# D-lightful sunshine disrupted: Vitamin D deficiency as a method for the reconstruction of changes in sociocultural practices due to industrialisation in 17th - 19th century Netherlands

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# 6 Four possible cases of osteomalacia: The value of a multidisciplinary diagnostic approach

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## ABSTRACT

Rickets and residual rickets are often encountered in Dutch archeological skeletal samples. However, no archeological Dutch paleopathological case of adult osteomalacia has been described in literature to date. This paper describes the first four archeological Dutch paleopathological cases of osteomalacia and assesses the value of the various modalities (macroscopic assessment, radiology and histology) that may be used for diagnosis. The skeletal remains investigated originate from the Meerenberg psychiatric hospital cemetery in Bloemendaal, the Netherlands, and date from 1891 – 1936. The remains of 69 adult individuals were inspected for macroscopic lesions which may be associated with osteomalacia. In cases suspect for osteomalacia, complimentary radiological and histological

investigations (BSE-SEM and light microscopy) were performed. Macroscopically, four individuals presented with lesions (highly) suggestive of osteomalacia. Histological examination (both BSE-SEM and light microscopy) provided valuable information to come to an eventual diagnosis of osteomalacia in all four cases. Light microscopy proved to be an feasible alternative for BSE-SEM. The added value of radiological analyses was limited. The individuals identified were most likely patients in the psychiatric hospital, and the reason for their institutionalization and/or the regime in the institution may have played a role in the development of the osteomalacia observed.

## INTRODUCTION

Juveniles with rickets, and/or adults presenting with lesions indicative of residual rickets, are relatively often encountered in Dutch archeological skeletal samples (e.g. Baetsen, 2001; Janssen and Maat, 1999; Maat et al., 1998; Maat et al., 2002; Veselka et al., 2015). However, osteomalacia, a disorder closely related to rickets, is rarely described in paleopathology records. Although the number of archeological population studies describing osteomalacia steadily increased over the past two decades, with the majority coming from England (Brickley et al., 2007; Brickley and Ives, 2008; Ives and Brickley, 2014; Schamall et al., 2003a), no archeological Dutch case of osteomalacia has been described in literature to date.

Osteomalacia can be described as a metabolic bone disease characterized by impaired osteoid mineralization (Francis and Selby, 1997; Vigorita, 2008). Although several inherited and acquired disorders have been linked to the condition, the most common cause is a vitamin D deficiency, which results from a prolonged lack of exposure to sunlight, a diet deficient of vitamin D and/or intestinal malabsorption (Francis and Selby, 1997; Reginato and Coquia, 2003). Consequently, the prevalence of the condition is much dependent on cultural factors such as clothing, diet and socio-economic status, and environmental factors such as latitude and atmospheric pollution. It is therefore a valuable source of information when encountered in an archeological context (Brickley et al., 2007; Brickley and Ives, 2008; Reginato and Coquia, 2003).

The diagnosis of osteomalacia in paleopathology has increasingly been subject of interest during the past two decades (Brickley et al., 2005; Brickley et al., 2007; Ives and Brickley, 2014; Mensforth, 2002; Schamall et al., 2003b), but nevertheless remains challenging. This is at least partially due to the non-specificity of some of the features that may indicate osteomalacia in skeletal remains. The often-encountered fragility and fragmentation of the remains of those affected impedes the diagnosis even further (Brickley and Ives, 2008).

The diagnosis of osteomalacia in skeletal remains has long been mainly dependent upon the evaluation of gross macroscopic deformation of the ribs, vertebral column, pelvis and sternum. These skeletal elements have the

highest metabolic rate and are therefore suspected to be the most affected by the poor mineralization of newly deposited osteoid (Ortner, 2003). These macroscopic changes were supported by the radiological identification of (often elusive) pseudofractures, especially of the ribs, femoral neck, pubic rami and the lateral border of the scapulae (Ortner, 2003; Vigorita, 2008). Only recently attention has focused on the description of more subtle macroscopic lesions (Brickley et al., 2007; Ives and Brickley, 2014), and on the histological diagnosis of the condition. For the latter, diagnostic features, as may be observed in dry bone material, were linked to the histopathological manifestation of osteomalacia in modern specimens (Brickley et al., 2007; De Boer and Van der Merwe, 2016). These recent developments increased the awareness for osteomalacia in paleopathology and improved the chances of an accurate diagnosis in skeletal remains.

It has been well described that the prevalence of osteomalacia is substantially increased amongst institutionalized patients (Francis and Selby, 1997). Therefore, the excavation of one of the cemeteries of the Meerenberg psychiatric hospital in Bloemendaal, the Netherlands, dating from 1891 to 1936, provided an apt opportunity to search for skeletal evidence of osteomalacia in the Dutch archeological record.

The aim of this study was to describe the first four paleopathological cases of osteomalacia in the Netherlands and their historical context, and to assess the value of the various modalities (macroscopic assessment, radiology and histology) that may be used for diagnosis.

## HISTORICAL BACKGROUND

The skeletal remains that were investigated in this study originate from the Catholic cemetery of the Meerenberg psychiatric hospital in Bloemendaal, the Netherlands, which, according to historical documents, were in use between 1891 and 1936. The Meerenberg psychiatric hospital was opened in 1849 and was one of the first 'modern' provincial mental asylums in the Netherlands, with as its main purpose the treatment of impoverished psychiatric patients. By 1890, after several expansions, the asylum housed over a thousand patients and was continuously overcrowded. By the end of the 19th century, treatment of psychiatric patients had evolved from a moral and religious training approach to a more empirical treatment policy, which included the regular submission of (deceased) patients to scientific research in order to search for the neurological, physiological and anatomical grounds to the various psychiatric illnesses observed (Vijselaar, 1997). That such research was also performed on patients in Meerenberg was supported by the large number of skeletons presenting with traces of autopsy procedures such as craniotomies and opening of the rib cage and spinal canal.

During the initial operative years of Meerenberg, mental illness was generally regarded as a disease of the brain

with a strong predisposing hereditary component. As such, mental disease would frequently be seen as a part of the biological constitution of the patient. Consequently, patients were often institutionalized for an extensive period of time (Vijselaar, 1982; Vijselaar, 1997). Approximately 30% of the patients being treated suffered from so-called 'passing psychiatric illnesses' such as melancholy, mania, periodical insanity or neurosis and were admitted for an average of one to two years. The remaining 70% which allegedly suffered from conditions such as paranoia, dementia, epilepsy or idiotism, were regarded as incurable, and thus were rarely discharged (Vijselaar, 1982). Patients who died in the institution were buried in one of the cemeteries on the hospital grounds. There were two cemeteries on the Meerenberg grounds, a catholic- and jewish cemetery and a general cemetery. These cemeteries were mostly, but not exclusively, used for patient burials, as they were also used for the occasional interment of deceased staff members (Vijselaar, 1982; Van Twuyver, 2000).



## MATERIALS AND METHODS

The accidental disturbance of a section of the Catholic cemetery of the Meerenberg psychiatric hospital, due to property development, led to the excavation of 195 graves of which a large number were empty or extremely disturbed. The remainder contained mainly single interments. All available skeletal remains that were complete enough to allow for reliable evaluation were included in the study. Juveniles, individuals only represented by crania and commingled remains were excluded from further analysis.

The demographic information of a large number of the individuals included in this study has been previously estimated and where possible adopted from Hagg et al. (2017). In all instances sex was estimated using an aggregate of methods, i.e. the standards described by the Workshop of European Anthropologists (1980) and the methods described by Walker (2008) and Phenice (1969). Age at time of death was estimated by combining the outcomes of the assessment of degenerative changes of the pubic symphysis (Brooks and Suchey, 1990), the morphological changes of the sternal rib ends (İşcan et al., 1984a; İşcan et al., 1984b; İşcan et al., 1985) and the degree of ectocranial suture obliteration (Buikstra and Ubelaker, 1994). Individuals were divided into four age groups; young adults (20–35 years), middle adults (36–50 years) and old adults (50+ years) as described by Buikstra and Ubelaker (1994). The individuals for which age could not be estimated, due to incompleteness or fragmentation, were categorized as 'adult'. The total investigated skeletal sample consisted of 69 adult individuals of which 28 were male and 39 were female. In two individuals sex was indeterminate.

All individuals were analyzed for macroscopic lesions which may be associated with osteomalacia according to Brickley and Ives (2008) and Ives and

Brickley (2014), and allocated to one of three diagnostic groups. The first group consisted of individuals with lesions 'highly suggestive' for osteomalacia and included individuals with (a) unilateral or bilateral pseudofracture(s) of the inferior lateral margin of the scapular spine(s), healing rib (pseudo)fractures and/or (pseudo)fractures of the pelvis. The second group consisted of individuals with 'possible' osteomalacia and included individuals with lesions on the scapular spine in the absence of any of the other lesions described as 'strongly diagnostic' or 'diagnostic' (see Brickley and Ives (2008) for a description). Individuals without scapular lesions, but presenting with possible pseudofractures of the ribs, pelvis and/or femur in combination with some of the more 'general features' described by Brickley and Ives (2008), were also regarded as presenting with 'possible' osteomalacia. The third and last group consisted of individuals with no or only 'general' signs of pathology such as pitting and/or porosity of the cranial bones, ante mortem tooth loss, kyphosis or scoliosis and ventral angulation of the sacrum. As these lesions might be associated with osteomalacia, but also numerous other pathological conditions, they were regarded as non-diagnostic for osteomalacia. Only individuals with 'highly suggestive' or 'possible' osteomalacia were subjected to further radiological and histological analysis.

Radiological analysis consisted of conventional radiography of the scapulae, ulnae, radii, pelvis, ribs and proximal femur, based on availability of these skeletal elements. The radiographs were analyzed to verify the macroscopically observed pseudofractures and to identify pseudofractures that were not seen during the macroscopic assessment. Radiologically a pseudofracture (in radiology referred to as a 'Looser's zone') was defined as a radiolucent linear band, sometime bordered by a sclerotic margin, orientated perpendicular to the cortex, and not extending across the entire diameter of the bone shaft (Adams, 2005; Brickley et al., 2007; Pitt, 1996; Steinbach et al., 1954). The presence of these lesions is considered pathognomonic for defective bone mineralisation (Adams, 2005; Brickley et al., 2007). Various studies have shown that pseudofractures associated with osteomalacia often occur bilaterally and on specific locations on the abovementioned skeletal elements (Adams, 2005; Brickley et al., 2005; Brickley et al., 2007; Pitt, 1996; Steinbach et al., 1954). All elements were imaged in anteroposterior and medio-lateral direction. Scapulae were also imaged in a cranio-caudal direction. All radiographs were performed at the Academic Medical Centre, Amsterdam using an Oldelft Digital Imaging Triathlon DR. The parameters were manually set to 50 kV, with an exposure level of 8.0 mAs.

For the purpose of histological analysis, two bone samples, each of 5mm thickness, were taken from the anterior proximal femoral diaphysis (approximately 4 cm under the lesser trochanter), a midsagittal cross-section of a well preserved lumbar vertebral body

and a crosssection of a rib shaft. These locations were chosen to compare the observed histological features in skeletal elements with different metabolic rates.

For BSE-SEM, one of each set of two bone samples was imbedded in Araldite 20/20, cut transversely with a circular saw, ground to a thickness of 10 µm and polished with a 3 µm aluminum oxide abrasive. The thus created histological sections were subsequently vacuum coated with carbon and examined using a Zeiss Sigma 300 Field Emission Scanning Electron Microscope operated in a backscattered electron (SEM-BSE) mode working at 7 keV beam voltage. All sections were assessed for previously described histological features of osteomalacia, namely traces of extensive bone resorption, incomplete mineralization of newly formed bone, regions of rapidly formed immature bone, large osteocyte lacunae with poorly mineralized walls and defectively mineralized bone adjacent to cement lines (Brickley et al., 2007).

The use of BSE-SEM is rather costly and labor intensive. Light microscopy might pose a less expensive and more accessible alternative and therefore its potential value in the diagnostic process was evaluated. For this purpose, the remaining bone samples were prepared for light microscopy according to the method described by De Boer et al. (2013). Histological sections were embedded in LX-112 embedding resin and cut transversally into a 2mm thick slide. One side of the section was hand ground, polished and mounted on a glass slide, also using the LX-112 embedding resin. The other side of the section was subsequently hand ground and polished until the section had a thickness of approximately 50–80 µm. After staining of the section with haematoxylin it was covered with a glass slip, again using the LX-112 embedding resin.

The sections were analyzed under plain and polarized light, with emphasis on the same diagnostic features as described for the BSE-SEM assessment.

## RESULTS

Four individuals presented with macroscopic lesions either 'highly suggestive' for, or 'possible' osteomalacia, and were included for further radiological and histological analysis. The remains of all four individuals were very fragile and substantially fragmented. A summary of the macroscopic, radiological and histological findings linked to osteomalacia in these four individuals can be found in Table 1.

### *Macroscopic observations*

MeB S2039 V084 (individual 1) consisted of the relatively complete but extremely fragile and lightweight skeletal remains of an old-adult female. The cranium had a cardboard-like consistency and had been subjected to craniotomy. All but two dental elements were lost antemortem. Multiple complete, well-healed rib fractures were observed (seven on the right, and two on the left side) and multiple ribs showed lateral straightening (Figure 1a) of their midsections. A possible

**Table 1.** A summary of the macroscopical, radiological and histological findings possibly associated with osteomalacia as observed in four individuals from the Meerenberg collection.[a]

| | Individual 1 | Individual 2 | Individual 3 | Individual 4 |
|---|---|---|---|---|
| Sex | Female | Female | Female | Female |
| Estimated age | Old-Adult | Adult | Adult | Middle-Adult |
| Evidence of autopsy | Yes | Yes | Yes | – |
| Macroscopic features | | | | |
| **Cranium** | | | | |
| Low weight | Yes | Yes | Yes | – |
| Cardboard like consistency | Yes | Yes | No | – |
| AMTL | Yes | Yes | Yes | – |
| **Scapulae** | | | | |
| Pseudofracture scapular spine with spicule formation | Yes (L)(U)[b] | Yes (B) | No | Yes (R)[b] |
| Pseudofracture scapular spine without bone formation | No | No | Yes (L)(U)[c] | No |
| **Ribs** | | | | |
| Healed fractures | Yes (B)[b] | – | Yes (B)[b] | No |
| Pseudofracture with bone spicules | No | – | Yes (B) | No |
| Lateral straightening | Yes (B) | – | – | – |
| **Long bones** | | | | |
| Poorly mineralized bone accumulations ulna | No | Yes (B) | No | Yes (B) |
| Poorly mineralized bone accumulations femur | No | No | No | Yes (B) |
| **Pelvis** | | | | |
| Pseudofracure inferior ischiopubic ramus | No | No | – | Yes (R) |
| Peudofracture anterior inferior iliac spine | Yes (L)[b] | No | No | No |
| **Radiological features** | | | | |
| **Ribs** | | | | |
| Healed fractures | Yes (B) | – | Yes (B) | – |
| Pseudofracture | No | – | Yes (B) | – |
| **Pelvis** | | | | |
| Peudofracture anterior inferior iliac spine | Yes (L) | No | No | No |
| **Scapulae** | | | | |
| Pseudofracture scapular spine | Yes (L) | – | No | Yes (R) |
| **Long bones** | | | | |
| Healed fracture distal ulna | Yes (R) | No | No | No |
| **BSE-SEM and light microscopy features** | | | | |
| Increased Howships lacunae | Yes (F) | Yes (F, V) | Yes (R) | Yes (F, Ri, V) |
| Islands of poorly mineralized bone | Yes (Ri, V) | Yes (Ri, V) | Yes (Ri, V) | Yes (Ri, V) |
| Incomplete mineralization of layers of bone | Yes (Ri, V) | Yes (Ri, V) | Yes (Ri, V) | Yes (Ri, V) |
| Enlarged osteocyte lacunae | Yes (F, Ri, V) | Yes (F, Ri, V) | Yes (Ri, V) | Yes (F, Ri, V) |
| Defectively mineralized bone adjacent to cement lines | Yes (F, Ri, V) | Yes (F, Ri, V) | Yes (F, Ri, V) | Yes (F, Ri, V) |

Individual 1 – MeB S2039 V084, Individual 2 – MeB S2034 V138, Individual 3 – MeB S2037 V148, Individual 4 – MeB S1047 V217. Only lesions/characteristic observed in one or more of the four individuals investigated are mention. AMTL – ante mortem tooth loss, L – left, R – right, B – bilateral, U – unilateral, F – femoral sample, Ri–rib sample, V–vertebral sample. 'Hyphen' denotes that a lesion was unobservable. 'No' refers to no pathological lesions present. 'Yes' refers to pathological lesions present.

[a] List of lesions possibly associated with osteomalacia as described by Brickley and Ives (2008).
[b] Fracture confirmed during radiological assessment.
[c] Fracture could not be confirmed during radiological assessment.

healed pseudofracture was observed on the ischial spine of the left os coxa. A second possible pseudofracture was observed on the anterior inferior iliac spine of the same skeletal element. This fracture presented with spiculated new bone formation along its margins but was damaged postmortem resulting in the fracture being extended further into the ilium (Figure 1b). A pseudofracture with spiculated new bone formation was present on the inferior lateral margin of the left scapular spine (Figure 1b), the scapular spine on the right was not affected. Apart from these lesions that may be associated with osteomalacia, individual 1 also presented with a healed fracture of the distal right ulna and a healed fracture of the left femoral neck with pseudoarthrosis and exostosis

development. It is plausible that the possible healed pseudofracture of the left ischial spine may be related to the hip fracture. Even though healing has occurred, which may suggest that the lesions are not associated with the 'active' osteomalacia being investigated, it cannot be excluded that the trauma observed may be related to prior episodes of the condition. There was severe vertebral osteoarthritis in the vertebral column with ankyloses of the facet joint of C2 and C3, whilst advanced degenerative disc disease resulted in ankyloses of the 5th and 6th, and the 7th and 8th thoracic vertebral bodies.

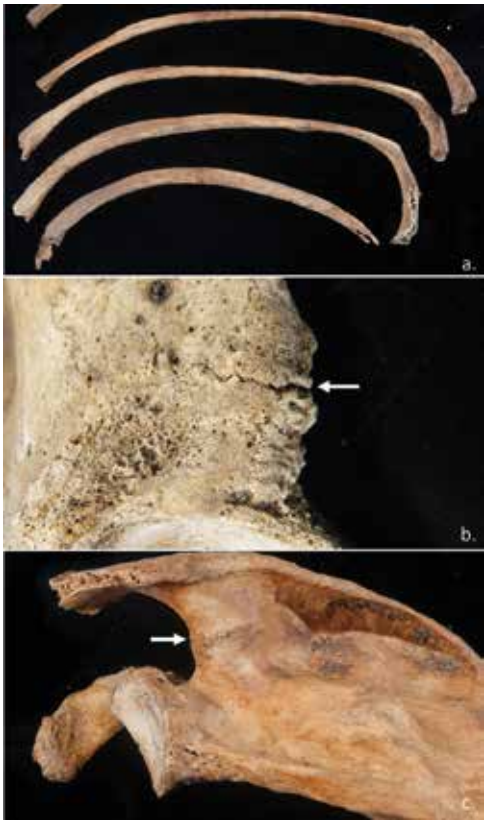


**Figure 1.** A composite figure illustrating some of the possible osteomalacia related lesions observed in Individual 1 (MeB S2039 V084). a. Lateral straightening of the ribs and several healed rib fractures. b. A possible pseudofracture of the left anterior inferior iliac spine (arrow). c. A pseudofracture of the lateral inferior border of the left scapular spine (arrow).

The skeletal remains of MeB S2034 V138 (individual 2) consisted of the extremely fragmented and incomplete skeletal remains of an adult female. The cranium had been subjected to craniotomy, and was very light weight with thinning of the left parietal bone, suggestive of osteoporosis. All teeth were lost antemortem. The vertebrae, sacrum, sternum and ribs were too incomplete and fragmented for reliable assessment. Both ulnae showed areas of poorly mineralized bone accumulations on their proximal surface. Both scapulae showed possible pseudofractures of the spine which were completely fractured post mortem. The fracture margins on the lateral inferior border of the scapular spine were rounded off and subtle traces of reactive bone growth could be observed alongside its edges. It should be stressed that the fragility and fragmentation of the skeletal elements severely hampered the diagnostic process. Apart from lesions that might be associated with osteomalacia, a well-healed fracture of the left ischial tuberosity was observed.

MeB S2037 V148 (individual 3) consisted of the fragmented but relatively complete skeletal remains of an adult female. The skull, vertebrae and ribs showed signs of autopsy, namely craniotomy and opening of the rib cage. All teeth were lost antemortem. Four rib fragments showed linear ridges of irregular spiculated bone formations suggestive of pseudofractures, eight rib

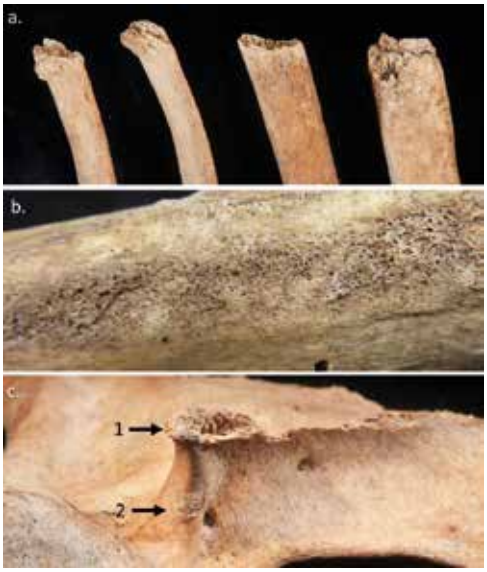


**Figure 2.** A composite figure illustrating some of the observed lesions possibly related to osteomalacia. a. Complete rib fractures presenting with signs of healing at the fractured ends in Individual 3 (MeB S2037 V148). b. Bone accumulations as observed bilaterally on the proximal femora, and (c.) postmortem damage (arrow 1) and a pseudofracture (arrow 2) of the lateral inferior border of the left scapular spine in Individual 4 (MeB S1047 V217).

fragments showed complete fractures with signs of healing (Figure 2a). A possible pseudofracture was present on the inferior lateral margin of the spine of the left scapula. The right scapula was too fragmented for reliable assessment. Apart from the lesions that may be associated with osteomalacia, advanced stages of degenerative disc disease and vertebral osteoarthritis were present in all vertebral regions.

MeB S1047 V217 (individual 4) consisted of the incomplete and fragmented postcranial skeletal remains of a middle-adult female. A possible healed pseudofracture was present on the inferior ischiopubic ramus of the right os coxa. Areas of poorly mineralized bone accumulations were present on the proximal femur, bilaterally on the intertrochanteric line and gluteal tuberosity, and on the ulnae. These bone accumulations were observed on locations where enthesophyte formation could be expected, but were more unorganized and delicate than would normally be expected for enthesophytes (Figure 2b). A pseudofracture with spiculated new bone formation was noted on the inferior lateral margin of the spine of the right scapula (Figure 2c). The left scapula was not available for analysis.

None of the four individuals presented with lesions associated with residual rickets.

### *4.2. Radiological observations*

Radiological analysis confirmed the several healed or healing rib fractures in individuals 1 and 3, and the fracture of the left anterior inferior iliac spine in individual 1 (Figure 3a). Pseudofractures of the inferior lateral margins of the scapular spine in individuals 1 and 4 were also confirmed (Figure 3b and c).

Discrepancies with the macroscopic analysis occurred when the alleged pseudofractures of the ischial spine in individual 1 and of the inferior lateral margins of the scapular spine in individual 3 did not show Looser's zones. The radiological analysis furthermore showed a Looser's zone on the neck of a left rib in individual 3 (Figure 3d), while no signs of pseudofracture were initially seen macroscopically. On reassessment the pseudofracture (which was characterized by an extremely

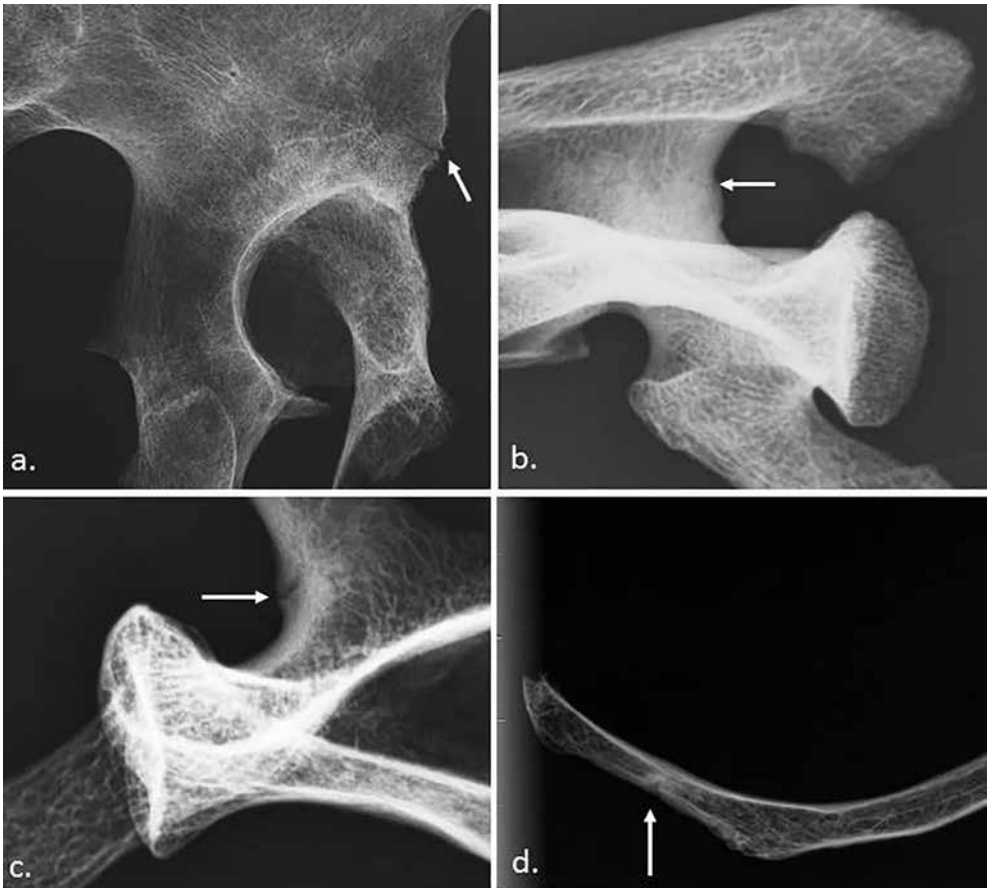




**Figure 3.** A composite figure presenting a selection of the possible osteomalacia related lesions observed in conventional radiography. a. A fracture of the left anterior inferior iliac spine in Individual 1 (MeB S2039 V084). Pseudofractures of the inferior lateral margins of the scapular spine as observed in Individual 1 (b) and Individual 4 (MeB S1047 V217)(c). d. A pseudofracture on the neck of a left rib as observed in Individual 3 (MeB S2037 V148).

subtle break in the cortex and marginal reactive bone growth) could however be macroscopically identified with the help of a magnifying glass.

### *4.3. Microscopic observations (BSE-SEM)*

All samples, especially the femur samples, showed some degree of taphonomic alteration such as tunneling, remineralization and fragmentation. This however did not preclude the analysis of the bone tissue architecture for diagnostic purposes.

All four individuals showed poor and incomplete mineralization of bone, highly indicative of osteomalacia. This was observable as areas of defective mineralization along cement lines, adjacent to Haversian canals or around (enlarged) osteocyte lacunae (Figure 4). An increase of the number of Howship's lacunae, and enlarged osteocyte lacunae were also noted. The mineralization defects, and the pathological separation of bone lamellae due to the post-mortem

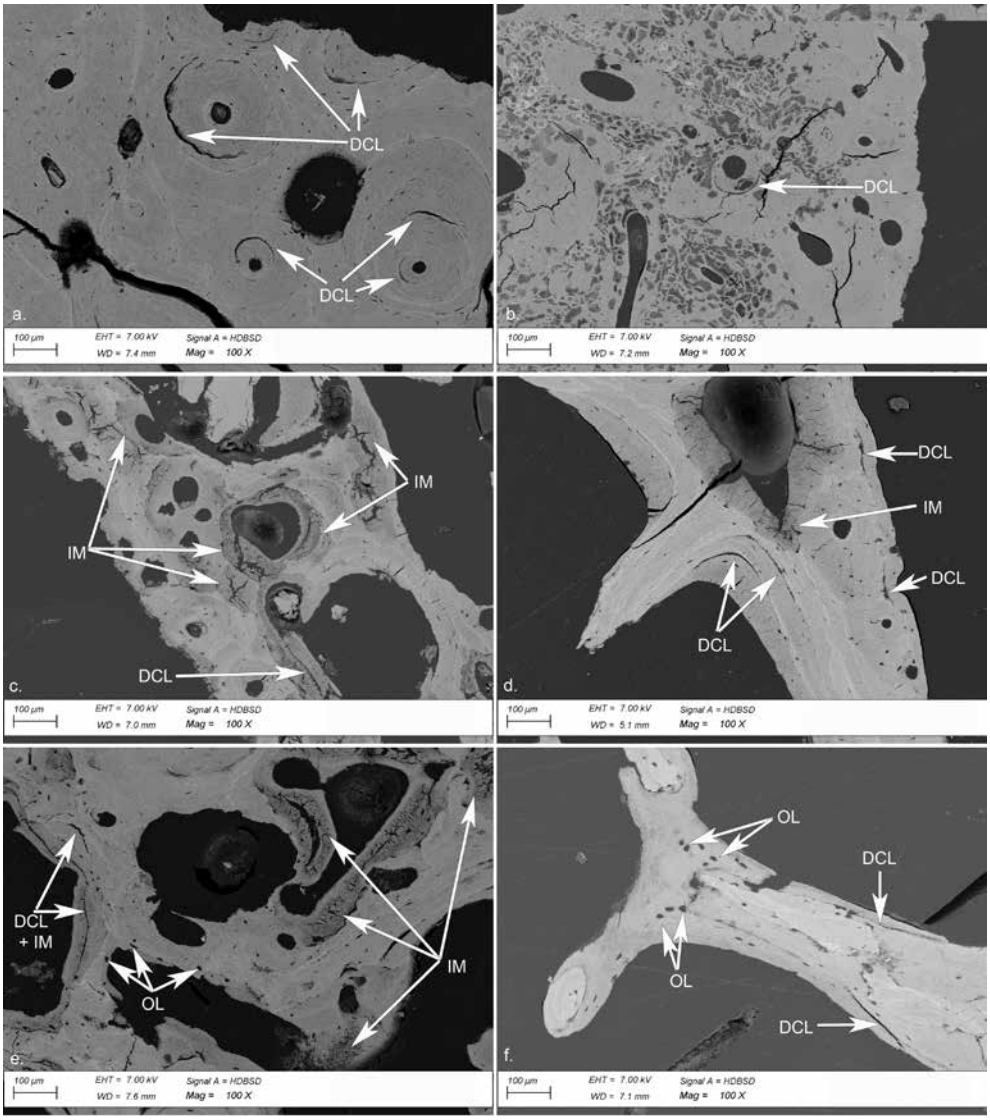


**Figure 4.** Composite figure of BSE-SEM images of the femoral, costal and vertebral bone samples taken from Individual 2 (MeB S2034 V138) (a., c. and e.) and Individual 3 (MeB S2037 V148) (b., d. and f.). Defective cement lines (DCL) are visible in all the sections. Note the difference in abundancy of mineralization defects when comparing the femoral (a. and b.) to the costal (c. and d.) and vertebral (e. and f.) samples. Layers and/or islands of incomplete mineralization (IM) and enlarged osteocyte lacunae (OL) were mainly observed in the costal and vertebral samples. Also note the similarity in appearance of the histological features when compared with light microscopy (Figure 6). (See the Appendix online for more detailed versions of the pictures included).

loss of osteoid could be distinguished from post-mortem damage by their appearance and distribution (Figure 5). The pathological features were characterized by a fine granular, delicate appearance and were 'anatomically' distributed (i.e. as could be expected from their pathogenesis). In contrast, postmortem damage and artefacts were much more irregular and coarse, and were haphazardly, 'non-anatomically' distributed.

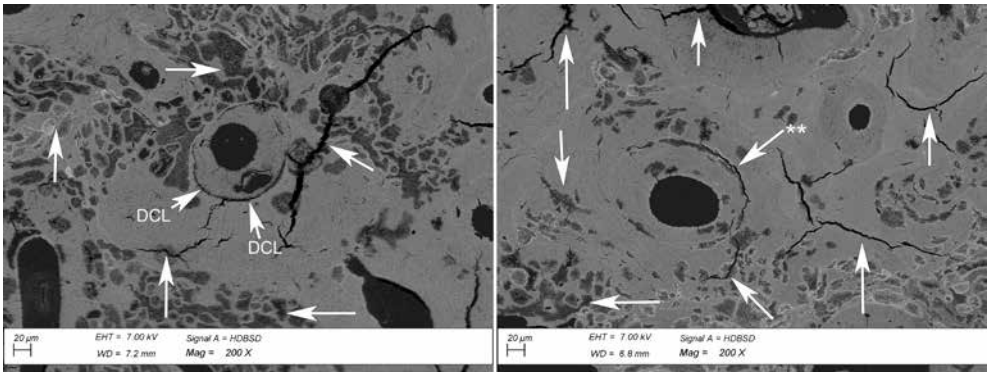


**Figure 5.** A composite figure illustrating the difference between mineralization defects and post-mortem changes (diagenetic changes) as observed during SEM investigation. Mineralization defects (DCL) were characterized by a fine granular, delicate appearance and were 'anatomically' distributed. In contrast, post-mortem damage and artefacts, as indicated by the remainder of the arrows, were much more irregular and coarse and were haphazardly, 'non-anatomically' distributed. Note the difference between the fine granular defective cement line and the coarser post-mortem damage indicated with **.

A clear difference in severity and intensity of histological lesions were observed when comparing the femoral, costal and vertebral samples per individual. The costal and vertebral sections were more frequently affected, whereas the pathological changes in the femoral samples were less pronounced. Defectively mineralized bone adjacent to cement lines and around large osteocyte lacunae was observed in all skeletal elements (Figure 4). Complete layers/islands of incomplete mineralization of newly formed bone and traces of extensive bone resorption were mainly observed in the costal and vertebral samples.

When comparing the relative extensiveness of the lesions between individuals, it became apparent that individual 1 and 2 presented with more mineralization defects in comparison to individual 3 and 4. In individual 1 and 2, the lesions were more widespread, affected larger areas of the bone and were already clearly visible at low magnification. Individual 3 appeared to be the least affected and presented with comparatively fewer defects most of which could only be accurately identified using higher magnification.

### *Microscopic observations (light microscopy)*

The light microscopic observations were highly similar to those with BSE-SEM (Figure 6). All sections were affected by some degree of taphonomic alteration, especially the femoral sections of individual 3 and 4. This however did not hamper analysis considerably.

All individuals showed mineralization defects, especially adjacent to cement lines and Haversian canals. Poorly mineralized bone tissue was also found directly adjacent to enlarged osteocyte lacunae. In all sections, post mortem loss of osteoid led to separation of bone lamellae, most often surrounding cement lines in cortical and cancellous bone. As with the BSE-SEM, the pathological changes could be distinguished from

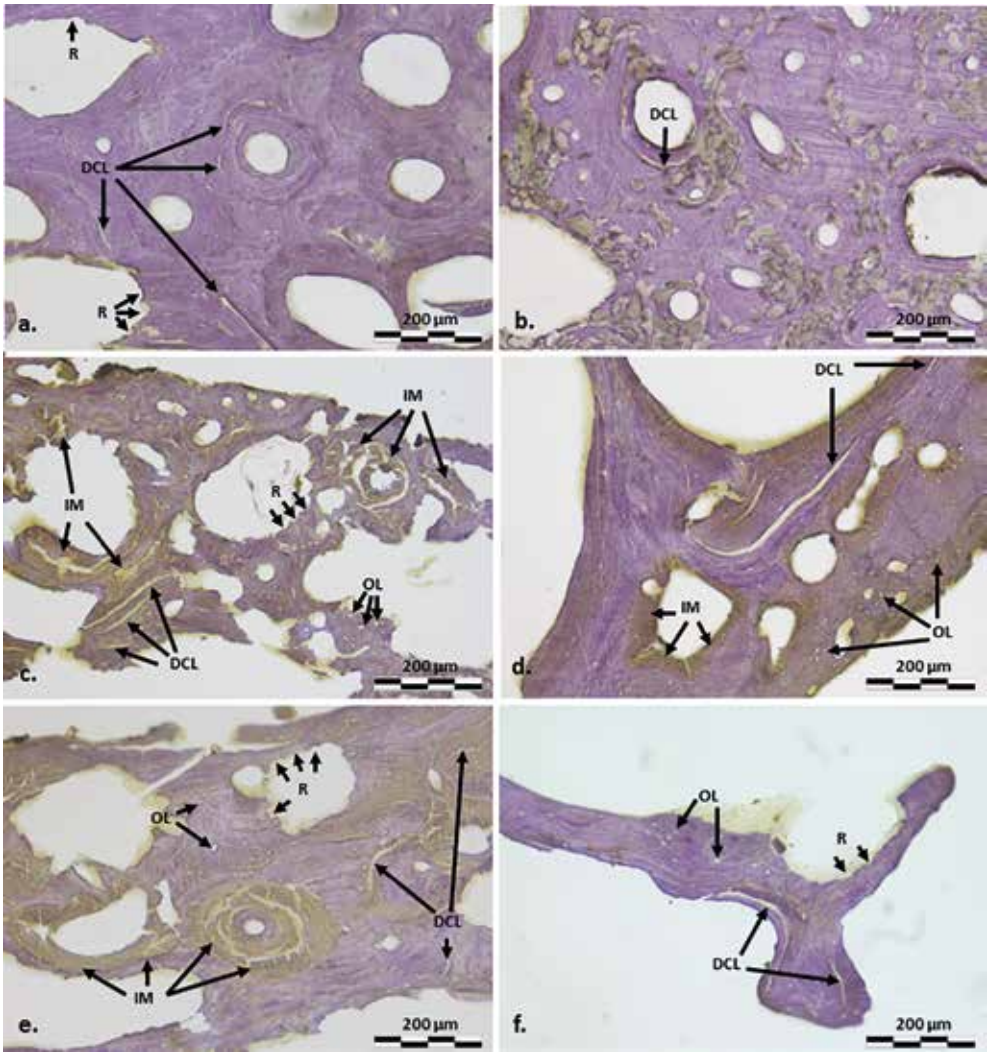


**Figure 6.** Composite figure of micrographs of undecalcified, haematoxylin stained, hand-ground sections of femoral, costal and vertebral bone samples from individual 2 (MeB S2034 V138) (a., c. and e.) and individual 3 (MeB S2037 V148) (b., d. and f.). All sections show defective cement lines (DCL). Furthermore, various samples show layers or islands of incomplete mineralization (IM), resorption bays (R) and/or enlarged osteocyte lacunae (OL). Note the difference in severity of the mineralization defects between the femoral (a. and b.) and the costal (c. and d.) and vertebral (e. and f.) samples. Also note the similarity in appearance of the histological features when compared with BSE-SEM (Figure 4).

post mortem damage on the basis of their fine, granular appearance and their 'anatomical' distribution.

The pathological changes were most pronounced in the costal and vertebral sections and in addition to mineralization defects, these sections also showed an increase in Howship's lacunae (Figure 6). The femoral sections were least affected, especially in the periosteal one-third of the cortex. As in the BSE-SEM images, individual 1 and 2 presented with relatively more lesions when compared to individual 3 and 4, whereas individual 3 was least affected.

## 5. DISCUSSION

This paper reports the first four paleopathological cases of adult osteomalacia in the Netherlands. No medical reports were available to corroborate these diagnoses. However, the combination of the findings and the similarities between the results of our combined macroscopic, radiological and histological diagnostic approach and the well-documented diagnostic criteria of Brickley and Ives (2008) and Ives and Brickley (2014) are such that the diagnosis becomes almost certain.

None of the four individuals described presented with the serious macroscopic lesions exhibited as characteristic for osteomalacia in historical pathology museum collections and described in paleopathology textbooks such as Ortner (2003). As was also noted by Ives and Brickley (2014) the museum specimens, which often include cases with severe bending of the long bones and pelvis and buckling of the axial skeleton, represent the most extreme changes which may be encountered and were most likely selected due to their severity. The macroscopic changes observed in this study were much less severe and mainly concerned the formation of pseudofractures. Deformation of bones were only observed in one individual and concerned the lateral straightening of the midsections of the ribs in individual 1.

The outcome of our multidisciplinary approach shows that all affected individuals had macroscopically identifiable scapular and/or costal (pseudo)fractures as described by Ives and Brickley (2014). The identification of these pseudofractures therefore seems a reliable way to macroscopically diagnose osteomalacia. The severity and duration of the condition however influences the occurrence of the lesions (Brickley et al., 2007; Francis and Selby, 1997) and as a result, not all individuals with osteomalacia necessarily present with pseudofractures (Steinbach et al., 1954). The distribution of pseudofractures across the skeleton and their stage of healing serves to differentiate them from non-osteomalacia related fractures. However, the fragility and associated fragmentation of skeletal remains of individuals with osteomalacia may severely hamper this differentiation during macroscopic analysis, ultimately precluding a definitive diagnosis purely base on macroscopic analysis. In our sample, this especially was noted in individual 2.

Brickley et al. (2007) suggest using radiological imaging as a primary supportive diagnostic modality in individuals suspected of osteomalacia. The radiological counterpart of a pseudofracture (Looser's zones) is considered pathognomonic for osteomalacia, and a reliable diagnosis would thus be possible without performing destructive histological investigation. A recent study also suggested that the visibility and appearance of pseudofractures during radiological assessment may shed light on the underlying cause and chronicity of osteomalacia (Jennings et al., in press). The added value of

radiological investigations in our study was limited. It did not reveal significantly more pseudofractures; all but one were already identified during macroscopic analysis. This was in part due to the severe fragmentation of the remains (especially ribs and scapulae). Pseudofractures in these fragments could only be accurately identified by meticulous macroscopic assessment.

Interestingly, radiology did prove useful to corroborate macroscopically suspected pseudofractures; in two out of the four individuals, at least one macroscopically observed possible pseudofracture could not be confirmed by radiology. As mentioned by Ives and Brickley (2014) the diagnosis of osteomalacia based on macroscopic assessment only is highly dependent on the identification of the characteristic fracture pattern associated with the condition. Unfortunately, the macroscopic presentation of the pseudofractures can be rather subtle, especially when healing has occurred. Supporting the macroscopic identification of 'possible' pseudofractures with radiological investigation will assist in guarding the quality of macroscopic pseudofracture pattern descriptions.

BSE-SEM investigation proved extremely valuable in the diagnostic process. The diagnostic pathological features such as layers or islands of poorly mineralized bone and defectively mineralized bone next to cement lines were easily identifiable. The pathological changes were clearly more pronounced in the vertebral and costal samples. This was an expected finding, since these skeletal elements have higher rates of bone turnover and will thus be affected more by systemic metabolic bone disease. Given these results we advise to limit histological analysis to bone elements with high metabolic rates in cases where sampling cannot be performed freely. This will increase the chances of observing microscopic lesions related to osteomalacia. The use of rib samples seems most sensible, since ribs are generally abundantly available and are often already fragmented. In our series, the rib samples also proved easier to process and analyze than vertebral samples.

Light microscopy showed to be a feasible, cheaper and less laborintensive alternative to BSE-SEM investigation. Histological features associated with osteomalacia as described for BSE-SEM investigations were also easily observed in the undecalcified, hand-ground bone sections. Please note that the extremely fragile bone samples should be embedded, since the diagnosis is based on minute details that will almost certainly be lost in unembedded samples. It is further imperative that the histological slides are surface stained with haematoxylin. In unstained sections, due to its thickness, the analysis will be severely hampered by the obscuring effect of the underlying portions of the bone tissue that are out of focus. Staining also enhances the contrast between the bone tissue and the surrounding resin, which improves the visibility of poorly mineralized areas, resorption bays and defective cement lines.

The presence of the condition in the four described individuals was somewhat anticipated given the origin of the skeletal remains, namely a cemetery of a psychiatric institution. The signs of autopsy make it highly likely that at least three of the four individuals were patients, rather than personnel, of the institution. As stated in historical documents, autopsies were often performed, with consent of the deceased family members, in an attempt to gather information on the possible anatomical, physiological and/or neurological grounds for the mental diseases observed. Autopsy as a means to determine cause of death only became mandatory in the Netherlands in 1955 (Das and van der Wal, 2001). The reason for their institutionalization and/or the regime in the institution may have played a role in the development of the osteomalacia observed.

As mentioned in the introduction, several disorders may result in osteomalacia (Vigorita, 2008). However, the condition is primarily related to a vitamin D deficiency which results from a prolonged lack of exposure to sunlight, a diet deficient of vitamin D and/or intestinal malabsorption (Francis and Selby, 1997; Reginato and Coquia, 2003). The meals in Meerenberg were simple and comprised mainly of mashed potatoes and vegetables, brown beans with bacon, pearl barley with syrup, beer, and bread. Since these meals contain low amounts of Vitamin D, patients would be mainly dependent on sunlight exposure to maintain sufficient levels of Vitamin D (Vijselaar, 1982; Vijselaar, 1997).

For approximately two-thirds of the patients in the institution that were compelled to perform physical labour, such as farming or gardening on the 32 ha surrounding the institution (Vijselaar, 1997), sufficient sunlight exposure would have not been problematic during the summer months. However, by the end of the 19th century the treatment of psychiatric patients could include prolonged periods of bed rest or lukewarm bath therapy (Vijselaar, 1997). This type of treatment would be reserved for the more difficult, unruly and/or unpredictable individuals. According to historical descriptions approximately a third of the patients at the Meerenberg institution were almost continuously kept indoors because of such treatment (Vijselaar, 1997).

These patients, or those considered too sick or unpredictable to venture or labor outside would be predisposed to developing a vitamin D deficiency due to insufficient sunlight exposure. As a matter of fact, a similar combination of low dietary intake of Vitamin D and low concentrations of (sun-derived) vitamin D precursor is visible in modern institutionalized and house- or bedbound patients (Brickley and Ives, 2008; Gough et al., 1986; Lui et al., 1997; Meunier and Chapuy, 2005; Plehwe, 2003). This might also partly explain the high crude prevalence of the condition in our small patient-based sample (4 of 69 individuals, 5.8%) if compared to the prevalence in other much larger, non-hospital bound archeological studies (e.g. Brickley et al., 2007; Ives and Brickley, 2014).

It is known that patients with seizure disorders taking chronic anticonvulsant medication often present with osteomalacia due to vitamin D deficiency (Francis and Selby, 1997; Gough et al., 1986; Malik, 2008; Meunier and Chapuy, 2005). It has been suggested that anticonvulsants (such as phenobarbital and phenytoin) accelerate the catabolism of vitamin D to a biologically inactive metabolite (Reginato and Coquia, 2003). However, these drugs were only developed in the early 20th century (Brodie, 2010).

Other causes for the observed osteomalacia, such as renal disease, hepatobilliary disease and dietary calcium and/or phosphate deficiencies cannot be excluded. As mentioned by Ives and Brickley (2014), the differentiation between these causative disorders is extremely challenging, if not impossible in an archeological setting.

Osteomalacia generally affects females more often than males, and is more prevalent in the elderly (Meunier and Chapuy, 2005). This tendency was also suggested by results obtained in archeological samples (Ives and Brickley, 2014). Unfortunately, the Meerenberg sample was unfit to confirm this pattern, as the sample was small, the biological profile distribution of the complete sample was rather skewed towards middle-aged and old adult females, and in two of the four cases age could only be estimated to 'adult'. It should also be kept in mind that reaffirming this tendency in archeological samples will most likely be hampered by the difficulties encountered when reconstructing a biological profile in the often severely fragile and fragmented skeletal remains that are associated with osteomalacia.

## 6. CONCLUSION

This study reports the first four paleopathological cases of osteomalacia in the Netherlands. These diagnoses are based on a comprehensive diagnostic approach including macroscopy, radiology, BSE-SEM and light microscopy. Macroscopy and histology proved very valuable diagnostic modalities. The added value of radiological imaging was limited. Light microscopy proved to be a feasible alternative for BSE-SEM for researchers with limit access to SEM facilities. All four individuals originate from a cemetery related to a psychiatric institution, which is likely to have contributed to their risk in the development of osteomalacia.

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## APPENDIX A. SUPPLEMENTARY DATA

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ijpp.2018.03.004.

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