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**Infant mortality and isotopic complexity: new approaches to stress, maternal health and weaning**

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## ABSTRACT

### Objectives

Studies of the carbon and nitrogen stable isotope ratios ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) of modern tissues with a fast turnover, such as hair and fingernails, have established the relationship between these values in mothers and their infants during breastfeeding and weaning. Using collagen from high-resolution dentine sections of teeth which form in the perinatal period we investigate the relationship between diet and physiology in this pivotal stage of life.

### Materials and Methods

Childhood dentine collagen  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  profiles were produced from horizontal sections of permanent and deciduous teeth following the direction of development. These were from two 19<sup>th</sup>-century sites ( $n = 24$ ) and a small number ( $n = 5$ ) of prehistoric samples from Great Britain and Ireland.

### Results

These high-resolution data exhibit marked differences between those who survived childhood and those who did not, the former varying little and the latter fluctuating widely.

### Discussion

Breastfeeding and weaning behavior have a significant impact on the morbidity and mortality of infants and the adults they become. In the absence of documentary evidence, archaeological studies of bone collagen of adults and juveniles have been used to infer the prevalence and duration of breastfeeding. These interpretations rely on certain assumptions about the relationship between isotope ratios in the bone collagen of the adult females and the infants who have died. The data from this study suggest a more complex situation than previously proposed and the potential for a new approach to the study of maternal and infant health in past populations.

Carbon and nitrogen stable isotope ratios from archaeological bone and dental tissues have been used for more than 20 years to investigate breastfeeding and weaning practices. Early studies, which revolutionized the use of bone collagen stable isotopes, identified patterns within the tissues of fetuses (less than 28 weeks gestation), neonates (28–40 weeks gestation) and infants (28 days to 1 year post-birth)(Lewis and Gowland 2007) which revealed the potential to investigate the duration of breastfeeding and weaning in past populations (e.g. Fogel et al. 1989; Katzenberg et al. 1993; Schurr 1997; White and Schwarcz 1994). Katzenberg (1996) reviewed previous interpretations of the method and cautioned against the potential effect of using the tissues of dead infants without knowing the cause of death and the potential for stress, bone turnover and growth to alter the isotope ratios of both mother and infant. Subsequently, a simplified method, based on a mathematical model of the isotope ratio variations which should be associated with a dietary change from breastmilk to the prevailing diet of the adult population, was proposed and widely accepted (Schurr 1997; Millard, 2000; Jay, 2008). This method has since been applied to data from modern, historical and archaeological tissues to estimate the timing of weaning and whether breastfeeding occurred over prolonged periods. The mathematical model has been used pragmatically despite the often-acknowledged fact that it makes several assumptions about the relationships of mother and infant body tissues, their diets, and the comparability of individual members of a population with the overall community. These assumptions are: that the isotope ratios of maternal bone collagen will be within one standard deviation of the adult female mean for the population; that fetal bone collagen will have the same isotope ratio range as this; that the  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values from the bone collagen of infants represent diet at approximately the time of death; and that the infants who died are representative of the diet and physiology of the whole population at that age. With regard to the final assumption, it is important to recognize that the ‘Osteological Paradox’ (Wood et al. 1992), which suggests that the infants who have died may not be representative, is ignored.

These assumptions may not hold in many cases. In most isotope studies of weaning, the bone collagen of infants at the time of death has been measured and the assumption made that this represents an averaged value for  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  which may be determined to a greater or lesser degree by the rate of bone turnover. Recent developments have meant that much smaller samples can now be measured accurately, opening the way for researchers to analyse tissues which develop in an incremental, and hence time-bound, manner. This study uses a new approach to the interpretation of isotope profiles with high-temporal resolution from incremental dentine samples. Choosing teeth from individuals who died during childhood and especially individuals with known dietary stress (i.e. from a Famine cemetery) allows a better interpretation of the unknown values: by comparing these with individuals who survived the period of tooth growth it is possible to re-examine the issues raised above and consider how such data can be used in the assessment of maternal and infant health, as well as breastfeeding and weaning behaviors.

### **MATERNAL AND INFANT HEALTH AND WEANING**

It has been demonstrated that the health of mothers and their babies not only affects the morbidity and mortality of the juveniles in a population, but has long-term effects on the health of the adults they become (Barker 1998; Harris 2001). This influence begins *in utero* and continues during the important stages of breastfeeding and weaning. Adequate nutrition may be defined as the availability of all the essential dietary components required for growth, development, maintenance, repair and function of an individual. Factors which control those requirements include the individual's activity levels, energy expenditure to maintain body temperature, diseases and their frequency and duration, and periods of physiological stress such as growth and pregnancy (Gopalan 1992).

‘Undernutrition’ is where the intake of nutrients is insufficient for the requirements of the individual (Shetty 2006), and the human body will react to minimize the effects (Osmani 1992). In the short-term, the body can make changes to improve metabolic efficiency by recycling and releasing stored nutrients which can be returned to normal status when adequate nutrition is restored. This return to homeostasis means that short periods of undernutrition would not have long-term measurable effects on the body (Srinivasan 1992), whereas adaptation to long-term chronic undernutrition will result in permanent changes in metabolic efficiency, in physical dimensions, and in behavior. Barker (1998) showed how nutritional deprivation *in utero* can result in babies of low birth weight and that this can have life-long effects on the individual’s response to nutrition. The effect of undernutrition during early life can affect not only the health outcomes of the adult but also potentially the offspring of that individual (Heijmans et al 2008; Champagne 2013). Fogel (1986, 271f) states that “mean heights reflect the accumulated past nutritional experience of an individual over all of his growing years including the fetal period”. Thus, ‘stunting’ can be the result of a period when growth is disturbed by undernutrition. As an illustration, Floud et al. (1990), in a study of British men joining the armed forces, showed that the height of the adult male can be linked to their nutrition as a juvenile and Sharpe (2012) connected poor nutrition and disease with low stature in nineteenth-century England. Data collected in 2003-2009 shows the extent of stunting in children under the age of five years and women from developing countries (Dewey and Begum 2011). Studies of modern individuals by the Maternal and Child Undernutrition Group (Black et al 2008; Victora et al. 2008) demonstrate the connections between maternal health, slow growth in early childhood and consequent poor health and educational/economic underachievement in modern populations.

It is well established that breastmilk is a hygienic source of the nutrients required by neonates and promotes immunity in the early months of life (Lönnerdal 2000), with the introduction of alternative or contaminated food and drinks to infants being long-recognized as a potential source of malnutrition, pathogens and poisoning (Howarth 1905; Motarjemi et al. 1993). However, the pattern and duration of breastfeeding, and the choice and timing of the introduction of other sources of nutrition, may be dictated by cultural practices, economic factors and availability (Fildes 1986; Quandt, 1995, 127-138) and will have effects on the health of both mother and infant. For example, in Britain, improvements in infant mortality rates predated the major public health measures of the mid-nineteenth century, and this was linked to a reduction in birth rates and female mortality (Fildes 1986; Harris 2001). Rather than employ wet-nurses, it became fashionable for mothers to feed their own children. Colostrum (the milk produced in the first 3-4 days post-partum) has an important role in transferring maternal immunity against local pathogens to the baby, but prior to the 1740s mothers were encouraged to discard this (Fildes 1986) and some modern societies still do so (Rogers et al. 2011). Early weaning was also advocated in the mid-eighteenth century (Fildes 1986). During the nineteenth century, evidence from urban centers such as Manchester show that breastfeeding rates reduced when women were employed in the factories. During periods such as the Cotton Famine in the 1860s, when women were forced to stay at home, infant mortality fell while mothers could suckle their infants and rose again when cotton production resumed (Fildes, 1995, 108). However, data collected in the 1890s shows that even in urban areas over 80% of women still breastfed, although for the majority the duration was only 3 months (Fildes 1995, 108-109).

Breastfeeding also improves maternal health, reducing the incidence of common illnesses such as mastitis and milk fever in the mother; in addition, continued frequent suckling has contraceptive effects and therefore reduces family size and depletion of maternal

resources (Lawrence and Lawrence 2011; Short 1987). The improvement in maternal health and the reduction in the number of pregnancies will have concomitant impacts on the overall health of the population.

### **ISOTOPE STUDIES OF WEANING**

It has been established from modern studies that there is a trophic level shift of between 2-4‰ in the nitrogen isotope ratio ( $\delta^{15}\text{N}$ ) between the hair and fingernail keratin of infants relative to the keratin  $\delta^{15}\text{N}$  values of their mothers during breastfeeding (Fogel et al. 1989; Fuller et al. 2006a). Once other foods are introduced to the diet, this offset should reduce until breastfeeding ceases completely (Fogel et al. 1989; Fuller et al. 2006a; Jay et al. 2008; Millard 2000), depending on if the supplementary foods are from a similar trophic level to those in the mother's diet. In addition, the carbon isotope ratio ( $\delta^{13}\text{C}$ ) of body tissues will give further information about the constituents of the diet of mother and child. While the trophic level effect on  $\delta^{13}\text{C}$  is small, in combination with  $\delta^{15}\text{N}$  the values permit discrimination between terrestrial and marine foods (Richards and Hedges 1999; Schoeninger and DeNiro 1984), and between plants with different photosynthetic pathways, such as  $\text{C}_3$  and  $\text{C}_4$  plants (van der Merwe and Vogel 1978).

The method currently used in archaeological studies to estimate the timing of weaning behavior is based on the assumptions that the mean value (plus or minus 1 standard deviation) of the  $\delta^{15}\text{N}$  values of collagen from the adult females in a burial assemblage defines the maternal range, and that infants have the same isotope ratio as their mother at birth because the mother is the source of all nutrition for the infant via the placenta during pregnancy (Jay et al. 2008). If the infant is subsequently breastfed, it is assumed that a trophic level rise will be visible in the infant bone collagen (as seen in the modern hair and nail studies) and measuring



the  $\delta^{15}\text{N}$  values of infants who died at different ages can be used to estimate when and whether breastfeeding and weaning occurred in an archaeological population (Fuller et al. 2003; Jay et al. 2008; Richards et al. 2002). The female mean bone collagen  $\delta^{15}\text{N}$  is plotted as a horizontal line parallel to age at death on the x-axis, and the individual bone collagen  $\delta^{15}\text{N}$  of the neonates and infants is plotted on the y-axis according to the osteological age at death (see Figure 1). The highest value for juvenile  $\delta^{15}\text{N}$  (allowing for the time-lag between changes in the diet and the expression of these in the bone) is interpreted as the age at which exclusive breastfeeding ceases, and the decreasing values for older individuals are deemed to represent the period of weaning and supplementary feeding until the  $\delta^{15}\text{N}$  value reaches +1 SD of the female mean. At this point, breastfeeding is considered to have ceased and the individuals to be fully weaned. Because of the importance of breastfeeding and weaning behavior to the health of infants and the control of fertility and birth spacing, many studies of bone collagen from burial populations where juveniles were present have used the model above to estimate weaning age as part of their discussion of the wider dietary information from the site (e.g. Oelze et al. 2011; Triantaphyllou et al. 2008; Turner et al. 2007).

There is evidence from published studies that may cast doubt on the reliability of this method:  $\delta^{15}\text{N}$  values which are higher than 1 SD above the female mean (and therefore interpreted as representing the consumption of breastmilk) have been recorded in fetal and neonatal individuals at British sites including medieval Wharram Percy, Yorkshire (Richards et al. 2002), eighteenth- and nineteenth-century Spitalfields, London, England (Nitsch et al. 2011) and the sub/late-Romano British site of Queenford Farm, Oxfordshire, England (Fuller et al. 2006b). Kinaston et al. (2009), in a study of a 3000-year-old site in Vanuatu, South Pacific, noted high  $\delta^{15}\text{N}$  values in fetal and perinatal individuals who were too young to have been breastfed and suggested that these values may be reflecting maternal stress during pregnancy.

Pearson et al. (2010) compared weaning ages at two Neolithic sites in Anatolia, Turkey, and interpreted high neonatal  $\delta^{15}\text{N}$  as evidence that inaccurate age estimation had under-aged individuals or that they were the result of nutritional stress in the adult females. Infants with lower than expected bone collagen  $\delta^{15}\text{N}$  values have been suggested to have had little or no access to breastmilk (Jay et al. 2008; Nitsch et al. 2011). However, the bone collagen  $\delta^{15}\text{N}$  value of a rapidly growing fetus or neonate is related to the  $\delta^{15}\text{N}$  values of the mother which may vary during pregnancy due to changes in diet and physiology (de Luca et al. 2012; Derbyshire 2011, 95; Fuller et al. 2004; Fuller et al. 2006a). This may not be the same as the long-term, life-time averaged bone collagen values obtained from the actual or putative mothers or the wider cemetery population. Bone collagen takes time to turn over (Hedges et al. 2007; Valentin 2003) and even for neonates, whose tissue growth is much faster than in adults, it will take time to record changes in the isotope values of the food consumed. Thus, the recording of an unexpectedly high or low  $\delta^{15}\text{N}$  in perinatal bone collagen could be interpreted as an *in-utero* value. It is also possible that the  $\delta^{15}\text{N}$  values of the collagen of infants at any age may be the result of influences other than their diet (Katzenberg and Lovell 1999; Reitsema 2013). It has been shown that  $\delta^{15}\text{N}$  in the body tissues will rise in response to nutritional stress or as a result of high demand on the body through illness and growth (Guthrie and Picciano 1995; Hobson et al. 1993; Mekota et al. 2006).

Increasing the time resolution of the analyzed skeletal material by using incremental tissues forming in the perinatal and childhood periods of life may help to explain the different roles of diet, stress and maternal health in the changes seen in  $\delta^{15}\text{N}$  values. There have been a number of recent studies which use this approach. Howcroft et al. (2012) analyzed collagen from dentine sections and bone to obtain temporal resolution for the variation in  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  in juveniles from Iron Age Sweden. Fuller et al. (2003) analyzed up to five serial dentine

sections from deciduous and permanent teeth to ascertain the likely duration of breastfeeding in medieval Wharram Percy, England. Eerkens et al. (2011) used analysis of incremental dentine collagen from first permanent molars (which form between 38 weeks *in utero* and  $9.5 \pm 0.5$  years of age) from six individuals to investigate this important period of life. The latter paper demonstrates that the  $\delta^{15}\text{N}$  profiles produced vary over time in a similar pattern in all six teeth, and discusses differences between individuals accorded high and low-status burials. Eriksson and Lidén (2013) used the collagen from dentine samples taken from the crown of deciduous teeth and the cervical area of permanent teeth and from compact bone to investigate diachronic changes in diet in Mesolithic and Neolithic sites in northern Europe. They include a one-year old with extremely high crown dentine  $\delta^{15}\text{N}$  values relative to the bone collagen of the adult female in the same grave (Eriksson and Lidén 2013). Burt and Garvie-Lok (2013) have developed a method for sampling modern deciduous dentine before and after the neonatal line (which denotes birth). Using modern teeth from Canadian children they collected three dentine samples: pre-birth, post-birth and from the growing edge of the tooth (representing dentine from the highest age available in developing or resorbing teeth), and suggest that the  $\delta^{15}\text{N}$  values of the post-birth samples sort into two groups: one breastfed, the other bottle-fed. However, the actual dietary history for these modern individuals was not recorded.

### **Secondary dentine**

It is important to demonstrate that the apparent differences in  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  profiles between those who died and those who survived childhood is not caused by a subsequent turnover of tissue within the dentine. Although primary dentine, once formed, does not remodel, the odontoblasts lining the pulp chamber retain the ability to produce new dentine throughout life (Nanci 2003, 194, 236). Secondary dentine forms at a very slow rate on the internal surface of the pulp chamber of the tooth and is a normal feature of both deciduous and permanent teeth.

It has been known for over 60 years that secondary dentine is not observable in the teeth of young adults, i.e. below the age of 28 (Gustafson 1950). More recent studies have confirmed these early findings. Following the work of Lamendin et al. (1992), Kvaal et al. (1995) used the regular deposition of secondary dentine as a technique for the age estimation of adults using the proportion of root to pulp chamber as measured on dental radiographs. The study by Kvaal et al. (1995), and others using variations on their technique, showed that there is insufficient depth of new dentine to measure reliably under the age of 29 years (Camereriere et al. 2013; Karkhanis et al. 2013) and is not recommended as a method of age estimation for individuals under the age of 25 years (Meinl et al. 2007). The odontoblasts can also form tertiary dentine, a more rapid production of new dentine in the pulp chamber as a response to damage to the tooth such as caries or attrition. This property of the tooth has been used in clinical treatments to encourage the “walling-off” of the live pulp tissue from a cavity in the crown of the tooth (Smith et al. 2012).

All of the individuals sampled in this incremental dentine study were under the age of 25 at death apart from KUW 1 (23-37 years) and HPCS 101(25-40 years). All the teeth were caries-free and had little or no attrition. Secondary and tertiary dentine is visibly different from primary dentine even *in vivo*, and if present in archaeological teeth can be identified and removed by reaming the root canal or abrading back to the primary dentine: this was carried out in both individuals over the age of 25. Thus the contribution of secondary dentine to the isotope values in this study will be negligible.

In this current study we present incremental dentine collagen  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  profiles at high spatial and temporal resolution from the permanent and deciduous teeth of individuals from archaeological sites in Ireland, England and Scotland who have died during tooth formation and compare these with individuals who survived childhood. These dentine collagen

data are used to test the assumptions behind the method currently employed to estimate breastfeeding behavior.

## MATERIALS

The dentine samples are from four sites: the Famine cemetery at Kilkenny Union workhouse (Ireland, AD 1847-1852); the cemetery of the Catholic Mission of St Mary and St Michael, Lukin Street (London, AD 1843-1854); High Pasture Cave (Isle of Skye, Scotland, middle Iron Age, first- to second-century AD); and previously published data for Neolithic humans from the Sumburgh cist (Shetland, Scotland, 3510 to 2660 BC) (Montgomery et al. 2013) (see Figure 2). References for site and osteological reports are presented in Table 1.

### Incremental dentine collagen

The teeth selected for this study were: the first permanent molar (M1) which initiates just prior to birth with apex completion at 9.5 years  $\pm$  0.5 years; the first deciduous molar (DM1) which initiates at -0.3 years with apex completion at 2.5 years  $\pm$  0.5 years; the second deciduous molar (DM2) which initiates at -0.3 years with apex completion at 3.5 years  $\pm$  0.5 years (AlQahtani 2009). Two second permanent molar teeth (M2) from individuals from Kilkenny Union workhouse were included; although they develop from 2.5 to 15.5 years  $\pm$  0.5 years (AlQahtani 2009). The M2 teeth are unlikely to include the period of weaning; however they were known to have died during a period of extreme nutritional stress.

A total of 29 individuals were included in the study, with a single tooth from each (see Table 2): eight completed M1s; nine developing M1s; two developing M2s; two complete DM2s; three developing DM1s; and five developing DM2s. The 19<sup>th</sup>-century individuals were selected to represent the range of bulk bone  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values from the two sites. In addition, published bone collagen values from Lukin Street and Kilkenny Union workhouse (Beaumont et al. 2013a) are used in the discussion.

Table 1 here
Figure 2 here

## Methods

The teeth were sectioned using either method 1 or 2 in Beaumont et al. (2013b) (noted in Table 2): any identified secondary or tertiary dentine was removed from the internal surface of the pulp chamber using a slow-speed tungsten-carbide rose-head bur, and an approximate age was assigned to each dentine segment as described. These methods allow isotopic changes over short periods (approximately 4 months in deciduous and 8-9 months in permanent teeth) to be investigated. Comparison of the two methods show no differences in the profiles produced (Beaumont et al. 2013b). Collagen was prepared using the modified Longin method (Brown et al. 1988; O'Connell and Hedges 1999). Surface debris was removed by air-abrasion and samples were demineralized in 0.5 M hydrochloric acid at 4°C. Samples were rinsed with de-ionized water, placed in sealed tubes with pH 3 acidified water at 70°C for 48 hours, allowing the collagen fibrils to denature. No filtration was carried out on the small incremental dentine samples to prevent size reduction. The samples were then freeze dried. They were measured in duplicate in the University of Bradford Stable Light Isotope Laboratory and compared with laboratory and international standards that were interspersed throughout each analytical run. The international standards were: IAEA 600, CH6, CH7, N1 and N2. The laboratory standards, fish gelatin and bovine liver, were calibrated against the international standards. The dentine samples were combusted in a Thermo Flash EA 1112 and the separated N<sub>2</sub> and CO<sub>2</sub> was introduced to a Delta plus XL via a Conflo III interface. This instrument was used for the incremental dentine samples because it can analyze smaller (0.5 mg) masses.

The C:N ratios obtained from each dentine collagen sample are within the range of 2.9 – 3.6 recommended by Ambrose (1993) and 3.1 – 3.5 suggested by van Klinken (1999). This indicates well-preserved collagen that has not undergone diagenetic alteration. The collagen yields from dentine were between 10-19% by weight after demineralization: the

value generally accepted as indicative of well-preserved collagen being above 1% (van Klinken 1999).

The results for the dentine collagen are expressed using the delta ( $\delta$ ) notation in parts per thousand (per mil or ‰) relative to international standards. When calibrated against international and laboratory standards the analytical error was determined at  $\pm 0.2\text{‰}$  (1 SD) or better.

## RESULTS

### Incremental dentine collagen

The results for incremental dentine collagen are given in Table 2. Figure 3 presents the incremental dentine collagen profiles for  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values for all M1s which had completed apices (i.e. the individual lived beyond the age of  $9.5 \pm 0.5$  years)( $n = 8$ ). The profiles generally show little variation in consecutive  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values throughout the formation of the tooth, apart from SUMB 45 and KUW 9. The maximum range of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values for any individual is 2.4 ‰.

The profiles for the individuals who died during root formation (Figure 4)( $n = 9$ ) display a wider variation than the individuals who survived this period of life with a maximum range of 6 ‰ in  $\delta^{15}\text{N}$  values for a single individual. As with the  $\delta^{15}\text{N}$  profiles, the  $\delta^{13}\text{C}$  profiles vary: LUK 413, 419 and 259 present relatively flat profiles resembling those of the teeth with closed apices shown in Figure 4. The remaining individuals with uncompleted roots show greater changes between consecutive increments, with  $\delta^{13}\text{C}$  rising or falling by between 1.5 ‰ and 4 ‰ overall and in some cases both rising and falling during the lifetime of the individual.

The  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  dentine profiles for the deciduous teeth are shown in Figure 5 ( $n = 10$ ). The DM1s and DM2s begin to form *c.* 30 weeks *in utero*, so that the earliest forming

figures 3 and 4 and 5 here

sub-occlusal or sub-incisal dentine increment will include some tissue formed before birth.

These infants all died before root completion. SUMB 39 shows the flattest  $\delta^{15}\text{N}$  profile with a range of 1 ‰ during life, and LUK 923 the widest range of 3.4 ‰.

The  $\delta^{13}\text{C}$  profiles show two main patterns: either a slight rise after birth or a drop. The longest surviving individuals, LUK 1033, 923, 724 and SUMB 39 achieve a flatter profile after the age of one year, while the other  $\delta^{13}\text{C}$  profiles appear to still be falling at time of death.

Figure 6 shows the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  profiles of KUW 4, an M2 which begins to form at  $2.5 \pm 0.5$  years and completes at  $15.5 \text{ years} \pm 0.5$  years. The  $\delta^{13}\text{C}$  profile increases by more than 5 ‰ with the main rise beginning at about the age of 8 years, while the  $\delta^{15}\text{N}$  profile decreases at the age of 4 years, this is followed by a peak at age 6-7 years. The KUW 14 M2 (Figure 7), by comparison, has a relatively flat  $\delta^{15}\text{N}$  profile but a rise of 3 ‰ in  $\delta^{13}\text{C}$  between the age of 6 and death at about the age of 9.5 years.

Figures 6 and 7 here

## DISCUSSION

### **Assumptions inherent in the current breastfeeding and weaning estimation method**

#### ***Mother and fetal bone collagen will have the same isotope ratio at birth.***

A recent study found a systematic difference in the hair samples of mother and infant pairs with infant  $\delta^{15}\text{N}$  values being approximately 0.9‰ higher than the mother and infant  $\delta^{13}\text{C}$  values approximately 0.4‰ higher (de Luca et al. 2012). Higher infant  $\delta^{15}\text{N}$  values were also reported at birth in the majority of mother/infant pairs by Fuller et al. (2006a). This could be a normal physiological offset: the difference in  $\delta^{15}\text{N}$  values could be due to short-term changes in diet or in the physiology of the mother during the pregnancy (Derbyshire 2011, 83; Fuller et al. 2004; Fuller et al. 2005). For example, a mother and fetus pair from the Lukin Street site



displayed an offset in  $\delta^{15}\text{N}$  values of ‰ (mother 12.4‰, fetus 13.4‰)(Beaumont et al. 2013). There is epigraphic and documentary evidence for the health of the Lukin Street mother during her pregnancy: she was Georgiana Neale, who died at the age of 23 while pregnant with her second child. Her death certificate records the cause of death as consumption (tuberculosis) and the census record for AD 1851 shows she was born and died in the same district of London. This suggests that the chronic condition which caused her death may have resulted in a short-term rise in  $\delta^{15}\text{N}$  values which had little or no impact on her bone collagen values, but has been recorded by the bone collagen of her fetus. It is therefore possible that the data from mother/fetus pairs provide evidence that short-term changes in the isotope ratios of the pregnant mother are not visible in the bone collagen due to the slow rate of turnover, but are recorded by fetal bone due to its increased responsiveness as a result of rapid growth.

The data in this study demonstrates a wide range of  $\delta^{15}\text{N}$  values measured in the first increment of dentine from both M1 and deciduous teeth of individuals from Lukin Street. This earliest-forming dentine will include some tissue which has formed *in utero*, and thus could be reflecting a wider range of maternal  $\delta^{15}\text{N}$  values than suggested by the population adult female mean (and see below, Estimating Maternal Health).

These data suggest that  $\delta^{15}\text{N}$  values of infants at birth may differ from the bone collagen values of their mother more often than is assumed by the current weaning studies. This may be due to an intrinsic offset between mother and infant as seen in the modern hair study by de Luca (2012) or a short-term change in nitrogen balance in the mother which could cause high or low values relative to the averaged value in her bone collagen. Furthermore, maternal physiology changes during pregnancy. Fat stores are built up during the first two trimesters to be available for the fetus during the third trimester (Butte 2000) and this raises blood lipid levels which may in turn alter the mother's  $\delta^{13}\text{C}$ . By comparison, protein metabolism adapts throughout

pregnancy to meet the needs of mother and fetus (Kalhan 2000). It has been shown that  $\delta^{15}\text{N}$  values can be raised during a period of nutritional stress such as morning sickness, and where the dietary intake of protein is insufficient for the growth requirements of the fetus the recycling of proteins in the body causes a negative nitrogen balance (catabolism) (Fuller et al. 2005; Kalhan 2000; Waters-Rist and Katzenberg 2010). The opposite can also be true: where sufficient dietary protein is available, during periods of rapid fetal growth  $\delta^{15}\text{N}$  values of the mother can be lower because of a reduction in the excretion of nitrogen which causes a positive nitrogen balance (anabolism) (Fuller et al. 2004; Waters-Rist and Katzenberg 2010). Neonates with low  $\delta^{15}\text{N}$  bone collagen values could be recording a low maternal *in-utero* value. The study by Nitsch et al. (2010) using nineteenth-century individuals from Spitalfields compared the  $\delta^{15}\text{N}$  bone collagen values of females for whom there was documentary evidence of frequent and multiple childbearing with the adult female mean and found no evidence for a lower value. This lack of evidence for a positive nitrogen balance was interpreted as resulting from a slow bone turnover rate in adults.

The data obtained from the deciduous teeth of modern Canadian children by Burt and Garvie-Lok (2013) shows that the range of  $\delta^{15}\text{N}$  values in pre-neonatal-line deciduous dentine is more than 7‰, compared with a 3‰ range for dentine formed after the age of 12 months. This is similar to the putative *in utero* range of  $\delta^{15}\text{N}$  values seen in the Lukin Street population and suggests a wider range of values can result from fluctuations in maternal diet and/or physiology during pregnancy than from the range for the childhood diet in the population.

***The  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values from the bone collagen of infant burials represent diet at the time of death.***

If the final increment of dentine from a tooth which is still forming is taken to represent tissue forming around the time of death, then the isotope ratios of the collagen should represent the diet and physiology of the individual at that time. Figure 8 shows the difference between bone collagen and the final increment of dentine for  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values of the 17 individuals from Lukin Street and Kilkenny Union workhouse. With the exception of LUK 1033 and LUK 316, the bone collagen  $\delta^{15}\text{N}$  values differ from the dentine collagen  $\delta^{15}\text{N}$  values by 0.3‰ or more. Almost all of the  $\delta^{15}\text{N}$  values for dentine collagen are higher than for bone; the exceptions to this are LUK 316 where there is no difference and LUK 413 where the  $\delta^{15}\text{N}$  value of the dentine collagen is 2‰ lower than the bone collagen. The  $\delta^{13}\text{C}$  difference is greater than 0.3‰ in 11/17 individuals. When the individuals are placed in age order from left to right; LUK 955 to LUK 1033 (n = 10) are all under the age of 3.5 years and thus are individuals who would be included in any plot constructed using the current method to investigate weaning behavior .

Figure 8 here

These data suggest that the bone collagen, even in very young juveniles, is not turning over fast enough to be representative of the diet and physiology at the time of death, and thus the data represent an average over a period of time. Furthermore, it appears from the data that there are some individuals for whom the  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values of the bone collagen cannot be an average of the dentine collagen values because the latter are consistently higher even though, based on age at death, the formation times of these two tissues must have overlapped. An explanation for this may be found in the differential growth of bone and teeth. For example, it has been shown that human teeth will continue to grow at the same rate regardless of nutritional status (Elamin and Liversidge 2013), whereas bone formation may be arrested if nutritional status is poor. The World Health Organization (WHO) defines juvenile chronic malnutrition in terms of the Body Mass Index (BMI) and Height for Age (2006) which can be interpreted as a measure of stunting. Moreover, a review of animal studies found that protein malnutrition and

food deprivation affect the epiphyseal plates, reducing bone growth and long bone length (de Carvalho da Silva et al. 2013). If the mean  $\delta^{15}\text{N}$  values for dentine are consistently higher than those of the bone collagen, one explanation may therefore be that there is a threshold level of undernutrition below which new bone collagen is not synthesized (Hatch 2012, 344-347 and 353-354). If growth is arrested, then the bone collagen from that period will not represent the true  $\delta^{15}\text{N}$  value, while the dentine collagen continues to form and record the higher values. All six of the juveniles from Kilkenny who died during tooth (and therefore bone) formation, and who were likely to have been chronically undernourished, have higher mean dentine collagen  $\delta^{15}\text{N}$  value than bone collagen. Clearly, these individuals are an extreme case where malnourishment is documented; more work would be needed to establish if such processes are relevant in less stressed populations.

***Deceased infants are representative of the diet and physiology of the total infant population***

Wood et al. (1992) made the point in their paper “The Osteological Paradox” that the health of an individual during life cannot be inferred from the human remains which are excavated: the juveniles in a cemetery population may have died during childhood because they were at highest risk for an unknown reason.

Documentary evidence shows that the Kilkenny Union workhouse cemetery was used during the Great Irish Potato Famine (1845-1852) for the burial of inmates (Geber 2011). The lower socio-economic groups which would have to resort to the workhouse were those who would have been eating a very monotonous diet of potatoes (a  $\text{C}_3$  plant) supplemented only by buttermilk (Clarkson and Crawford 2001) prior to the Famine. John Peel, the British Prime Minister, imported maize (a  $\text{C}_4$  plant) for a period of about two years as a relief food and it formed a large part of the calories in the workhouse diet for that period (Clarkson and Crawford

2001). Potatoes and maize, because of their different photosynthetic pathways, have very different  $\delta^{13}\text{C}$  values which would lead to different values in the collagen of consumers (van der Merwe and Vogel 1978). The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  dentine profiles from the M2 of two juveniles who were buried in the Kilkenny Union workhouse cemetery (KUW 4, Figure 6 and KUW 14, Figure 7) show that this short-term dietary shift is identifiable. KUW 14 (Figure 7) shows a rising trend for  $\delta^{13}\text{C}$  which becomes steeper at the age of 6 years and continues until death. The early  $\delta^{13}\text{C}$  values at age 2.5 years are consistent with a  $\text{C}_3$  plant-based diet and towards the end of life these values are consistent with a  $\text{C}_4$  plant-based diet. The  $\delta^{15}\text{N}$  values for this individual are consistent with a low trophic level plant-based diet and the profile remains fairly flat throughout life. By contrast, the dentine  $\delta^{15}\text{N}$  profile of KUW 4 (Figure 6) has a peak at the age of 6-7 years, just prior to the introduction of the maize and the rise in  $\delta^{13}\text{C}$ . This is very unlikely, given the historical and documentary evidence, to be caused by the introduction of a short-term protein-rich higher trophic level input into the diet, and so can be interpreted as a short-term period of stress related to the shortage of food which preceded the switch to maize, the Famine relief food. It appears that the  $\delta^{13}\text{C}$  values are a robust indicator of the dietary input to the dentine collagen, but that  $\delta^{15}\text{N}$  values are likely recording diet in addition to periods of physiological stress. A similar pattern of elevated  $\delta^{15}\text{N}$  values without a corresponding rise in  $\delta^{13}\text{C}$  values has also been observed in the incremental dentine profiles of Neolithic inhabitants of the Shetland Isles and stress resulting from crop failure was suggested as a possible explanation (Montgomery et al. 2013).

It can be seen from Figures 3 and 4 that the  $\delta^{15}\text{N}$  profiles of the M1s from juveniles who have died during the formation of the tooth and those who survived beyond it are different. Some of the juveniles with incomplete M1s have  $\delta^{15}\text{N}$  profiles which mimic the expected curve seen in Figure 1, but there is a range of different patterns, including flat profiles. Those who

have survived beyond childhood do not show any evidence for the expected breastfeeding peak in their  $\delta^{15}\text{N}$  dentine collagen profiles, and if the current interpretation method was used to explain these data, it would suggest that the individuals who survived childhood were not breastfed, whilst some of those who died during childhood were breastfed. Such an interpretation would be contrary to all the evidence that breastfeeding is healthier for infants.

The profiles from the deciduous teeth, from individuals who all died during the formation of these teeth, show more resemblance to Figure 1, although there are still individuals who do not match the breastfeeding and weaning pattern. The  $\delta^{15}\text{N}$  profiles from individuals from Neolithic and Iron Age Scotland (SUMB 41, 45 and HPCS 101, Figure 3 and SUMB 39, Figure 4) demonstrate that similar dietary information can be obtained from teeth from earlier archaeological periods and that differences between those who died during tooth formation and those who survived are not restricted to burials from nineteenth-century England and Ireland.

Given that each section of dentine from the M1 represents 8-9 months of life, it is possible that any rise in isotope ratios resulting from breastfeeding is lost due to averaging if the duration of breastfeeding is short or the range of variation in  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  is small. Each increment in the deciduous teeth represents about 4 months and thus short-term or small variations in  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values will be visible. In those cases where there is a clear change in the  $\delta^{15}\text{N}$  values over time, it is important to interpret this in light of the  $\delta^{13}\text{C}$  profile. Where the latter also shows a trophic level shift, this may be interpreted as breastfeeding. However, where  $\delta^{13}\text{C}$  values suggest that the changes in  $\delta^{15}\text{N}$  profile are not related to diet, then the effects of growth or stress must be considered. The complexity of the changes in maternal values during pregnancy discussed above will also affect the pre-natal portion of the dentine and, of course, changes in the mother's diet and physiology will continue to affect the breastfed infant via her milk. For example, a low *in-utero*  $\delta^{15}\text{N}$  value from a healthy pregnancy could be cancelled out

by the offset between mother/infant tissues observed at birth by de Luca et al. (2012), and a high *in-utero*  $\delta^{15}\text{N}$  value may produce a falling  $\delta^{15}\text{N}$  dentine profile as the post-natal diet changes over time which mimics the breastfeeding curve in Figure 1, even when there is no breastfeeding. Given the high turnover rate of juvenile bone (Valentin 2003) it is possible that the infants who are dying during early childhood are recording short-term elevations in  $\delta^{15}\text{N}$  bone collagen values as a result of the stress of childbirth, or their own experience of disease or malnutrition, *in addition* to any trophic level increase in the nitrogen isotope ratio of their mother's breastmilk. For the group of infants with varying  $\delta^{15}\text{N}$  dentine collagen profiles, these could be exhibiting changes which are not due to diet alone, particularly if the  $\delta^{13}\text{C}$  values do not co-vary. In the case of the individuals with flat profiles who have died, these may be juveniles whose dietary variation has not been sufficient to affect the  $\delta^{15}\text{N}$  mean for each increment, whose  $\delta^{15}\text{N}$  values have not been affected by stress and who have died accidentally or succumbed to an acute illness. When compared with the profiles of those who survived childhood, it appears that some of the individuals who have died in infancy and childhood are not representative of the collagen  $\delta^{15}\text{N}$  values of the total population at these ages.

### **Re-evaluating previous studies**

A re-examination of the previous studies which have used dentine sections to examine breastfeeding and weaning (Burt and Garvie-Lok 2013; Eerkens et al. 2011; Eerkens and Bartelink 2013; Henderson et al., 2014 Fuller et al. 2003) shows that their data also demonstrate considerable isotope variability during infancy. Fuller et al. (2003) use the means for each dentine section to match the current interpretation model, but if the data for each individual are analyzed, for five of the 21 deciduous teeth and two of the eight permanent teeth, the differences in  $\delta^{15}\text{N}$  values between the breastfeeding/weaning period and dentine formed after this period are either within analytical error, or the post-weaning value is higher. A different interpretation

of the data from Eerkens et al. (2011) could be that the individual who appears to have been breastfed for the shortest period (according to current interpretations of isotope data) is in fact a high-status individual who demonstrates a low  $\delta^{15}\text{N}$  value and possibly the lowest level of stress at the earliest age, in other words, a well-nourished and healthy infant. Eerkens and Bartelink (2013) produce dentine collagen profiles for 17 prehistoric individuals from California, including one (burial 180) who appears to have rising  $\delta^{15}\text{N}$  and falling  $\delta^{13}\text{C}$  values between 6 and 9 years of age. This was interpreted as early childhood diet, but could be a period of low trophic level foods (indicated by the low  $\delta^{13}\text{C}$ ) and nutritional distress (high  $\delta^{15}\text{N}$ ) during a period of hardship similar to the Irish Famine victim, KUW 4 (Figure 6). Of the 28 modern individuals whose deciduous teeth were sampled pre- and post-neonatal-line by Burt and Garvie-Lok (2013), 18 exhibit a downward shift in  $\delta^{15}\text{N}$  values after birth while 10 other individuals displayed elevated  $\delta^{15}\text{N}$  values or their values remained level. In their study the largest increase in  $\delta^{15}\text{N}$  values in one tooth was 5.1‰, and the largest decrease 3.9‰. This supports the hypothesis that there are changes in the  $\delta^{15}\text{N}$  values around birth in healthy individuals which are large enough to mask the changes in  $\delta^{15}\text{N}$  values due to breastfeeding. Burt and Garvie-Lok (2013) also note that these modern individuals achieve the expected population  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values at around the age of 12 months. Henderson et al. (2014) show potential sex differences in the dentine  $\delta^{15}\text{N}$  values between males and females, but acknowledge that this may be due to differences either in breastfeeding/weaning behavior, or an increased male susceptibility to nutritional deprivation. This may also be the case for primates, as similar sex differences have been described by Fahy et al. (2014) in the isotopic profiles in the dentine of wild chimpanzees.

In summary, because of the complexity of maternal physiology, the relationship between the isotope ratios of mother and infant, and the potential effects of stress on  $\delta^{15}\text{N}$  values, the



interpretation of breastfeeding and weaning behavior using the bone collagen stable isotope ratios of female adults and infants/juveniles is not as straightforward as assumed by the often-used mathematical model. Whilst it appears that the information contained in the dentine collagen profiles of the survivors and non-survivors of childhood do not always support the current weaning curve interpretation method, they offer new opportunities to estimate the health of pregnant mothers and juveniles in the archaeological record.

### **Estimating maternal health**

If the bone collagen of fetal/neonatal individuals is recording the mother's short-term diet and physiology during pregnancy, then this could be compared to the adult female mean in a population to estimate the health and dietary status of pregnant women.

Dentine in deciduous teeth starts to grow before birth and, like fetal bone, contains collagen which formed *in utero*. Thus, the first incremental sample of dentine (representing approximately four months of life) could be used as a proxy for the maternal short-term  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values. The first increment of the M1s used in this study contains a small amount of perinatal collagen, but will also contain up to nine months of post-natal collagen. The averaging of perinatal and post-natal collagen in this first increment will be the same for all individuals, so that the comparison between infant remains and adults who have survived childhood and still have an M1 present can give useful information about the health of the mothers of those children who died in childhood compared to the mothers whose children survived. The upper plot in Figure 9 shows the  $\delta^{15}\text{N}$  values of the first dentine increment of the deciduous teeth from Lukin Street compared with the adult female bone collagen mean  $\delta^{15}\text{N}$  value for the population and connects this to previously published bone collagen values at death (Beaumont et al. 2013a). From this plot it can be seen that all of the first increments are more than 1 SD higher

than the adult female bone collagen mean  $\delta^{15}\text{N}$  values, suggesting that all the mothers of these individuals had a short-term rise in their  $\delta^{15}\text{N}$  values. The upper plot in Figure 9 also shows how the bone collagen  $\delta^{15}\text{N}$  value is, in most cases, lower than the birth values from the first dentine increment which would confirm that a rise in the values due to breastfeeding is not visible for this group. The lower plot in Figure 9 compares the  $\delta^{15}\text{N}$  values from the first dentine increment for the M1s of the childhood survivors and non-survivors with their bone collagen (for adults the bone collagen value is shown as age 18). All of the survivors have first increment dentine collagen  $\delta^{15}\text{N}$  values within the adult female mean  $\pm 1\text{SD}$ , and the link to their bone collagen suggests remarkable homeostasis in their  $\delta^{15}\text{N}$  values throughout life. Non-survivors are more variable, and it is possible that some of the deaths may be due to susceptibility to disease because their childhood  $\delta^{15}\text{N}$  values suggest stress either from their mother during pregnancy or during the early perinatal period.

Figure 9 here

### **Is this a 19<sup>th</sup>-century phenomenon?**

The majority of the data presented in this paper are from two well-dated mid-19<sup>th</sup>-century British and Irish sites which were specifically chosen because of the short duration of cemetery use and the documentary evidence for the populations having suffered extreme dietary stresses. It is possible that the patterns seen are a result of both these extreme conditions, and the relatively short duration of breastfeeding reported in low socio-economic groups of this period. However, it is striking that similar patterns are also seen amongst the prehistoric humans presented for comparison.

## **CONCLUSION**

Whilst it is acknowledged that the dataset presented is small and the variations seen may be location or period specific it is clear that the results of this study do not support the current

method of interpreting isotope data for the estimation of the duration and timing of breastfeeding and weaning in cemetery populations using the bone collagen of adult females and juveniles. By increasing the time resolution of sampling, incremental dentine data indicate that the variables affecting the isotopic relationship between mother and child are ubiquitously more complex than the current interpretation method allows for. The findings of this study strongly suggest that a re-evaluation of previous studies would be useful to establish whether the interpretations based on bone collagen  $\delta^{15}\text{N}$  values to estimate breastfeeding and weaning behavior are robust. The dentine  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  profiling of a population for which a weaning curve has been produced from infant and female adult bone collagen could establish how isotope ratios vary in the juveniles through their whole life span, alongside a comparison with the childhood profiles of those who achieved adulthood. The evaluation of the patterns seen in the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of the individuals in this study requires further work with other populations and periods to exclude factors such as the consumption of high-trophic-level foods during pregnancy, and to build up a library of survivors and non-survivors of childhood to compare the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values at the same ages in the same populations. The effect on the stable isotope values of filtration of the bone collagen but not the dentine should also be evaluated.

A more focused study evaluating an archaeological population where a stable diet and healthier circumstances can be assumed could help to clarify the presence or absence of the overlying effects of stress on the  $\delta^{15}\text{N}$  values during childhood. Future work should also focus on modern, healthy, well-nourished children whose breastfeeding history is known as this could confirm the presence or absence of a recognizable breastfeeding and weaning profile in incrementally-sampled teeth. Incremental dentine analysis offers new opportunities to investigate the health of a population at a pivotal time of life: the perinatal period. By

establishing  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values at this point, the potential dietary and physiological status of mothers during pregnancy, together with childhood survivors and non-survivors, can be considered.

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Table 1 Information about tooth samples in this study

Site	Sample type	Sample number	Period	References	
<b>Lukin Street</b>			AD 1843-1854	Miles et al. (2013)	
	M1	LUK 1404			
	M1	LUK 419			
	M1	LUK 413			
	M1	LUK 1459			
	M1	LUK 47			
	M1	LUK 1212			
	M1	LUK 259			
	M1	LUK 695			
	DM1	LUK 567			
	DM1	LUK 316			
	DM1	LUK 431			
	DM2	LUK 613			
	DM2	LUK 923			
	DM2	LUK 1033			
	DM2	LUK 724			
	DM2	LUK 517			
DM2	LUK 955				
<b>Kilkenny Union workhouse</b>			AD 1847-1852	Geber (2012)	
	M1	KUW 1			
	M1	KUW 9			
	M1	KUW 12			
	M1	KUW 13			
	M1	KUW 16			
	M2	KUW 4			
	M2	KUW 14			
<b>Sumburgh cist</b>			Neolithic 3510 to 2660 BC	Montgomery et al. (2013)	
	M1	SUMB 41			Walsh et al. (2012)
	M1	SUMB 45			
	M1	SUMB 46			
	DM2	SUMB 39			
<b>High Pasture Cave</b>			Middle Iron Age, 1st-2nd century AD	Birch and Wildgoose (2010)	
M1	HPCS 101				

Table 2 Data and quality parameters for dentine samples in this study

Method 1	Sample and section number	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	Amt%C	Amt%N	C:N
*	LUK 1459 M1 1	13.1	-19.8	40.1	14.6	3.2
	LUK 1459 M1 2	12.5	-20.1	40.6	13.8	3.4
	LUK 1459 M1 3	12.3	-19.9	40.3	14.8	3.2
	LUK 1459 M1 4	12.1	-20.0	41.2	15.0	3.2
	LUK 1459 M1 5	12.4	-19.7	40.8	15.0	3.2
	LUK 1459 M1 6	11.9	-19.5	39.3	14.4	3.2
	LUK 1459 M17	11.7	-19.4	43.0	15.9	3.2
	LUK 1459 M1 8	11.7	-19.5	42.3	15.5	3.2
	LUK 1459 M1 9	11.7	-19.4	41.0	15.4	3.1
	LUK 1459 M1 10	11.6	-19.6	40.6	14.5	3.3
	LUK 1459 M1 11	11.4	-19.6	40.0	14.7	3.2
	LUK 1459 M1 12	11.3	-19.7	40.0	14.8	3.2
	LUK 1459 M1 13	11.1	-19.8	40.8	15.0	3.2
	LUK 1459 M1 14	11.1	-19.8	42.5	15.6	3.2
	LUK 1459 M1 15	11.4	-19.9	40.8	14.8	3.2
	LUK 1459 M1 16	11.3	-20.0	40.7	14.5	3.3
	LUK 1459 M1 17	11.4	-19.7	40.7	15.0	3.2
	LUK 1459 M1 18	11.3	-19.9	41.2	15.0	3.2
	KUW 16 M1 1	11.1	-18.3	31.3	11.7	3.1
	KUW 16 M1 2	10.8	-19.6	57.4	20.0	3.3
	KUW 16 M1 3	10.6	-19.8	55.0	20.4	3.1
	KUW 16 M1 4	10.6	-20.3	56.4	20.9	3.2
	KUW 16 M1 5	10.6	-21.0	55.1	19.5	3.3
	KUW 16 M1 6	10.7	-20.6	50.3	18.5	3.2
	KUW 16 M1 7	11.6	-19.3	52.5	19.2	3.2
	KUW 16 M1 8	11.8	-19.5	50.1	18.3	3.2
	KUW 9 M1 1	11.4	-20.8	11.2	4.5	2.9
	KUW 9 M1 2	11.3	-20.7	20.7	8.1	3.0
	KUW 9 M1 3	11.1	-20.8	26.8	10.5	3.0
	KUW 9 M1 4	11.0	-20.8	20.5	8.0	3.0
	KUW 9 M1 5	11.3	-20.8	6.6	2.6	3.0
	KUW 9 M1 6	11.5	-20.7	17.3	7.0	2.8
	KUW 9 M1 7	11.6	-20.7	16.7	6.7	2.9
	KUW 9 M1 8	11.5	-20.6	9.5	3.7	2.9
	KUW 9 M1 9	11.4	-20.5	25.6	10.1	3.0
	KUW 9 M1 10	11.0	-20.4	13.6	5.7	2.8
	KUW 9 M1 11	10.5	-20.5	16.5	6.6	2.9

KUW 9 M1 12	10.0	-20.6	13.6	5.6	2.8
KUW 9 M1 13	9.5	-20.7	7.9	3.4	2.7
KUW 9 M1 14	10.2	-20.4	11.9	5.0	2.8
KUW 13 M1 1	12.3	-18.3	31.8	11.5	3.2
KUW 13 M1 2	9.8	-16.4	37.8	13.7	3.2
KUW 13 M1 3	10.4	-16.5	28.3	10.4	3.2
KUW 13 M1 4	10.7	-17.6	27.9	10.3	3.2
KUW 13 M1 5	11.8	-18.8	34.3	12.6	3.2
KUW 13 M1 6	13.0	-19.7	49.8	18.4	3.2
KUW 13 M1 7	14.3	-19.9	35.3	12.8	3.2
LUK 419 M1 1	12.4	-19.4	41.3	14.4	3.3
LUK 419 M1 2	12.0	-19.1	41.4	15.0	3.2
LUK 419 M1 3	11.9	-19.1	42.1	15.4	3.2
LUK 419 M1 4	11.7	-19.1	39.6	14.4	3.2
LUK 419 M1 5	11.8	-19.3	42.4	15.4	3.2
LUK 419 M1 6	11.9	-19.3	43.1	15.7	3.2
LUK 419 M1 7	11.9	-19.4	42.4	15.4	3.2
LUK 419 M1 8	12.0	-19.5	40.8	14.9	3.2
LUK 419 M1 9	12.1	-19.5	41.2	15.0	3.2
LUK 419 M1 10	12.1	-19.5	41.6	15.1	3.2
LUK 419 M1 11	12.1	-19.8	40.5	14.6	3.2
LUK 419 M1 12	12.7	-19.7	39.2	14.3	3.2
*					
LUK 1404 M1 1	-19.7	12.5	38.4	13.8	3.2
LUK 1404 M1 2	-19.6	12.6	38.6	13.9	3.2
LUK 1404 M1 3	-19.7	12.6	39.9	14.4	3.2
LUK 1404 M1 4	-19.7	12.4	40.9	14.7	3.2
LUK 1404 M1 5	-19.6	12.4	37.6	13.7	3.2
LUK 1404 M1 6	-19.5	12.1	38.6	14.0	3.2
LUK 1404 M1 7	-19.6	11.7	39.8	14.6	3.2
LUK 1404 M1 8	-19.5	11.2	40.4	14.8	3.2
LUK 1404 M1 9	-19.6	10.9	38.7	14.2	3.2
LUK 1404 M1 10	-19.6	10.8	37.9	13.9	3.2
LUK 1404 M1 11	-19.7	10.8	36.1	13.3	3.2
LUK 1404 M1 12	-19.6	11.3	38.0	13.9	3.2
LUK 1404 M1 13	-19.7	11.3	38.8	14.1	3.2
LUK 413 M1 1	12.3	-19.7	36.5	12.3	3.5
LUK 413 M1 2	12.4	-19.5	37.9	13.9	3.2
LUK 413M1 3	12.2	-19.4	40.1	14.6	3.2
LUK 413M1 4	12.4	-19.0	38.8	14.4	3.1
LUK 413M1 5	12.3	-19.3	39.7	14.7	3.2
LUK 413 M1 6	11.9	-19.4	35.4	13.3	3.1



LUK 413 M1 7	11.7	-19.3	39.2	14.4	3.2
LUK 413 M1 8	11.5	-19.4	35.8	13.2	3.2
LUK 413 M1 9	11.5	-19.4	37.1	13.7	3.2
LUK 413 M1 10	11.6	-19.4	41.2	15.2	3.2
LUK 413 M1 11	11.6	-19.4	36.9	13.6	3.2
LUK 413 M1 12	11.7	-19.4	60.5	22.4	3.2
LUK 413 M1 13	11.6	-19.4	39.9	14.6	3.2
LUK 413 M1 14	11.0	-19.2	35.9	13.2	3.2
* LUK 47 M1 1	12.3	-20.0	39.3	12.9	3.5
LUK 47 M1 2	12.5	-20.4	40.6	14.8	3.2
LUK 47 M1 3	11.8	-20.1	40.5	14.5	3.3
LUK 47 M1 4	11.7	-20.0	41.8	15.2	3.2
LUK 47 M1 5	11.7	-20.0	36.0	13.0	3.2
LUK 47 M1 6	11.8	-20.0	62.5	22.7	3.2
LUK 47 M1 7	11.9	-20.0	40.3	14.7	3.2
LUK 47 M1 8	11.9	-19.9	35.9	13.0	3.2
LUK 47 M1 9	11.9	-19.9	33.5	11.0	3.5
LUK 47 M1 10	11.9	-19.9	37.2	13.5	3.2
LUK 47 M1 11	12.0	-19.8	36.9	13.4	3.2
LUK 47 M1 12	12.0	-19.8	40.0	14.5	3.2
LUK 47 M1 13	12.2	-19.9	39.7	14.1	3.3
LUK 47 M1 14	12.0	-19.8	39.2	14.1	3.2
LUK 47 M1 15	12.1	-19.9	40.6	14.8	3.2
LUK 47 M1 16	12.1	-20.0	41.1	15.0	3.2
KUW 12 M1 1	11.1	-19.7	43.7	15.2	3.4
KUW 12 M1 2	11.2	-19.6	43.3	15.2	3.0
KUW 12 M1 3	10.7	-19.4	36.5	13.6	2.9
KUW 12 M1 4	10.7	-19.3	36.5	13.4	3.0
KUW 12 M1 5	10.4	-19.1	47.0	17.5	3.1
KUW 12 M1 6	10.3	-18.6	38.3	14.2	3.2
KUW 12 M1 7	10.4	-18.8	50.4	19.0	3.2
KUW 12 M1 8	11.1	-19.7	33.1	12.3	3.5
KUW 1 M1 1	11.9	-20.2	41.2	14.9	3.2
KUW 1 M1 2	12.2	-19.7	39.5	14.6	3.2
KUW 1 M1 3	11.7	-20.0	49.4	18.0	3.2
KUW 1 M1 4	11.5	-19.9	48.8	17.8	3.2
KUW 1 M1 5	11.5	-19.9	42.2	15.5	3.2
KUW 1 M1 6	11.3	-20.0	38.4	14.2	3.2
KUW 1 M1 7	11.2	-20.0	41.0	15.0	3.2
KUW 1 M1 8	11.2	-20.1	45.4	16.7	3.2
KUW 1 M1 9	11.5	-19.9	41.7	15.4	3.2
KUW 1 M1 10	11.5	-19.9	40.4	15.0	3.2

	KUW 1 M1 11	11.4	-20.0	45.6	16.9	3.2
	KUW 1 M112	11.6	-19.9	47.1	17.5	3.1
	KUW 1 M1 13	11.5	-20.2	65.8	24.7	3.1
	KUW 1 M114	11.4	-20.4	41.9	15.5	3.2
*	LUK 1212 M1 1	15.4	-19.7	59.6	21.8	3.2
	LUK 1212 M1 2	13.1	-20.4	66.4	24.4	3.2
	LUK 1212 M1 3	11.2	-20.9	66.4	24.6	3.2
	LUK 1212 M1 4	10.4	-21.5	69.6	25.7	3.2
	LUK 1212 M1 5	10.0	-21.3	73.0	26.9	3.2
	LUK 1212 M1 6	9.7	-21.2	56.7	20.9	3.2
	LUK 1212 M1 7	9.4	-20.9	59.2	22.0	3.1
	LUK 1212 M1 8	9.6	-20.1	61.1	22.4	3.2
	LUK 1212 M1 9	10.0	-19.5	58.5	21.7	3.2
	LUK 1212 M1 10	10.7	-19.1	76.5	28.3	3.2
*	LUK 259 M1 1	13.4	-19.0	42.0	15.8	3.1
	LUK 259 M1 2	15.8	-18.8	41.0	15.4	3.1
	LUK 259 M1 3	15.6	-18.9	40.7	15.3	3.1
	LUK 259 M1 4	15.2	-18.9	42.1	16.0	3.1
	LUK 259 M1 5	13.9	-19.1	41.5	15.8	3.1
	LUK 259 M1 6	13.5	-19.3	43.7	16.7	3.1
	LUK 695 M1 1	16.2	-18.2	30.6	11.0	3.3
	LUK 695 M1 2	16.6	-18.0	40.9	15.1	3.2
	LUK 695 M1 3	15.8	-18.3	38.2	14.2	3.1
	LUK 695 M1 4	13.8	-18.9	52.2	19.5	3.1
	LUK 695 M1 5	13.5	-19.1	41.6	15.4	3.1
	LUK 695 M1 6	12.9	-19.1	61.5	23.1	3.1
	LUK 695 M1 7	12.6	-19.2	42.5	16.1	3.1
	LUK 695 M1 8	12.4	-19.3	40.9	15.4	3.1
	LUK 695 M1 9	12.3	-19.3	41.5	15.5	3.1
	LUK 695 M1 10	12.4	-19.3	41.7	15.7	3.1
	LUK 695 M1 11	12.4	-19.5	41.2	15.3	3.1
	LUK 695 M1 12	12.4	-19.5	41.4	15.4	3.1
	LUK 695 M1 13	12.4	-19.7	40.8	15.1	3.1
	LUK 695 M1 14	12.4	-19.7	40.6	15.0	3.2
	LUK 695 M1 15	12.6	-19.6	40.6	15.0	3.2
	LUK 695 M1 16	12.7	-19.5	40.4	14.9	3.2
*	LUK 1404 M1 1	12.5	-19.7	38.4	13.8	3.2
	LUK 1404 M1 2	12.6	-19.6	38.6	13.9	3.2
	LUK 1404 M1 3	12.6	-19.7	39.9	14.4	3.2
	LUK 1404 M1 4	12.4	-19.7	40.9	14.7	3.2
	LUK 1404 M1 5	13.4	-17.6	37.8	14.3	3.1

LUK 1404 M1 6	12.1	-19.5	38.6	14.0	3.2
LUK 1404 M1 7	11.7	-19.6	39.8	14.6	3.2
LUK 1404 M1 8	11.2	-19.5	40.4	14.8	3.2
LUK 1404 M1 9	10.9	-19.6	38.7	14.2	3.2
LUK 1404 M1 10	10.8	-19.6	37.9	13.9	3.2
LUK 1404 M1 11	10.8	-19.7	36.1	13.3	3.2
LUK 1404 M1 12	11.3	-19.6	38.0	13.9	3.2
LUK 1404 M1 13	11.3	-19.7	38.8	14.1	3.2

Method 2	Sample and section number	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	Amt%C	Amt%N	C:N
	LUK 316 DM1 1	14.6	-20.1	42.5	13.0	3.8
	LUK 316 DM1 2	15.8	-18.6	42.7	15.2	3.3
	LUK 316 DM1 3	16.1	-18.4	42.8	15.4	3.2
	LUK 316 DM1 4	16.3	-18.3	43.1	15.5	3.2
	LUK 316 DM1 5	16.4	-18.1	42.3	15.4	3.2
	LUK 316 DM1 6	16.1	-18.1	42.2	15.3	3.2
	LUK 316 DM1 7	16.1	-18.0	41.9	15.2	3.2
	LUK 316 DM1 8	15.2	-18.5	41.1	14.5	3.3
	LUK 567 DM1 1	15.2	-18.0	42.1	15.3	3.2
	LUK 567 DM1 2	15.5	-18.0	40.9	15.1	3.2
	LUK 567 DM1 3	15.3	-17.9	42.7	15.7	3.2
	LUK 567 DM1 4	14.9	-17.8	43.1	15.8	3.2
	LUK 567 DM1 5	14.9	-17.7	42.2	15.4	3.2
	LUK 567 DM1 6	15.2	-17.6	41.6	15.2	3.2
	LUK 567 DM1 7	15.2	-18.0	42.5	15.4	3.2
	LUK 431 DM1 1	0.0	0.0	0.0	0.0	0.0
	LUK 431 DM1 2	16.4	-18.3	41.0	15.1	3.2
	LUK 431 DM1 3	16.4	-18.2	40.7	15.2	3.1
	LUK 431 DM1 4	15.6	-18.3	48.3	18.1	3.1
	LUK 431 DM1 5	15.4	-18.6	41.5	15.6	3.1
	LUK 431 DM1 6	14.8	-18.8	42.0	15.8	3.1
	LUK 431 DM1 7	14.3	-19.1	41.6	15.6	3.1
	LUK 431 DM1 8	14.2	-19.6	42.2	15.4	3.2
	LUK 517 DM2 1	15.6	-17.5	43.1	15.9	3.2
	LUK 517 DM2 2	15.4	-18.1	43.0	15.8	3.2
	LUK 517 DM2 3	14.2	-18.5	41.0	15.0	3.2
	LUK 517 DM2 4	14.5	-18.5	42.0	15.3	3.2
	LUK 517 DM2 5	14.5	-18.5	41.8	15.3	3.2
	LUK 517 DM2 6	14.5	-18.5	43.2	15.9	3.2
	LUK 724 DM2 1	14.6	-19.0	42.0	15.5	3.2

LUK 724 DM2 2	13.7	-19.3	42.3	15.6	3.2
LUK 724 DM2 3	13.1	-19.5	41.7	15.4	3.2
LUK 724 DM2 4	12.8	-19.7	43.4	16.1	3.1
LUK 724 DM2 5	12.6	-19.8	41.8	15.5	3.1
LUK 724 DM2 6	12.6	-19.8	43.3	16.0	3.1
LUK 724 DM2 7	12.5	-19.7	42.5	15.8	3.1
LUK 724 DM2 8	12.6	-19.5	42.7	15.8	3.2
LUK 724 DM2 9	12.7	-19.8	46.0	16.9	3.2
LUK 613 DM2 1	14.6	-18.6	24.3	9.0	3.1
LUK 613 DM2 2	14.3	-18.7	42.1	15.9	3.1
LUK 613 DM2 3	13.9	-18.7	41.8	15.8	3.1
LUK 613 DM2 4	13.2	-18.8	42.0	16.0	3.1
LUK 613 DM2 5	12.8	-18.8	42.2	16.0	3.1
LUK 613 DM2 6	12.0	-18.9	42.4	16.1	3.1
LUK 613 DM2 7	11.7	-19.1	41.1	15.5	3.1
LUK 613 DM2 8	11.6	-19.1	42.6	16.0	3.1
LUK 613 DM2 9	11.4	-19.0	41.8	15.8	3.1
LUK 613 DM2 10	11.4	-19.0	41.6	15.7	3.1
LUK 613 DM2 11	11.3	-18.9	41.7	15.7	3.1
LUK 613 DM2 12	11.4	-19.1	41.8	15.7	3.1
LUK 923 DM2 1	15.7	-18.5	41.8	15.3	3.2
LUK 923 DM2 2	15.4	-18.3	38.2	14.3	3.1
LUK 923 DM2 3	14.2	-19.0	44.7	16.8	3.1
LUK 923 DM2 4	13.0	-19.6	41.7	15.6	3.1
LUK 923 DM2 5	12.6	-19.5	39.6	14.8	3.1
LUK 923 DM2 6	12.5	-19.6	44.6	16.6	3.1
LUK 923 DM2 7	12.5	-19.5	38.3	14.2	3.1
LUK 923 DM2 8	12.4	-19.5	41.4	15.6	3.1
LUK 923 DM2 9	12.6	-19.7	42.4	15.9	3.1
LUK 955 DM2 1	16.8	-17.9	43.9	15.8	3.2
LUK 955 DM2 2	17.2	-17.8	40.6	14.6	3.2
LUK 955 DM2 3	17.6	-17.9	44.9	16.3	3.2
LUK 955 DM2 4	16.8	-17.9	44.3	16.0	3.2
LUK 955 DM2 5	17.8	-18.1	44.4	16.0	3.2
LUK 955 DM2 6	18.2	-18.1	44.6	16.2	3.2
LUK 955 DM2 7	18.6	-18.1	45.1	16.3	3.2
HPCS 101 M1 1	11.8	-21.6	40.9	15.3	3.1
HPCS 101 M1 2	11.4	-21.6	40.1	15.1	3.1
HPCS 101 M1 3	11.1	-21.7	41.7	15.7	3.1
HPCS 101 M1 4	11.0	-21.4	41.6	15.4	3.2
HPCS 101 M1 5	11.0	-21.8	41.5	15.6	3.1

HPCS 101 M1 6	10.5	-21.2	42.0	15.8	3.1
HPCS 101 M1 7	10.7	-21.2	41.8	15.8	3.1
HPCS 101 M1 8	10.7	-21.1	43.1	16.1	3.1
HPCS 101 M1 9	10.4	-21.1	40.4	15.0	3.1
HPCS 101 M1 10	10.6	-21.0	42.0	15.7	3.1
HPCS 101 M1 11	10.7	-20.9	41.9	15.6	3.1
HPCS 101 M1 12	10.7	-21.1	42.7	15.8	3.2
HPCS 101 M1 13	10.9	-20.9	41.7	15.4	3.2
HPCS 101 M1 14	11.0	-21.0	43.6	16.0	3.2
HPCS 101 M1 15	11.1	-21.1	42.7	15.5	3.2
HPCS 101 M1 16	11.3	-21.0	40.7	14.7	3.2
HPCS 101 M1 17	11.4	-21.2	42.1	15.3	3.2
HPCS 101 M1 18	11.2	-20.9	41.8	15.1	3.2
HPCS 101 M1 19	11.0	-21.0	42.9	15.4	3.2
HPCS 101 M1 20	11.3	-21.5	43.8	15.2	3.4
LUK 1033 M1 1	15.1	-19.0	53.7	19.6	3.2
LUK 1033 M1 2	15.7	-19.1	57.3	21.4	3.1
LUK 1033 M1 3	15.2	-19.3	58.7	21.9	3.1
LUK 1033 M1 4	14.2	-19.3	58.6	22.0	3.1
LUK 1033 M1 5	13.9	-19.4	60.2	22.7	3.1
LUK 1033 M1 6	13.6	-19.6	58.8	22.0	3.1
LUK 1033 M1 7	13.5	-19.4	58.8	22.1	3.1
LUK 1033 M1 8	13.5	-19.3	59.6	22.4	3.1
LUK 1033 M1 9	13.6	-19.2	56.5	21.1	3.1
LUK 1033 M1 10	13.6	-19.3	60.0	22.4	3.1
LUK 1033 M1 11	13.6	-19.3	57.8	21.5	3.1
KUW 14 M2 1	11.3	-20.6	40.7	15.0	3.2
KUW 14 M2 2	11.2	-20.0	41.1	15.2	3.2
KUW 14 M2 3	10.9	-19.7	41.5	15.3	3.2
KUW 14 M2 4	11.2	-20.0	41.4	15.3	3.2
KUW 14 M2 5	11.0	-19.2	41.4	15.2	3.2
KUW 14 M2 6	11.1	-18.1	41.9	15.3	3.2
KUW 14 M2 7	11.1	-17.0	41.7	15.1	3.2
KUW 4 M2 1	10.1	-21.0	59.9	22.2	3.1
KUW 4 M2 2	10.1	-20.9	58.3	21.9	3.1
KUW 4 M2 3	9.2	-21.3	58.2	21.9	3.1
KUW 4 M2 4	9.8	-21.4	59.2	22.2	3.1
KUW 4 M2 5	10.5	-21.3	57.4	21.6	3.1
KUW 4 M2 6	10.7	-21.2	58.0	21.8	3.1
KUW 4 M2 7	10.2	-21.0	56.2	21.1	3.1
KUW 4 M2 8	10.1	-21.2	55.2	20.6	3.1
KUW 4 M2 9	9.9	-20.6	67.3	25.1	3.1

KUW 4 M2 10	9.7	-20.1	61.0	22.9	3.1
KUW 4 M2 11	9.9	-19.0	57.9	21.5	3.1
KUW 4 M2 12	10.0	-17.6	59.9	22.6	3.1
KUW 4 M2 13	9.9	-15.5	58.2	21.8	3.1
KUW 4 M2 14	10.0	-14.7	58.7	21.4	3.2

- Denotes data originally published in Beaumont et al.(2013b)

Figure 1 Isotopic trend for infants and young children showing expected pattern for bone collagen  $\delta^{15}\text{N}$  when plotted against age for a period of breastfeeding followed by weaning. The dotted line represents the average value for adult females (after Schurr 1997; Millard 2000 ; Jay et al . 2008)

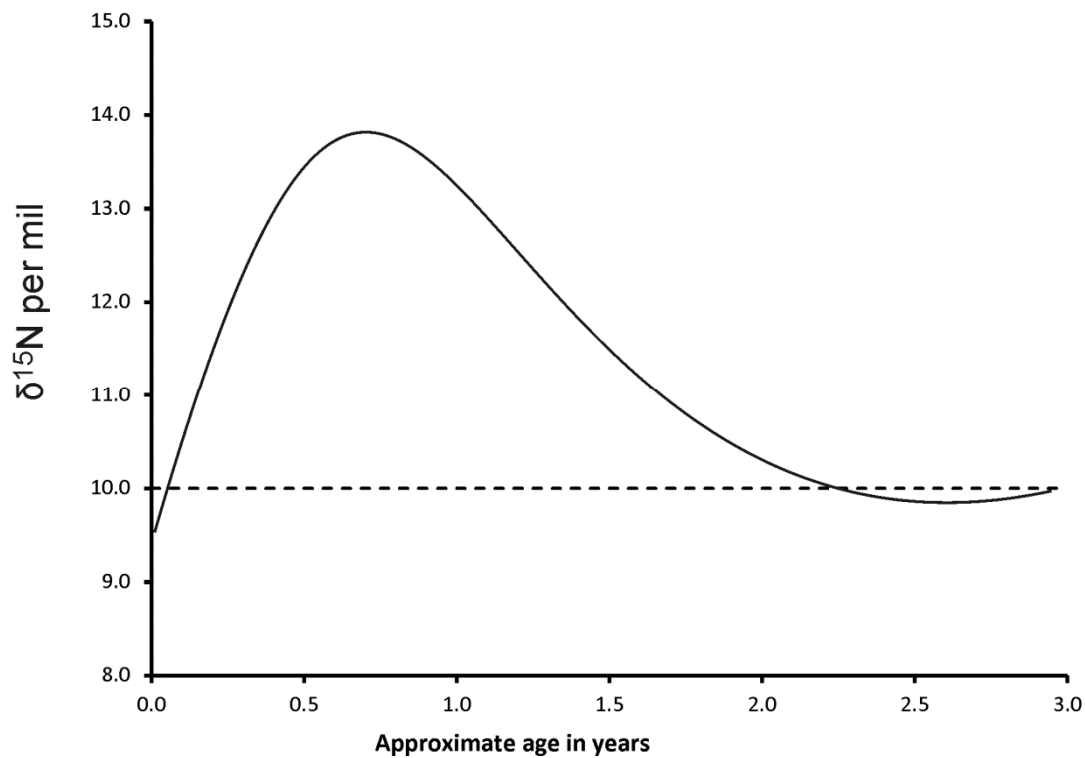




Figure 2 Map showing British and Irish sites included in this study.

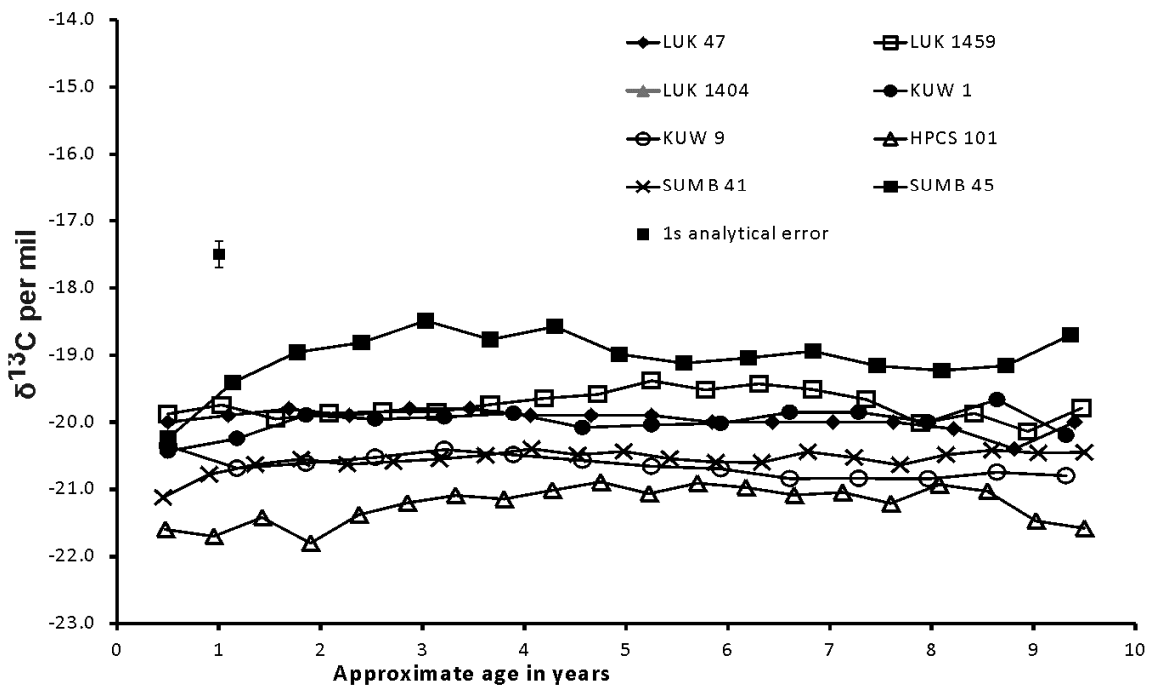
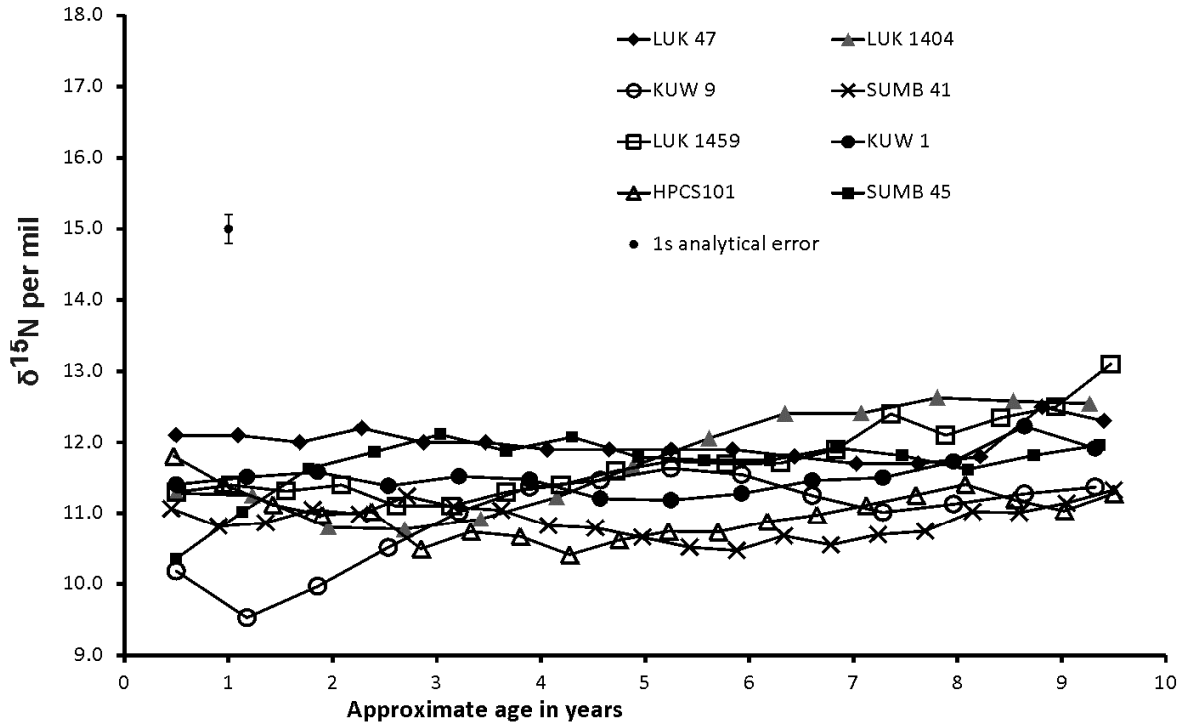


Figure 3  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values of incremental dentine collagen against age for M1 of individuals who lived beyond root completion. Data from Sumburgh Cist, Lukin Street, Kilkenny Union workhouse and High Pasture Cave



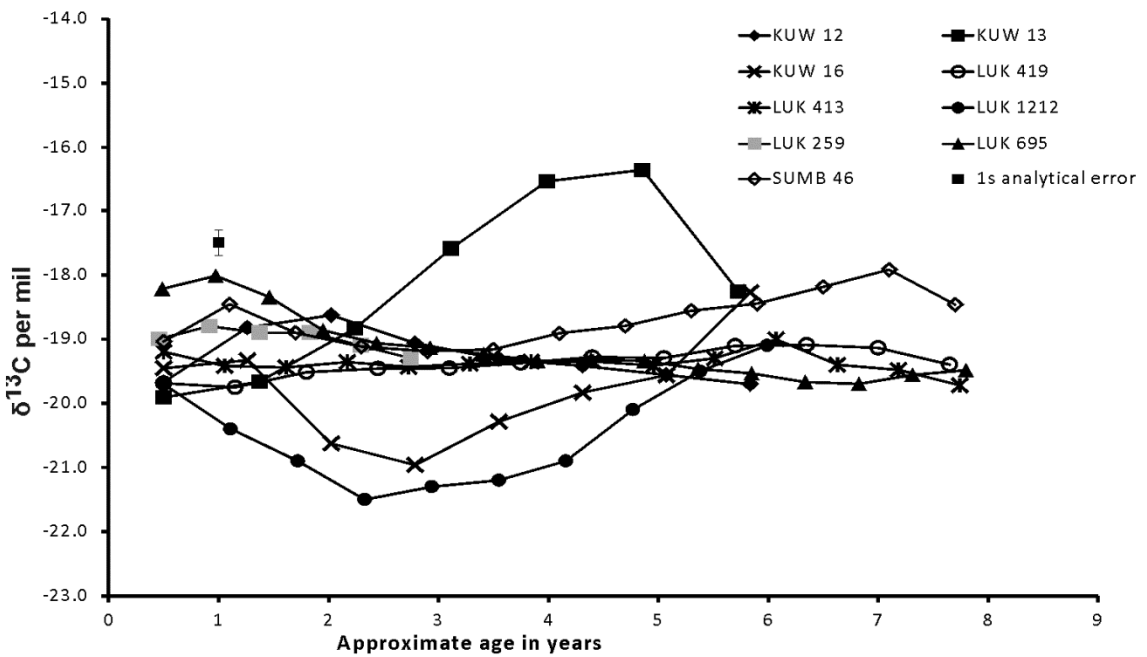
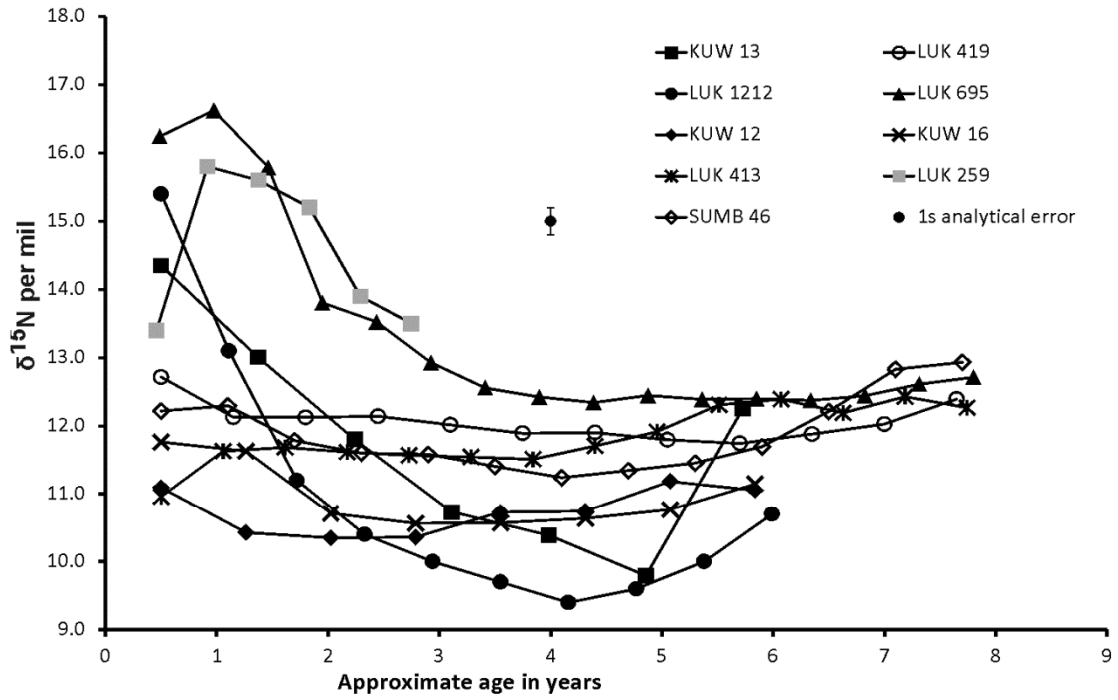


Figure 4  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values of incremental dentine collagen against age for M1 of individuals who died before root completion. Data from Sumburgh Cist, Lukin Street and Kilkenny Union workhouse

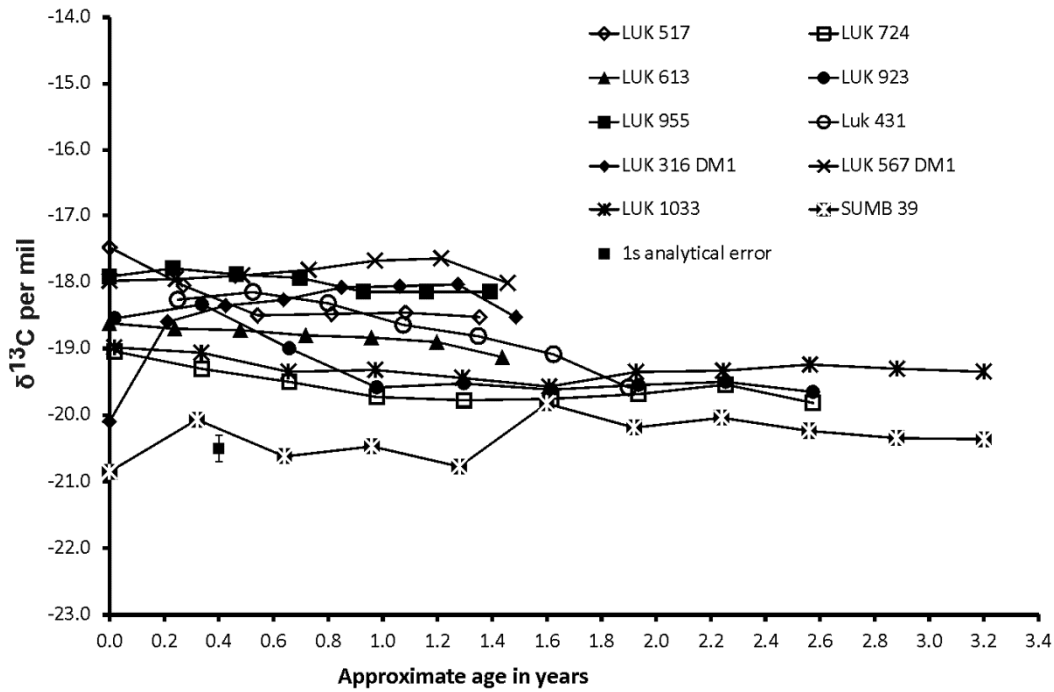
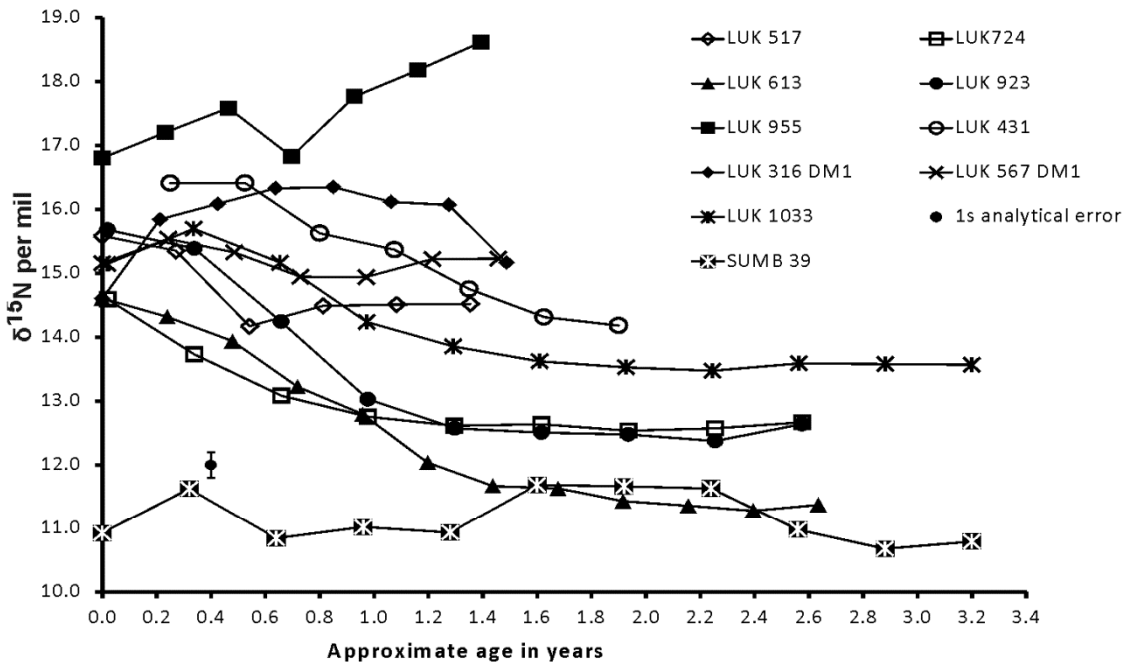


Figure 5  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values of incremental dentine collagen against age for deciduous teeth of individuals who died before root completion. Data from Sumburgh Cist and Lukin Street

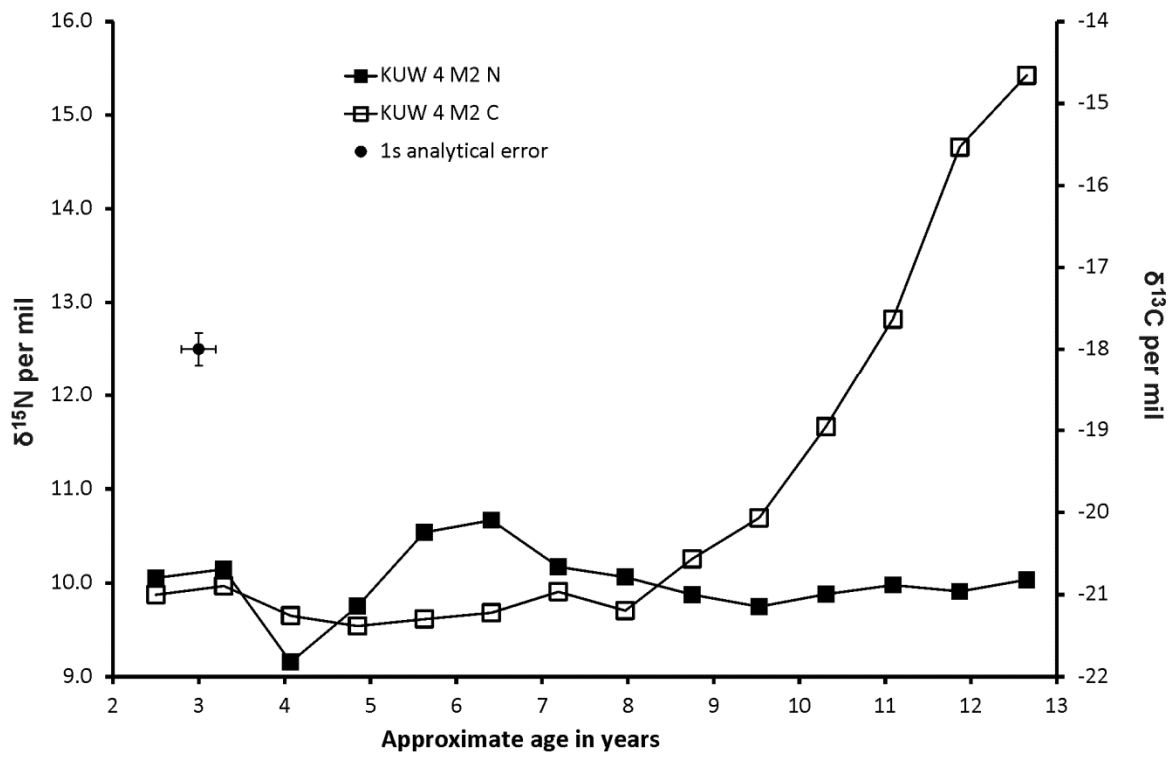


Figure 6  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of dentine sections against age for M2, KUW 4

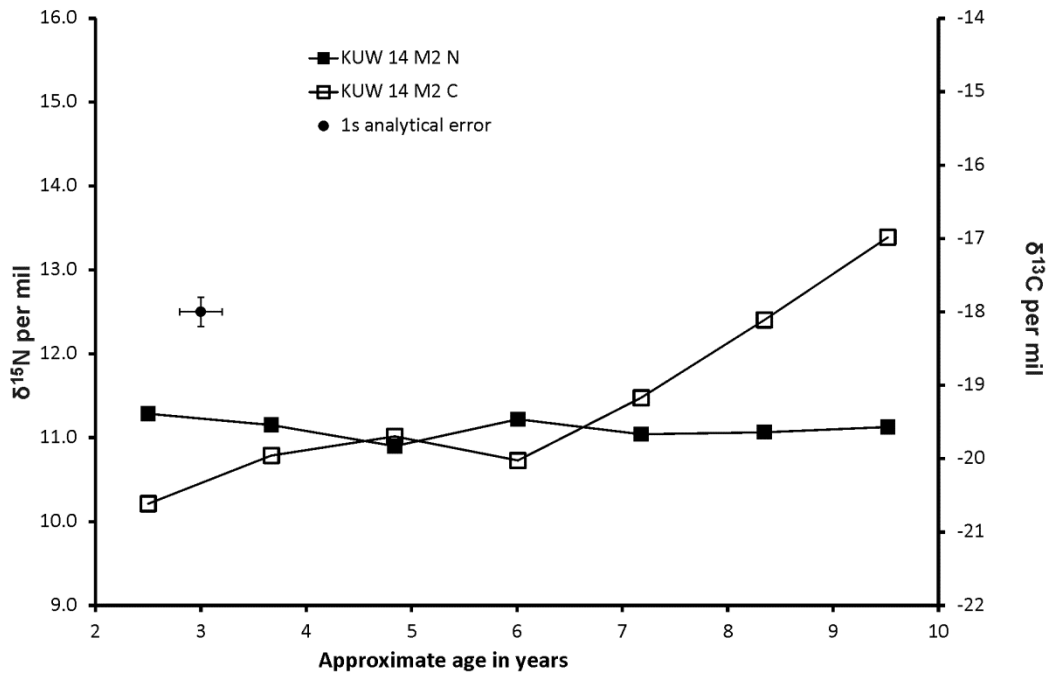


Figure 7 δ<sup>13</sup>C and δ<sup>15</sup>N values of dentine sections against age for M2, KUW 14

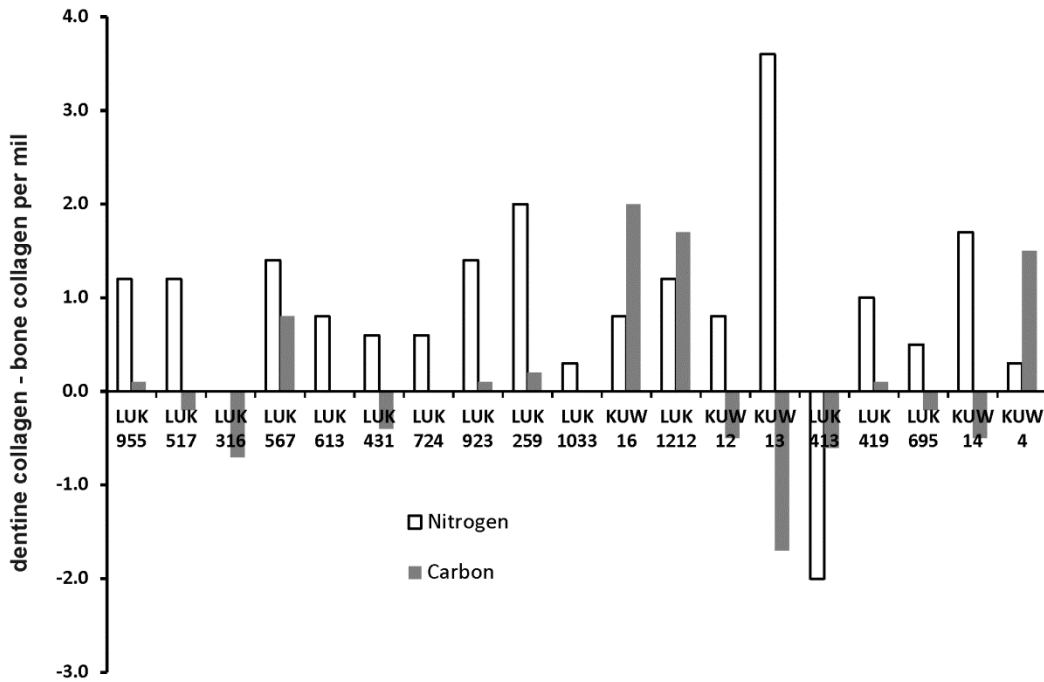


Figure 8 Plot showing the difference in δ<sup>13</sup>C and δ<sup>15</sup>N collagen values between the final dentine increment and bone collagen for juveniles from Lukin Street and Kilkenny Union workhouse

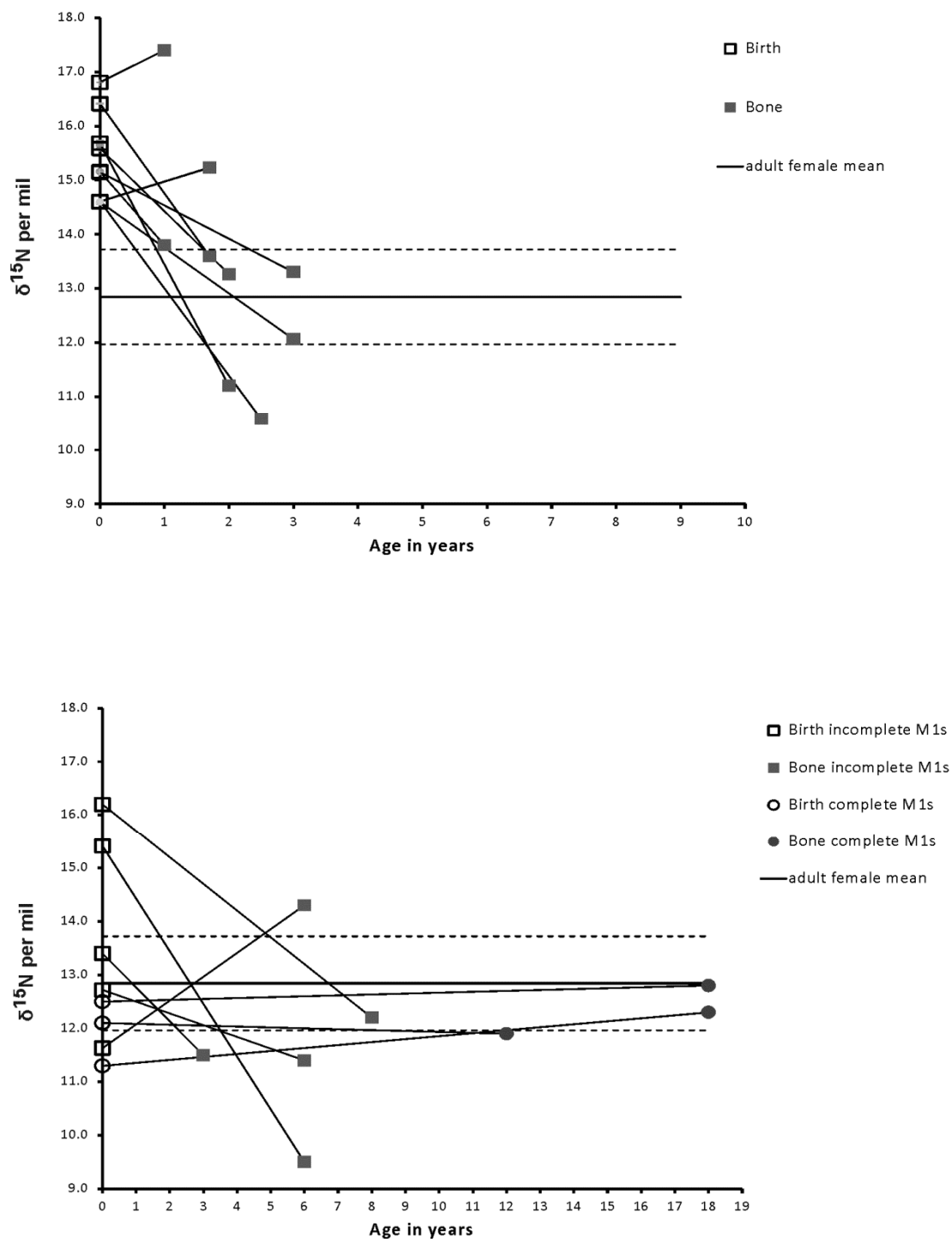


Figure 9 Plots showing  $\delta^{15}\text{N}$  birth values and bone collagen  $\delta^{15}\text{N}$  values against age for individuals from Lukin Street. Shaded area denotes adult female mean and 1sd bone collagen values. In the upper plot birth is represented by the first dentine increment from the deciduous molar, in the lower plot birth is represented by the first dentine increment from M1.