Bone deep: variation in stable isotope ratios and histomorphometric measurements of bone remodelling within adult humans

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Abstract

Stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope studies of ancient human diet increasingly sample several skeletal elements within an individual. Such studies draw upon differences in bone turnover rates to reconstruct diet during different periods of time within an individual's lifetime. Rib and femoral bone, with their respectively fast and slow remodeling rates, are the bones most often sampled to reconstruct shorter and longer term signals of diet prior to death. It is poorly understood if $\delta^{13}C$ and $\delta^{15}N$ vary between bone types within a single individual, or if this variation corresponds with bone turnover rate (BTR). Here, we determined $\delta^{13}C$ and $\delta^{15}N$ for ten different bones from ten adult human skeletons (n=5 males; n=5 females). Isotope values were compared to the rate that each bone remodeled, calculated from osteon population (OPD) density. Results reveal that isotope ratios varied within each skeleton ($\delta^{13}C$: max= -1.58‰; $\delta^{15}N$: max= 3.05‰). Humeri, metacarpals, and ribs had the highest rate of bone remodelling; the occipital bone had the lowest. A regression analyses revealed that higher rates of bone remodeling are significantly and negatively correlated with lower $\delta^{15}N$. Our results suggest that the occipital bone, with its slow rate of bone renewal, may prove useful for isotopic studies that reconstruct diet over longer periods of time within an individual's lifetime. Isotope studies that compare individual skeletal elements between populations should standardize their methodology to bones with either a slow or fast turnover rate.

Highlights

- We present stable carbon and nitrogen isotope ratios and bone remodelling rates for ten different bones in ten adult human skeletons.
- Humeri, ribs and metacarpals had the fastest bone turnover.
- Occipital had the slowest bone turnover.
- Bones with higher turnover rates generally had lower $\delta^{15}N$.

Keywords

Stable isotopes. Bone remodelling.

1. Introduction

Stable isotope analyses of biological tissues can provide a long-term record of diet (Deniro & Epstein 1978; Rundel et al. 2016). Because of this, stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope analyses of bone and dentin collagen have become a standard approach in archaeological science for reconstructing dietary ecology of past modern human populations (Ambrose & DeNiro 1989; Deniro & Epstein 1978; DeNiro & Epstein 1981; Hedges & Law 1989; Reynard & Hedges 2008), with applications extending to non-human primates and fossilized remains (Bocherens et al. 1999; Fahy et al. 2013; Fahy et al. 2014; Fahy et al. 2015; Sponheimer et al. 2013). Increasingly, such studies incorporate isotopic signals from several skeletal elements to reconstruct ancient diet during different periods of time within an individual's lifetime (Sealy et al. 1995; Cox & Sealy 1997; Schroeder et al. 2009; Pollard et al. 2012; Chenery et al. 2012; Lamb et al. 2014). The adult human rib and femur are the skeletal elements most commonly sampled because of apparent differences in bone turnover rates (see Section 1.3). However, little is known about relationships between $\delta^{13}C$ and $\delta^{15}N$ and remodelling in other skeletal elements. Here we 1) explore variation in $\delta^{13}C$ and $\delta^{15}N$ in ten different bones from ten archaeological human skeletons and 2) identify associations between these ratios and histomorphometric measurements of bone remodelling.

1.1 Stable carbon and nitrogen isotopes

Ratios of heavy to light stable isotopes of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) display distinctive patterns of distribution that enable them to be employed in the interpretation of various aspects of life history. Body tissue isotopic composition is highly influenced by food and drink consumed in life (Sealy et al., 1995), variation in food sources (Hopkins & Ferguson 2012) and water availability (Stewart et al. 1995; Amundson 2003; Swap & Aranibar 2004); consequently isotopic analyses of body tissues can offer clues to aspects of diet and lifestyle. The main source of terrestrial carbon is atmospheric CO_2 whereas the main source of marine carbon is dissolved CO_2 and biocarbonate ions (HCO_{3-}). These sources of carbon express $\delta^{13}C$ of -7.5 and +1.5‰, respectively (Lee-Thorp et al. 1989; Van Klinken 1991). The differences then continue up the food chain from primary producers to apex predators (Lee-Thorp et al. 1989; Van Klinken 1991). This expression is dependent on the biochemical mode of photosynthesis with most plants utilizing the C_3 cycle (expressing $\delta^{13}C$ around -26‰) compared to those few utilizing the C_4 pathway (expressing $\delta^{13}C$ around -12‰) (Smith & Epstein 1971). Nitrogen incorporation into plant biomolecules can occur in three different ways: direct nitrogen fixation from air, ammonium or nitrate in soil water,

recycled organic nitrogen from soil (Lee-Thorp 2008). Similar to $\delta^{13}C$, there is a stepwise increase in $\delta^{15}N$ with trophic level (DeNiro & Epstein 1981). Isotope data from bone collagen have long been shown to largely reflect the protein component of an individual's diet (Ambrose & Norr 1993; Lee-Thorp et al. 1989; Schoeninger et al. 1997; Schoeninger et al. 1998; Schroeder et al. 2009).

1.2 Bone remodeling rates

Human bones form through intramembranous and endochondrial ossification. Bone modeling commences in utero and continues until the early teenage years, depending upon the bone type (Pitfield et al., 2017). Bone remodelling occurs throughout the whole human lifespan (Burr & Allen 2014; Katsimbri 2017; Robling et al. 2006; Peacock 2010) as osteoclasts resorb old tissue and osteoblasts produce new tissue (Robling et al. 2008; Miszkiewicz & Mahoney 2016). Metabolic activity, including the exchange of nutrients, calcium, oxygen and mechanical signaling (Miszkiewicz & Mahoney 2016), along with targeted remodeling, maintains and repairs bone (Burr 2002; Robling et al. 2001). As new bone forms, it incorporates the isotopic composition of an individual's diet (Fry & Arnold 1982). However, the rate that different bone within a skeleton remodel is not consistent. Age, health, biological sex, mechanical loading, and genetic predisposition can all regulate the rate at which Bone Multicellular Units (BMUs) add or remove bone (Burr 2002; Sealy et al. 1995; Pfeiffer et al. 2006; Hedges et al. 2007; Pollard et al. 2012; Robling et al. 2001; Wolff 1899).

Evidence of remodelling is retained in bone as basic structural and somewhat independent functional units, as secondary osteons. Osteon population density (OPD) is a measure of complete and fragmentary secondary osteons per section area, which together represent past remodeling events (Frost 1994; Gocha & Agnew 2016). As such, OPD can represent a measure of bone remodeling dynamics, or accrued bone density (Miszkiewicz 2015). Increasing OPD is closely associated with advancing age, and eventually an asymptote is reached where new secondary osteon formations begin to remove traces of earlier osteons (Stout & Lueck 1995). When age-at-death is controlled for, OPD variation may indicate differences in bone structure and response to mechanical stress (Britz et al. 2009; Schlecht et al. 2012), dietary changes (e.g., Pfeiffer, S. K., & Lazenby 1994; Paine & Brenton 2006), or health status (e.g., Martin & Armelagos 1979; Storm et al. 1993), or general human lifestyle (Miszkiewicz & Mahoney 2016).

An estimated rate of remodelling varies across bone types, because of surface to volume ratio differences in bone shape and size (Parfitt 2002). For example, a cancellous

bone sample (~135 μm thick) from a modern adult human ilium remodels at an average rate of 17.7% per year, whereas a turnover rate for a cortical sample (~1225 μm thick) from the same individual would remodel at approximately 7.7% per year (Parfitt 2002). When considering cortical bone only, its renewal varies quite substantially throughout the skeleton (Hobson & Clark 1992; Klinken & Mook 1990). For example, ribs are bones are never at rest due to the load arising from respiration (Skedros et al. 2013); with a greater surface area to volume ratio ribs have a relatively fast cortical turnover rate, which is approximately 4% a year after age 50 (Frost 1969; Hill & Orth 1998). The dense cortical bone of the femoral shaft is thought to have a slow turnover rate relative to rib bone (Hill & Orth 1998; Hedges et al. 2007; Skedros et al. 2013).

1.3 Human bone remodelling and isotope variation

Dietary reconstruction using standard isotope methodology tries to account for variation in bone remodelling. Studies compare various skeletal elements between individuals; usually only one bone type is sampled, though sometimes one bone is substituted for another (e.g. Fahy et al. 2015). Multiple sampling of bone (and teeth) is increasingly utilised to reconstruct diet during different periods of time from an individual's lifetime (e.g. Lamb et al. 2014). For example, it is thought that the slower turnover of femoral bone collagen, isotopically, reflects a longer-term and average dietary signal, which may be more than ten years prior to death (Hedges et al. 2007). In contrast, ribs, with faster turnover rates, may represent diet from a more recent period prior to death (e.g. Cox & Sealy 1997).

Olsen et al. (2014) directly compared $\delta^{13}C$ and $\delta^{15}N$ to an inferred rate of remodelling for different bones within 59 adult human skeletons. While they suggest that paleodiet researchers should avoid sampling collagen close to pathological lesion sites due to differing isotope values, they state that normal, non-pathological bone show limited intraskeletal variation in $\delta^{13}C$ and $\delta^{15}N$. Similarly, DeNiro & Schoeniger (1983) examined the mean isotopic composition of collagen extracted from mink humeri and femora and found that it did not differ significantly for either $\delta^{13}C$ or $\delta^{15}N$, leading them to suggest that differences in the isotopic composition of collagen extracted from different bones of an individual are small. Research by Larson & Longstaffe (2007) on deer, Brady et al. (2008) on sheep and Luz & Kolodny (1985) on rat bone, looked at the relationship between $\delta^{13}C$ and $\delta^{18}O$ and osteon lacunar density, and research by Balasse et al. (1999) examined the intra-individual variability in $\delta^{13}C$ and $\delta^{15}N$ of mineralized tissues in modern steers. All of these studies reported significant variation in isotopic ratios for different bones from the same individual.

2. Materials and methods

2.1 Samples

Ten human skeletons, dating to the early medieval period, from St Gregory's cemetery in Canterbury, England, were selected (Hicks and Hicks 2001). Historical texts state that burials were from a single socio-economic group that lived and worked in Canterbury, and represent non-catastrophic mortality (Brent 1879; Duncombe 1785; Somner 1703). We selected complete individuals without skeletal signs of pathology. This collection is curated in the Skeletal Biology Research Centre, University of Kent, UK. All sectioning adhered to the British Association of Biological Anthropology and Osteoarchaeology code of practice (2014), and guidelines for invasive sampling (Mays, et al., 2013). No permits were required as these are archaeological samples from before the 19th Century AD.

2.2 Collagen extraction and IRMS

Bone samples were taken from the same location on each bone from ten skeletons. Sampled bones were femur, tibia, rib (right 5th), humerus, metacarpal, occipital, pelvis, clavicle, radius and thoracic vertebrae. Samples were taken from the anterior mid shaft region of the tibia and humerus, the posterior mid shaft region of the femur, and from the mid-shaft metacarpal, radius, left 5th rib, clavicle, and the planum region of the occipital. An attempt was made to separate cortical and cancellous bone for isotope ratios, but this proved difficult for cancellous-rich bones such as the rib. Prior to sampling, bone surfaces were cleaned by air abrasion with Al_2O_3; approximately 100-300mg of bone was sampled. Collagen extraction was done following Longin (1971), Brown et al. (1988) and Richards & Hedges (1999). Isotopic measurements were carried out using Elemental Analysis - Isotope Ratio Mass Spectrometry (EA-IRMS) by Iso Analytical Limited (UK). The analytical precision, calculated from repeated analysis of internal and international standards, was better than 0.2‰ (1σ) for $\delta^{13}C$ and $\delta^{15}N$.

2.3 Histological sample preparation and analysis

Standard histological methods were used (e.g., Crowder & Stout 2011; Miszkiewicz 2015; Miszkiewicz 2016). Dry un-decalcified transverse thin sections (each section was approximately 0.7 ±0.2cm thick) were removed from the anterior mid shaft region of the tibia and humerus, the posterior mid shaft region of the femur, and complete sections were

198 removed from the mid-shaft metacarpal, mid-shaft radius, mid-shaft left 5th rib, mid-shaft
199 clavicle, and occipital. Sections were taken adjacent to isotope sampling locations in all
200 cases. All sections were removed using an electronic drill (Dremel Rotary Tool®) with a
201 diamond wafering blade. Sections were embedded in epoxy resin (Buehler EpoxiCure®),
202 further reduced to 0.3 ±0.1cm using a Buehler Isomet 4000 precision saw, and fixed to glass
203 microscope slides (Evo Stick® resin). Each section was ground (Buehler EcoMet® 300),
204 polished with a 0.3 mm aluminum oxide powder (Buehler® Micro-Polish II), cleaned in an
205 ultrasonic bath, dehydrated in 95-100% ethanol, cleared (Histoclear®), and mounted with a
206 coverslip using a xylene-based mounting medium (DPX®).

207 2.4 Microscopy

208 Imaging and histomorphometric procedures followed standard methods (e.g., Villa &
209 Lynnerup 2010; Miszkiewicz & Mahoney 2016). Imaging was undertaken using an Olympus
210 BX51 compound microscope with an Olympus DP25 microscope camera. Images were
211 obtained from five regions of interest (ROIs) from each bone using CELL® Live Biology
212 Imaging software. Each ROI was positioned adjacent to the periosteum within the anterior
213 cortex, with the exception of the femur (sub-perisotealy within the posterior cortex), ribs and
214 occipital (sub-periostealy within the external cortex). The number of secondary osteons and
215 secondary osteon fragments were counted in each ROI at a magnification of 10x, meeting the
216 current standards of data representing 25-50 osteons per section (Stout, S. D., & Crowder
217 2012) (Stout and Crowder, 2011) . Secondary osteons were identified by the presence of an
218 intact cement line and complete Haversian canal (Currey 2012) and fragments were identified
219 as partial secondary osteons with >10% of the Haversian canal remodeled. All osteons which
220 had their Haversian canals within or touching the border of the ROI were included (Britz et
221 al. 2009). These osteon counts formed the OPD, which was calculated by dividing the
222 number of osteons and fragments by the area of ROI (2.24mm^2). OPD was calculated for
223 cortical bone only. It was not possible to consistently calculate OPD for cancellous bone in
224 our sample because of differential preservation. Thus, OPD was not calculated for the
225 vertebrae and pelvis which has a high proportion of cancellous bone.

226 2.5 Age and sex

227 Biological sex estimation was carried out using multiple standard methods to increase the
228 accuracy of the determination (Buikstra & Ubelaker 1994; Martin, Harrod, & Pérez 2013).
229 We relied upon standard morphological characteristics of the pelvis and occipital. The pelvic
230 methods included the three Phenice characteristics (Phenice 1969), and the greater sciatic

notch described in Buikstra & Ubelaker (1994). Cranial features included the mastoid process, supraorbital margin, mental eminence, and nuchal crest (Buikstra & Ubelaker 1994). When determinations from cranial and pelvic features conflicted, priority was given to the pelvic criteria (White et al. 2012). Differences between males and females are not one of the main focuses of this study.

Only young adults were selected. We estimated age from the morphology of the pubic symphysis, and the auricular surface of the pelvis (e.g. Meindl & Lovejoy, 1985; Lovejoy et al., 1985). All samples were between 25-35 years old, falling into classic anthropological age-at-death categories (Buikstra & Ubelaker 1994).

2.6 Statistical analyses

Statistical analysis was undertaken using IBM Statistics SPSS 22 (2014). First, we combine data for the ten skeletons and examine variation in isotopic ratios, and bone turnover rates, between the different bone types when subdivided by sex. These log-transformed data are then analysed using linear regression analysis. We present the r^2 value (coefficient of determination) which measures the proportion of explained variation, and the r value (correlation coefficient) which measures the strength and direction of the relationship between isotope ratios and OPD. Following this, we examine variation in isotopic ratios and bone turnover rates within each skeleton using a non-parametric Spearman's Rho.

3. Results

3.1 Isotopic variation between bone types

When data for the 10 skeletons are combined, mean $\delta^{13}C$ ranged between -19.4‰ in the radius to -19.1‰ in the ribs and pelvis (Table 1). Mean $\delta^{15}N$ ranged from 11.2‰ in the radius, to 12.2‰ in the thoracic vertebrae.

3.1.1 Males vs females

Slightly different trends emerge when $\delta^{13}C$ and $\delta^{15}N$ are subdivided into males and females. Amongst the males, mean $\delta^{13}C$ ranged between -19.6‰ to -19.4‰ in the long bones (femur and radius) to -18.9‰ for the rib. Females also showed depleted mean $\delta^{13}C$ of -19.7‰ in the long bones (radius), but had a relatively higher value of -19.1‰ in the occipital. The $\delta^{15}N$ for males ranged between 11.2‰ in the radius, to 12.4‰ in the thoracic vertebrae and pelvis. Amongst the females, $\delta^{15}N$ ranged from 11.4‰ in the radius, to 12.5‰ in the occipital.

Table 1: Mean $\delta^{13}C$ and $\delta^{15}N$ isotopic (‰) ratios for each bone type

Bone	$\delta^{13}C$			$\delta^{15}N$		
	All (n=10)	Males (n=5)	Females (n=5)	All (n=10)	Males (n=5)	Females (n=5)
Femur	-19.4	-19.6	-19.2	11.5	11.3	11.6
Tibia	-19.2	-19.2	-19.1	11.9	11.7	12.1
Rib	-19.1	-19.0	-19.2	12.0	12.2	11.7
Radius	-19.4	-19.5	-19.3	11.3	11.2	11.4
Occipital	-19.3	-19.4	-19.1	12.2	11.8	12.5
Metacarpal	-19.3	-19.4	-19.1	11.7	11.5	11.8
Humerus	-19.2	-19.3	-19.2	11.6	11.6	11.7
Thoracic vertebrae	-19.2	-19.2	-19.2	12.2	12.4	12.0
Pelvis	-19.1	19.1	-19.1	12.1	12.4	11.9
Clavicle	-19.3	19.4	-19.2	11.7	11.7	11.7

3.2 Isotopic variation within each skeleton

Table 2: Maximum change in $\delta^{13}C$ and $\delta^{15}N$ isotopic (‰) within each skeleton

Males (n=5)			Females (n=5)			Males (n=5)			Females (n=5)		
All bones						Femur to Rib					
Sk	**$\delta^{13}C$**	**$\delta^{15}N$**	**Sk**	**$\delta^{13}C$**	**$\delta^{15}N$**	**Sk**	**$\delta^{13}C$**	**$\delta^{15}N$**	**Sk**	**$\delta^{13}C$**	**$\delta^{15}N$**
1	-0.8	1.7	**6**	-0.4	1.5	**1**	0.7	1.1	**6**	-0.1	0.4
2	-1.2	1.0	**7**	-0.8	1.4	**2**	0.6	0.1	**7**	0.0	0.7
3	-0.4	1.3	**8**	-0.7	1.2	**3**	0.3	0.6	**8**	-0.7	-0.7
4	-0.5	1.2	**9**	-1.0	1.3	**4**	0.2	0.2	**9**	0.7	0.7
5	-1.6	3.1	**10**	-1.6	1.9	**5**	0.9	2.4	**10**	-0.2	-0.2
Mean	**-0.9**	**1.7**		**-0.9**	**1.5**		**0.5**	**0.9**		**-0.1**	**0.2**

Variation in $\delta^{13}C$ and $\delta^{15}N$ within each skeleton was broadly similar for males and females (Table 2). On average $\delta^{13}C$ differed by -0.9‰ within all skeletons. Mean $\delta^{15}N$ differed by 1.7‰ within the female skeletons, compared to 1.5‰ for males. When skeletons are considered individually, $\delta^{13}C$ changed from -18.6‰ in the occipital to -20.2‰ in the pelvis of female skeleton number 10. $\delta^{15}N$ ranged between 1.0‰ to 3.1‰ in male skeleton number 5 (Sk5) (Fig. 1), who also had the greatest change in $\delta^{13}C$. The femur of each male skeleton was consistently depleted in $\delta^{13}C$, and $\delta^{15}N$, when compared to the rib. Differences between these bones in females were inconsistent.

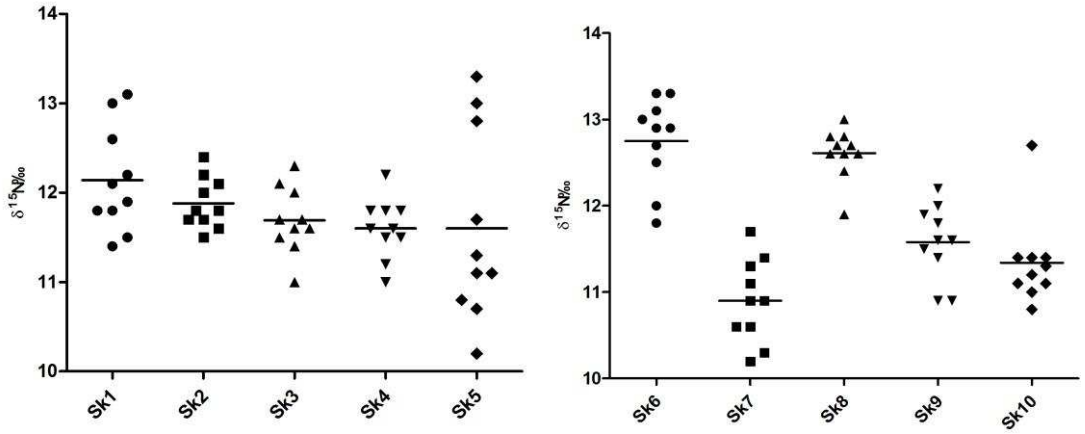

Fig. 1. : $\delta^{15}N$ for the 10 bones from each skeleton (males = Sk1 – Sk5; females = Sk6 – Sk10)

3.3. Variation in bone turnover rate between bone types

Table 3: Mean OPD for each bone type

Bone[1]	All (n=10)	Males (n=5)	Females (n=5)
Humerus	15.10	14.32	15.89
Metacarpal	14.06	12.20	15.93
Rib	13.90	11.83	15.98
Femur	13.48	11.36	15.60
Tibia	12.54	12.60	12.49
Radius	12.23	10.20	14.26
Clavicle	11.82	10.89	12.76
Occipital	4.23	5.01	3.46

1=Ordered by fastest to slowest turn over.

When data for the 10 skeletons are combined, and OPD is used as proxy for the amount of bone produced and, by extension, past evidence of bone remodelling, mean values are highest in the humerus, metacarpals, and ribs. Values were lowest in the occipital. Relative to the other bones, the femur, and tibia have medium to high remodelling rates (Table 3).

3.3.1 Males vs females

Table 3 illustrates differences in mean OPD between males and females. Generally, females in our sample display higher mean OPD values for bones with faster turnover rates (humerus, metacarpal, rib), when compared to males. This variation in bone turnover rates between the sexes could relate in part to differences in activity due to occupation (Pitfield et al., 2017), or instead, it may reflect a relationship between the underlying histology and overall size or robusticity of the sampled bone (Miszkiewicz and Mahoney, 2017). Our sample sizes are

small, so it is difficult to draw firm conclusions, but future research can explore this variation further using larger sample sizes.

3.4. Relationship between isotope ratios and bone turnover compared between bone types

Table 4: Mean $\delta^{13}C$ and $\delta^{15}N$ and OPD data for each bone type

Bone	$\delta^{13}C$			$\delta^{15}N$			OPD		
	All (n=10)	Males (n=5)	Females (n=5)	All (n=10)	Males (n=5)	Females (n=5)	All (n=10)	Males (n=5)	Females (n=5)
Femur	-19.4	-19.6	-19.2	11.5	11.3	11.6	13.48	11.36	15.60
Tibia	-19.2	-19.2	-19.1	11.9	11.7	12.1	12.54	12.60	12.49
Rib	-19.1	-19	-19.2	12	12.2	11.7	13.90	11.83	15.98
Radius	-19.4	-19.5	-19.3	11.3	11.2	11.4	12.23	10.20	14.26
Occipital	-19.3	-19.4	-19.1	12.2	11.8	12.5	4.23	5.01	3.46
Metacarpal	-19.3	-19.4	-19.1	11.7	11.5	11.8	14.06	12.20	15.93
Humerus	-19.2	-19.3	-19.2	11.6	11.6	11.7	15.10	14.32	15.89
Thoracic vertebrae	-19.2	-19.2	-19.2	12.2	12.4	12			
Pelvis	-19.1	19.1	-19.1	12.1	12.4	11.9			
Clavicle	-19.3	19.4	-19.2	11.7	11.7	11.7	11.82	10.89	12.76

Average $\delta^{13}C$ and $\delta^{15}N$ and OPD data for each bone type is presented in Table 4. When all skeletons are combined, a linear regression analysis of log-transformed data indicates that there is a significant and negative correlation between $\delta^{15}N$ and bone turnover rates (slope = -1.986, intercept =3.171, r = -0.231; r^2=0.053, p=0.050). Figure 1 illustrates the negative relationship between these variables. The occipital bone is highlighted in the figure to illustrate the low bone turnover rates associated with this bone type. When the analysis was repeated on $\delta^{13}C$ and OPD, there was no significant relationship between the variables (r=0.064, p=0.571).

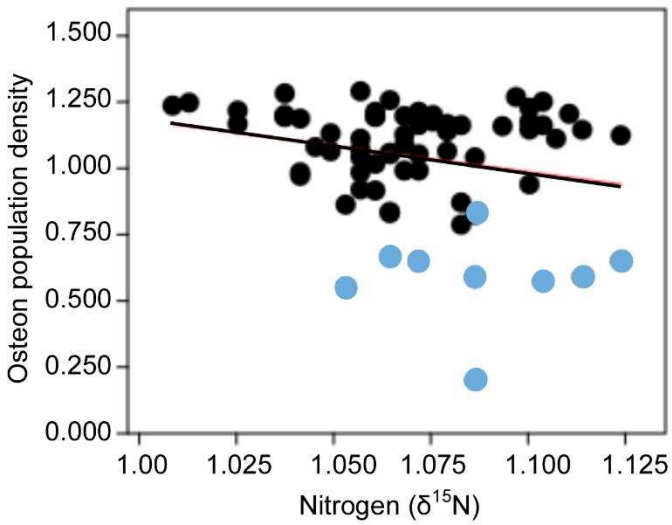

Fig. 2. Linear regression analyses of log-transformed $\delta^{15}N$ against log-transformed osteon population density. Blue circles = occipital bone. Black circles are data for all other bone types[1]. Excluding Sk 5 which showed a positive correlation between the variables, and the greatest variation in $\delta^{15}N$ of any skeleton: see Fig 1.

3.4.1 Relationships between isotope ratios and bone turnover rates within each skeleton

When each skeleton is considered separately, $\delta^{15}N$ and products of bone remodelling are negatively correlated for eight of the 10 skeletons (Table 5). For one male (SAC89), the negative relationship is significant (p=0.007). For the five females the relationship is not significant (p>0.05) but all of the r values are negative. Thus, higher $\delta^{15}N$ values are generally associated with lower products of remodelling, within each skeleton. When each skeleton is considered separately $\delta^{13}C$ are OPD are positively correlated for eight of the 10 skeletons (Table 5). For one skeleton (SAC 92), this relationship is significant.

Table 5: Spearman's Rho analyses of $\delta^{15}N$ and OPD, and $\delta^{13}C$ and OPD within each skeleton. *Significant

	$\delta^{15}N$		$\delta^{13}C$	
Sk	**r**	**p**	**r**	**p**
Males				
SAC 88	0.168	0.691	0.025	0.954
SAC 89	-0.855	0.007*	-0.12	0.778
SAC 90	-0.036	0.932	0.409	0.314
SAC 91	-0.133	0.754	0.703	0.053
SAC 92	0.431	0.286	0.952	0.000*
Females				
SAC 93	-0.539	0.168	0.501	0.206
SAC 94	-0.602	0.114	0.458	0.254
SAC 95	-0.659	0.076	0.05	0.906
SAC 96	-0.494	0.213	0.564	0.146
SAC 97	-0.586	0.127	-0.17	0.688

4. Discussion

When the different bone types are compared to each other, the rib, humeri and metacarpals all have a high mean OPD. This high OPD indicates increased remodelling, suggesting these skeletal elements are all suitable to gain insights into an individual's diet during a relatively recent period prior to death, compared to bones with a slower rate of remodelling. The occipital bone had the lowest mean OPD, implying that this skeletal element had the slowest rate of remodelling of all bone types in our sample. The slower remodelling of the occipital suggests that this bone might provide a dietary record for a longer period of time from an individual's lifetime, compared to other bone types. $\delta^{15}N$ were also clearly elevated in the occipital (Table 1). When considered together, these results support current isotopic methodological practice that samples human ribs to access diet from a period that is relatively near to the point of death (Section 1.3). Results suggest that the humerus is an appropriate substitute for the rib, when the rib is not available for sampling.

Our findings suggest that current isotopic sampling strategies can be modified to incorporate the occipital, rather than the femur, to access a longer-term dietary signal. Our data does not support the idea that the femur has a slow rate of turnover when compared to the rib. Mean bone turnover rates of 13.48 (SD: 3.05) of the femur did not differ significantly when compared to the mean turnover rate of 13.90 (SD: 3.69) for the rib (Mann Whitney U= 51.000; p= 0.940; Table 3). In contrast, mean OPD of the rib differed significantly when compared to the occipital (mean=4.23, SD=1.31; U=0.000; p=0.000). This latter finding is inconsistent with the long standing idea that a slower turnover of femoral bone collagen reflects a longer-term dietary signal (Hedges et al. 2007) when compared to a faster turnover of rib bone collagen that represents a more recent period prior to death (Cox & Sealy 1997).

Previous studies have reported varying results in terms of isotopic differences between bones of the same skeleton. Olsen et al. (2014) analysed $\delta^{13}C$ and $\delta^{15}N$ in four bones (rib, metacarpal, fibula, vertebrae) of the skeleton, with sample sizes that were similar in size to the current study. They found limited variation in either $\delta^{13}C$ (0.0 ± 0.1‰) or $\delta^{15}N$ (-0.1 ± 0.4‰). Similarly a study by Pollard et al. (2012) found that variation in $\delta^{13}C$ didn't exceed analytical error; variation in $\delta^{15}N$ was slightly higher, but not statistically significant. Olsen et al. (2014) also reported significant intra-skeletal variation in nitrogen values related to non-specific disease. Skeletons selected for our study did not retain any evidence of non-specific disease, though we cannot rule out the presence of active diseases at the point of death that do not leave a record on bone (Wood et al. 1992).

Pollard et al. (2012) found rib $\delta^{15}N$ to be higher compared to femora by an average of ~0.5 - 1‰ in a group of tenth-century young males. A similar trend was observed by Chenery et al. (2012) who reported elevated rib $\delta^{15}N$ in comparison to femora by 0.9 – 1.2‰ in 31 individuals analysed. In contrast, Jørkov et al. (2007) reported no measureable rib-femora isotopic difference in 58 individuals from a static community from Holbæk, Denmark. We found negligible difference between average $\delta^{15}N$ rib (11.7‰) and femora (11.6‰) in females, but there was a 0.9‰ difference between average $\delta^{15}N$ rib (12.2‰) and femora (11.3‰) in males (table 1). Hedges et al. (2007) suggest that male adolescent collagen turnover rates are higher than in female adolescents. The differences observed between males and females were related to femoral stable isotope values that reflect a substantial portion of collagen synthesized during adolescence, when the rate of turnover is thought to be higher in males (Hedges et al. 2007). Although we found a negligible difference in OPD in our samples between the rib and the femur, it is possible that the difference in $\delta^{15}N$ between males and females reflects increased BTR during adolescence.

The lack of a measurable difference in $\delta^{13}C$ is likely indicative of a typical diet based primarily on a C_3-photosynthetic system. Pollard et al. (2012) suggest potential explanations for the lack of variation they observed in $\delta^{13}C$ compared to $\delta^{15}N$: 1) the lack of a systematic shift in $\delta^{13}C$ may stem from increased consumption of marine resources as adults and 2) that a change in metabolic activity may have been brought about as a result of increased stressful, activity as adults. For our sample, it is possible, given the origin of the samples (Canterbury, United Kingdom), that there was some level of increased marine resource consumption in adulthood, at least for the male skeletons, which may account for the variation in $\delta^{15}N$. However, while plausible, this idea is not strongly supported as there is no corresponding alteration in $\delta^{13}C$. Additionally the female skeletons appear to have consistently high $\delta^{15}N$ in their cranial bone, suggesting a consistent long-term diet with little change in adulthood.

The variation in $\delta^{13}C$, and particularly in $\delta^{15}N$, across different bones, warrants further discussion. This may perhaps be linked to the proportion of cancellous to cortical bone in the isotopic samples. Brady et al. (2008) reported significantly different $\delta^{13}C$ and $\delta^{18}O$ for compact and cancellous bone, illustrating the relationship between bone remodelling and isotopic heterogeneity in bone. Research by Hill & Orth (1998) suggests that cancellous bone with higher surface-to-volume ratios tends to turnover at a faster rate. Therefore, even with a similar cortical OPD's, bones with proportionally more cancellous bone than cortical bone, such as the rib, metacarpal, clavicle, could still reflect different ages compared to bones with

more cortical bone such as the femur and tibia, which could ultimately have impacted upon our isotopic results.

Our study highlights that caution should be applied when substituting one bone for another in isotope studies that compare single skeletal elements between individuals or when sampling a small population of individuals for individual dietary interpretations. Our $\delta^{15}N$ ranged from 10.2‰ to 13.3‰ in male Sk5, and $\delta^{13}C$ changed from -18.6‰ in the occipital to -20.2‰ in the pelvis in female Sk10. Thus, comparing different bone types between individuals can potentially introduce additional variation into analyses, clouding diet-isotope relationships. However, more freedom is allowed if the sample population is larger and the goal is a population-wide dietary interpretation as interestingly, while individual $\delta^{15}N$, and to some extent $\delta^{13}C$, vary greatly among individuals depending on the type of bone that is sampled, when taken as a group these differences disappear for $\delta^{13}C$ (females = -19.2±0.6‰; males = -19.3±0.5‰) and $\delta^{15}N$ (females = 11.8±0.9‰; males = 11.8±0.6‰).

5. Conclusion

Our study sampled ten bones from ten individuals to examine the range of variation in $\delta^{13}C$ and $\delta^{15}N$ across the skeleton and to determine relationships between $\delta^{13}C$ and $\delta^{15}N$ and static indicators of bone remodelling. Lower $\delta^{15}N$ were significantly correlated with higher values of remodelling products when compared between individuals. Given that many studies utilize the differences in turnover rates to demonstrate dietary changes in individuals and populations, and that much emphasis is put on $\delta^{15}N$ and potential high or low protein diets, we suggest that future stable nitrogen isotope studies of diet should standardize bone sampling, to bones with either high or low turnover rates.

Acknowledgements

This project was funded by a University of Kent, School of Anthropology & Conservation Small Research Grant.

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Appendix 1

Supp. Table 1: Stable carbon and nitrogen isotope data and OPD data for each bone sampled.

MALES					FEMALES				
Lab #	Bone	$\delta^{13}C$	$\delta^{15}N$	OPD	Lab #	Bone	$\delta^{13}C$	$\delta^{15}N$	OPD
SAC88F	Femur	-19.2	11.5	10.42	SAC93F	Femur	-18.5	12.9	16.07
SAC88T	Tibia	-18.4	12.1	7.44	SAC93T	Tibia	-18.5	13.0	13.99
SAC88R1	Rib	-18.5	12.6	8.71	SAC93R1	Rib	-18.6	13.3	13.33
SAC88R2	Radius	-18.6	11.4	8.33	SAC93R2	Radius	-18.8	11.8	14.58
SAC88O	Occipital	-18.7	11.8	4.46	SAC93O	Occipital	-18.9	13.3	4.48
SAC88M	Metacarpal	-18.7	11.8	9.82	SAC93M	Metacarpal	-18.6	12.5	18.60
SAC88H	Humerus	-18.5	11.9	15.03	SAC93H	Humerus	-18.5	12.7	17.86
SAC88TV	Thoracic vertebrae	-18.6	13.0		SAC93TV	Thoracic vertebrae	-18.9	13.1	
SAC88P	Pelvis	-18.5	13.1		SAC93P	Pelvis	-18.5	12.9	
SAC88C	Clavicle	-18.5	12.2	11.01	SAC93C	Clavicle	-18.6	12.0	13.84
SAC89F	Femur	-20.1	12.0	14.73	SAC94F	Femur	-19.1	10.3	17.71
SAC89T	Tibia	-20.2	11.5	15.77	SAC94T	Tibia	-18.8	11.7	12.28
SAC89R1	Rib	-19.4	12.1	14.56	SAC94R1	Rib	-19.1	10.9	15.70
SAC89R2	Radius	-20.6	11.8	11.31	SAC94R2	Radius	-19.2	10.6	14.73
SAC89O	Occipital	-19.7	12.2	6.70	SAC94O	Occipital	-19.5	11.3	3.57
SAC89M	Metacarpal	-19.8	11.6	18.10	SAC94M	Metacarpal	-18.7	10.9	19.20
SAC89H	Humerus	-19.9	11.8	15.18	SAC94H	Humerus	-19.2	10.2	17.26
SAC89TV	Thoracic vertebrae	-19.6	12.4		SAC94TV	Thoracic vertebrae	-19.5	11.1	
SAC89P	Pelvis	-19.9	11.7		SAC94P	Pelvis	-19.3	11.4	
SAC89C	Clavicle	-20.0	11.7	15.77	SAC94C	Clavicle	-19.3	10.6	16.52
SAC90F	Femur	-19.4	11.0	9.38	SAC95F	Femur	-18.4	12.6	16.96
SAC90T	Tibia	-19.4	11.5	8.26	SAC95T	Tibia	-18.6	12.8	12.95
SAC90R1	Rib	-19.1	11.6	6.79	SAC95R1	Rib	-19.1	11.9	15.89
SAC90R2	Radius	-19.4	11.4	9.66	SAC95R2	Radius	-18.5	12.6	14.00
SAC90O	Occipital	-19.5	11.6	4.64	SAC95O	Occipital	-18.5	13.0	3.90
SAC90M	Metacarpal	-19.4	12.1	6.14	SAC95M	Metacarpal	-18.6	12.4	14.43
SAC90H	Humerus	-19.2	11.7	13.39	SAC95H	Humerus	-18.5	12.6	15.03
SAC90TV	Thoracic vertebrae	-19.2	12.0		SAC95TV	Thoracic vertebrae	-18.4	12.7	
SAC90P	Pelvis	-19.0	12.3		SAC95P	Pelvis	-18.5	12.8	
SAC90C	Clavicle	-19.4	11.7	9.82	SAC95C	Clavicle	-18.6	12.7	14.55
SAC91F	Femur	-19.5	11.6	11.31	SAC96F	Femur	-20.0	10.9	15.63
SAC91T	Tibia	-19.4	11.8	15.48	SAC96T	Tibia	-19.9	11.6	11.46
SAC91R1	Rib	-19.3	11.8	15.58	SAC96R1	Rib	-19.3	11.4	19.54
SAC91R2	Radius	-19.5	11.0	9.67	SAC96R2	Radius	-20.0	10.9	15.92
SAC91O	Occipital	-19.6	12.2	3.87	SAC96O	Occipital	-19.8	12.2	1.59
SAC91M	Metacarpal	-19.8	11.2	13.57	SAC96M	Metacarpal	-19.6	11.8	16.37
SAC91H	Humerus	-19.4	11.5	15.63	SAC96H	Humerus	-19.7	11.5	16.37
SAC91TV	Thoracic vertebrae	-19.7	11.8		SAC96TV	Thoracic vertebrae	-19.3	11.9	
SAC91P	Pelvis	-19.8	11.5		SAC96P	Pelvis	-19.0	11.6	
SAC91C	Clavicle	-19.6	11.6	6.86	SAC96C	Clavicle	-19.7	12.0	11.61

SAC92F	Femur	-19.6	10.7	10.94	SAC97F	Femur	-19.8	11.2	11.61
SAC92T	Tibia	-18.5	11.7	16.07	SAC97T	Tibia	-19.9	11.4	11.76
SAC92R1	Rib	-18.6	13.0	13.53	SAC97R1	Rib	-20.0	11.0	15.42
SAC92R2	Radius	-19.6	10.2	12.05	SAC97R2	Radius	-19.8	11.1	12.05
SAC92O	Occipital	-19.8	11.3	5.36	SAC97O	Occipital	-18.6	12.7	3.75
SAC92M	Metacarpal	-19.5	10.8	13.36	SAC97M	Metacarpal	-20.1	11.4	11.03
SAC92H	Humerus	-19.5	11.1	12.35	SAC97H	Humerus	-19.9	11.4	12.95
SAC92TV	Thoracic vertebrae	-18.9	12.8		SAC97TV	Thoracic vertebrae	-19.9	11.1	
SAC92P	Pelvis	-18.3	13.3		SAC97P	Pelvis	-20.2	10.8	
SAC92C	Clavicle	-19.7	11.1	11.01	SAC97C	Clavicle	-20.0	11.3	7.30

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