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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

61

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  $\text{CO}_2$  whereas the main  
89 source of marine carbon is dissolved  $\text{CO}_2$  and bicarbonate ions ( $\text{HCO}_3^-$ ). 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  $\text{C}_3$  cycle (expressing  $\delta^{13}\text{C}$   
94 around -26‰) compared to those few utilizing the  $\text{C}_4$  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  $\mu\text{m}$  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  $\mu\text{m}$  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 19<sup>th</sup> 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 5<sup>th</sup>), 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 Al<sub>2</sub>O<sub>3</sub>; 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 δ<sup>13</sup>C and δ<sup>15</sup>N.

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 5<sup>th</sup> 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 (2.24mm<sup>2</sup>). 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

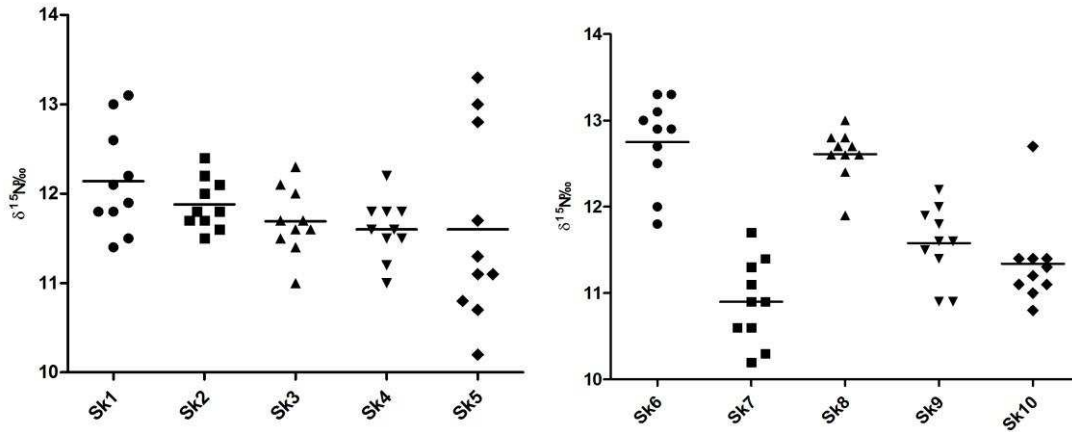


Fig. 1. :  $\delta^{15}\text{N}$  for the 10 bones from each skeleton (males = Sk1 – Sk5; females = Sk6 – Sk10)

### 3.3. Variation in bone turnover rate between bone types

Table 3: Mean OPD for each bone type

| Bone <sup>1</sup> | 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.

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

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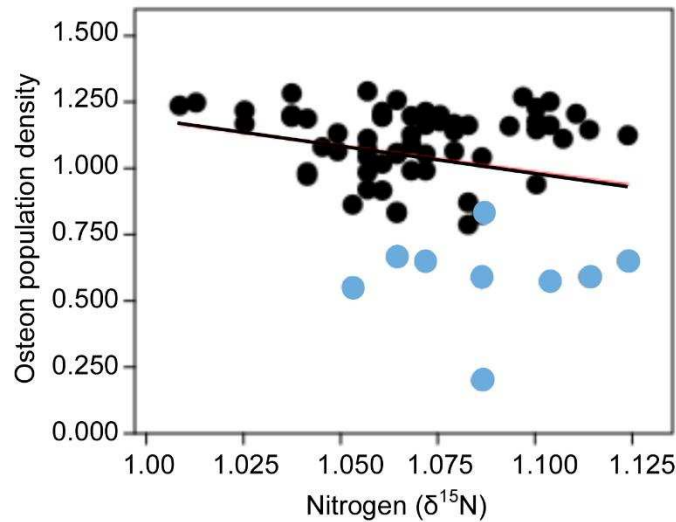
304

**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         |

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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 ( $r=0.064$ ,  $p=0.571$ ).



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

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

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When each skeleton is considered separately,  $\delta^{15}\text{N}$  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  $\delta^{15}\text{N}$  values are generally associated with lower products of remodelling, within each skeleton. When each skeleton is considered separately  $\delta^{13}\text{C}$  are OPD are positively correlated for eight of the 10 skeletons (Table 5). For one skeleton (SAC 92), this relationship is significant.

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**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      |
| <b>Males</b>   |                       |        |                       |        |
| 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* |
| <b>Females</b> |                       |        |                       |        |
| 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  |

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#### 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  $0.4\text{‰}$ ). 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 ( $11.7\text{‰}$ ) and femora ( $11.6\text{‰}$ ) in  
369 females, but there was a  $0.9\text{‰}$  difference between average  $\delta^{15}\text{N}$  rib ( $12.2\text{‰}$ ) and femora  
370 ( $11.3\text{‰}$ ) 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  $\text{C}_3$ -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 =  $-19.2 \pm 0.6\text{‰}$ ;  
408 males =  $-19.3 \pm 0.5\text{‰}$ ) and  $\delta^{15}\text{N}$  (females =  $11.8 \pm 0.9\text{‰}$ ; males =  $11.8 \pm 0.6\text{‰}$ ).

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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