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Human soft tissue preservation in the Cova des Pas site (Minorca Bronze Age)☆

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ABSTRACT

The preservation process of soft tissues in an archeological context is mainly unknown because they occur only in truly exceptional situations. The Cova des Pas is a Bronze Age site in Minorca where the special conditions enabled the preservation of some soft tissues associated with 66 individuals. This finding allows the study of the preservation process that took place by means of the analysis of the histological and chemical characteristics of the tissues. Our results show that the preservation mechanism was the adipocere, because the fatty acids profile shows higher concentration of saturated than unsaturated fatty acids. The evidence indicates that the kind of funerary ritual and the environmental conditions favored this preservation.

1. Introduction

References to preserved prehistoric human soft tissues in Europe are scarce. Hitherto, the most ancient tissue reported belongs to the Tyrolean Iceman (Tyrolean Ötztal Alps, Italy), that dates back to 5300 B.P. (Seidler et al., 1992) and has been preserved by cold conditions. Two semi-mummified individuals from Galera (Granada, Spain), (Molina et al., 2003), with an antiquity of 3500 years, were found in 1997. Also, in the prehistoric context, the site of Cova des Pas (Minorca, Spain, 3000 B.P.) can be added to this group due to the exceptional recovery of human soft tissues. The funerary complex of Cova des Pas is located in a cave in the Trebalúger ravine (south of Minorca). This is a small cave, approximately 7 m wide and 4.5 m long, located in the cliff wall about 15 m above the ground (Fullola et al., 2008). The collective burial contains a minimum of 66 individuals found in a strongly flexed position (Armentano et al., 2012). The first burials were deposited around 1100 B.C. at the end of the Bronze Age, although the largest number of inhumations took place between 900 and 800 B.C., during the first Iron Age (Van Strydonck et al., 2010). The funerary rite involved a primary inhumation of individuals, which presented maximum flexion of the upper and lower limbs. Furthermore, they were wrapped in shrouds and tied up with ropes. The corpses were deposited in several layers, piled up among previously buried individuals. According to Armentano et al. (2012), the good preservation of soft tissues (intracranial, intrathoracic, abdominal cavities and among others soft tissues) could be related to the overlapping of the corpses, since there were a large number of bodies buried in such a small space.

Soil analysis of the Cova des Pas showed the presence of high amounts of nitrates and sulfates, as well as calcium, iron and aluminum. The salts were associated with the presence of gypsum,

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quartz and sodium nitrate. The ions were the product of weathering of a clay mineral soil and calcite (Van Strydonck et al., 2010). Cabanes and Albert (2011) concluded that the presence of highly soluble minerals, such as sodium nitrate and gypsum, suggests stable dry conditions inside the cave. These minerals would have helped to absorb the humidity, facilitating the natural preservation of the corpses, the phytoliths and the vegetal remains.

Therefore, there are two possible explanations for the preservation of the organic remains in the Cova des Pas: a process of saponification, caused by the compacting and superimposition of individuals, and a process of mummification due to a dry environment.

Adipocere is the soap-like substance that can be formed from the neutral fats of decomposing bodies (Forbes et al., 2005a). Their formation is initiated by intrinsic lipases, which convert the triacylglycerides (TAG) into their corresponding saturated and unsaturated fatty acids, including myristic, palmitic and stearic acids (Liu et al., 2010). Hydroxyl forms are regularly identified in adipocere; however their presence appears to be dependent on the decomposition environment (Bereuter et al., 1996; Forbes et al., 2005b).

Although adipocere is typically regarded as a product of a damp environment, it can be formed in a variety of contexts, including dry environments and corpses in submersion (Quigley, 1998; O'Brien and Kuehner, 2007; Forbes et al., 2011; Ubelaker and Zarenko, 2011).

Nowadays, a "mummy" refers to any naturally or artificially preserved body, or soft tissue, where desiccation has prevented its decomposition (David, 1997). Dry environment, air circulation and elevated temperatures could lead to mummification of human tissue by means of desiccation (Makristathis et al., 2002). Taking into account that water is essential for the enzymatic activity and bacterial growth, as well as for arthropod colonization, dehydration of the tissues is a straightforward way to achieve mummification (Lynnerup, 2007).

The main goal of this work is to test both hypotheses of preservation, and to understand the taphonomical process responsible for the preservation of soft tissue. For this purpose, the characterization of these tissues through their microstructure and their fatty acids profiles was carried out.

2. Materials and methods

Nineteen samples belonging to 9 individuals were analyzed. They were, thus, representative samples of several soft tissues of different individuals located in different zones of the cave. The histological analysis was carried out on 15 samples belonging to these 9 individuals. They included soft tissues from intracranial, intrathoracic and abdominal regions, as well as some other tissues externally adhered to bones, and also bony tissue itself. Eleven samples were subjected to biochemical analysis (fatty acid profile) (Fig. 1) (Table 1).

2.1. Histological processing

Small pieces of each specimen (8–10 mm) were rehydrated in Sandison solution (Sandison, 1955) for 1–2 h. For bony samples, the tissue was decalcified for 1 h in 5% nitric acid prior to rehydration. Samples were then fixed in 10% formalin and immersed in multiple baths of increasing concentrations of ethanol and xylene. The specimens were then embedded in paraffin wax and microsectioned at 3–5 μm. Given the uniqueness and scarcity of samples, the uses of more general and informative stains (Hematoxylin–Eosin and Masson Trichrome) were used first, in order to obtain a comprehensive view of the soft tissue.

2.2. Biochemical analysis

Gas chromatography-mass spectrometry analysis was performed using 70 mg of tissue taken from the different individuals. Lipid extraction was performed following the method of Makristathis et al. (2002). In brief, each sample was mechanically homogenized with a mortar, and lipids were saponified using 8 M sodium hydroxide and methanol (1:1). Afterward, methylation of the fatty acids was performed using 6 M aqueous hydrochloric acid and methanol (54:46), and was then extracted into n-hexane and t-butylethylether (1:1). The organic extract was cleaned up by adding 0.3 M sodium hydroxide. The extracts were subjected to analysis by GC and GC–MS. Experimental conditions for GC were as follows: gas chromatograph Hewlett Packard 6890 series II GC System Agilent Technologies; capillary column HP5- MS 30 m × 0.2 mm × 0.25 mm film thickness; FID detector. Chromatographic volume was 1 μL with an injection temperature of 275 °C. Program temperature was as follows: initial 120 °C 2 min, ramp of 2 °C/min until 220 °C; the second ramp was 10 °C/min until 270 °C, and final ramp was 30 °C/min until 300 °C, which was maintained for 15 min. Total run time: 73 min.

The FA analyzed were the most informative acids in adipocere formation (Takatori, 2001; Dent et al., 2004; Ubelaker and Zarenko, 2011).

2.3. Data analysis

In order to understand the relationship between the fatty acid profiles of the specimens, box-plots were represented and a principal components analysis (PCA) was performed. Ten selected fatty acids from specimens from our study were compared to data from 21 specimens preserved in different environments, and 17 fresh tissue specimens from previous projects (Varmuza et al., 2005) (Table 2). The fatty acid profile of the Cova des Pas (CdP) was also analyzed and compared between tissues. The statistical analyses were performed using Spss 15.0.

3. Results

3.1. Paleohistology

Macroscopically, the soft tissues from the Cova des Pas were brittle and dry, and easily pulverized under soft pressing. The intracranial samples were found adhered to the inner surface of the cranium on the decubitus area. These tissues were shapeless and shrunken, about 5 × 5 cm and *duramater* was not readily identified (Fig. 2). The intrathoracic tissues appeared totally collapsed in the decubitus zone of the costal margin. They formed a homogenous and flattened layer of about 10 × 7 cm and with a low weight (Fig. 3). In contrast, abdominal mass was located in the hypochondriac region. Tissues showed an amorphous shape and a homogenous aspect (6 × 4 cm) with a high density. Finally, the soft tissues attached to bones were distributed among all bony remains. They consisted of thin layers of variable sizes with a bright cover and salt deposits (Fig. 4).

Microscopically, intracranial soft tissue specimens showed the reticular and homogeneous eosinophylic background characteristic of brain tissues, indicating the presence of remnants of cytoplasmic structures (Eklektos et al., 2006). Furthermore, some larger spaces contained concentric structures, reminiscent of vessels inside the cortex. In some instances, abundant round empty spaces (20–40 μm) were observed, suggesting neuronal locations in the brain cortex (see Prats-Muñoz et al., 2012) (Fig. 5).

Some intrathoracic specimens showed typical characteristics of pulmonary parenchyma, since a thin connective tissue layer

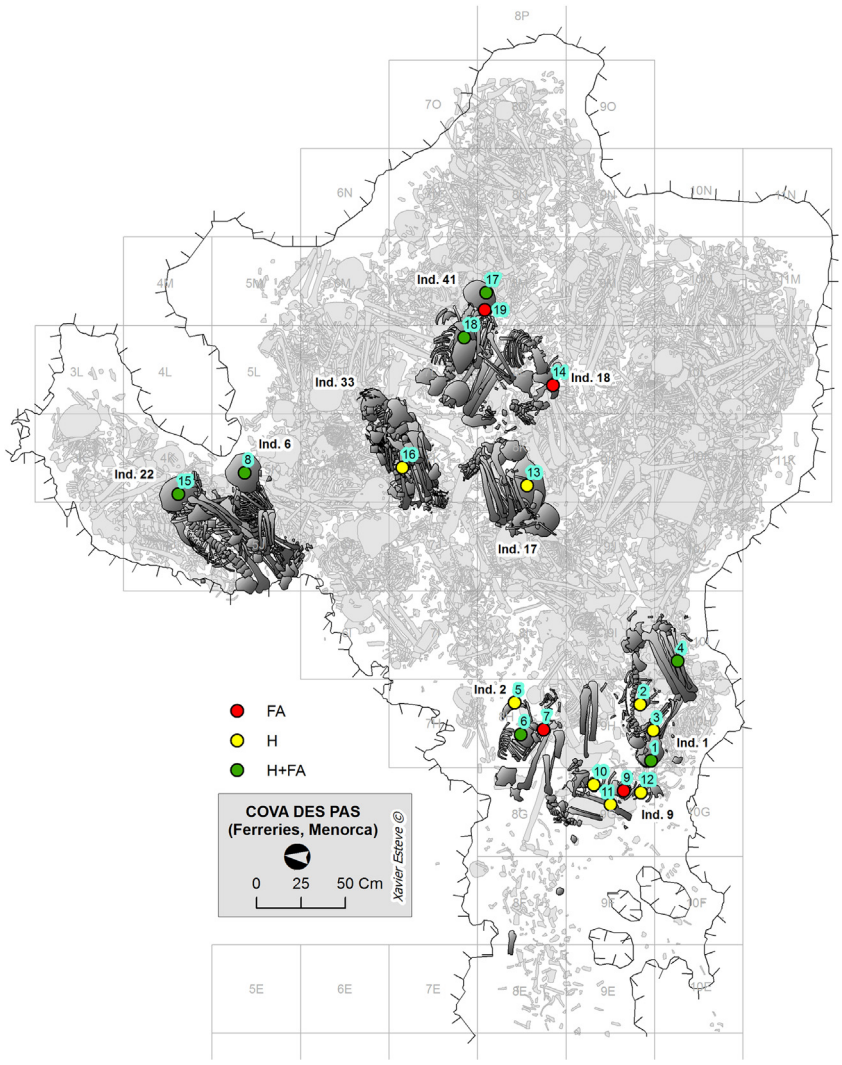

Fig. 1. Location of the analyzed individuals in the surface of the cavity.

Table 1
Samples of Cova des Pas used for histological (H) and fatty acid (FA) analyses.

Id	Individual	Anatomical location	Cave location	H	FA
1	1	Intracranial	9H	X	X
2		Intrathoracic	9H	X	
3		Mandible	10H	X	
4		Tibia	10I	X	X
5	2	Intracranial	8H	X	
6		Intrathoracic	8H	X	X
7		Radius	8H		X
8	6	Intracranial	5K	X	X
9	9	Intrathoracic	9G		X
10		Abdominal zone	9G and 9I0	X	
11		Femur	9G	X	
12		Rib bone	9G	X	
13	17	Intrathoracic	8K	X	
14	18	Coxal	8L		X
15	22	Intracranial	4K	X	X
16	33	Femur	7K	X	
17	41	Intracranial	8M	X	X
18		Intrathoracic	8L and 7M	X	X
19		Skull	8M		X

surrounded and separated spaces in some areas, consistent with collapsed alveoli. Also, one specimen from the surface showed a thick layer of connective tissue, suggesting that it may be the visceral pleura. Finally, some accumulations of black granular deposits were observed, suggesting anthracosis (Fig. 6).

Soft tissues specimens initially interpreted within the abdominal cavity revealed high content of vegetal structures (Fig. 7). Due to the number of organs within the abdomen, it was difficult to differentiate between them after the compacting process.

With regard to tissue adhered to bone, bundles of fibers appeared discernible, although the cellular architecture, including

Table 2
Human conserved tissues used to compare.

Identifier	Name	Samples	Burial time	Reference
T	Tyrolen Iceman	4	5200	Varmuza et al., 2005
G	Glacier	9	<100	
L	Lake	3	<100	
P	Permafrost	2	2200–2500	
H	High mountain	2	500	
D	Desert	1	1000	
F	Fresh	17	–	
CdP	Cova des Pas	11	3500	Present study

Fig. 2. Skull from individual No. 1, a material shapeless and shrunken attached in the right parietal.

Fig. 3. Intrathoracic cavity from individual No. 5, showing a homogenous and flatten soft tissue located in the decubitus zone of the costal arches.

striation, was not observed. This morphology was consistent with striated muscle (Fig. 8).

Microscopical section of ribs showed a typical matrix of cortical bone with holes that were consistent with Haversian canals. Furthermore, small gaps compatible with osteocyte lacunes and remains of bone marrow were observed (Fig. 9).

3.2. Fatty acids profile

The fatty acid composition of the Cova des Pas (CdP) specimens was evaluated. Table 3 shows the concentrations of the 10 fatty

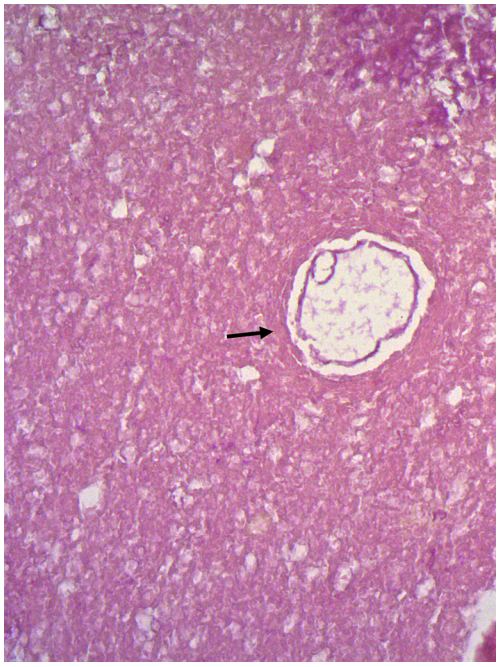

Fig. 5. Histological section of intracranial material from individual No. 1, showing a reticular tissue with a round space consistent with a vessel (arrow). HE, ×200.

acids selected for the analysis. In general, concentrations of saturated fatty acids (SFA) were higher than unsaturated fatty acids (UFA). The highest concentrations of SFA corresponded to palmitic ($\bar{x}$ 22.11%) and stearic acids ($\bar{x}$ 13.5%), whereas the highest concentration of UFA was that of oleic acid ($\bar{x}$ 9.12%). The presence of the 10-dyhydroxy stearic acid, with a $\bar{x}$ of 4.83% of the total amount of FA, must be highlighted.

Boxplots were used to compare the concentration of 5 relevant fatty acids in specimens with different types of preservation (Fig. 10). The other 7 FAs were not shown because they have a high degree of variation in all the specimen types, and no pattern could be observed. All specimens had similar levels of myristic acid, except for the specimens from the lake (L), which had high concentrations of this fatty acid. Oleic acid showed higher concentrations in fresh and desert samples. OH-Hydroxy stearic acid was absent in fresh samples (F), while CdP and high mountain samples contained low concentrations of it. Conversely, the samples of our study contained a slightly higher amount of pentadecanoic acid. Specimens belonging to CdP, Tyrolean Iceman (T), permafrost (P), and high mountain (H) showed slightly higher concentrations of stearic acid than the other groups.

The PCA analysis was performed to compare the fatty acid composition of specimens preserved in different environments. In

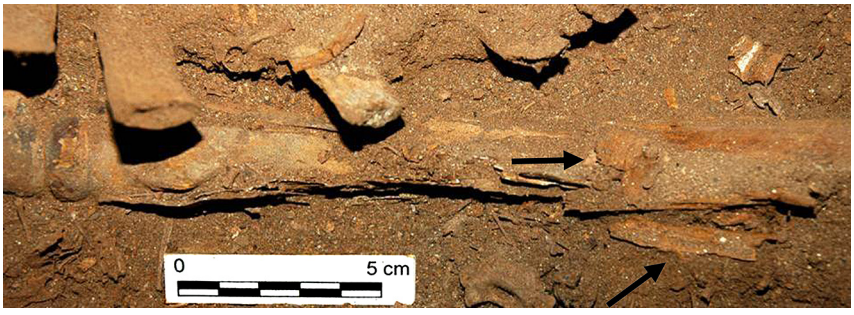

Fig. 4. Soft tissue attached to radius bone (arrow) from Individual No. 2.

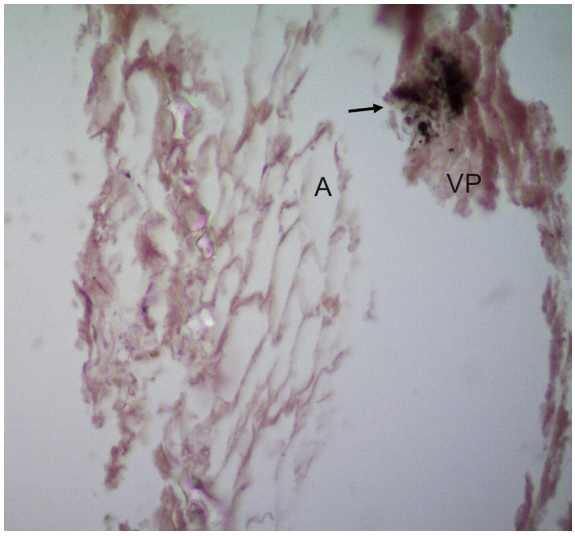

Fig. 6. Histological section of intrathoracic material from Individual No. 2, showing remains of lung parenchyma, such as alveoli (A) or visceral pleura (VP). Anthracotic pigment was also seen in the latter (arrow). HE, ×200.

the loading plot, three groups can be observed (Fig. 11). The first group, which is located on the negative quadrant, includes unsaturated fatty acids (oleic, linoleic and palmitoleic acid). On the positive range of component 1, there are two groups; the first one includes all SFA, and the second one contains the hydroxyl forms. The latter were clearly separated in component 2. These associations reflected different clusters in the score plot which consisted of: 1) fresh and desert samples; 2) glaciers (G), Tyrolean Iceman and lake specimens, and 3) CdP and high mountain samples. Fresh and desert samples constitute the cluster located in the negative side of both components (PC1 and PC2). These samples are characterized by high concentrations of oleic, linoleic and palmitoleic acids. On the other hand, there are two clusters which are mainly separated by component 2. The specimens from la Cova des Pas, high mountain, and one specimen of permafrost are located on the negative quadrant. These specimens are mainly influenced by low levels of SFA. The other cluster (positive quadrant), including glacier, Tyrolean Iceman, and lake is more influenced by a high concentration of the hydroxyl forms.

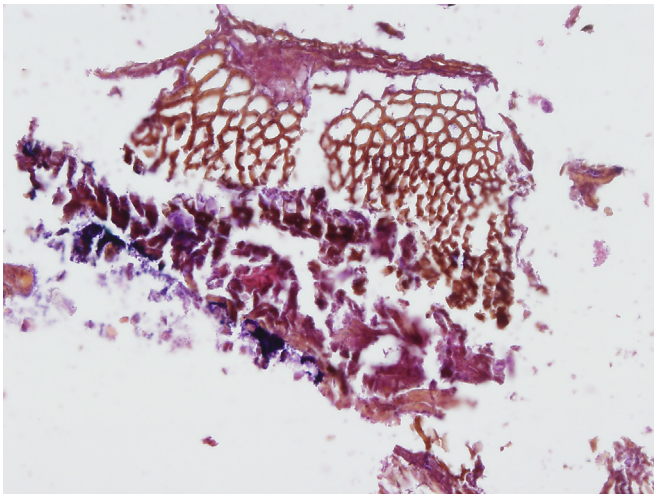

Fig. 7. Histological section of abdominal material from individual No. 9, showing a vegetal tissue with typical polyhedral structures and thick walls. HE, ×200.

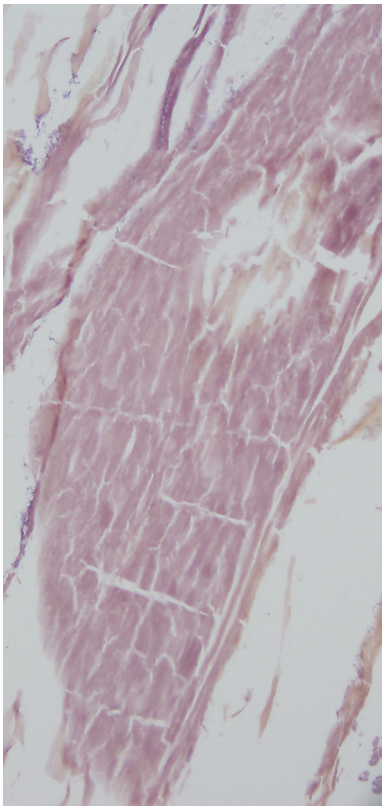

Fig. 8. Histological section of soft tissue attached to long bone from individual No. 9. It showed a tissue organized in fragmental bundles of fibers consistent with striated muscle. HE, ×200.

To study the variability between the tissues from CdP, a PCA analysis using 8 FA of 11 specimens was performed (Fig. 12). The two first components of PCA explain 63% of the variability. The score plot considering each tissue showed dispersion in the obtained values. All specimens were mixed, indicating that there were no specific differences in the preservation state depending on the type of tissue. However, a cluster was defined for brain. The corresponding loading plot indicates that the stearic, myristic and pentadecanoic acids were the main influences in this cluster, as

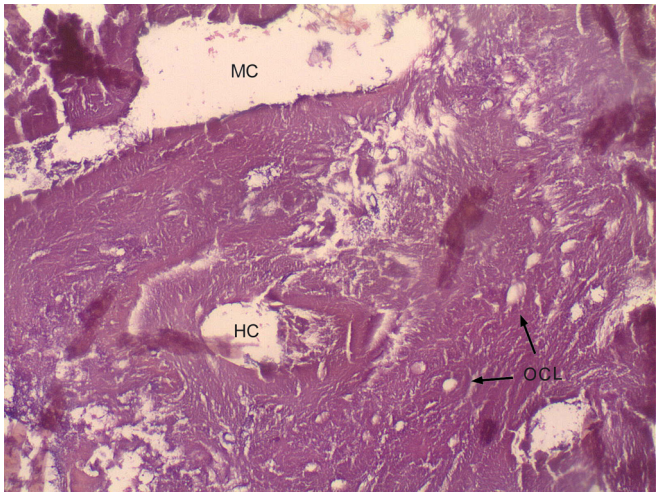

Fig. 9. Histological section of rib from individual No.9 showed a typical matrix of cortical bone with Haversian canals (HC) and medullary cavities (MC). Also, osteocyte lacunae (OCL) are observed. HE, ×1000.

Table 3
Fatty acid profile.

Tissues	Individual	Lauric acid 12:0	Myristic acid 14:0	Pentadecanoic acid 15:0	Palmitoleic acid 16:1	Palmitic acid 16:0	Linoleic acid 18:2	Oleic acid 18:1	10 Hydroxy palmitic acid 16:0 10 OH	Stearic acid 18:0	10-Hydroxy- stearic acid 18:0 10 OH
Brain	6	0,00	2,05	1,37	0,00	6,99	1,42	4,29	0,00	16,40	2,00
Brain	22	1,23	3,86	2,10	0,00	15,84	3,71	7,81	0,00	15,67	2,21
Brain	41	1,28	3,70	2,43	0,00	19,07	6,13	9,84	1,18	14,46	3,69
Lung	1	1,35	2,61	0,00	1,57	29,12	1,15	7,44	0,00	7,39	7,90
Lung	2	1,32	2,88	0,00	3,32	25,11	3,53	18,50	0,00	9,13	2,63
Lung	9	1,42	5,33	2,43	0,00	18,95	1,14	4,27	1,02	26,05	4,85
Lung	41	0,00	2,84	1,06	0,00	42,50	1,26	7,70	0,00	9,43	4,60
Muscle	1	2,09	3,60	1,63	3,73	13,22	2,78	16,58	0,00	5,15	2,58
Muscle	2	1,28	3,78	1,01	1,89	17,34	1,96	13,00	1,17	11,45	17,02
Muscle	18	1,81	5,86	2,25	0,00	29,04	1,75	7,11	2,71	10,74	2,22
Skin	41	3,61	6,41	1,64	0,00	26,00	1,67	3,77	1,19	22,59	3,46
$\bar{x}$		*1,4*	*3,91*	*1,44*	*0,96*	*22,11*	*2,41*	*9,12*	*0,66*	*13,5*	*4,83*

well as a higher concentration of linoleic acid. Interestingly, two specimens were placed in an unexpected location in the PCA due to their high UFA concentration (positive quadrant), suggesting a different preservation process.

4. Discussion

Preservation of soft tissues that are more than 3000 years old is rarely seen in Europe, when no artificial methods are used to enhance it. European prehistoric mummies are scarce, and come from different extreme environments, such as cold locales in the case of Ötzi (Seidler et al., 1992), arid environments such as Galera Man (Molina et al., 2003), or bogs (Ravn, 2010). Nevertheless, in some historical periods more preserved bodies can be found over the entire European territory (Lynnerup, 2010; Papageorgopoulou et al., 2010; O'Connor et al., 2011). Mummified bodies dating from medieval times, such as Charles V (Ordi et al., 2006), or King Ferdinand I of Aragon (Fornaciari et al., 1999), have been studied. The discoveries of mummified remains increased in more modern times (Schotsmans et al., 2011), with a higher incidence between the 17th and the 19th centuries (Panzer et al., 2010). Therefore, natural preservation of bodies in prehistoric times is almost unknown, especially when it affects a large number of individuals. The Cova des Pas remains are among the few preserved bodies available from prehistoric time in Europe, with some soft tissues preserved in an exceptional manner. Two questions are central in the discussion about the preservation phenomenon in this archaeological site: What process led to the extraordinary preservation of the organic remains? And, what factors helped to preserve the corpses inside Cova des Pas?

The preserved tissues of CdP show the characteristic image of soft ancient tissues. Tissue remnants were collapsed and had lost their macroscopic characteristics. Histological sections showed soft tissue without its distinctive architecture, and cells without nucleus, as well as a low capacity to retain stain. Notwithstanding, the preservation state of different tissues is extraordinary. Brain samples show a relatively good preservation of the reticular tissue and some vessels, but no special conditions, like pathologies, can be observed. Conversely, the analyses of intrathoracic masses enable us to see pulmonary tissue where anthracite particles covered pleural walls. In Paleopathology, anthracosis has been attributed in different historical and cultural frames (Eskimos, Egyptian, Medieval Ages, etc.) to life-long exposure to open fires, for heating and cooking (Zimmerman, 2012). Another finding related to life style comes from the analysis of abdominal masses where vegetal remains were found. These remains demonstrated a consumption of vegetables.

Either way, all the preserved soft tissues in this archaeological site are highly modified due to a decomposition process. Bone material from CdP does not contain enough collagen to perform a C14 analysis (Van Strydonck et al., 2010). Also, molecular analyses from teeth and bones show a low efficiency in the DNA recovery and amplification (Simón et al., 2012). Therefore, these analyses demonstrate a poor preservation of biomolecules. The same environmental conditions that destroyed organic molecules and acted as PCR inhibitors (Maillard products, humic and fulvic acids, iron, damaged DNA, among others) enabled the preservation of soft tissues.

As regards the preservation process, the histological analysis does not allow the mechanism of preservation in the CdP individuals to be determined. This may be related to the histological techniques use, because the standard methods, instead of specific analytical methods such as histology of fat remains, were applied (Mekota and Vermehren, 2005). This area clearly deserves further research and methodological improvements.

Paleoclimatic and sedimentologic studies also provide knowledge about the environmental conditions of CdP, showing stable, dry and warm conditions at the time of use of the cave. Specifically, this archaeological site presents 1) a stable acid pH (Van Strydonck et al., 2010), due to the high amount of nitrates and sulfates coming from the decomposition of the bodies, together with the presence of gypsum; and 2) the presence of relatively soluble minerals, such as sodium nitrate and gypsum, which regulated the humidity of the cave. In this regard, Cabanes and Albert (2011) claim that, once formed, sodium nitrate helps to preserve remains through humidity regulation and preventing bacterial activity (Bailey et al., 2002). Furthermore, geological data suggest that the entrance of the cave was first protected by a projection of the roof, being narrower than it is today. Blocks fell and the cliff stepped back, and so the entrance was enlarged. This dynamic could have modified the environmental conditions inside the cave, exposing it to fluctuations of the external environment.

According to Cabanes and Albert (2011), these dry conditions facilitated the natural mummification of the corpses and the preservation of vegetal remains. However, our chemical analyses indicated that saponification took place in soft tissues, and was possibly followed by dehydration. The fatty acid profile of the specimens of our study indicated, in all cases, a higher concentration of SFA than UFA. The most predominant fatty acid among the saturated acids is palmitic acid, followed by stearic acid, which is usually the second more abundant fatty acid in adipocere formation (O'Brien and Kuehner, 2007). OH- stearic and myristic acids are also found. All these SFA are identified as the main constituents of adipocere described in experimental conditions (Forbes et al., 2005a, Forbes, et al., 2005b, Forbes, et al., 2005c), as well as in ancient specimens (Varmuza et al., 2005). As regards UFA, only oleic acid is found in a slightly high concentration (Table 3).

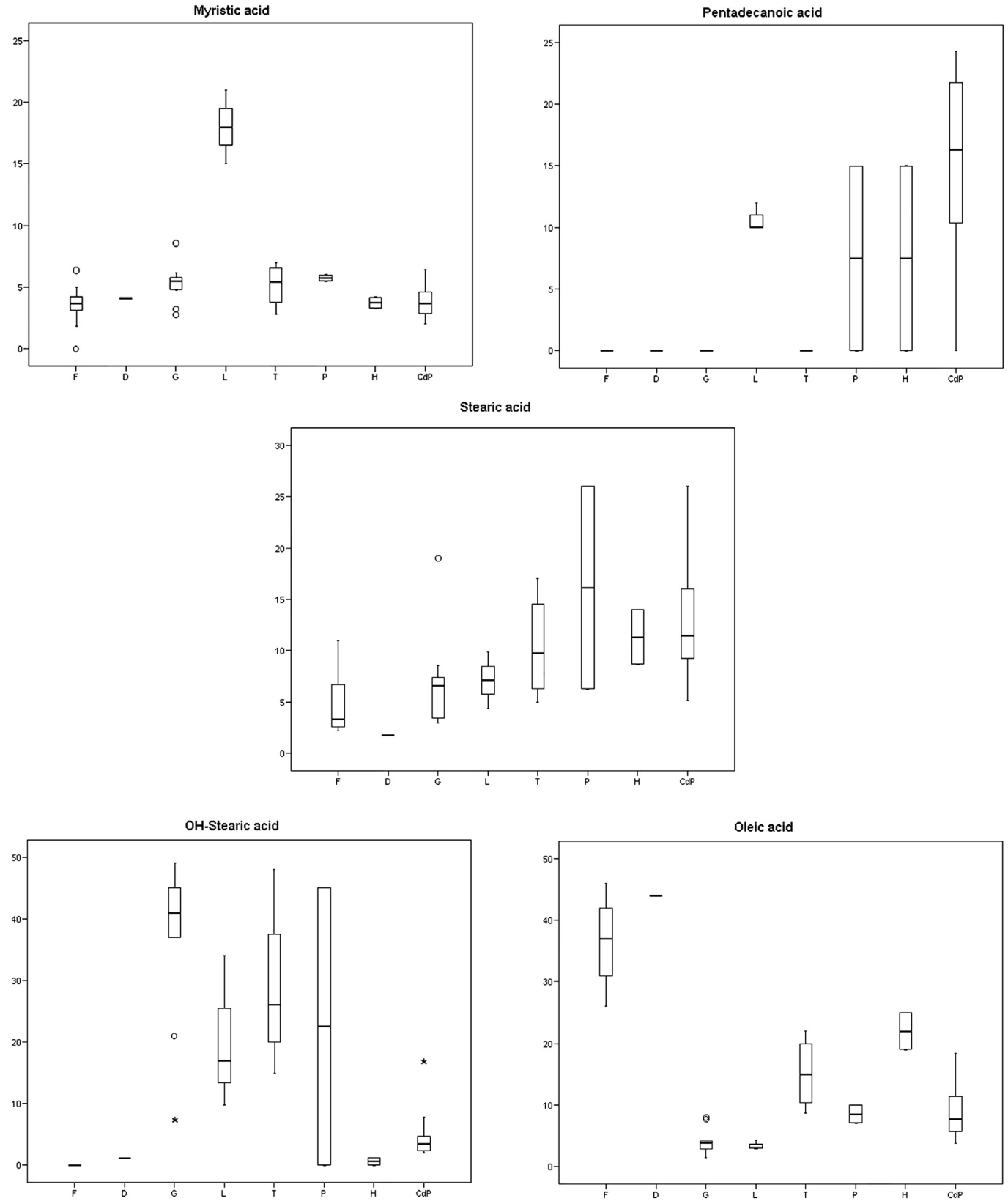

Fig. 10. Box-plots for 6 fatty acids. Ordinates show concentration ranges of the compounds in percent of total fatty acids (F: Fresh; D: Desert; G: Glacier; L: Lake; T: Tyrolean Iceman; P: Perfmafrost; H: High mountain and CdP: Cova des Pas).

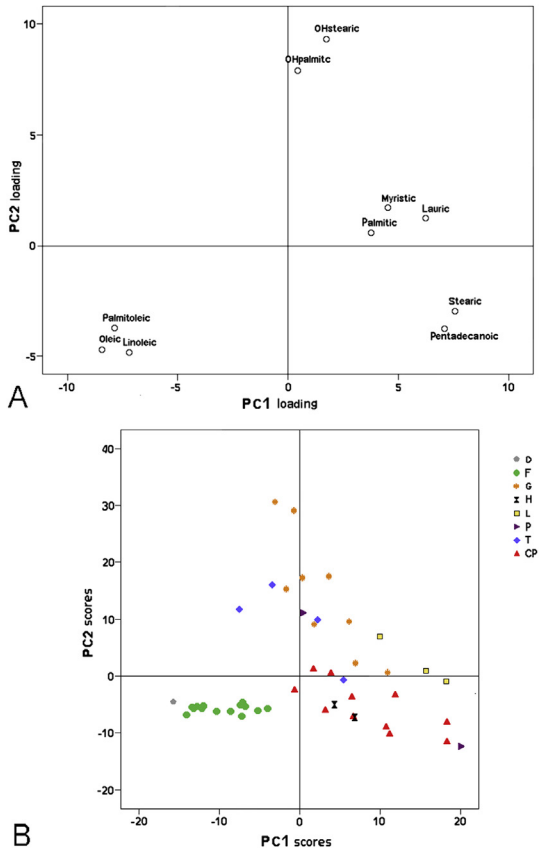

Fig. 11. Principal components analysis (PCA) to analyze samples from different origins. The first and second components explain 42% and 19% of total variance, respectively. **A**. Loading plot showing the fatty acids that are characteristic for the clusters in the score plot; **B**. Score plot showing clustering of the 49 samples (F: Fresh; D: Desert; G: Glacier; L: Lake; T: Tyrolean Iceman; P: Perfmafrost; H: High mountain and CdP: Cova des Pas).

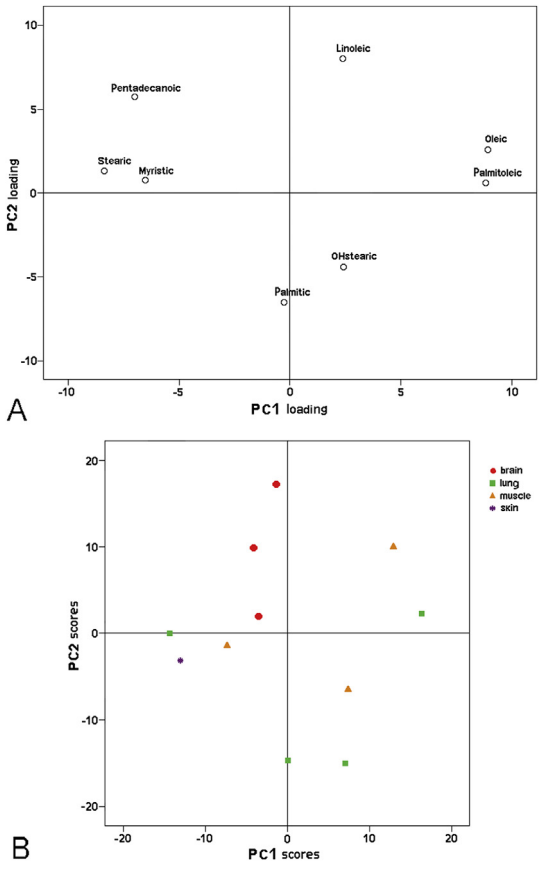

Fig. 12. Principal components analysis (PCA). The first and second components explain 43% and 21% of total variance, respectively. **A**. Loading plot showing the fatty acids that are characteristic for the clusters in the score plot; **B**. Score plot showing clustering of the 11 samples.

Saponification and mummification have different requirements. Mummification involves a complete desiccation of the body. The lack of water inhibits microorganisms from both the body and the soil, so they cannot carry out the putrefaction process. In the case of saponification, the presence of water is needed to hydrolyze triglycerides to free FA. Moreover, this process requires a decrease in bacterial growth, but not a complete inhibition, because some of these bacteria are involved in the formation of adipocere (Takatori, 2001; Pfeiffer et al., 1998).

Acidic pH is the characteristic that mainly enhanced the preservation in the Cova des Pas. In a very acidic pH environment, the bacterial growth is slowed down, and therefore there is a lower concentration of bacteria in the environment, so the rate of decomposition also decreases. However, in the Cova des Pas the pH was 4.95 (Van Strydonck et al., 2010). According to Forbes et al. (2005a) the soil with a pH between 5 and 9 enhances adipocere formation. In this range of pH, the environments are not completely inhospitable to bacteria, allowing enough bacterial activity to achieve the fatty acid composition characteristic of adipocere.

On the other hand, several authors indicate that the anaerobic conditions are essential to induce the adipocere. Armentano et al. (2012) suggested that the compression and overlaying of such a great number of bodies buried in such a small space may be responsible for the preservation. This site could be similar to a mass grave inhumation, where an anaerobic environment is generated. Enough water could be retained to induce the adipocere formation thanks to clothes that improved the absorption and storage of moisture (Fiedler and Graw, 2003; Mant, 1987).

The comparison of Cova des Pas with other conservation series (Varmuza et al., 2005) supports the above mentioned theory. In the PCA, individuals of the Cova des Pas are located in the same quadrant as the corpses of high mountain and of the permafrost. The three series show a low level of hydroxyl forms, which play important roles in the formation of adipocere.

According to Varmuza (2005), the high mountain samples belong to individuals that do not have water availability and were desiccated by winds in a zone of eternal snow. On the other hand, the specimen from permafrost was found in a wooden coffin filled with ice and without evident thawing and therefore, in similar conditions to those of a dry environment with low water supply.

Takatori (2001) demonstrated that specific FA, such as OH-stearic and OH-palmitic, are synthesized by microbial enzymes of bacteria *Clostridium perfringens or Micrococcus luteus*. The oleic and palmitic acid suffer a beta-oxidation, followed by hydration of their double bond, turning into their saturated forms. Afterward, both of them are transformed to hydroxyl forms, through hydrogenation of this same bond. Therefore, low concentrations of these forms may be explained by a limitation of microbial activity and/or availability of water that caused that the hydroxylation these acids were not possible.

In relation to the differential conservation of tissues, it is interesting to highlight the separation between the brain cluster and the rest of tissues of Cova des Pas (Fig. 12). The corresponding loading plot indicates that it is mainly the high amount of linoleic acid what provokes this clustering. Although lipid analysis of degraded brain tissues from archaeological contexts is uncommon,

Papageorgopoulou et al. (2010) observed a typical profile of fatty acids of adipocere in brain tissues, but the 18:2 was not analyzed. In other the studies (Melton et al., 2010; O'Connor et al., 2011) the absence of phospholipids was almost complete and only a trace of cholesterol was detected, and amounts of free FA are not mentioned. On the other hand, Varmuza (2005) compared different sorts of tissues, observing slight differences among them, but no specimens of brain were compared.

Fresh central nervous system has the second greatest concentration of lipids of the body, immediately after adipose tissue. Brain contains very high amounts of polyunsaturated fatty acids, particularly arachidonic acid (AA; 20:4 n−6) and docosahexaenoic acid (DHA; 22:6 n−3). However, these acids have not been detected in this study. This may be explained by the fact that these FA are unstable, even if the corpse is well preserved (Makristathis et al., 2002). Arachidonic acid and DHA are the major constituents of neural cell membrane phospholipids, and are derived from two dietary precursors: linoleic acid (18:2 n−6) and a-linolenic acid (18:3 n−3), respectively (Benjamins et al., 2012). Vertebrates are unable to synthesize these two essential polyunsaturated fatty acids, so they must be provided by the diet. Consequently, this could be the biochemical interpretation of the high amounts of linoleic acids in specimens of the Cova des Pas.

On the other hand, there are two singular concentrations of two specimens, that had high levels of oleic and palmitoleic acids (UFA) (Fig. 12). These tissues do not present a typical profile of fatty acids saponification. The fact that these individuals were placed at the beginning of the cave and they were the last ones to be placed, could explain these UFA concentrations. The rate of transformation of oleic acid to other products can be used to determine the time of death. The low percentage of oleic acid indicates an advanced state of decay but adipocere formation did not undergo a complete process due to their location (Yan et al., 2001; Frund and Schoenen, 2009).

5. Conclusions

In conclusion, our results suggest that a wide number of factors interacted, leading to the conservation of soft human tissues in the Cova des Pas. The high number of overlapping corpses in decomposition and their wrapping could create an anaerobic and moisture environment, promoting the formation of adipocere. Therefore, saponification could play an important role in the preservation of soft tissues. Afterward, the environmental conditions probably changed, enhancing a dehydration of the remains due to the increase of temperature and the decrease of humidity. Finally, this study offers new insights into adipocere formation in different ancient tissues, and reflects the complexity of the factors that act in the conservation of archaeological remains.

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References

Armentano, N., Esteve, X., Nociarová, D., Malgosa, A., 2012. Taphonomical study of the anthropological remains from Cova Des Pas (Minorca). Quaternary International 275, 112–119.

Bailey, R.A., Clark, H.M., Ferris, J.P., Krause, S., Strong, R.L., 2002. The Environmental Chemistry of Some Important Elements, Chemistry of the Environment, second ed. Academic Press, San Diego, pp. 347–414.

Benjamins, J.A., Hajra, A.K., Agranoff, B.W., 2012. Lipids. In: Siegel, G.J., Albers, R.W., Brady, S.T., Price, D.L. (Eds.), Basic Neurochemistry. Principles of Molecular, Cellular, and Medical Aspects. Academic Press-Elsevier, London, pp. 33–49.

Bereuter, M.T.L., Lorbeer, E., Reiter, C., Seidler, H., Unterdorfer, H., 1996. Post-mortem alterations of human lipids — part I: evaluation of adipocere formation and mummification by desiccation. In: Spindler, K., Wilfing, H., Rastbichler-Zissernig, E., Zur Nedden, D., Nothdurfter, H. (Eds.), Human Mummies: a Global Survey of Their Status and the Techniques of Conservation. Springer-Verlag Wien GmbH, New York, pp. 265–273.

Cabanes, D., Albert, R.M., 2011. Microarchaeology of a collective burial: cova des Pas (Minorca). Journal of Archaeological Science 38, 1119–1126.

David, A.R., 1997. Disease in Egyptian mummies: the contribution of new technologies. Lancet 349, 1760–1763.

Dent, B.B., Forbes, S.L., Stuart, B.H., 2004. Review of human decomposition processes in soil. Environmental Geology 45, 576–585.

Eklektos, N., Dayal, M.R., Manger, P.R., 2006. A forensic case study of a naturally mummified brain from the Bushveld of South Africa. Journal of Forensic Sciences 51, 498–503.

Fiedler, S., Graw, M., 2003. Decomposition of buried corpses, with special reference to the formation of adipocere. Naturwissenschaften 90, 291–300.

Forbes, S.L., Dent, B.B., Stuart, B.H., 2005a. The effect of soil type on adipocere formation. Forensic Science International 154, 35–43.

Forbes, S.L., Stuart, B.H., Dent, B.B., 2005b. The effect of the burial environment on adipocere formation. Forensic Science International 154, 24–34.

Forbes, S.L., Stuart, B.H., Dent, B.B., 2005c. The effect of the method of burial on adipocere formation. Forensic Science International 154, 44–52.

Forbes, S.L., Wilson, M.E.A., Stuart, B.H., 2011. Examination of adipocere formation in a cold water environment. International Journal of Legal Medicine 125, 643–650.

Fornaciari, G., Marchetti, A., Pellegrini, S., Ciranni, R., 1999. K-ras Mutation in the tumour of King Ferrante I of Aragon (1431–1494) and environmental mutagens at the Aragonese court of Naples. International Journal of Osteoarchaeology 9, 302–306.

Frund, H.C., Schoenen, D., 2009. Quantification of adipocere degradation with and without access to oxygen and to the living soil. Forensic Science International 188, 18–22.

Fullola, J.M., Guerrero, V.M., Petit, M.A., Calvo, M., Malgosa, A., Armentano, N., Arnau, P., Cho, S., Esteve, X., Fadrique, T., Galtés, I., García, E., Fornés, J., Jordana, X., Pedro, M., Riera, J., Sintes, E., Zubillaga, M., 2008. La Cova des Pas (Ferreries, Menorca): un jaciment cabdal en la prehistòria de les Balears. Unicum 7, 10–20.

Liu, C., Park, H.M., Monsalve, M.V., Chen, D.D.Y., 2010. Free fatty acids composition in adipocere of the Kwaday Dan Ts'inchi ancient remains found in a glacier. Journal of Forensic Sciences 55, 1039–1043.

Lynnerup, N., 2007. Mummies. American Journal of Physical Anthropology Suppl 45, 162–190.

Lynnerup, N., 2010. Medical imaging of mummies and bog bodies – a mini-review. Gerontology 56, 441–448.

Makristathis, A., Schwarzmeier, J., Mader, R.M., Varmuza, K., Simonitsch, I., Chavez, J.C., Platzer, W., Unterdorfer, H., Scheithauer, R., Derevianko, A., Seidler, H., 2002. Fatty acid composition and preservation of the Tyrolean Iceman and other mummies. Journal of Lipid Research 43, 2056–2061.

Mant, A.K., 1987. Knowledge acquired from post-war exhumations. In: Boddington, A., Garland, A.N., Janaway, R.C. (Eds.), Death, Decay and Reconstruction: Approaches to Archaeology and Forensic Science. Manchester University Press, Manchester, pp. 65–78.

Mekota, A.M., Vermehren, M., 2005. Determination of optimal rehydration, fixation and staining methods for histological and immunohistochemical analysis of mummified soft tissues. Biotechnic and Histochemistry 80, 7–13.

Melton, N., Montgomery, J., Knüsel, C.J., Batt, C., Needham, S., Parker Pearson, M., Sheridan, A., Heron, C., Horsley, T., Schmidt, A., Evans, A., Carter, E., Edwards, H., Hargreaves, M., Janaway, R., Lynnerup, N., Northover, P., O'Connor, S., Ogden, A., Taylor, T., Wastling, V., Wilson, A.C., 2010. Gristhorpe Man: an Early Bronze Age log-coffin burial scientifically defined. Antiquity 84, 796–815.

Molina, F., Rodríguez-Ariza, M.O., Jiménez, S., Botella, M., 2003. La sepultura 121 del yacimiento Argárico de el Castellón Alto (Galera, Granada). Trabajos de prehistoria 60, 153–158.

O'Brien, T.G., Kuehner, A.C., 2007. Waxing grave about adipocere: soft tissue change in an aquatic context. Journal of Forensic Sciences 52, 294–301.

O'Connor, S., Ali, E., Al-Sabah, S., Anwar, D., Bergstrom, E., Brown, K.A., Buckberry, J., Buckley, S., Collins, M., Denton, J., Dorling, K.M., Dowle, A., Duffey, P., Edwards, H.G.M., Faria, E.C., Gardner, P., Gledhill, A., Heaton, K., Heron, C., Janaway, R., Keely, B.J., King, D., Masinton, A., Penkman, K., Petzold, A., Pickering, M.D., Rumsby, M., Schutkowski, H., Shackleton, K.A., Thomas, J., Thomas-Oates, J., Usai, M.R., Wilson, A.S., O'Connor, T., 2011. Exceptional preservation of a prehistoric human brain from Heslington, Yorkshire, UK. Journal of Archaeological Science 38, 1641–1654.

Ordi, J., Alonso, P.L., de Zulueta, J., Esteban, J., Velasco, M., Mas, E., Campo, E., Fernandez, P.L., 2006. The severe gout of Holy Roman Emperor Charles V. The New England Journal of Medicine 355, 516–520.

Panzer, S., Zink, A.R., Piombino-Mascali, D., 2010. Radiologic evidence of anthropogenic mummification in the Capuchin Catacombs of Palermo, Sicily. Radiographics 30, 1123–1132.

Papageorgopoulou, C., Rentsch, K., Raghavan, M., Hofmann, M.I., Colacicco, G., Gallien, V., Bianucci, R., Ruhli, F., 2010. Preservation of cell structures in a medieval infant brain: a paleohistological, paleogenetic, radiological and physicochemical study. NeuroImage 50, 893–901.

Pfeiffer, S., Milne, S., Stevenson, R.M., 1998. The natural decomposition of adipocere. Journal of Forensic Sciences 43, 368–370.

Prats-Muñoz, G., Malgosa, A., Armentano, N., Galtés, I., Esteban, J., Bombi, J.A., Tortosa, M., Fernández, E., Jordana, X., Isidro, A., Fullola, J.M., Petit, M.A.,

Guerrero, V.M., Calvo, V.M., Fernández, P.L., 2012. A paleoneurohistological study of 3000-year-old mummified brain tissue from the Mediterranean Bronze Age. Pathobiology 79, 239–246.
Quigley, C., 1998. Modern Mummies: the Preservation of the Human Body in the Twentieth Century. McFarland & Company, Inc, Jefferson.
Ravn, M., 2010. Bronze and Early Iron Age bog bodies from Denmark. Acta Archaeologica 81, 112–123.
Sandison, A.T., 1955. The histological examination of mummified material. Biotechnic and Histochemistry 30, 277–283.
Schotsmans, E.M.J., de Voorde, W.V., De Winne, J., Wilson, A.S., 2011. The impact of shallow burial on differential decomposition to the body: a temperate case study. Forensic Science International 206, E43–E48.
Seidler, H., Bernhard, W., Teschlernicola, M., Platzer, W., Nedden, D.Z., Henn, R., Oberhauser, A., Sjovold, T., 1992. Some anthropological aspects of the prehistoric Tyrolean ice Man. Science 258, 455–457.
Simón, M., González-Ruiz, M., Prats-Muñoz, G., Malgosa, A., 2012. Comparison of two DNA extraction methods in a Spanish Bronze Age burial cave. Quaternary International 247, 358–362.
SPSS 15.0, 2006. Software for Windows. SPSS Inc, Chicago.
Takatori, T., 2001. The mechanism of human adipocere formation. Legal Medicine (Tokyo, Japan) 3, 193–204.
Ubelaker, D.H., Zarenko, K.M., 2011. Adipocere: what is known after over two centuries of research. Forensic Science International 208, 167–172.
Van Strydonck, M., Boudin, M., Guerrero, V.M., Calvo, M., Fullola, J.M., Petit, M.A., 2010. The necessity of sample quality assessment in (14)C AMS dating: the case of Cova des Pas (Menorca – Spain). Nuclear Instruments & Methods in Physics Research Section B-beam Interactions with Materials and Atoms 268, 990–994.
Varmuza, K., Makristathis, A., Schwarzmeier, J., Seidler, H., Mader, R.M., 2005. Exploration of anthropological specimens by GC–MS and chemometrics. Mass Spectrometry Reviews 24, 427–452.
Yan, F., Mcnally, R., Kontanis, E.J., Sadik, O.A., 2001. Preliminary quantitative investigation of postmortem adipocere formation. Journal of Forensic Sciences 46, 609–614.
Zimmerman, M.R., 2012. The analysis and interpretation of mummified remains. In: AL, G. (Ed.), A Companion to Paleopathology. Wiley-Blackwell, Oxford, pp. 152–169.