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RESEARCH PAPER



A new method for investigating the relationship between diet and mortality: hazard analysis using dietary isotopes

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ABSTRACT

Background: The population of Roman Britain are renowned for having elevated nitrogen ($\delta^{15}\text{N}$) stable isotope values, which have been interpreted as evidence for the increased consumption of marine products. However, such results are now understood to also reflect episodes of stress and disease, suggesting that new interpretations are warranted.

Aim: To test a novel approach which combines hazard mortality analysis and stable isotope data to determine whether there is a relationship between age-at-death, elevated $\delta^{15}\text{N}$ values and mortality risk.

Subjects and methods: This study used published osteological and dietary stable isotope data for nitrogen ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$) of 659 1st–5th century AD individuals aged >12 years old excavated from Roman cemeteries in Britain. The relationship between diet and mortality risk was assessed using the Gompertz hazard model, and differences in median reported isotope values between the sexes was determined using a Mann Whitney test.

Results: It was discovered that higher $\delta^{15}\text{N}$ levels are associated with elevated risks of mortality, whereas the opposite pattern was observed for $\delta^{13}\text{C}$, and males had higher median $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values.

Conclusion: This study successfully demonstrated that stable isotope data can be integrated into hazard models, allowing one to connect diet and mortality in past populations. It supports the findings of other isotope studies which have established that individuals with childhood stress/trauma will have different isotope patterns.

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Introduction

Diet and health are deeply intertwined; with nutritional studies showing that a person's dietary requirements to maintain health vary according to age, body size, sex, genetic traits, growth, illness, lifestyle, pregnancy and lactation (Brown et al. 2013). The clinical literature has shown that diet, especially during childhood, can be directly linked to adult morbidity and mortality risk (Adler and Ostrove 1999). These risks can be buffered by socio-economic status, with higher status individuals having a lower risk, but studies of both modern and archaeological populations show that low status during childhood does impact health in adulthood, regardless of later acquired or inherited social status (Adler et al. 1994; Wadsworth and Kuh 1997; Buzon 2006; Power et al. 2007; Cohen et al. 2010).

Studies of both modern and ancient individuals have sought to tease out the relationship between diet and health. The stable isotope ratios of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) in the body tissues of an animal or human reflect the food and drink ingested by the individual at the time the tissue is growing. Depending on which tissue is analysed,

isotope ratios can act as records of diet at different times during life. For example, scalp hair grows at ~1 cm per month and does not remodel, human dentine (deciduous and permanent) grows at a regular rate and does not remodel, while human bone may grow during childhood and adolescence, but is continuously remodelling; and various skeletal elements have different turnover rates (Bell et al. 2001; Hedges and Reynard 2007). Thus, measuring the collagen or keratin in hair, bone and teeth can allow an estimate of the protein element in a diet with a varying degree of temporal resolution, whilst taking offset values, the difference between the dietary and body tissue values, into consideration (Sealy et al. 1995; O'Connell et al. 2012; Beaumont et al. 2013b).

$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ from bone collagen have been the subject of many palaeodietary studies, and it has been well-established that these values, when compared with dietary sources, can allow distinctions between high and low-trophic level foods ($\delta^{15}\text{N}$), marine and terrestrial resources ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$), as well as defining the introduction of plants with different carbon pathways (C_3 and C_4 plants) as new sources of

food or imports (Vogel and van der Merwe 1977; van der Merwe and Vogel 1978; Schoeninger and DeNiro 1984; Richards and Hedges 1999). As the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in bone collagen represents an average of the diet over a period of time, it has been possible to compare individuals within a cemetery population in order to infer differences in diet depending on, for example, status and geographical origin (Müldner et al. 2011; Müldner 2013).

Careful baseline studies are required to ensure that the stable isotope changes are due to dietary change alone and not to the source and treatment of the foods ingested. For example, soil that has been treated with manure will contain recycled nitrogen and $\delta^{15}\text{N}$ values will rise in the plants growing there (Bogaard et al. 2007; Fraser et al. 2011). $\delta^{15}\text{N}$ will also rise in plants grown in conditions of aridity, anoxia and salinity (Heaton 1987; Britton et al. 2008) and leaching where rainfall is exceptionally high (Ambrose 1991). Animals feeding on the plants in such environments will also have higher $\delta^{15}\text{N}$ in their tissues (Müldner et al. 2014).

The $\delta^{15}\text{N}$ will also respond to changes in body physiology in humans. When there is no shortage of nutrients, the body tends toward homeostasis, balancing the incoming nutrition with the requirements for energy and growth/repair and the nitrogen balance will be affected by *anabolic* and *catabolic* changes. When the body's nutritional requirements are greater, for example during growth, an anabolic state is reached when there is a positive nitrogen balance: less nitrogen is excreted and $\delta^{15}\text{N}$ in the body tissues will fall (Waters-Rist and Katzenberg 2010). However, during nutritional stress, if there is insufficient intake of protein and calories, a catabolic state is reached and nitrogen within the body is recycled to make new proteins, a trophic level effect occurs and the $\delta^{15}\text{N}$ in the body tissues will rise. For example, changes during pregnancy can demonstrate both these states of nitrogen balance. During the anabolic state of rapid foetal growth, a fall in $\delta^{15}\text{N}$ can be seen in a pregnant mother (Fuller et al. 2005), but a catabolic state is found in mothers experiencing severe morning sickness (Fuller et al. 2004). Although it was suggested that chronic illness may cause changes in the $\delta^{15}\text{N}$ values of pathological bone (Katzenberg and Lovell 1999), short-term changes in physiology will be masked by the slow turnover of bone collagen and the averaging of values this produces.

Analysis of tissues with a much faster turnover such as hair and fingernails in modern studies, and increasingly the use of incremental dentine in archaeological populations have allowed the identification of short-term changes in diet, the identification of stress markers and periods of extreme nutritional stress, such as raised $\delta^{15}\text{N}$ values of body tissues (Hobson et al. 1993; Guthrie 1995; Neuberger et al. 2013). Mekota et al. (2006) showed how body mass index (BMI) was related to changes in the $\delta^{15}\text{N}$ values of individuals deliberately depriving themselves of food, while Duška et al. (2007) demonstrated a negative nitrogen balance (catabolism) in acute starvation in a clinical setting; Hatch et al. (2006) advised the use of isotopic values from hair as a diagnostic tool for anorexia and bulimia. Powanda and Beisel (2003) noted that the metabolic effects of infection on nitrogen

balance were masked or reduced when protein and calorie intake was increased, suggesting that the effects of any illness on nitrogen balance would be greater when nutrition was also insufficient.

In archaeological populations, investigations into the relationship between health and stable isotope results are few in number, and a small number of publications have concerned themselves with exploring these discrepancies further for conditions such as osteoporosis (White and Armelagos 1997), diffuse idiopathic skeletal hyperostosis (Spencer 2010) and physical abuse (Richards and Montgomery 2012). One study by Arcini et al. (2014) also explored the relationship between diet and stature in Medieval and post-Medieval samples from Sweden. They concluded that sex and temporal changes, but not stable isotope data, explained the decrease in femoral length over time, because the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ derived from bone collagen were not a sensitive enough gauge of dietary change (Arcini et al. 2014).

The influence of nutritional stress and starvation on $\delta^{15}\text{N}$ has also been attested in archaeological populations from the Neolithic, Iron Age and 19th century in Britain, where incremental isotope analysis of dentine collagen showed an increase in $\delta^{15}\text{N}$ during periods of nutritional stress (Beaumont et al. 2013a, 2015; Montgomery et al. 2013; Armit et al. 2015; Beaumont and Montgomery 2016). Incremental dental sampling can provide this nuanced result, because bone has a slower turnover rate and does not capture episodic events, rather the average of several years before death (Humphrey 2014). Work by Beaumont et al. (2015) has also shown that individuals with compromised nutritional intake during infancy and childhood may have different dietary isotope patterns compared to their peers who reached adulthood. A recent study using the isotope ratios of carbon and nitrogen from incremental dentine samples from an British Anglo-Saxon population (Raunds Furnells) who have osteological evidence for metabolic diseases and growth stunting, found identifiable patterns within the tissues for periods of nutritional stress (Beaumont et al. 2018). There was also a correlation between the level of growth stunting and the maximum measurement for $\delta^{15}\text{N}$ of dentine collagen in this population. However, there are individuals in the Raunds Furnells study and in other archaeological bone/dentine collagen studies where there is evidence for an individual's bone not recording the extreme isotope ratios found in the dentine, possibly because it stopped growing (Burt 2013, 2015; King et al. 2017; Beaumont et al. 2018).

A recent development in the literature has been the discussion of the putative relationship between nutritional stress and $\delta^{13}\text{C}$. It has been suggested that when $\delta^{15}\text{N}$ rises as the result of a catabolic state, $\delta^{13}\text{C}$ values will remain the same, or even fall as the result of the breakdown of body fat deposits, which are $\sim 3\text{‰}$ lower than other body tissues such as muscle (Tiezen and Fagre 1993). This is the case when free fatty acids are obtained from adipose tissue (Casper 1996). This phenomenon has been noted in studies of human hair (Mekota et al. 2006; Neuberger et al. 2013; Lehn et al. 2015), the blood plasma levels of fasting King

Penguins (Cherel and LeMaho 1985) and in the dentine of famine victims (Beaumont and Montgomery 2016).

In her review of this topic, Reitsema (2013) suggested that bone may still be a useful source of information, if micro-sampling techniques and a holistic approach were applied, which considered the wider context of life-experience of the individual. It is still possible that prolonged and/or repeated episodes of stress may eventually have an influence on both the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of bone collagen, as shown in the individual suffering a prolonged wasting disease published by Katzenberg and Lovell (1999).

In summary, high $\delta^{15}\text{N}$ bone collagen values have mainly been interpreted in the past as a sign of higher-trophic-level foods, and in Roman Britain, related to higher-status food sources (see Richards et al. 1998). This may imply that the individuals with high $\delta^{15}\text{N}$ measurements had a lower level of nutritional stress because of their access to these high-status foods. However, isotope data are not a fail-safe solution, because current methods using these isotopes are unable, for example, to detect small contributions of marine foods in the diet (see, amongst others, Craig et al. 2013; Montgomery et al. 2013; Webb et al. 2016). The complexities of the contribution of food and physiology to the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in the tissues of an individual continue to present challenges to the archaeologist.

Due to the ever-increasing use of stable isotope research in the analysis of archaeological human remains, and the results of numerous clinical studies demonstrating that diet significantly impacts health and disease (Reitsema and McIlvaine 2014), we were motivated to determine whether these two datasets could be joined in order to gain a more holistic and integrated understanding of past health and mortality risk. Consequentially, we devised a novel approach that allows osteological and stable isotope data to be combined using hazard analysis and tested it using Romano-British dietary data obtained from bone samples, as this is the largest temporal dataset available from the UK. Our hypothesis is that those who have lived through prolonged periods of stress will have higher $\delta^{15}\text{N}$ bone collagen values than those who have not and that the lifetime experience of stress may affect each individual's risk of dying.

Hazard analysis allows for the assessment of mortality and survival patterns using archaeologically-derived skeletal samples, which are often relatively small with missing data and can be more informative than other statistical approaches, such as Chi-Square tests. However, to the authors' knowledge, this approach has not been applied to the analysis of stable isotope data for diet ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$).

Archaeological context

Britain became part of the Roman Empire following the Claudian invasion of 43AD and remained so until the 5th century AD (Mattingly 2006). This period saw the introduction of urban settlements, new foodstuffs and population migration from Europe, meaning that lifestyles and foodways were very diverse, reflecting both a person's occupation (e.g. military), social status (e.g. enslaved) and living

environment (rural or urban) (Eckardt 2010; Mattingly 2006). The most significant change observed isotopically and in the environmental record is the increased consumption of marine resources (Jay and Richards 2006; Locker 2007; Jay 2008; Redfern et al. 2010, 2012; Stevens et al. 2012; Müldner 2013).

Sex differences in diet have also been reported for some populations (Müldner 2013), but it is not clear the extent to which these are mediated by other factors, such as social age, status, enslavement and occupation. Age-related differences are also attested with respect to sub-adults, whereby there are inter- and intra-regional and cemetery-population variations in the duration of breastfeeding, choice of weaning foods and the period of weaning itself (Fuller et al. 2006; Cummings 2008; Redfern et al. 2010; Nehlich et al. 2011; Cheung et al. 2012; Powell et al. 2014).

In comparison with later periods of British history, the Roman period did not experience significant climatic decline or episodes of famine, unlike, for example, Medieval populations during the late 13th–14th century AD (Jones 1996). Therefore, these should not be factors biasing the dietary evidence from this period. The primary sources pertaining to Roman Britain are very limited and are mainly concerned with Britain's conquest and the first few decades of colonisation (Mattingly 2006). The extant epigraphic and inscription evidence is weighted towards the military and administrative activities, rather than descriptions of agricultural activities or the environment (Tomlin 2011). In Tacitus' record of his father-in-law's participation in the conquest of Britain in the 1st century AD, the climate is described as being 'unpleasant, with frequent rain and mist ... crops grow quickly but ripen slowly. This is due to the high rainfall and dampness of the soil' (Tacitus 1973, p. 12). However, neither these nor the evidence for climate and environment from pollen records and ice cores provide evidence for periods of agricultural collapse or famine, akin to those seen in Medieval England, although increased levels of rainfall, flooding and colder temperatures are attested from the mid-3rd century AD and, in combination with the Roman transformation of the agricultural economy, it is posited that agricultural output would have fallen from earlier levels (Jones 1996). Nevertheless, this does not mean that there were food shortages, as huge quantities of food-stuffs were imported into Britain from the Continent during this period (Jones 1996; Cool 2006).

Materials and methods

Osteological data

This study uses a total of 659 individuals aged >12 years old (356 males, 231 females and 72 sub-adults) and dating from the 1st to 5th centuries AD. These skeletonised human remains are derived from formal extra-mural cemeteries, excavated in England over the past 50 years (Figure 1). The osteological and stable isotope data were taken from published site reports, journal articles and unpublished theses. Only a portion of the entire sample available could be used for hazards analysis, because many individuals did not have age-estimates. For analysis of $\delta^{13}\text{C}$, the sample included 601 individuals aged >12 years (322 males, 207 females, and 72

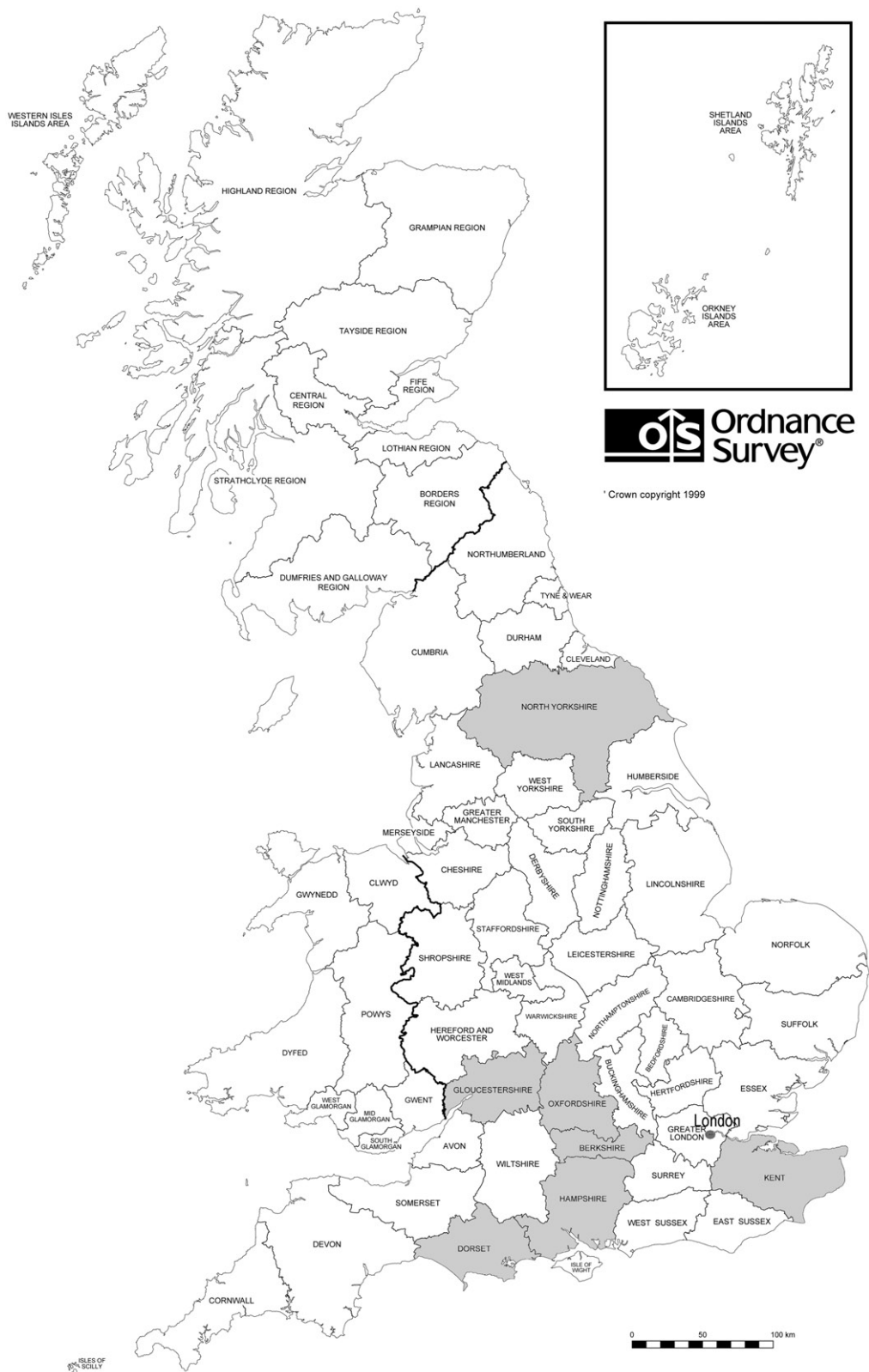


Figure 1. Location by county within England of the sites used in the study (see Table 1) (after Ordnance Survey's open data).

sub-adults). For analysis of $\delta^{15}\text{N}$, the sample included 600 individuals aged >12 years (322 males, 206 females, and 72 sub-adults) (Table 1).

We acknowledge that relying on human bone data published over the past 30 years does raise concerns about the comparability and reliability of data, particularly concerning

the methods and morphological features used to determine age-at-death and estimate sex (Roberts and Cox 2003). Furthermore, in the Romano-British period, stable isotope research has revealed significant age-related differences in diet during childhood and early adulthood (Fuller et al. 2006; Cummings 2008; Redfern et al. 2010; Nehlich et al. 2011;

Table 1. Sites (with corresponding sample sizes and references) used in this study.

Site name and county	Number of adult males	Number of adult females	Number of sub-adults (12–20 years old)	References
Albert Road (Dorset)	4	1	–	Redfern et al. (2010)
Alchester (Oxfordshire)	6	7	1	Cumming (2008); Smith et al. (2010)
Alington Avenue (Dorset)	8	4	–	Redfern et al. (2010)
Andover Road (Hampshire)	2	1	–	Bonsall and Pickard (2015)
Asthall (Oxfordshire)	2	4	–	Cumming (2008); Booth (1997)
Atlantic House (London)	–	–	1	Powell et al. (2014); Watson (2003)
Bainesse Farm (Yorkshire)	9	7	1	Chenery et al. (2011); Wilson (2002)
Blossom Street (Yorkshire)	8	6	2	Müldner and Richards (2007)
Broad Street (London)	–	–	1	Powell et al. (2014); MoLA pers.comm
Calvert's Brewery (London)	–	1	–	Research Laboratory for Archaeology and the History of Art, Oxford University (C14/2913); MoL (15SKS80)
Catterick Bridge (Yorkshire)	7	1	–	Chenery et al. (2011); Wilson (2002)
Chester Street (Hampshire)	–	3	–	Bonsall and Pickard (2015)
Cirencester (Gloucestershire)	87	45	10	Cumming (2008); McWhirr et al. (1982)
Cotswold Community (Gloucestershire)	13	6	–	Cheung et al. (2012)
Courage Brewery (London)	–	–	1	Powell et al. (2014); MoL (COSE84)
Dere Street (Yorkshire)	2	–	–	Chenery et al. (2011); Wilson (2002)
6 Driffeld Terrace (Yorkshire)	13	–	–	Müldner et al. (2011)
Eton Rowing Lake (Berkshire)	–	1	–	Stevens et al. (2012)
Fordington Bottom (Dorset)	1	–	–	Redfern et al. (2010)
Gambier Parry Lodge (Gloucestershire)	12	12	6	Cumming (2008); Cameron and Roberts (1984)
Gravesend (Kent)	4	2	–	Pollard et al. (2011)
Great Dover Street (London)	1	–	2	Powell et al. (2014); MacKinder (2000); Research Laboratory for Archaeology and the History of Art, Oxford University (C14/2913)
1–4 Giltspur Street (London)	2	2	3	Powell et al. (2014); MoL (WES89); Research Laboratory for Archaeology and the History of Art, Oxford University (C14/2913)
Gussage All Saints (Dorset)	1	–	–	Redfern et al. (2010)
Haydon Street (London)	–	–	1	Powell et al. (2014); Barber and Bowsher (2000)
Honeypot Road (Yorkshire)	2	–	–	Chenery et al. (2011); Wilson (2002)
Hooper Street (London)	2	–	–	Research Laboratory for Archaeology and the History of Art, Oxford University (C14/2913); MoL (HOO88)
Horcutt Quarry (Oxfordshire)	16	5	–	Cheung et al. (2012)
Hucclecote (Gloucestershire)	1	6	1	Cumming (2008); Thomas et al. (2003)
Hyde Road (Hampshire)	1	1	–	Bonsall and Pickard (2015)
Hyde Street (Hampshire)	1	1	–	Bonsall and Pickard (2015)
Lankhills Schools (Hampshire)	49	42	10	Booth et al. (2010)
Lant Street (London)	4	4	3	Ridgeway et al. (2014)
Maiden Castle Road (Dorset)	3	2	–	Redfern et al. (2010)
Mansell Street (London)	–	3	1	Powell et al. (2014); Barber & Bowsher (2000)
Old Vicarage (Dorset)	4	2	–	Redfern et al. (2010)
Oxford Road (Oxfordshire)	9	5	5	Cheung et al. (2012)
Poundbury Camp (Dorset)	21	14	1	Redfern et al. (2012)
Queensford Farm (Oxfordshire)	16	17	6	Fuller et al. (2006)
Race Course (Yorkshire)	1	1	–	Chenery et al. (2011); Wilson (2002)
Spitalfields Ramp Project (London)	–	2	1	Powell et al. (2014); MoLA pers. comm (SRP98)
St Bartholomew's Hospital (London)	–	–	3	Powell et al. (2014); Bentley & Pritchard (1982)
Stanton Harcourt (Oxfordshire)	13	11	3	Cumming (2008); McGavin (1980)
Sycamore Terrace (Yorkshire)	–	1	–	Müldner (2013)
Tolpuddle Ball (Dorset)	1	1	–	Redfern et al. (2010)
Trentholme Drive (Yorkshire)	25	9	7	Müldner and Richards (2007)
Trinity Street (London)	–	1	1	Powell et al. (2014); Pre-Construct Archaeology pers.comm (2013)
Total <i>n</i> in study	356	231	72	

MoL: Museum of London; MoLA: Museum of London Archaeology.

Cheung et al. 2012; Powell et al. 2014). Therefore, in order to best mitigate these biases, we only included individuals if they were >12 years old, because dietary stable isotope research on Romano-British sub-adults has shown that, by this age, the breast-feeding signature and the weaning process should have been eliminated by bone remodelling (e.g. Redfern et al. 2012; Powell et al. 2014), because they were eating an 'adult' diet. The individuals' age-at-death was determined by calculating the mean of their age-group assigned by the original osteologist, as studies have found no statistical significance between observers in the application of a broad range of macroscopic methods (Galera et al.

1995). We did accept the sex estimation given for those over the age of 20 years old in the original publication, because studies have found that there is low inter-observer error in identifying skeletons as being either male or female (Walrath et al. 2004).

Differences between dentine and bone $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ have been reported in the literature (Hedges and Reynard 2007; Brown and Brown 2011) and this could be explained by the different time periods represented by these two tissues; however, contrary datasets also exist that suggest that bone collagen may not be formed during periods of extreme stress while dentine will still grow (and see above, Beaumont

et al. 2018). The reasons for this difference are not entirely understood at present. Therefore, to limit potential sources of error we only used $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ reported from bone samples, which in the majority of cases were rib bones that reflect the last 3–5 years of life (Beaumont et al. 2013a; Bell et al. 2001).

Statistical methods

We assessed the relationship between diet and risk of mortality using hazards analysis. Hazard models specify the time until a certain event (e.g. death) occurs. For this study, we fit a parametric model of mortality, the Gompertz model, to our data. Models such as the Gompertz model that have few parameters can be applied to small samples, and they smooth the random variation in mortality data that is an artefact of small samples without imposing any particular age pattern on the data (Gage 1988). Hazard models also accommodate missing data, which is particularly important when we use conventional age estimation methods that do not provide estimates for the oldest adult ages.

The Gompertz model is a parsimonious two-parameter model of human mortality that represents the typical pattern of human adult mortality, i.e. an exponentially increasing risk with senescence (Gage 1988):

$$h(a) = \alpha e^{\beta a}$$

The α parameter represents the force of mortality associated with senescent causes and β represents the rate at which this increases with increasing age. Initial analyses using the three-parameter Gompertz-Makeham model, which includes a parameter representing an age-independent component of mortality, indicated that including this additional parameter did not improve the fit of the model to the data. This is unsurprising given that estimating the age-independent component is often difficult using paleodemographic data (Herrmann and Konigsberg 2002, Nagaoka et al. 2006).

Isotope values were modelled as a covariate affecting the baseline Gompertz model of mortality. The isotope covariate was modelled using a proportional hazard specification:

$$h(a|x_i\rho) = h(a)e^{x_i\rho}$$

where the baseline hazard $h(a)$ is the Gompertz hazard, x_i is the isotope covariate and ρ is the parameter representing the effect of the covariate on the baseline hazard. A positive estimate for the parameter representing the effect of the covariate on the hazard would suggest that higher isotope values are associated with higher risks of mortality. Alternatively, a negative estimate for the parameter representing the effect of the covariate on the hazard would suggest that higher isotope values are associated with lower risks of mortality. The fit of the full model that included the parameter representing the isotope covariate, compared to a reduced model in which the value of the parameter representing the isotope covariate was set equal to 0, was assessed using a likelihood ratio test (LRT). The LRT tests the null hypothesis that isotope values are not associated with elevated or decreased risks of death. The LRT was computed

as follows: $\text{LRT} = -2[\ln(L_{\text{reduced}}) - \ln(L_{\text{full}})]$, where LRT approximates a χ^2 distribution with $df = 1$.

Model parameters, and their 95% confidence intervals, were estimated using maximum likelihood analysis with the programme *mle* (Holman 2005). Analyses were conducted separately for carbon and nitrogen isotopes. Previous research has shown that maximum likelihood estimates are increasingly biased with decreasing sample sizes (Mueller et al. 1995; Shouman and Witten 1995), though maximum likelihood estimation can perform satisfactorily with samples of less than 100 for models with relatively few parameters, such as the Gompertz model used in this study (El-Sherpieny et al. 2013).

Mann Whitney tests

Differences in median reported isotope values between adult males and females ($\delta^{13}\text{C}$: females, $n = 231$; males, $n = 355$; $\delta^{15}\text{N}$: females, $n = 230$; males, $n = 354$) were assessed using Mann Whitney tests with SPSS version 25. The Mann Whitney test was used because it does not require that the isotope values are normally distributed.

Results

Table 2 shows the estimated value of the parameter representing the effects of the carbon and nitrogen isotope covariates (and the 95% confidence intervals) on the Gompertz hazards and the results of the likelihood ratio tests. The estimated value of the parameter representing the carbon covariate effect is negative (and the confidence interval for that parameter includes only negative values). The result of the likelihood ratio test indicates that inclusion of the covariate improves the fit of the model. This result suggests that higher $\delta^{13}\text{C}$ is associated with lower risks of mortality. The estimated value of the parameter representing the $\delta^{15}\text{N}$ covariate effect is positive (and the confidence interval for that parameter includes only positive values). The results of the likelihood ratio tests indicate inclusion of the covariate improves the fit of the model. These results suggest that higher $\delta^{15}\text{N}$ is associated with elevated risks of mortality, an opposite pattern to that observed for $\delta^{13}\text{C}$.

The results of the Mann Whitney tests are shown in Table 3. Median $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were higher in males.

Table 2. The effect of the isotope covariates ($\delta^{13}\text{C}$, carbon; $\delta^{15}\text{N}$, nitrogen) on the Gompertz model, the associated confidence intervals, and the results of the likelihood ratio tests (LLR).

Covariate	Gompertz hazard		
	Effect of covariate	95% CI	LLR
$\delta^{13}\text{C}$	−0.15	−0.15, −0.14	6.27 ($p = 0.01$)
$\delta^{15}\text{N}$	0.10	0.08, 0.11	8.27 ($p = 0.004$)

Table 3. Mann-Whitney tests of sex differences in reported isotope values ($\delta^{13}\text{C}$, carbon; $\delta^{15}\text{N}$, nitrogen).

	Female	Male	p -value
median reported $\delta^{13}\text{C}$	−19.70	−19.60	0.05
median reported $\delta^{15}\text{N}$	9.8	10.2	0.006

Discussion

The perspective gained from the statistical analyses of these data is one of increased mortality risk for those with high bone collagen $\delta^{15}\text{N}$, suggesting that these people experienced insufficient levels of protein, causing their body tissue to be recycled and creating a protein-stressed individual (Reitsema 2013). The key to this result may be to establish if both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ are covarying, suggesting a trophic level shift in diet (or not), implying that the $\delta^{15}\text{N}$ is really reflecting increased stress and that the $\delta^{13}\text{C}$ may be reduced because of utilisation of adipose tissue and borne out by the lower risk of mortality with elevated carbon in this study. Both results when viewed from a bioarchaeological perspective are unlikely to be a 'best fit' for all of the individuals in this study and alternatives must be explored.

In Roman Britain, environmental archaeology and material culture studies have shown that there are distinct dietary differences between site types (i.e. rural vs urban, military vs civilian) in diet: domestic vs wild species consumed and presence of imported foodstuffs (Cool 2006). These subtle differences will have contributed to the results observed in this study, but because we only have the average bulk isotope dietary data and are reliant on published datasets, it is a bias that we can only at the present time acknowledge as potentially influential, but to an unknown degree. Layered on this variation is the 'standard' interpretation for Romano-British populations—that increased nitrogen values are caused by the increased consumption of marine resources in this period (Müldner 2013), a trend first observed in the material culture and environmental evidence (Cool 2006; Locker 2007). This may be true for some individuals, particularly those of a higher social status in certain cemeteries (Richards et al. 1998), where these dietary choices reflected their wealth, but this association has not been identified across the different regions of Britain (Cheung et al. 2012) and, crucially, for other time periods in populations with known histories, where such results were caused by famine and poor health (Beaumont and Montgomery 2016). Therefore, the shift to marine resources cannot be the only explanation.

This standard interpretation obfuscates a myriad of experiences and outcomes experienced by people living in Roman Britain, reflecting the incredibly complex and diverse life experiences of the individuals used in this study. First, the burial context and funerary treatment of many individuals shows that they were of high status (e.g. use of embalming and/or burial in a lead coffin) or associated with the military (e.g. York) and from primary sources and bioarchaeological evidence, such status groups are unlikely to have experienced compromised nutrition (Cool 2006). We do not discount the role that individual choices or attested cultural practices played, as some researchers have suggested from their reading of primary texts that the Emperors and other elites regularly practiced bulimia (Crichton 1996, Ziolkowski 1996).

Furthermore, Roman Britain was inhabited by many enslaved individuals who the primary sources describe as being fed a restricted diet if they were of low status. For example, Cato the Elder stated that agricultural chain gangs

should receive four pounds of bread a day in the winter, rising to five when they dug the vines, which decreased to four again once the figs had ripened (Cato the Elder 2018, *De Agri Cultura Libra*, cited in Bradley 1984). These people came from a variety of sources: natal slaves, within the Empire, those captured from surrounding territories or newly conquered provinces and transported across the Empire—a process which would have caused immense physiological and psychological distress (Redfern 2018). Enslavement in this period was a diverse experience, with roles and occupations ranging from mine-workers to doctors (Joshel 2010), but it is reasonable to suggest that, for some people, the protein stress does indeed reflect a reality of inadequate nutrition, illness and physiological stress due to their restricted diet and the work they were expected to do, such as this description of workers in a bakery, 'These emaciated men ... they were hideous with jaundice, and their eyelids were so gnawed away by the dark smoke ... they could hardly see' (Apuleius, *Metamorphoses*).

Enslaved people could be from any status group and primary source evidence shows that people's social status and access to resources frequently rose and fell throughout their lifetime (Joshel 2010) and we know that maternal and childhood health influences long-term health outcomes (Gowland 2015). Therefore, the increased mortality risk for those with this higher $\delta^{15}\text{N}$ may indicate a coming-together of different stressors in a person's life that their underlying health could not cope with. For example, for a person enslaved from birth but with a free low-status adulthood, the resulting nutritional insufficiencies and poor health could have created this elevated risk of death. Alternatively, it could also indicate an enslaved childhood but a free, higher-status adulthood, where the person had sufficient resources to access high trophic-level foods.

An important finding was the sex differences in median values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, confirming that this is a national trend, rather than a pattern limited to a particular cemetery population. Thus, this result is likely to reflect the biological impact of the Roman life course and its social values, where, regardless of status, females were always less than males, because of their basic biology (Hemelrijk 2015). Dietary studies elsewhere in the Roman Empire have found sex-differences in diet (Prowse 2011) and this must have played a role in these findings. However, because diet and health are interlinked, additional work is needed to establish how both datasets connect with each other, as earlier work by two of the authors (Redfern and DeWitte 2011) found that, in one region of Britain, males had an increased mortality risk in this period and higher rates of disease, compared to the preceding Iron Age; this work has not been undertaken elsewhere in Britain or the Empire, so it may be a local result.

Overall, the results of our approach show that $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ data can be successfully integrated into a hazards model, enabling us to establish the important connection between diet and mortality in past populations. Importantly, our study supports the findings of isotope studies that used incremental dental or hair samples in individuals who have osteological and/or primary source evidence for childhood

stress and trauma to have different stable isotope patterns (Wheeler et al. 2013; Beaumont et al. 2015). Our study has been able to demonstrate that such insights can also be obtained from bone collagen samples. Usefully, this means that the relationship between diet and mortality risk can be gauged in samples of human remains that lack mummified tissue and dentition. Additionally, it means that in samples with a more robust determination of age-at-death, this method could be used to establish the age-groups for which compromised nutrition posed the greatest risk.

Conclusions

Our ability to understand the lives of past peoples relies on the methods and techniques we employ. Stable isotope analysis and hazard modelling provide unique perspectives on past communities, and our new method allows us to combine these two sources to further explore the crucial relationship between diet and health. We propose that our investigative method allows researchers to explore this relationship in populations where there is an absence of primary sources or limited environmental evidence for diet. Further studies are being undertaken using incremental dentine analysis of archaeological populations of infants with osteological evidence for stress to further investigate this phenomenon.

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