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TECHNICAL NOTE

The impact of unburned bone antimere asymmetry and burned bone preservative procedures on osteodensitometric studies

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ABSTRACT

Heat-induced bone changes are being increasingly studied. Namely, it is necessary to evaluate some relevant assumptions regarding experimental investigations involving burned bones. This study intends to assess potential bilateral asymmetry between antimeres and the effect that the application of the Primal SF-016 preservative may have in the inorganic portion of the bone as well as its possible impact on osteodensitometric studies. Therefore, femora, patellae and first metatarsals were selected from five female skeletons and five male skeletons from the 21st Century

Identified Skeletal Collection of the University of Coimbra. This sample was analysed with Dual X-ray Absortionmetry in order to compare the right antimere with the left antimere and to assess possible variation between the pre- and post-application of Primal SF-016 on the same antimere. Results showed important bilateral asymmetry between antimeres, not allowing for the extrapolation of results from one antimere to the other which is a procedure often followed in osteodensitometric studies. Bone mineral density variations between pre- and post- preservative applications suggest the occurrence of bone crystallinity changes caused by Primal SF-016 thus biasing osteodensitometric analyses. Therefore, caution is required when implementing this kind of analyses, especially when applied to burned bones.

Keywords: Forensic anthropology, Bioarchaeology, DXA, bilateral asymmetry, Primal SF-016.

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RESUMO

As alterações provocadas pela queima no esqueleto são um tema cada vez mais estudado, tornando-se necessário averiguar alguns pressupostos relevantes no âmbito de investigações com ossos experimentalmente queimados. Este estudo pretende averiguar a potencial assimetria bilateral entre antímeros e o efeito que a aplicação do consolidante Primal SF-016 pode ter na fracção inorgânica do osso e o seu respectivo impacto em estudos osteodensitométricos. Para tal, foram selecionados os fémures, patelas e primeiros metatársicos de cinco esqueletos femininos e cinco esqueletos masculinos da Coleção de Esqueletos Identificados do Século XXI da Universidade de Coimbra. Esta amostra foi analisada com recurso a densitometria bifotónica de modo a comparar o antímero direito com o esquerdo. Além disso, compararam-se os valores obtidos para o osso antes e após a aplicação do consolidante. Foi detetada uma importante assimetria bilateral entre antímeros, o que obsta à extrapolação dos resultados de um antímero para o outro, procedimento frequentemente adoptado em estudos osteodensitométricos. As variações na densidade mineral óssea pré- e pós-consolidante sugerem que a aplicação do Primal SF-016 provoca alterações na cristalinidade do osso enviesando assim as análises osteodensitométricas. Este estudo alerta por isso para alguns cuidados a ter neste tipo de análises, especialmente quando aplicados a ossos queimados.

Palavras-chave: Antropologia Forense, Bioarqueologia, DXA, assimetria bilateral, Primal SF-016.

Introduction

The study of bones experimentally subjected to high temperatures is essential to better understand this taphonomic process, and several investigations have long been focused on both the macro- and microstructure of bone undergoing thermal changes (Binford, 1963; Herrmann, 1977; Shipman *et al.*, 1984; McKinley, 1993; Thompson, 2004, 2005; Thompson *et al.*, 2009; Castillo *et al.*, 2013; Gonçalves *et al.*, 2013; Ellingham *et al.*, 2015; Reidsma *et al., 2016*; Krap *et al.*, 2019*)*.

Identified human skeleton collections have been amassed for more than one century (Ferreira *et al.*, 2014), positively impacting anthropological research and their benefits over animal proxies are self-evident. However, collections of experimentally burned human skeletal remains are scarce. To our knowledge only one is currently available, namely the 21st Century Identified Skeletal Collection (CEI/XXI) housed at the Laboratory of Forensic Anthropology of the University of Coimbra (Ferreira *et al.*, 2014; Gonçalves, 2016). Given its uncommonness and typical fragile nature, it is important to ensure that these experimentally burned skeletons maintain their preservation so that they can continue to be used in future research. In that collection, just one of the two antimeres of each individual is experimentally burned so that the unburned antimere can be used as a comparative reference. It is assumed that the latter can act as an approximate representative of the pre-burn data of the burned antimere. Although this may hold true for several parameters, it is necessary to verify whether the differences between antimeres are negligible or not. Bilateral asymmetry is very common in biological structures (Gawlikowska *et al.*, 2007; Krishan, 2011; Hart *et al.*, 2016; Kurki, 2017; Perchalski *et al.*, 2018) so it is important to ascertain if extrapolation from one antimere to the other is a reliable procedure.

Additionally, it is important to determine if the data obtained on the burned antimere are reliable as well. Experimentally burned skeletons are strengthened with consolidants to enhance their ability to sustain damage (Makhoul *et al.*, 2015). The impact of consolidants on the bones is unknown and researchers must assess if they alter bone properties or bone analyses. The impact of these potential problems extends to archaeological materials, since analyses often focus on one of the antimeres or on consolidated bone.

One bone property is investigated in this research: bone mineral density. This feature has not been previously studied in burned skeletal remains although it has been proved useful for anthropological studies since it has several applications (Kranioti *et al.*, 2019), such as sex and age-at-death estimations (Wheatley, 2005; Castillo and Ruiz, 2011; Meeusen *et al.*, 2015; Curate *et al.*, 2017; Navega *et al.*,2018; Kranioti *et al.*, 2019).

The aim of this study was to investigate the potential bilateral asymmetry in human remains in terms of bone mineral density as well as the influence of consolidants application in these two parameters.

If no considerable differences are found to be present, this would mean that measurements on only one of the sides of the skeleton can be taken with confidence, and regarding burning experiments the unburned side of the skeleton can be used as an indicator of pre-burn values. Also, if the application of consolidant does influence bone mineral density, this kind of analysis should be performed prior to the consolidation of bones.

Material and Methods

The femora, patellae and first metatarsals of ten individuals (five females and five males) of the 21st Century Identified Skeletal Collection (CEI/XXI) were selected (Figure 1).

These bone elements were chosen either due to the extensive research on femora using Dual X-ray Absorptiometry (DXA) (Curate, 2014) and to the usual good preservation of the patella and the first metatarsal. Also, they are easily identified in both archaeological and forensic contexts involving burned bones (Gonçalves, 2012; McKinley, 2015).

It is also important to mention that the available skeletons for this study exhibit an advanced age (Table 1), which entails some problems to their analysis since there is a high probability of them showing lower BMD. Moreover, the sample used here lacks representativeness regarding age distribution because it is under-represented by individuals with younger ages. However, this problem could not be overcome because younger ages are under-represented on the CEI/XXI itself.

For each individual, both antimeres were evaluated through DXA to assess potential bilateral asymmetry. Afterwards, the right antimere of each individual was subsequently submitted to an experimental burning process. On the burned antimere, DXA analysis was repeated to assess the potential influence of the application of the consolidant on bone mineral density.

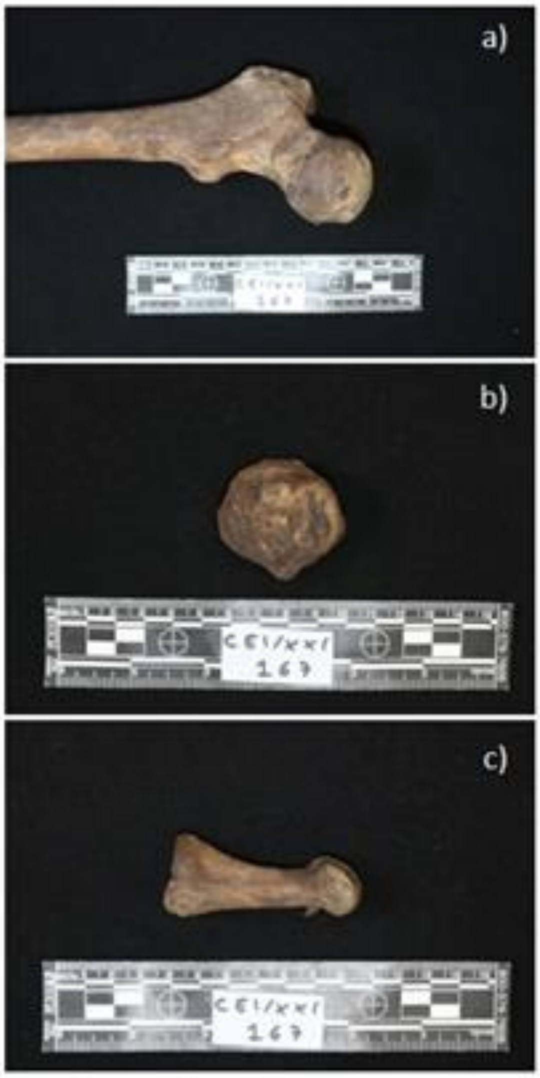

Figure 1 - Example of unburned bones of individual CEI/XXI_167 selected for this study: a) right femur; b) right patella; and c) first metatarsal.

Bilateral Asymmetry

Bone mineral density was analysed using DXA with a Hologic QDR4500 elite osteodensitometer at the University Hospital of Coimbra. The bones were placed anatomically in a box with rice since it mimics soft tissue density (Curate, 2011).

Table 1 – Sample characterization.

	Sex	Age-at-death (years)
CEI/XXI_11	Male	61
CEI/XXI_13	Female	68
CEI/XXI_63	Male	64
CEI/XXI_75	Female	86
CEI/XXI_105	Female	89
CEI/XXI_167	Female	82
CEI/XXI_249	Male	85
CEI/XXI_269	Female	78
CEI/XXI_274	Male	93
CEI/XXI_276	Male	90

Femoral analysis was performed using the *Left Hip* and *Right Hip* scans, corresponding to the left and right femurs, respectively (Curate, 2011; Curate *et al.*, 2017). However, since there is no specific scan protocol for the patella and the first metatarsal, it was necessary to use one of the existing scans, and thus the results for these bones were obtained using the *Lumbar Spine* scan. Bone elements were positioned sequentially to simulate the position of the vertebrae in a vertebral column. As this scan evaluates each vertebra individually, in this situation it also evaluated each bone piece individually.

Femur examinations measured area values in cm^2, bone mineral content (BMC) in g and bone mineral density (BMD) in g/cm^2 in the regions of interest (ROI) total area ("Total"), femoral neck ("Neck"), trochanter ("Troch"), intertrochanteric region ("Inter") and Ward area ("Ward"). In the patellar and first metatarsal exams, BMD and BMC values were obtained for the total area of the bone portion (Figure 2).

Preservative application

Burns were performed in a 14A three-phased Barracha K-3 electric furnace at maximum temperatures of 600ºC, 700ºC, 800ºC, 900ºC and 1000ºC, which took four hours to reach those temperatures. Only the right antimere of each skeleton was burned. Immediately after the desired temperature was reached, bones were left to cool down. Only one antimere of paired bones was burned, in order to maintain information from its non-burned antimere.

After burning, bones showed a good state of preservation despite their fragility (Figure 3) and DXA was again performed on the burned bone elements of interest. Afterwards, these same bones were consolidated with Primal SF-016 (100%), and again submitted to densitometry analysis.

Statistical analyses were performed with the *Statistical Package for the Social Sciences* (SPSS) version 23 software (SPSS Inc, Chicago IL). Wilcoxon signed ranks tests were used to assess statistically significant differences between antimeres and between pre- and post-consolidant application. Bilateral differences and pre- to post-consolidant differences were also evaluated by calculating relative differences between them, *i.e.* in percentage values.

Results

Bilateral Asymmetry

Statistically significant bilateral differences, i.e. with a *p* smaller than 0.01, were not found between both antimeres in any of the analysed parameters (Table 2).

The "Neck", "Total" and "Ward" parameters on the femoral area presented relative differences smaller than 5% on most cases (Supplementary Material - Table 1). However, BMD and BMC showed relative differences larger than 5% on approximately half of the cases. The smallest coefficients of variations within all individuals were found for the "Neck" and "Ward" parameters of the femoral area.

The potential influence of bone position on the osteodensitometer was evaluated through the technical error of measurement (TEM) and the relative technical error of measurement (TEMr), presented in another study (Curate, 2011). Using data acquired from Curate (2011), mean relative differences between measurements were estimated for the "Neck" and "Total" features, in order to assess the variation of values resulting from variable bone positioning (Table 3). The relative differences were calculated and compared to the relative differences obtained in the antimere study. The differences found between antimeres due to bone positioning variation during the analyses were small (TEM = 0.021 and 0.004; TEMr = 2.72% and 0.42%, respectively at "Neck" and "Total" BMD). Also, the position of the femur showed a relative difference of 1.09% between scans at "Neck" and 0.26% at "Total", thus being smaller than the relative differences found between antimeres (mostly higher than 5%).

Preservative application

According to Wilcoxon signed ranks tests (Table 4), statistically significant differences were found for the femoral "Inter" and "Total" areas (Z=50.00; p=0.02 e Z=44.00; *p*=0.01; respectively), BMD (Z=49.50; *p*=0.03; on both) and BMC (Z=55.00; *p*=0.01 e Z=28.00; *p*=0.02, respectively). Regarding the patellae, the Wilcoxon test indicated that post-consolidated areas were significantly larger than pre-consolidated areas (Z=5.00; *p*=0.04) while the post-consolidated BMD was significantly larger than the pre-consolidated BMD (Z=36.00; *p*=0.01). For the first metatarsal, only the post-consolidated area was significantly larger than the pre-consolidated area (Z=0, *p*=0.01).

Relative differences measured between pre- and post-consolidated bones showed that the majority of differences larger than 5% was found in the same parameters, *i.e.* "Inter" and "Total" of the femoral area, BMD and BMC (Supplementary Material – Table 2). Although a few large differences were also found in other parameters, those were unusual. Regarding the patella, the area and the BMD showed again differences larger than 5% in the majority of the cases. However, maximum relative differences in the metatarsal seemed to be scattered throughout the various parameters. Again, the lowest coefficients of variation within all individuals were found for the "Neck" and "Ward" parameters of the femoral area.

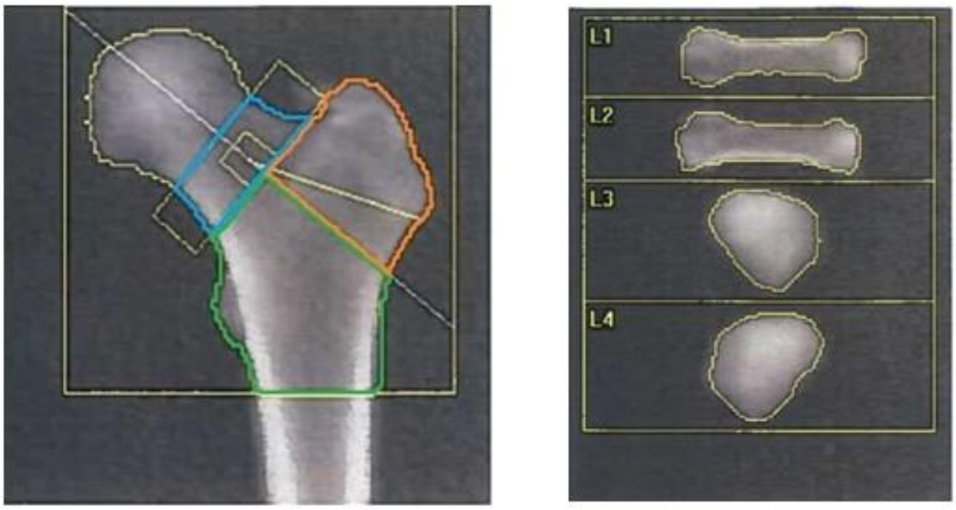

Figure 2 - Example of DXA analysis before burning, with focus on the studied areas: a) Femur with the femoral neck region of interest (ROI) in blue, trochanter ROI in orange, intertrochanteric region ROI in green and total area as the sum of all the previous areas; b) total area analysis of metatarsals and patellae.

Figure 3 - Bones burned: a) at the lowest temperature temperature investigated in this research (600ºC) and b) at the highest temperature temperature investigated in this research (1000º) showing good overall preservation.

Table 2: Descriptive statistics and Wilcoxon signed ranks test results of differences between unburned antimeres.

			Left						Right						Wilcoxon	
Bone	**Parameter**	**Region**	**n**	**Mean**	**Standard Deviation**	**Median**	**Range**	**Variation Coeficient**	**n**	**Mean**	**Standard Deviation**	**Median**	**Range**	**Variation Coeficient**	**Z**	**Sig.**
Femur	**Area (cm²)**	**Neck**	10	4.99	0.36	4.93	1.07	7.21%	10	5.10	0.43	5.26	1.25	8.43%	44.00	0.09
		Troch	10	11.90	1.68	12.09	5.36	14.12%	10	11.60	1.55	11.74	4.94	13.36%	18.00	0.33
		Inter	10	20.23	3.33	19.52	11.45	16.46%	10	20.26	3.06	20.02	8.84	15.10%	28.00	0.96
		Total	10	37.12	4.73	37.42	15.43	12.74%	10	36.96	4.51	36.81	12.21	12.20%	24.00	0.72
		Ward	10	1.08	0.04	1.08	0.15	3.70%	10	1.09	0.04	1.10	0.13	3.67%	25.00	0.77
	DMO (g)	**Neck**	10	0.64	0.17	0.58	0.41	26.56%	10	0.63	0.17	0.57	0.42	26.98%	23.00	0.64
		Troch	10	0.57	0.15	0.53	0.44	26.32%	10	0.57	0.16	0.51	0.48	28.07%	13.00	0.26
		Inter	10	0.92	0.21	0.87	0.58	22.83%	10	0.90	0.21	0.83	0.60	23.33%	17.00	0.29
		Total	10	0.77	0.18	0.70	0.51	23.38%	10	0.76	0.19	0.68	0.46	25.00%	18.00	0.33
		Ward	10	0.41	0.14	0.35	0.36	34.15%	10	0.41	0.13	0.39	0.39	31.71%	29.00	0.88
	CMO(g/cm²)	**Neck**	10	3.19	0.91	2.73	2.16	28.53%	10	3.22	0.89	2.69	2.21	27.64%	29.00	0.88
		Troch	10	6.89	2.32	5.91	7.23	33.67%	9	6.16	1.94	5.21	4.94	31.49%	14.00	0.31
		Inter	10	18.85	6.35	15.11	16.94	33.69%	10	18.34	5.88	15.20	15.34	32.06%	15.00	0.20
		Total	10	28.93	9.29	22.90	22.76	32.11%	10	28.30	9.19	22.83	23.42	32.47%	12.00	0.11
		Ward	10	0.44	0.15	0.37	0.37	34.09%	10	0.44	0.14	0.43	0.36	31.82%	22.00	0.57
Patella	**Area (cm²)**		10	12.30	1.77	12.27	5.38	14.39%	9	12.32	1.65	11.89	4.73	13.39%	12.00	0.21
	DMO (g)		10	0.48	0.08	0.47	0.29	16.67%	9	0.51	0.06	0.50	0.20	11.76%	29.00	0.44
	CMO(g/cm²)		10	5.96	1.47	5.54	4.82	24.66%	9	5.57	0.99	5.55	3.11	17.77%	17.00	0.51
MT1	**Area (cm²)**		10	10.49	1.24	10.60	3.43	11.82%	10	10.40	1.30	10.90	3.31	12.50%	25.00	0.80
	DMO (g)		10	0.35	0.06	0.37	0.17	17.14%	9	0.33	0.05	0.34	0.15	15.15%	11.50	0.19
	CMO(g/cm²)		10	3.68	0.77	3.67	2.52	20.92%	9	3.36	0.69	3.60	2.01	20.54%	13.00	0.26

Table 3 - Technical error of measurement (TEM), relative technical error of measurement (TEMr) and relative differences for the "Neck" and "Total" parameters of BMD (N=50). The influence of bone positioning during DXA analysis was negligible given that relative differences between antimeres were generally higher than 5%.

Region	TEM	TEMr (%)	Relative differences (%)
DMO (Neck)	0.021	2.72	1.09
DMO (Total)	0.004	0.42	0.26

Discussion

Although no statistically significant different osteodensitometric mean values were found between antimeres, individual bilateral variations may still be relevant from a practical point of view thus hindering reliable extrapolation from one to the other.

Several authors suggest that these differences may be due to: i) variable bone positioning during DXA analysis; ii) genetic predisposition; iii) the dominance of one of the antimeres; and iv) pathologies (Lilley *et al*., 1992; Faulkner *et al*., 1995; Petley *et al*., 2000; Hamdy *et al*., 2006; Mounach *et al*., 2012; Afzelius *et al*., 2017). In this study, pathological conditions, at least those with macroscopic manifestations, can apparently be dismissed as an influent factor because the presence of visible lesions was one of the excluding criterion during sample selection. The influence of bone positioning during DXA analysis can also be excluded because it showed small intraobserver errors, which were smaller than the relative differences found between antimeres. Previous studies evaluating the presence of bilateral asymmetry within clinical contexts have not always reported results in agreement with ours. Hamdy *et al.* (2006) noticed that antimere femora presented significant differences despite showing similar mean values. In another study, Hwang *et al*. (2012) reported statistically significant differences for the "Neck" and "Troch" parameters, but those were in turn not found for the "Inter" and "Total" parameters. Lopes *et al*. (2009) reported a high correlation for BMD between antimeres for the "Neck" and "Total" parameters. However, Schwarz *et al*. (2011) only found a moderate correlation for the "Neck" parameter between femora.

As for the differences found in osteodensitometric values between the pre- and post-consolidation procedure, a possible cause may be related to the positioning of the femur during DXA analysis. However, as stated before, relative differences found here were mostly larger than intraobserver errors. Also, statistically significant differences were observed in the same areas ("Inter" and "Total") for all parameters, suggesting these may not be random.

Table 4: Descriptive statistics and Wilcoxon signed ranks test results on differences between pre- and post-consolidant values on the burned bones.

			Pre-consolidant						Post-consolidant						Wilcoxon	
Bone	**Parameter**	**Area**	**n**	**Mean**	**Standard Deviation**	**Median**	**Range**	**Variation Coeficient**	**n**	**Mean**	**Standard Deviation**	**Median**	**Range**	**Variation Coeficient**	**Z**	**Sig.**
Femur	**Area (cm^2)**	**Neck**	10	4.67	0.34	4.78	1.01	7.28%	10	4.59	0.39	4.65	1.35	8.50%	17.50	0.55
		Troch	10	9.91	1.64	9.47	5.37	16.55%	10	9.97	1.45	9.94	4.67	14.54%	32.00	0.65
		Inter	10	17.08	2.46	16.22	7.81	14.40%	10	18.87	3.45	17.51	9.89	18.28%	50.00	**0.02**
		Total	10	31.66	3.86	30.76	12.90	12.19%	10	33.42	4.68	32.07	14.05	14.00%	51.00	**0.02**
		Ward	10	1.09	0.06	1.07	0.25	5.50%	10	1.09	0.06	1.07	0.20	5.50%	8.50	0.67
	DMO (g)	**Neck**	10	0.72	0.20	0.69	0.68	27.78%	9	0.76	0.18	0.74	0.52	23.68%	25.00	0.77
		Troch	10	0.65	0.17	0.63	0.57	26.15%	9	0.62	0.14	0.60	0.37	22.58%	36.00	0.11
		Inter	10	0.99	0.22	0.96	0.68	22.22%	10	1.02	0.23	1.01	0.70	22.55%	49.50	**0.03**
		Total	10	0.84	0.19	0.81	0.58	22.62%	10	0.87	0.20	0.84	0.62	22.99%	49.50	**0.03**
		Ward	10	0.50	0.13	0.50	0.34	26.00%	10	0.50	0.15	0.52	0.45	30.00%	35.50	0.42
	CMO(g/cm^2)	**Neck**	10	3.38	0.99	2.98	3.11	29.29%	9	3.52	0.98	2.90	2.46	27.84%	21.00	0.86
		Troch	10	6.55	2.63	5.09	7.57	40.15%	9	6.04	1.86	5.14	5.12	30.79%	30.00	0.37
		Inter	10	16.95	4.62	14.03	11.66	27.26%	10	19.31	6.10	16.81	15.90	31.59%	55.00	**0.01**
		Total	10	26.87	8.03	21.91	20.98	29.88%	8	25.59	6.23	22.13	14.75	24.35%	28.00	**0.02**
		Ward	10	0.54	1.14	0.56	0.37	211.11%	9	0.57	0.12	0.63	0.32	21.05%	21.00	0.24
Patella	**Area (cm^2)**		10	11.07	2.15	10.72	7.67	19.42%	9	10.83	1.96	10.54	5.02	18.10%	5.00	**0.04**
	DMO (g)		10	0.55	0.14	0.53	0.53	25.45%	8	0.58	0.08	0.58	0.20	13.79%	36.00	**0.01**
	CMO(g/cm^2)		10	5.95	1.46	5.70	4.79	24.54%	10	5.93	1.44	5.66	4.63	24.28%	25.00	0.80
MT1	**Area (cm^2)**		10	9.50	1.35	9.11	4.55	14.21%	10	8.95	1.35	8.90	4.60	15.08%	0	**0.01**
	DMO (g)		10	0.40	0.10	0.39	0.31	25.00%	9	0.38	0.07	0.39	0.26	18.42%	26.00	0.68
	CMO(g/cm^2)		10	3.75	0.93	3.60	3.10	24.80%	10	3.60	0.93	3.61	3.53	25.83%	17.00	0.28

Consolidant application has the purpose of minimizing the usual fragility of burned bones. Being commonly used in materials from archaeological contexts, the *Primal* acrylic consolidant makes bones more resistant to fractures and grants them a shiny look (López-Polín, 2012). Several consolidants are used with the same purpose for burned bones (Rossi *et al.*, 2004; Siegert, 2016; Topoleski e Christensen, 2019), yet their consequences, especially regarding microstructural features, have not been comprehensively assessed.

Although it is not a very well explored topic, there have been attempts to understand the effects of consolidants on bone. Still, no study focused on the consolidant used in this experiment. As for other consolidants, Rossi *et al*. (2004) reported that *Acryloid B-72* is recommended for the consolidation of burned remains when the objective is to analyze bone microstructure, as is the case for histological samples. However, Chadefaux *et al.* (2008) used scanning electron microscope (SEM) and X-ray diffraction (XRD) to conclude that *Acryloid B-72* (as well as *Rhodopas M*) did not change bone porosity but modified its crystallinity. Also, Chaumat *et al*. (2011) reported an increase in animal bone cristallinity when using a diluted solution of azelaic acid for consolidation purposes. More recently, Siegert *et al*. (2020) found that *Acryloid B-72*, among other consolidants (*Acrysol WS-24*, *Rhoplex B-60A* and *Butvar B-98*) was the most suitable for burned bone. However, they did not test for crystallinity alterations so no direct comparison with our results can be established.

Variations found on the pre- and post-consolidant results suggest a similar effect to that found by Chadefaux *et al.* (2008). Nevertheless, those authors used different consolidants (*Paraloid B-72* and *Rhodopas M*) – so alterations observed by them may not necessarily be replicated with *Primal SF-016*.

Our results indicate that it is necessary to continue exploring the effect that consolidants have on bone and therefore assess what implications eventual changes may have regarding their analyses. Ideally, such investigations must rely on larger samples and on other methodologies besides DXA, which may contribute with more information about bone microstructure.

This is also an important topic regarding archaeological materials. Consolidation of archaeological bone has long been a common procedure to facilitate its study, which otherwise would have been more difficult to achieve. However, some of the negative influences of consolidation have been reported (López-Polín, 2012), namely its interference on the analysis of bone topography (Fernández-Jalvo and Monfort, 2008), DNA (Eklund and Thomas, 2010), radiocarbon dating (Takahashi *et al.*, 2002) and stable isotopes analyses (Moore *et al.*, 1989; Stephan, 2000; Takahashi *et al.*, 2002). There are advantages and disadvantages for each consolidant so their use depends on the objectives of each study. This research reports that Primal SF-016 brings one such disadvantage, namely regarding the study of bone mineral density. Other parameters associated to it, such as crystallinity, may be similarly affected and must be further researched.

Conclusion

Bilateral bone mineral density asymmetry, although widely researched in clinical context for osteoporosis diagnosis, is still not very well studied regarding its application to archaeological or forensic settings. When applying DXA as a contribution to the assessment of ancestry, sex or age at death, there should be a special attention to this potential drawback. Bone mineral density analyses may need more systemic approaches, *i.e.* should probably be focused on both antimeres instead of focused in only one as a representative of the individual. Our results strongly suggest that the existing bilateral asymmetry does not allow the extrapolation of data from one antimere to the other.

Also, consolidation techniques can have negative effects not only on burned bone but also on unburned archaeological materials, as was also seen in other investigations. Because the application of consolidants reportedly alters the crystallitiny of bone (Chadefaux *et al.* 2008; Chaumat *et al.*, 2011), it does not allow for research involving this parameter after its application. Research on the crystallinity of bone after its consolidation will possibily be biased by such alterations. Ideally, bone sampling or bone analysis should probably focus on bone regions not affected by consolidation such as the endocortex. However, this parameter it still not completely understood and therefore more research is advised.

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Supplementary Material Table 1 – Relative bilateral asymmetry between antimeres according to each parameter and each individual.

(BMD – Bone Mineral Density; BMC – Bone Mineral Content)

Individual CEI/XXI 105

Maximum Temperature: 600ºC, Female

	Femur															Patella			MT1		
	Area					BMD					BMC					Area	BMD	BMC	Area	BMD	BMC
	Neck	Troch	Inter	Total	Ward	Neck	Troch	Inter	Total	Ward	Neck	Troch	Inter	Total	Ward						
Left	4.46	11.59	15.95	32.00	1.06	0.588	0.433	0.899	0.687	0.324	2.62	5.02	14.34	21.99	0.34	9.87	0.481	4.74	8.48	0.380	3.23
Right	4.51	10.54	15.33	30.38	1.11	0.550	0.394	0.830	0.637	0.309	2.48	4.15	12.72	19.35	0.34	10.48	0.449	4.71	8.66	0.301	2.60
Bilateral asymmetry (%)	1.12	-9.06	-3.89	-5.06	4.72	-6.46	-9.01	-7.68	-7.28	-4.63	-5.34	-17.33	-11.30	-12.01	0.00	6.18	-6.65	-0.63	2.12	-20.79	-19.50

Individual CEI/XXI 249

Maximum Temperature: 600ºC, Male

	Femur															Patella			MT1		
	Area					BMD					BMC					Area	BMD	BMC	Area	BMD	BMC
	Neck	Troch	Inter	Total	Ward	Neck	Troch	Inter	Total	Ward	Neck	Troch	Inter	Total	Ward						
Left	4.91	13.64	27.40	45.95	1.05	0.827	0.662	1.071	0.924	0.550	4.06	9.04	29.35	42.44	0.58	14.90	0.460	6.86	11.91	0.420	5.00
Right	5.31	12.39	24.17	41.87	1.12	0.801	0.734	1.133	0.973	0.565	4.25	9.09	27.39	40.73	0.63	15.21	0.496	7.55	11.29	**0.510***	**5.76***
Bilateral asymmetry (%)	8.15	-9.16	-11.79	-8.88	6.67	-3.14	10.88	5.79	5.30	2.73	4.68	0.55	-6.68	-4.03	8.62	2.08	7.83	10.06	-5.21	21.43	15.20

Individual CEI/XXI 269

Maximum Temperature: 700ºC, Female

	Femur															Patella			MT1		
	Area					BMD					BMC					Area	BMD	BMC	Area	BMD	BMC
	Neck	Troch	Inter	Total	Ward	Neck	Troch	Inter	Total	Ward	Neck	Troch	Inter	Total	Ward						
Left	4.92	9.99	17.77	32.67	1.00	0.577	0.467	0.841	0.687	0.354	2.84	4.67	14.94	22.45	0.35	12.42	0.504	6.26	10.06	0.246	2.48
Right	5.21	9.73	18.81	33.74	1.11	0.520	0.463	0.787	0.653	0.361	2.71	4.51	14.80	22.02	0.40	11.84	0.495	5.86	9.38	0.241	2.26
Bilateral asymmetry (%)	5.89	-2.60	5.85	3.28	11.00	-9.88	-0.86	-6.42	-4.95	1.98	-4.58	-3.43	-0.94	-1.92	14.29	-4.67	-1.79	-6.39	-6.76	-2.03	-8.87

Individual CEI/XXI 276

Maximum Temperature: 700ºC, Male

	Femur															Patella			MT1		
	Area					BMD					BMC					Area	BMD	BMC	Area	BMD	BMC
	Neck	Troch	Inter	Total	Ward	Neck	Troch	Inter	Total	Ward	Neck	Troch	Inter	Total	Ward						
Left	4.93	11.67	18.32	34.92	1.05	0.860	0.719	1.223	1.003	0.640	4.24	8.39	22.41	35.03	0.68	12.23	0.540	6.60	11.84	0.322	3.82
Right	4.73	12.09	19.18	36.00	1.00	0.840	0.736	1.229	1.012	0.643	3.98	8.90	23.57	36.44	0.64	12.50	0.520	6.49	11.11	0.356	3.96
Bilateral asymmetry (%)	-4.06	3.60	4.69	3.09	-4.76	-2.33	2.36	0.49	0.90	0.47	-6.13	6.08	5.18	4.03	-5.88	2.21	-3.70	-1.67	-6.17	10.56	3.66

Individual CEI/XXI 75

Maximum Temperature: 800ºC, Female

	Femur															Patella			MT1		
	Area					BMD					BMC					Area	BMD	BMC	Area	BMD	BMC
	Neck	Troch	Inter	Total	Ward	Neck	Troch	Inter	Total	Ward	Neck	Troch	Inter	Total	Ward						
Left	4.86	13.16	20.71	38.73	1.15	0.453	0.445	0.738	0.603	0.279	2.20	5.86	15.28	23.34	0.32	11.49	0.421	4.84	11.88	0.371	4.41
Right	5.16	11.39	20.87	37.42	1.13	0.515	0.445	0.739	0.619	0.266	2.66	5.07	15.43	23.16	0.30	10.56	0.526	5.55	10.68	0.337	3.60
Bilateral asymmetry (%)	6.17	-13.45	0.77	-3.38	-1.74	13.69	0.00	0.14	2.65	-4.66	20.91	-13.48	0.98	-0.77	-6.25	-8.09	24.94	14.67	-10.10	-9.16	-18.37

Individual CEI/XXI 274

Maximum Temperature: 800ºC, Male

	Femur															Patella			MT1		
	Area					BMD					BMC					Area	BMD	BMC	Area	BMD	BMC
	Neck	Troch	Inter	Total	Ward	Neck	Troch	Inter	Total	Ward	Neck	Troch	Inter	Total	Ward						
Left	5.51	12.89	19.04	37.44	1.11	0.453	0.451	0.652	0.553	0.282	2.50	5.81	12.41	20.72	0.31	13.89	0.363	5.04	10.76	0.257	2.76
Right	5.55	12.66	17.99	36.20	1.04	0.459	0.444	0.670	0.558	0.295	2.55	5.62	12.05	20.21	0.31	13.32	**0.345***	4.59	11.62	0.263	3.06
Bilateral asymmetry (%)	0.73	-1.78	-5.51	-3.31	-6.31	1.32	-1.55	2.76	0.90	4.61	2.00	-3.27	-2.90	-2.46	0.00	-4.10	-4.96	-8.93	7.99	2.33	10.87

Individual CEI/XXI 167

Maximum Temperature: 900ºC, Female

	Femur															Patella			MT1		
	Area					BMD					BMC					Area	BMD	BMC	Area	BMD	BMC
	Neck	Troch	Inter	Total	Ward	Neck	Troch	Inter	Total	Ward	Neck	Troch	Inter	Total	Ward						
Left	5.32	12.09	19.99	37.40	1.04	0.453	0.493	0.677	0.586	0.298	2.41	5.96	13.53	21.90	0.31	12.31	0.468	5.76	10.21	0.345	3.52
Right	5.47	11.28	23.87	40.62	1.07	0.426	0.462	0.627	0.554	0.257	2.33	5.21	14.96	22.50	0.28	11.89	0.474	5.64	11.81	0.333	3.93
Bilateral asymmetry (%)	2.82	-6.70	19.41	8.61	2.88	-5.96	-6.29	-7.39	-5.46	-13.76	-3.32	-12.58	10.57	2.74	-9.68	-3.41	1.28	-2.08	15.67	-3.48	11.65

Individual CEI/XXI 63

Maximum Temperature: 900ºC, Male

	Femur															Patella			MT1		
	Area					BMD					BMC					Area	BMD	BMC	Area	BMD	BMC
	Neck	Troch	Inter	Total	Ward	Neck	Troch	Inter	Total	Ward	Neck	Troch	Inter	Total	Ward						
Left	5.24	13.61	22.03	40.87	1.11	0.833	0.875	1.236	1.064	0.593	4.36	11.90	27.22	43.48	0.66	14.45	0.657	9.49	10.54	0.407	4.29
Right	5.41	13.81	23.15	42.37	1.07	0.773	0.874	1.145	1.009	0.516	4.18	**12.07***	26.51	42.77	0.55	14.19	0.647	**9.19***	11.62	0.367	4.27
Bilateral asymmetry (%)	3.24	1.47	5.08	3.67	-3.60	-7.20	-0.11	-7.36	-5.17	-12.98	-4.13	1.43	-2.61	-1.63	-16.67	-1.80	-1.52	-3.16	10.25	-9.83	-0.47

Individual CEI/XXI 13

Maximum Temperature: 1000ºC, Female

	Femur															Patella			MT1		
	Area					BMD					BMC					Area	BMD	BMC	Area	BMD	BMC
	Neck	Troch	Inter	Total	Ward	Neck	Troch	Inter	Total	Ward	Neck	Troch	Inter	Total	Ward						
Left	4.44	8.28	17.80	30.52	1.10	0.547	0.575	0.812	0.709	0.343	2.43	4.76	14.45	21.64	0.38	9.52	0.491	4.67	8.60	0.388	3.34
Right	4.30	8.87	16.99	30.16	1.09	0.580	0.553	0.825	0.710	0.418	2.49	4.91	14.02	21.42	0.46	**8.73***	0.509	4.44	8.50	0.339	2.88
Bilateral asymmetry (%)	-3.15	7.13	-4.55	-1.18	-0.91	6.03	-3.83	1.60	0.14	21.87	2.47	3.15	-2.98	-1.02	21.05	-8.30	3.67	-4.93	-1.16	-12.63	-13.77

Individual CEI/XXI 11

Maximum Temperature: 1000ºC, Male

	Femur															Patella			MT1		
	Area					BMD					BMC					Area	BMD	BMC	Area	BMD	BMC
	Neck	Troch	Inter	Total	Ward	Neck	Troch	Inter	Total	Ward	Neck	Troch	Inter	Total	Ward						
Left	5.31	12.09	23.32	40.72	1.11	0.802	0.619	1.051	0.890	0.400	4.26	7.48	24.52	36.26	0.45	11.95	0.445	5.32	10.66	0.366	3.90
Right	5.35	13.22	22.27	40.84	1.11	0.848	0.601	0.985	0.843	0.448	4.54	7.95	21.93	34.41	0.50	10.90	0.485	5.29	9.30	0.395	3.67
Bilateral asymmetry (%)	0.75	9.35	-4.50	0.29	0.00	5.74	-2.91	-6.28	-5.28	12.00	6.57	6.28	-10.56	-5.10	11.11	-8.79	8.99	-0.56	-12.76	7.92	-5.90

* Outliers

Supplementary Material Table 2 – Relative differences pre- and post-consolidant for each parameter in each individual.

(BMD – Bone Mineral Density; BMC – Bone Mineral Content)

Individual CEI/XXI 105

Maximum Temperature: 600ºC, Female

	Femur															Patella			MT1		
	Area					BMD					BMC					Area	BMD	BMC	Area	BMD	BMC
	Neck	Troch	Inter	Total	Ward	Neck	Troch	Inter	Total	Ward	Neck	Troch	Inter	Total	Ward						
Pre-	4.29	9.44	15.45	29.18	1.00	0.585	0.405	0.848	0.666	0.327	2.51	3.82	13.11	19.44	0.33	10.76	0.433	4.66	8.63	0.327	2.83
Post-	4.25	9.43	16.85	30.52	1.11	0.606	0.418	0.881	0.700	0.352	2.58	3.94	14.84	21.35	0.39	10.05	0.463	4.65	8.19	0.353	2.89
Relative difference (%)	-0.93	-0.11	9.06	4.59	11.00	3.59	3.21	3.89	5.11	7.65	2.79	3.14	13.20	9.83	18.18	-6.60	6.93	-0.21	-5.10	7.95	2.12

Individual CEI/XXI 249

Maximum Temperature: 600ºC, Male

	Femur															Patella			MT1		
	Area					BMD					BMC					Area	BMD	BMC	Area	BMD	BMC
	Neck	Troch	Inter	Total	Ward	Neck	Troch	Inter	Total	Ward	Neck	Troch	Inter	Total	Ward						
Pre-	5.11	12.90	19.94	37.95	1.07	0.829	0.755	1.090	0.941	0.575	4.23	9.74	21.73	35.70	0.62	14.95	0.485	7.25	11.72	0.475	5.57
Post-	5.11	11.98	24.60	41.69	1.06	0.835	0.756	1.194	1.024	0.596	4.26	9.06	29.38	**42.70***	0.63	13.37	0.550	7.35	11.07	0.394	4.36
Relative difference (%)	0.00	-7.13	23.37	9.86	-0.93	0.72	0.13	9.54	8.82	3.65	0.71	-6.98	35.20	19.61	1.61	-10.57	13.40	1.38	-5.55	-17.05	-21.72

Individual CEI/XXI 269

Maximum Temperature: 700ºC, Female

	Femur															Patella			MT1		
	Area					BMD					BMC					Area	BMD	BMC	Area	BMD	BMC
	Neck	Troch	Inter	Total	Ward	Neck	Troch	Inter	Total	Ward	Neck	Troch	Inter	Total	Ward						
Pre-	4.81	8.96	15.40	29.17	1.07	0.600	0.531	0.845	0.708	0.455	2.89	4.76	13.01	20.65	0.49	11.45	0.488	5.59	9.22	0.267	2.47
Post-	4.62	9.37	16.64	30.63	1.07	0.595	0.520	0.838	0.704	0.453	2.75	4.87	13.94	21.56	0.49	11.54	0.505	5.83	8.86	0.240	2.12
Relative difference (%)	-3.95	4.58	8.05	5.01	0.00	-0.83	-2.07	-0.83	-0.56	-0.44	-4.84	2.31	7.15	4.41	0.00	0.79	3.48	4.29	-3.90	-10.11	-14.17

Individual CEI/XXI 276

Maximum Temperature: 700ºC, Male

	Femur															Patella			MT1		
	Area					BMD					BMC					Area	BMD	BMC	Area	BMD	BMC
	Neck	Troch	Inter	Total	Ward	Neck	Troch	Inter	Total	Ward	Neck	Troch	Inter	Total	Ward						
Pre-	4.36	11.48	15.89	31.73	1.05	0.870	0.781	1.244	1.025	0.662	3.80	8.97	19.76	32.53	0.69	12.45	0.515	6.41	10.89	0.326	3.55
Post-	4.79	10.98	18.17	33.95	1.05	0.869	0.789	1.268	1.057	0.674	4.16	8.67	23.05	35.88	0.71	12.35	0.521	6.43	10.36	0.351	3.63
Relative difference (%)	9.86	-4.36	14.35	7.00	0.00	-0.11	1.02	1.93	3.12	1.81	9.47	-3.34	16.65	10.30	2.90	-0.80	1.17	0.31	-4.87	7.67	2.25

Individual CEI/XXI 75

Maximum Temperature: 800ºC, Female

	Femur															Patella			MT1		
	Area					BMD					BMC					Area	BMD	BMC	Area	BMD	BMC
	Neck	Troch	Inter	Total	Ward	Neck	Troch	Inter	Total	Ward	Neck	Troch	Inter	Total	Ward						
Pre-	4.49	9.50	15.50	29.50	1.09	0.654	0.536	0.896	0.743	0.391	2.94	5.09	13.89	21.92	0.43	9.59	0.620	5.95	9.63	0.377	3.63
Post-	4.79	9.96	14.71	29.46	1.09	0.606	0.553	0.945	0.758	0.385	2.90	5.50	13.91	22.32	0.42	8.71	0.664	5.78	9.58	0.387	3.71
Relative difference (%)	6.68	4.84	-5.10	-0.14	0.00	-7.34	3.17	5.47	2.02	-1.53	-1.3	8.06	0.14	1.82	-2.33	-9.18	7.10	-2.86	-0.52	2.65	2.20

Individual CEI/XXI 274

Maximum Temperature: 800ºC, Male

	Femur															Patella			MT1		
	Area					BMD					BMC					Area	BMD	BMC	Area	BMD	BMC
	Neck	Troch	Inter	Total	Ward	Neck	Troch	Inter	Total	Ward	Neck	Troch	Inter	Total	Ward						
Pre-	5.14	9.23	21.20	35.57	1.07	0.421	0.469	0.668	0.581	0.341	2.16	4.33	14.16	20.65	0.37	13.33	0.337	4.49	10.98	0.308	3.38
Post-	4.30	9.99	24.54	38.82	1.09	**0.317***	0.466	0.649	0.565	0.220	**1.36***	4.66	15.92	21.93	**0.24***	13.50	**0.340***	4.59	9.97	0.321	3.20
Relative difference (%)	-16.34	8.23	15.75	9.14	1.87	-24.70	-0.64	-2.84	-2.75	-35.48	-37.04	7.62	12.43	6.20	-35.14	1.28	0.89	2.23	-9.20	4.22	-5.33

Individual CEI/XXI 167

Maximum Temperature: 900^{o}C, Female

	Femur															Patella			MT1		
	Area					BMD					BMC					Area	BMD	BMC	Area	BMD	BMC
	Neck	Troch	Inter	Total	Ward	Neck	Troch	Inter	Total	Ward	Neck	Troch	Inter	Total	Ward						
Pre-	4.75	8.48	16.55	29.78	1.25	0.547	0.588	0.786	0.691	0.417	2.60	4.99	13.01	20.59	0.52	10.62	0.535	5.68	8.99	0.410	3.69
Post-	4.62	8.51	16.76	29.88	1.25	0.561	0.595	0.804	0.707	0.452	2.59	5.06	13.48	21.13	0.56	8.90	0.602	5.36	8.18	0.438	3.58
Relative difference (%)	-2.74	0.35	1.27	0.34	0.00	2.56	1.19	2.29	2.32	8.39	-0.38	1.40	3.61	2.62	7.69	-16.20	12.52	-5.63	-9.01	6.83	-2.98

Individual CEI/XXI 63

Maximum Temperature: 900^{o}C, Male

	Femur															Patella			MT1		
	Area					BMD					BMC					Area	BMD	BMC	Area	BMD	BMC
	Neck	Troch	Inter	Total	Ward	Neck	Troch	Inter	Total	Ward	Neck	Troch	Inter	Total	Ward						
Pre-	4.82	11.70	18.35	34.87	1.07	0.905	0.974	1.344	1.159	0.654	4.36	11.39	24.67	40.42	0.70	10.68	0.869	9.28	8.74	0.580	5.06
Post-	4.95	12.10	21.04	38.08	1.05	0.926	**0.991***	1.351	1.181	0.624	4.58	**11.99***	28.42	**44.99***	0.66	10.54	**0.872***	9.19	7.92	**0.713***	5.65
Relative difference (%)	2.70	3.42	14.66	9.21	-1.87	2.32	1.75	0.52	1.90	-4.59	5.05	5.27	15.20	11.31	-5.71	-1.31	0.35	-0.97	-9.38	22.93	11.66

Individual CEI/XXI 13

Maximum Temperature: 1000^{o}C, Female

	Femur															Patella			MT1		
	Area					BMD					BMC					Area	BMD	BMC	Area	BMD	BMC
	Neck	Troch	Inter	Total	Ward	Neck	Troch	Inter	Total	Ward	Neck	Troch	Inter	Total	Ward						
Pre-	4.13	7.53	13.39	25.05	1.11	0.732	0.676	1.029	0.874	0.540	3.02	5.09	13.78	21.89	0.60	7.28	0.620	4.51	7.17	0.498	3.57
Post-	3.76	7.43	16.44	27.64	1.07	0.741	0.692	1.076	0.927	0.591	2.79	5.14	17.69	25.62	0.63	**7.02***	0.650	4.56	6.47	0.496	3.21
Relative difference (%)	-8.96	-1.33	22.78	10.34	-3.60	1.23	2.37	4.57	6.06	9.44	-7.62	0.98	28.37	17.04	5.00	-3.57	4.84	1.11	-9.76	-0.40	-10.08

Individual CEI/XXI 11

Maximum Temperature: 1000°C, Male

	Femur															Patella			MT1		
	Area					BMD					BMC					Area	BMD	BMC	Area	BMD	BMC
	Neck	Troch	Inter	Total	Ward	Neck	Troch	Inter	Total	Ward	Neck	Troch	Inter	Total	Ward						
Pre-	4.80	9.87	19.14	33.81	1.11	1.099	0.745	1.166	1.034	0.613	5.27	7.35	22.33	34.95	0.68	9.54	0.598	5.71	8.99	0.414	3.72
Post-	4.68	9.91	18.92	33.51	1.05	1.077	0.747	1.189	1.043	0.643	5.04	7.41	22.50	34.95	0.68	8.48	0.652	5.52	8.94	0.408	3.65
Relative difference (%)	-2.50	0.41	-1.15	-0.89	-5.41	-2.00	0.27	1.97	0.87	4.89	-4.36	0.82	0.76	0.00	0.00	-11.11	9.03	-3.33	-0.56	-1.45	-1.88

*Outliers