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Early Holocene preservation differences between cortical and trabecular bone proteomes

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**A R T I C L E   I N F O**

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Skeletal remains
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ZooMS

**A B S T R A C T**

Skeletal sample selection for evolutionary palaeoproteomic studies is currently based on the availability of material, without taking into account the influence of bone biology on the proteome composition of living bone. A prime example concerns the cortical and trabecular bone that is present simultaneously in many skeletal elements, including those recovered from archaeological sites. These two bone components are formed and maintained in different manners and at different rates. In palaeoproteomics there is currently no empirical data demonstrating differences in composition and/or preservation of cortical and trabecular bone proteomes. Here we analyse pairs of cortical and trabecular bone from six Early Holocene ungulate rib specimens from the site of La Draga, Spain, using shotgun proteomics. We observe generally larger proteomes, a larger number of peptides, and lower rates of degradation, for the cortical bone samples compared to the trabecular bone samples. These results suggest that in degraded proteomic contexts, preference should be given to the sampling of cortical bone in order to maximise the retrieval of larger and better-preserved bone proteomes.

1. Introduction

The palaeoproteomic analysis of faunal and hominin specimens is a fast-growing and developing field, largely based on the extraction of proteins from skeletal remains, and resulting in important and exciting insights into the evolutionary history of hominins as well as in a range of archaeological contexts (Cappellini et al., 2018; Hendy, 2021; Hendy et al., 2018; Warinner et al., 2022). In palaeoanthropological contexts, this has led to the application of several high- and medium-throughput methods for faunal taxonomic identifications, such as Zooarchaeology by Mass Spectrometry (ZooMS) and Species by Proteome INvestigation (SPIN), as well as methods to study whole proteomes of skeletal specimens (Buckley et al., 2009; Rüther et al., 2022; Sawafuji et al., 2017). These analyses rely either on visibly destructive sampling of skeletal remains, for example through cutting, drilling, or grinding of a bone surface, although “minimally” destructive sampling of bone surfaces exists (Coutu et al., 2021; Evans et al., 2023; McGrath et al., 2019; Multari et al., 2022; van Doorn et al., 2011). As most studies utilise destructive approaches to remove a bone sample from a skeletal specimen, there is a need to ensure that samples are taken in such a way that informative morphological characteristics and bone surface modifications are retained (Hansen et al., 2024; Sinet-Mathiot et al., 2021). Furthermore, for evolutionary studies the location of sampling should be optimised in such a way that proteome size and protein sequence coverage are maximised, especially for highly-degraded specimens. However, there is little empirical data to inform the palaeoproteomic suitability of sampling locations located across and within the mammalian skeleton.

The mammalian bone proteome is rather large, consisting of more than 1,000 proteins in modern material (Alves et al., 2011). Time and preservation conditions have a tremendous effect on the proteome, resulting in a much smaller identifiable proteome in archaeological specimens. In palaeoproteomic analysis the surviving bone proteome is generally considered to be homogenous across the skeleton, implying that the same proteins can be acquired regardless of where in the skeleton a sample is taken.

Many bones are composed of two types of bone, cortical and trabecular bone, that are different in structure and function in the living
bone. The rate of bone turnover (the cellular mechanism of bone remodelling through resorption and replacement of new bone (Sansalone et al., 2021)), varies between the two types of bone, with much slower rates in cortical bone (3–10 % per year) than in trabecular bone (20–30 % per year) (Deftos, 1998; Parfitt, 2002, 1994; Parfitt et al., 1996). The bone turnover rate also varies depending on the skeletal element concerned, individual aspects such as age, health, and biological sex, as well as differences in mechanical loading between bones (Beresheim et al., 2020; Fahy et al., 2017). In addition, trabecular bone has a lower bone mineral density and higher water content compared to cortical bone (Beresheim et al., 2020; Faraldi et al., 2022; Gong et al., 1964; Haverfeld et al., 2023; Lehtinen et al., 2004).

Proteomic studies comparing cortical and trabecular bone proteomes are rare, but suggest that there are differences in proteome composition (Faraldi et al., 2022). These differences observed in the living skeleton and animal models have not been studied systematically in archaeological bone. In addition to the potential proteomic difference based on skeletal formation and maintenance, the depositional environment also plays a big role in the surviving ancient proteomes, while various environmental conditions affect the proteome preservation in distinct ways (Demarchi et al., 2016; Hendy et al., 2018; Warinner et al., 2022).

We hypothesise that these differences in bone turnover as well as bone mineral density influence both proteome composition and proteome preservation across depositional environments. How these differences are affected by the passing of time is largely unknown. Here we compare the proteome composition and preservation differences between cortical and trabecular bone of six Early Holocene ungulate ribs. We observe that cortical bone proteomes are generally larger and better-preserved compared to trabecular bone proteomes. This finding enables evidence-based decision making for future sampling of bone specimens containing both trabecular and cortical bone.

2. Materials and methods

2.1. Material

Six rib specimens from La Draga, Spain, containing both cortical and trabecular bone, were selected for this study (Table 1, SI Fig. 1). All specimens had been tentatively assigned as Caprinae based on morphology. ZooMS analysis subsequently allowed us to determine the taxonomic identity more precisely. Five out of these six specimens are rib fragments (specimens 1 to 5), while the sixth specimen is a near-complete rib. All ribs are from adult individuals based on fusion status.

La Draga is an Early Neolithic site (7,250–6,750 years cal. BP), in Northeastern Spain, with sedimentary conditions allowing for the excellent preservation of wooden objects, basketry, as well as plant and faunal remains, among others (Palomo et al., 2017). The site consists of four contemporaneous sectors: Sector A (terrestrial), Sector B and D (waterlogged), and Sector C (aquatic) (Andreaki et al., 2022). Other studies have demonstrated intra-site variability in Bos sp. humeri protein preservation between sectors A and B, with the waterlogged environment of sector B providing better conditions for protein survival than the “terrestrial” environment of sector A (Le Meilloux et al., 2024). The six specimens studied here are from La Draga Sector D, which is waterlogged, and derive from three different sedimentary units (Table 1). The sedimentary units are: “VI”, which is composed of greyish terrigenous clay beneath a travertine stone pavement; “Vla”, which fills up collapsed wooden structures and is composed of dark grey clay containing an abundance of charred particles; and “VII”, which is located below the collapsed wooden structures and is composed of dark organic clay (Revelles et al., 2016). This heterogeneity of sedimentary conditions in Sector D suggests that observations between sectors of the site are not directly comparable in terms of proteome preservation.

2.2. Methods

2.2.1. Protein extraction

Bone powder was generated from each specimen by separating cortical and trabecular bone followed by powdering of each separately to a homogenous powder, using a mortar and pestle. For each sample, 12–20 mg of power was generated for both the cortical and the trabecular bone based on material availability (Table 1) and placed in microtubes (Protein LoBind Tubes, Eppendorf). All instruments were cleaned between the sampling of different samples, first with 5 % bleach followed by 70 % ethanol.

Protein extraction was performed in-solution as described by Jensen and colleagues (2023). Samples were demineralised in UltraPure 0.5 M EDTA pH 8.0 (Invitrogen, Thermo Fisher Scientific) for 48 h at room temperature under mild agitation. A laboratory blank was added at this stage to measure laboratory and cross sample contamination. Protein digestion was carried out by adding 0.8 μg sequence grade Trypsin (Promega, V5111) to the EDTA solution and incubated at 37 °C under mild agitation for 18 h. After digestion the peptides were cleaned and desalted using EvoTip C18 tips (EV-2011, EvoSep).

2.2.2. LC-MS/MS

For liquid chromatography tandem-mass spectrometry (LC-MS/MS) analysis, the peptides were separated using an EvoSep One (EvoSep) with the 60 samples-per-day (SPD) method on a 21 min gradient. The gradient occurred at a flow rate of 1 μl/min using two mobile phases: A) which consisted of 5 % acetonitrile (ACN; LC-MS grade ACN, VWR), 95 % deionised water, and 0.1 % formic acid (FA; LC-MS grade FA, Thermo Fisher Scientific); and B) comprising 100 % ACN and 0.1 % FA. The chromatography system utilises an in-house made 15 cm long silica tube, with a 150 μm inner diameter, packed with represil C18 particles of 1.9 μm diameter and pores measuring 120 Å (ReproSil-Pur, C18-AQ, Dr. Maisch). The column wasattached to an easySpray source with a column oven set at 60 °C with a source voltage of +2,000 V, and an ion transfer tube maintained at 275 °C. An Orbitrap Exploris 480 mass spectrometer (Thermo Fisher Scientific) was utilised, operating in data-dependent acquisition (DDA) mode, with the first MS1 scan at a resolution of 60,000 in the mass range of 350 to 1,400 m/z. The top twelve ions, with an intensity above 2e5 and with a charge state ranging from two to six, were selected for fragmentation. MS2 for the selected ions was acquired using higher-energy collision dissociation (HCD) at a resolution of 15,000, a normalised collision energy of 30 % and 1.3 m/z of isolation width. Prior to the analysis of the samples, a quality control was performed utilising 100 ng of HeLa cells which displayed 4,984 unique peptides, 1,236 protein groups, and 5.5 % of repeated sequencing when searched against the full human proteome with

Table 1

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Archaeological ID</th>
<th>ZooMS ID</th>
<th>Square</th>
<th>Sedimentary unit</th>
<th>Cortical bone sample weight (mg)</th>
<th>Trabecular bone sample weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1422</td>
<td>Capra sp.</td>
<td>JE-80</td>
<td>VI</td>
<td>23</td>
<td>12</td>
</tr>
<tr>
<td>2</td>
<td>1444</td>
<td>Sus sp.</td>
<td>JE-80</td>
<td>VI</td>
<td>19</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>1959</td>
<td>Capra sp.</td>
<td>JE-79</td>
<td>VII</td>
<td>17</td>
<td>16</td>
</tr>
<tr>
<td>4</td>
<td>2139</td>
<td>Ovis sp.</td>
<td>JA-81</td>
<td>Vla</td>
<td>21</td>
<td>19</td>
</tr>
<tr>
<td>5</td>
<td>2353</td>
<td>Bos/Bison</td>
<td>JB-81</td>
<td>Vla</td>
<td>26</td>
<td>16</td>
</tr>
<tr>
<td>6</td>
<td>2512</td>
<td>Sus sp.</td>
<td>JG-80</td>
<td>VII</td>
<td>19</td>
<td>20</td>
</tr>
</tbody>
</table>
oxidation (M) as a variable modification in MaxQuant.

2.2.3. ZooMS

From the leftover peptides not used for LC-MS/MS, 50 µL was desalted and purified using a C18 Hypersep® plate (Thermo Fisher). Eluted samples were then spotted in triplicate on an MTP 384 target MALDI-plate ground steel BC (Bruker). Spots consisted of 1 µL eluted peptide and 1 µL α-cyano-4-hydroxycinnamic acid matrix solution (Welker et al., 2015).

Employing a Bruker timsTOF fleX MALDI-tims-Q-ToF in reflector mode, positive polarity, laser intensity of 40 %, and a mass range of 800–4,000 m/z the mass spectra were acquired. Samples were calibrated against three spots containing a mixture of six peptides (des-Arg1 Bradykinin m/z = 904.681, Angiotensin I m/z = 1,295.685, Glu1-Fibrinopeptide B m/z = 1,750.677, ACTH (1–17 clip) m/z = 2,093.086, ACTH (18–39 clip) m/z = 2,465.198 and ACTH (7–38 clip) m/z = 3,657.929).

2.2.4. Data and statistical analysis

2.2.4.1. LC-MS/MS data. All raw files, including the laboratory blank, were analysed using MaxQuant (version 2.1.3.0) (Cox and Mann, 2008). The search was performed against the reference proteomes of Sus scrofa (UP000008227, 22,786 entries), Bos taurus (UP000009136, 23,836 entries), Capra hircus (UP000029100, 21,149 entries) and Ovis aries (UP00002356, 21,218 entries), based on species identification obtained through ZooMS (downloaded from Uniprot.org on January 24th 2024). The search was run in semi-specific Trypsin/P mode with oxidation (P), deamidation (QA), Glu (Q) -> pyro-Glu, and Glu (E) -> pyro-Glu as variable modifications. For quantification all peptides were selected. Other settings were left as default. The internal MaxQuant contaminant list was used. The mass spectrometry proteomics data, as well as MaxQuant output, have been deposited to the ProteomeXchange Consortium via the PRIDE (Perez-Riverol et al., 2022) partner repository with the dataset identifier PXD047126.

Laboratory blanks were used to determine the presence of laboratory or cross sample contamination. Laboratory blanks were excluded from further analysis. Protein groups matching only with the internal MaxQuant contamination list were excluded from the data analysis, as were proteins identified as any form of keratin.

The six sample pairs were composed of different amounts of cortical and trabecular bone leading to difference in sample weight for the protein extraction. A strong linear relationship between sample weight (mg) and protein intensities for COL1A1 and COL1A2 were observed (linear model, p = 0.021 and p = 0.019 for COL1A1 and COL1A2 respectively; SI Fig. 2). A non-significant relationship was observed for peptide counts for COL1A1 and COL1A2 (linear model, n.s.; SI Fig. 2). To prevent a weight bias between samples, the protein intensities and peptide abundances were adjusted for sample weight. The following protein and peptide count results are based on this correction per mg of sample. Analysis of deamidation and peptide length distribution were exempt from these adjustments as they are based on relative expressions of abundance and therefore not influenced by sample weight.

Data analysis was performed in the software R (version 4.2.2) (R Core Team, 2022), using R Studio (version 2022.02.4) (RStudio Team, 2022) using the following packages: janitor (version 2.2.0) (Firke, 2023), tidyverse (version 2.0.0) (Wickham et al., 2019), and ggpubr (version 0.6.0) (Kassambara, 2023). For statistical analysis ggpmisc (version 0.5.4.1) (Aphalo, 2023) was used for linear models, vegan (version 2.6–4) was used for PERMANOVA (Oksanen et al., 2022), rstatix (version 0.7.2) (Kassambara, 2023) was used for paired t-tests, and for two sample t-tests an in-house-made function was made using R. Deamidation was quantified for collagen type 1 (COL1), both the alpha 1 and 2 chains, based on spectral intensities following Mackie and colleagues (2018).

2.2.4.2. ZooMS data. The spectral triplicates were merged according to Le Meillour and colleagues (2024) in the software R (version 4.2.2) (R Core Team, 2022), using R Studio (version 2022.02.4) (RStudio Team, 2022) and processed using MALDQquant (version 1.22.1) (Gibb and Franceschi, 2024) and MALDQquantForeign (version 0.14) (Gibb and Franceschi, 2024). Glutamin deamidation values were calculated using the Betacalc3 package (Wilson et al., 2012). No statistical analysis was performed due to the limited observations. Raw MALDI spectra have been deposited to Zenodo with dataset identifier https://doi.org/10.5281/zenodo.10849105.

3. Results

The bone proteome from the six rib specimens is dominated by COL1, a number of other collagens (n = 11), and further includes a range of non-collagenous proteins (n = 7; Fig. 1). The non-collagenous proteins include a range of typical bone proteins, such as matrix gla protein (MGP), osteocalcin (BGLAP), osteomodulin (OMD), and biglycan (BGN). These non-collagenous proteins have previously been identified in Pleistocene and Holocene bone proteomes (Wadsworth and Buckley, 2014). In addition to COL1, the collagens include a range of other proteins that are typically observed in bone proteomes, such as collagen type II (COL2A1) and collagen type V (COL5A1 and COL5A2). The dominance of COL1 in these proteomes mirrors the observations made in the original study describing the protein extraction method used (Jensen et al., 2023).

We find a strong, significant linear relationship between sample weight (mg) and protein intensities (COL1A1 p = 0.021 and COL1A2 p = 0.019; SI Fig. 2). No relationship between sample weight (mg) and peptide abundance was detected (linear model n.s.; SI Fig. 2). Due to this, protein and peptide intensities, and peptide abundances for consistency, were adjusted for sample weight. Deamidation and peptide length distributions are relative expressions of abundance per extraction and are therefore not corrected for sample weight.

There are some notable differences in proteome composition, and preservation, among the six specimens analysed. For example, COL1 and COL2A1, are the only proteins present in all twelve extractions (Fig. 1). Specimens 2 and 3 only yielded collagen identifications, without any non-collagenous proteins securely identified. Five out of six specimens (specimens 1 to 5) present larger numbers of collagens, non-collagenous proteins, and higher collagen peptide counts, for cortical bone in comparison to trabecular bone (Figs. 1 and 2). For the sixth specimen, proteome size and peptide counts are almost identical when comparing cortical and trabecular bone. A significant difference in peptide counts between cortical and trabecular bone is observed when comparing all samples (paired t-test, df = 5, p = 0.014). Similarly, for specimen 6 almost all nine peptide markers regardless of bone type could be accounted for based on the ZooMS spectra (Fig. 2). For the remaining five specimens, more peptide markers (8.6 ± 0.89 SD) could be observed from the cortical samples compared to the trabecular portion (1.2 ± 1.79 SD).

These observations on proteome composition and size are corroborated by a principal component analysis based on protein intensities adjusted by sample weight (mg) per sample (Fig. 3). With the exception of specimen 6, there is a clustering of cortical and trabecular sampling locations, as opposed to a clustering of bone specimens or taxonomy. In this principal component analysis, PC1 is the major driver of differentiation, explaining 27.35 % of the variation for all specimens, and 25.01 % of the variation when specimen 6 is excluded, through a relative difference of intensities per mg for COL5A1 and COL2A1. No statistical relationship was detected when estimating the effect of bone type on the variation seen in the PCA (PERMANOVA F-value = 0.9571, Pr(F>F) = 0.351).

Since COL1 is the dominant protein in the identified bone proteome for all specimens, we restricted subsequent analysis only to peptides matching COL1A1 and COL1A2. Here, we observe that protein sequence
Fig. 1. Presence and absence of proteins identified. X-axis labels indicate specimen (1–6) and cortical and trabecular bone.

Fig. 2. Peptide abundance of collagenous and non-collagenous proteins (NCPs) per mg of sample, for all six specimens studied. A) Mean number of peptides based on all specimens. Error bars are ± 1 SD. B) Total number of peptides per specimen. C) Number of ZooMS peptide markers identified for each specimen, out of a maximum of 9.
coverage is, on average, slightly higher for both COL1A1 and COL1A2 in cortical bone samples compared to trabecular bone samples (Table 2), but no statistically significant relationship is detected for either COL1A1 or COL1A2 (paired t-test, n.s.). For amino acid positions present in the corresponding pairs of cortical and trabecular bone samples we observe that cortical bone peptide intensities are generally higher (Figs. 4 and 5; SI Figs. 3 and 4). No statistical relationship was detected between unique COL1A1 and COL1A2 peptides per mg for cortical bone compared to trabecular bone (paired t-test, n.s.).

The length of the identified COL1 peptides is on average shorter for trabecular bone samples compared to cortical bone samples with the majority of peptides observed in trabecular bone composed of 15 or fewer amino acids, while the majority of cortical bone peptides are composed of 18 amino acids or fewer (Fig. 6; SI Fig. 5). We observe significantly longer COL1 peptides in all cortical bone samples compared to trabecular bone (paired t-test, df = 5, p = 0.0095).

Finally, we looked at differences in glutamine and asparagine deamidation of COL1 peptides. As a diagenetic modification, deamidation has been extensively characterised in palaeoproteomic studies in an attempt to quantify the relative degradation of proteins (Chen et al., 2019; Mackie et al., 2018; Nair et al., 2023; Ramøe et al., 2020; Schroeter and Cleland, 2016; Welker et al., 2016). Across our dataset, it appears that both asparagine (N) and glutamine (Q) deamidation are slightly more advanced in trabecular bone compared to cortical bone (two sample t-test, n.s.; Fig. 7). A statistically significant difference is observed for both asparagine and glutamine deamidation between cortical and trabecular bone for all specimens (two sample t-test, p > 0.001 for specimens 1–6; Fig. 7). The same observations are true for the glutamine deamidation values derived from the ZooMS spectra, which were calculated for the peptide marker COL1α1 508–519 and which contains a single glutamine. Deamidation values could only be calculated for one sample pair, specimen 6, where trabecular bone deamidation is slightly more advanced than in the cortical bone. Deamidation values could be calculated for cortical bone for three other samples, specimens 2, 4, and 5, but not for other trabecular bone samples. We conclude that the bone proteome preserved in trabecular bone is more degraded than that of cortical bone. This is observable by a reduction in proteome size as well as in peptide properties such as amino acid length and peptide intensity, and amino acid properties, such as the extent of deamidation.

4. Discussion

The information about past lives hidden within the skeleton has been expanded with the recent increases in analytical techniques applied to this material. However, the skeletal archaeological record is a finite resource. One of the biggest challenges of the palaeoproteomics field is determining ideal sampling locations that optimise proteomic information retrieval and minimise material destruction. For that, we require

Table 2
Average number of amino acids covered for COL1A1 and COL1A2 for cortical and trabecular bone for all specimens (1–6), adjusted for sample weight. AA = Amino acids.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Bone</th>
<th>Coverage (AA/mg sampled)</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>COL1A1</td>
<td>Cortical</td>
<td>36.95</td>
<td>6.01</td>
</tr>
<tr>
<td></td>
<td>Trabecular</td>
<td>34.22</td>
<td>4.71</td>
</tr>
<tr>
<td>COL1A2</td>
<td>Cortical</td>
<td>30.59</td>
<td>7.74</td>
</tr>
<tr>
<td></td>
<td>Trabecular</td>
<td>26.42</td>
<td>9.57</td>
</tr>
</tbody>
</table>

Fig. 3. PCA of proteome composition, based on protein intensities per mg of sample. A) A PCA including all specimens (1–6) where the cortical and trabecular bone pair for specimen 6 is connected by a grey line. B) A PCA including specimens 1–5, where each cortical and trabecular bone pair is connected by a grey line.
empirical data on optimal locations within and between skeletal elements.

Sampling strategies have been well established within many subdivisions of biomolecular archaeology, e.g. ancient DNA (aDNA) and stable isotope research, based on sampling ethics and molecular preservation within the (human) skeleton. Within aDNA research, sampling strategies favour locations with high mineral and/or cellular density, such as the petrous pyramid and dental cementum (Alberti et al., 2018; Damgaard et al., 2015; Hansen et al., 2017; Kontopoulos et al., 2019; Parker et al., 2020; Pinhasi et al., 2015), and locations that undergo minimal bone remodelling after the initial ossification, like in the case of the petrous pyramid (Harvig et al., 2014; Jørgkov et al., 2009;
Fig. 6. Relative abundance of COL1 peptides by peptide length, for A) all specimens (1–6), and B) for specimens 1–5. The number of peptides per length is adjusted to the total number of peptides for both cortical and trabecular bone per specimen. The grey area indicates 95% confidence intervals for each bone type.

Fig. 7. Deamidation of asparagine (N) and glutamine (Q) of COL1 for A) all extracts combined, and B) for each specimen separately (1–6). Peptides belonging to other proteins are not included here. C) Deamidation of the COL1α1 508–519 peptide for specimen 6, measured in the ZooMS analysis. For all panels, 100 % indicates complete deamidation of asparagine and glutamine, respectively, while 0 % indicates no deamidation. Error bars are ± 1 SD.
The favouring of denser bone locations is also observed in our results where the cortical bone yielded a higher number of protein groups and higher peptide abundance from the LC-MS/MS data, as well as more peptide markers in the ZooMS data.

In our results, we observe that within trabecular bone there are fewer and shorter peptides for COL1 suggesting that bone turnover, alongside taphonomic processes, is a potential factor in determining the abundance and number of identifiable peptides in archaeological material. Similar observations have been made in sampling strategies for stable isotope research using collagen isotopes. Isotopic signatures are incorporated into the bone during ossification, allowing for a snapshot of various life events, such as the individual’s diet at the time. The isotopic signature can be overwritten with bone turnover, through which new biomolecular “information” may be incorporated (Fry and Arnold, 1982).

As a result, some studies have demonstrated a significant difference in $\delta^{13}$C values between cortical and trabecular femoral pairs, while no significant difference was observed for $\delta^{15}$N (Hedges et al., 2008). However, this was only observed among adults, as they have a slower turnover rate as compared to children and adolescents (Hedges et al., 2006). As with the effect of turnover on isotopic signals, we observe similar effects on the proteome composition and its preservation, suggesting that renewal of bone during life affects the archaeological proteome.

Within palaeoproteomics, Zooarchaeology by Mass Spectrometry (ZooMS) has received significant attention over the past decade as a quick proteomic method to assign taxonomic identities to COL1-rich tissues (Buckley et al., 2009; Richter et al., 2022). This method relies on the preservation of COL1, the most abundant bone protein, with its robust structure allowing for it to be stable over time in a variety of conditions (Buckley, 2018). COL1 contains single amino acid polymorphisms, SAPs, that are phylogenetically informative (Buckley et al., 2009).

Within the ZooMS literature, it is generally not explicitly stated whether cortical or trabecular bone was sampled (e.g. Brown et al., 2021; Morin et al., 2023; Rubens et al., 2023, 2022; Sinet-Mathiot et al., 2023, 2019). While the aforementioned studies cover a large temporal and geographic scale, their success rate of taxonomic assignments ranges from ~77% to over 95%. Whether this difference is solely due to spatiotemporal differences affecting preservation, or if the preservation difference between cortical and trabecular bone are influencing the results, or a combination of both, is unclear. Based on the results in this study, cortical bone would potentially be more suitable for ZooMS as the peptide markers needed are more likely to be preserved and/or observed. A similar recommendation applies to the nascent addition of high-throughput LC-MS/MS approaches such as SPIN, which likewise aims to provide taxonomic identifications. For highly-degraded proteomes SPIN almost entirely relies on the recovery of peptides derived from COL1 (Miyopotamitaki et al., 2023; Rüther et al., 2022). Finally, our observation of differences in glutamine deamidation between trabecular and cortical bone samples from the same bone specimen has implications for the within-site comparisons of such values for large cohorts of bone specimens, especially if both cortical and trabecular bone samples have been studied.

Palaeoproteomic studies utilising bone and dentine proteomes rely on the recovery of comparable proteomes from skeletal specimens to draw phylogenetic, physiological, or archaeological conclusions. As a result, the recovered proteomes should be as large as possible, and ideally composed of identical proteomes. Our results demonstrate that in contexts with relatively poor bone proteome preservation, the bone protein fragments surviving in cortical and trabecular bone sample pairs are different. Specifically, we observe larger proteomes, longer and more abundant peptide fragments, and lower rates of amino acid degradation in the cortical bone samples compared to the trabecular bone samples. This preservation-driven difference in proteome composition in favour of cortical bone is likely driven by its increased mineral density, which is known to be responsible for the enhanced protein preservation of mineral-binding protein regions in dental enamel and eggshell (Cappellini et al., 2019; Demarchi et al., 2022, 2016). Palaeoproteomic studies with a phyloproteomic aim can therefore optimise their study design by preferentially using cortical bone samples. Future work could explore whether similar sampling recommendations also apply to archaeological skeletal material from more well-preserved contexts and/or contexts of different chronological ages.

5. Conclusion

Sample selection strategies in skeletal palaeoproteomics are currently underdeveloped, relying mainly on specimen availability and rarely on empirical data. Although there are known differences in cortical and trabecular bone formation, turnover rate, bone density, and water content, the possible proteomic implications of these have not been tested empirically in a degraded archaeological sample set. By studying cortical-trabecular sample pairs from six Early Holocene rib fragments from La Draga, Spain, we demonstrate that cortical bone proteomes are larger and less degraded compared to trabecular bone proteomes recovered from the same bone specimen.

CRediT authorship contribution statement

Ragnheidur Dilja Asmundsdottir: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. Jakob Hansen: Writing – review & editing, Methodology, Investigation, Formal analysis. Zandra Fagernas: Writing – review & editing, Formal analysis. Gaudry Troché: Writing – review & editing, Investigation. Jesper V. Olsen: Resources. Maria Saña Seguí: Writing – review & editing, Resources, Funding acquisition. Frido Welker: Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Proteomic data are available via ProteomeXchange with identifier PXD047126. Raw MALDI spectra have been deposited to Zenodo with dataset identifier https://doi.org/10.5281/zenodo.10849105.

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Appendix A. Supplementary material

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References


