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X-ray, CT and DXA study of bone loss on medieval remains from North-West Italy

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Abstract

Purpose

The aim of this study was to investigate whether the population differences in osteoporosis observed nowadays is a reflection of the times and modern lifestyle factors, or whether they were also present in the past.

Materials and methods

The study was performed on the skeletal remains of medieval and post-medieval populations from a burial ground in the North–West of Italy. Some individuals had been buried inside the church (privileged subjects), others outside in the parvis (members of rural population), and others still to the north of the church. X-ray, computed tomography and dual-energy X-ray absorptiometry studies were carried out on the lumbar spines and/or femurs of 27 male and 28 female individuals to determine any associations between cortical index, bone mineral density (BMD), gender, age and social status.

Results

No statistically significant differences were observed in cortical index values according to gender, age or place of burial. Conversely, statistically significant differences in average BMD values were observed according to place of burial; in particular, among those buried inside the church, a lower BMD was observed compared to the parvis group (1.09 vs. 1.42, $p < 0.001$) and the north group (1.09 vs. 1.49, $p < 0.001$).

Conclusions

The differences observed in the BMD values may be related to the different lifestyle of the rural population, i.e. more dietary calcium intake, more sun exposure and vigorous physical activity, compared to that of the privileged individuals.

Keywords

X-ray Computed tomography DXA Cortical index BMD Italian medieval population Bone loss Osteoporosis

Introduction

Osteoporosis is an increasingly important issue in today's society and a major public health problem afflicting western populations, especially postmenopausal women once oestrogen secretion falls. The multifactorial issues associated with modern life are also known to take a toll, such as sedentary lifestyle, low calcium intake and vitamin D deficiency [1]. The study of osteoporosis incidence in subjects with different lifestyles is today useful to provide information on the role of socio-economic status, environmental conditions, bio-cultural context and genetics play in influencing bone loss.

With this aim in mind, a study on osteoporosis on ancient populations, who led very different lifestyles, was carried out to determine if this is a *new or old* pathological condition. A number of paleopathological studies have been performed on skeletons excavated from archaeological sites and demonstrated that osteoporosis is not simply an issue of modern lifestyle, but was also present in ancient human populations [2–11].

The literature on ancient or modern individuals which evaluated cortical bone thickness using diagnostic techniques, like bones and sites, has reported differences between gender and age [9, 12–17] and that cortical thickness directly correlates with bone strength and fracture risk [15, 18–20]. Mechanical stress is also important in bone modelling and various activities, such as weight-bearing exercises, may enhance cortical femur strength [15, 20, 21].

Dual-energy X-ray absorptiometry (DXA) is now a well-established method routinely used to measure bone mineral density (BMD) and an accurate method for the clinical diagnosis of osteoporosis in modern populations. The last two decades have witnessed an ongoing trend in research on DXA as the elective method to assess age- and gender-related changes in bone mineral density in archaeological populations. Moreover, as DXA is a noninvasive, nondestructive method to assess osteopenia and osteoporosis, it does not damage or destroy ancient bones [22, 23] and is the current technique of choice to monitor BMD in clinical practice, facilitating comparisons between ancient and modern individuals. The best sites for DXA scanning are the trabecular bone of the proximal femur and the vertebral body.

However, there is ongoing controversy as to there being a strong correlation between cortical thickness, osteoporotic bone loss and different lifestyle factors of ancient and modern populations [9, 16, 24].

The aim of this study was to investigate whether the population differences in osteoporosis observed nowadays are a reflection of the times and modern lifestyle factors, or whether they were also present in the past. For this purpose, the study describes the cortical index and bone mineral

density of skeletons buried in the medieval cemetery of San Michele's Church in Trino Vercellese, and explores associations between individual characteristics including gender, age, place of burial and bone status.

Materials and methods

Analyses were performed on skeletal remains excavated from the burial grounds at San Michele's Church in Trino Vercellese (Piedmont, North–West Italy), dating back to the Medieval and post-medieval Ages (8th–13th and 17th centuries) and presently held in the osteological collections of the Museum of Anthropology and Ethnography of Turin (Italy). The archaeological excavations were made by the University of Turin [25] in different burial grounds, where a total of more than 700 skeletons of adults, adolescents and infants of both genders were found (Fig. 1). The gender and age-at-death of all individuals were estimated by standard anthropological methods [26].

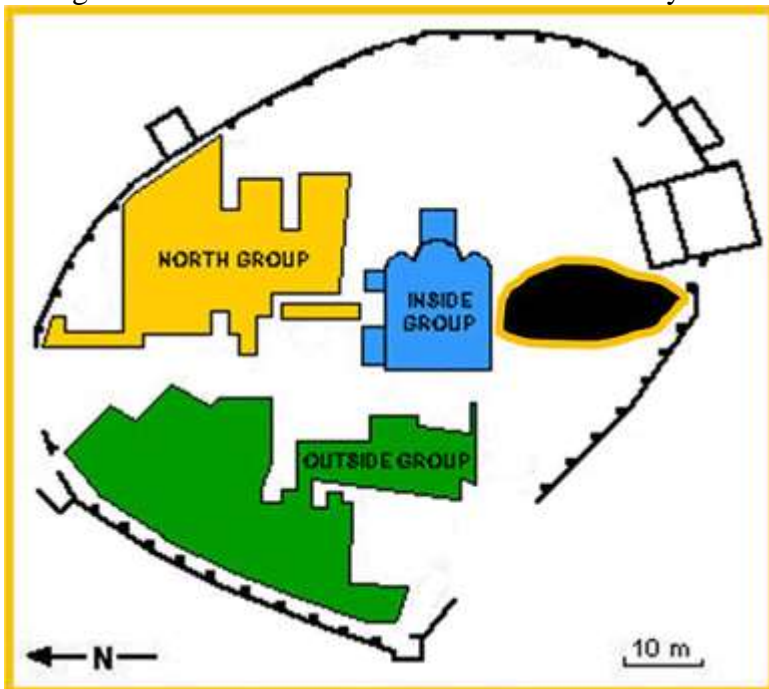


Fig. 1

Plan of the Cemetery of San Michele's Church in Trino Vercellese (Piedmont, North–West Italy) the different burial places

The analyses of the spatial organisation and the typology of the burials and graves indicated that there were different social groups [25], as follows:

1. 1.

The inside group: 273 skeletons, buried inside or along the walls of the Church. The custom of burying members of the clergy and privileged families inside churches has been documented as from the Early Middle Ages and the archaeological evidence from Trino Vercellese indicates that there is no reason to doubt the presence of noble families inside San Michele's Church;

2. 2.

The outside group (parvis): 219 skeletons buried outside the church in the parvis, close to the building. During the Medieval period, this burial area was dedicated to a plebeian cemetery: in the case of San Michele, this space was dedicated to rural population burials;

3. 3.

The north group: 99 skeletons buried outside the church, in an area located to the north of the building; these burials were not very close to the church and may accommodate different groups, such as military personnel or servants, and were most likely not dedicated to the local population.

All the materials included in the study were dependent on its state of preservation. Indeed, all the bones analysed were undamaged (Fig. 2) and they showed neither pathological features nor signs of soil erosion and/or infiltration, which might have hindered the accuracy of the measurements.



Fig. 2

An undamaged femur of the study

On the basis of these criteria, lumbar vertebral bodies (L1–L4) and/or femurs from 55 adult individuals (27 males and 28 females) were deemed suitable specimens for the present study. A radiological study was performed in two planes (frontal and lateral) with a remote-controlled system (Opera GMM, Seriate (BG), Italy). The femurs were positioned directly on a 35 × 43 cm CR plate IP cassette, FCR Fujifilm, Tokyo Japan, at 150 cm focus-film distance (FFD). Exposure parameters were 55 KVp, 100 mA, 160 ms. A CT scan was performed with a 4-row CT scanner (Brightspeed GE Healthcare, Fairfield CT USA), using a dedicated protocol with a bone reconstruction algorithm so as to obtain a volumetric acquisition of the whole femur. The tube voltage was 120 KVp, with a current of 130 mA and a slice thickness of 1.25 mm for the reconstructed axial images. All the X-ray and CT images were stored in the picture archiving and communications system (PACS) within the Radiodiagnostic Unit of the CTO/Maria Adelaide (Azienda Ospedaliera Universitaria Città della Salute e della Scienza di Torino). DXA scanning was performed by a fan-beam bone densitometer (Discovery Hologic, Waltham MA, USA), with a precision coefficient below 1 % in vivo and about 0.5 % in vitro. Before scanning, the bones were placed into a padded box to standardise their position. There was an X-ray generator below the bone and a detector was positioned above it. As the detector passed slowly over the area being scanned, it generated images on a computer monitor. The femurs and spines were positioned on the frontal plane (femurs as they were placed on the condyles and the greater trochanter). Each sample was placed in dry rice to simulate the presence of soft tissue around bones, in line with best results of previous studies [9, 27].

The cortical index was calculated both on the frontal X-ray image (Fig. 3) and the axial CT image (Fig. 4). The total femoral width (TW) and medullar width (MW) were measured at the midshaft [9,

[18](#), [21](#), [28](#)]. The measurements were taken with fixed visualisation parameters on the PACS-stored images. The linea aspera was identified in the axial CT scan and considered the most posterior point: the total width and the medullar width were measured perpendicular to the line passing through the linea aspera. Each measurement was repeated twice and the average value of two measurements was taken into consideration. The cortical index was calculated using the TW and MW values obtained on the X-ray and CT images, applying the formula proposed by Garn [\[18\]](#):

$$\text{Corticalindex} = \frac{\text{TW} - \text{MW}}{\text{TW}} \times 100$$

Individual characteristics were described using medians and the first and the third quartiles for the continuous variables. Percentage frequencies were used for the categorical variables. A multivariable linear regression model was used to analyse the effects of individual characteristics, i.e. gender, age and burial place, on the cortical index, reporting crude and adjusted marginal effects for each variable.



Fig. 3

Frontal X-ray image of a femur the total width (TW) and the medullar width (MW) were measured at the midshaft

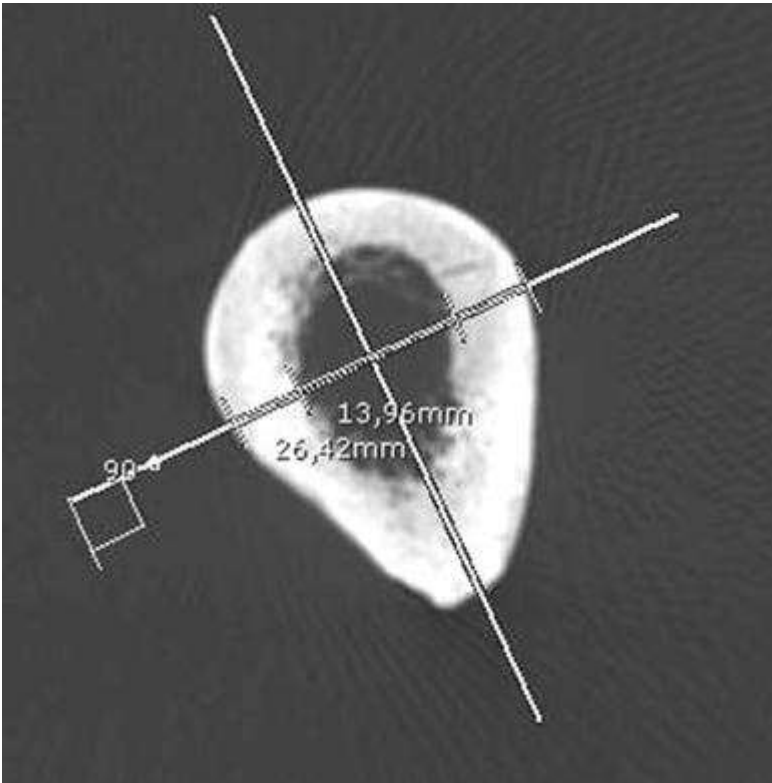


Fig. 4

Axial computed tomography (CT) scan of a femoral midshaft the total width (TW) and the medullar width (MW) were measured perpendicular to the line passing through the linea aspera

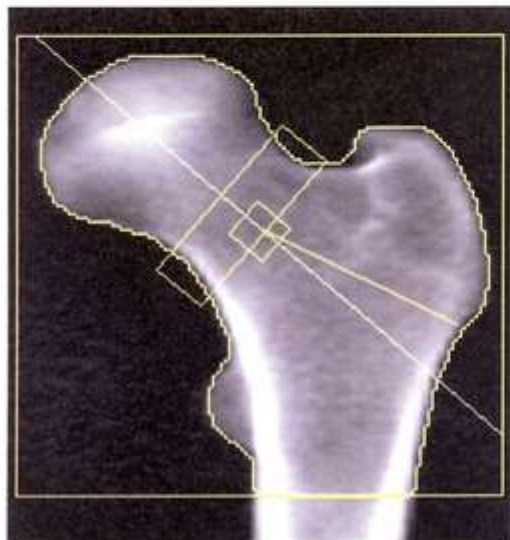
Data on BMD were obtained by DXA scans (Fig. 5) and the BMD averages were calculated [95 % confidence intervals (CI)] for the lumbar spine and femoral neck. The missing data for the lumbar spine BMD were estimated as a function of femoral BMD using a linear regression model. As sensitivity analysis, missing data were imputed using bootstrap method.

Name: 20 TSM
 Patient ID:
 DOB: 01 January 1958

Sex: Male
 Ethnicity: White

Height: 171.0 cm
 Weight:
 Age: 53

Referring Physician:



k = 1.158, d0 = 69.4
 122 x 116
 NECK: 49 x 15
 DAP: 3.1 cGy*cm²

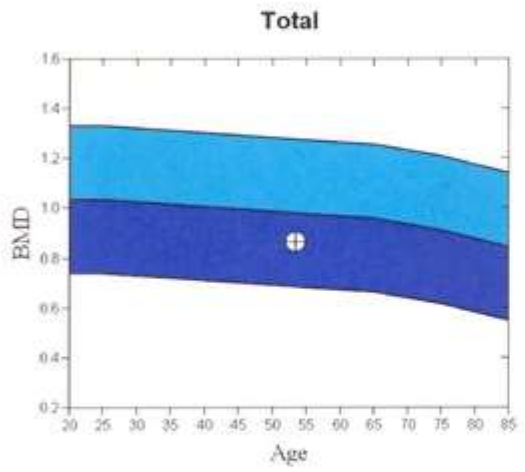
Scan Information:

Scan Date: 13 May 2011 ID: A0513110T
 Scan Type: a Left Hip
 Analysis: 13 May 2011 10:07 Version 13.2:5
 Hip
 Operator:
 Model: Discovery Wi (S/N 85418)
 Comment:

DXA Results Summary:

Region	Area (cm ²)	BMC (g)	BMD (g/cm ³)	T - score	Z - score
Neck	5.69	3.76	0.661	-2.0	-1.2
Troch	17.23	12.17	0.706	-0.6	-0.3
Inter	28.12	28.28	1.006	-1.0	-0.8
Total	51.04	44.22	0.866	-1.1	-0.7
Ward's	1.25	0.75	0.599	-1.3	0.1

Total BMD CV 1.0%, ACF = 1.026, BCF = 1.009, TH = 1.557



Comment:

T-score vs. White Male; Z-score vs. White Male. Source: BMDCS/NHANES

Fig. 5

Dual-energy X-ray absorptiometry (DXA) scan of a femoral neck with the bone mineral density (BMD) measurement

Similarly to the analysis of individual characteristic effects on the cortical index, a multivariable linear regression model was used, reporting crude and adjusted marginal effects for each variable to investigate into any association between BMD, gender, age and burial place.

Statistical analyses were performed by Stata 11.2 (StataCorp LP, College Station, TX, USA).

Results

The characteristics of the individuals are described in Table 1.

Table 1

Individual characteristics according to burial place

	Total (N = 55)		Burial place					
			Inside church (N = 20)		Parvis (N = 18)		North group (N = 17)	
	No.	%	No.	%	No.	%	No.	%
Gender								
Female	28	50.91	8	40	10	55.56	10	58.82
Male	27	49.09	12	60	8	44.44	7	41.18
Age (years)								
20–30	17	30.91	2	10	8	44.44	7	41.18
30–40	13	23.64	4	20	4	22.22	5	29.41
40–50	12	21.82	5	25	3	16.67	4	23.53
>50	13	23.64	9	45	3	16.67	1	5.88
Age (years) ^a	40	30–50	46.5	37–53	36.5	25–50	40	30–50
Height (cm) ^b	163.0	155.0–168.0	168.5	158.5–172.0	162.5	153.0–166.5	157.5	153.5–167.0
Period								
X–XI	6	10.91	6	30	0	0	0	0
XII–XIII	3	5.45	3	15	0	0	0	0
XVI–XVII	9	16.36	9	45	0	0	0	0
Not available	37	67.27	2	10	18	100	17	100

^aMedian (I quartile–III quartile)

^bHeight is estimated for 47 individuals (18 Inside Church, 15 Parvis, 14 North Group)—median (I quartile–III quartile)

Information on the cortical index was available in 38 skeletons (17 males and 21 females). There were no statistically significant differences in X-ray or CT cortical indexes according to individual characteristics: as shown in Table 2, there were no variations in the averages according to gender, age or burial place. Incidental findings were observed in six of the femur X-rays and CT scans: one small round radiolucent lesion of the neck and five calcified areas inside the medullary canal.

Table 2

Marginal effect on cortical index according to gender, age and burial place

	Observed cortical index (X-ray)								Observed cortical index (CT)							
	Crude estimates				Adjusted estimates				Crude estimates				Adjusted estimates			
	N	Margi n	95 % CI	p valu e	N	Margi n	95 % CI	p valu e	N	Margi n	95 % CI	p valu e	N	Margi n	95 % CI	p valu e
Gender																
Female	21	56.22	53.93 – 58.51	ref	21	56.56	54.12 – 58.99	ref	21	53.18	50.56 – 55.80	ref	21	53.73	50.99 – 56.46	ref
Male	17	56.38	53.83 – 58.93	0.928	17	55.96	53.22 – 58.70	0.763	17	52.70	49.78 – 55.61	0.812	17	52.01	48.94 – 55.09	0.441
Age (years)																
20–30	11	56.51	53.44 – 59.59	ref	11	56.53	52.7– 60.36	ref	11	52.59	49.03 – 56.16	ref	11	51.64	47.34 – 55.93	ref
30–40	8	53.96	50.36 – 57.57	0.299	8	53.93	50.05 – 57.8	0.351	8	52.11	47.93 – 56.29	0.865	8	52.59	48.25 – 56.94	0.758
40–50	9	55.31	51.91 – 58.71	0.610	9	55.32	51.69 – 58.95	0.671	9	51.07	47.13 – 55.01	0.577	9	51.59	47.52 – 55.66	0.988
>50	10	58.79	55.56 – 62.01	0.324	10	58.78	54.85 – 62.72	0.477	10	55.75	52.01 – 59.49	0.239	10	55.95	51.53 – 60.37	0.229
Period																
X–XI	6	58.01	53.08 – 62.94	ref					6	54.09	48.50 – 59.67	ref				ref
XII– XIII	3	58.00	50.20 – 65.79	0.998					3	55.76	46.94 – 64.59	0.758				
XVI– XVII	9	56.51	52.83 – 60.18	0.639					9	52.98	48.84 – 57.14	0.759				
Burial place																
Inside church	18	56.99	54.50 – 59.48	ref	18			ref	18	53.69	50.92 – 56.46	ref	18	52.99	49.74 – 56.24	ref
Parvis	9	56.13	52.61 – 59.65	0.696	9	56.49	53.60 – 59.39	0.855	9	54.50	50.58 – 58.41	0.742	9	55.05	50.60 – 59.49	0.506
North	11	55.27	52.09 – 58.45	0.409	11	55.99	52.02 – 59.96	0.907	11	50.52	46.98 – 54.06	0.176	11	51.21	47.30 – 55.12	0.527

DXA studies were performed on 21 lumbar spines and 15 proximal femurs from males (seven showed both lumbar spine and femurs) and 19 lumbar spines and 21 proximal femurs from females (nine showed both lumbar spine and femurs). There were no statistically significant differences in BMD according to gender: the average lumbar spine BMD was 1.31 (95 % CI, 1.17–1.45) in

females and 1.35 (95 % CI, 1.25–1.45) in males and femoral BMD was 1.20 (95 % CI, 1.07–1.33) and 1.14 (95 % CI, 1.03–1.24), respectively, in females and males (Table 3). Both lumbar spine and femoral BMD were higher in younger subjects. When both observed BMD (Table 3) and estimated BMD (Table 4) were taken into consideration, there was a statistically significant difference according to burial place, i.e. the inside group had a lower BMD than the other two groups (1.09 vs. 1.42, $p < 0.001$ and 1.09 vs. 1.49, $p < 0.001$). These results did not change when the bootstrap method was used to impute the missing data. The period was not included in the multivariable model because the availability of information is collinear to burial place.

Table 3

Mean of BMD (lumbar spine, femoral neck and femoral) according to gender, age and burial place

	Lumbar spine BMD				Femoral neck BMD				Femoral BMD			
	N	Mean	Standard deviation	95 % CI	N	Mean	Standard deviation	95 % CI	N	Mean	Standard deviation	95 % CI
Gender												
Female	19	1.31	0.30	1.17–1.45	21	0.98	0.25	0.86–1.09	21	1.20	0.28	1.07–1.33
Male	21	1.35	0.23	1.25–1.45	15	0.95	0.20	0.85–1.06	15	1.14	0.19	1.03–1.24
Age (years)												
20–30	14	1.42	0.19	1.31–1.53	11	1.15	0.19	1.02–1.28	11	1.36	0.23	1.24–1.52
30–40	8	1.37	0.29	1.13–1.61	7	0.90	0.15	0.76–1.04	7	1.13	0.19	0.96–1.31
40–50	9	1.37	0.26	1.17–1.57	8	0.93	0.20	0.76–1.10	8	1.13	0.23	0.94–1.32
>50	9	1.12	0.25	0.93–1.32	10	0.84	0.22	0.69–0.99	10	1.02	0.19	0.88–1.16
Period												
X–XI	6	1.07	0.08	0.98–1.16	5	0.85	0.18	0.63–1.07	5	1.04	0.21	0.77–1.30
XII–XIII	2	1.13	0.22	0.83–3.09	2	0.89	0.10	0.02–1.75	2	1.12	0.04	0.79–1.44
XVI–XVII	5	0.97	0.22	0.70–1.25	9	0.78	0.18	0.65–0.92	9	0.94	0.14	0.82–1.05
Not available	27	1.47	0.17	1.40–1.53	20	1.09	0.19	1.00–1.18	20	1.32	0.20	1.22–1.41
Burial place												
Inside church	14	1.07	0.18	0.96–1.17	18	0.83	0.17	0.75–0.92	18	1.01	0.17	0.92–1.09
Parvis	13	1.38	0.16	1.29–1.48	9	1.18	0.21	1.02–1.34	9	1.41	0.22	1.24–1.58
North group	13	1.56	0.13	1.25–1.41	9	1.03	0.16	0.90–1.15	9	1.26	0.14	1.16–1.25

Table 4

Marginal effect on BMD estimate (lumbar spine) according to gender, age and burial place

	Lumbar spine BMD estimate							
	Crude estimates				Adjusted estimates			
	N	Margin	95 % CI	p value	N	Margin	95 % CI	p value
Gender								
Female	28	1.32	1.22–1.41	ref	28	1.28	1.21–1.35	ref
Male	27	1.32	1.23–1.42	0.907	27	1.36	1.29–1.43	0.118
Age (years)								
20–30	17	1.45	1.34–1.57	ref	17	1.37	1.29–1.46	ref
30–40	13	1.32	1.20–1.45	0.140	13	1.31	1.22–1.41	0.377
40–50	12	1.31	1.18–1.44	0.110	12	1.31	1.21–1.41	0.373
>50	13	1.15	1.02–1.28	0.001	13	1.26	1.16–1.36	0.130
Period								
X–XI	6	1.07	0.94–1.20	ref				
XII–XIII	3	1.16	0.98–1.35	0.422				
XVI–XVII	9	1.01	0.91–1.12	0.487				
Burial place								
Inside church	20	1.08	1.00–1.15	ref	20	1.09	1.01–1.18	ref
Parvis	18	1.42	1.34–1.51	<0.001	18	1.42	1.33–1.50	<0.001
North group	17	1.50	1.41–1.58	<0.001	17	1.49	1.40–1.57	<0.001

In conclusion, significant differences were observed according to burial place in the distribution of BMD, but not in the distribution of the cortical index (Fig. 6).

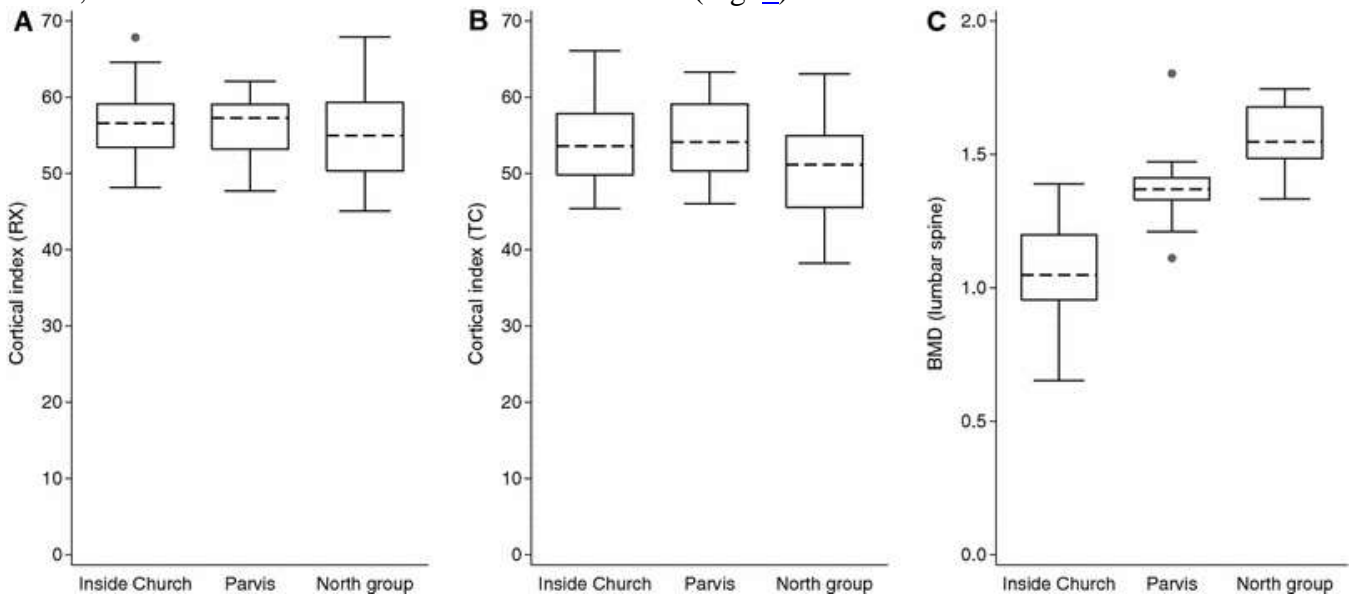


Fig. 6

Boxplot of the cortical index measured by X-ray (a), the cortical index measured by CT (b) and the BMD (c) measured by DXA according to the different burial places

Discussion

Previous anthropological and anthropometric studies have reported intrapopulation variations that could be related to differences in social status [26, 29, 30]. Therefore, this study compared groups of assumed different social status. As most of the female skeletons examined were of young adults and only 4/55 were of women over 50 years of age, we may reasonably assume that the population was homogenous for sex hormonal status.

Large bones, such as the femur rather than small bones, e.g. metacarpi, were privileged for the evaluation of cortical index by X-ray and CT scan as femurs are usually in a better state of preservation. Moreover, a careful selection was made to ensure the use of bones that were as intact as possible on gross examination so as to rule out, as far as possible, the possibility that diagenetic changes had altered bone composition and accordingly density.

CT was also used to evaluate the cortical index so as to identify the limit between the cortical bone and the endosteal trabecular component, using the correct visualisation parameters and an accurate measurement technique [28, 31, 32]. CT images make for an easy identification of the linea aspera, which is the posterior point of the diaphysis, allowing for precise geometrical measurements of both the medial and lateral cortical thickness [33].

No statistically significant differences were observed in the cortical index measured by X-ray and CT between gender, age or burial place, demonstrating an overall limited bone loss. Therefore, it may be asserted that the individuals studied showed no signs of diseases known to lead to macroscopic alterations of the cortical bone: in particular, there was no evidence of serious bone remodelling, such as rachitism, osteomyelitis or post-traumatic signs.

An interesting collateral finding of the radiological studies was the presence of some focal lesions that were not visible at gross inspection of the femurs: one small round radiolucent lesion of the neck and five calcified areas inside the medullary canal. Sporadic reports of similar lesions can be found in paleopathological studies [34, 35]; it might well be of interest to compare these findings with the focal lesion patterns of contemporary populations.

However, when using DXA on ancient bones, the possibility that bone diagenesis may affect DXA readings cannot be ignored. Therefore, any bones with gross post-depositional damage, such as erosion of the external surface, were excluded from the study. In buried skeletal material, soil may infiltrate the bone structure meaning that buried bone often shows microstructural changes caused by soil-dwelling micro-organisms. Consequently, the material used for this study was selected according to its state of preservation to avoid significant reduction of DXA diagnostic accuracy and all the bones scanned were in good condition with normal morphological features. Any damaged bones and/or those with artifacts, such as metal deposition or pathological features that might have interfered with the accuracy of the measurement, were excluded from this study.

This study identified differences among the assumed different social groups both in males (only lumbar spine BMD data available) and females (both at lumbar spine and proximal femur). In particular, individuals buried inside the church (members of the clergy and privileged families) had statistically significantly lower average BMD values than the individuals buried outside the church (likely members of rural population, militaries or servants). These differences may be related to the different lifestyle led by the more privileged subjects compared to the less privileged classes who had a higher dietary calcium intake (milk and dairy products), more sun exposure and vigorous physical activity. Our findings support the results obtained from an earlier study on intrapopulation variation in stature and body proportions in relation to social status and gender differences, performed by other authors on the same samples of population from Trino Vercellese [32].

As the findings on the cortical index did not differ greatly among the groups, the differences in the BMD between burial sites seem to be of greater significance as it is a direct effect of changes in the mineral content of cancellous bone, which is more sensitive than is compact bone to osteopenic modifications.

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Conflict of interest

The authors declare no conflict of interest.

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