Journal of Archaeological Science: Reports 36 (2021) 102792

Contents lists available at ScienceDirect

Journal of Archaeological Science: Reports

journal homepage: www.elsevier.com/locate/jasrep

Bone mineral density through history: Dual-energy X-ray absorptiometry in archaeological populations of Norway

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A R T I C L E I N F O

Keywords:
Bone mineral density
Femur neck
dual X-ray absorptiometry
Patterns of bone loss
Trabecular bone
Late Iron Age
Medieval
post-Reformation
Archaeology
Paleopathology

A B S T R A C T

Information regarding bone mineral density (BMD) and related variations through prehistoric and historic time periods in Norway is scarce. We present results of BMD measurements of 222 individuals from four rural and urban burial sites representing the medieval and post-Reformation period using osteological analysis and dual-energy X-ray absorptiometry. Existing BMD data from 137 individuals dating to the Late Iron Age and medieval period were incorporated. Young medieval females have the highest mean BMD of all time periods, including the modern female population, and significant higher mean BMD than young females from the Late Iron Age ($p = 0.02$; $q = 0.093$). Mean BMD increased significantly from the Late Iron Age to the medieval period ($p = 0.0002$) followed by a significant decline from the medieval to the post-Reformation period ($p = 0.014$). The overall results reveal significant BMD variation through prehistoric and historic time periods in Norway. The patterns of age-related bone loss observed in the archaeological record are diverse with substantial temporal changes suggesting a transition towards a modern pattern. The bone loss often exceeds that observed in the population today. This study sheds light on long-term historical trends and patterns in Norway by examining BMD variation and age-related bone loss in adult life of males and females within three archaeological time periods and compared to present populations.

1. Introduction

Reduction of bone mass is a natural part of the ageing process in both sexes, but is especially marked in women after menopause (Agarwal and Stout, 2003; Aufderheide and Rodríguez-Martín, 1998). While bone loss is an age-related and postmenopausal phenomenon, the risk of developing osteoporosis is greatly affected by independent factors, such as heredity, physical activity, parity and lactation (Agarwal and Glencross, 2010) and also shows genetic variability (Cauley, 2011; Pothiwala et al., 2006). Osteoporosis is an important and frequent primary bone disease affecting modern societies, with especially high prevalence in Western and Asian populations. The major impact on rates of morbidity and mortality, has led to substantial research to identify possible causes and the history of this condition (Mays et al., 2006a). While written historical sources are of little use (Gass and Dawson-Hughes, 2006; Mays, 1999), a number of paleopathological examinations of archaeological skeletal remains have elucidated the historical prevalence of bone loss. Research on European skeletal material has uncovered various degrees of bone loss in past populations, but the findings have been inconsistent. It has been unclear to what extent the patterns of age-related bone loss in the past mirror those seen in modern populations (Agarwal and Grynpas, 1996; Mays, 1999; Mays et al., 1998). Research on archaeological skeletal material from Norway (Holck, 2007; Mays et al., 2006a; Turner-Walker et al., 2001) has focused mainly on the medieval period and lead to few conclusive results regarding long-term trends and patterns of changes from prehistoric to modern times. This study addresses this knowledge gap and uncovers characteristics of BMD variations throughout history in Norway, seeking possible temporal patterns. Osteological analysis and DXA (dual-energy X-Ray absorptiometry) are combined in this study in an attempt to describe skeletal characteristics from prehistoric times to the present. The time horizon is expanded by examining long term trends in material from the Late Iron Age

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https://doi.org/10.1016/j.jasrep.2021.102792
Received 7 February 2020; Received in revised form 27 December 2020; Accepted 2 January 2021
Available online 1 February 2021

(750–1030 CE), medieval period (1030–1536 CE) and post-Reformation period (1537 CE-present). The novel DXA measurements of bones from the post-Reformation period provide a vital historical link to the population today. Results are seen in relation to comparative paleopathological studies of femur neck BMD and age-related bone loss, both within Scandinavia (Bennike and Bohr, 1990; Ekenman et al., 1995; Holck, 2007; Mays et al., 2006a; Poulsen et al., 2001; Turner-Walker et al., 2000b, 2001) and in Europe in general (Curate and Tavares, 2018; Hammerl et al., 1990; Kneissel et al., 1994; Lees et al., 1993; Mays et al., 2006b; Mays, et al., 1998; Mays, 1996, 2006).

The amount of bone mineral acquired from birth to adulthood follows distinct age- and sex-specific patterns. Total skeletal mass peaks a few years after fusion of the long bone epiphyses (Heaney et al., 2000). Peak bone mass refers to the maximum amount of bone an individual accrued during young adulthood (Weaver et al., 2016), and tends to be higher in men than in women. Before puberty, boys and girls acquire bone mass at similar rates. After puberty, however, men tend to acquire greater bone mass than women (NIH, 2015), apparently due to a prolonged bone maturation period (Bonjour et al., 1994). Modern women tend to experience little change in total bone mass between age 30 and menopause, but then a progressive bone loss occurs, starting prior to cessation of estrogen production. This rapid bone loss then slows, but continues throughout the postmenopausal years (NIH, 2015). In men, however, a more gradual bone loss occurs throughout their adult life (Clarke and Khosla, 2010). Age-related bone loss also occurs secondary to most chronic diseases, high alcohol consumption, smoking, reduced physical activity (Falch et al., 1993; Hollenbach et al., 1993; Naessen et al., 1989; Paganini-Hill et al., 1991; Turner-Walker, et al., 2001b), vitamin D deficiency, and steroid use (Holck, 2007; Meyer et al., 1995).

2. Material

The skeletal material included in this study is a part of the Schreiner Collection at the Division of Anatomy, University of Oslo. This collection consists of about 8500 skeletal finds. Remains from a large number of inhumation burial sites were considered for DXA analysis; cohorts examined and enrolled for DXA analysis are shown in Fig. 1. Skeletal material from medieval Oslo is well represented in the Schreiner Collection – however, remains of any significant size from rural Norway proved difficult to obtain. Skeletal material from the post-Reformation period in Norway is limited in size due to a lack of legal protection. (Sellevold, 2006). These remains are therefore seldom recovered during archaeological excavations. The final study sample for DXA analysis included skeletal material from medieval and post-Reformation burial sites in Eastern Norway dating from the eleventh to nineteenth centuries CE. The geographic location of burial sites are shown in Fig. 2.

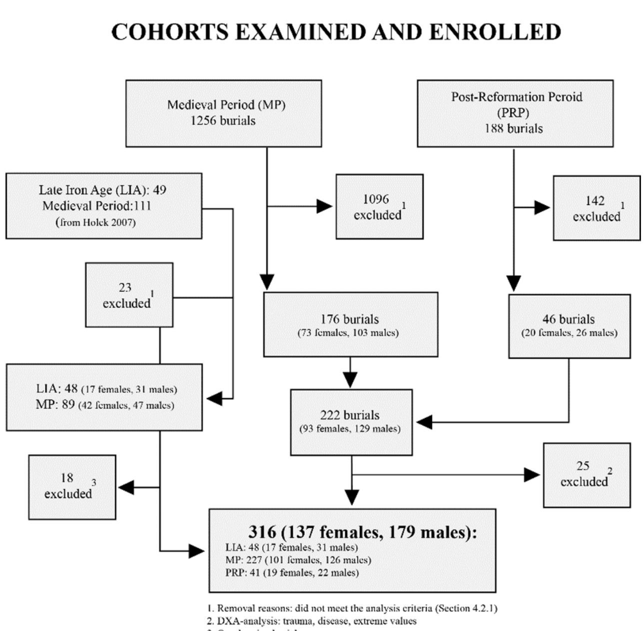

Fig. 1. Cohorts examined and enrolled in the study, including incorporated sample (Holck, 2007) and sample analyzed with DXA.

2.1. Burial sites

2.1.1. The Church of St. Mary, Oslo

The Church of St. Mary (ca. 1050–1540 CE) was likely intended as a burial place for the Norwegian royal family, much like Westminster Abbey in London and St. Denis outside Paris (Nedkvitne and Norseng, 2000). Apart from the kings and their descendants, it is likely that the nobility and clergy constituted the largest part of the burials at the cemetery (Roaldset, 2000). This burial site was therefore classified as having high socioeconomic status (see 3.1. Osteological data collection for information on the identification of social status). The skeletal material from this site is previously examined (Brødholt and Holck, 2012; Brødholt, 2006, 2007), and is generally well preserved with good contextual information. Considerable parts of the material consist of crania and commingled postcranial remains. Sixty-nine of 308 adult individuals met the inclusion criteria (see below, 3.2.3. DXA analysis inclusion criteria). Bedrock topographic maps display that this specific part of the Oslo region is characterized by somewhat calcareous bedrock with areas of neutral bedrock (Geological Survey of Norway, 2020). Geochemical analyses of the cultural layers at the cemetery of the Church of St. Mary detected neutral to weakly alkaline soils (pH 7–8), poor preservation and degradation of organic substances (Bye Johansen et al., 2009).

2.1.2. Hamar Cathedral, Hamar

Hamar Cathedral is thought to have been completed by the beginning of the twelfth century CE (Sæther, 1998), and it continued to function for another 30 years or so after the Reformation in 1536–1537 CE (Sellevold, 2001). It has been debated whether the cathedral may have functioned as a parish church or whether its use was reserved for the ecclesiastical community (Müller, 1986; Sæther, 1992). The age and sex distribution at this site was considered atypical of either an ecclesiastical or a parish churchyard, and it was therefore hypothesized that the church had served both. Calculations of the supporting population of the churchyard suggests that it served laypersons from the whole diocese of Hamar, which comprised a large part of eastern Norway (Sellevold, 2001), and this burial site was therefore classified in the parish population category regarding socioeconomic status. Osteological data were provided by Sellevold (2001) and the Schreiner Collection's database. Of 482 burials, 322 were considered adult individuals, of which 107 individuals were included in this analysis. There is considerable local variation in calcium content in the bedrock in Hamar (Geological Survey of Norway, 2020), but this area of Norway is mainly characterized by calcareous soil (Stensrud and Selstad, 1991) which is generally advantageous for the preservation of bone (Historic England, 2016).

2.1.3. Christiania Tukthus, Oslo

The social institution known as *Christiania Tukthus* was founded in 1741 CE as a work and correctional facility. The conditions were dire; it was overcrowded and hygiene was dismal (Holck, 1990a), resulting in many deaths and, consequently, an urgent need for a cemetery. According to the institution's records of deaths and funerals, it is estimated that about 10% of inmates died each year between 1758 and the closing of the latest cemetery around 1830, with extreme mortality rates during the impoverished 1770s and the Napoleonic War c. 1807–1814 CE (Holck, 1990a). The inmates consisted mostly of women, a pattern which dominated until the 1780s when the institution started taking in criminals. The proportion of male inmates increased steadily until the institution became a regular prison in 1813 (Daa, 1908; Holck, 1990a,

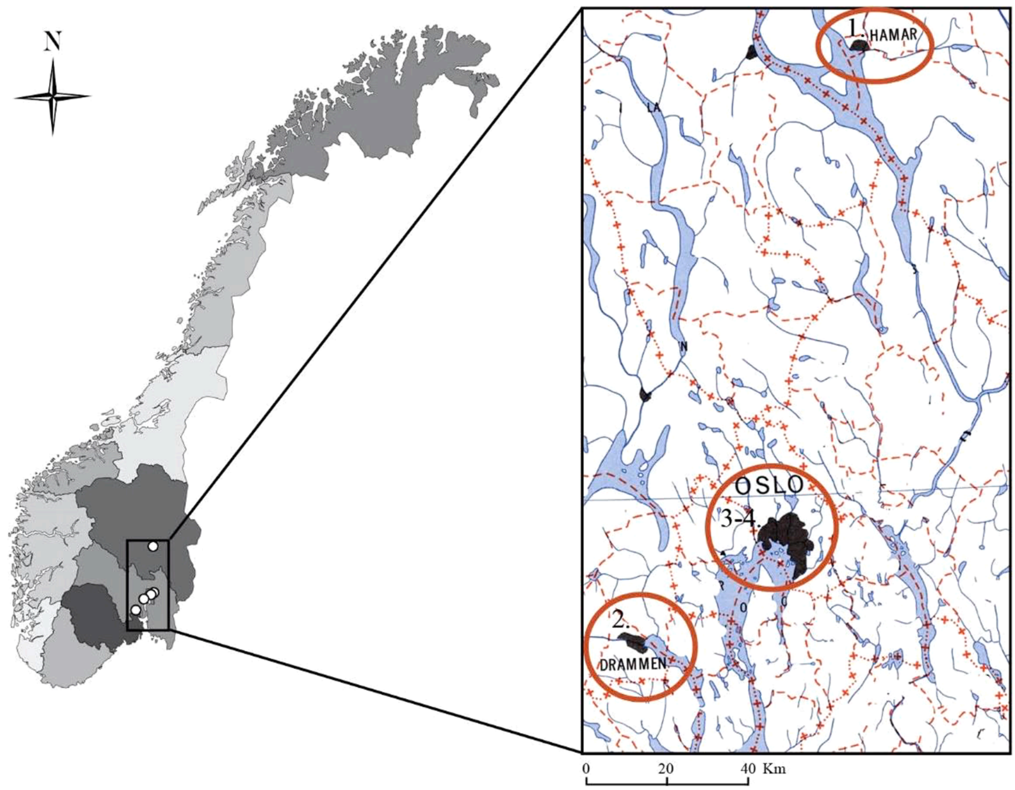

Fig. 2. Map of Norway with the four burial sites providing skeletal material for the DXA-analysis: Hamar Cathedral, Innlandet (early-12th century – c. 1565/1570 CE; 2. Tangen Church, Viken (1696–1850 CE); 3. Church of St. Mary, Oslo (ca. 1050–1540 CE); 4. Christiania Tukthus, Oslo (1741–1830 CE).

1990b). Accordingly, the burial from this site was classified as having low socioeconomic status. The remains were highly fragmented, commingled, often poorly preserved, and covered with blue marine clay on the bone surface and in the medullary cavity. Bone preservation in clay can be good or bad, depending on the pH (Brothwell, 1972). Only 28 individuals met the inclusion criteria. For information on bedrock topography in the Oslo region, see 2.2.1 (Artsdatabanken; Geological Survey of Norway, 2020; Historic England, 2016).

2.1.4. *Tangen Church, Drammen*

In 1914, a large number of coffins were removed from the crypt under the floor of Tangen Church, due to their strong odour and suspicion of posing a health hazard. These graves were thought to have been moved from the old church built in 1696 CE when the new church was erected in 1850–1853 CE. The remains were mummified and the remaining facial features, hair/wigs, elaborate clothing, and flowers are extremely well preserved. The burials date to ca. 1700–1850 CE and constitute remains of the upper class in the post-Reformation society of Drammen, thereby placing them in the high status category regarding socioeconomic status. Inscriptions on some of the coffins revealed that these were individuals of nobility (Svenkerud Fresvik, 2013). The skeletal material consisted mostly of complete skeletons and was in excellent condition, never having been in the soil but mummified in the crypt. Three adult individuals were excluded due to known identity, while 18 of the remaining 20 adults met the inclusion criteria.

2.2. *Incorporated burial sites*

Skeletal material previously analysed with DXA by Holck (2007), dating from the Late Iron Age (48 individuals) and medieval period (89 individuals), was included in the study for comparison. The location and distribution of these burials are shown in Fig. 3. The material from the Late Iron Age consists of few and scattered burials, as Christian churchyards did not exist in Norway until after the middle of the eleventh century CE. Many of the burials are richly furnished and considered characteristic of the upper social strata at the time. Accordingly, these burials were placed in the high status category regarding socioeconomic status. The medieval rural material comes from the churchyard of Prestgardskirken, in the small community of Heidal in the southern inland of Norway (Holck, 2007), which was in use until 1531 CE (Schreiner, 1939). This burial site was therefore placed in the parish population category regarding socioeconomic status. The medieval urban material comes from three locations in Oslo. St. Clemens Church is a parish church that operated throughout the medieval period and thus contains some of the oldest Christian burials found in Norway, dating to 980–1030 CE (Nedkvitne and Norseng, 2000). Accordingly, these burials were placed in the parish population category regarding socioeconomic status. The medieval monastic material comes from the south wing of St. Olav's Monastery, founded in 1239 CE when a group of Dominicans were given a plot of land in Oslo. It operated until the Reformation in 1537 CE (Holck, 2007). This burial site was used by other citizens in addition to the friars (Ekstrøm, 2006; Hommedal, 1987), and was therefore classified as having mixed socioeconomic status. Ekstrøm (2006)For description of the material from the Church of St. Mary, see section 2.2.1.

3. Methods

3.1. *Osteological data collection*

Demographic data of the skeletal material included are displayed in Table 1. Complete profiles on the Late Iron Age material and the skeletal material from the Church of St. Mary were obtained from previous analyses (Brødholt, 2006, 2007, 2016). Separate osteological examinations were performed on the material from Christiania Tukthus and Tangen Church. These examinations were conducted according to traditional methods given by Buikstra and Ubelaker (1994); the sexing of crania was performed according to the scoring system by Acsádi and Nemeskéri

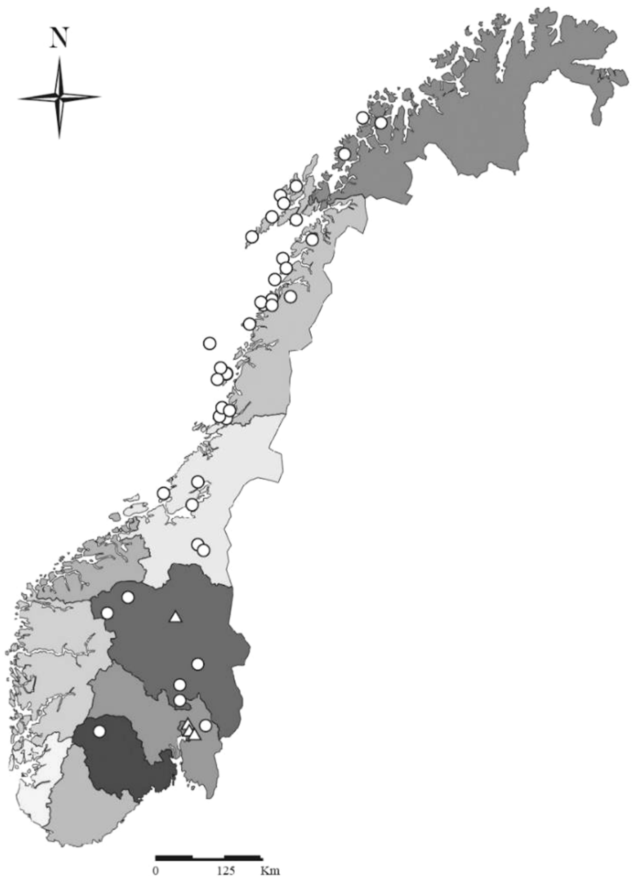

Fig. 3. Location and distribution of burial sites in incorporated sample. Selection of sites analyzed with DXA by Holck (2007). Circles indicate scattered Late Iron Age burials. Triangles indicate burials from the medieval period: 1. Church of St. Clemens, Oslo (980–1030 CE); 2. Church of St. Mary, Oslo (ca. 1050–1540 CE); 3. St. Olav's Monastery, Oslo (1239–1537 CE); 4. Prestgardskirken, Innlandet (1000/1050–1531 CE).

(1970) and the assessment of pelvic features was performed according to Phenice (1969). The estimation of age-at-death was performed by scoring the degree of suture closure, presented in Meindl and Lovejoy (1985), and the pubic symphysis according to Todd (1921a), Todd (1921b) and Brooks and Suchey (1990), Bass (1987), Buikstra and Ubelaker (1994). The following age groups were applied: young (20–35 years old), middle (35–50 years), and old adult (50 +) as per (Buikstra and Ubelaker, 1994). Socioeconomic status was identified based on information from literature and (previous) osteological analyses, and classified using the following categories: 1) high, 2) mixed, 3) parish population, 4) low. Stature was estimated according to Trotter and Gleser (1952), Trotter and Gleser (1958). Pathological conditions and trauma were identified according to characteristics described in Aufderheide and Rodríguez-Martín (1998) and Ortner (2003). (Aufderheide and Rodríguez-Martín, 1998; Ortner, 2003)

3.2. *Dual-Energy X-Ray absorptiometry*

DXA is the most utilised and most thoroughly validated technique for assessing BMD and bone mineral loss in a clinical context (WHO, 2007), facilitating comparisons between archaeological and today's skeletons (Mays, 1999). Modern DXA scanners offers high precision and can detect subtle changes in bone (GE, 2014). DXA bone mineral measurements are taken by measuring the attenuation of the X-ray beam in the bone tissue, which shows the amount of calcium in the bone. After calibration, the BMC (bone mineral content) in the examined area is calculated. BMD (g/cm^2) is calculated by dividing the BMC value by the size of the total area measured (Falch, 2003).

The BMD measurements in this study were obtained with a Lunar iDXA (GE Healthcare Lunar, Madison, WI, USA). BMD was measured at the femur neck (*collum femoris*), defined as the region of interest (ROI) (Fig. 4). The difference between the mean BMD value for young adults and measured BMD, expressed in standard deviations (SD), is defined as the T-score. When calculating the T-score in the present case, an individual's BMD is compared to the young adult reference mean value for the femur neck, using data on Caucasian women aged 20–29 years (GE, 2014; WHO, 2007). Normative data from the NHANES (National Health and Nutrition Examination Survey) reference database constitutes the international standard for the description of osteoporosis in postmenopausal women and men older than 50 years (Kanis et al., 2008; Kanis, 2002; WHO, 2007). A T-score between −1.0 and −2.5 SD is defined as low bone mass (osteopenia), which is usually a precursor to osteoporosis. The World Health Organization's (WHO) definition of osteoporosis by DXA is a femur neck BMD equal to or lower than −2.5 standard deviations (SD) below the young female adult mean (Genant et al., 1999; WHO, 2007; Kanis, et al., 2008; Kanis, 2002; GE, 2014)

3.2.1. *BMD data for incorporated skeletal sample*

BMD data from the DXA-analysis by Holck (2007) were retrieved from the database associated with the Lunar Prodigy (GE Healthcare Lunar, Madison, WI), located at Lovisenberg Diakonale Hospital, Oslo. Holck examined BMD at the femur neck, and one femur from each skeleton was analysed. A bag of rice was placed beneath the bone, acting as a soft-tissue substitute. In a conversation with our co-author (Holck, P 2019, oral communication, 8th June), it was stated that the measurements were taken by a technician at Lovisenberg Diakonale Hospital, Oslo, and performed according to traditional methods for DXA-analysis. One hundred thirty-seven individuals, with scans in accordance with the DXA analysis criteria were included in the study (Figs. 1 and 3).

3.2.2. *Soft tissue substitute and standardisation*

The DXA analysis method has been modified in order to be applied to dry bones in paleopathological studies. Direct comparison with measurements of living individuals is not possible due to the lack of soft tissue and bone marrow in skeletal remains (Chappard et al., 2004; Lees, et al., 1993). It has been customary to use bags of rice, immerse the specimen in water, or use other substitute material to simulate the presence of soft tissue in these samples (Agarwal and Stout, 2003). The use of rice, however, is not recommended with the Lunar iDXA since the high-resolution images will portray the grains of rice. Instead, this study tested a combination of water and plastic boards as a soft-tissue substitute. A specially constructed frame with plastic boards inside and a container of water on top was used, and was created to allow the positioning of a femur between the boards and the water. The water level in the container was set to 14.5 cm. An angled mirror placed in the proximal end facilitated the horizontal orientation of the proximal femur (Fig. 5). The weight was set according to estimated stature and the soft tissue substitutes were adjusted in order to obtain the required thickness of soft tissue and a BMI (body mass index) within the normal range, 18.5–24.9 kg/m^2 (WHO, 2018).

The femur was placed with the anterior surface up, and angled so that the femur neck lay horizontal and the femur diaphysis was oriented parallel to the scanner's axis (Fig. 5). BMD was measured at the femur neck, and both femora from each individual were measured if available. A QA (quality assurance) procedure was performed daily, using a calibration block consisting of tissue-equivalent material with three bone-simulating chambers of known BMD content. This daily QA procedure calibrated the machine as well as performing quality control measurements. A phantom (simulating L2–L5) was also used as a separate control measure and quality control in addition to the QA (GE, 2014). Each femur was scanned three times consecutively, in order to examine the precision and repeatability of the measurements.

Table 1
Demographic data total sample.

Time Period	Burial site, County	Socioeconomic status	n	Sex	Age YA	MA	OA	Total
Medieval Period (MP) (1030–1536 AD)	Church of St. Mary, Oslo	High		Females	8	8	3	19
				Males	16	20	14	50
			Total					69
	Hamar Cathedral, Innlandet	Parish		Females	9	16	29	54
				Males	25	15	13	53
			Total					107
	Total MP			Females	17	24	32	73
				Males	41	35	27	103
			Total					176
Post-Reformation Period (PRP) (1537–1880 AD)	Christiania Tukthus, Oslo	Low		Females	11	1	0	12
				Males	7	8	1	16
			Total					28
	Tangen Church, Viken	High		Females	2	4	2	8
				Males	3	4	3	10
			Total					18
	Total PRP			Females	13	5	2	20
				Males	10	12	4	26
			Total					46
Total				Females	30	29	34	93
				Males	51	47	31	129
			Total					222
HOLCK (2007)								
Late Iron Age (LIA) (750–1030 AD)	Scattered burials	High		Females	11	4	2	17
				Males	7	12	12	31
	Total LIA							48
Medieval Period (MP) (1030–1536 AD)	Church of St. Mary, Oslo[1]	High		Females	2	5	1	8
				Males	5	2	3	10
			Total					18
	St. Clemens Church, Oslo	Parish		Females	4	2	4	10
				Males	5	2	2	9
			Total					19
	St. Olav's Monastery, Oslo	Mixed		Females	5	2	2	9
				Males	5	4	3	12
			Total					21
	Prestgardskirken, Innlandet	Parish		Females	2	5	8	15
				Males	2	4	10	16
			Total					31
	Total MP			Females	13	14	15	42
				Males	17	12	18	47
			Total					89
Total Holck (2007)				Females	24	18	17	59
				Males	24	24	30	78
								137
TOTAL STUDY SAMPLE				Females	54	47	51	152
				Males	75	71	61	207
			Total					359[2]

[1]Overlapping burials excluded after cross-calibration.
[2]25 burials excluded after DXA-analysis.

3.2.3. DXA analysis inclusion criteria

The following criteria were required to be included in the DXA analysis. 1) All individuals should have a known dating and context. 2) Remains from adult individuals only (>20 years old). 3) All skeletal remains should permit unambiguous sex and age estimation as well as an estimation of stature. 4) At least one femur from each individual should be present to permit DXA measurement. 5) The measured femora should be complete/approximately complete. 6) The external surface of the femur neck (*compacta*) should be preserved and intact. 7) The femora should not portray any pathologies known to influence BMD.

3.2.4. Validation of precision of DXA analyses

Each femur in the DXA sample was scanned three times in order to assess precision. According to the manufacturer of the Lunar iDXA, expected precision error for repeated measurement of femur BMD $\leq$ 1.0% (% CV), or $\leq$ 0.010 g/cm^2 (GE, 2014). Observed precision error was 0.7% or 0.007 g/cm^2.

3.3. Compatibility of measurements and cross-calibration

Absolute BMD values obtained on equipment from different manufacturers cannot be directly compared because of technical differences (Boonen et al., 2003; Genant et al., 1994; Hui et al., 1997; Mays et al., 2006a). It is essential to compare data among instruments before data are combined for research, since differences in DXA models may affect results (Morrison et al., 2016). The ISCD (International Society for Clinical Densitometry) recommends undertaking cross-calibration before comparing BMD results (Shepherd et al., 2006). This is not recommended, however, when the mean difference is less than 0.02 g/cm^2 (GE, 2004). The BMD measurements obtained by the Lunar Prodigy and the Lunar iDXA are, according to Genant et al. (1994), approximately identical (1–2%, in vivo). The high correlation ($r > 0.999$) in femur neck BMD measurements between these densitometers is supported by a later study (Krueger et al., 2012). To assess the compliance of measurements between these two studies a control group was set up. Fifteen femora from the Church of St. Mary, previously scanned with the Lunar Prodigy, were scanned with the Lunar iDXA.

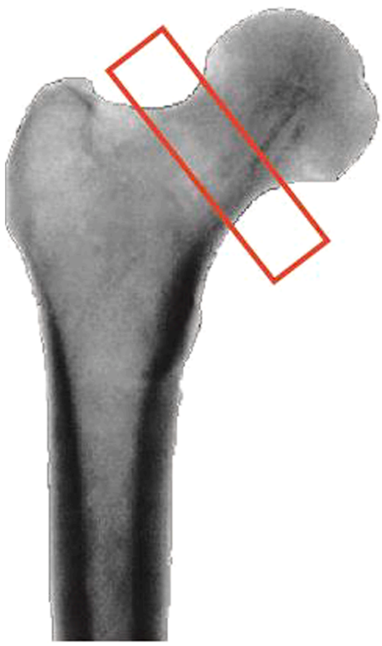

Fig. 4. Region of interest (ROI) in the proximal femur: the femur neck. Right femur, ventral aspect: collum femoris.

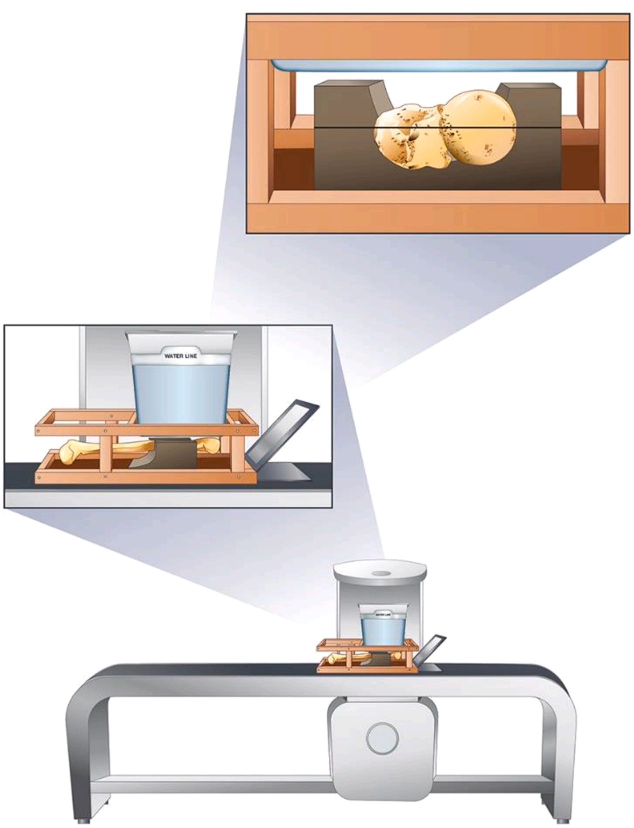

Fig. 5. Orientation of the proximal femur (top) and standardization of the femur diaphysis (middle and bottom).

3.4. Statistical analysis

A multivariate linear regression model was fitted to the complete dataset, with mean BMD as the dependent variable and time period, age group, age group, sex and socioeconomic status as independent variables. In this model, the effect on mean BMD is estimated simultaneously for all independent variables in the model. The interpretation of the model is thus that the estimated effect of e.g. the medieval period is the increase or decrease in mean BMD (g/cm2) compared to the Late Iron Age when the other explanatory variables remains constant, i.e. for the same sex, age and socioeconomic status. To compare the mean BMD between time periods for specific age groups for females or males or between age groups of females or males within a time period, two-sample *t*-test were used. One-sample *t*-test was applied to compare the mean BMD levels from the Late Iron Age, medieval and post-Reformation period samples to the modern reference values for each sex and age group (calculated from the manufacturer's reference values for USA/Northern Europe (GE, 2014). When performing many statistical tests, the problem of multiple testing needs to be addressed. We applied the Benjamini-Hochberg procedure to control the false discovery rate at the 10% level, and thus comparisons with an FDR q-value less than 0.10 were considered significant. The statistical analysis was performed using R (R Core Team, 2014).

4. Results

4.1. Osteology

Applying our criteria for inclusion (see Methods, section 3.2.3) resulted in a final study sample of 222 individuals (93 females and 129 males) distributed among the different burial sites and time periods. The demographic data for the skeletal material we examined with DXA are shown in the first half of Table 1.

4.2. Dual-energy X-Ray absorptiometry

4.2.1. Cross-calibration

Assessment of BMD data in the control group (15 femora) and comparison of data from the two DXA analyses (Lunar Prodigy versus Lunar iDXA) showed that the BMD measurements given by Holck (2007) were somewhat higher than in the present study, with a mean difference of 0.0233 g/cm^2 (SD 0.023), using a Bland-Altman plot (Fig. 6). The BMD values previously obtained (Lunar Prodigy) were adjusted by subtracting 0.0233 g/cm^2 to reduce any systematic differences in average BMD measurements between scanners.

4.3. Total skeletal sample

Demographic data of the combined skeletal material from the Late Iron Age, medieval period and post-Reformation period are shown in Table 1, presenting 359 individuals (152 females and 207 males). Twenty-five individuals were excluded from further study due to the possibility that measured BMD could be affected by underlying bone pathology (including systemic diseases) and/or trauma to weight-bearing bones.

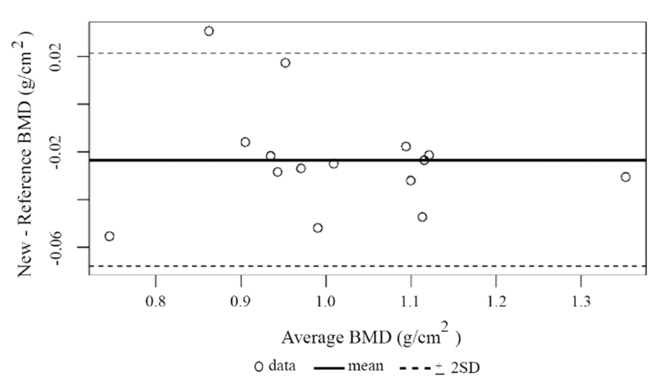

Fig. 6. Bland-Altman comparison of densitometers.

4.3.1. BMD in archaeological periods

To examine BMD from a long-term perspective, we calculated the femur neck mean BMD values for both sexes and all age categories for each time period (Fig. 7 A-B and Table 2). The results of the statistical analyses are shown in Tables 3 and 4. The mean BMD in the medieval period was significantly higher than in both the Late Iron Age and the post-Reformation period. The estimated effects in the multiple linear regression models (Table 3) show that the increase in mean BMD from the Late Iron Age to the medieval period is 0.152 g/cm^2 ($p = 0.0002$), and the decline from the medieval period to the post-Reformation period is −0.105 g/cm^2 ($p = 0.014$). Males had a mean BMD value that was 0.102 g/cm^2 higher than that of females. As shown in Table 4, young adult females in the medieval period had significantly higher mean BMD values than young adult females in the Late Iron Age ($p = 0.02$, q-value 0.093; two-sample *t*-test, Benjamini-Hochberg procedure). No other significant differences were found between young adult females or males between the different time periods. Non-similar socioeconomic status had a significant impact on the mean BMD (Table 4); the parish population had significantly lower BMD than individuals of high socioeconomic status ($p = 0.032$). This association is further explored by the authors (Brødholt et al., 2021). We have estimated the occurrence of osteopenia and osteoporosis in the various time period, although not subject to further discussion in this study. In the Late Iron Age, 31.6% of the females display osteopenia, while 5.3% display osteoporosis. 50% of the males from this period display osteopenia, while 13.6% display osteoporosis. In the medieval period, 35.6% of the females display osteopenia, while 10.9% display osteoporosis. 29.4% of the males from this period display osteopenia, while only 1.6% display osteoporosis. In the post- Reformation period, 35% of the females display osteopenia, while neither of these display osteoporosis. 30.8% of the males from this period display osteopenia, while 3.8% display osteoporosis.

4.3.2. BMD in archaeological periods compared to the modern reference population

Compared to modern reference levels (Table 4) middle adult males in the Late Iron Age had lower mean BMD ($p = 0.01$, q-value 0.12; one sample *t*-test, Benjamini-Hochberg procedure), young adult females in the medieval period had higher mean BMD ($p = 0.02$, q-value 0.13) and old adult males in the post-Reformation period had lower mean BMD ($p = 0.01$, q-value 0.12). However, none of these results was significant after adjusting for multiple testing.

4.3.3. Age-related BMD variations in the archaeological periods

Calculated mean BMD values for females in each time period are shown in Fig. 7A, and corresponding values for males in Fig. 7B, in addition to an overview in Table 2. The changes are given as percent of the mean BMD in the Young Adult age category. The mean BMD was significantly lower in middle and old adulthood compared to young adulthood, with estimated differences of 0.10 and 0.16 g/cm^2, respectively. In the Late Iron Age, females displayed a marked, but not significant reduction in mean BMD from young to middle adulthood (from 0.936 to 0.825 g/cm^2). The mean BMD appeared to be increasing slightly from middle to old adulthood. The number of individuals here is low and therefore provides uncertain basis for comparison. As shown in Table 4, the Late Iron Age males displayed a significant reduction (from 1.099 to 0.905 g/cm^2) in mean BMD from young to middle adulthood ($p = 0.03$, q-value 0.09; two-sample *t*-test, Benjamini-Hochberg procedure). The mean BMD appeared to be increasing slightly from middle to old adulthood. In the medieval period, females showed significant early bone loss (from 1.051 to 0.901 g/cm^2), $p = 0.0014$, q-value 0.025. The mean BMD was reduced to 0.830 g/cm^2 by old adulthood, a non-significant reduction. The age-related bone loss in men in the medieval period was significant both early and late in life ($p = 0.02$, q-value 0.09 and $p = 0.017$, q-value 0.09 respectively). Females in the post-Reformation period showed minimal early bone loss (from 0.991 to 0.967 g/cm^2) and marked late bone loss (0.967 to 0.816 g/cm^2). However, neither of these losses was significant. Men in the post-Reformation period displayed little early bone loss (from 1.057 to 0.985 g/cm^2) and significant late bone loss (from 0.985 to 0.856 g/cm^2; $p = 0.03$, q-value 0.09).

4.3.4. BMD variations in archaeological compared to modern populations

In the Late Iron Age, the mean BMD value for middle adult females had declined to 88% of that for young females, clearly lower than that reported for modern females (96%). Mean BMD for older females was 89%, a value not very different from that reported for modern females (86%). Mean BMD for middle-aged males in the Late Iron Age was reduced to 82% of the value in the young adult group, a decline much greater than that reported for modern men (94%). For older men, mean BMD was 85%, a bone mass reduction similar to that observed in modern men (87%). In the medieval period, BMD for middle-aged females was only 86% of that for young females, much lower than observed in modern females (96%). Mean BMD for older females had declined to 79%, considerably less than that observed in modern females (86%). For middle-aged men from this period, mean BMD declined to 93%, while older males presented a BMD of 86% compared to young males. The corresponding values for modern men are 94% and 87%, respectively, indicating a quite similar pattern of bone loss in these two groups. In the post-Reformation period, mean BMD for middle-aged females fell to 98%, little different from that observed in modern women (96%). The mean BMD value for older females in this period was 82%, moderately lower than that reported for modern women (86%). Mean BMD values for middle-aged men from the post-Reformation period fell to 93%, quite similar to that of the modern reference group (94%). For older men, the mean BMD value was 81%, somewhat less than that observed in the modern group (87%).

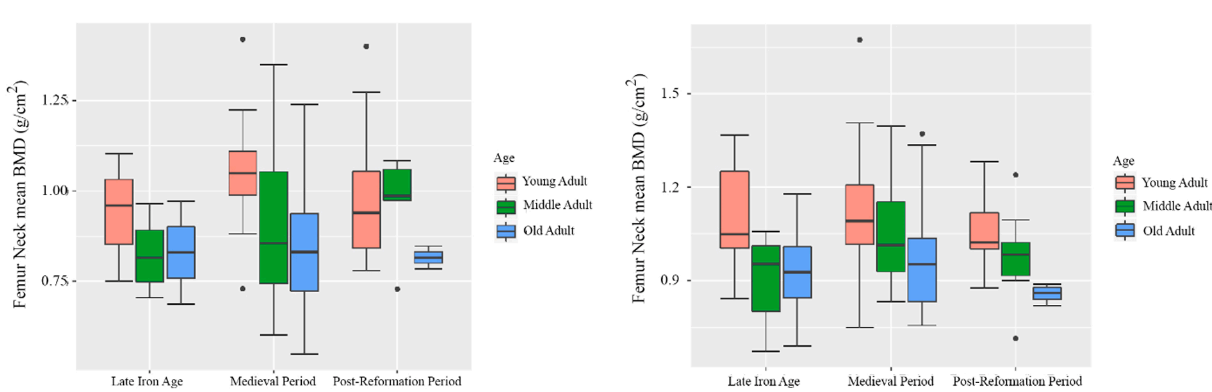

Fig. 7. A. Femur Neck BMD for females in the examined time periods. B. Femur Neck BMD for males in the examined time periods.

Table 2
Femur neck mean BMD values and SD for each sex, age group and time period. BMD values given in percent of the value in the Young Adult group.

		LIA				MP				PRP				M	
		n	BMD g/cm^2	SD	%	n	BMD g/cm^2	SD	%	n	BMD g/cm^2	SD	%	BMD g/cm^2	%
F	Young Adult	11	0.936	0.12	100	26	1.051	0.14	100	12	0.991	0.19	100	0.985	100
	Middle Adult	4	0.825	0.12	88	31	0.901	0.20	86	5	0.967	0.14	98	0.943	96
	Old Adult	2	0.830	0.20	89	44	0.830	0.15	79	2	0.816	0.04	82	0.843	86
M	Young Adult	7	1.099	0.19	100	49	1.116	0.17	100	9	1.057	0.13	100	1.080	100
	Middle Adult	12	0.905	0.13	82	38	1.039	0.14	93	9	0.985	0.14	93	1.020	94
	Old Adult	12	0.929	0.14	85	39	0.959	0.15	86	4	0.856	0.03	81	0.940	87

F= females, M= males. LIA= Late Iron Age, MP= Medieval period, PRP= post-Reformation period, M= modern.

Table 3
Results of the multivariate regression model. Significant p-values in red.

	Estimated effect	p-value
Intercept	0.897	1.60E-51
Age 42.5	-0.102	1.83E-06
Age 50	-0.163	2.27E-13
Male	0.102	1.52E-08
Medieval period*	0.152	1.65E-04
Post-Reformation period*	0.047	4.19E-01
Socio.ec. status Mixed	0.057	1.44E-01
Socio.ec. status Parish	-0.053	3.17E-02
Socio.ec. status Low	0.068	1.61E-01
Post-Reformation period**	-0.105	1.39E-02

*Estimated effect with Late Iron Age as reference level.
**Estimated effect in refitted model with Medieval period as reference level.

5. Discussion

This study, combining DXA analysis of skeletal remains and osteological analyses, spans ca 1300 years, from the sixth to nineteenth century AD in Norway. To our knowledge, it represents the most extensive study of its kind and provides a number of new insights, for the first time mapping long-term trends in BMD variations from the Late Iron Age to the post-Reformation period.

5.1. Temporal variations in BMD

In this study, the mean BMD increased significantly from the Late Iron Age to the Medieval Period, followed by a significant decrease from the Medieval Period to the Post-Reformation Period. Although direct comparisons between the various examinations undertaken are somewhat hampered by different methodological and analytical approaches, our results supports uniformly the notion that BMD has varied considerably through prehistoric and historic time periods in Norway. We interpret this variation as the result of the interplay of complex and exogenous variables influencing bone mineral density in the specific populations. Temporal BMD variations in Scandinavia has been examined in two previous studies (Bennike and Bohr, 1990; Holck, 2007); however, the pattern of BMD variation are variable. Holck (2007) found no significant differences in femur neck mean BMD between the prehistoric, Viking Age and medieval material in Norway ($p = 0.151$, one-way ANOVA). The results were interpreted to indicate similar physical strains experienced in these time periods. Only the medieval bones showed a significantly higher mean BMD than the modern reference population ($p = 0.001$, one-sample *t*-test). Bennike and Bohr (1990), however, found the highest values in the Neolithic and the lowest in the medieval period measuring BMC of the femur diaphysis in a Danish skeletal cohort spanning from 4200 BCE to 1536 CE. Compared with modern autopsy cases, the BMC values in these time periods were significantly higher and lower, respectively. Possible explanations for these findings were not further discussed by the authors, but were later interpreted by Poulsen, et al. (2001, pp. 456) as lack of support for the hypothesis of "a consistent millennial trend toward lower BMD in the Scandinavian population".

The current study revealed that only young adult females in the Medieval period had higher mean BMD ($p = 0.02$, q-value 0.13) than the modern population, while middle adult males in the Late Iron Age and old adult males in the Post-Reformation Period had lower mean BMD ($p = 0.01$, q-value 0.12 and $p = 0.01$, q-value 0.12, respectively). The results may be representative, but were not significant after adjusting for multiple testing. The few other studies on Scandinavian skeletal material measured BMD in a single time period, specifically the medieval period, and compared these values to the contemporary population. The overall results pointed to a higher BMD in medieval men than in modern men, while the medieval women displayed a more diverse pattern compared to their modern counterparts. Poulsen et al. (2001) examined the remains from a Danish Christian cemetery (1000–1250 CE) and detected significantly higher BMD in medieval men ($p = 0.02$, two-sided *t*-test) of all age categories when compared with the contemporary Danish population. Medieval women displayed a significantly lower BMD ($p = 0.04$) than their modern counterparts, but this relationship was reversed for women who survived to older ages. The difference observed between the men was explained by men's higher level of physical activity in the medieval period, while the difference between women was explained by fertility-related factors. Ekenman et al. (1995) detected slightly higher bone density at the femur diaphysis in men in their skeletal material from medieval Stockholm, Sweden (1300–1530 CE) compared to present-day Stockholm, but detected no difference in women. For the medieval men, the higher values were interpreted as being caused by daily physical activity, which included frequent standing and walking. The lack of information regarding socioeconomic status in this population makes it difficult to draw comparative conclusions in relation to the Norwegian findings.

5.2. Temporal age-related bone mass variations

Our results showed that the patterns of age-related BMD variations in the archaeological periods were diverse and indicated substantial change with time. The overall pattern showed that the Late Iron Age was characterised by marked early bone loss for both sexes (although only significant for the males). In the medieval period significant early bone loss occurred in both sexes, while significant late bone loss occurred in males. The post-Reformation period was characterised by marked late bone loss for both sexes, but this was only significant for males. The temporal pattern and variation observed in this study lack parallels in previous research on archaeological populations, rendering it challenging to contextualize our results. Osteoporosis and age-related fractures are closely associated and research has shown that low BMD heightens the risk of almost all types of fractures (Cooper et al., 2011). Fracture rates are positively correlated with socioeconomic status, level of education and health (Cauley et al., 2014) and presumably linked to the (adoption of a) Western industrial lifestyle (Rosengren et al., 2017).

Table 4
Results of two sample and one sample t-tests. Benjamini-Hochberg procedure applied. Comparisons with an FDR q-value less than 0.10 considered significant. Significant p- and q-values in green and red respectively.

	t-value	p-value	FDR q-value
Two sample t-test			
Young adult females LIA vs Young adult females MP	-2.520	0.020	0.093
Young adult females LIA vs Young adult females PRP	-0.830	0.419	0.58
Young adult females MP vs Young adult females PRP	0.980	0.342	0.513
Young adult males LIA vs Young adult males MP	-0.080	0.936	0.979
Young adult males LIA vs Young adult males PRP	0.630	0.542	0.697
Young adult males MP vs Young adult males PRP	1.200	0.253	0.455
Two sample t-test			
LATE IRON AGE			
Young adult females vs Middle adult females	1.620	0.160	0.320
Middle adult females vs Old adult females	-0.030	0.979	0.979
Young adult males vs Middle adult males	2.530	0.031	0.093
Middle adult males vs Old adult males	-0.420	0.677	0.812
MEDIEVAL PERIOD			
Young adult females vs Middle adult females	3.370	0.0014	0.025
Middle adult females vs Old adult females	1.680	0.098	0.220
Young adult males vs Middle adult males	2.360	0.021	0.093
Middle adult males vs Old adult males	2.440	0.017	0.093
POST-REFORMATION PERIOD			
Young adult females vs Middle adult females	0.290	0.779	0.876
Middle adult females vs Old adult females	2.140	0.086	0.220
Young adult males vs Middle adult males	1.120	0.278	0.455
Middle adult males vs Old adult males	2.560	0.029	0.093
One sample t-test			
MEDIEVAL PERIOD vs. MODERN			
Young adult females vs 0,985	2.470	0.021	0.126
Middle adult females vs 0,943	-1.180	0.249	0.640
Old adult females vs 0,843	-0.570	0.574	0.849
Young adult males vs 1,080	1.510	0.138	0.497
Middle adult males vs 1,020	0.880	0.384	0.849
Old adult males vs 0,940	0.800	0.428	0.849
LATE IRON AGE vs. MODERN			
Young adult females vs 0,985	-1.330	0.213	0.639
Middle adult females vs 0,943	-2.050	0.133	0.497
Old adult females vs 0,843	-0.090	0.941	0.941
Young adult males vs 1,080	0.420	0.689	0.874
Middle adult males vs 1,020	-2.970	0.013	0.117
Old adult males vs 0,940	-0.290	0.777	0.874
POST-REFORMATION vs. MODERN			
Young adult females vs 0,985	0.100	0.919	0.941
Middle adult females vs 0,943	0.370	0.728	0.874
Old adult females vs 0,843	-0.860	0.548	0.849
Young adult males vs 1,080	-0.530	0.613	0.849
Middle adult males vs 1,020	-0.730	0.486	0.849
Old adult males vs 0,940	-5.360	0.013	0.117

Research on hip fractures in Norway and Sweden has demonstrated a secular increase in fracture rates in both sexes since the 1960 s (Falch, et al., 1993; Naessen, et al., 1989; Turner-Walker, et al., 2001b). We interpret our results to indicate that the observed pattern of age-related bone loss shifts towards a more modern pattern and we hypothesize that this transition is related to changes in societal and economic conditions, such as improved living conditions, increased life expectancy and altered reproductive pattern.

For women in the Late Iron Age and medieval period, the age-related BMD variations portrayed a pattern of distinct early bone loss, exceeding that observed in the modern reference population. Such pre- or *peri*-menopausal bone loss as evident in these time periods are observed and discussed in several previous studies (Agarwal and Grynpas, 1996; Curate, 2014; Mays, 2008). Research involving skeletal material from medieval Norway (Mays et al., 2006a; Turner-Walker et al., 2000b, 2001) has led to the hypothesis that age-related bone loss in women started earlier than today, and the early bone loss was seen in connection with a different practice regarding childbearing (start of onset), with high parity and late weaning compared to modern populations. Low BMD in young females in archaeological populations has been interpreted as the result of the stresses of pregnancy and lactation coupled with insufficient nutrition (Holck, 2007; Mays, et al., 2006b; Poulsen, et al., 2001; Turner-Walker, et al., 2001b). In a recent DXA-study of 78 young women (maternal deaths vs. other causes of death and married/widowed women vs. single women) from the Coimbra Identified Skeletal Collection (20th century), Curate and Tavares (2018) advised against a strict reproductive interpretation of early bone loss.

The pattern of late rather than early bone loss in women in the post-Reformation period in this study, similar to that observed in present-day European populations, might indicate a different practice regarding childbearing and lactation in this time period, perhaps coupled with other societal changes. Such postmenopausal bone loss, similar to (or even greater than) the one observed in modern populations has been observed in a number of studies, albeit none involving skeletal material from Norway. The examination of a Spitalfields sample, UK, dated to 1729–1852 CE (Lees, et al., 1993), revealed little premenopausal but significant postmenopausal bone loss in women. It was hypothesized that physical activity and the effect of parity in conserving bone density could be plausible explanations for the observed pattern, while dietary factors alone, were deemed an unlikely explanation. Late rather than early bone loss in women is not a recent trait, as it has been observed in skeletal populations of far greater age than our study population from the post-Reformation period: in a multi-method study of material from the Early Bronze Age (4000 BP) in Austria (Kneissel, et al., 1994), in a 3rd-4th century CE population from Ancaster, UK (Mays, 2006) and in a Merovingian population (5th-7th century CE) from Bockenheim, Germany (Hammerl, et al., 1990).

The pattern of age-related bone loss in males in the examined archaeological periods was somewhat different from the pattern for women and equally indicative of temporal changes. The significant early bone loss occurring in men in the Late Iron Age, which far exceeds that observed in modern populations, is remarkable. This is a pattern that lacks parallel in the archaeological record and in the modern population, and is difficult to explain without further examination of risk factors in this population. The presence of marked early reduced BMD in both sexes during this time period (significant in males), may indicate that the same factor(s) influencing bone mass affected both sexes in the Late Iron Age society. This pattern of early bone loss may partially be correlated with the shorter life expectancy in this society, which resulted in few individuals reaching advanced old age (Mays, et al., 1998; Mays, 1996). Arduous work, poor (childhood) nutrition and generally hard living conditions could be other possible explanatory factors.

Males in the medieval and post-Reformation period displayed a pattern of age-related bone loss similar to the modern population, characterized by significant late bone loss. The significant late bone loss in men at medieval Wharram Percy (Mays, et al., 1998), which was similar to or even exceeded the bone loss in modern subjects, was taken to support the hypothesis that lifestyle factors may be less important than previously thought. In addition to late bone loss, the medieval men in our study also showed significant early bone loss, which is an unprecedented finding. We interpret these patterns to indicate that multiple factors, some of which we have not identified, may have influenced BMD and bone loss in these later periods, compared to the Late Iron Age society. Since the factors involved are multiple, complex and often symbiotic, simplistic and unicausal explanations should be avoided, *sensu* Weaver (1998).

5.3. Limitations of the study

Remains obtained from the different burial sites were heterogeneous with regard to sample size and selection, therefore rendering it challenging to compare the different sites. Our interpretations and conclusions are therefore tentative. The limited number of skeletal remains from certain time periods (e.g. the Late Iron Age) and varying degrees of preservation of bone has posed a challenge with regard to the statistical analysis. We have tried to partly account for post depositional changes to the bones by strict DXA analysis inclusion criteria; the femora should be complete/approximately complete and the external surface preserved and intact. Fragmented remains with visible soil intrusion were excluded from the DXA analysis. The lack of relevant information from few previous research studies and data on BMD in consecutive archaeological populations made it difficult to put the results in context. We regard this study as a first mapping of long-term trends and patterns of BMD changes in Norway presenting possible directions for future research.

There are many diagenetic processes that potentially could affect bone in the soil, such as soil environment and post depositional time (López-Costas et al., 2016) and thereby the (measurement of) bone mineral density. Soil rich in calcium minerals enhances the preservation of skeletal remains. Although soil conditions often vary between sites, accumulation of skeletons from inhumations in a cemetery often result in well preserved skeletons, even when the surrounding soil may be acidic, since the ground water within the cemetery becomes practically saturated with dissoluble bone minerals. This explains why skeletal remains from medieval graveyards often are well preserved (Sjøvold, 1982). Research by Turner-Walker et al. (2000b) demonstrated that post mortem changes of bone in two medieval populations (considerable microbial reworking vs. perfect preservation) did not influence BMD measurements significantly. Similarly, research on mobilisation of bone apatite and redistribution of mineral in skeletal remains has indicated that the measured BMD are close representations of those found in vivo (Turner-Walker et al., 2000a).

The R^2 value of the fitted linear regression model is 0.28, which suggests that far from all variability is captured by the significant explanatory variables in the model. This could be due to unknown sample variations, preanalytical and/or measurement errors in the mean BMD values. In addition, the interplay of explanatory variables not included in the model, may have affected the results.

6. Conclusion

The present DXA analysis of skeletal remains spanning from the sixth to nineteenth century in Norway resulted in a number of new insights. This work constitutes a major step in mapping long-term trends and patterns of BMD changes from the Late Iron Age to the post-Reformation period, and the data presented here indicate considerable variation. The pattern of age-related bone loss revealed substantial temporal changes and suggested a transition towards a modern pattern. The overall results demonstrated that the age-related bone loss in these prehistoric and historic periods was no less, but often exceeded, comparable bone loss in populations today. There were, however, some marked differences occurring between the sexes and the various time periods. A future avenue to explore is how the temporal bone mass variations and patterns of age- and sex-related bone loss in these archaeological populations can be attributed to characteristics of and changes in these societies. These complex questions require further investigation in a broader context, considering osteological, paleopathological, archaeological and historical data.

Funding Sources

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CRediT authorship contribution statement

Elin T. Brødholt: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Visualization. **Clara-Cecilie Günther:** Validation, Formal analysis, Investigation, Writing - original draft, Writing - review &

editing, Visualization. **Kaare M. Gautvik:** Supervision, Writing - original draft, Writing - review & editing. **Torstein Sjøvold:** Supervision, Writing - review & editing. **Per Holck:** Supervision, Writing - review & editing.

Declaration of competing interest

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

Acknowledgements

We would like to acknowledge the contribution of the following: Department of Molecular Medicine, Institute of Basic Medical Sciences, UiO, for granting access to the skeletal material at The Schreiner Collection. Principal supervisor Trygve Brauns Leergaard for keeping an overview of the thesis work, contributing to article revisions as well as offering practical advice. The Department of Nutrition, Institute of Basic Medical Sciences, UiO, for granting access to the Lunar iDXA. Knut Rekdahl at the Mechanical Workshop, Institute of Basic Medical Sciences, UiO, for creating and adjusting the set up for DXA-scanning of dry bones; Pål Wangen at Scanex Medical Systems AS for advice in constructing and testing the set up for DXA-scanning, as well as answering questions related to the Lunar iDXA scanner. Anna Saither at Geological Survey of Norway (NGU) for providing us with data on fluoride and calcium concentrations in water as well as the distribution of calcareous and non-calcareous bedrock in Norway. We thank our reviewer for a thorough evaluation of the manuscript, insightful comments and constructive suggestions. We would also like to thank Carina V. S. Knudsen at the Section of Internal Service, IMB, UiO, for invaluable effort with article illustrations.

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