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

3

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31 **Abstract**

32 Stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope studies of ancient human diet increasingly
33 sample several skeletal elements within an individual. Such studies draw upon differences in
34 bone turnover rates to reconstruct diet during different periods of time within an individual's
35 lifetime. Rib and femoral bone, with their respectively fast and slow remodeling rates, are the
36 bones most often sampled to reconstruct shorter and longer term signals of diet prior to death.
37 It is poorly understood if $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ vary between bone types within a single individual,
38 or if this variation corresponds with bone turnover rate (BTR). Here, we determined $\delta^{13}\text{C}$ and
39 $\delta^{15}\text{N}$ for ten different bones from ten adult human skeletons (n=5 males; n=5 females).
40 Isotope values were compared to the rate that each bone remodeled, calculated from osteon
41 population (OPD) density. Results reveal that isotope ratios varied within each skeleton
42 ($\delta^{13}\text{C}$: max= -1.58‰; $\delta^{15}\text{N}$: max= 3.05‰). Humeri, metacarpals, and ribs had the highest rate
43 of bone remodelling; the occipital bone had the lowest. A regression analyses revealed that
44 higher rates of bone remodeling are significantly and negatively correlated with lower $\delta^{15}\text{N}$.
45 Our results suggest that the occipital bone, with its slow rate of bone renewal, may prove
46 useful for isotopic studies that reconstruct diet over longer periods of time within an
47 individual's lifetime. Isotope studies that compare individual skeletal elements between
48 populations should standardize their methodology to bones with either a slow or fast turnover
49 rate.

50

51 **Highlights**

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

57

58 **Keywords**

59 Stable isotopes. Bone remodelling.

60

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62

63 **1. Introduction**

64 Stable isotope analyses of biological tissues can provide a long-term record of diet (Deniro &
65 Epstein 1978; Rundel et al. 2016). Because of this, stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$)
66 isotope analyses of bone and dentin collagen have become a standard approach in
67 archaeological science for reconstructing dietary ecology of past modern human populations
68 (Ambrose & DeNiro 1989; Deniro & Epstein 1978; DeNiro & Epstein 1981; Hedges & Law
69 1989; Reynard & Hedges 2008), with applications extending to non-human primates and
70 fossilized remains (Bocherens et al. 1999; Fahy et al. 2013; Fahy et al. 2014; Fahy et al.
71 2015; Sponheimer et al. 2013). Increasingly, such studies incorporate isotopic signals from
72 several skeletal elements to reconstruct ancient diet during different periods of time within an
73 individual's lifetime (Sealy et al. 1995; Cox & Sealy 1997; Schroeder et al. 2009; Pollard et
74 al. 2012; Chenery et al. 2012; Lamb et al. 2014). The adult human rib and femur are the
75 skeletal elements most commonly sampled because of apparent differences in bone turnover
76 rates (see Section 1.3). However, little is known about relationships between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$
77 and remodelling in other skeletal elements. Here we 1) explore variation in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in
78 ten different bones from ten archaeological human skeletons and 2) identify associations
79 between these ratios and histomorphometric measurements of bone remodelling.

80

81 1.1 Stable carbon and nitrogen isotopes

82 Ratios of heavy to light stable isotopes of carbon ($^{13}\text{C}/^{12}\text{C}$) and nitrogen ($^{15}\text{N}/^{14}\text{N}$) display
83 distinctive patterns of distribution that enable them to be employed in the interpretation of
84 various aspects of life history. Body tissue isotopic composition is highly influenced by food
85 and drink consumed in life (Sealy et al., 1995), variation in food sources (Hopkins &
86 Ferguson 2012) and water availability (Stewart et al. 1995; Amundson 2003; Swap &
87 Aranibar 2004); consequently isotopic analyses of body tissues can offer clues to aspects of
88 diet and lifestyle. The main source of terrestrial carbon is atmospheric CO_2 whereas the main
89 source of marine carbon is dissolved CO_2 and bicarbonate ions (HCO_3^-). These sources of
90 carbon express $\delta^{13}\text{C}$ of -7.5 and +1.5‰, respectively (Lee-Thorp et al. 1989; Van Klinken
91 1991). The differences then continue up the food chain from primary producers to apex
92 predators (Lee-Thorp et al. 1989; Van Klinken 1991). This expression is dependent on the
93 biochemical mode of photosynthesis with most plants utilizing the C_3 cycle (expressing $\delta^{13}\text{C}$
94 around -26‰) compared to those few utilizing the C_4 pathway (expressing $\delta^{13}\text{C}$ around -
95 12‰) (Smith & Epstein 1971). Nitrogen incorporation into plant biomolecules can occur in
96 three different ways: direct nitrogen fixation from air, ammonium or nitrate in soil water,

97 recycled organic nitrogen from soil (Lee-Thorp 2008). Similar to $\delta^{13}\text{C}$, there is a stepwise
98 increase in $\delta^{15}\text{N}$ with trophic level (DeNiro & Epstein 1981). Isotope data from bone collagen
99 have long been shown to largely reflect the protein component of an individual's diet
100 (Ambrose & Norr 1993; Lee-Thorp et al. 1989; Schoeninger et al. 1997; Schoeninger et al.
101 1998; Schroeder et al. 2009).

102

103 1.2 Bone remodeling rates

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

117 Evidence of remodelling is retained in bone as basic structural and somewhat
118 independent functional units, as secondary osteons. Osteon population density (OPD) is a
119 measure of complete and fragmentary secondary osteons per section area, which together
120 represent past remodeling events (Frost 1994; Gocha & Agnew 2016). As such, OPD can
121 represent a measure of bone remodeling dynamics, or accrued bone density (Miskiewicz
122 2015). Increasing OPD is closely associated with advancing age, and eventually an asymptote
123 is reached where new secondary osteon formations begin to remove traces of earlier osteons
124 (Stout & Lueck 1995). When age-at-death is controlled for, OPD variation may indicate
125 differences in bone structure and response to mechanical stress (Britz et al. 2009; Schlecht et
126 al. 2012), dietary changes (e.g., Pfeiffer, S. K., & Lazenby 1994; Paine & Brenton 2006), or
127 health status (e.g., Martin & Armelagos 1979; Storm et al. 1993), or general human lifestyle
128 (Miskiewicz & Mahoney 2016).

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

131 bone sample (~135 μm thick) from a modern adult human ilium remodels at an average rate
132 of 17.7% per year, whereas a turnover rate for a cortical sample (~1225 μm thick) from the
133 same individual would remodel at approximately 7.7% per year (Parfitt 2002). When
134 considering cortical bone only, its renewal varies quite substantially throughout the skeleton
135 (Hobson & Clark 1992; Klinken & Mook 1990). For example, ribs are bones are never at rest
136 due to the load arising from respiration (Skedros et al. 2013); with a greater surface area to
137 volume ratio ribs have a relatively fast cortical turnover rate, which is approximately 4% a
138 year after age 50 (Frost 1969; Hill & Orth 1998). The dense cortical bone of the femoral shaft
139 is thought to have a slow turnover rate relative to rib bone (Hill & Orth 1998; Hedges et al.
140 2007; Skedros et al. 2013).

141

142 1.3 Human bone remodelling and isotope variation

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

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

165 **2. Materials and methods**

166

167 2.1 Samples

168 Ten human skeletons, dating to the early medieval period, from St Gregory's cemetery in
169 Canterbury, England, were selected (Hicks and Hicks 2001). Historical texts state that burials
170 were from a single socio-economic group that lived and worked in Canterbury, and represent
171 non-catastrophic mortality (Brent 1879; Duncombe 1785; Somner 1703). We selected
172 complete individuals without skeletal signs of pathology. This collection is curated in the
173 Skeletal Biology Research Centre, University of Kent, UK. All sectioning adhered to the
174 British Association of Biological Anthropology and Osteoarchaeology code of practice
175 (2014), and guidelines for invasive sampling (Mays, et al., 2013). No permits were required
176 as these are archaeological samples from before the 19th Century AD.

177

178 2.2 Collagen extraction and IRMS

179 Bone samples were taken from the same location on each bone from ten skeletons. Sampled
180 bones were femur, tibia, rib (right 5th), humerus, metacarpal, occipital, pelvis, clavicle, radius
181 and thoracic vertebrae. Samples were taken from the anterior mid shaft region of the tibia and
182 humerus, the posterior mid shaft region of the femur, and from the mid-shaft metacarpal,
183 radius, left 5th rib, clavicle, and the planum region of the occipital. An attempt was made to
184 separate cortical and cancellous bone for isotope ratios, but this proved difficult for
185 cancellous-rich bones such as the rib. Prior to sampling, bone surfaces were cleaned by air
186 abrasion with Al₂O₃; approximately 100-300mg of bone was sampled. Collagen extraction
187 was done following Longin (1971), Brown et al. (1988) and Richards & Hedges (1999).
188 Isotopic measurements were carried out using Elemental Analysis - Isotope Ratio Mass
189 Spectrometry (EA-IRMS) by Iso Analytical Limited (UK). The analytical precision,
190 calculated from repeated analysis of internal and international standards, was better than
191 0.2‰ (1σ) for δ¹³C and δ¹⁵N.

192

193 2.3 Histological sample preparation and analysis

194 Standard histological methods were used (e.g., Crowder & Stout 2011; Miskiewicz 2015;
195 Miskiewicz 2016). Dry un-decalcified transverse thin sections (each section was
196 approximately 0.7 ±0.2cm thick) were removed from the anterior mid shaft region of the tibia
197 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; Miskiewicz & 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-periosteally within the posterior cortex), ribs and
214 occipital (sub-periosteally 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 (2.24mm²). 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

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

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

240

241 2.6 Statistical analyses

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

250

251 3. Results

252 3.1 Isotopic variation between bone types

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

256

257 3.1.1 Males vs females

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

264

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

Bone	$\delta^{13}\text{C}$			$\delta^{15}\text{N}$		
	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

265

266 3.2 Isotopic variation within each skeleton

267

268

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

Males (n=5)			Females (n=5)			Males (n=5)			Females (n=5)		
All bones						Femur to Rib					
Sk	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	Sk	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	Sk	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	Sk	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
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

269

270 Variation in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ within each skeleton was broadly similar for males and females
 271 (Table 2). On average $\delta^{13}\text{C}$ differed by -0.9‰ within all skeletons. Mean $\delta^{15}\text{N}$ differed by
 272 1.7‰ within the female skeletons, compared to 1.5‰ for males. When skeletons are
 273 considered individually, $\delta^{13}\text{C}$ changed from -18.6‰ in the occipital to -20.2‰ in the pelvis of
 274 female skeleton number 10. $\delta^{15}\text{N}$ ranged between 1.0‰ to 3.1‰ in male skeleton number 5
 275 (Sk5) (Fig. 1), who also had the greatest change in $\delta^{13}\text{C}$. The femur of each male skeleton
 276 was consistently depleted in $\delta^{13}\text{C}$, and $\delta^{15}\text{N}$, when compared to the rib. Differences between
 277 these bones in females were inconsistent.

278

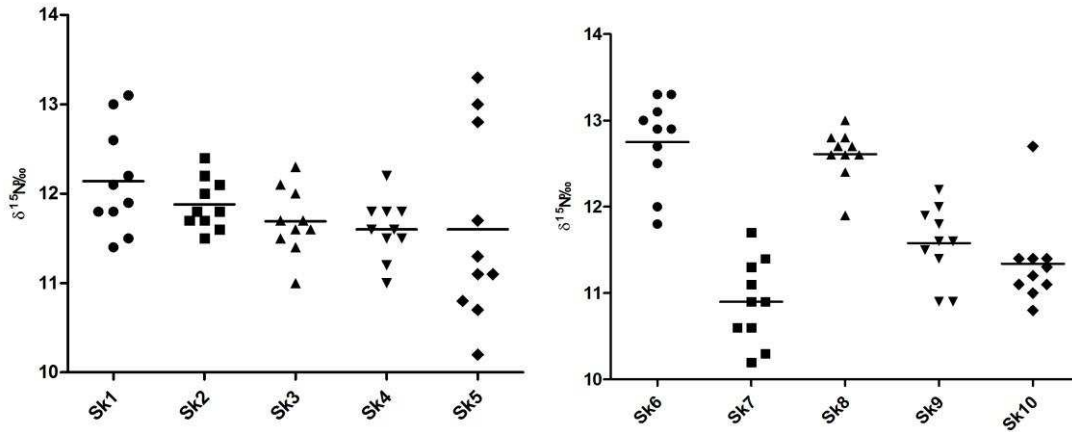


Fig. 1. : $\delta^{15}\text{N}$ for the 10 bones from each skeleton (males = Sk1 – Sk5; females = Sk6 – Sk10)

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

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

301

302 3.4. Relationship between isotope ratios and bone turnover compared between bone types

303

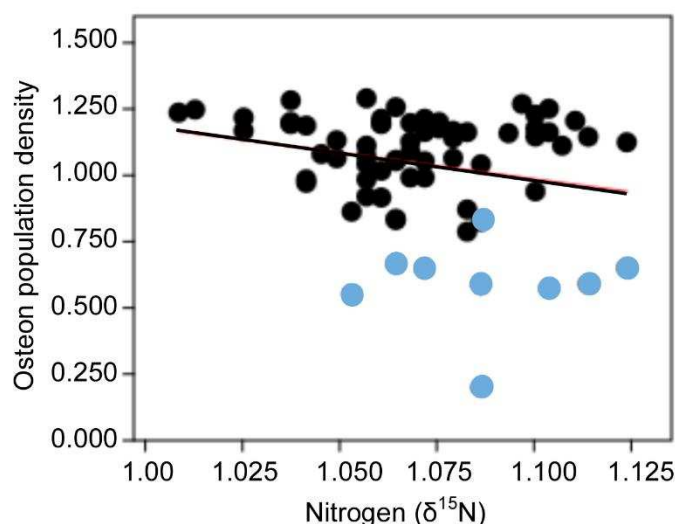
304

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

Bone	$\delta^{13}\text{C}$			$\delta^{15}\text{N}$			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

305

306 Average $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ and OPD data for each bone type is presented in Table 4. When all
 307 skeletons are combined, a linear regression analysis of log-transformed data indicates that
 308 there is a significant and negative correlation between $\delta^{15}\text{N}$ and bone turnover rates (slope = -
 309 1.986, intercept =3.171, $r = -0.231$; $r^2=0.053$, $p=0.050$). Figure 1 illustrates the negative
 310 relationship between these variables. The occipital bone is highlighted in the figure to
 311 illustrate the low bone turnover rates associated with this bone type. When the analysis was
 312 repeated on $\delta^{13}\text{C}$ and OPD, there was no significant relationship between the variables
 313 ($r=0.064$, $p=0.571$).



314
 315 **Fig. 2.** Linear regression analyses of log-transformed $\delta^{15}\text{N}$ against log-transformed osteon population density.
 316 Blue circles = occipital bone. Black circles are data for all other bone types¹ Excluding Sk 5 which showed a
 317 positive correlation between the variables, and the greatest variation in $\delta^{15}\text{N}$ of any skeleton: see Fig 1.
 318

319 3.4.1 Relationships between isotope ratios and bone turnover rates within each skeleton

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

327
 328 **Table 5:** Spearman's Rho analyses of $\delta^{15}\text{N}$ and OPD, and $\delta^{13}\text{C}$ and OPD within each skeleton. *Significant

Sk	$\delta^{15}\text{N}$		$\delta^{13}\text{C}$	
	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

329

330 4. Discussion

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

343 Our findings suggest that current isotopic sampling strategies can be modified to
344 incorporate the occipital, rather than the femur, to access a longer-term dietary signal. Our
345 data does not support the idea that the femur has a slow rate of turnover when compared to
346 the rib. Mean bone turnover rates of 13.48 (SD: 3.05) of the femur did not differ significantly
347 when compared to the mean turnover rate of 13.90 (SD: 3.69) for the rib (Mann Whitney U=
348 51.000; p= 0.940; Table 3). In contrast, mean OPD of the rib differed significantly when
349 compared to the occipital (mean=4.23, SD=1.31; U=0.000; p=0.000). This latter finding is
350 inconsistent with the long standing idea that a slower turnover of femoral bone collagen
351 reflects a longer-term dietary signal (Hedges et al. 2007) when compared to a faster turnover
352 of rib bone collagen that represents a more recent period prior to death (Cox & Sealy 1997).

353 Previous studies have reported varying results in terms of isotopic differences
354 between bones of the same skeleton. Olsen et al. (2014) analysed $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in four bones
355 (rib, metacarpal, fibula, vertebrae) of the skeleton, with sample sizes that were similar in size
356 to the current study. They found limited variation in either $\delta^{13}\text{C}$ ($0.0 \pm 0.1\text{‰}$) or $\delta^{15}\text{N}$ ($-0.1 \pm$
357 0.4‰). Similarly a study by Pollard et al. (2012) found that variation in $\delta^{13}\text{C}$ didn't exceed
358 analytical error; variation in $\delta^{15}\text{N}$ was slightly higher, but not statistically significant. Olsen et
359 al. (2014) also reported significant intra-skeletal variation in nitrogen values related to non-
360 specific disease. Skeletons selected for our study did not retain any evidence of non-specific
361 disease, though we cannot rule out the presence of active diseases at the point of death that do
362 not leave a record on bone (Wood et al. 1992).

363 Pollard et al. (2012) found rib $\delta^{15}\text{N}$ to be higher compared to femora by an average of
364 $\sim 0.5 - 1\text{‰}$ in a group of tenth-century young males. A similar trend was observed by Chenery
365 et al. (2012) who reported elevated rib $\delta^{15}\text{N}$ in comparison to femora by $0.9 - 1.2\text{‰}$ in 31
366 individuals analysed. In contrast, Jørkov et al. (2007) reported no measurable rib-femora
367 isotopic difference in 58 individuals from a static community from Holbæk, Denmark. We
368 found negligible difference between average $\delta^{15}\text{N}$ rib (11.7‰) and femora (11.6‰) in
369 females, but there was a 0.9‰ difference between average $\delta^{15}\text{N}$ rib (12.2‰) and femora
370 (11.3‰) in males (table 1). Hedges et al. (2007) suggest that male adolescent collagen
371 turnover rates are higher than in female adolescents. The differences observed between males
372 and females were related to femoral stable isotope values that reflect a substantial portion of
373 collagen synthesized during adolescence, when the rate of turnover is thought to be higher in
374 males (Hedges et al. 2007). Although we found a negligible difference in OPD in our samples
375 between the rib and the femur, it is possible that the difference in $\delta^{15}\text{N}$ between males and
376 females reflects increased BTR during adolescence.

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

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

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

398 Our study highlights that caution should be applied when substituting one bone for
399 another in isotope studies that compare single skeletal elements between individuals or when
400 sampling a small population of individuals for individual dietary interpretations. Our $\delta^{15}\text{N}$
401 ranged from 10.2‰ to 13.3‰ in male Sk5, and $\delta^{13}\text{C}$ changed from -18.6‰ in the occipital to
402 -20.2‰ in the pelvis in female Sk10. Thus, comparing different bone types between
403 individuals can potentially introduce additional variation into analyses, clouding diet-isotope
404 relationships. However, more freedom is allowed if the sample population is larger and the
405 goal is a population-wide dietary interpretation as interestingly, while individual $\delta^{15}\text{N}$, and to
406 some extent $\delta^{13}\text{C}$, vary greatly among individuals depending on the type of bone that is
407 sampled, when taken as a group these differences disappear for $\delta^{13}\text{C}$ (females = $-19.2\pm 0.6\%$;
408 males = $-19.3\pm 0.5\%$) and $\delta^{15}\text{N}$ (females = $11.8\pm 0.9\%$; males = $11.8\pm 0.6\%$).

409

410 **5. Conclusion**

411 Our study sampled ten bones from ten individuals to examine the range of variation in
412 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ across the skeleton and to determine relationships between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ and
413 static indicators of bone remodelling. Lower $\delta^{15}\text{N}$ were significantly correlated with higher
414 values of remodelling products when compared between individuals. Given that many studies
415 utilize the differences in turnover rates to demonstrate dietary changes in individuals and
416 populations, and that much emphasis is put on $\delta^{15}\text{N}$ and potential high or low protein diets,
417 we suggest that future stable nitrogen isotope studies of diet should standardize bone
418 sampling, to bones with either high or low turnover rates.

419

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423

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MALES					FEMALES				
Lab #	Bone	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	OPD	Lab #	Bone	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	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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