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Cribra and trace elements in the Prat de la Riba Necropolis (Tarragona, Spain, 3rd-5th centuries AD)

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Abstract The different kinds of *cribra* are manifestations of porotic hyperostosis on the orbital roof (*cribra orbitalia*), on the femur neck (*cribra femoralis*) or on the humerus head (*cribra humeralis*). The aetiology of *cribra* is not clear. Some authors attribute the presence of *cribra* to anaemia (infectious or genetic) while others consider it the consequence of nutritional deficit. The analysis of trace elements in human bones may help to reveal the aetiology of *cribra*. This study analyzes the prevalence of *cribra* related to trace element levels in a Spanish late Roman necropolis (3rd–5th centuries AD). The results show no association between the different kinds of *cribra* and nutritional deficiency.

Key words Paleopathology; *cribra;* trace elements; Tarragona; Late Roman Necropolis.

Resumo Os diferentes tipos de *cribra* são manifestações de hiperostose porótica localizadas nas fossas orbitárias (*cribra orbitalia*), no colo femoral (*cribra femoralis*) ou na cabeça do úmero (*cribra humeralis*). A etiologia da *cribra* não é clara. Alguns autores atribuem a presença de *cribra* a anemia (infecciosa ou genética), enquanto outros a consideram resultante de um défice nutricional. A análise de oligoelementos nos ossos humanos pode contribuir para revelar a etiologia da *cribra*. Esta metodologia foi utilisada no espólio de uma necrópole Tardo-Romana Espanhola dos séculos III-V d.C.. Os resultados obtidos mostraram não haver associação entre a incidência dos diferentes tipos de *cribra* e a deficiência nutricional.

Palavras-chave Paleopatologia; *cribra;* oligoelementos; Necrópole Tardo-Romana; Tarragona.

Introduction

Trace elements are defined as those occurring in amounts of less than 0.01% of the human body mass (Gilbert, 1985). They can contribute significantly to paleopathological analyses because of their associations with the biological conditions of humans groups, prehistoric diets and the aetiology of different illnesses. Multi-elemental analyses are employed in paleopathology both to discover the relationship between nutrition and illness, and to estimate the effect of chemical deficiencies or excesses in the organism (Carlson *et al.*, 1974; Fornaciari *et al.*, 1981; Von Endt and Ortner, 1982; Gilbert, 1985; Subirà *et al.*, 1992; Gleń-Haduch *et al.*, 1997). Normal growth and development, and the maintenance of good health, depend upon an adequate supply of the essential trace elements (Waldron, 1987). Excess or deficiency of those elements in the diet could play a negative part in the development of whole populations (Smrčka *et al.*, 1989).

Although paleopathology is aimed at evaluation of disease in excavated skeletons, the detectable macro- or microscopic symptoms are often not very specific. Plausibility of diagnosis is increased if other background information is available. We therefore combine two methods – paleopathology and chemical analyses of excavated skeletons – to identify the causes of disease in past populations (Hühne-Osterloh and Grupe, 1989).

In this study we analyse three pathological lesions: *cribra orbitalia, cribra femoralis* and *cribra humeralis*. The different kinds of *cribra* are considered to be markers of either nutritional deficiencies or physiological stress (Hengen, 1971; Carlson *et al.*, 1974; El-Najjar *et al.*, 1975; El-Najjar, 1976; El-Najjar *et al.*, 1976; El-Najjar and Robertson, 1976; Lallo *et al.*, 1977; Stuart-Macadam, 1982, Cohen and Armelagos, 1984; Campillo, 1993; 2001). Other authors attributed the *cribra* to genetic or infectious anaemia (Moore, 1929; Smith, 1954; Angel, 1966; 1971; 1984; Weinberg, 1974; Stuart-Macadam, 1982; 1992).

Cribra orbitalia is a form of porotic hyperostosis present on the orbital roof, whose aetiology is still rather obscure, although most authors accept that it is related to genetic or infectious anaemia.

Stuart–Macadam (1992) has reported three major trends in the occurrence of porotic hyperostosis: temporal, geographic and ecological. *Cribra femoralis* is another form of porotic hyperostosis, observed on a specific zone in the femur neck; it seems to be more frequent in sub-adults. The third form, *cribra humeralis*, has been described only recently in the paleopathological literature (Baxarias, 2001). This form is located in the internal anterior region of humerus head, near the metaphysis.

For this study we analized the population of Prat de la Riba, a great necropolis which is very well suited for this kind of study, not only for the good state of bone preservation, but also for the high number of skeletons recovered and for their careful excavation and prior study. The necropolis of Prat de la Riba is located in the city of Tarragona (Spain) and it is part of a huge cementerial area called Necropolis del Francolí. In the Prat de la Riba necropolis were found 220 burials which contained 243 skeletons. The site was dated using the Keay typological systematization classification of the amphoric containers. It was occupied for a long period of time during the Late Roman period, from the end of the 3rd century a.C. to the end of the 5th century a.C. (Foguet and Vilaseca, 1995).

The main goal of this study is to examine the relationship between the lesions of *cribra orbitalia*, *cribra femoralis* and *cribra humeralis* and the concentration of selected trace elements in human skeletons from the Prat de la Riba necropolis.

Material and method

For this study, we submitted human bone samples from 118 individuals, both pathological and non-pathological, for chemical analysis of trace elements (Table 1). The chemical elements chosen for this study were: calcium (expressed in percentage in one bone gram), strontium, barium, zinc, copper and magnesium (quantified in parts per million, ppm). Iron would have been a very interesting element to include in this type of study because of its relationship to iron deficiency anaemia. However, this element is very difficult to quantify using standard trace elements methodology because it is very subject to diagenesis, and so it was excluded (Lambert and Weydert-Homeyer, 1993; Price and Kavanagh, 1982). For this analysis we sampled the right femur of each of the 118 individuals because it was more often present than other bones in the skeletal remains. Before the chemical analysis, the surface of each bone sample was removed to eliminate possible contamination. The chemical method used for bone analysis was the one established in the *Unitat d'Antropologia* in the Biology Department of the *Universitat Autònoma de Barcelona* (Subirà, 1993). The trace elements were quantified using an ICP/AES Spectrometer at the *Serveis Científico-Tècnics* of the *Universitat de Barcelona.* A complete anthropological analysis, which includes anthropometric, demographic, nutritional and paleopathological data, was previously performed for the skeletal individuals in the *Museu d'Arqueologia de Barcelona.*

Table 1. Distribution of the human bone samples studied.

Sex/Age	Infantile I	Infantile II	Juvenile	Adult	Mature	Senile	TOTAL
Female	0	5	17	18	15	3	58
Male	1	6	7	14	18	3	49
Indeterminate	6	4	1	0	0	0	11
TOTAL	7	15	25	32	33	6	118

The range ages are: Infantile I: 0-6 years; Infantile II: 7-12 years; Juvenile: 13-20 years; Adult: 21-40 years; Mature: 41-60 years; Senile: 61- 80 years. For estimation of sex we used the skull and pubis morphology for the adults and the jaw and pubis morphology for the sub-adults (Schutkowsky, 1993).

The distributions of the concentrations of trace elements were first analysed by Kolmogorov–Smirnov tests (SPSS 11.5.1). The concentrations of barium, strontium, magnesium, copper and calcium (but not zinc) were normally distributed, so they were further analysed using parametrical tests. For zinc, a non-parametrical test was employed.

Results and discussion

Before comparing the levels of trace elements with the presence/absence of *cribra* it is necessary to know the demographic profile of the sampled population, to determine if different concentrations of trace elements are associated with sex or age in the sample. The presence of different elemental concentrations can be attributed either to physiology or to diet. No relationship was found between the levels of barium, calcium, copper, magnesium, zinc and strontium and the sex of the specimens when ANOVA analysis was applied, although the males have slightly higher levels of all elements except calcium. As for age, all elements showed higher levels in sub-adults than in adults, except for calcium. Magnesium initially showed a significant difference in concentration between age groups (P=0,037) but when a Post Hoc test (Tukey) was applied in order to evaluate in greater detail the inter-group variation, the differences were no longer significant. However, significant differences in concentrations between the age groups for copper remained consistent, detected through ANOVA analysis (Table 2). Infants and juveniles showed higher copper levels than mature and elderly adults. These different age-related patterns probably reflect both physiology and diet. Neonates and infants show a generalized tendency towards higher tissue levels of essential elements which gradually decline with age towards the adult levels. This temporal change in tissue levels is reflected in the concentrations in the skeleton (Waldron, 1987). When infants are weaning they may be fed additional foods like cow's milk and cereal gruels (Hühne-Osterloh and Grupe, 1989). Cereals are very rich in copper, and their consumption may explain the higher levels of copper found in the infantile group. This same pattern has been reported at other sites such as Can Martorell (Barcelona, Spain) and Can Reinés (Mallorca, Spain) (Garcia and Subirà, 2001; Subirà and Garcia, 2003).

Table 2. Statistical values of trace elements by age groups.

T. E.	Age groups	N	Mean	Standard deviation	F	P
Ba	Infantile I	7	799,947	411,580	0,507[1]	0,771
	Infantile II	15	659,833	229,722		
	Juvenile	25	670,418	371,425		
	Adult	32	756,005	379,473		
	Mature	33	659,130	337,660		
	Senile	6	622,750	217,288		
	TOTAL	**118**	**694,386**	**342,418**		
Cu	Infantile I	7	107,452	28,541	8,045[1]	**0,000**
	Infantile II	15	87,453	37,888		
	Juvenile	25	80,718	23,200		
	Adult	32	63,730	21,204		
	Mature	33	56,577	21,956		
	Senile	6	77,717	13,757		
	TOTAL	**118**	**71,649**	**28,150**		
Mg	Infantile I	7	1672,225	127,009	2,469[1]	0,037
	Infantile II	15	1700,267	299,411		
	Juvenile	25	1514,600	182,707		
	Aadult	32	1613,672	247,411		
	Mature	33	1584,606	181,321		
	Senile	6	1806,917	352,966		
	TOTAL	**118**	**1608,861**	**233,089**		
Sr	Infantile I	7	670,884	124,952	1,410[1]	0,226
	Infantile II	15	808,533	217,391		
	Juvenile	25	750,472	184,095		
	Adult	32	835,922	204,415		
	Mature	33	782,726	211,132		
	Senile	6	912,250	313,146		
	TOTAL	**118**	**793,550**	**208,407**		
Ca	Infantile I	7	29,969	3,139	1,941[1]	0,093
	Infantile II	15	29,541	2,109		
	Juvenile	25	30,364	2,182		
	Adult	32	31,461	1,918		
	Mature	33	30,242	2,661		
	Senile	6	29,789	1,511		
	TOTAL	**118**	**30,470**	**2,336**		
Zn	Infantile I	7	169,204	35,640	2,942[2]	0,709
	Infantile II	15	176,893	44,567		
	Juvenile	25	192,192	49,766		
	Adult	32	180,242	45,738		
	Mature	33	193,453	77,596		
	Senile	6	244,633	112,703		
	TOTAL	**118**	**297,824**	**26,571**		

T.E. - trace elements; Ba - barium; Cu - cooper; Mg - magnesium; Sr - strontium; Ca - calcium; Zn - zinc.
(1) ANOVA Test; (2) Kruskal Wallis Test.

Among the late Roman remains from Prat de la Riba, 10% of the specimens displayed *cribra orbitalia* (12/124 cases observed). There was no difference between males and females in the frequency of occurrence of this pathology. However, *cribra orbitalia* was more frequently observed in skulls of individuals less than twenty years old (Baxarias, 2001). Trace elements levels do not differ significantly between individuals with *cribra orbitalia* and without the lesions when analyzed by Student's t-test (Table 3).

Table 3. Statistical values of trace elements and *cribra orbitalia* and Student's t-test.

T. E.	*cibra orbitalia*	N	Mean	Standard deviation	Student's t-test	P
Ba	No	102	699,522	341,325	0,638	0,525
	Yes	12	633,321	326,676		
Cu	No	102	69,831	26,114	-1,444	0,152
	Yes	12	81,363	26,676		
Mg	No	102	1607,158	220,776	0,129	0,897
	Yes	12	1597,958	325,584		
Sr	No	102	785,214	208,104	-0,734	0,465
	Yes	12	831,804	207,129		
Ca	No	102	30,485	2,407	-0,026	0,979
	Yes	12	30,504	2,062		
Zn	No	102	191,193	64,402	500,5 (1)	0,303
	Yes	12	172,200	34,929		

[1] U Mann-Whitney Test

In the studied population, *cribra femoralis* is present in 27% of the skeletons (31/113 cases observed). No relationship between the presence or absence of *cribra femoralis* and the sex of the specimens was found. On the other hand, in this necropolis there exists an association between *cribra orbitalia* and *cribra femoralis* (Baxarias, 2001). When a Student's t-test was applied, a significantly higher level of copper was found in the skeletons with *cribra femoralis* (Table 4). Ninety-four percent of the individuals with this pathology are infants and juveniles, who also show higher levels of copper than do the adults in the sample. When we only consider the sub-adult group, no differences were found in the level of copper between the individuals with and without this pathology.

Table 4. Statistical values of trace elements and *cribra femoralis* and Student's-Test.

T. E.	*Ccribra femoralis*	N	Mean	Standard deviation	Student's t-test	P
Ba	No	82	672,192	328,568	-0,997	0,321
	Yes	31	743,468	366,429		
Cu	No	82	66,303	26,321	-2,831	0,006
	Yes	31	81,706	24,384		
Mg	No	82	1618,043	246,242	0,538	0,592
	Yes	31	1591,451	199,301		
Sr	No	82	780,024	212,915	-0,827	0,410
	Yes	31	816,710	203,420		
Ca	No	82	30,733	2,537	1,597	0,113
	Yes	31	29,943	1,734		
Zn	No	82	189,349	66,569	1215,500 (1)	0,721
	Yes	31	182,205	47,227		

[1] U Mann-Whitney Test

In the Prat de la Riba population, 3% of the individuals show *cribra humeralis* (3/112 cases observed). One case also shows evidence of chronic osteomyelitis. There is one hundred percent statistical association between *cribra femoralis* and *cribra humeralis*. We found no difference in the levels of any particular element between specimens with pathological changes and healthy specimens, using Student's t-test analysis (Table 5).

Table 5. Statistical values of trace elements and *cribra humeralis* and Student's t-test.

T. E.	*cribra humeralis*	N	Mean	Standard deviation	Student's t-test	P
Ba	No	109	690,931	340,873	-0,281	0,779
	Yes	3	747,267	401,600		
Cu	No	109	69,951	26,858	-0,869	0,386
	Yes	3	83,517	11,582		
Mg	No	109	1607,211	228,609	0,679	0,499
	Yes	3	1517,166	39,863		
Sr	No	109	781,550	199,273	-0,685	0,495
	Yes	3	861,833	244,717		
Ca	No	109	30,542	2,378	0,731	0,466
	Yes	3	29,523	2,457		
Zn	No	109	188,222	62,490	121,000 (1)	0,468
	Yes	3	159,150	37,153		

[1] U Mann-Whitney Test

If now we consider the three different forms of *cribra* to be pathologies with the same aetiology (Miquel-Feucht *et al.*, 2000) 32% of the individuals show some kind of *cribra* (35/108 cases observed). We also observed significantly higher levels of copper in the specimens with *cribra*, as related to the age of the individuals, through Student's t-test analysis (Table 6).

Table 6. Statistical values of trace elements and *cribra* in general and Student's-Test.

T. E.	*cribra*	N	Mean	Standard deviation	Student's t-test	P
Ba	No	73	678,172	331,354	-0,582	0,562
	Yes	35	719,130	363,617		
Cu	No	73	65,631	25,896	-3,178	**0,002**
	Yes	35	82,399	25,169		
Mg	No	73	1600,063	220,076	-0,060	0,952
	Yes	35	1602,886	243,132		
Sr	No	73	767,059	199,317	-1,028	0,306
	Yes	35	809,376	201,898		
Ca	No	73	30,765	2,573	1,453	0,149
	Yes	35	30,050	1,951		
Zn	No	73	192,042	69,680	1161,500 (1)	0,446
	Yes	35	179,953	45,210		

[1] U Mann-Whitney Test

One of the possible aetiologies attributed to *cribra* is anemia, either of genetic, infectious, or nutritional origin. In this study, it seems likely that the aetiology of the *cribra* lesions is not anaemia because in the Prat de la Riba population trace element deficiencies are not present. Moreover, in the sub-adult group, individuals with and without *cribra* all present higher levels of copper, related to the physiology and diet of this age group.

Conclusions

In the Prat de la Riba population, a statistical association was evident between the age of the specimens and the levels of only one element: copper. However, the significantly higher level of copper in young subadults (94% of the individuals with *cribra femoralis*) can be explained by physiology and/or by a diet rich in cereals during the weaning period. The study showed no associations between *cribra* and lower levels of strontium, barium, magnesium, calcium and zinc.

Therefore, it does not appear that *cribra* was related to nutritional deficiencies, at least not those reflected by the trace elements under examination. Further studies are necessary to search for a relationship with population genetics (through DNA analysis), infectious conditions, or other processes which involve bone marrow hyperplasia.

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