Mercury and Stable Isotope Analysis of Human Bone from a Late Neolithic/Chalcolithic Ditched Enclosure at Perdigões, Portugal

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Project Summary

Numerous ditched enclosures of late Neolithic and Chalcolithic ages have been documented throughout Europe including the Iberian Peninsula. The large enclosure at Perdigões, south-central Portugal, has been excavated since 1997 and includes at least eight funerary features (pits, ditches and *tholoi* tombs; Valera et al. 2014). Depositions of human remains (including cremations), animal remains, and a diversity of archaeological materials were deposited in pits, ditches or tombs at the site. Here, 21 samples of human bone were analyzed for total mercury concentration using a Tri-cell Direct Mercury Analyzer (DMA-80). The samples were from a variety of proveniences including Pit 11, 16, Tomb 1 and Tomb 2 (Fig. 1) with radiocarbon dates that span the known chronology of the site. The samples produced a high variation in mercury content, ranging from 0.06 to 115.62 ppm; samples from Tomb 2 had the highest levels. Our original hypothesis was that mercury levels would correlate with diet as individuals feeding at higher trophic levels would be more exposed via biomagnification. However, stable isotope analysis ($\delta^{13}C$ and $\delta^{15}N$) of the same 21 bone samples revealed a weak negative relationship with $\delta^{15}N$. These results imply a possible age and/or sex bias in exposure to mercury, possibly through the use of cinnabar as a pigment in funerary rituals, body paintings, pottery or other cultural uses.

Figure 1. Map of the Iberian Peninsula (top) showing the location of Perdigões (Portugal) and the cinnabar mine at Almaden, Spain. Bottom: aerial view of Perdigões showing location of ditches from magnetometry survey, as well as the pits and tombs where human remains were recovered.

Methods

Mercury analysis was completed by first washing the bone in running distilled water and scrubbing with a brush to remove any surface contaminants. The outer surface of the bone was then removed with a pneumatic drill to expose clean inner compact bone. After wiping the drill and counter clean with ethanol, inner bone was removed onto a clean piece of aluminum foil with the drill until enough powdered bone was obtained for stable isotope and mercury analyses (0.02-0.04 g). The samples for mercury analysis were weighed and analyzed for total mercury using a Tri-Cell DMA-80 at UNCW. Stable isotope analyses were completed by W. Patterson at the Sakatchewan Isotope Laboratory.

Figure 2. Pneumatic drill (top) used to sample human bone. Bottom: the Direct Mercury Analyzer (DMA-80) used to measure total mercury from bone samples.

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Results

Preliminary results on 21 individuals from four different burial pits at Perdigões indicate a high level of variation in both stable isotope (δ15N, 3.6 – 15.5 ‰) and mercury values (range 0.06 – 106.65 ppm; Table 1). While initially it was expected that mercury exposure would be minimal, the results were quite surprising in indicating that most individuals had extremely high levels of total mercury in bone (>10 ppm in 12 individuals). As this was the first time that mercury had been analyzed in Neolithic/Chalcolithic human remains, the unexpectedly high level of exposure revealed by this analysis quickly became the main focus of this research to determine the source of the mercury and effect it may have had on highly exposed individuals. In addition, there was a weak negative correlation ($R^2 = 0.114$) between $\delta^{15}N$ and Hg, indicating no relationship with diet and mercury levels in the samples (Fig. 3).

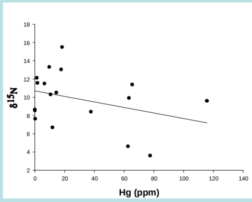

Figure 3. Human bone mercury and nitrogen content showing week negative relationship ($R^2 = 0.114$). These results indicate that diet is not related to mercury levels in the bone.

Table 1. Total mercury in human bone samples from Perdigões.

Human Bone Provenience	Hg (ppm, dw)	Date (BP)*	Notes
pit 16 #90.2 rib fragment	1.40	3990 +/- 40	Adult
pit 16 #74.2 rib fragment	0.41	3990 +/- 40	Adult
pit 7 114 #20 distal left fibula	0.26	4430 +/- 40	ad female
pit 11 #76 proximal right humerus	0.06	4370 +/- 40	juv. male
pit 11 #77 diaphysis of right femur	0.06		juv. male
pit 11 #78 diaphysis of right femur	0.12		juv. male
tomb 1 F7 #42 97 Chamber diaphysis of femur	18.24	4030 +/- 40	Adult
tomb 1 B5 #59 97 Chamber diaphysis of right femur	6.43	4060 +/- 30	Adult
tomb 1 #1754 447 302 Chamber diaphysis of femur	17.63	4130 +/- 30	Adult
tomb 1 #117 63 Chamber diaphysis of left femur	1.83		Adult
tomb 1 E7 #105 Chamber diaphysis of left femur	32.23		Adult
tomb 2 Bag 2278 #1823 Chamber diaphysis L. femur	9.64	3840 +/- 30	Adult
tomb 2 Bag 1236 #429 Chamber diaphysis R. femur	11.82	4090 +/- 30	Adult
tomb 2 Bag #429 1804 Chamber diaphysis R. femur	77.39	3890 +/- 30	Adult
tomb 2 Bag 1133 #429 Chamber diaphysis R. femur	37.76	3970 +/- 30	Adult
tomb 2 Bag 1339 #402 Chamber diaphysis L. femur	65.48		Adult
tomb 2 Bag 3955 #108 Atrium diaphysis L. femur	62.58		Adult
tomb 2 Bag 3043 #614 Atrium diaphysis L. femur	115.62		Adult
tomb 2 Bag 2631 #486 Atrium diaphysis R. femur	10.57		Adult
tomb 2 Bag 638 #227 Atrium diaphysis L. femur	14.49		Adult
tomb 2 Bag 1370 #239 diaphysis R. femur	63.20		Adult

*Dates are from Valera et al. 2014 SPAL 23: 11-26

Discussion

Two major questions have thus emerged: how did the mercury enter the bone tissue, and were the levels high enough to have caused mercury poisoning to the prehistoric people? With diet unlikely as a source of the high mercury levels in bone, answering the first question includes analysis of soil samples to determine if mercury leached into the bone after deposition (the diagenetic hypothesis). Some of the burial pits had high levels of ochre and cinnabar sprinkled over the human remains after deposition (Fig. 4 and Table 2). Moreover, cinnabar has been documented at other Neolithic sites in Europe (Mioc et al. 2004), indicating it was widely available. Cinnabar mining by the Inca and Spanish also has contributed to high levels of mercury in lake sediments dating over the past 3500 years in Peru (Cooke et al. 2009). Could cinnabar sprinkled over burials enter bone tissue via leaching, or did cultural use of cinnabar as shown by its presence with burials result in mercury exposure to individuals using it in life (the cultural use hypothesis)? Both of these questions are currently being tested with analysis of soil samples, human remains from pits with and without cinnabar associated with the remains, and Hg isotope analysis of raw cinnabar samples obtained with a collaborator (M. Hunt-Ortiz) from known mines and sources of this mineral in Spain. With these analyses, it will be possible to answer the first question on how mercury entered the bone tissue, as well as tracing its original source (see application of this relatively new methodology in Gehrke et al. 2011). Once this question is addressed, we will be able to begin answering the second question and whether or not mercury poisoning was impacting prehistoric human health.

Figure 4. Ochre or cinnabar was found in association with human remains in Tombs I and II at Perdigões (top). Bottom: a sample of cinnabar in its raw form from Almaden.

Table 2. Results of geochemical analyses of ochre and cinnabar found with human remains in Tombs I and II at Perdigões.

Amostra	Unidade	Sep	Cor	Resultados EDXRF	Outros elementos	Interpretação EDRXF	Raman
4574	29	1	Rosa	Fe	Hg, Ti	Óxidos Ferro	Vermelhão/ cinábrio
7246	201	1	Amarelo	Fe	K, S, Ca	Óxidos Ferro	Oxido ferro III
9250	175	1	Castanho	Fe	Ca, Ti, Si	Óxidos Ferro	-
9268	310	1	Rosa	Hg	Si, Fe	Cinábrio	Vermelhão/ Cinábrio
9275	311	1	Castanho	Fe	K, Si	Óxidos Ferro	Hematite/ red earths
9561	310	1	Avermelhado	Hg + Fe	Ca, Si		Cinábrio
9573	175	1	Rosa	Hg	Si, Fe, S	Cinábrio	Cinábrio
9716	173	1	Laranja	Hg	Si, Fe, S	Cinábrio	Vermelhão
10261	408 A	2	Castanho	Hg + Fe	Ca, Si		-
10339	407	2	Rosa	Hg + Fe	Ca, Si		Cinábrio
10437	430	2	Amarelo	Fe	K	Óxidos Ferro	-
11042	429	2	Rosa	Hg + Fe	Ca, Si		Cinábrio

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