



Differential skeletal preservation between sexes: a diachronic study in Milan over 2000 years

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Abstract

A recurring observation in the literature is that females tend to be less represented than males in osteological collections. How can we explain such observations? Do female skeletons preserve less well than their male counterparts? In this study, 200 skeletons from the *Collezione Antropologica LABANOF* (CAL), equally divided between sexes, were selected from four archaeological sites of Milan, representing a continuum of about 2000 years. The state of preservation was analyzed according to three criteria: quantity, quality, and integrity. Linear model and ANOVA statistical analyses were performed using R software. As a result, females tend to be less complete than males, regardless of age-at-death or post-mortem interval (PMI). Preservation also showed lower values as PMI increased, except for the medieval sample, which may be explained by soil taphonomy and/or general poor bone health. This is one of the few studies to demonstrate a differential skeletal preservation between sexes, supporting bone mineral density as a major factor of bone survival. Further studies may strengthen our results and confirm the trends observed in this paper.

Keywords Biological anthropology · Skeletal preservation · Skeletal conservation · Skeletal fragmentation · Diachronic study · Gender differences

Introduction

The state of preservation of skeletal remains is dependent upon both intrinsic and extrinsic factors. While extrinsic influences represent environmental determinants such as the chemical and biological characteristics of the soil, the temperature and humidity of the surrounding environment, the effects of flora and fauna, as well as anthropogenic processes, intrinsic factors correspond to the sex and age of the individual, bone type, bone size, and pathological conditions. The recovery of well-preserved skeletal material is essential for a thorough anthropological analysis, as methods for sex, age, and population affinity estimations rely on

specific anatomical areas. Similarly, pathological and traumatic analyses may be hindered by taphonomic alterations (Symes et al. 2014; Schotsmans et al. 2017; Biehler-Gomez and Cattaneo 2021).

Studies examining the state of preservation of skeletal remains have mainly focused on the analysis of the survival of osteological elements, and on the differential preservation between immature and adult skeletal remains (Walker et al. 1988; Willey et al. 1997; Stojanowski et al. 2002; Bello et al. 2003) (among others). The bones most susceptible to deterioration are those with the highest proportion of spongy bone, such as the sternum, ribs, vertebrae, and the bones of the hands and feet; by opposition, the bones with the highest cortical density tend to preserve better, such as long bone shafts (Stojanowski et al. 2002; Manifold 2012). Thus, survival and preservation of osteological elements are correlated to bone mineral density (Willey et al. 1997), which explains why the skeletal remains of the juveniles and old adults tend to be less well-represented and less well-preserved than those of the young and middle-aged adults (Walker et al. 1988; Stojanowski et al. 2002; Bello et al. 2003; Bello and Andrews 2006). While most studies have seen no difference in preservation between sexes (Walker et al. 1988; Stojanowski et al. 2002),

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Bello et al. (Bello and Andrews 2006; Bello et al. 2006) noted that among immature individuals, female skeletons were less well-preserved with bones less well-represented than their male counterparts. In her PhD thesis, Bello (2001) described a better preservation of male individuals with respect to females (without finding any statistical significance), suggesting that it might be related to a higher robusticity of male bodies. Additionally, Walker et al. (1988) remarked that “there are nearly always more males than females in skeletal collections from archeological sites” and explains this differential preservation between sexes by a more rapid deterioration of lightly built female skeletons, which is especially pronounced in postmenopausal women due to osteoporosis. Indeed, osteoporosis is characterized by increased skeletal fragility, due to impairment of the bone microarchitecture, low bone mass, and reduced bone strength. Although osteoporosis may affect all age groups and populations of both sexes, it is most common in Caucasians, older age groups, and women (Rachner et al. 2011; Sozen et al. 2017).

The current study will present the results of the examination of the state of preservation of 200 skeletons from four different archaeological excavations in Milan (Italy) representing a continuum of 2000 years, according to three criteria: quantity (i.e., anatomical representation), quality of preservation, and integrity of the remains (i.e., state of fragmentation).

Materials and methods

For this study, 200 skeletons were extracted from the CAL (*Collezione Antropologica LABANOF*), considering only individuals for which a reliable sex estimation was possible. The CAL osteological collection of Milan is under study at the LABANOF (*Laboratorio di Antropologia e Odontologia Forense*), housed in the Department of Biomedical Sciences for Health, University of Milan (Italy). No cremated remains were included in the sample. The 200 skeletons were taken in even numbers from four different sites and equally divided between males and females:

- 50 skeletons (25 females and 25 males) from the excavations below the *Università Cattolica* dated back to the Imperial age (second–fifth century AD). The soil filling the burials was composed of a uniform matrix of silt and clay (darker in color in the case of cremations).
- 50 skeletons (25 females and 25 males) from the emergency excavations for the construction of the new M4 underground metropolitan line at the Sant’Ambrogio basilica, dating back to the Middle Ages (sixth–fourteenth century AD). The soil filling the burials was composed of a uniform matrix of silt and clay.

- 50 skeletons (25 females and 25 males) in anatomical connection from the emergency excavation of *Viale Sabotino* were mass grave burials probably due to an epidemic or a hospital burial (middle of the seventeenth century AD). Sandy soil filled the burials of this site.
- 50 skeletons (25 female and 25 male) from the CAL Milano Cemetery Skeletal Collection (Cattaneo et al. 2018), consisting of unclaimed skeletal remains of individuals who died in the twentieth century. These individuals were buried in coffins in the main cemeteries of Milan for 10 to 15 years before being exhumed by cemetery workers before entering the collection.

Only individuals for which a reliable sex estimation was possible were selected. Sex estimation was based on the morphometric characteristics of the pelvis (Phenice 1969; Walker 2005; Klales et al. 2012; Selliah et al. 2020) on fused innominate bones as well as cranial morphological traits (Walker 2008) and postcranial measurements (Purkait 2003; Spradley and Jantz 2011) for adult individuals. Age-at-death estimations were performed based on dental eruption (AlQahtani et al. 2010), epiphyseal fusion (Scheuer and Black 2004), changes at the pubic symphysis (Brooks and Suchey 1990), auricular surface (Lovejoy et al. 1985; Buckberry and Chamberlain 2002) and acetabulum (Rougé-Maillart et al. 2009), sternal end of the fourth rib (Iscan and Loth 1986), and radiological measurements of the dental pulp (Kvaal et al. 1995). The age ranges were then classified into the following age categories: 16–20, 21–30, 31–45, 46–60, 61–80, and > 80 years.

The burials selected did not show any notable difference related to sex, age-at-death, or social status. According to historical and archaeological data, the individuals buried in these necropolises belonged to the lower-middle classes of the Milanese society.

The state of preservation of the skeletal remains was evaluated before sex estimation and according to three criteria: *quantity*, *quality*, and *integrity* of the remains. Completeness of the skeleton, or “quantity,” representing the quantity of osseous material present, and “quality” which refers to the quality of the preservation of the cortical surface, was assessed using the Anatomical Preservation Index and Quality Bone Index respectively (McKinley and Smith 2004; Bello et al. 2006). To evaluate the state of preservation of entire skeletal remains, the methods were adapted by shifting the terminology from “bone preserved” (as formulated in the original research) to “skeletal system” for the purpose of this paper (Biehler-Gomez et al. 2021).

Quantity was thus divided in four categories:

- 1–24% of the skeletal system is preserved
- 25–49% of the skeletal system is preserved
- 50–74% of the skeletal system is preserved

- 75–100% of the skeletal system is preserved

Quality was similarly scored in four categories:

- 1–24% of sound cortical surface is present on the entire skeleton
- 25–49% of sound cortical surface is present on the entire skeleton
- 50–74% of sound cortical surface is present on the entire skeleton
- 75–100% of sound cortical surface is present on the entire skeleton

However, these indices do not translate the reality of a skeleton that is almost complete (quantity: 75–100%) with a sound cortical surface (quality: 75–100%) but with bones that are fragmented (i.e., broken in various pieces and/or missing pieces). Such reality would not represent a well-preserved skeleton, yet the indices cannot account for the state of fragmentation of the remains. Therefore, we added a criterion for evaluation which would classify the state of conservation of the skeleton or “integrity” in four categories:

- 1–24% of the bones are intact (i.e., not fragmented)
- 25–49% of the bones are intact (i.e., not fragmented)
- 50–74% of the bones are intact (i.e., not fragmented)
- 75–100% of the bones are intact (i.e., not fragmented)

For *quality*, *quantity*, and *integrity*, a forward stepwise regression considering all interactions between age, sex, and site was carried out to find the minimum predictive model by comparing each other using ANOVA (Faraway 2005). Linear model and ANOVA statistical analyses were performed using R software. In detail, different multiple linear regressions were carried out computing the statistical significance of each categorical variable, growing the complexity of the model, up to also consider the interaction among variables.

With this strategy, for each model, we verified statistical differences among categorical data within each variable.

In detail, to predict the outcome variable y based on a predictor variable x , we can simply write the regression equation as $y = b_0 + b_1 * x$ in which b_0 and b_1 are the regression beta coefficients that represents the intercept and the slope, respectively. For example, investigating the differences in *quality* based on gender, we have 1 if a skeleton is male, 0 if a skeleton is female. Using these variables as predictor in our regression, we obtain the following model: $b_0 + b_1$ if skeleton is male, b_0 if skeleton is female. In this context, we can interpret coefficients as follows: b_0 is the average *quality* among females, $b_0 + b_1$ is the average *quality* among males, and b_1 is the average difference in *quality* between males and females. Then, performing ANOVA among models, we selected the minimal models able to explain *quality*, *quantity*, and *integrity* basing our decision on p -value. The p -value is used to test the validity of the null hypothesis that two models are equal. The p -value (Prob > F) lower than 0.05 implies that the averages are not all equal.

As per the design of the study, the samples were equally divided between sexes and archaeological sites (50 skeletons per site/period = 25 males + 25 females, 200 skeletons total). The distribution of the results for age-at-death showed a peak for the 31–60 years and with all age groups represented. While age-at-death for females peaked in the 31–45 years age category (24 skeletons), the highest frequency was at 46–60 years for males (34 skeletons) (Fig. 1).

Results

The results for *quantity* and *quality* progressively increased per category. Indeed, 13 skeletons had less than 25% of the bones represented (6.5%) and 101 individuals presented over 75% of skeletal completeness (50.5%), evidencing that most of the skeletons were mainly complete (Fig. 2).

Fig. 1 Age-at-death distribution

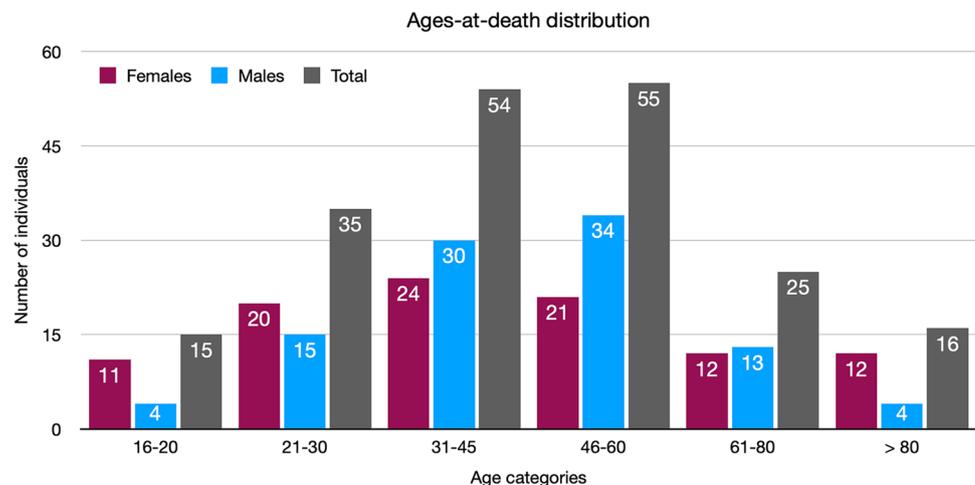
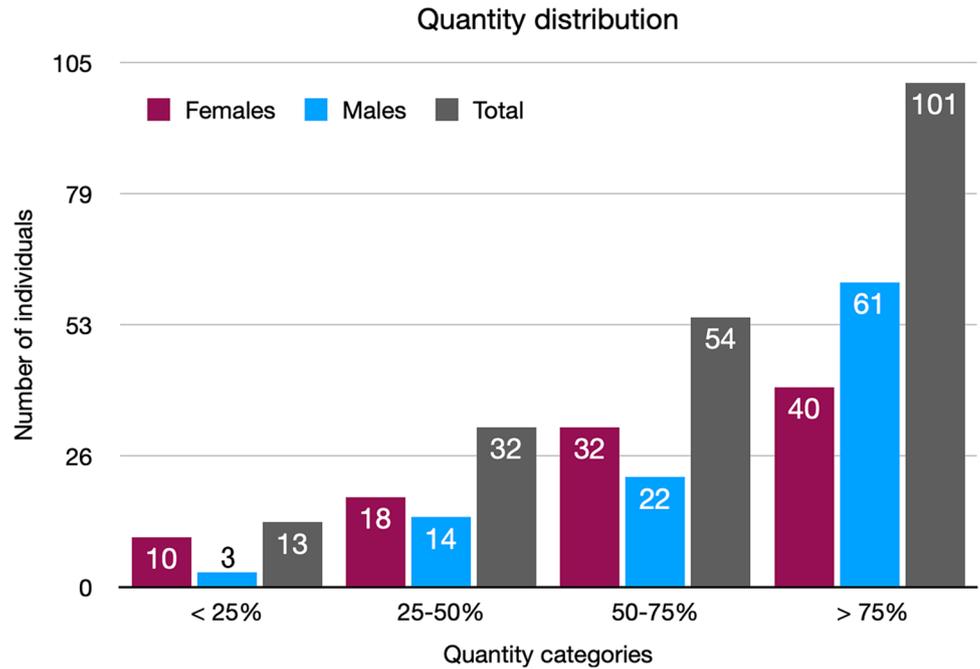


Fig. 2 Distribution of the variable *quantity*



A total of 162 skeletons (81%) had a *quality* of preservation of the remains above 50% and 77 (38.5%) above 75%, showing a generally good conservation of the cortical bone (Fig. 3). Regarding the *integrity* of the remains, 69 individuals (34.5%) had less than 25% of the bones intact and 57 skeletons (28.5%) had more than 75% of the bones intact, placing the highest frequencies in the extreme categories (Fig. 4). This shows that the state of fragmentation of the remains varied considerably in the dataset.

The statistical linear model analyzing the variable *quantity* with respect to sex in the entirety of the dataset (200 skeletons) showed a strongly significant difference between

sexes ($p < 0.01$), whereas the variable *quality* gave a weakly significant difference ($p < 0.1$), and the variable *integrity* reported no significant difference between sexes (Table 1 and Supplementary Materials).

Analysis of variance showed that the inclusion of age-at-death as a variable in the *quantity*, *quality*, and *integrity* linear models did not provide any other significant data. However, by considering the site/period, more variability in the data could be explained ($p < 0.001$ — by comparison with models without site/period). *Quantity* was significantly better in the contemporary sample than in the other sites ($p < 0.001$), as well as in the Roman age with respect to the

Fig. 3 Distribution of the variable *quality*

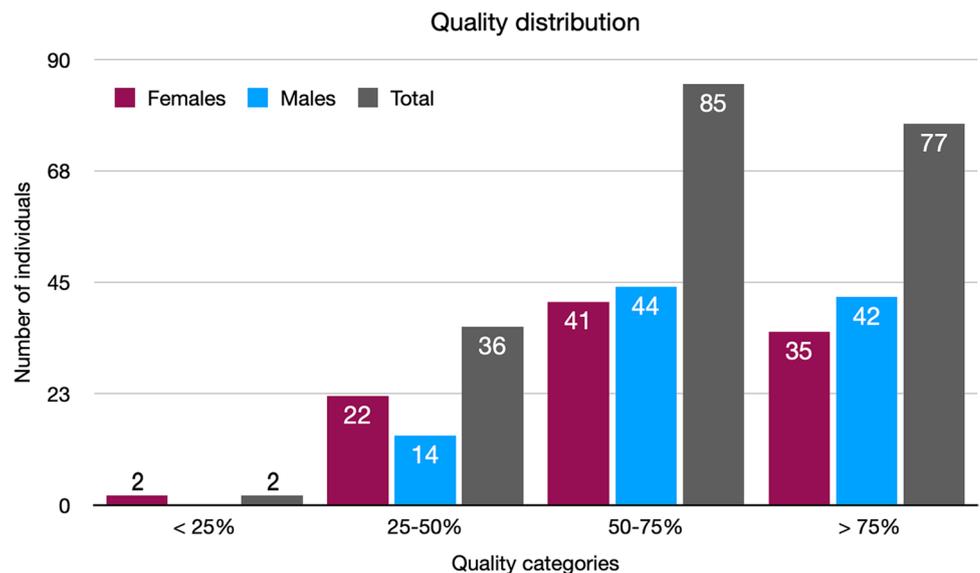
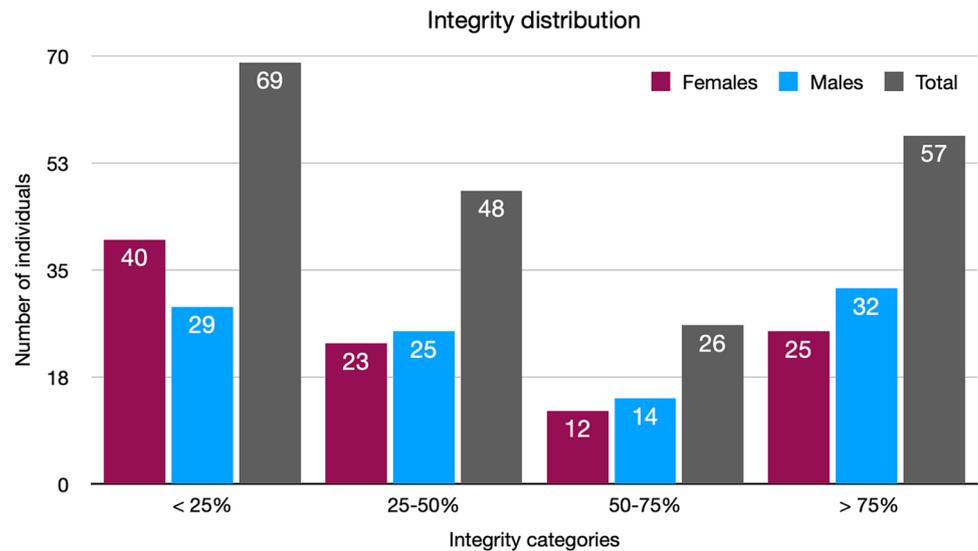


Fig. 4 Distribution of the variable *integrity*

Medieval and Modern eras ($p < 0.05$) without any significant difference between the latter two. *Quality* was significantly better in the contemporary ($p < 0.001$) and modern ($p < 0.05$) samples, with respect to the other periods, whereas no significant difference was observed between the Roman and Medieval samples. *Integrity* showed more variability in the dataset; with respect to the Roman period, it was significantly better in the Contemporary and Modern samples ($p < 0.001$) as well as in the Medieval period ($p < 0.01$).

Discussion

In this study, skeletal preservation was examined for a sample of 200 skeletons, equally divided between males and females, from four archaeological sites in Milan, each representing a different historical period, thus spanning a total of 2000 years. Apart from the selection of an equal number of individuals per sex with fused coxal bones for a reliable sex estimation, the skeletons were randomly selected per archaeological site. These urban necropolises of the city of Milan, dated to the Roman, Medieval, Modern, and Contemporary ages, regroup the middle and lower classes of the population. The burials selected did not present any distinctive feature that could justify a difference in preservation or social status.

As a result, skeletons of males are more complete than those of females, regardless of post-mortem interval and age-at-death (Table 1). The results of our study indicate that whether they died in the second century AD, or 30 years ago, female skeletons are consistently and significantly less well-preserved than male skeletons in terms of skeletal completeness. This cannot be justified by taphonomy only or burial as there was no notable difference between the burials selected for the study and all sites gave statistically significant results for completeness between sexes.

A factor that may influence bone recovery is the type of excavation. Indeed, emergency excavations may be rushed and force archaeologists to not recover all the bones of the skeleton. Nevertheless, this would not explain why female burials were systematically less completely recovered across different archaeological sites. Reduced bone mineral density could explain a poorer bone preservation/stability and hence a greater degree of bone degradation in skeletal remains, such as osteoporosis, particularly frequent in post-menopausal women (Rachner et al. 2011). Nonetheless, analysis of variance showed that inclusion of age-at-death as a variable did not provide any meaningful result, showing no statistical significance between age groups. To better understand this differential conservation, other aspects of skeletal preservation can be investigated, for instance, *quality*, or the degree of preservation of the cortical bone, and *integrity*, that is, the state of fragmentation of the remains. The results of statistical analyses regarding the variables *quality* and *integrity* showed no significant difference between sexes, which means that the variation of completeness between male and female individuals does not seem to be related to the conservation of the cortical shell of the bones or to the state of fragmentation of the remains. This shows that extrinsic factors are not the sole reason for our observations and that intrinsic factors must have played a role as well, as no aspect of taphonomy would be responsible for the loss of skeletal elements without also impacting the quality of preservation of the cortical bone or their state of fragmentation. Intrinsic differences between males and females are apparent in bone mass, which may later impact skeletal preservation. Indeed, males have a higher peak bone mass, which means that they gain more bone during growth. In addition, because there is no male equivalent for menopause, men lose less bone during aging than females (Callewaert et al. 2010). This means that in general and throughout their life, females

Table 1 Summary of results of statistical analyses (*CAT*, Roman site of Cattolica; *MED*, Medieval site of Sant’Ambrogio; *MOD*, Modern site of Viale Sabotino; *CIM*, Contemporary cemetery skeletons). Significant results (at $p \leq 0.05$) are indicated in bold

Variables	Linear model p -value	ANOVA p -value
Quantity ~ Sex	0.01	
Quantity ~ Sex + Site	0.001	
SiteCAT vs SiteCIM	0.001	
SiteCAT vs SiteMED	0.05	
SiteCAT vs SiteSAB	0.05	
Quantity ~ Sex \times Site	0.01	
SiteCAT vs SiteCIM	0.001	
SiteCAT vs SiteMED	0.05	
SiteCAT vs SiteSAB	0.05	
Quantity ~ Sex vs Quantity ~ Sex + Site		0.001
Quantity ~ Sex + Site vs Quantity ~ Sex + Age + Site		> 0.1
Quantity ~ Sex + Age + Site vs Quantity ~ Sex \times Age + Site		> 0.1
Quality ~ Sex	0.1	
Quality ~ Sex + Site	0.05	
SiteCAT vs SiteCIM	0.001	
SiteCAT vs SiteMED	> 0.1	
SiteCAT vs SiteSAB	0.05	
Quality ~ Sex \times Site	> 0.1	
SiteCAT vs SiteCIM	0.001	
SiteCAT vs SiteMED	> 0.1	
SiteCAT vs SiteSAB	> 0.1	
Quality ~ Sex vs Quality ~ Sex + Site		0.001
Quality ~ Sex + Site vs Quality ~ Sex + Age + Site		0.01
Quality ~ Sex + Age + Site vs Quality ~ Sex \times Age + Site		0.01
Integrity ~ Sex	> 0.1	
Integrity ~ Sex + Site	0.05	
SiteCAT vs SiteCIM	0.001	
SiteCAT vs SiteMED	0.01	
SiteCAT vs SiteSAB	0.001	
Integrity ~ Sex \times Site	> 0.1	
SiteCAT vs SiteCIM	0.001	
SiteCAT vs SiteMED	0.01	
SiteCAT vs SiteSAB	0.001	
Integrity ~ Sex vs Integrity ~ Sex + Site		0.001
Integrity ~ Sex + Site vs Integrity ~ Sex + Age + Site		> 0.1
Integrity ~ Sex + Age + Site vs Integrity ~ Sex \times Age + Site		> 0.1

have a lower bone mineral density than males (Warming et al. 2002), and as previously mentioned, there is a high correlation between bone mineral density and bone survival (Willey et al. 1997). Consequently, this inequality in bone mineral density between sexes could, at least in part, explain the observations of some authors on the differential skeletal preservation between males and females (Walker et al. 1988; Kemkes-Grottenthaler 2005; Bello and Andrews 2006; Bello et al. 2006) and the results of the present study.

Regarding the analysis per site/period, our results indicate that the skeletons from the CAL Milano Cemetery Skeletal Collection, representing our contemporary sample, showed

the best preservation for all three variables and were better preserved than the skeletons from the other periods. It is important to specify that although these individuals died recently (mainly in the 1990s), they were not exhumed and retrieved by forensic archaeologists and anthropologists but by cemetery workers by means of heavy machinery (Cattaneo et al. 2018). Therefore, the state of completeness of these remains constitutes a worst-case scenario as no personnel with an anthropology or archaeology background was present during the exhumations. The good preservation of these skeletons could be justified by the small post-mortem interval that characterizes the contemporary sample (48

skeletons died between 1990 and 1997, representing 96% of the sample with a post-mortem interval of 24–31 years and two skeletons with a post-mortem interval of 52 and 94 years) and the consequent limited influence of taphonomy. The medieval sample (Sant’Ambrogio archaeological site) presented the lowest values of skeletal preservation and was statistically less well-preserved than the other sites. These results could be explained by specific taphonomic conditions of that site (e.g., soil type and pH) more destructive to bone and/or bone health. Indeed, the medieval skeletons presented more stress markers and signs of poor health than the other sites of the dataset (unpublished data) and poor health could induce a lower bone mineral density and increase the risk of skeletal degradation. One of the main characteristics of the silt and clay soils is water retention, which could explain a poorer skeletal preservation in the Sant’Ambrogio site than in the Sabotino site, constituted of sandy soil, characterized by a limited water retention capacity and high permeability. Nonetheless, both Cattolica and Sant’Ambrogio sites had soils of silt and clay, yet a significantly better preservation was observed in the Cattolica skeletons, dated further back to the Roman era. Overall, the skeletons from the Modern era seem better preserved than the roman sample, which may be the result of the different post-mortem interval between the two periods (about 1200–1500 years difference, 1600–1900 years for the roman sample, and about 400 years for the modern one). Further investigations of the quality of the soil (including soil type and pH) of all the sites considered in this study could shed more light on the differences between samples. In addition, an increase in sample size and the inclusion of skeletons from various historical periods but from another geographical location could confirm and strengthen the trends observed in this study.

Conclusion

In this study, 200 skeletons from four archaeological sites of Milan, equally divided between sexes and historical periods, were considered, spanning a total of almost 2000 years. The state of preservation was examined according to three criteria: *quantity* or completeness of the skeleton, *quality* or degree of preservation of the cortical bone, and *integrity* or state of fragmentation of the remains. Linear model and ANOVA statistical analyses were performed considering these variables as well as sex, age-at-death, and site/period using R.

This study showed that male skeletons are more complete than female skeletons regardless of age-at-death or time since death. Considering that these results are consistent in all archaeological sites, no difference was noted between burials, and *quality* and *integrity* were not as impacted; taphonomy cannot be the sole responsible party. Intrinsic

differences in bone mineral density between sexes may play a role in the differential conservation of the remains after death. In addition, the state of preservation of the remains tended to worsen as the post-mortem interval increased, with the exception of the medieval sample for which the variables *quality* and *quantity* were as bad if not worse than those of the roman sample. This may be the results of taphonomic conditions of the soil (such as type and pH) and/or the consequence of poor bone health (i.e., conditions that may lead to bone weakening, rendering them fragile and prone to fractures) leading to an increased bone degradation (as supported by paleopathological analyses of the remains). This research, performed in a diachronic perspective over 2000 years in a single place, Milan, an urban context and a major city in Europe throughout History, is one of the few to demonstrate a differential skeletal preservation between sexes. Additional studies are needed to further investigate the trends observed in the present paper.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s12520-022-01616-0>.

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Declarations

Conflict of interest The authors declare no competing interests.

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