humans with ‘local’ $^{87}\text{Sr}/^{86}\text{Sr}$ isotope values from West Heslerton and 8 other UK sites (Chenery et al. 2010: 153, 161). The same mean value of $17.7\text{‰}\pm 1.4\text{‰}$ (2σ ; $n=615$) was recently reported in Evans et al. (2012), with the corollary that further sub-population control groups, eastern and western Britain, can be characterised by means of $17.2\text{‰}\pm 1.3\text{‰}$ (2σ ; $n=83$) and $18.2\text{‰}\pm 1.0\text{‰}$ (2σ ; $n=40$) respectively, due to differences in rainfall levels. It should be noted however, that Chenery et al. utilised a different conversion equation from that used here, favouring that of Levinson et al.

(1987) and including a 1.4‰ ‘method bias correction’ due to the difference between mean reported values of the now commercially-unavailable standard material NBS120b in Levinson’s study and that of those determined at NIGL (Chenery et al. 2010: 160). Although justified in this instance, given that new studies are unlikely to incorporate this exhausted standard, such method bias corrections (which could be expected to vary between different laboratories and with different precipitation techniques) would be difficult to determine. However, as Chenery et al. note, the $\delta^{18}\text{O}_{\text{dw}}$ values calculated using the Levinson et al. (1987) equation (including corrections) are very similar to the values computed using the equations published by Daux et al. 2008 (Chenery et al. 2010: 160 and Figure A.1). Furthermore, Daux et al. (2008) report values for commercially-available standard material NBS120c, allowing any method bias to be corrected, where necessary, through the preparation of this standard material alongside archaeological materials. In light of the above, and the agreement between the predicted $\delta^{18}\text{O}_{\text{p}}$ ‘local’ values calculated here and those values $\delta^{18}\text{O}_{\text{p}}$ values measured in bioapatite from proximal sites, we advocate the use of Daux et al.’s equation (2008: 1143, equation 6). Therefore, encompassing both previously-measured values of individuals with local $^{87}\text{Sr}/^{86}\text{Sr}$ values from the Anglo-Saxon site of West Heslerton ($17.1 \pm 0.4\text{‰}$) and the predicted local bioapatite values calculated here (16.4-17.0‰), local and regional bioapatite ($\delta^{18}\text{O}_{\text{p}}$) values for humans drinking local water at Wharram Percy might be expected to lie between 16.4‰ and 17.5‰.

METHODS

Tooth and bone samples were prepared for isotopic analysis in the Archaeological Chemistry Laboratories, Max Planck Institute for Evolutionary Anthropology (Department of Human Evolution). Rib bones were sampled using a

diamond-coated circular drill bit and split; and interior and exterior surfaces were cleaned prior to grinding using air abrasion, including the removal of cancellous bone. Individual samples were ground and homogenized using an agate pestle and mortar. Bone samples were then prepared for oxygen isotope analysis following methods described in Tütken et al. (2006) modified after O'Neil et al. (1994) and Dettman et al. (2001: Appendix, GSA Data Repository item 20018). Due to the high organic content of bone (including archaeological bone), it was necessary to pre-treat samples to remove organics (including oxygen-bearing organic components). In order to remove organics, samples were agitated in 30% hydrogen peroxide (H_2O_2 ; $40\mu\text{l}/1\text{mg}$ of bone powder) at room temperature for 48 hours in microcentrifuge tubes sealed with Parafilm[®] M and pierced in order to prevent gas accumulation. Powdered bone samples were rinsed in (4x) Milli-Q (ultrapure) water and dried (24hrs, 50°C) prior to dissolving in 2M HF.

Dried samples ($\sim 10\text{mg}$) were dissolved in 0.8ml 2M HF and agitated in solution for 24 hours at room temperature. The resultant phosphate solution and the residue (comprised of CaF_2) were separated by centrifugation, and the phosphate solution was pipetted into fresh microcentrifuge tubes that had been previously weighed. In each instance, the CaF_2 residue was washed with $\sim 0.1\text{ml}$ of Milli-Q (ultrapure) water after the phosphate solution had been pipetted off, these were then centrifuged with the additional solution being added to the phosphate solution to maximise recovery of soluble components. A single drop of indicator (Bromothymol Blue, or BTB), was added to each sample (yellow: acidic solution). In order to neutralise each sample, 25% ammonia solution (NH_4OH) was added using a Microliter[™] fixed needle syringe (Hamilton Bonaduz AG, Switzerland) drop for drop until a colour change (yellow to green) was observed ($\sim 180\mu\text{l}$). Then 0.8ml of 2M silver nitrate (AgNO_3) solution was

added to each sample, forming a yellow silver phosphate (Ag_3PO_4) precipitate. The precipitate was separated by centrifugation, and the liquid fraction was discarded. The precipitate was rinsed 4x with Milli-Q (ultrapure) water, dried (24hrs, 50°C) and weighed into silver capsules for oxygen isotope analysis. Un(pre)treated samples of NBS120c were also prepared alongside the samples. NBS120c, although not certified for oxygen isotope values, is an international standard material commonly used in many laboratories as a reference material during phosphate oxygen isotope analysis as its composition and matrix is more similar to bio-phosphates than other reference materials (Chenery et al. 2010: 159), and has a broadly accepted value of $\sim 21.7\text{‰}$ (Chenery et al. 2010 and references therein)

Phosphate $\delta^{18}\text{O}$ values were determined by CF-IRMS, measured with a Thermo-Quest TC-EA connected to a Thermo-Quest Delta Plus XL mass spectrometer, at the Department of Geology, University of Tübingen. Mean values and standard deviations (1σ) were provided by the analysing laboratory, calculated from the analysis of each sample in triplicate (Table 1 and Table 2). In some instances, these values were provided from duplicate measurements, due to sample size, sample loss, loss of sample integrity or through internal data quality control checks in Tübingen (for exceptions, see Table 1 and Table 2). Long-term laboratory reproducibility was reported as $\pm 0.3\text{‰}$ (1σ). Samples were calibrated to $\delta^{18}\text{O}$ values relative to V-SMOW using internal standards, including TU-1 ($\delta^{18}\text{O}=21.1\text{‰}$); TU-2 ($\delta^{18}\text{O}=5.4\text{‰}$) and 130-0.5-1 ($\delta^{18}\text{O}= -1.1\text{‰}$) (Vennemann et al. 2002). Repeat analysis of prepared samples of NBS120c ($n=5$) gave a mean $\delta^{18}\text{O}_p$ value of $21.6\pm 0.4\text{‰}$ (1σ), which is the same as previously reported values (e.g. Tütken et al. 2006) and within error of the mean reported values from 19 previous publications (cited in Chenery et al. 2010: 161, Table A3; $21.7\pm 0.5\text{‰}[1\sigma]$) and a weighted mean value reported in a recent inter-laboratory

calibration ($21.79 \pm 0.15\text{‰}$ [1σ]; Halas et al. 2011: 582). Therefore, we deem it not necessary to apply a method correction bias to our data (see ‘Establishing local isotope ranges’).

RESULTS AND DATA INTERPRETATION

Bone oxygen isotope data analysed from Wharram Percy are shown in Table 1, and $\delta^{18}\text{O}_p$ values from tooth enamel phosphate are shown in Table 2. The total range of bone $\delta^{18}\text{O}_p$ values exhibited varies from 15.1‰ to 19.7‰ (total range: $\Delta 4.6\text{‰}$), with the highest value observed in an infant (individual WCO104 [s-eva-16808]) and the lowest in an adult female (individual G760 [s-eva-16852]). The mean $\delta^{18}\text{O}_p$ value for the infants (<3.5 yrs; $n=32$) is $17.7 \pm 0.8\text{‰}$ (1σ), the mean for children aged 4 to 12 yrs ($n=12$) is $16.9 \pm 0.4\text{‰}$ (1σ) and the mean value for all adults (males and females, >18 yrs, $n=17$) is $16.5 \pm 0.6\text{‰}$ (1σ) and therefore $\sim 1\text{‰}$ lower than the mean infant $\delta^{18}\text{O}_p$ value. Tooth enamel phosphate values range from 16.7‰ to 18.5‰ , with the mean value for infant deciduous second molars being 18.2‰ ($\pm 0.2\text{‰}$, 1σ), mean values for permanent canines and third molars being 17.3‰ ($\pm 0.6\text{‰}$, 1σ) and 17.2‰ ($\pm 0.3\text{‰}$, 1σ), respectively.

Comparison of $\delta^{18}\text{O}$ (bone and enamel phosphate) to $\delta^{15}\text{N}$ (bone collagen) in infants and juveniles (<12 years)

Figure 2 shows the infant and juvenile (<12 yrs) bulk bone phosphate $\delta^{18}\text{O}$ data (primary y-axis) plotted against age (years; x-axis), with $\delta^{15}\text{N}$ data obtained from bone collagen from the same individuals plotted on a secondary y-axis ($\delta^{15}\text{N}$ bone collagen data from Richards et al. 2002). The range of estimated local water values (see above), converted to predicted bioapatite values for local humans, are also depicted, along with

previously-measured 'local' bioapatite values. The most elevated $\delta^{18}\text{O}_p$ values are observed in some of the youngest individuals, and there is a non-linear correlation between $\delta^{18}\text{O}_p$ and age ($r^2=0.32$, polynomial regression). Notably, the perinates/neonates (G492, NA220, G470 and G579; aged 41-46 weeks *in utero*), exhibit a mix of values, two within the normal adult range and two with bone $\delta^{18}\text{O}_p$ values slightly higher than the mean adult range. While it might be anticipated that individuals *in utero* should exhibit values in the normal adult range, with the foetus forming in equilibrium with the mother's blood values, this may evidence the influence of seasonal or other compositional changes in the $\delta^{18}\text{O}$ of drinking water (and therefore, the mother's body water $\delta^{18}\text{O}$).

In order to explore differences in mean $\delta^{18}\text{O}$ values between individuals under the age of three (excluding the four perinates/neonates) and older children, a *t*-test was used, confirming that these differences are statistically significant ($t=4.87$, $p<0.01$). After the age of approximately 3 years, $\delta^{18}\text{O}_p$ values are lower, within the expected regional range and comparable to the adult mean value. The correlation between higher $\delta^{15}\text{N}$ bone collagen values and age also shown in Figure 2 ($r^2=0.57$) has been documented previously at Wharram Percy (Mays et al. 2002; Richards et al. 2002) and elsewhere (Fogel et al. 1989; Jay et al. 2008), and has been attributed to ^{15}N enrichment in infancy during the period of breastfeeding. Enrichment in ^{18}O in tooth enamel (carbonate) formed in childhood has also been attributed to breastfeeding (Wright and Schwarcz 1998, 1999). The limited number of deciduous teeth sampled from Medieval individuals at Wharram Percy in this study demonstrate similar enrichment (Figure 3), and are ^{18}O -enriched compared to teeth formed later in childhood (canines and third molars from different individuals, Figure 3) and adult bone phosphate (Figures 3-4). The higher values observed here in infant bone ($\sim+1.2\%$, compared to adult bone

values) are consistent with previous studies on tooth enamel (Wright and Schwarcz 1998, 1999; White et al. 2004) and a single limited study on infant bone phosphate (White et al. 2004; $n=3$). We propose that the higher values observed in infant bone phosphate (compared to Medieval adult values, and predicted 'local' values) from Wharram Percy, along with the deciduous molars, presented in this study are also attributable to the consumption of breast milk. The timing in the decrease in $\delta^{18}\text{O}_p$ values of bone phosphate and that of $\delta^{15}\text{N}$ values of bone collagen measured in the same materials are comparable but the period in which $\delta^{18}\text{O}_p$ values decrease in all individuals is a little more extended. After the age of ~2 years, all bone collagen $\delta^{15}\text{N}$ values of children are within the adult range (Fig. 2; Mays et al. 2002; Richards et al. 2002), which appears to be in agreement with limited Medieval documentary evidence of weaning practices (Fildes 1986: 45-58, 66). However, although elevated $\delta^{18}\text{O}_p$ values peak in early infancy (between 4 and 12 months), between the ages of 2 and 3 years there are still a small number of individuals with $\delta^{18}\text{O}_p$ values higher than the adult range. These differences may be consistent with a slower rate of turnover in the mineral phase due to the synthesis of organic matrix being faster than the mineralization process during ontogeny (Tsutaya and Yoneda 2013: e72327[2]), metabolic/physiological factors (which may influence $\delta^{15}\text{N}_{\text{coll}}$ values in infants, see review in Beaumont et al. 2013), or the fact that these two isotope ratios reflect different inputs (primarily protein source in the case of $\delta^{15}\text{N}_{\text{coll}}$ and ingested water in the case of $\delta^{18}\text{O}_p$) and are not interdependent. Figure 5 shows bulk bone phosphate oxygen isotope values ($\delta^{18}\text{O}_p$) plotted with bulk bone collagen nitrogen isotope values ($\delta^{15}\text{N}$) from the same individuals in three groups (infants <3.5 yrs, children 4-12 yrs and adults 18+ yrs). Although both $\delta^{18}\text{O}_p$ and $\delta^{15}\text{N}_{\text{coll}}$ are, on average, elevated in the infants compared to the other individuals, the correlation coefficient between $\delta^{18}\text{O}_p$ and $\delta^{15}\text{N}_{\text{coll}}$ is low

($r^2=0.02$). This value is far lower in the children over four years of age ($r^2<0.01$) and negligible in adults ($r^2<0.0001$), suggesting no significant correlation between $\delta^{18}\text{O}_p$ and $\delta^{15}\text{N}_{\text{coll}}$ values in early or later life. This lack of agreement between elevated $\delta^{15}\text{N}_{\text{coll}}$ and $\delta^{18}\text{O}_p$ values is also exemplified by some individual cases, for example, infant G327 (s-eva-16824; 1.5 yrs) in which one of the highest $\delta^{15}\text{N}_{\text{coll}}$ values in this study was determined (12.3‰) while the $\delta^{18}\text{O}_p$ value was similar to the adult mean (16.6‰). This serves to reiterate that, despite an apparent *general* shared trend in elevated $\delta^{18}\text{O}_p$ and $\delta^{15}\text{N}_{\text{coll}}$ in early infancy (pre-weaning), the two isotope ratios measured are independent systems, reflecting different inputs – in infancy as well as in adulthood. Furthermore, as the values of some perinates/neonates demonstrate, birth timing and timing of death (in terms of season) could reasonably influence $\delta^{18}\text{O}_p$ in perinates/neonates and young infants undergoing rapid growth/tissue mineralization.

Bone phosphate $\delta^{18}\text{O}$ in Medieval adults

As shown in Figure 4, after the age of approximately 3 years, $\delta^{18}\text{O}_p$ values in both children and adults from Medieval Wharram Percy are similar ($16.9\pm 0.4\text{‰}[1\sigma]$, and $16.5\pm 0.6\text{‰}[1\sigma]$, respectively). These values correspond well with both the predicted ‘local’ bioapatite range of 16.4‰ to 17.0‰ (based on contours in Darling et al. 2003; and Daux et al. 2008: 1143, equation 6), and values from the proximal early Medieval site of West Heslerton ($17.1\pm 0.4\text{‰}[2\sigma]$), published in Budd et al. (2004), recalculated by Chenery et al. (2010: 161). Slight differences are observable in the adult male and female mean bone $\delta^{18}\text{O}_p$ values ($16.8\pm 0.5\text{‰}[n=7]$ and $16.3\pm 0.6\text{‰}[n=10]$, respectively). It is also notable that two females (G760 and G635) have bone $\delta^{18}\text{O}_p$ values less than 16‰, which is unusual for the UK. These differences could be due to slight variations in the treatment/type of dietary water, lifetime mobility or

metabolic/physiological differences. However, given the small sample size, and a lack of statistical significance (*t*-test assuming equal variance; $t=1.78$, $p=0.1$), care should be taken not to over-interpret any inter-sex difference. In light of errors associated with conversion equations (used to predict 'local' $\delta^{18}\text{O}_p$; see above and Pollard et al. 2011), similar caution should be applied to the interpretation of data from marginal outliers.

In the case of Medieval Wharram Percy, after early childhood enrichment (likely due to a residual breastfeeding signal in the bone, see above), human bone phosphate $\delta^{18}\text{O}$ values generally correlate well with both values predicted from modern local precipitation, and also with values measured in 'local' enamel bioapatite. A notable exception is individual NA185 (s-eva-17034), an unsexed individual of around 16.5 years. This high value of 19.6‰ is more than 3‰ above the mean adult value at the site ($16.5 \pm 0.6\text{‰}[1\sigma]$), and beyond both the expected local range and the predicted range of British values (as defined in Chenery et al. 2010; Evans et al. 2012). Although visual assessment did not suggest the sample should be excluded from analysis due to bacterial action and collagen yielded from the same sample met quality indicators (suggesting good preservation), post-mortem chemical (diagenetic) alteration of the sample could account for this high value. Alternatively, this individual may be a recent migrant to the area from a region with ^{18}O -enriched water values, where bone phosphate has not yet adjusted to the local values. A further explanation could be that this particular individual's drinking water was consistently influenced by some particular culinary practice prior to ingestion. Recently, new experimental data have demonstrated that culturally-mediated behaviours – such as boiling, stewing and fermentation – can influence the isotopic composition of ingested fluids and thus potentially serve to influence body water (and, consequently, body tissue) values, causing them to deviate from local expected drinking water values (Brettell et al. 2012).

Experimentally stewed, boiled and brewed liquids have all demonstrated a resultant enrichment in the heavier isotope, to varying degrees (Brettell et al. 2012). Where such products contributed to, or even dominated fluids ingested, a subsequent enrichment should be anticipated in co-forming tooth enamel or bone bioapatite. Other fermented beverages, such as wine could induce a similar enrichment, along with ruminant milk (sheep/goats and cows) which is also ^{18}O -enriched relative to local water by $\sim 2\text{-}4\%$ (e.g. D'Angela and Longinelli 1990; Fricke and O'Neil 1996; Lin et al. 2003; Camin et al. 2008). However, bone collagen $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values from this individual (8.4% and -19.6% , respectively) fit well with adult mean values at Wharram Percy ($\delta^{15}\text{N}=8.6\pm 1.3\%$, $\delta^{13}\text{C}=-19.7\pm 0.4\%$), and do not suggest a dietary difference (at least in terms of source of dietary protein). Further study of this individual, for example utilising oxygen and strontium isotope analyses of tooth enamel or sulphur isotope analysis of bone collagen (both useful in identifying potential immigrants) may help to further illuminate the migration history of this individual.

DISCUSSION

The results of this study strongly suggest that the analysis of oxygen isotope ratios in bone phosphate can be used as an indicator of approximate weaning age in archaeological populations, and are consistent with evidence from limited previously-published studies (i.e. White et al. 2004). Unlike the nitrogen isotope analysis of bone collagen, this approach does not rely on the preservation of proteins and could potentially be applied where collagen cannot be yielded from archaeological bones (e.g. at older sites, or sites in warmer regions where collagen is not preserved). Although, we propose the lower $\delta^{18}\text{O}_p$ values observed from the age of approximately 2-3 years in the infants of Medieval Wharram Percy does ultimately represent the cessation of

breastfeeding (suggesting weaning commenced before this point), there is some discrepancy between the bone $\delta^{18}\text{O}_p$ values and bone $\delta^{15}\text{N}_{\text{coll}}$ data. A (predicted) slower rate of turnover in the mineral phase, due to the synthesis of the organic matrix being faster than the mineralization process during ontogeny (Tsutaya and Yoneda 2013: e72327[2]), could account for this disparity. Beyond this, however, although both elevated $\delta^{15}\text{N}_{\text{coll}}$ and $\delta^{18}\text{O}_p$ values are found in younger individuals (associated with age) and are both likely associated with weaning not yet being complete, it must be noted that the mechanisms that induce elevated values in these two systems are different (reflecting primary protein source, and primary water source respectively) and are not mutually dependant. Nitrogen isotope values of bone collagen reflect the source of dietary protein, so could vary with the introduction of new weaning foods, for example, while breastfeeding may still be ongoing (inducing lower $\delta^{15}\text{N}$ values, but still elevated $\delta^{18}\text{O}_p$ values). Inter-individual variation in when (and what type) of complementary foods were introduced, along with when breastfeeding finally ceased to be the primary source of fluids, could compound any discrepancies in $\delta^{15}\text{N}_{\text{coll}}$ and $\delta^{18}\text{O}_p$ values due to differences in bone collagen/bone mineral tissue formation times. Patterns of variation in lactation and complementary feeding prior to weaning are well-documented in modern human groups, both between and within different natural fertility/non-industrialised populations (e.g. Sellen 2001; Kennedy 2005). Some Medieval literary sources hint at variation in practices, for example, recommending that boys be weaned from the breast 6-12 months later than girls (Fildes 1986: 45-58, 66). The continuation of breastfeeding may or may not preclude the consumption of other foods at any point after the first few months of life, and this could also account for such within group variation. The nature and length of the weaning process itself, therefore, must be considered as this could induce a more gradual or more rapid isotopic shift in either or

both systems, as could the nature of the peri- and post-weaning diet itself. There is evidence to suggest, for example, that the consumption of ruminant milk was recommended for the young in the Medieval period (Brettell et al. 2012: 782 and references therein), which is also ^{18}O -enriched relative to local water (Lin et al. 2003) and could induce elevated $\delta^{18}\text{O}_p$ values, particularly if heated (Brettell et al. 2012: 782). Often consumed with plant-based foods, such as bread or cereals (for example, as pap; Brettell et al. 2012: 782), this could be matched with a subsequent decrease in bone collagen $\delta^{15}\text{N}$. Finally, regarding $\delta^{15}\text{N}$, factors other than diet have been demonstrated to influence tissue $\delta^{15}\text{N}$ values, such as physiological or nutritional stresses, which can effect protein metabolism and nitrogen elimination, and therefore influence the $\delta^{15}\text{N}$ values of developing proteins in growing infants in the months before death (Fuller et al. 2004, 2005; Hauber et al. 2005; Huelsemann et al. 2009; Mekota et al. 2006). While the results of this study demonstrate an apparent shared trend in elevated $\delta^{18}\text{O}_p$ and $\delta^{15}\text{N}_{\text{coll}}$ in early infancy (pre-weaning), factors that dictate the $\delta^{15}\text{N}$ values of growing tissues are entirely different systems from those that result in ^{18}O -enrichment in co-forming bioapatites.

Similarly, although bone $\delta^{18}\text{O}_p$ values represent averaged long-term inputs, given that body water $\delta^{18}\text{O}$ (and therefore breast milk $\delta^{18}\text{O}$) could reasonably be influenced by seasonal changes in the $\delta^{18}\text{O}$ of drinking water, birth timing and time of death could influence $\delta^{18}\text{O}$ in perinates/neonates and young infants undergoing rapid growth/tissue mineralization. The range of bone $\delta^{18}\text{O}_p$ values in the perinates/neonates may be due to such a seasonal influence, which may also account for the range of values observed in the infants, and the slightly higher standard deviation in this group ($\pm 0.8\text{‰}$) compared to the older children ($\pm 0.4\text{‰}$) and adults ($\pm 0.6\text{‰}$). Although an adult's bone $\delta^{18}\text{O}_p$ represents years of averaged input, a mother's blood during pregnancy and *post-*

partum (during lactation) could be liable to change seasonally, depending on geographical location and water source, influencing the body water $\delta^{18}\text{O}$ of prenatal or neonatal offspring.

While the ‘bulk’ sampling of bone provides a longer-term averaged isotopic value, serial-sampling of teeth is being increasingly employed to reconstruct more detailed time-series or seasonal trends in isotopic inputs in archaeological humans and animals. Although not undertaken as part of this study, such an approach to human tooth enamel (in combination with phosphate oxygen isotope analysis) has the potential to provide a higher resolution record of weaning age in archaeological individuals. Analysis of a small number of deciduous and adult teeth from selected individuals confirm that high $\delta^{18}\text{O}$ values in early childhood is likely due to infants consuming ^{18}O -enriched breast milk, as previously suggested and observed in other studies on tooth enamel carbonate (e.g. Wright and Schwarcz 1998, 1999). However, given the relatively small difference between mean infant (<3.5 yrs) and adult bone phosphate values at Medieval Wharram Percy (~1.2‰), and the fact that meteoric water at any given location varies seasonally (and pronouncedly, especially at mid- to high-latitude regions), it seems likely that – unless a water source is isotopically-constant across all seasons – natural seasonal variation in a particular region may serve to obscure the more subtle differences in enamel $\delta^{18}\text{O}_\text{p}$ due to weaning. Few sequential oxygen isotope studies have been undertaken on human teeth to date, compared to herbivores for example, and more studies would be required to better understand how sequentially-sampled human enamel reflects seasonal ‘baseline’ differences in water before these methods could be employed with greater confidence to identify weaning age in archaeological teeth. For example, the sequential-sampling and oxygen isotopic analysis of tooth enamel from multiple successively-forming teeth (e.g. whole tooth

rows) from single individuals, along with bone, might help to illuminate this. Co-analysis of $\delta^{18}\text{O}$, both sequential and 'bulk', in herbivore tooth enamel (from obligate drinkers, such as horse or cattle) from the same site in such a study could also serve to provide a seasonal and local 'baseline' and highlight any anthropogenic modifications of drinking water.

However, one clear constraint associated with the $\delta^{18}\text{O}$ analysis of human tooth enamel that must be highlighted is the lack of human birth seasonality. Whereas birth season (which of course influences the trend of peaks and troughs in intra-tooth $\delta^{18}\text{O}$ patterns) can be reasonably predicted in wild and domestic herbivores (aside from in instances of certain animal husbandry practices, e.g. Balasse et al. 2003; Balasse et al. 2012a, 2012b; Blaise and Balasse 2011), this is not the case with humans. Furthermore, the periodicity, directionality and structural geometry of human crown mineralisation is currently poorly understood and likely presents additional challenges to the gaining of time-series isotopic information compared to hypsodont teeth. These uncertainties also have implications for enamel sampling procedures in mobility/palaeoclimatic studies using oxygen isotope analysis, in both humans and other species. Unless the periodicity of tooth mineralisation can be reasonably estimated, and the amount of enamel sampled is representative of a sufficiently long (i.e. annual) period of growth (and thus isotopic inputs), a 'seasonal bias' (with consequently low or high $\delta^{18}\text{O}$) could lead to the false identification of lifetime mobility or a lack thereof. Similarly, in palaeoclimatic studies, such an approach would result in falsely low or high 'mean annual' temperature reconstructions and/or a high amount of inter-sample variability. The slight differences in the $\delta^{18}\text{O}$ of canines and third permanent molars from the same individuals observed in this study (as well as inter-individual differences in the same teeth, see Figure 3 and Table 2) are likely the product of such seasonal or inter-annual

differences in isotopic inputs, 'fixed' in tissues forming over a restricted period which do not undergo modification (i.e. enamel). Further studies, exploring inter-individual differences, as well as intra-tooth differences in the same individual, will provide useful additional data and help to define the parameters/constraints for sampling human tooth enamel for weaning, mobility or palaeoclimatic studies.

In addition to suggesting the use of $\delta^{18}\text{O}$ in bone phosphate to reconstruct breastfeeding and weaning habits in archaeological populations, the data from adults at Wharram Percy also demonstrate the potential for the method to be used as a palaeoclimatic proxy indicator, as adult bone phosphate values at the site largely correlate with those predicted from local patterns of precipitation and those suggested from other regional studies. However, although most of the adult Medieval samples from Wharram Percy are consistent with being local, the single adolescent outlier does highlight an important issue. Given that recent studies have indicated that culture-specific culinary and dietary practices can serve to influence the values of ingested water (Daux et al. 2008; Brettell et al. 2012), thus potentially influencing the stable oxygen isotope composition of resultant tissues, such outliers cannot simply be presumed to be non-local. For example, Daux et al., argue that solid food consumption (using modern cooking processes) influence the $\delta^{18}\text{O}$ of ingested water by up to 1.2‰ compared to drinking water (2008: 1145). Similarly, Brettell et al. (2012: 782) predict ingested fluids comprising 20% ale, 10% (boiled) 'teas' and 20% stews could influence bioapatite values by +2.3‰. In light of this, while it is unlikely this single individual had a unique diet, were elevated values (beyond that expected from the range of local water values) observed in more or all adult individuals at a site, this may not evidence a high rate of immigration from more exotic climes but rather a community drinking culturally-modified fluids (i.e. local water prepared in a manner as to significantly alter

its oxygen isotope ratio, and consumed in sufficient quantities relative to plain water to influence body tissue values). Such considerations urge caution in the use of human bone (or indeed, tooth) phosphate, as a palaeoclimatic proxy indicator or to determine provenance in archaeological or modern populations. Furthermore, sources of oxygen isotope fractionation as part of the respiratory cycle have been demonstrated to be influenced by certain physiological, metabolic and environmental factors including habitual smoking (Epstein and Zeiri 1998), engaging in regular exercise (Zanconato et al. 1992; Epstein and Zeiri 1998; Widory 2004), and iron-deficiency and sickle-cell anaemia (Zanconato et al. 1992; Epstein and Zeiri 1998; Reitsema and Crews 2011). Although the majority of bodily oxygen is gained from drinking water (~55% as experimentally determined in rats, Podlesak et al. 2008), the potential for these or other (particularly metabolic) conditions to influence the tissue oxygen isotope chemistry of an individual is unknown and more research is evidently required. Infant WCO104 (s-eva-16808) is the only other individual analysed to demonstrate such elevated $\delta^{18}\text{O}_p$ values, exceeding the adult mean and also elevated compared to other infants, which may suggest the influence of some physiological or metabolic condition in this individual. It should be noted that both individuals, NA185 and WCO104, exhibit $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values within 1σ of the adult and infant means respectively, not evidencing any unusual dietary or metabolic conditions (or, at least not any that are evidenced in bone collagen $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values). It is recommended that, ahead of further interpretation, whether or not these individuals were potential immigrants should first be excluded through co-analysis with other isotopes, such as strontium in the case of tooth enamel or sulphur isotope analysis in bone collagen.

Lastly, although in this instance both the infant and adult bone phosphate data (and their correlation with tooth phosphate and regional environmental oxygen isotope

values) suggest that the oxygen isotope measurements are indicative of *in vivo* oxygen isotope values at Medieval Wharram Percy, the potential for diagenetic alteration in bone phosphate samples remains problematic for the broader application of this technique and elicits caution. While mineral integrity suggests diagenetic alteration in samples analysed from Wharram Percy is unlikely (Mays 2003), histological analysis suggests some microbial attack (Nielsen-Marsh and Hedges 2000), and there are no widely-used and universally-accepted quality control indicators or precautions for the avoidance of diagenetically-altered samples currently employed for this method akin to those employed for bone collagen studies (Ambrose 1990; van Klinken 1999). The success of further applications may be dependent on the further development of standardised protocols to identify and exclude potentially compromised samples, or the application of existing experimental approaches; for example, the systematic (albeit potentially species-variable) oxygen isotope partitioning between phosphate and carbonate in bioapatite (e.g. Bryant et al. 1996b; Iacumin et al. 1996; Zazzo et al. 2004b; Pellegrini et al. 2011; France and Owsley 2013). Carbonate and phosphate oxygen isotope data in modern mammalian bone are highly correlated ($r^2=0.98$; Iacumin et al. 1996), and deviations could indicate diagenetic change. Future analysis of oxygen isotope ratios (whole bone and tooth enamel, carbonate) of the samples included in this study will likely provide useful comparative data and illuminate this issue.

CONCLUSIONS

In this study, elevated oxygen isotope values were observed in bone phosphate in young infants from the Medieval site of Wharram Percy, England; after the age of approximately 3 years old, child bone $\delta^{18}\text{O}_p$ values are $>1\%$ lower and close to the adult mean value. On the whole, bone $\delta^{18}\text{O}_p$ values of adults from Medieval Wharram

Percy reflect the range that could be expected for local individuals. The $\delta^{18}\text{O}_p$ changes occurring in early childhood are attributed to the cessation of breastfeeding, as the main source of ingested water shifts from breast milk to local drinking water. Although uncertainties concerning the rate of bone turnover and the length/nature of the Medieval weaning process in early childhood necessitate a degree of unavoidable (and perhaps, in light of the complex nature of the human process being explored, advisable) imprecision, the results of this study strongly suggest that the analysis of oxygen isotope ratios in bone phosphate can be used as an indicator of approximate weaning age in archaeological populations. Although the disparities between 'bulk' bone $\delta^{15}\text{N}_{\text{coll}}$ and $\delta^{18}\text{O}_p$ indications of weaning age are not fully understood, we suggest that $\delta^{18}\text{O}_p$ data provides important alternative lines of enquiry, and likely evidences different aspects of the weaning process. Wherever possible, we would advocate the determination of both $\delta^{15}\text{N}$ values of bone collagen and bone $\delta^{18}\text{O}_p$ values in the same individuals in future studies, in order to provide corroborating lines of evidence and to better explore the complexity of the weaning process, through the examining both protein source ($\delta^{15}\text{N}$) and source of ingested fluids ($\delta^{18}\text{O}$). However, the results of this study do confirm that the investigation of past weaning practices using archaeological bone samples need not be dependent on protein preservation (as with $\delta^{15}\text{N}_{\text{coll}}$ analysis) and that bone $\delta^{18}\text{O}_p$ analysis can be applied where collagen cannot be yielded (e.g. at older sites, or sites in warmer regions where collagen is often poorly preserved).

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Figure Legends:

Figure 1: Map of Yorkshire showing location of the deserted Medieval village Wharram Percy (based on Beresford and Hurst 1990: 16, Figure 1).

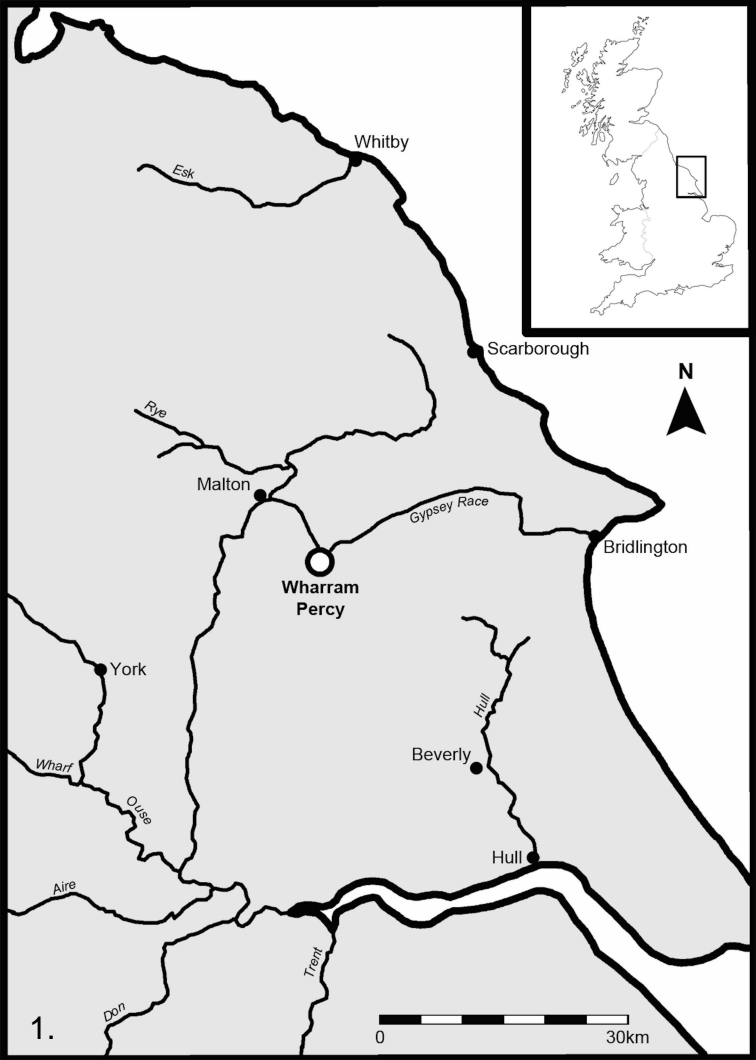
Figure 2: Oxygen isotope composition of bone phosphate (y -axis) in infants and children under 12 years of age (x -axis) at Wharram Percy, showing nitrogen isotope composition of bone collagen from the same individuals (secondary y -axis; collagen data from Richards et al. 2002). Polynomial (non-linear) regressions and correlation coefficients are shown ($\delta^{18}\text{O}$ and age=solid line; $\delta^{15}\text{N}$ and age=dotted line). Local water range is depicted in the area between solid horizontal lines and is taken from Darling et al. (2003), converted to estimated bioapatite values following recommendations in Daux et al. (2008: 1143, equation 6). The mean value 'local' human tooth enamel ($\pm 2\sigma$), measured at the proximal Anglo-Saxon site of West Heslerton is depicted in the area between dashed horizontal lines (from Budd et al. 2004, recalculated in Chenery et al. 2010: 161).

Figure 3: Tooth and bone oxygen phosphate data from selected individuals from Wharram Percy, including deciduous second molars and rib bone from three children, and permanent canines, third molars and rib bone from three adults. Approximate crown formation times for teeth are shown (Garn et al. 1962; Gustafson and Koch 1974; Anderson et al. 1976; Levesque et al. 1981). Burial References: 1=WCO97; 2=NA79; 3=NA31A; 4=G443; 5=G635; 6=G643 (connecting lines and arrows denote materials from the same individuals). Local water range is depicted in the area between dashed lines and is taken from Darling et al. (2003), converted to estimated bioapatite values following recommendations in Daux et al. (2008: 1143, equation 6). The mean value

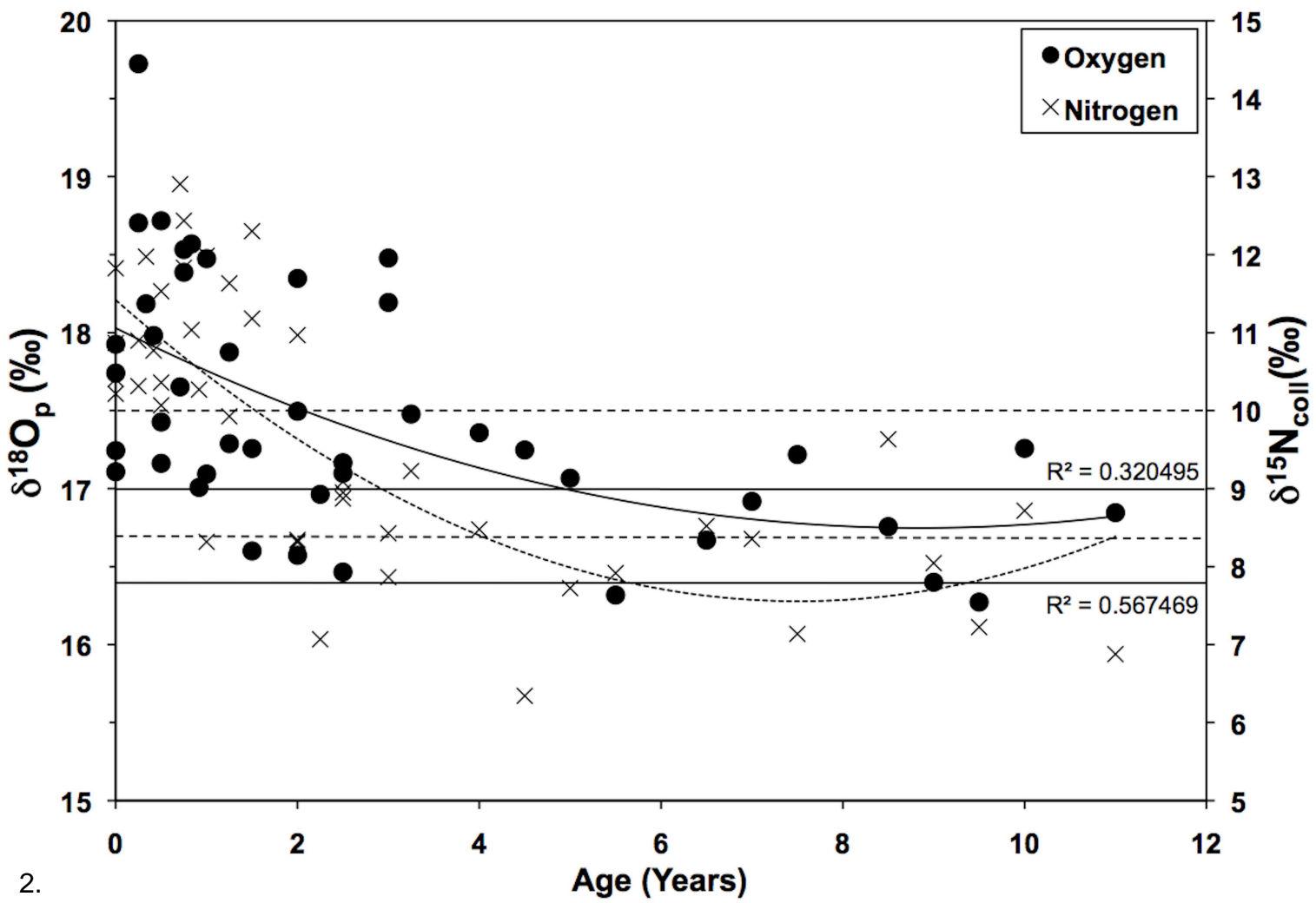
'local' human tooth enamel ($\pm 2\sigma$), measured at the proximal Anglo-Saxon site of West Heslerton is depicted in the area between dashed horizontal lines (from Budd et al. 2004, recalculated in Chenery et al. 2010: 161).

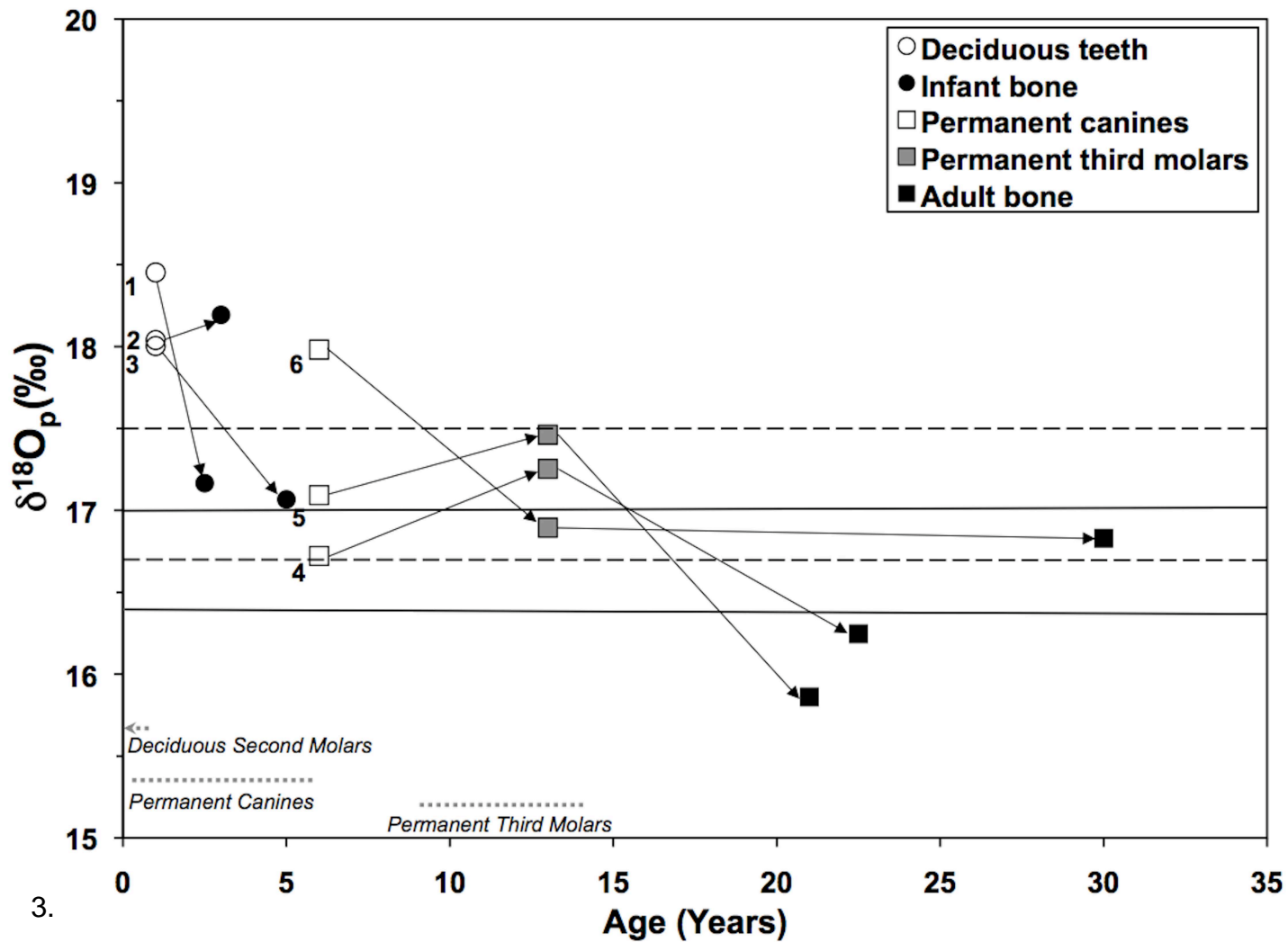
Figure 4: Oxygen isotope composition of bone phosphate of infants, children, unsexed juveniles, adult females and adult males from Wharram Percy, plotted against age (years). Local water range is depicted in the area between dashed lines and is taken from Darling et al. (2003), converted to estimated bioapatite values following recommendations in Daux et al. (2008: 1143, equation 6). The mean value 'local' human tooth enamel ($\pm 2\sigma$), measured at the proximal Anglo-Saxon site of West Heslerton is depicted in the area between dashed horizontal lines (from Budd et al. 2004, recalculated in Chenery et al. 2010: 161).

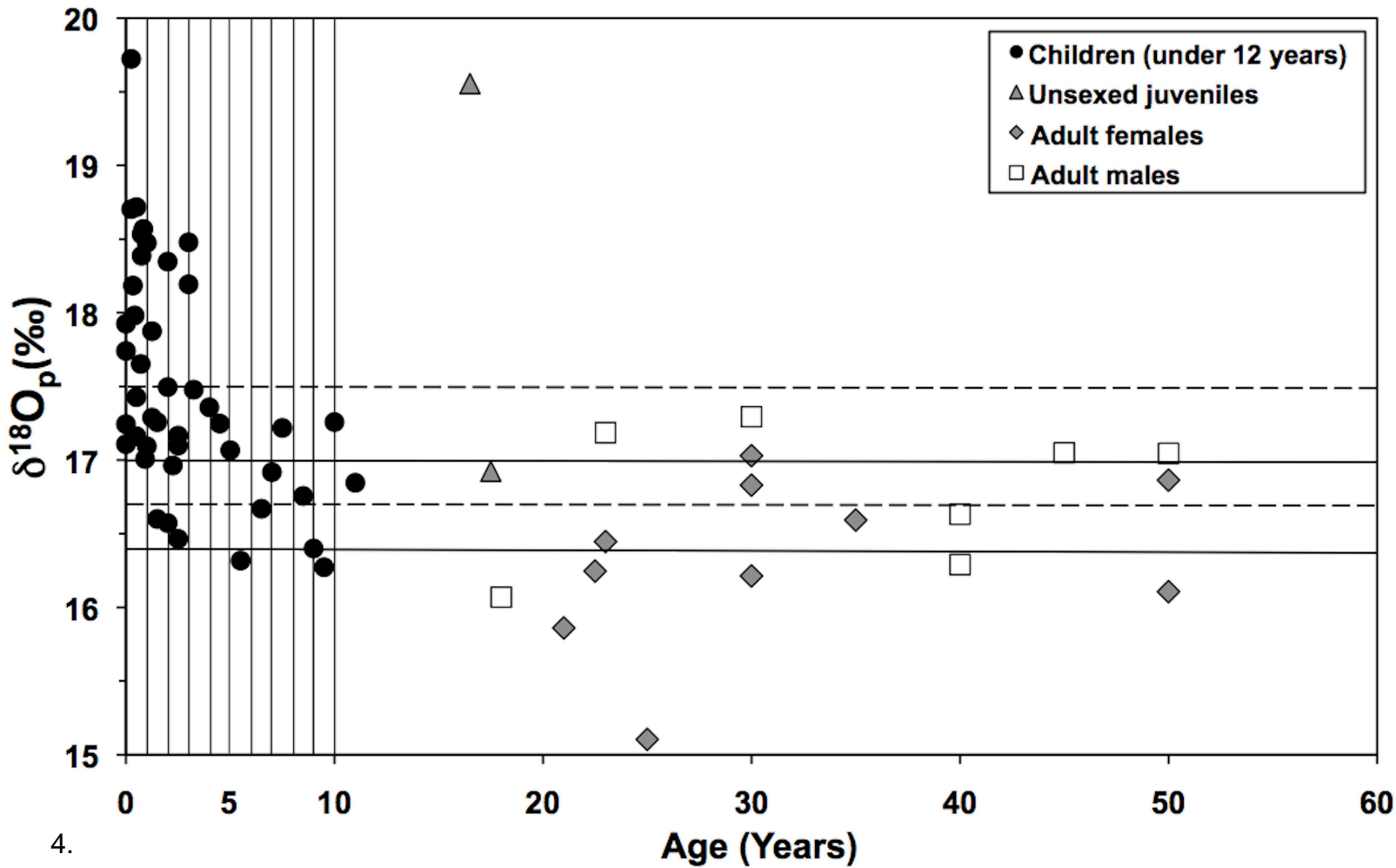
Figure 5: Nitrogen isotope composition of bone collagen (x -axis) and oxygen isotope composition of bone phosphate (y -axis) of infants (<3.5 yrs), children (4-12 yrs) and adults (>18 yrs) from Wharram Percy. Linear regressions and correlation coefficients ($\delta^{15}\text{N}$ and $\delta^{18}\text{O}$) are shown for each data series (infants=black line; children=dashed line; adults=grey line).



1.







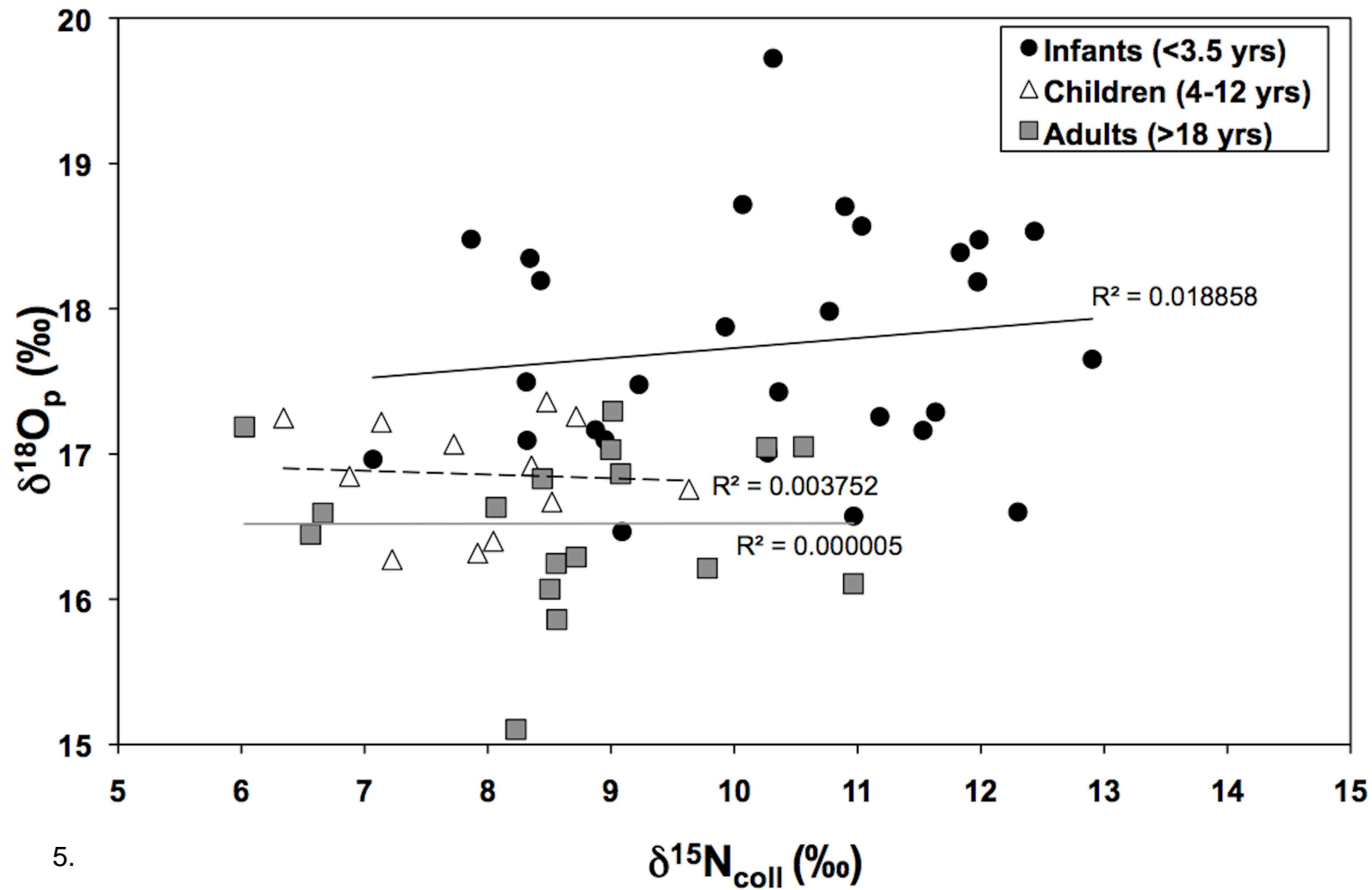


Table 1. Stable oxygen isotope values ($\delta^{18}\text{O}_p$ [‰]) for bone phosphate from Medieval Wharram Percy. Note: asterisk (*) denotes where mean values and standard deviations are calculated from duplicate rather than triplicate measurements. Stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) data for bone collagen from the same individuals are also presented (collagen previously-published as data in figures in Richards et al. 2002).

Burial No.	S-Eva No.	Age	Age (yrs)	m/f	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	Mean $\delta^{18}\text{O}_p$ (‰)	Stdev ($\pm 1\sigma$)	
G492	16802	41 weeks (<i>in utero</i>)	0.0	-	-20.1	10.4	17.1	0.1	
NA220	16803	42 weeks (<i>in utero</i>)	0.0	-	-19.2	10.2	17.7	0.2	
G470	16804	44-46 weeks (<i>in utero</i>)	0.0	-	-18.8	10.9	17.9	0.2	
G579	16805	46-48 weeks (<i>in utero</i>)	0.0	-	-18.5	11.8	17.2	0.1	
WCO104	16808	3 months	0.3	-	-19.8	10.3	19.7	0.1	*
NA191	16809	3 months	0.3	-	-19.4	10.9	18.7	0.2	
G534	15327	3-5 months	0.3	-	-19.6	12.0	18.2	0.2	*
NA194	16810	4-6 months	0.4	-	-18.8	10.8	18.0	0.0	*
WCO042	16812	6 months	0.5	-	-19.8	10.1	18.7	0.2	*
NA119	16813	6 months	0.5	-	-20.5	10.4	17.4	0.1	*
NA231	16814	6 months	0.5	-	-18.2	11.5	17.2	0.2	*
NA050	16815	8-9 months	0.7	-	-19.8	12.9	17.7	0.0	*
EE068	15326	9 months	0.8	-	-18.9	12.4	18.5	0.0	*
NA107	16816	9 months	0.8	-	-18.8	11.8	18.4	0.1	
V31	16817	10 months	0.8	-	-19.7	11.0	18.6	0.1	*
EE048	16818	10-12 months	0.9	-	-19.1	10.3	17.0	0.1	*
WCO085	16819	1 year	1.0	-	-19.1	8.3	17.1	0.3	
NA164	16820	1 year	1.0	-	-20.0	12.0	18.5	0.0	*
G522	16822	15 months	1.3	-	-19.7	11.6	17.3	0.0	*
NA037	16823	1-1.5 years	1.3	-	-20.6	9.9	17.9	0.0	*
NA028	16821	1.5 years	1.5	-	-19.2	11.2	17.3	0.1	
G327	16824	1.5 years	1.5	-	-19.1	12.3	16.6	0.1	*
EE057	16825	2 years	2.0	-	-20.1	8.3	17.5	0.1	*
G298	16826	2 years	2.0	-	-19.4	11.0	16.6	0.2	*
WCO072	16827	2 years	2.0	-	-19.7	8.3	18.3	0.1	*
SA055	16828	2-2.5 years	2.3	-	-20.0	7.1	17.0	0.2	*
G339	16829	2.5 years	2.5	-	-19.9	9.1	16.5	0.0	*

G363	16830	2.5 years	2.5	-	-19.6	8.9	17.1	0.4	
NA031A	16831	2.5 years	2.5	-	-20.2	8.9	17.2	0.1	*
NA079	16832	3 years	3.0	-	-20.2	8.4	18.2	0.0	*
WCO002	16833	3 years	3.0	-	-19.2	7.9	18.5	0.1	*
G576	16834	3-3.5 years	3.3	-	-20.4	9.2	17.5	0.0	*
NA219	16835	4 years	4.0	-	-19.5	8.5	17.4	0.1	*
EE069	16836	4-5 years	4.5	-	-19.9	6.3	17.2	0.2	*
WCO97	16837	5 years	5.0	-	-19.3	7.7	17.1	0.2	
G614	16838	5-6 years	5.5	-	-20.2	7.9	16.3	0.3	*
G424	16840	6-7 years	6.5	-	-19.8	8.5	16.7	0.1	*
NA23	16811	7 years	7.0	-	-19.1	8.4	16.9	0.3	*
EE065	16841	7-8 years	7.5	-	-20.6	7.1	17.2	0.2	
EE066	16842	8-9 years	8.5	-	-20.2	9.6	16.8	0.1	*
WCO140	16843	9 years	9.0	-	-20.3	8.0	16.4	0.2	
G500	15329	9-10 years	9.5	-	-20.4	7.2	16.3	0.2	*
EE072	16844	10 years	10.0	-	-20.9	8.7	17.3	0.2	
G658	15328	11 years	11.0	-	-20.1	6.9	16.8	0.2	*
NA185	17034	16.5 years	16.5	-	-19.6	8.4	19.6	0.1	*
WCO092	17036	17-18 years	17.5	-	-19.6	7.7	16.9	0.3	*
EE036	16845	18 years	18.0	m	-19.6	8.5	16.1	0.3	
G635	16846	21years	21.0	f	-19.6	8.6	15.9	0.2	
G443	16848	21-24 years	22.5	f	-19.4	8.6	16.2	0.0	*
G597	15330	21-25 years	23.0	f	-20.1	6.6	16.4	0.1	*
SA002	16850	22-24 years	23.0	m	-20.1	6.0	17.2	0.1	*
G760	16852	25 years	25.0	f	-19.9	8.2	15.1	0.1	
EE003	16856	25-35 years	30.0	m	-20.4	9.0	17.3	0.1	
G643	16857	25-35 years	30.0	f	-20.2	8.4	16.8	0.0	*
SA014	16858	25-35 years	30.0	f	-19.2	9.0	17.0	0.1	*
G416	16859	25-35 years	30.0	f	-19.6	9.8	16.2	0.1	
G746	16861	30-40 years	35.0	f	-19.3	6.7	16.6	0.1	
NA059	16860	35-45 years	40.0	m	-20.2	8.7	16.3	0.2	*
EE067	16863	30-50 years	40.0	m	-19.6	8.1	16.6	0.1	
V51	16864	40-50 years	45.0	m	-19.2	10.6	17.0	0.0	*

NA046	16869	50+ years	50.0	f	-20.1	11.0	16.1	0.1	*
G636	16870	50+ years	50.0	f	-19.9	9.1	16.9	0.1	
SA034	16871	50+ years	50.0	m	-18.7	10.3	17.0	0.1	*

Table 2. Stable oxygen isotope values ($\delta^{18}\text{O}$ [‰]) for tooth phosphate from Medieval Wharram Percy. Note: asterisk (*) denotes where mean values and standard deviations are calculated from duplicate rather than triplicate measurements.

Burial No.	S-Eva No.	Tooth	Mean $\delta^{18}\text{O}$ (‰)	Stdev ($\pm 1\sigma$)	
WCO97	17915	Deciduous Second Molar	18.5	0.2	*
NA079	17918	Deciduous Second Molar	18.0	0.1	*
NA031A	17919	Deciduous Second Molar	18.0	0.1	*
G443	17908	Permanent Canine	16.7	0.1	
	17907	Third Molar	17.3	0.2	*
G635	17910	Permanent Canine	17.1	0.0	*
	17909	Third Molar	17.5	0.0	
G643	17912	Permanent Canine	18.0	0.3	*
	17911	Third Molar	16.9	0.2	*