bradscholars

The whole tooth and nothing but the tooth: or why temporal resolution of bone collagen may be unreliable

Item Type	Article
Authors	Beaumont, Julia
Citation	Beaumont J (2020) The whole tooth and nothing but the tooth: or why temporal resolution of bone collagen may be unreliable. Archaeometry. 62(3): 626-645.
DOI	https://doi.org/10.1111/arcm.12544
Rights	© 2020 Wiley This is the peer reviewed version of the following article: Beaumont J (2020) The whole tooth and nothing but the tooth: or why temporal resolution of bone collagen may be unreliable. Archaeometry. 62(3): 626-645, which has been published in final form at https://doi.org/10.1111/arcm.12544. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Self-Archiving.
Download date	2025-05-21 03:30:17
Link to Item	http://hdl.handle.net/10454/17648

Beaumont Julia (Orcid ID: 0000-0002-7166-5857)

The whole tooth and nothing but the tooth: or why temporal resolution of bone collagen may be unreliable.

Author: Dr Julia Beaumont[1]

[1]School of Archaeological and Forensic Sciences, University of Bradford, UK

ABSTRACT

The carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratios of human bone collagen have been used extensively over the last 40 years to investigate the diet of past populations. It has become apparent that bone collagen can give an unreliable temporal dietary signature especially in juveniles. With higher temporal resolution sampling of collagen from tooth dentine, it is possible to identify short-term changes in diet previously invisible in bone. This paper discusses the inherent problems of using bone collagen for dietary studies and suggests better sample choices which can make our interpretations more robust, using breastfeeding and weaning as an example.

KEYWORDS: isotopes, temporal resolution, cohort, bone collagen, dentine, stress, weaning

INTRODUCTION

As bioarchaeologists, we aim to use the data which we collect to investigate the life-histories of individuals and populations in the past in the same way as modern human biologists. However we have some inherent problems with our datasets. Public health researchers and social scientists have been aware for the last 80 years (Bell and Jones 2013) that they combine data from both individuals and populations: and that there are effects on the data and health outcomes dependent on when an individual was born. This can be thought of as "age-period-cohort" effects as described in Suzuki (2012). Not only does the age of an individual have an effect on the biological data, so does the period in which they are living, and the cohort with which they grow up: ideally all would be taken into account (Bell and Jones 2013). An historical example would be people who died during the Black Death (1347-1351) in 14th century London. All age groups are represented within the cemetery population, and they are buried within the same short period of the plague epidemic. However, older individuals will be a cohort who experienced the Great Famine of 1315-1317, which killed as much as 10 percent or more of the population of England (Kershaw 1973) and was followed by the Great Bovine Pestilence (1319-1320), which caused decades-long milk scarcities (DeWitte and Slavin 2013; Kershaw 1973): their diet and health at that period may have affected their likelihood to survive the later epidemic compared with younger individuals with a better-nourished childhood (DeWitte 2015).

Our ability to know these three parameters in any detail will be limited to the recovered skeletal material to which we have access. Age is usually an estimated value based on biological features of the bone and is less accurate in older individuals (Buckberry 2016). The period we are examining can be the whole duration of cemetery usage, from a few years to many hundreds. The cohort from whom each individual is drawn could be very different, depending on their life experiences such as famine, epidemic infections, wars and migration events. Buried together without epigraphic or reliable dating evidence, we have little chance of separating individuals into period and cohort groups, and even then, we cannot be sure that the small, opportunistic samples we find are truly representative of the population we are studying. This paper discusses the current practice of using bone collagen isotope ratio data to infer population diet and behaviour, the potential offered by using tooth dentine instead, and suggests better sample choices which can make our interpretations more robust.

CHALLENGES IN BONE ISOTOPIC STUDIES

In a review paper Lee-Thorp (2008) summarised the pathway research had taken over the preceding 30 years leading to established methods and interpretations of $\delta^{13}C$ and $\delta^{15}N$ in studies of human nutrition in the past. The advantage of analysing the skeletal and dental remains of humans is that "it reflects the foods actually eaten by and individual, or group of individuals" (Lee-Thorp 2008). It is tempting to think that the early researchers had found universal isotopic values for foodstuffs of different types, and that all the values which were found in bone collagen of fauna and humans would follow a simple relationship: thus their diet could be identified by simply looking at their relative position on a graph representing the trophic level shifts in a food web (Richards and Hedges 1999)(and see Figure 1). However, it is obvious that the actual relationship between diet and bone collagen $\delta^{15}N$ and $\delta^{13}C$ is more complex: the results do not always reflect the archaeological evidence (although this may be biased by the available materials) (Craig et al. 2009; Montgomery et al. 2013; Richards and Hedges 1999), and there is a huge amount of variation between individuals within a population who appear to have consumed the same diet. Human bone collagen datasets from a given population will produce a "cloud" of data from the subjects, which suggests that individuals will choose to consume a different diet (Turner and Thompson 2013), have differential entitlement to foods (Müldner and Hedges 2007; Müldner et al. 2009; Sen 1981), or because of unknown biological variation the food they eat is processed in a different way by each person, or a combination of these. Our interpretations depend on knowing whether the cross-sectional data truly represent the population, and the accurate identification of outliers: are these migrants to the area, or are there missing data points between them and the other individuals which would mean their inclusion in the population? Making the wrong assumptions could mean a skewed mean value and alter the interpretations of the data. There is an argument for revisiting the established methods in order to produce a more nuanced interpretation of the data.

Temporal resolution

The study of diet in the past through the analysis of bone collagen from adults in a population has been based on some assumptions about the timing of collagen deposition. Bone collagen $\delta^{15}N$ and $\delta^{13}C$ reflects the protein portion of the diet (Ambrose and Norr 1993; Lee-Thorp et al. 1989; Tieszen and Fagre 1993) and is best used in context with analysis of faunal remains from the same site and period (Huelsemann et al. 2013). However, $\delta^{13}C$ and $\delta^{15}N$ in bone collagen reflect the main sources of protein consumed at the time that *new* bone is forming (Ambrose and DeNiro 1986; Hedges and

Reynard 2007; Sealy et al. 1995; van der Merwe and Vogel 1978). Reference values for bone turnover rates show that collagen will record varying times of life depending on the bone type and age of the individual (Valentin 2003). Sealy *et al.* (1995) analysed collagen from five African individuals from different periods, using two teeth forming at different times of life, along with collagen from bones which have different rates of turnover, long bone and rib (Sealy et al. 1995). In combination with enamel strontium isotope ratios, these were used these to produce "lifeways" for the individuals, giving insight into their possible migratory and dietary histories. Other studies have also used parts of the skeleton which have differing turnover rates to achieve temporal resolution (Bell et al. 2001; Hedges et al. 2007).

Physiological changes will also have an effect on the isotope ratios of new collagen. The recorded values may vary depending upon the use of the protein during a period of growth and the recycling of body tissues (catabolism) where there is undernutrition. A study by Lidén and Angerbjörn of the isotopic effects of maternal dormancy on the tissues of cave bear cubs (1999) demonstrates the importance of understanding the effects of growth and metabolism on the tissues of offspring. Waters-Rist and Katzenberg (2010) discussed the potential effects of growth, pregnancy and nutritional/physiological stress on both positive and negative nitrogen balance (catabolism and anabolism). They compared the $\delta^{13}C$ and $\delta^{15}N$ of bone collagen taken from the epiphyses (areas of bone which were actively growing) with areas of compact bone from the diaphyses (where turnover would be slower) of juvenile long bones, and found that the expected effects of growth (a reduction in $\delta^{15}N$ values) could not be identified using bone collagen (Waters-Rist and Katzenberg 2010). Nitsch et al.(2010) investigated the effect of pregnancy on adult bone nitrogen balance. A study on modern individuals by Fuller *et al.*(2004) demonstrated that during normal pregnancies a reduction in $\delta^{15}N$ in hair of mothers could be detected. Nitsch *et al.* (2010) found that they could not identify changes in $\delta^{15}N$ of bone collagen from mothers who were known from the burial records to have had multiple pregnancies. Both studies (Nitsch et al. 2010; Waters-Rist and Katzenberg 2010) conclude that the bone turnover rate in adults is too slow to record the transitory, short-term changes in the isotope values which were identified in hair studies and more recently in incremental dentine (Beaumont and Montgomery 2016). Katzenberg and Lovell (1999) investigated the differences between healthy and pathological bone in modern humans, with differences identifiable only in a patient who suffered a long-term wasting condition.

One potential explanation is that bone may cease growing under some circumstances (Cameron 2012, 51-53), meaning any dietary changes will not be recorded in a period of arrested growth, and as an adult gets older, by osteopenia and osteoporosis (Lane 2006). Smith and Rennie (2007) found that bone collagen synthesis speeded up during feeding in humans which may affect the rate of incorporation of

new proteins. In adults suffering the nutritional disease pellagra, the lack of new bone growth has been used as a diagnostic histological feature (Brenton and Paine 2007; Paine and Brenton 2006). In juveniles, where co-forming dentine and bone do not appear to be recording the same isotopic values, it is possible that there is a "threshold" of stress (where nutrition is inadequate for the prevailing conditions, be they intrinsic or extrinsic) above which bone ceases to grow while teeth, because they are evolutionarily important and buffered (Elamin and Liversidge 2013) continue to form and record isotopic changes (Beaumont et al. 2018). This would also explain the lack of isotopic differences in pathological bone, or the epiphyses of growing bones, as new bone may only form in favourable low-stress conditions. Stunting in archaeological juveniles can be identified where there is a difference between the skeletal age assessment and age determined from dental development (rather than eruption which is less reliable (Manjunatha and Soni 2014)) suggesting again that bone collagen production will be reduced in these individuals, and catch-up growth takes place when nutrition improves (Norgan et al. 2012, 145). Both of these can confound estimation of the period of life represented by the isotope ratios in the bone.

Recent statistical applications to the published bone collagen data have provided evidence that chronic physiological changes do appear to be recorded in the isotope ratios. Hazard analysis applied to 650 adult bone collagen $\delta^{13}C$ and $\delta^{15}N$ values from Roman Britain suggest that high $\delta^{15}N$ and low $\delta^{13}C$ are associated with mortality risk (Redfern et al. 2019). The changes in the isotopic values found in the individual with a prolonged wasting disease by Katzenberg and Lovell may be another example, as may the elevated $\delta^{15}N$ in 19th century London populations when compared to other contemporaneous English sites (Beaumont et al. 2013a).

Bone collagen in juveniles

For some isotopic studies, bone collagen from juveniles was avoided as it was considered likely to be affected by breastfeeding, or may represent a juvenile rather than adult diet. The assumption of an effect from breastfeeding has, however, allowed researchers to attempt to estimate breastfeeding and weaning practices in past populations using the bone of neonates and infants (e.g. (Jay et al. 2008; Mays et al. 2002; Nitsch et al. 2011) although this has relied upon certain assumptions about the mother during pregnancy, and that the dead infants from whom the bone is sampled represent the population as a whole (Beaumont et al. 2015; DeWitte and Stojanowski 2015; Wood et al. 1992)(and see later in this paper).

Given the disadvantages inherent in bone collagen isotopic analyses (bone turnover rates, the effect of undernutrition and growth) and combining cross-sectional data from individuals in a population who may have experienced very different lifeways, the use of incremental dentine samples offers some solutions.

Temporal resolution

We are able to analyse a tissue that forms in a regular, incremental way (Dean and Scandrett 1995) at a time of life which we can estimate much more accurately than the bone. We know that teeth continue to form even under conditions of undernutrition (Elamin and Liversidge 2013) and that the timing of initiation and duration of growth of human teeth is reliable for all periods and geographical origins (AlQahtani et al. 2010; AlQahtani et al. 2014; Dean et al. 2014). We can also analyse the data from an individual throughout the formation period of the tooth to identify any changes in diet and physiology (Beaumont et al. 2014; Beaumont and Montgomery 2016; Craig-Atkins et al. 2018; Eerkens et al. 2011; Fuller et al. 2003; Henderson et al. 2014) and relate these to the approximate age at which they experienced these. Of especial interest is the first forming 0.5mm of dentine in deciduous teeth which is laid down during the third trimester of pregnancy and thus records the maternal/infant diet and physiology (Beaumont et al. 2018). The methods used by all researchers still include some blurring of the data because of the overlapping developmental layers sampled by horizontal sectioning of the teeth (Beaumont et al. 2013b; Eerkens et al. 2011; Henderson et al. 2014; King et al. 2018), but is a great improvement on the problems associated with bone turnover.

Cohort and longitudinal study using dentine collagen

Using incremental dentine collagen isotope profiles will address some of the requirements of a human biological perspective. Each individual is their own longitudinal study, and where these data can be linked to known events, those with matching profiles can form a cohort. For example, the children who died in the Kilkenny Workhouse, Ireland, during the Great Irish Famine experienced a similar pattern of nutritional distress followed by the introduction of a maize-based diet. This was visible in the variations in δ^{13}C which allowed their dentine profiles to be arranged in chronological order using the known parameters of the introduction of maize and the end use of the cemetery. Furthermore, it was apparent that the δ^{13}C and δ^{15}N followed a distinct pattern of opposing covariance just prior to the relief food, similar to those seen in the hair of starvation victims (D'Ortenzio et al. 2015; Fuller et al. 2005; Neuberger et al. 2013)(Figure 2). When compared with the

dentine collagen isotope profiles of the adults from the same cemetery, the differences in not only diet, but the variability of the isotope ratios could be seen (Beaumont and Montgomery 2016). The adults had experienced a much less varied diet and little or no variation attributable to nutritional distress, apart from one individual who had markers for both nutritional deprivation and the introduction of maize, but who died as an adult. Historical research showed that maize had been introduced for a short period into the Kilkenny area 30 years earlier during a less well-known period of crop failure. By contrast, the rib bone of the adults in this population had all started to show changes related to the maize diet, but without the dentine evidence, it would have been difficult to understand how much the δ^{13}C was altered from the usual potato-based diet during childhood (Beaumont and Montgomery 2016). The identification of a particular pattern in the δ^{13}C profiles of the juveniles marks them out as a contemporaneous cohort.

A more recent study of a rural Irish cemetery in Crookstown, County Kildare, Ireland, reported incremental dentine collagen isotope data from 5 juveniles excavated in 2015 by Rubicon Heritage Ltd. (Foxton 2019). Radiocarbon dates from bone collagen of five adults and 2 juveniles from the burial ground gave a range of cal AD 1529-1942, historical documents show the burial ground was in use until 1867, and the δ^{13}C and δ^{15}N for bone collagen from one of the juveniles (MA 16) suggested a maize-based diet. Incremental dentine analysis of the 5 juveniles reveals that there is evidence for maize consumption in two of these (MA 6 and MA 16). If the δ^{13}C dentine collagen profiles are matched to the same historical year axis as used for the Kilkenny study, it appears that both MA 6 and MA16 died around the time of the Great Irish Famine (Figure 3). This provides the first evidence for maize consumption in a rural burial ground in Ireland (Foxton 2019), but also allows us to assign these individuals to the same cohort as the Kilkenny children: furthermore, we can refine the date of death of these two children to within a few years of 1850, rather than the 96-year range (cal AD 1799-1895 at best) from the C14 dating of the bone collagen. Further examples of cohorts visible in incremental dentine comparisons include medieval monastic populations such as those studied by Kancle et al. (2018) and the commingled remains from St. Stephen's chapel, Westminster (Beaumont et al. in press).

Age estimation

More recent studies hint at other life events which may be visible in the incremental dentine: the individuals excavated from the Palace Green at Durham in 2013 were found to be Scottish prisoners held at the Cathedral following the battle of Dunbar in 1650, who died and were buried there shortly after (Gerrard et al. 2018). Detailed dentine collagen isotope analysis of the canine and third molar from 12 of these individuals provided life histories from the age of 6 months to about 23 years, or in

the case of 3 individuals, until their death while the tooth was still forming (Beaumont and Montgomery 2018). All but one of the individuals had a wide range of $\delta^{13}C$ and $\delta^{15}N$ values in their dentine profiles, indicating changes of diet and/or stress during their lives: all were young adult males who died before the age of 25 years. The exception was the single older adult whose dentine profiles suggested a stable childhood with little variation of diet or nutritional stress. The evidence matches with the turbulent history of Scotland at the time, when young males were conscripted into the wars at an early age and lived through periods of deprivation as a result of the political unrest (Gerrard et al. 2018). Those who died during the formation of the third molar or shortly after have profiles with isotopic markers for stress (opposing covariance of $\delta^{13}C$ and $\delta^{15}N$) towards the last forming tissues, consistent with their recent experience of warfare (Beaumont and Montgomery 2018). "Wiggle-matching" of $\delta^{13}C$ and $\delta^{15}N$ profiles from co-forming teeth can be used to refine the age at which individual dentine increments have formed. This was shown in the Iron Age female from High Pasture Cave, Scotland, where the profiles of a supernumerary tooth and permanent M1 were shown to match (Beaumont and Montgomery 2015). This technique was applied to the age estimation of M3 (which have a much more variable age of initiation than other permanent teeth) in the Palace Green individuals (Beaumont and Montgomery 2018).

WEANING STUDIES: USING INCREMENTAL DENTINE

The sections above give examples of problems inherent in the assignment of age to skeletal bone, and the effect of physiological stress on $\delta^{13}C$ and $\delta^{15}N$: however researchers are still measuring the isotope values of bulk bone samples taken from the remains of infants and attempting to estimate the age at which a population ceases exclusive breastfeeding (the weaning process) and the age of cessation of breastfeeding (weaning completed).

For many reasons, changes in infant feeding practices are important for the mortality, morbidity and long-term health of the individual, and also to the birth-spacing in a family (for a summary of previous studies see Tsutaya and Yoneda 2015). The $\delta^{15}N$ is plotted against the estimated ages of the infants at death to create a pattern which is compared with the mean of the female population in the same cemetery and compared to a model used in early bone collagen weaning studies (Jay et al. 2008; Millard 2000). The method of interpretation does appear unscientific. The latest age at which exclusive breastfeeding ceases is determined in the cases above (and many others) as the age of the

oldest individual with a high δ¹⁵N, and cessation of breastfeeding (weaning complete) as the age at which the bone collagen values align with the estimated maternal population diet. Taken to the extreme, this would include an individual in the Clayton study (2006) with the highest δ¹⁵N as the age of 4.9 years, or at birth in the Wetwang study (Jay et al. 2008). If these values are deemed to be unlikely given the age of the individuals and/or evidence for an unusual maternal diet, then it is difficult to justify why the authors choose to rely on the data from the other individuals, and often ignore the range of δ¹⁵N values below these highest/oldest. Some studies have found high levels of δ¹⁵N in the bone of perinates, and interpreted this as early birth and breastfeeding, when a more likely explanation is that the bone is recording maternal stress in utero. An elegant example can be found in Siebke et al. (2019) where high δ¹⁵N values were found in infants who had no neonatal line visible in their dental enamel, suggesting that they had died before or very soon after birth, and thus never breastfed.

The δ¹³C is often ignored in estimating the weaning behaviour, unless it is used to identify the introduction of supplementary diet. This could be a better method for finding the age at which exclusive breastfeeding ceases, but against this argument are the findings of the breastmilk study by Herrscher et al. (2017) where maternal physiology in the later stages of breastfeeding includes the mobilization of fat stores and a fall in the δ¹³C of breastmilk and thus the infant tissues.

Evidence for thresholds of stress affecting the growth of bone

A number of studies using the co-forming incremental dentine collagen and bone collagen from the same juveniles have shown that the δ¹³C and δ¹⁵N do not match, and do not overlap, with the δ¹⁵N higher in dentine than in bone (Beaumont 2013; Beaumont et al. 2018; Burt 2013; Burt 2015; King et al. 2018). This implies that the dentine is recording something different in the collagen isotope ratios than the bone. If the higher δ¹⁵N could be attributable to diet *plus* catabolic recycling of amino acids, this suggests that the bone has ceased forming, while the dentine continues to grow and records the higher values. In these cases, the collagen isotope ratios of the two tissues cannot be used interchangeably, and any assignment of age to the bone collagen values of juveniles without corroborating evidence from dentine is questionable. One exception to this may be the auditory ossicles, which grow during pregnancy and (other than a period of mineralisation during the second year of life) do not remodel. They are also likely to be buffered from the effects of stress on growth in the same way as dentine. Leskovar et al. (Leskovar et al. 2019) compared the δ¹³C and δ¹⁵N

isotope ratios in this bone with the incremental dentine profiles from co-forming dentine and suggest that the bone could be used as a biomarker for diet and/or physiology during infancy in the same way as deciduous dentine.

Given what we now know about the likelihood of the arrest of bone growth during periods of nutritional stress, and of the elevated $\delta^{15}N$ values in collagen during starvation, it is difficult to be sure that the bone is still growing at the time of death, and that the isotope data represents diet alone. Indeed, survivors of childhood often seem to have the least perturbation in their childhood $\delta^{15}N$ dentine profiles (Beaumont et al. 2013a; Beaumont and Montgomery 2016; Beaumont and Montgomery 2018; Montgomery et al. 2013) which again suggests different cohort experiences between victims and survivors of childhood.

Sample size and interpretation

Reynard and Tuross (2015) addressed many of these issues in their paper but it appears that the idea of using the data persists. As an example of the limitations of the data, Table 1 compares the total number of bone samples in children under the age of 3 years, under the estimated age of weaning and the duration of the cemetery, for the studies quoted in Haydock et al.(2013), and prehistoric studies from Britain (Jay 2005) and South Africa (Clayton et al. 2006)(Table 1). While those cemeteries which were only in use for a very short period may contain a likely cohort with the same childhood experience (Beaumont et al. 2013a), it is difficult to argue that any meaningful interpretations about weaning behaviour can be deduced from the bone collagen isotope ratios of 6 juveniles over a period of 150 years (Privat et al. 2002) or 20 bone and dentine collagen isotope ratios over a period of 5500 years (Clayton et al. 2006). Furthermore, the well-recognised problem of the "osteological paradox" must be considered (DeWitte and Stojanowski 2015; Wood et al. 1992): these bone samples are from infants who died, how representative will they be of the whole population?

Attempts have been made to assign an age at weaning using a statistical model, WARN (Tsutaya and Yoneda 2013). However, the development of this was based on published papers from the past with all the built-in problems of both data and interpretations, and is being utilised for very small datasets from cemeteries in use for long periods: for example, King et al. (2018) used WARN to estimate the difference in weaning behaviour between two periods in the Atacama desert, Chile. The first, earlier

period covers a date range of 450-1450 AD (8 bone samples) and the later period 1450-1600 AD (4 bone samples), so it is difficult to feel that the results are in any way meaningful.

Why high resolution dentine sampling?

Although bulk sampling of dentine would give a more reliably time-bound isotopic collagen value than bulk bone, the same cross-sectional problems would apply as with bone collagen. A single sample of dentine collagen from any of these individuals would not allow the observer to determine the breastfeeding history of that infant, it is the profile as a whole which gives the history. It is also important to consider using teeth from those who died during the breastfeeding and weaning period and those who survived to compare life-histories at the same biological ages.

Two studies of Anglo-Saxon infants from England (Beaumont et al. 2018; Craig-Atkins et al. 2018) used incremental dentine collagen isotope profiles to investigate the dietary changes from in utero through to death or completion of the tooth root, and establish whether weaning patterns were visible in this tissue. These were the first to use the pre-natal values from deciduous dentine to give a measure of the diet and physiology of the mother during the first trimester of pregnancy. They found that most infants were born with $\delta^{13}C$ and $\delta^{15}N$ already much higher than the putative maternal value from female bone collagen in the populations.

They identified 4 main patterns in the dentine profiles, including the expected breastfeeding and weaning curve suggested by Millard (2000), but also curves consistent with higher $\delta^{15}N$ (breastfeeding plus stress), falling profiles suggestive of in utero stress followed by lower $\delta^{15}N$ feeding, and flat or rising profiles where breastfeeding does not appear to have taken place: in some cases the $\delta^{15}N$ rises and $\delta^{13}C$ falls towards death, the pattern of opposing covariance consistent with perimortem stress, but without the earlier profile data, could be misinterpreted as prolonged breastfeeding. In some studies the mean of the dentine collagen datapoints for each individual have been combined to produce an overall population profile: given all the cohort and period mixing that we are almost bound to have in our archaeological material, this must be meaningless. It is far better to spend time understanding the individual experiences and comparing those, rather than combining them.

Modern data: initial results

In a study aimed at establishing which patterns in the dentine profiles truly reflect the changes in isotope ratios, >200 modern deciduous teeth, donated by children in Bradford, England, were sampled for incremental dentine collagen isotope ratios and compared to the known breastfeeding history. All were between 4 and 8 years at the time of tooth extraction allowing them to be considered a cohort.

The data from the first 47 analyses (Figure 4 and Table 2) shows the difference between the breastfed and non-breastfed individuals in this population. The non-breastfed children consume a homogenous formula-milk diet until the weaning process starts, evident in the lack of variation in the first 9 months of life. In contrast, the breastfed children have a wider range of isotope ratios (reflecting maternal dietary variations) and more variable patterns in their profiles. All individuals have started weaning by the age of 6 months. It is interesting that some breastfed individuals continue to have high $\delta^{15}N$ past the age at which weaning commences, an unexpected finding in this modern population, and in light of the expectation from the isotopic models that these values will fall once other foods are introduced. A further 200 individuals have donated teeth to the study: once the total dataset is produced, this will be matched to the dietary, medical and social history data supplied by the parents/guardians to provide a more detailed interpretation of the data shown.

DISCUSSION

While it has been a useful tool for investigating the diet of past populations, the current understanding of the problems associated with bone collagen, when it is laid down and what it records make it mandatory that we, as archaeological scientists, apply rigorous standards to our sample choices, and our interpretations of the stable isotope ratios we measure.

An obvious requirement is that we compare like with like: choosing a bone sample from the same skeletal element and part of that element in all cases, and where this is impossible, understanding the differences that this will make. The cross comparison of values from bone and dentine collagen should be undertaken with the understanding that they may not be recording the same values even when they should be co-forming, and further studies are required to establish whether there is a structural difference between the collagen in the two tissues which may alter the values.

Where we have the problems associated with small sample sizes spread over a long period of cemetery use, we have a duty to make this clear in our interpretations, by remembering that these are not necessarily a population. Thus, outliers may be migrants, individuals who have made different lifestyle choices, have different status or we may not have found other individuals whose isotope ratios come between them and the majority. Including them or excluding them from the calculations of the mean of our data is fine, provided that we clarify how we are treating their data.

Statistics have become an extremely powerful tool for researchers who are dealing with large datasets. However, to use these correctly and infer population behaviour, we need to be sure that any tool is based on good data, and that we are using good and valid data in high enough numbers to be sure of the output.

CONCLUSIONS

The use of incremental dentine collagen isotope ratios has allowed us to be critical of the bone collagen data that we have been using for many decades. The time has come to apply that critical knowledge to the biological and anthropological questions which we wish to answer, and the interpretations we make of that data.

While we have always tried to understand human behaviour at a population level, maybe it is time to focus on the individuals that we have the privilege to study, understand their life histories and focus on the outliers as well as those approaching the mean. Incremental dentine gives us the opportunity to produce lifeways for the childhood and adolescence of individuals, compare those who die with those who survive at the same biological age, and find cohorts of comparable individuals. The main drawback is that teeth have all formed by about 23 years, so information about more recent diet and physiology still has to be drawn from the bone collagen, and active research is under way to achieve this by applying microsampling techniques. Further research using individual amino acid isotope measurements would also be a way of teasing out more detail from the collagen proteins from both bone and dentine, particularly where we suspect the recycling of body tissues.

ACKNOWLEDGEMENTS

BIBLIOGRAPHY

AlQahtani SJ, Hector MP, and Liversidge HM. 2010. Brief Communication: The London Atlas of HumanTooth Development and Eruption. American Journal of Physical Anthropology 142:481-490.

AlQahtani SJ, Hector MP, and Liversidge HM. 2014. Accuracy of dental age estimation charts: Schour and Massler, Ubelaker and the London Atlas. American Journal of Physical Anthropology 154(1):70-78.

Ambrose SH, and DeNiro MJ. 1986. Reconstruction of African human diet using bone collagen carbon and nitrogen isotope ratios. Nature 319:321-324.

Ambrose SH, and Norr L. 1993. Experimental evidence for the relationship of the carbon isotope ratios of whole diet and dietary protein to those of bone collagen and carbonate. In: Lambert JB, and G.Grupe, editors. Prehistoric human bone: archaeology at the molecular level. Berlin: Springer-Verlag. p 1-37.

Beaumont J. 2013. An Isotopic and Historical Study of Diet and Migration During the Great Irish Potato Famine 1845-1852 [PhD]. Bradford: University of Bradford.

Beaumont J, Atkins E-C, Buckberry J, Haydock H, Horne P, Howcroft R, Mackenzie K, and Montgomery J. 2018. Comparing apples and oranges: Why infant bone collagen may not reflect dietary intake in the same way as dentine collagen. American Journal of Physical Anthropology 167(3):524-540.

Beaumont J, Bekvalac J, Harris S, and Batt C. in press. Painting a Picture of Lives at St Stephen's Chapel Using Bones: Osteological, Isotopic and Radiocarbon Dating of Five Individuals from an Assemblage beneath St Stephens's Chapel, Westminster. In: Ayers T, editor. St Stephens Chapel, Westminster.

Beaumont J, Geber J, Powers N, Wilson AS, Lee-Thorp J, and Montgomery J. 2013a. Victims and survivors: Stable isotopes used to identify migrants from the Great Irish Famine to 19th century London. American Journal of Physical Anthropology 150(1):87-98.

Beaumont J, Gledhill A, Lee-Thorp J, and Montgomery J. 2013b. Childhood diet: a closer examination of the evidence from dental tissues using stable isotope analysis of incremental human dentine. Archaeometry 55(2):277-295.

Beaumont J, Gledhill A, and Montgomery J. 2014. Isotope analysis of incremental human dentine: towards higher temporal resolution. Bulletin of the International Association for Palaeodontology 8(2):212-223.

Beaumont J, and Montgomery J. 2015. Oral histories: a simple method of assigning chronological age to isotopic values from human dentine collagen. Annals of Human Biology 42(4):407-414.

Beaumont J, and Montgomery J. 2016. The Great Irish Famine: Identifying Starvation in the Tissues of Victims Using Stable Isotope Analysis of Bone and Incremental Dentine Collagen. PLoS ONE
11(8): e0160065.

Beaumont J, and Montgomery J. 2018. Messages from the mouth: life histories recorded in the teeth. In: Gerrard C, Graves P, Millard A, Annis R, and Caffell A, editors. Lost Lives , New Voices. Oxford: Oxbow Books. p 88-91.

Beaumont J, Montgomery J, Buckberry J, and Jay M. 2015. Infant mortality and isotopic complexity: New approaches to stress, maternal health, and weaning. American Journal of Physical Anthropology 157(3):441-457.

Bell A, and Jones K. 2013. The impossibility of separating age, period and cohort effects. Social Science & Medicine 93:163-165.

Bell LS, Cox G, and Sealy A. 2001. Determining Isotopic Life History Trajectories Using Bone Density Fractionation and Stable Isotope Measurements: A New Approach. American Journal of Physical Anthropology 116:66-79.

Brenton BP, and Paine RR. 2007. Reevaluating the Health and Nutritional Status of
Maize-Dependent Populations: Evidence for the Impact
of Pellagra on Human Skeletons from South Africa. Ecology of Food and Nutrition 46(5):345-360.

Buckberry J. 2016. The (mis)use of adult age estimates in osteology. Annals of Human Biology 42(4):323-331.

Burt NM. 2013. Stable isotope ratio analysis of breastfeeding and weaning practices of children from medieval Fishergate House York, UK. American Journal of Physical Anthropology 152(3):407-416.

Burt NM. 2015. Individual dietary patterns during childhood: an archaeological application of a stable isotope microsampling method for tooth dentin. Journal of Archaeological Science 53:277-290.

Cameron N. 2012. the Curve of Human Growth. In: Cameron N, and Bogin B, editors. Human Growth and Development. 2nd ed. London: Academic Press.

Clayton F, Sealy J, and Pfeiffer S. 2006. Weaning Age Among Foragers at Matjes River Rock Shelter, South Africa, From Stable Nitrogen and Carbon Isotope Analyses. American Journal of Physical Anthropology 129:311-317.

Craig-Atkins E, Towers J, and Beaumont J. 2018. The role of infant life histories in the construction of identities in death: An incremental isotope study of dietary and physiological status among children afforded differential burial. American Journal of Physical Anthropology 167(3):644-655.

Craig OE, Biazzo M, O'Connell. T. C., Garnsey. P., Martinez-Labarga C, Lelli R, SaLvadei L, Tartaglia G, Nava A, Reno L and others. 2009. Stable Isotopic Evidence for Diet at the Imperial Roman Coastal Site of Velia (1st and 2nd Centuries AD) in Southern Italy. American Journal of Physical Anthropology 139:572-583.

D'Ortenzio L, Brickley M, Schwarcz H, and Prowse T. 2015. You are not what you eat during physiological stress: Isotopic evaluation of human hair. American Journal of Physical Anthropology 157(3):374-388.

Dean MC, Liversidge HM, and Elamin F. 2014. Combining radiographic and histological data for dental development to compare growth in the past and the present. Annals of Human Biology 41(4):336-347.

Dean MC, and Scandrett AE. 1995. Rates of Dentine Mineralization in Permanent Human Teeth. International Journal of Osteoarchaeology 5:349-358.

DeWitte S, and Stojanowski C. 2015. The Osteological Paradox 20 Years Later: Past Perspectives, Future Directions. Journal of Archaeological Research:1-54.

DeWitte SN. 2015. Setting the stage for medieval plague: Pre-black death trends in survival and mortality. American Journal of Physical Anthropology 158(3):441-451.

DeWitte SN, and Slavin P. 2013. Between Famine and Death. Physiological Stress and Dairy Deficiency in England on the Eve of the Black Death (1315-50): New Evidence from Paleoepidemiology and Manorial Accounts. Journal of Interdisciplinary History 44:37-61.

Eerkens JW, Berget AG, and Bartelink EJ. 2011. Estimating weaning and early childhood diet from serial micro-samples of dentin collagen. Journal of Archaeological Science 38(11):3101-3111.

Elamin F, and Liversidge HM. 2013. Malnutrition has no effect on the timing of human tooth formation. PLoS ONE 8(8):e72274.

Foxton K. 2019. Isotopic Investigation of the Evidence of the Famine Relief Diet in a Rural Population During the Great Irish Famine. School of Archaeological and Forensic Science. Bradford: University of Bradford.

Fuller BT, Fuller JL, Sage NE, Harris DA, O'Connell TC, and Hedges REM. 2004. Nitrogen balance and d15N: why you're not what you eat during pregnancy. Rapid communications in mass spectrometry 18:2889-2896.

Fuller BT, Fuller JL, Sage NE, Harris DA, O'Connell TC, and Hedges REM. 2005. Nitrogen balance and δ15N: why you're not what you eat during nutritional stress. Rapid Communications in Mass Spectrometry 19(18):2497-2506.

Fuller BT, Richards M, and Mays SA. 2003. Stable Carbon and Nitrogen Isotope Variations in Tooth Dentine Serial Sections from Wharram Percy. Journal of Archaeological Science 30:1673-1684.

Gerrard C, Graves P, Millard A, Annis R, and Caffell A. 2018. Lost Lives, New Voices. Oxford: Oxbow books.

Haydock H, Clarke L, Craig-Atkins E, Howcroft R, and Buckberry J. 2013. Weaning at Anglo-Saxon Raunds: Implications for Changing Breastfeeding Practice in Britain Over Two Millenia. American Journal of Physical Anthropology 151:604-612.

Hedges REM, Clement JG, Thomas DL, and O'Connell TC. 2007. Collagen Turnover in the Adult Femoral Mid-Shaft: Modeled From Anthropogenic Radiocarbon Tracer Measurements. American Journal of Physical Anthropology 133:808-816.

Hedges REM, and Reynard LM. 2007. Nitrogen isotopes and the trophic level of humans in archaeology. Journal of Archaeological Science 34:1240-1251.

Henderson RC, Lee-Thorp J, and Loe L. 2014. Early life histories of the London poor using δ13C and δ15N stable isotope incremental dentine sampling. American Journal of Physical Anthropology 154(4):585-593.

Herrscher E, Goude G, and Metz L. 2017. Longitudinal Study of Stable Isotope Compositions of Maternal Milk and Implications for the Palaeo-Diet of Infants. BMSAP Bulletins et mémoires de la Société d'anthropologie de Paris.

Huelsemann F, Koehler K, Braun H, Schaenzer W, and Flenker U. 2013. Human dietary δ15N intake: Representative data for principle food items. American Journal of Physical Anthropology 152(1):58-66.

Jay M. 2005. Stable isotope evidence for British Iron Age diet: Inter- and intra-site variation in carbon and nitrogen from bone collagen at Wetwang in East Yorkshire and sites in East Lothian, Hampshire and Cornwall. Bradford: University of Bradford.

Jay M, Fuller BT, Richards MP, Knüsel CJ, and King SS. 2008. Iron Age Breastfeeding Practices in Britain: Isotopic Evidence From Wetwang Slack, East Yorkshire. American Journal of Physical Anthropology(136):327-337.

Kancle L, Montgomery J, Grocke DR, and Caffell A. 2018. From field to fish: Tracking changes in diet on entry to two medieval friaries in northern England. Journal of Archaeological Science: Reports 22:264-284.

Katzenberg MA, and Lovell NC. 1999. Stable Isotope Variation in Pathological Bone. International Journal of Osteoarchaeology 9(316-324).

Kershaw I. 1973. The Great Famine and Agrarian Crisis in England 1315-1322. Past and Present 59(1):3-50.

King CL, Millard AR, Gröcke DR, Standen VG, Arriaza BT, and Halcrow SE. 2018. A comparison of using bulk and incremental isotopic analyses to establish weaning practices in the past. STAR: Science & Technology of Archaeological Research:1-9.

Lane NE. 2006. Epidemiology, etiology, and diagnosis of osteoporosis,. American Journal of Obstetrics and Gynecology, 194(2):S3-S11.

Lee-Thorp JA. 2008. On Isotopes and Old Bones. Archaeometry 50:925-950.

Lee-Thorp JA, Sealy JC, and van der Merwe NJ. 1989. Stable carbon isotope ratio differences between bone collagen and bone apatite, and their relationship to diet. Journal of Archaeological Science 16(6):585-599.

Leskovar T, Beaumont J, Lisic N, and McGalliard S. 2019. Auditory ossicles: a potential biomarker for maternal and infant health in utero. Annals of Human Biology:1-11.

Lidén K, and Angerbjörn A. 1999. Dietary change and stable isotopes: a model of growth and dormancy in cave bears. Proceedings of the Royal Society B 266(1430).

Manjunatha BS, and Soni NK. 2014. Estimation of age from development and eruption of teeth. Journal of forensic dental sciences 6(2):73-76.

Mays S, Richards MP, and Fuller BT. 2002. Bone stable isotope evidence for infant feeding in Mediaeval England. Antiquity 76:654-658.

Millard AR. 2000. A Model for the Effect of Weaning on Nitrogen Isotope Ratios in Humans. In: Goodfriend GA, Collins MJ, Fogel M, Macko SA, and Wehmiller JF, editors. Perspectives in Amino Acid and Protein Geochemistry. Oxford: Oxford University Press.

Montgomery J, Beaumont J, Jay M, Keefe K, Gledhill AR, Cook GT, Dockrill SJ, and Melton ND. 2013. Strategic and sporadic marine consumption at the onset of the Neolithic: increasing temporal resolution in the isotope evidence. Antiquity 87(338):1060-1072.

Müldner G, and Hedges REM. 2007. Diet and Diversity at Later Medieval Fishergate: The Isotopic Evidence. American Journal of Physical Anthropology 134:162-174.

Müldner G, Montgomery J, Cook G, Ellam R, Gledhill A, and Lowe C. 2009. Isotopes and individuals: diet and mobility among the medieval Bishops of Whithorn. Antiquity 83:1119-1133.

Neuberger FM, Jopp E, Graw M, Püschel K, and Grupe G. 2013. Signs of malnutrition and starvation: Reconstruction of nutritional life histories by serial isotopic analyses of hair. Forensic Science International 226(1-3):22-32.

Nitsch EK, Humphrey LT, and Hedges REM. 2010. The effect of parity status on δ^{15}N: looking for the "pregnancy effect" in 18th and 19th century London. Journal of Archaeological Science 37(12):3191-3199.

Nitsch EK, Humphrey LT, and Hedges REM. 2011. Using Stable Isotope Analysis to Examine the Effect of Economic Change on Breastfeeding Practices in Spitalfields, London, UK. American Journal of Physical Anthropology 146:619–628.

Norgan NG, Bogin B, and Cameron N. 2012. Nutrition and Growth. In: Cameron N, and Bogin B, editors. Human Growth and Development. 2nd ed. London: Academic Press.

Paine RR, and Brenton BP. 2006. The paleopathology of pellagra: investigating the impact of prehistoric and historical dietary transitions to maize Journal of Anthropological Sciences 84:125-135.

Privat K, O'Connell TC, and Richards MP. 2002. Stable Isotope Analysis of Human and Faunal Remains from the Anglo-Saxon Cemetery at Berinsfield, Oxfordshire: Dietary and Social Implications. Journal of Archaeological Science 29:779-790.

Redfern RC, DeWitte SN, Beaumont J, Millard AR, and Hamlin C. 2019. A new method for investigating the relationship between diet and mortality: hazard analysis using dietary isotopes. Annals of Human Biology:1-42.

Reynard LM, and Tuross N. 2015. The known, the unknown and the unknowable: weaning times from archaeological bones using nitrogen isotope ratios. Journal of Archaeological Science 53:618-625.

Richards MP, and Hedges REM. 1999. A Neolithic revolution? New evidence of diet in the British Neolithic. Antiquity 73:891-897.

Sealy J, Armstrong R, and Schrire C. 1995. Beyond Lifetime Averages: Tracing Life Histories Through Isotopic Analysis of Different Calcified Tissues from Archaeological Human Skeletons. Antiquity 69:290-300.

Sen A. 1981. Ingredients of famine analysis: availability and entitlements. The Quarterly Journal of Economics 96(3):433-464.

Siebke I, Moghaddam N, Cunningham CA, Witzel C, and Lösch S. 2019. Those who died very young—Inferences from $\delta15N$ and $\delta13C$ in bone collagen and the absence of a neonatal line in enamel related to the possible onset of breastfeeding. American Journal of Physical Anthropology 0(0).

Smith K, and Rennie MJ. 2007. New approaches and recent results concerning human-tissue collagen synthesis. Current Opinion in Clinical Nutrition & Metabolic Care 10(5):582-590.

Suzuki E. 2012. Time changes, so do people. Social Science & Medicine 75(3):452-456.

Tieszen LL, and Fagre T. 1993. Effect of diet quality and composition on the isotopic composition of respiratory CO2, bone collagen, bioapatite, and soft tissues. In: Lambert JB, and Grupe G, editors. Prehistoric human bone: archaeology at the molecular level. Berlin: Springer-Verlag. p 121-155.

Tsutaya T, and Yoneda M. 2013. Quantitative reconstruction of weaning ages in archaeological human populations using bone collagen nitrogen isotope ratios and approximate Bayesian computation. PLoS ONE 8(8):8e72327.

Tsutaya T, and Yoneda M. 2015. Reconstruction of breastfeeding and weaning practices using stable isotope and trace element analyses: A review. American Journal of Physical Anthropology 156:2-21.

Turner BL, and Thompson AL. 2013. Beyond the Paleolithic prescription: incorporating diversity and flexibility in the study of human diet evolution. Nutrition Reviews 71(8):501-510.

Valentin J, editor. 2003. Basic Anatomical and Physiological Data for Use in Radiological Protection: Reference Values. ICRP publication 89 ed: International Commission on Radiological Protection.

van der Merwe NJ, and Vogel JC. 1978. [13]C Content of human collagen as a measure of prehistoric diet in woodland North America. Nature 279:815-816.

Waters-Rist AL, and Katzenberg MA. 2010. The Effect of growth on Stable Nitrogen Isotope Ratios in Subadult Bone Collagen. International Journal of Osteoarchaeology 20:172-191.

Wood JW, Milner GR, Harpending HC, Weiss KM, Cohen MN, Eisenberg LE, Hutchinson DL, Jankauskas R, ÄŒesnys G, Katzenberg MA and others. 1992. The Osteological Paradox: Problems of Inferring Prehistoric Health from Skeletal Samples [and Comments and Reply]. Current Anthropology 33(4):343-370.

Figure 1 Diagram showing the relative positions within a carbon and nitrogen plot for trophic levels for a terrestrial C3 plant-based food chain and marine food chain, and the relative position of C4 plants. The axes have no values as these vary depending on archaeological period, geographical location and climate.

Figure 2 Incremental dentine carbon (δ13C) isotope ratio profiles for juveniles from Kilkenny Union Workhouse aligned with the estimated calendar year of life: the blue vertical line denotes the introduction of maize as a C4 relief food in March 1846 (after Beaumont and Montgomery 2016).

Figure 3 Incremental dentine carbon (δ13C) isotope ratio profiles for juveniles from Crookstown Upper, Ireland, aligned with the estimated calendar year of life: the blue vertical line denotes the introduction of maize as a C4 relief food in March 1846 (after Foxton 2019).

Figure 4 Incremental dentine nitrogen (δ15N) isotope ratio profiles of modern deciduous teeth donated to the Tooth Fairy project, showing values from third trimester of pregnancy to approximately 9 months after birth for breastfed and non-breastfed individuals.

Table 1 Period, cemetery duration and sample sizes for infant bone collagen from weaning studies from prehistory to 19th century (after Beaumont et al. 2013; Clayton et al. 2006; Fuller et al. 2006; Haydock et al. 2013; Jay et al. 2008; Mays and Beavan 2012; Nitsch et al. 2011; Privat et al. 2002).

Study	period (with dates)	duration of cemetery use	juveniles under 3 years	juveniles under quoted age for cessation of exclusive breastfeeding
Clayton et al. 2006	South African prehistoric foragers (7500-2000BC)	5500 years	20	13
Jay et al. 2008	Iron Age (4th-2nd century BC)	approx. 200 years	21	21
Fuller et al. 2006	Romano-British (350-450 approx)	>100	22	20
Mays et al. 2002	Medieval (10th to 16th century)	>500 years	39	30
Privat et al. 2002	early Anglo-Saxon (450-700)	approx.150 years	4	6
Haydock et al. 2013	late Anglo-Saxon (978-1040)	62 years	32	12
Nitsch et al. 2011	Post-Medieval (1760-1844)	84 years	38	14
Beaumont et al. 2013	Post-Medieval (1843-1854)	11 years	34	9

Table 2 Incremental dentine carbon (δ13C) and nitrogen (δ15N) isotope ratios of modern deciduous teeth donated to the Tooth Fairy project

Tooth Fairy ID and section number breastfed	δ15N ‰	δ13C ‰
TF 001-1	11.5	-20.8
TF 001-2	11.1	-21.2
TF 001-3	11.6	-20.8
TF 001-4	12.1	-20.5
TF 001-5	12.2	-20.6
TF 004-1	11.6	-20.5
TF 004-2	11.5	-20.7
TF 004-3	11.7	-20.8
TF 004-4	11.7	-20.9
TF 004-5	11.7	-20.9
TF 006-1	11.4	-20.7
TF 006-2	12.4	-20.6
TF 006-3	11.7	-20.4
TF 006-4	11.0	-20.0
TF 006-5	10.9	-20.0
TF 009 1	13.4	-20.3
TF 009-2	13.6	-20.6
TF 009 3	14.1	-20.6
TF 009 4	13.8	-20.6
TF 009 5	13.6	-20.6
TF 010-1	12.2	-19.0
TF 010-2	12.5	-19.2
TF 010-3	12.5	-19.2
TF 010-4	11.9	-19.0
TF 010-5	11.5	-18.9
TF 011-1	13.5	-20.4
TF 011-2	14.0	-20.4
TF 011-3	14.2	-20.3
TF 011-4	13.7	-20.3
TF 011-5	14.5	-20.1
TF 023-1	12.5	-21.9
TF 023-2	12.8	-20.8
TF 023-3	13.2	-20.8
TF 023-4	12.1	-20.4

TF 023-5	11.7	-20.3
TF 028-1	11.4	-21.0
TF 028-2	11.8	-21.2
TF 028-3	11.9	-21.3
TF 028-4	12.1	-20.8
TF 028-5	12.2	-20.7
TF 026-1	12.6	-21.1
TF 026-2	12.4	-21.4
TF 026-3	12.7	-21.5
TF 026-4	12.3	-21.7
TF 026-5	11.6	-21.3
TF 027-1	11.9	-20.8
TF 027-2	11.9	-20.7
TF 027-3	12.3	-20.6
TF 027-4	12.1	-20.4
TF 027-5	12.4	-20.5
TF 030-1	11.8	-20.6
TF 030-2	11.7	-20.4
TF 030-3	11.6	-20.3
TF 030-4	12.0	-20.2
TF 030-5	12.0	-20.2
TF 034-1	11.6	-21.0
TF 034-2	11.5	-21.3
TF 034-3	11.9	-21.9
TF 034-4	12.1	-21.5
TF 034-5	12.2	-21.1
TF 048-1	12.8	-20.2
TF 048-2	12.2	-20.1
TF 048-3	11.6	-19.5
TF 048-4	11.3	-19.5
TF 048-5	11.1	-19.5
TF 049-1	12.1	-20.2
TF 049-2	11.7	-20.0
TF 049-3	11.7	-20.0
TF 049-4	11.5	-20.1
TF 049-5	11.6	-20.0
TF 060-1	12.6	-20.8
TF 060-2	12.0	-20.7

TF 060-3	11.3	-21.9
TF 060-4	12.2	-20.9
TF 060-5	11.5	-20.9
TF 067-1	10.5	-17.9
TF 067-2	10.7	-18.2
TF 067-3	11.5	-18.7
TF 067-4	12.0	-19.2
TF 067-5	11.9	-19.4
TF 070-1	12.8	-20.7
TF 070-2	13.2	-20.6
TF 070-3	12.7	-20.6
TF 070-4	11.4	-20.9
TF 070-5	11.4	-20.9
TF 077-1	10.4	-18.8
TF 077-2	10.1	-18.8
TF 077-3	10.7	-18.0
TF 077-4	10.9	-18.4
TF 077-5	10.9	-18.9
TF 078-1	12.3	-20.5
TF 078-2	11.9	-20.5
TF 078-3	11.4	-20.4
TF 078-4	10.9	-20.5
TF 078-5	10.5	-20.5
TF 079-1	12.2	-18.0
TF 079-2	11.7	-19.6
TF 079-3	12.0	-19.5
TF 079-4	11.8	-19.8
TF 079-5	11.2	-20.5
TF 085-1	12.9	-20.7
TF 085-2	12.3	-20.5
TF 085-3	12.1	-20.5
TF 085-4	12.0	-20.5
TF 085-5	12.0	-20.4
TF 108-1	11.9	-21.2
TF 108-2	12.1	-21.0
TF 108-3	12.1	-20.6
TF 108-4	11.9	-20.5
TF 108-5	11.7	-20.5

TF 139-1	11.8	-20.4
TF 139-2	11.5	-20.5
TF 139-3	11.3	-20.7
TF 139-4	11.3	-20.7
TF 139-5	11.3	-20.7
TF 145-1	12.2	-20.4
TF 145-2	13.0	-20.7
TF 145-3	13.7	-20.6
TF 145-4	12.5	-20.7
TF 145-5	12.1	-20.8
TF 251-1	11.1	-20.8
TF 251-2	11.3	-20.6
TF 251-3	11.1	-20.7
TF 251-4	11.4	-20.1
TF 251-5	11.9	-19.8
non-breastfed		
TF 002-1	11.9	-20.9
TF 002 2	11.9	-20.9
TF 002-3	12.0	-20.7
TF 002-4	11.8	-20.3
TF 002-5	11.7	-20.2
TF 003-1	11.7	-20.5
TF 003 2	11.6	-20.8
TF 003-3	11.6	-20.8
TF 003-4	11.6	-20.6
TF 003-5	11.1	-20.7
TF 005-1	11.8	-20.7
TF 005-2	11.0	-20.6
TF 005-3	10.8	-20.6
TF 005-4	11.0	-20.6
TF 005-5	11.0	-20.8
TF 014-1	12.2	-20.5
TF 014-2	12.3	-21.0
TF 014-3	11.9	-21.1
TF 014-4	12.0	-20.9
TF 014-5	11.6	-20.6
TF 019-1	12.4	-20.4
TF 019-2	12.0	-21.1

TF 019-3	12.1	-21.1
TF 019-4	12.0	-21.2
TF 019-5	12.2	-21.0
TF 020-1	12.4	-20.8
TF 020-2	12.3	-21.4
TF 020-3	12.3	-21.5
TF 020-4	12.4	-21.3
TF 020-5	12.1	-21.2
TF 024-1	11.4	-21.1
TF 024-2	11.2	-20.8
TF 024-3	11.3	-20.3
TF 024-4	11.1	-20.1
TF 024-5	11.4	-20.1
TF 025-1	11.9	-21.3
TF 025-2	12.0	-21.6
TF 025-3	12.1	-21.8
TF 025-4	11.7	-21.8
TF 025-5	11.6	-21.8
TF 045-1	12.1	-21.8
TF 045-2	11.8	-21.7
TF 045-3	11.7	-20.8
TF 045-4	11.5	-21.1
TF 045-5	11.6	-20.9
TF 050-1	12.2	-20.9
TF 050-2	11.8	-20.7
TF 050-3	11.9	-20.5
TF 050-4	11.5	-20.4
TF 050-5	11.4	-20.3
TF 066-1	11.9	-20.9
TF 066-2	12.5	-21.2
TF 066-3	12.3	-20.7
TF 066-4	12.1	-20.5
TF 066-5	11.3	-20.2
TF 068-1	11.8	-21.1
TF 068-2	11.3	-20.6
TF 068-3	11.5	-20.4
TF 068-4	11.6	-20.2
TF 068-5	11.8	-20.2

TF 069-1	12.4	-20.6
TF 069-2	12.0	-20.8
TF 069-3	12.1	-20.9
TF 069-4	11.9	-20.4
TF 069-5	11.8	-20.4
TF 071-2	10.9	-20.6
TF 071-3	10.9	-20.6
TF 071-4	11.1	-20.5
TF 071-5	11.3	-20.6
TF 072-1	12.3	-20.0
TF 072 2	12.2	-20.9
TF 072 3	12.2	-21.2
TF 072 4	12.1	-21.1
TF 072 5	12.1	-21.1
TF 081-1	11.4	-20.3
TF 081-2	11.1	-20.4
TF 081-3	11.3	-20.4
TF 081-4	11.3	-20.8
TF 081-5	11.2	-21.0
TF 082-2	11.7	-20.0
TF 082-3	11.9	-19.7
TF 082-4	11.7	-20.0
TF 082-5	11.7	-20.5
TF 088-1	12.4	-20.4
TF 088 2	12.1	-20.3
TF 088 3	11.6	-20.5
TF 088 4	12.2	-20.4
TF 088 5	12.1	-21.0
TF 090-1	12.0	-21.0
TF 090 2	11.9	-21.3
TF 090 3	12.0	-21.4
TF 090 4	12.0	-20.9
TF 090 5	12.2	-21.0
TF 109-1	12.2	-20.6
TF 109-2	11.8	-20.8
TF 109-3	12.0	-20.9
TF 109-4	12.0	-20.8

TF 109-5	11.6	-20.7
TF 144-2	11.8	-20.4
TF 144-3	11.9	-20.8
TF 144-4	11.8	-20.9
TF 144-5	11.6	-21.0
TF 146-1	11.9	-20.9
TF 146-2	11.7	-20.5
TF 146-3	11.9	-20.2
TF 146-4	11.6	-20.2
TF 146-5	11.2	-20.4