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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D-LIGHTFUL SUNSHINE DISRUPTED:
VITAMIN D DEFICIENY AS A METHOD FOR THE RECONSTRUCTION OF CHANGES IN SOCIOCULTURAL PRATICES DUE TO INDUSTRIALISATION IN 17TH-19TH CENTURY NETHERLANDS

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1 Introduction

AIMS AND OBJECTIVES OF THIS RESEARCH

Many aspects of daily life changed in the Netherlands over the course of the 17th to 19th centuries. Some of these changes had important repercussions for health and disease. Although the Netherlands did not experience the Industrial Revolution in the same way the United Kingdom or other European countries, the process of industrialisation did significantly affect Dutch populations, especially in the 19th century (De Vries 2000; De Vries and Van der Woude 1997; Mokyr 1976; 2000; Wintle 2000). Historic sources provide us with data on the economic and political changes that various Dutch centres experienced. Indeed, industrialisation must have affected both urban and rural centres by intensification of production processes and task specialisation (De Vries 2000; Falger et al. 2012; Sypkens Smit 1964; Van der Bent et al. 2011; Van Gastel 1995; Wintle 2000). This in turn, must have influenced sociocultural practices in the various communities in response to these new demands. However, the way industrialisation impacted daily life and how sociocultural practices changed are poorly understood, especially in smaller towns and rural villages.

The use of just historic sources to assess the influence of industrialisation on the various communities in the Netherlands provides an incomplete understanding. Most of these sources describe general trends, some aspects of elite lifestyle, or are focussed on inhabitants from large, highly industrialised urban centres (Clerx 1985; De Vries and Van der Woude 1997; De Vries 2000; Drukker and Tassenaar 1997;

Mokyr 2000; Wintle 2000). Information on rural populations and individuals from small urban centres, as well as and women and children in general, is lacking, even though political and economic changes of industrialisation must have affected these groups. To improve our understanding of the impact of industrialisation on various Dutch communities, both rural and urban, the analysis of human skeletal remains can be undertaken. Human skeletal remains provide direct information on individuals and communities from the 17th - 19th centuries, and may improve our insights into behavioural changes which were integral to the industrialisation process.

A way to obtain information about behavioural changes is by looking at vitamin D deficiency. It is a pathological condition caused by inadequate levels of vitamin D. Cutaneous synthesis under the influence of ultraviolet B (UVB) radiation in sunlight is the most effective way of acquiring vitamin D (Holick 2006). The amount of vitamin D, however, that is produced in the skin is dependent on several variables. These include geographic latitude, age, occupation, behavioural factors related to social status and gender, cultural traditions, and religious practices that influence sunlight exposure. In addition, the contribution of vitamin D from dietary sources, while low in non-fortified foods from the past (Holick 2006), must be considered. Therefore, the study of vitamin D deficiency may provide information on sociocultural practices, as has been observed in modern (e.g. Van der Meer et al. 2006; Underwood and Margetts 1987; Yassin and Lubad 2010) and archaeological populations (e.g. Giuffra et al. 2015; Palkovich 2012). Moreover, the analysis of vitamin D deficiency, as visible in the human skeleton, can provide information on lifestyle, tradition, gendered activities, and diet, that often cannot be obtained from historic sources (Brickley et al. 2014).

The aim of this research is to improve our understanding of the changes in sociocultural practices of 17th - 19th century Dutch populations before and during industrialisation by using osteoarchaeological data on vitamin D deficiency prevalence. This will (a) contribute to existing knowledge provided by historic sources on the influence of industrialisation, (b) assess ideas and assumptions about lifestyles in the past, (c) improve our knowledge of regional variation in the influence of industrialisation, and (d) decrease the paucity of research on rural populations and small urban centres in the Netherlands. The main research question is:

How does the occurrence of vitamin D deficiency in 17th to 19th century Dutch communities enhance our knowledge of changing sociocultural practices as a result of industrialisation?

This chapter will be arranged in four sections. Section 1 contains information about the economic and political frameworks of 17th to 19th century Netherlands. The functions and importance of vitamin D are described in section 2, and a description of the macroscopic, radiologic, and microscopic skeletal features that may occur as a result of vitamin D deficiency in various age groups is provided. Section 3 describes the demographic and historic characteristics of the skeletal collections that are used for this research. Research sub-questions are presented and a description of the dissertation's structure is provided in section 4. Finally, this chapter is concluded with an explanation of the importance and value of this research.

SECTION 1: THE NETHERLANDS IN THE 17TH TO 19TH CENTURIES

By the end of the 16th century, the Republic of the Seven United Netherlands, as the Netherlands was called in that time, consisted of eight sovereign states: Groningen, Friesland, Drenthe, Overijssel, Gelderland, Utrecht, Holland (Noord and Zuid), and Zeeland. Drenthe was a sovereign state without representation in the parliament and the remaining provinces of Noord-Brabant and Limburg were part of the Republic but were not sovereign states (De Vries and Van der Woude 1997). Roughly, the Republic could be divided into three major areas with similar economic characteristics: (1) urban, (2) modern agricultural, and (3) traditional rural (De Vries and Van der Woude 1997), as depicted in figure 1. The city of Amsterdam and the sites analysed in this study are also depicted in figure 1 and will be discussed in section 3.

At the beginning of the 17th century, the urban part of the Republic consisted of the provinces of Zeeland, Noord- and Zuid- Holland. This area was characterised by relatively large urban centres famous for their industry (e.g. the sugar-refining industry in Amsterdam), although agriculture did exist in these provinces and was dominated by horticulture and dairy farming (De Vries and Van der Woude 1997). The importance of Zeeland's seaports declined over the course of this century and agriculture became more important. By the end of the 18th century, Zeeland, Groningen and Friesland were considered to be modern agricultural areas, characterised by large-scale, specialised and market-oriented dairy farming and agriculture (De Vries and Van der Woude 1997). Rich alluvial soils typified the provinces of the urban and modern agricultural parts of the Republic, whereas the rest of the provinces (Drenthe, Overijssel, Gelderland, Utrecht, Noord-Brabant, and Limburg) had poor diluvial soils (De Vries and Van der Woude 1997; Mokyr 1976). The latter provinces were considered to be rural traditional, meaning the agriculture in this part of the Republic had a more local and regional character, and was focused on self-sufficiency instead of international trade (De Vries and Van der Woude 1997; Mokyr 1976).

In the 17th century, the Republic experienced an economic rise, population growth, and an explosive increase of international trade that heralded the start of the Golden Age (De Vries and Van der Woude 1997). The need for skilled and unskilled labour rose due to several economic developments. The process of land

Figure 1. Division of the Netherlands in urban, modern agricultural, and rural traditional areas.

reclamation (AD 1608 – 1640) gave rise to six major polder drainage projects in the province of Noord-Holland and more than 100 industrial windmills were built to enable this (De Vries and Van der Woude 1997). The VOC (Dutch East-India Company) and the WIC (Dutch West-India Company), that were founded in this century, employed a large number of individuals on ships and in the harbours (De Vries and Van der Woude 1997; Mokyr 2000). Population growth increased the need for agricultural products, and during this time, wages and the prices of commodities rose. The second phase of the Golden Age, AD 1623 – 1663, is characterised by stagnation in economic growth, partially due to the rising resistance of powerful competitors and the limited options for further expansion of Dutch international trade (De Vries and Van der Woude 1977). Although land reclamation provided room for the much needed agricultural and settlement expansions, it also led to the salinisation of surface water and gradual silting of the harbours (De Vries and Van der Woude 1977). The poor quality of the surface water caused many problems, whereby the increasing brackishness of the water gave way to mosquitos bearing malaria (Wintle 2000). In addition, several plague epidemics were followed by outbreaks of dysentery and small pox, responsible for a large number of deaths (De Vries and Van der Woude 1997; Wintle 2000). At the end of the Golden Age, three naval wars with England and the French invasion of the Republic in AD 1672 disrupted the international trade profoundly. A sharp reduction in population growth can be observed in these decades, and unemployment rose (De Vries and Van der Woude 1997).

The 18th century is characterised by further deterioration of the surface water quality in the coastal provinces affecting the industries dependent on water (e.g. breweries) and the living conditions of its inhabitants, whereby malaria epidemics increased (De Vries and van der Woude 1977); Wintle 2000). The herring fishery, that was an important contributing factor to the economic rise at the beginning of the Golden Age, suddenly collapsed as did the whaling industry. Prices of agricultural products began declining at the end of the 17th century and continued to drop during the 18th century causing an agricultural depression from AD 1650 – 1750 (De Vries and Van der Woude 1977). Several outbreaks of the cattle plague (AD 1714 – 1720, 1744 – 1754, and 1769 – 1784) caused a substantial drop in dairy production and a decline in the export of cheese (De Vries and Van der Woude 1997). England declared war on the Republic in AD 1780 and attacked the VOC. The growing political unrest in the Republic led to the Patriot Revolt in AD 1787 that was initially crushed by regent Willem V (De Vries and Van der Woude 1977). With the help of the French Revolutionary Army, the Patriots overthrew the government of Willem V in AD 1795 and founded the Batavian Republic, for which the Republic paid dearly in the form of high taxes putting further pressure on the economy (De Vries and Van der Woude 1977; Mokyr 1976). The only production sector that experienced growth was agriculture. Commercial farmers intensified their dairy and grain production causing increasing prosperity in a period characterised by urban crisis and general impoverishment (De Vries and Van der Woude 1997).

The beginning of the 19th century, is marked by the incorporation of the Batavian Republic into the French Empire in AD 1810 and in the years thereafter (AD 1811 – 1813) all that was left of the Dutch industry collapsed (De Vries and Van der Woude 1977). During the Batavian and French period, the Netherlands had undergone a thorough reorganisation of its tax system, the guild system was dissolved, a central government was established, whereby the Kingdom of the Netherlands emerged in AD 1814. Its economy remained stable until the 1840s, when several consecutive crop failures, and multiple outbreaks of the typhoid fever, and cholera in 1838 and 1848, brought about years of misery and hunger (De Vries and Van der Woude 1997;

Wintle 2000). The Industrial Revolution that began in England at the end of the 18th century and took place in other northern European countries at the beginning of the 19th century, was not observable as such in the Netherlands (De Regt 1977; Mokyr 1976). Industrial innovation took place in the remote area of Twente (province of Gelderland), and the province of Noord-Brabant which embraced new techniques in the production of textiles, whereas the maritime provinces in the west of the Netherlands did not experience industrialisation in that period (Mokyr 1976). Many factors are suggested to be responsible for the absence of a clearly defined Industrial Revolution in the Netherlands, including the already relatively modern economy during the Golden Age and the political and economic instability caused by many wars (De Vries 2000; Mokyr 2000; Wintle 2000).

Although the Golden Age brought about prosperity to the Republic in general, just a small percentage of individuals were postulated to be actually wealthy (De Vries and Van der Woude 1977). About half of the urban population consisted of individuals without steady work and often without an address (e.g. vagabonds, paupers, and beggars), individuals that on occasion earned some money (e.g. street peddlers, and market sellers), and numerous unskilled and semiskilled labourers (e.g. domestic servants, sailors, and soldiers) whose living conditions were poor and their income uncertain (Clerx 1985; De Regt 1977; De Vries and Van der Woude 1977). In rural areas, large differences in landownership or tenancy of agricultural land existed between local communities that caused marked dissimilarities in wealth between farmers (De Vries and Van der Woude 1977). Rising prices of agricultural products must have brought about prosperity for most farmers, whereas the agricultural depression of AD 1650 – 1750 likely impoverished many.

Most historic data on occupations are about male labour, but females and children also formed an important part of the labour force (De Vries and Van der Woude 1977; Van Cuyningen 2005; Schenkeveld 2008). Until the 19th century, the participation of females and children from the middle and low socioeconomic classes was considered to be normal (Clerx 1985; De Vries and Van der Woude 1977). Typical female activities were the preparation of dairy products, spinning, and all sorts of housework. Women also worked in the salt refinery, stacked and dried peat, and were employed to periodically turn the stored grain to prevent it from overheating (De Vries and Van der Woude 1977). Although females formed an important part of the labour force, their work was generally considered to be less important, stressed by the fact that they were not part of guilds and their wages tended to be lower (De Regt 1977; De Vries and Van der Woude 1977). Moreover, the men were considered to be head of the household and responsible for the family income, whereas the income of women was regarded as additional. During the 19th century, the participation of women in the labour market decreased. This may be partly explained by improved wages for the men, but foremost due to the increasing pressure of society that stressed that women were primarily needed at home to tend to their husband and children (De Regt 1977; Van Poppel et al. 2009). Although already before the 19th century this was the primary task of women, the situation at many households worsened during the difficult times of the 18th and beginning of the 19th century leading to an increase in the number of neglected or even abandoned children and poorly cleaned houses (Clerkx 1985; De Regt 1978). Since this was primarily the women's responsibility, pressure of society on women to stop working outside their home increased and the number of women participating in various jobs dropped (Van Poppel et al. 2009). Clerkx (1985) reports groups of neglected and orphaned children who wandered around the towns, begging and stealing. They were regarded as cheap labourers and were put to work in large numbers (Clerx 1985; De Vries and Van der Woude 1977). In urban centres, children were commonly employed in paper mills, brickwork factories, and performed various chores in the textile industry (De Vries and Van der Woude 1977). The children's part in agriculture and dairy farming was more important, whereby sometimes children were already put to work at the age of 6 years (Schenkeveld 2008).

Individuals working long hours indoors are postulated to have elevated risks of developing vitamin D deficiency, since their exposure to sunlight is decreased. Factors commonly attributed to the Industrial Revolution, such as tall densely-packed buildings and air pollution, would have further reduced the availability of sunlight even for individuals that were unemployed or otherwise free to be outside often. As outlined, the economic and political situations of the Netherlands in the 17th to 19th centuries suggest it is unlikely that high prevalences of vitamin D deficiency would be observed in most communities from that time period. If indeed vitamin D deficiency was prevalent, it is expected to only be observable in the urban area, the provinces of Noord- and Zuid-Holland, where most urbanisation and settlement expansion took place, whereas in the modern agricultural and rural traditional areas of the Netherlands, vitamin D deficiency is unlikely to be prevalent.

SECTION 2: VITAMIN D

For the main research question to be answered, information on vitamin D and its function in the human body is needed. Furthermore, a clear understanding of the consequences of inadequate vitamin D levels is required.

Vitamin D and its functions

Vitamin D is considered to be a hormone, rather than a vitamin, since it is produced in the human body as opposed to true vitamins (Brickley and Ives 2008; Holick 2005a; Holick and Adams 1990; Lockau and Atkinson 2017). Although there are several forms of vitamin D, the most common are vitamin D3 (cholecalciferol), which is produced in the skin, and vitamin D2 (ergocalciferol), that can be derived from plants (Holick 2005a). Vitamin D3 is produced in the skin under the influence of UVB radiation in sunlight that causes photolysis of 7-dehydrocholesterol to previtamin D3 (Holick 2003; 2005a; 2005b). Both vitamin D3 and D2 undergo two hydroxylations before the most active form of the vitamin, 1,25(OH)2D, is produced. The first hydroxylation takes place in the liver, and the second in the kidneys (DeLuca 1980; Holick 2005b; Horst et al. 2005; Wasserman 2005).

Vitamin D has several functions in the human body. Classic actions are those that influence calcium and phosphate homeostasis, while non-classic actions include the regulation of cell proliferation and differentiation, modulation of immune function, and vitamin D is known to have several endocrine functions (Remuzzi 2007). The most commonly known function of vitamin D is that of calcium homeostasis, whereby vitamin D is needed for the mineralisation of newly formed bone, osteoid. During growth (deposition of new bone; also called modelling) and remodelling (replacement of old and damaged bone), osteoid is deposited after which mineralisation is required to provide the bone with strength and hardness. This firmness is needed to withstand muscular tension and gravity. Vitamin D deficiency may lead to bending, flaring, and thickening deformities in the skeleton, because mineralisation of the newly formed osteoid is inadequate or impaired. Especially important for diagnosis are the weight bearing bones, such as the arm bones during periods of crawling and leg bones once an individual can walk. These bones will more easily bend due to gravity and/or muscular tension. This bent, poorly mineralised bone is also more susceptible to fracture. More osteoid is secreted to repair the damage from bending and/or fracture, but the accumulation of unmineralised osteoid in the joints will cause thickening, flaring, and cupping deformities of the metaphyses (unfused proximal and distal ends of the long bone) (Brickley et al. 2018; Brickley and Ives 2008; Ives and Brickley 2014; Mankin 1974). In nonadults (< 18 years of age), vitamin D deficiency lesions are referred to as rickets, whereas in adults (18+ years of age) vitamin D deficiency is referred to as osteomalacia. Remnant lesions of rickets still visible in the adult skeleton is referred to as residual rickets.

Macroscopic detection of vitamin D deficiency

Rickets

As described by Chesney (2001), and Pearce and Cheetham (2010), common clinical features that result from a deficiency in vitamin D in nonadults are: (1) large head with prominent frontal bossing due to softening of the cranial bones and a delay in the closure of the fontanelles, (2) rachitic rosary (thickening/swelling of sternal rib ends), (3) deformity of the chest resembling a pigeon chest, (4) flaring/thickening of wrist and knee, (5) presence of fractures, (6) bending of the lower limbs, (7) growth retardation, (8) increased prevalence of pneumonia, (9) stridor (a harsh noise when breathing), (10) muscle weakness and pain, (11) involuntary muscle contractions, especially in the hands and feet, and (12) waddling gait. Not all clinical features of rickets are visible in skeletal remains, as obviously only the skeletal lesions can be assessed.

Cranial lesions that can occur because of vitamin D deficiency include thinning of the cranial bones, frontal and parietal bossing, craniotabes (softening of the parietal bones), a square-shaped head, orbital roof porosity, and layers of irregular porous bone (Brickley et al. 2018; 2014; Hess 1930; Mays et al. 2006; Ortner and Mays 1998; Schatmann et al. 2016). These lesions are not strongly diagnostic for vitamin D deficiency and can also result from normal variation, or other pathological conditions such as scurvy and congenital syphilis (Brickley and Ives 2008). However, these lesions can occur and need to be scored to improve our understanding of lesion development of vitamin D deficiency in various affected individuals. Although delayed eruption of teeth (deciduous and permanent), enamel hypoplasia, and dental caries may result from a vitamin D deficiency (Berry et al. 2002; Brickley et al. 2014; Hess 1930; Mankin 1974), these features are not diagnostic and can be caused by various pathological conditions and nutritional stress (Brickley and Ives 2008). One feature of the skull that is considered quite diagnostic of vitamin D deficiency is medial angulation of the mandibular ramus. This deformity of the mandibular ramus is presumably caused by the pull of chewing muscles, whereby the weakened bone is not able to withstand the tension (Brickley et al. 2018; 2014; Mays et al. 2006; Ortner and Mays 1998; Schatmann et al. 2016).

In rickets, the vertebral column may show an abnormal forward (kyphosis) or sideways (scoliosis) curvature. (Brickley et al. 2014; Hess 1930; Mankin 1974). The ribs may display an alteration in the rib neck angle, lateral straightening of the shaft (narrowing of the chest), swelling of the costochondral rib ends (rachitic rosary), and/or pigeon-chest deformity (Brickley et al. 2018; 2014; Hess 1930; Mays et al. 2006; Ortner and Mays 1998; Schatmann et al. 2016). All of these features are considered diagnostic for rickets (Brickley and Ives 2008). Diagnostic features of the pelvis include a more pronounced medio-lateral curvature of the ilium, and more dorsally located acetabulae that may be angled anteriorly (Brickley et al. 2018; 2014; Hess 1930; Mays et al. 2006; Schatmann et al. 2016).

Long bones in rachitic affected individuals may display several diagnostic features that include flaring and swelling of the distal metaphyses, underlying porosity of the growth plate, cupping of the metaphyses and growth plates, bending in various directions, concave curvature porosity, angulation of the femoral neck, and thickening (Brickley et al. 2018; 2014; Hess 1930; Mays et al. 2006; Ortner and Mays 1998; Schatmann et al. 2016). The presence of underlying porosity of the growth plate is considered to be a macroscopic feature indicative of the active stage of rickets (Brickley et al. 2018; Mays et al. 2006). Angulation of the knees, stunted growth, and fractures may occur, but are not considered to be diagnostic for vitamin D deficiency because they have many other causes (Brickley and Ives 2008).

Residual rickets

In residual rickets, nonadult vitamin D deficiency was overcome. Growth and remodelling of the bones will usually have obliterated most skeletal lesions, such that only the more pronounced deformities will remain visible in the adult skeleton (Brickley et al. 2017a; 2010; Hess 1930). Residual frontal or parietal bossing and a square-shaped head may still be visible and the mandibular ramus may show medial angulation (Brickley et al. 2014). Scoliosis and/or kyphosis may be observable in the vertebral column (Brickley et al. 2018). Angulation of the rib neck, narrowing of the chest or a pigeon-chest deformity, and bending of the sternum may be present (Brickley et al. 2018; 2014). Lateral narrowing of the pelvis, abnormal curvature of the ilia, narrowing of the pelvic inlet, more dorsally located acetabulae, and anterior angulation of the sacrum are likely to remain and are diagnostic features of residual rickets (Brickley et al. 2018; 2014). Residual bending and thickening of the long bones and a more pronounced femoral neck angle may still be observed, and are also diagnostic features of residual rickets (Brickley et al. 2018; 2014).

Osteomalacia

Growth has ceased in the adult skeleton and only remodelling takes place. Therefore, clinical manifestations of adult vitamin D deficiency are almost always much subtler. Parfitt (1990) describes classic symptoms of adult vitamin D deficiency to be: (1) muscle weakness, (2) difficulty in walking, and (3) bone pain and tenderness. The features we may observe in the skeletal remains are described below.

In human skeletal remains, most macroscopic manifestations of osteomalacia are not pathognomonic for the disease. That includes all of the following lesions. The cranium may display diffuse porosity of the cranial vault and be of low weight, but this feature is not considered to be diagnostic for osteomalacia (Brickley and Ives 2008; Mankin 1974). The vertebra may show loss of body height, irregularity of the endplates, buckling of the body, multiple bodies may show biconcave compression, and kyphosis and/or scoliosis may be present (Brickley et al. 2018; 2014; 2007; 2005; Ives and Brickley 2014). The scapulae may display increased posterior curvature, and display a collapsed superior border (Brickley et al. 2018; 2014; 2007; 2005; Ives and Brickley 2014). The ribs may show a rib neck angulation and lateral straightening of the shafts (Brickley et al. 2014; 2005). The sternum may display bending in severe cases (Brickley et al. 2018; 2014; 2005; Ives and Brickley 2014). The ilia may protrude into the pelvic inlet, collapse, fold or fracture, the pubic rami may be dislocated or angled, and the sacrum may display extreme ventral angulation (Brickley et al. 2018; 2014; 2007; 2005; Ives and Brickley 2014). The femora may show anterolateral bending of the shafts, the knees may show medial bending, and the angle of the femoral neck may be reduced (Brickley et al. 2018; 2014; 2007; 2005; Ives and Brickley 2014).

Strongly diagnostic for osteomalacia are “pseudofractures” or “stressfractures”, which are small linear fractures that arise in defectively mineralised bone due to stress. The term “pseudofractures” may be problematic because it suggests that the observed lesions are not true fractures. However, it is a frequently used term in clinical and paleopathological literature (Berry et al. 2002; Brickley et al. 2018; 2014; 2007; 2005; Ives and Brickley 2014; Mankin 1974; Schamall et al. 2003) and therefore will also be used in this research. Pseudofractures are most commonly observed in the ribs, pubic rami, medial aspect of the ilium adjacent to the greater sciatic notch, femoral neck, lateral border of the scapula, and inferior lateral margin of the spinous process. Less frequently observed are pseudofractures in the ulna, radius, clavicle, humerus, tibia, fibula, metacarpals and metatarsals (Brickley et al. 2018; 2014; 2007; 2005; Ives and Brickley 2014; Mankin 1974; Schamall et al. 2003). Pseudofractures may develop into true fractures with irregularly formed fracture margins (Brickley et al. 2018; 2014; 2007; 2005; Ives and Brickley 2014).

Radiographic and microscopic detection of vitamin D deficiency

Radiographic features

Not all macroscopic lesions attributed to vitamin D are severe, and not all affected individuals develop clear macroscopic lesions (Brickley et al. 2017a; Hess 1930). The use of radiographic and microscopic methods for vitamin D deficiency assessment can aid in detecting additionally affected individuals and may support diagnoses. Radiographs of bone can provide further information on the stage of the disease. Porosity of the bone along the growth margin, thinning of the cortex, and fraying of the growth plate are radiographic features that are attributed to active pathological changes due to rickets, whereas healing changes observable on radiographs include thickening of the cortex, more defined growth plate margins, and trabecular coarsening in distal metaphyses due to mineralisation (Berry et al. 2002; Brickley and Ives 2008; Hess 1930; Pettifor and Daniels 1997). Radiographic features of residual rickets are few, since vitamin D deficiency in childhood was overcome and the process of growth and remodelling will have obliterated most features of rickets (Brickley and Ives 2008). Thickening of the cortical bone on the opposing side of the location were bending deformities occurred may be visible on radiographs (Hess 1930; Mankin 1974). Radiographic features of osteomalacia include generalised osteopenia (lower bone mineral density), pseudofractures in the ribs and sternum, the pelvis, long bones, scapulae, hands, and feet (Berry et al. 2002; Brickley and Ives 2008; Brickley et al. 2007; 2008; Hess 1930; Mankin 1974).

Radiographs taken from permanent molars enable assessment of the pulp chamber shape, whereby a constricted or chair-shaped pulp chamber is indicative of vitamin D deficiency (D'Ortenzio et al. 2017). Micro-CT scans of the teeth enable the assessment of interglobular dentine (IGD), a microscopic mineralisation defect attributed to vitamin D deficiency (Brickley et al. 2017b; D'Ortenzio et al. 2016). This method may provide supporting information for initial macroscopic diagnosis, but may also detect vitamin D deficient individuals without visible macroscopic lesions. Furthermore, assessment of IGD can provide information on the age of vitamin D deficiency onset and the number of deficient periods per individual.

Histological features

Microscopic assessment of bone can provide additional data that may support vitamin D deficiency diagnosis, although most microscopic features are not diagnostic for this deficiency. The cortical bone may show increased numbers of resorption sites, display increased cortical porosity, and may show mineralisation defects adjacent to the cement lines (Brickley and Ives 2008; Mankin 1974). The trabecular bone may display increased resorption, newly formed bone may appear separated from more mature sections, mineralisation defects may be adjacent to cement lines, and if mineralisation occurs the quality of the bone will appear poor (Brickley and Ives 2008; Mankin 1974). Histological analysis of teeth will enable assessment of IGD and may support initial macroscopic and radiological diagnosis. Similar to micro-CT analysis, it can detect affected individuals that do not display clear macroscopic lesions, and provides information on the age of vitamin D deficiency onset and the number of deficient periods per individual.

Etiology of vitamin D deficiency

The majority of individuals with skeletal deformities due to vitamin D deficiency had inadequate exposure to sunlight and/or diets low in vitamin D (Brickley et al. 2014; Mithal et al. 2009). Geographic characteristics, such as latitude and climate, influence the availability of sunlight and the wavelengths of UVB radiation required for the production of vitamin D (290 – 315 nm) (Holick et al. 1981). The Netherlands has a latitude between 50° and 53°N and production of vitamin D during the winter months is expected to be absent since at latitudes above 35°N and below 35°S, there is no production of vitamin D in the winter months (Jablonski and Chaplin 2013; Lee et al. 2008; Webb et al. 1988). In addition, the time of day also can influence the amount of vitamin D that can be produced in the skin, since the angle of sunlight changes during the day and a large number of UV photons are filtered in the atmosphere at oblique angles causing differences in cutaneous vitamin D

Table 1. Hereditary or acquired conditions causing rickets and/or osteomalacia.

Acquired or Inherited condition	Manifestations
Hereditary vitamin D-dependent rickets Type I	Defect in the hydroxylation that occurs in the kidneys to form active vitamin D
	Pseudo-vitamin D deficiency rickets
Hereditary vitamin D-dependent rickets Type II	Mutations in vitamin D receptor gene cause vitamin D resistant rickets
Hereditary vitamin D-depenedent rickets Type III	Resistant to the action of active vitamin D
	Vitamin D deficiency occurs despite normal intake of vitamin D
	Severe skeletal deformities
25-OHase-deficiency rickets	Defect in the hydroxylation that occurs in the liver, thus limiting amount of vitamin D passed to the kidneys
Fibroblast growth factor 23 (FGF23)	Defect in phosphorous homeostasis: failure or excessive production of FGF23
Autosomal dominant hypophosphatemic rickets	Mutation of the FGF23 gene
	Increased levels of phosphorous excretion
	Increased levels of growth hormone
	Decreased absorption of phosphorous from the diet
X-linked hypophosphatemic rickets	Hypophosphatemia and decreased intestinal absorption of calcium and phosphorous
	May increase expression of FGF23 affecting phosphorous metabolism

production during the day (Holick 1994; 2003; 2005b; Holick and Chen 2008). With age, 7-dehydrocholesterol levels decline and less vitamin D can be produced in older compared to younger individuals after the same exposure to sunlight (Holick 2003; Lee et al. 2008). In addition, skin thickness and kidney function decrease with age, markedly affecting vitamin D levels in older individuals (Chapuy and Meunier 1997; Halloran and Portale 1997). Other factors that affect the amount of vitamin D produced in the skin include clothing, spending time indoors, and sunscreen, although the latter will not have affected past populations (Brickley and Ives 2008; Brickley et al. 2014; Holick 1994).

There are a number of congenital or acquired pathological conditions that can cause vitamin D deficiency. Table 1 lists a number of these conditions as described by Berry et al. (2002), Glorieux (1999), Holick (2006), Liberman and Marx (1999), Nield et al. (2006), and Reginato and Coquia (2003), and White and Thakker (2005).

All of these conditions are very rare and therefore, it is unlikely that individuals in archaeological populations were affected by one of these conditions. Thus, it is assumed that bending deformities due to a shortage of vitamin D most likely are the result of insufficient dermal synthesis perhaps combined with suboptimal diets (Brickley et al. 2014; Mays 2008; Mithal et al. 2009).

SECTION 3: SKELETAL COLLECTIONS

For this research, six skeletal collections were selected from different parts of the Netherlands and their location is shown in figure 1 in Section 1. Table 2 provides an overview of the total number of individuals analysed divided into nonadults and adults with a further distinction between males (M) and females (F), site type, and average socioeconomic status.

The first and largest collection comes from Beemster, a predominantly 19th

Table 2. Number of nonadults and adult females and males assessed for vitamin D deficiency with socioeconomic status and site type (rural, urban or psychiatric institution) and size (small vs. large) for each site.

Sites	Nonadults						
Adults	Total Individuals					Average SES	Site type
		F	M	Total			
Beemster	95	100	100	200	295	M – L	Rural
Bloemendaal	0	39	28	67	67	M - L	Psychiatric hospital
Gouda	4	18	23	41	45	H	Small, urban
Rotterdam	3	16	18	34	37	M – L	Large, urban
Roosendaal	15	18	27	45	60	M – L	Small, urban
Hattem	28	47	53	100	128	M – L	Small, urban
TOTAL	145	238	249	487	632		

SES = socioeconomic status, F = female, M = male, M – L = middle to low, H = high.

century rural community in the province of Noord-Holland. The main activities of the inhabitants concerned cattle farming (Falger et al. 2012), and archival data show the individuals buried in this cemetery had a middle to low SES. The collection of Beemster is the largest of the sites and enables vitamin D deficiency assessment of all age groups and both sexes in a rural community from a province typified as urban (De Vries and Van der Woude 1977).

Bloemendaal is the second collection and the skeletal remains come from 19th century Meerenberg psychiatric hospital, where both deceased patients and staff were buried (Vijselaar 1997). This site was selected because of the risk of osteomalacia development was expected to be elevated due to confinement of the patients rather than factors attributed to industrialisation, and provided a unique opportunity to research and present the first data on osteomalacia in the Netherlands.

The third collection consists of individuals from Gouda that come from inside the Sint-Janskerk (St. John's church) from the 17th to 19th centuries. Burial records were available for the majority of individuals and indicated they had a high SES, some of them being regents, mayors and prefects (Van Dasselaar 2015). Generally, it is assumed that individuals with high SES did not experience vitamin D deficiency, as observed in the high SES collections from Alkmaar (Baetsen 2001) and Zutphen (Berk 2007), since their living conditions and diets are expected to be better than those of lower SES. This collection enables assessment of vitamin D deficiency in a high SES community to test this assumption.

Rotterdam, the fourth and smallest collection, was an urban centre in the province of Zuid-Holland, and already in the 17th century important in the sea faring trade (Van der Bent et al. 2011). The skeletal remains come from the cemetery surrounding the Laurenskerk (Laurence church) that are dated to the 17th century, and their SES is suggested to be middle to low. The analysis of vitamin D deficiency in the skeletal remains from this urban centre dating to the Golden Age improves our understanding of the influence of urbanisation, increasing prosperity, and other economic and political changes on vitamin D deficiency development.

The fifth skeletal collection, Roosendaal, comes from a cemetery surrounding the Sint -Janskerk (St. John's church) from the 17th to 19th centuries. This small urban centre in the province of Noord-Brabant was strategically positioned between the larger cities of Dordrecht and 's-Hertogenbosch, and its main activities were agriculture and a small-scale textile industry (Van Gastel 1995). Historic sources suggest the population buried outside the church consisted of individuals with a middle to low SES, although the majority are postulated to have been poor (Van Gastel 1995). The collection of Roosendaal enables assessment of vitamin D deficiency prevalence in a small urban centre from one of the poorer provinces that was typified as rural traditional (De Vries and Van der Woude 1977).

Hattem is the last collection. Historic sources report Hattem to have been a small, prosperous town during the 16th century as part of the Hanseatic League, but a number of attacks of the Spanish Army at the end of this century depleted Hattem's resources and destroyed large parts of the town (Sypkens Smit 1964). Several periods of French occupation in the 17th and 18th centuries caused further impoverishment (Sypkens Smit 1964). Hattem's main activities consisted of agriculture and the transportation of goods by ship such as peat (Koridon 1985; Scheper 1984). The individuals buried outside the Andreaskerk (Andrew's church) in the 17th to 19th centuries are expected to have had a middle to low SES. Hattem is a larger collection from the rural traditional area (De Vries and Van der Woude 1977) that further improves our understanding of vitamin D deficiency development in this part of the Netherlands.

Section 4. Sub-questions and dissertation organisation

The main research question is divided into three sub-questions that will be answered by determining vitamin D deficiency prevalence in six Dutch communities from the 17th to 19th centuries. These sites were selected based on differences in settlement type (rural vs. urban, including Bloemendaal from a psychiatric hospital), location (provinces of Gelderland, Noord- and Zuid-Holland, and Noord-Brabant), all SES groups (high vs. middle to low), all age groups, and both sexes. In this way, the influence of industrialisation on sociocultural practices (e.g. SES, clothing, and gendered activities) related to vitamin D deficiency development in these six communities can be assessed, which will provide a more complete understanding of vitamin D deficiency prevalence in the Netherlands and improve our understanding of the process of industrialisation in the Netherlands.

Sub-question 1: How did differences in population aggregation affect vitamin D deficiency prevalence in 17th to 19th century Netherlands?

Differences in population size, living conditions, activities, and availability of foods are likely to affect vitamin D deficiency frequency. Factors such as air pollution and tall buildings blocking the sunlight are commonly considered to be the main cause for high vitamin D deficiency prevalence (Baetsen 2001; Holick 2003; Maat et al. 2002). However, other factors are likely to be at play as demonstrated by several researchers for modern (Van der Meer et al. 2006; Underwood and Margetts 1987; Yassin and Lubad 2010) and archaeological populations (Giuffra et al. 2015; Palkovich 2012; Pētersone-Gordina et al. 2013). By examining communities from both rural and urban locations, changes in urban (factory) and rural (farming) industrialisation on vitamin D levels can be most effectively compared and contrasted. Combined with information from historic sources, the impact of settlement size, various activities, and the availability of foods can be assessed which will aid in understanding regional variation of industrialisation and its influence on the population, as will be demonstrated in Chapters 2, 4, and 5.

Sub-question 2: How is vitamin D deficiency prevalence influenced by age, sex, and gender in 17th to 19th century Netherlands?

Vitamin D deficiency prevalence between various age and sex groups will be compared. Differences in age-related prevalences will enhance our knowledge of behavioural patterns in various stages of life. Information on the effect of industrialisation on specific age groups is limited, in particular information on nonadults is virtually lacking from the historical record (Wintle 2000). Determining differences in age-related vitamin D deficiency prevalence will enhance our understanding of changes in behaviour and activity throughout life and will aid in decreasing the paucity of information on nonadults. In addition, differences in vitamin D deficiency prevalence that can be attributed to sex and gendered roles will provide information on traditions and community norms. This way, changes in gender-related behaviours will be informative on the effect of industrialisation on community life. Chapters 3 and 4 show the impact of gender-related activities on the prevalence of vitamin D deficiency in several rural and urban sites in post-Medieval Netherlands. The influence of age on the prevalence of vitamin D deficiency will be shown in Chapters 2, 4, and 5. In Chapter 6, the impact of other sociocultural practices will be demonstrated in a population from the psychiatric hospital of Meerenberg.

Sub-question 3: : How did socioeconomic status influence vitamin D deficiency prevalence in 17th to 19th century Netherlands?

It is commonly assumed that individuals of high socioeconomic status were less affected by vitamin D deficiency than those of lower socioeconomic status (Baetsen 2001; Maat et al. 2002). However, behavioural patterns, and the availability of foods that can be attributed to these statuses, need to be determined. By combining archival data with the results from osteoarchaeological analysis, socioeconomic status, activity and vitamin D deficiency frequency can be linked, thus testing this assumption and enhancing our understanding of differences in socioeconomic classes in past populations in the Netherlands. The influence of socioeconomic status will be demonstrated in Chapter 4.

Dissertation organisation

In Chapter 2, assessment of 95 nonadults from the rural community of Beemster is discussed. Chapter 3 demonstrates residual rickets prevalence in 200 Beemster adults and Chapter 4 provides an overview of vitamin D deficiency prevalence in five collections: Beemster, Gouda, Rotterdam, Roosendaal, and Hattem. In the study presented in Chapter 5, micro-CT and histological analysis of IGD in teeth is performed, and macroscopic, radiological, and histologic vitamin D detection methods are compared to observe the merits of each. Chapter 6 presents the results of the analysis of the skeletal remains from the Meerenberg psychiatric hospital from Bloemendaal focussing on the improvement of osteomalacia detection. Chapter 7 contains the overarching conclusion in which the main and sub-research questions will be answered.

CONCLUSION

The analysis of vitamin D deficiency in human skeletal remains can provide information on sociocultural practices and other variables related to sunlight exposure in past communities that cannot be obtained from historic sources alone. Although numerous studies describe the economic and political changes in 17th to 19th century Netherlands (De Vries 2000; De Vries and Van der Woude 1977; Mokyr 1976; 2000; Wintle 2000), the effect of important processes, such as industrialisation, on local communities is hard to assess without information about individuals from these past communities. This study intends to do just that and is the first to undertake a large-scale assessment of vitamin D deficiency in Dutch skeletal remains by evaluating vitamin D deficiency prevalence in six 17th to 19th century communities of different settlement size, region, occupation, and SES. Biological and cultural characteristics of individuals from all age groups combined with historic information facilitate a life course perspective to provide a more profound understanding of the range of factors increasing the risk of vitamin D deficiency development, including industrialisation. The use of the recently developed methods of IGD assessment in teeth via micro-CT and histology (Brickley et al. 2017; D'Ortenzio et al. 2016), generate novel data that cannot be obtained from macroscopic and radiographic analysis of bone, such as the age of rickets onset, and the number of vitamin D deficient periods per individual. The data from micro-CT and histological analysis aid in the reconstruction of a more accurate overview of vitamin D deficiency prevalence in 17th to 19th century

Netherlands, and facilitate a more nuanced understanding of vitamin D deficiency development within certain age groups. This study contributes to our understanding of the transformation that Dutch communities underwent in this period of significant economic, political, and social change via macroscopic, radiographic, and histologic assessment of vitamin D deficiency prevalence.

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2 Rural Rickets: Vitamin D Deficiency in a Post-Medieval Farming Community from the Netherlands

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ABSTRACT

Rickets is caused by vitamin D deficiency as a result of limited exposure to sunlight and inadequate diet. In the 19th century, rickets was endemic in most northern European cities. In post-Medieval Netherlands, rickets is documented in low frequencies in a few urban samples, but has not been studied in contemporaneous rural populations. Beemster is a rural farming community in the Netherlands that was established in the 17th century upon drained land, with the Middenbeemster cemetery in use until 1866 AD. Ninety-five individuals from the ages of 32 weeks *in utero* to 15 years were examined for rickets in order to understand factors that can cause vitamin D deficiency in rural, non-industrialized populations. To identify rickets in the Beemster sample, ten features were scored, with bending deformities of the lower limb and one other feature, or at least three non-bending features, having to be present in order for diagnosis. Nine individuals (9.5%) had evidence of rickets—a high prevalence, especially for a rural community where ample sunlight was available. The two and three year old Beemster infants were most heavily affected with an age-specific prevalence of 30.4%. Two three-month-old infants also had rickets. Some of the affected may have developed rickets secondarily, as a result of a different illness, but cultural practices including prolonged swaddling, occlusive clothing, and keeping the young indoors, are suggested to have contributed to this high rickets prevalence. Dietary variables including poor weaning foods and common episodes of malnutrition may have also contributed to vitamin D deficiency. This study demonstrates the value of careful analysis of pathological conditions in subadults and highlights that rickets was not only a disease of cities, but affected populations that would appear to have been at low risk, because of maladaptive cultural practices.

INTRODUCTION

Rickets is a metabolic bone disease with multiple causes. The most common cause is a vitamin D deficiency. Vitamin D is needed for mineralization of newly formed bone matrix, osteoid. When mineralization is impaired due to a prolonged vitamin D deficiency, the bones become soft and will bend due to weight bearing and muscular tension (Ortner, 2003; Brickley and Ives, 2008; Waldron, 2009). The manifestation of vitamin D deficiency in subadults is referred to as rickets, and in adults, osteomalacia. The most important source of vitamin D is dermal synthesis under the influence of UV-B in sunlight. Climate and latitude affect the amount of UV-B and thus the amount of vitamin D that can be produced in the skin. Another way of acquiring this vitamin is by consuming fatty fish (e.g. salmon and tuna) and some other foods (e.g. egg yolk), but the amount in food is low (Holick, 2003). Today, our foods are fortified with vitamin D, and the availability of supplements makes rickets very rare in developed nations (Brickley and Ives, 2008). Neither fortified foods nor supplements were available in the past so limited exposure to sunlight and inadequate diet led to vitamin D deficiency and rickets in many populations.

Figure 1. Map of the Netherlands divided into provinces (after http://nl.wikipedia.org/wiki/provincien_van_nederland).

Beemster is located in the province of North Holland, The Netherlands (Figure 1). The Netherlands have a latitude of 52°N, which almost entirely impedes dermal production of vitamin D from November to March (Holick, 2003). However, sufficient exposure to sunlight in the spring and summer months would provide stores for an entire year and replenish a deficiency (Holick, 2003). Therefore, climatic factors alone cannot adequately explain the occurrence of rickets in Dutch populations, and sociocultural variables must be considered.

Previous studies of post-Medieval urban collections in the Netherlands found crude rickets prevalences that varied from zero (Baetsen, 2001) to 4.4% (Maat *et al.*, 2002). Studies of rural Dutch communities from this time period are lacking. Comparatively few studies have reported evidence for rickets as caused by non-urban specific causes (i.e. Molla *et al.*, 2000; van der Meer *et al.*, 2006; Mays 2007; Hatun *et al.*, 2011; Palkovich, 2012). This is in part due to the excavation of more urban sites, but is also the result of the preconception that rickets was a disease of the cities, resulting from pollution, crowding, and too much time indoors. As this paper demonstrates, other cultural variables not tied to an urban versus rural juxtaposition, including child care practices, clothing, and diet, can play a major role in rickets prevalence. As well, less research has been done on rickets in past Dutch populations compared to past British populations. More studies of rickets in past peoples from mainland Europe are needed to better understand geographic similarities and differences in etiology.

The aims of this paper are two-fold. First, to present data on the Beemster individuals with rickets in order to draw more attention to the existence of rickets in rural communities, and in countries other than the United Kingdom. Second, to focus on maladaptive sociocultural and dietary variables that may have been interacting to cause vitamin D deficiency in subadults under the age of four.

MATERIALS AND METHODS

The Beemster was established in the 17th century when the former Beemster Lake was drained. The land was parceled out and a large number of farms and manorial estates were built, owned by rich merchants and governors from Amsterdam, and serving as summer-residences (de Jong, 1998). The farming land was meant for agriculture, but due to the high water table and composition of the soil, the land was converted to pastures for cattle breeding (de Jong, 1998). During the 17th and beginning of the 18th centuries, Beemster enjoyed prosperous times by trading its products of wool, butter, cheese, and cattle (de Jong, 1998). However, by mid-18th century, the population experienced more frequent and severe hardships. For example, episodes of rodent infestation in the mid-18th and 19th centuries destroyed pastures and crops, and their rummaging weakened the dams which frequently resulted in partial flooding of Beemster (Falger *et al.*, 2012). A rinder pest in 1744 AD killed twothirds of the cattle and another in 1769 AD killed about half, severely impoverishing the community (Falger *et al.*, 2012). From 1845 to 1847 AD, much of Western Europe, including the Netherlands, experienced potato, rye, and wheat crop failures (Bergman, 1967; Vanhaute *et al.*, 2007). Thus, periods of dietary inadequacy were likely to have affected the Beemster community.

The cemetery of Middenbeemster, located in the center of Beemster, was excavated in the summer of 2011. It was in use from 1617 to 1866 AD, although most individuals date from the 19th century according to archival sources. Approximately 450 individuals were excavated including both sexes and all ages, their preservation being very good. The sample for this research is based on assessment of 450 individuals, from which 95 individuals with adequate completeness and preservation fell into the relevant age range of fetal (youngest at 32 week *in utero*) to 15 years. Fifteen years served as the upper age limit because with epiphyseal fusion of long bones, growth of that area ceases, and osseous changes of rickets would be less visible.

Age was estimated using a combination of several methods: dental measurements of both deciduous and permanent teeth by Liversidge *et al.* (1998), dental development of deciduous teeth by Demirjian *et al.* (1973), dental development of permanent teeth by Moorrees *et al.* (1963), and dental eruption by Ubelaker (1979). For those individuals whose teeth were unobservable, age was estimated based on the stage of bone and epiphyseal fusion by Schaefer *et al.* (2009), long-bone length by Maresh (1970), and clavicle length by Black and Scheuer (1996). Age categories of one year were used, except for the neonates (less than one month of age) who were grouped together with the full-term fetuses (>37 weeks *in utero*) into a perinate category.

A form was developed scoring ten macroscopic features of rickets as described by Ortner and Mays (1998) and refined by Brickley and Ives (2008). Table 1 shows the scores for all

Table 1. Overview of affected individuals

Individual	MB11S032V082	MB11S038V026	MB11S046V023	MB11S062V071	MB11S165V242	MB11S189V332	MB11S314V655	MB11S316V641	MB11S343V732	Frequency of feature
Features										
Mean age	3.5 years	2.2 years	2.5 years	3.0 years	2.5 years	3.5 months	3.0 months	3.0 years	3.5 years	
Cranium porosity	A	-	A	P	-	A	P	A	A	28.6%(2/7)
Orbital roof porosity	P	-	A	-	P	P	-	P	A	66.7%(4/6)
Mandibular ramus angulation	A	-	P	-	A	-	-	A	A	20.0%(1/5)
Deformation arms	P	-	A	P	A	-	P	A	A	42.9%(3/7)
Deformation legs	P	P	P	P	P	P	A	P	P	88.9%(8/9)
Flaring of costochondral rib ends	A	P	P	P	-	-	P	A	A	57.1%(4/7)
Cortex of rib ends porous and irregular	A	P	A	P	-	-	P	A	A	42.9%(3/7)
Irregularities of metaphyses of long bones	P	-	P	P	P	P	P	P	P	100%(8/8)
Cortex of metaphyses irregular and porous	A	-	P	P	P	-	P	P	A	71.4%(5/7)
Thickening of long bones	P	-	P	P	P	P	P	A	P	87.5%(7/8)

Overview of affected individuals with each feature scored as either present (P), absent (A), or unobservable (-).

features for each affected individual. The feature 'Growth plate abnormality of long bones' as defined by Ortner and Mays (1998:46), mostly concerns irregularities of the epiphyseal surface and underlying porosity. However, since abnormality of the growth plate in this paper includes cupping and flaring, this feature is renamed to 'irregularities of the metaphyses of long bones'.

Other developmental and pathological processes can result in osseous changes that mimic those of vitamin D deficiency. Therefore, at least three diagnostic features needed to be present, or bending deformities of the long bones and one other feature, for the diagnosis to be rickets. In addition, a distinction was made between healed and active rickets based on the definition of Ortner and Mays (1998): active cases of rickets show porosity of cortical bone in the cranial or postcranial skeleton, and/or growth plate abnormality.

To provide context to the data, Beemster is explicitly compared to four sites from the United Kingdom, the best studied region in Europe. Broadgate and Spitalfields, both located in London (Pinhasi *et al.*, 2006), and St. Martin's in Birmingham (Brickley *et al.*, 2006), are urban sites that date to the same time period as Beemster. Wharram Percy in York (Mays, 2007) occurred earlier during the late-Medieval period but is included because it was a rural community.

RESULTS

Nine individuals have evidence of rickets. Figure 2 presents the distribution of affected individuals. No individuals aged 11, 13, or 15 years (with unfused long bone epiphyses) were encountered. It is not known why so few one-year olds were encountered (n = 2), but because of their underrepresentation, interpretations for this age group are not offered. Table 1 provides an overview of all affected individuals, their mean age, the scoring of each macroscopic feature as either present (P), absent (A), or unobservable (-), and the frequency of features observed. In Table 2, the distinction is made between active and healed rickets. Five cases were in an active state, and four were in a healed state. All healed cases occur in the three year olds, revealing a trend whereby only older infants moved into the healing process.

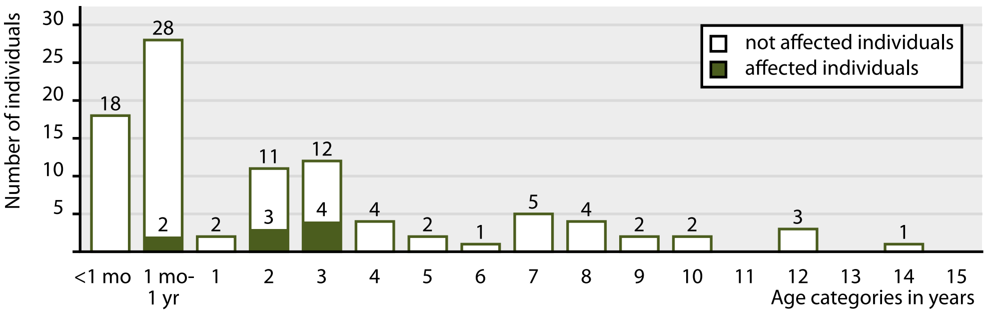

Figure 2. Total number of individuals and the total of individuals affected by rickets. Age is in years, unless stated; 3 denotes 3.00–3.99 and so on.

Table 2. Rickets phase

Individual	Mean age	Phase
S032V082	3.5 years	healed
S038V026	2.2 years	active
S046V023	2.5 years	active
S062V071	3.0 years	active
S165V242	2.5 years	active
S189V332	3.5 months	healed
S314V655	3.0 months	active
S316V641	3.0 years	healed
S343V732	3.5 years	healed

Figure 4. Rachitic rosary of rib 1 to rib 7 of individual MB11S062V071(photograph B. Veselka 2012).

Of the affected subadults, individual MB11S062V071 of 3.0 years +/ 12 months of age, suffered most clearly from active rickets. Cranial vault porosity and deformation of both upper and lower limbs are evident. All metaphyseal ends are enlarged and display thickening and deformation of which Figure 3 is an example. The sternal ends of the ribs were enlarged, resulting in a rachitic rosary (Figure 4). Individual MB11S032V082 of 3.5 years +/ 12 months of age also is a clear case of rickets, but in the healing phase, with pronounced bending deformities of the tibiae and fibulae (Figure 5) and upper limb bones, but lacking some other morphological changes such as porous and irregular metaphyseal cortices.

The crude rickets prevalence of the Beemster subadults is 9.5%. Age-specific rickets prevalences provide more information. Rickets prevalence in the two year olds is 27.3% and in the three year olds 33.3%. Finally, two young infants around three months of age also had rickets, for an age-specific prevalence of 7.1% in the under one-year group. Table 3 shows the age based comparison of Beemster to three of the British collections (Broadgate, Spitalfields, and Wharram Percy). The comparison of Beemster to the fourth British collection of St. Martin's in Birmingham (Brickley

Figure 3. Tibiae and fibulae of individual MB11S062V071 showingenlargement and bending deformities (photograph B. Veselka 2012).

Figure 5. Tibiae and fibulae of individual MB11S032V082 show marked bending deformities (photograph B. Veselka 2012).

Table 3. Comparison between Dutch and British collections

	Beemster			Broadgate*			Spitalfields*			Wharram Percy**		
Age	N	Na	P(%)	N	Na	P(%)	N	Na	P(%)	N	Na	P(%)
0	34	2	5.9	9	1	11.1	38	6	15.8	32	0	0
1	2	0	0	5	0	0	27	7	25.9	69	6	8.7
2	11	3	27.3	4	1	25.0	12	0	0	30	2	6.7
3	12	4	33.3	3	2	66.7	6	1	16.7	21	0	0
4	4	0	0	5	2	40.0	1	0	0	11	0	0
5	2	0	0	2	1	50.0	5	0	0	21	0	0
6	1	0	0	7	0	0	6	0	0	23	0	0
Total	66	9	13.6	35	7	20.0	95	14	14.7	207	8	3.9

N is the total of individuals; Na is the total of affected individuals; P is the prevalence.
*= data from Pinhasi et al. (2006),
**= data from Mays (2007)
Age in years; 3 denotes 3.00–3.99 and so on.

et al., 2006) is shown in Table 4 due to differently comprised age categories.

The frequency of each macroscopic feature of rickets is noted to provide a better understanding of its diagnostic value (Table 1). Deformation of the leg bones was observed commonly, in all cases but a single three-month-old infant. All individuals with observable metaphyses had irregularities, and many had thickening. The features that occurred least are those on the cranium: porosity and mandibular ramus angulation.

DISCUSSION

Differential diagnosis

Confounding factors in the diagnosis of rickets are, as Brickley and Ives (2008:105) point out, that many of the features are not pathognomonic. For example, orbital roof porosity is a feature that occurs in a number of pathological conditions and is therefore considered to be a non-specific stress marker. Thus, its high frequency in this sample could be because of rickets, but other conditions may be involved. Scurvy, which is caused by vitamin C deficiency, prevents osteoid from being secreted and causes features such as cranial vault porosity and swelling of the costochondral rib ends (Brickley and Ives, 2008:103–105). While it is possible scurvy could be contributing to the macromorphological changes seen in the Beemster subadults, it rarely results in bowing deformities (Ortner, 2003; Brickley and Ives, 2008; Waldron, 2009) so is unlikely to be the primary cause of all the Beemster cases, as they all have bowing of the upper or lower limb bones. Congenital syphilis can cause bending deformities, but has other pathognomonic markers that would be easily noted when assessing the entire skeleton (Waldron, 2009) and can be discounted as a cause. Other pathological causes of bending deformities in the lower limbs include Blount's disease and congenital defects (Waldron, 2009). Blount's disease is a rare, acquired, and progressive growth

Table 4. Comparison between Beemster and St. Martin's*

	Beemster			St. Martin's*		
Age	N	Na	P(%)	N	Na	P(%)
Infant	47	5	10.6	73	14	19.2
Child	36	4	11.1	52	6	11.5
Total	83	9	10.8	125	20	16.0

N is the total of individuals; Na is the total of affected individuals;
P is the prevalence.
*= data from Brickley et al. (2006)
Infant = birth - 3 years; Child = 4 - 12 years

disorder of the proximal tibial epiphyses and metaphyses, producing a sharp lateral bend that is usually asymmetric (Cheema *et al.*, 2003). The deformities of Blount's disease (tibia vara) differ from the ones reported by Brickley *et al.* (2010) where tibial deformities were observed in the proximal third of the shaft. Bending deformities of the Beemster tibiae occur medially in the proximal third of the shaft and are symmetric, thus ruling out Blount's disease.

Congenital bowing of the tibia is usually convex with the bending oriented posteriorly and medially. This does not match the bowing of the Beemster subadults. As well, congenital bowing is rare (Brickley *et al.*, 2010) and will not cause the other osseous changes included in our diagnostic method, whereby a minimum of three non-bending features, or bending deformities and one other feature, had to be present, thus ensuring the exclusion of congenital bowing.

Often some degree of bowing is present in children's lower limbs which could be mistaken for rickets (Brickley *et al.*, 2010). Bleck (1982) researched several forms of bowing in children's lower limbs and found most were due to normal developmental processes which usually resolved in the course of maturation. Brickley *et al.* (2010) compared several of the bending deformities examined by Bleck (1982) to the ones found in their study. One of the typical deformities was an anterior twist of the head and neck of the femur. The deformities of the femora noted by Brickley *et al.* (2010) differed by having an anterior curvature of the proximal third of the femoral shaft below the level of the lesser trochanter. In Beemster, the femora had similar bending deformities as the ones reported by Brickley *et al.* (2010). Thus, the observed bowed femora are likely not due to normal developmental processes.

Overall, the observed morphological changes in the affected Beemster individuals are most consistent with a diagnosis of rickets. Certain features may be more common in one sample than another. For example, Ortner and Mays (1998) found medial angulation of the mandibular ramus to be common, but this feature was encountered only rarely in the Beemster subadults, as in the studies of Pinhasi *et al.* (2006) and Mays *et al.* (2006). In the Beemster sample, the cranial traits were least common, while postcranial changes, especially those of the long bones were most common, particularly bending deformities of the legs and irregularities and porosity of the metaphyseal ends. These osseous changes are diagnostic for rickets (Ortner, 2003; Brickley and Ives 2008; Waldron, 2009). Assessing as many reliably characteristic features as possible will lead to a diagnosis that is clear-cut and accurate.

Etiology

While inadequate sunlight is by far the most common cause of rickets, there are other causes of vitamin D deficiency. There are inherited and acquired forms of rickets due to problems in the synthesis of vitamin D either by the liver or kidneys or alterations in mineral metabolism (Brickley and Ives, 2008), and hypophosphatia, which is a disorder of low blood phosphate levels, that causes rachitic changes (Brickley and Ives, 2008). All of these conditions are rare and therefore unlikely to be the cause of rickets in an archaeological sample.

For the Wharram Percy collection, Ortner and Mays (1998) suggested that the occurrence of rickets in only an active state could be partially explained if it formed secondarily. Historical sources and archival documents from the Beemster note the presence of many infectious diseases and episodes of food shortage which would have caused substantial infant morbidity and mortality (Bergman, 1967; Vanhaute *et al.*, 2007; Falger *et al.*, 2012), so it is possible that some rickets cases developed secondarily. However, rickets prevalence in Beemster is markedly higher than in Wharram Percy (Table 3) which implies that not all cases are likely to be secondary. Moreover, the mix of active and healed cases in the Beemster sample, and wider demographic spread of affected individuals, suggests rickets developed in a primary context as well.

Unlike in urban settings architecture, diminishing sunlight and smoke from industrial factories were not factors responsible for causing rickets in the Beemster subadults. As a rural community, abundant sunlight was readily available in the spring and summer months. With a latitude of 52°N, however, dermal synthesis of vitamin D is almost entirely impeded in winter and early spring (Holick, 2003). During that part of the year, the entire population would be dependent on bodily stores and diet for their required supply of vitamin D. Those with low vitamin D stores and/or an inadequate diet would have been especially vulnerable to developing rickets from November to March. Thus, the main consideration for why an individual developed rickets is cultural factors that led to low bodily stores of vitamin D.

As well, the contribution of dietary sources of vitamin D must be considered, because the foremost vitamin D containing foods can sometimes provide enough to prevent the development of rickets. Fatty fish and cod liver oil have the highest amount of vitamin D, with foods like egg yolk and beef liver having lower amounts (Holick, 2006). Yet, episodes causing food shortages such as crop failures, livestock epidemics, and low fish procurement, common throughout the mid-18th and 19th centuries in Holland (Bergman, 1967; van Poppel *et al.*, 2005; Vanhaute *et al.*, 2007), may have limited access to these foods. Regardless, the dietary contribution of vitamin D is comparatively minimal. Those that developed rickets likely had low preexisting vitamin D stores entering the winter months, possibly exacerbated by a diet with inadequate vitamin D. Infants born in the fall or winter would be reliant on fetal accumulation, breastmilk, and possibly early weaning foods (see below) for vitamin D. By about eight weeks after birth, transplacental stores of vitamin D are expired, and breastmilk is a poor source of vitamin D, especially in mothers with low levels (Henderson, 2005). Thus, rickets in the two Beemster three month olds may have been seasonal.

Most of the two year olds with rickets died while it was in an active phase, while most of the three year olds had evidence of healing (Table 2). This suggests that the vitamin D deficiency began at the age of two years, and that those who survived past this age were able to enter a phase of healing. What would have made two year olds most vulnerable? Poor weaning foods and cultural practices, including long periods of swaddling, occlusive clothing, and being kept indoors, all could have resulted in low vitamin D levels in two-year-old infants.

Weaning foods were likely quite similar among households, with rather homogenous options including cow or goat milk and paps made of grains such as wheat and rye. Weaning foods made with cow's milk are low in vitamin D, and also low in calcium compared to breast milk (Henderson, 2005). Inadequate calcium (hypocalcaemia) leads to an increase in vitamin D requirements to restore the unbound levels of calcium (Brickley and Ives, 2008), thus raising the risk of developing rickets. Weaning foods containing wheat or rye have a high level of phytic acid which inhibits iron and zinc absorption, reducing calcium levels, thus increasing vitamin D requirements (Coulibaly *et al.*, 2011). While there are several ways to prepare cereal-based foods that will reduce or neutralize phytic acid, such as germination and fermentation, these methods are not commonly used in the preparation of weaning foods (Coulibaly *et al.*, 2011). Clearly, common Beemster weaning foods could have contributed to vitamin D deficiency. In addition, the quality of drinking water in Dutch coastal areas in the 18th and 19th century was poor due to the high water table and gradual salinization (van Poppel *et al.*, 2005). The consumption of polluted water may have been a major cause of gastrointestinal illness, decreasing consumption and absorption of critical nutrients, the number one cause of infant mortality (van Poppel *et al.*, 2005). Thus, it is perhaps not surprising that only the infants in Beemster were affected by rickets, as commonly eaten foods either increased vitamin D requirements or caused illness that impacted food intake and/or absorption.

Prolonged swaddling, which implies swaddling over a period of more than six months, was practiced in some farming communities (de Leeuw, 1992) and would have diminished sunlight exposure. Swaddling was thought to ensure straight growth, keep infants warm, promote sleep, and prevent harm, and would also give caregivers more time to tend to other tasks (Gerard *et al.*, 2002). Although already in 1762 AD, philosopher Rousseau warned against swaddling clothes, family tradition, and community norms would have largely determined its duration (Lipton *et al.*, 1965). Van Poppel *et al.* (2005) suggested that mothers of lower socioeconomic status were more likely to swaddle their infants for a longer period of time. Obviously, rickets in the two three month olds from Beemster may have been caused or exacerbated by swaddling, but even rickets in the older two year olds may have been partially caused by low levels of vitamin D during the first year of life because of prolonged swaddling. The low number of infants of one year of age limits assessment of this possibility.

What factors could be limiting dermal synthesis of vitamin D for infants past the age of swaddling? Children younger than four or five years of age would not have been able to genuinely help their parents on the land. During periods of increased farm work, when mothers were needed to help on the land, infants and children were tended for by their grandmothers or older sisters (Schenkeveld, 2008). Tasked with domestic chores in and around the house, caregivers may have kept the young inside, thus inhibiting sunlight exposure. As such, gender-based labour norms could be influencing the amount of sunlight that individuals received. In post-Medieval Netherlands, the division of labour is thought to have been traditional: women doing work in and around the house, men working mostly in the fields (Haks, 1985). This labour division was put into effect at a young age with children older than five years beginning to perform various jobs: boys typically herded cattle or tended the land and girls typically did housework or childcare (Schenkeveld, 2008). This suggests that girls may have been more at risk of developing rickets. Yet, as rickets is absent in individuals older than four years, by this age, both girls and boys must have had sufficient sunlight exposure, and the risk of rickets seems more related to age than gender.

During the post-Medieval period in the Netherlands, children's clothing may have played an important role in limiting dermal synthesis of vitamin D. In the 19th century, children were considered to be small adults and were dressed as such (de Leeuw, 1992). They would be dressed in many layers of clothing covering as much skin as possible, a practice frequently depicted in contemporaneous art, such as David Artz' ‘Mother with children and a lamb’ (www.niceartgallery.com). Clearly, occlusive clothing and being kept indoors would inhibit sunlight exposure (Molla *et al.*, 2000; van der Meer *et al.*, 2006; Hatun *et al.*, 2011).

As mentioned, by the age of about five years, children became more independent and were likely required or permitted to spend more time outdoors. This transition roughly coincides with when rickets is no longer observed in the Beemster sample. While subadults aged four to fifteen years have no evidence of rickets, ongoing research has found residual rachitic changes in the older adolescents and adults. Thus, some individuals who contracted rickets, likely during infancy, healed and survived. There may be a lack of older subadults with detectable evidence of healed rickets in part because over time healing can diminish or erase some, though not all, rachitic changes (Brickley *et al.*, 2010).

Finally, it is pertinent to consider if differences in socioeconomic status could have influenced the occurrence of rickets in Beemster. At this time, the socioeconomic statuses of the families of affected individuals are not known, but archival data indicate the majority of households were engaged in cattle farming with statuses ranging from quite good (land owners) to rather poor (transitory labourers) (register of deaths, Beemster). One group may have been especially vulnerable. From 1680 AD into the 19th century, an orphanage existed next to the Middenbeemster church (Falger *et al.*, 2012). Complaints to the municipality about poorly fed children and children being late for work are on record. Child labour was common until 1902 AD when the public education law was put into effect (Schenkeveld, 2008). Orphaned infants could have been especially vulnerable to developing rickets because of an inadequate diet, and it is possible they spent a limited amount of time outside, although it is not well known if they were made to work in indoor or outdoor settings, nor the organization of the facility for outside access. Future research will focus on linking archival data to the skeletal data with a major goal being the assessment of socioeconomic status on health.

Population comparisons

In environments with sufficient sunlight, rickets is more rare in rural than urban communities; however, there are a few examples from archaeological contexts (Mays, 2007; Palkovich, 2012). For example, Palkovich (2012) suggested that malnutrition and family social dynamics resulted in cases of rickets in the ancestral Puebloan community of Arroryo Hondo, New Mexico, where ample sunlight was available on a year-round basis. Mays (2007) found several cases of rickets in the one and two year olds at Wharram Percy and suggested rickets to be a secondary condition. Thus, a variety of sociocultural and dietary factors have been proposed to explain the occurrence of rickets in rural populations from different geographic locations and temporal periods.

Previous studies of post-Medieval urban collections in the Netherlands found rickets prevalences for subadults that vary from zero (Baetsen, 2001 for the site of Alkmaar; Maat *et al.*, 1998 for the site of Dordrecht), to 4.4% at ‘s-Hertogenbosh (Maat *et al.*, 2002), compared to the Beemster's prevalence of 9.5%. Maat *et al.* (2002) note that epidemics of cholera (1830–1850 AD) and typhus (1850 AD), as well as other diseases more common in densely populated urban areas, was likely a major contributory factor to the development of rickets. None of the Dutch collections have a rickets frequency or age pattern that is similar to the Beemster, suggesting a different interaction of causes.

Comparison of Beemster to three contemporaneous British urban collections, Broadgate, Spitalfields, and St. Martin's, as well as the late-Medieval rural community of Wharram Percy, is made to enhance our knowledge of the impact of rickets in Western

Europe. At Broadgate, Pinhasi *et al.* (2006) examined subadults from the ages of birth to seven years and found agespecific rickets prevalences higher than those of Beemster for subadults from birth to age five (excluding the one year olds), clearly indicating insufficient vitamin D levels in the many of the subadults. At Spitalfields, subadults from birth to age two were most affected, and some at age three, but at levels usually lower than at the neighbouring site of Broadgate (Pinhasi *et al.*, 2006). Pinhasi *et al.* (2006) point out that individuals from Spitalfields were of higher socioeconomic status than those of Broadgate, which resulted in better living conditions and nutrition for more of the Spitalfields subadults. In the other urban site, St. Martin's, almost 20% infants from birth to age three had rickets, as well as some of the four to twelve year olds. As mentioned, at the rural site of Wharram Percy it was one and two year olds who were most affected (Mays, 2007). None of the British sites used in this comparison have a pattern of age-specific prevalence that is the same as the Beemster, where the highest frequencies occurred in the two and three year olds, with no cases occurring past this age and very few prior to this age. This suggests the way the factors responsible for vitamin D inadequacy in the Beemster subadults combined and interacted is different from what has been previously documented and makes clear the value of additional research on rickets in non-British and non-urban populations.

The etiology of rickets is complex because of the multiple practices that can affect sunlight exposure and diet. Unfortunately, there is a paucity of research on daily life in rural Dutch communities which limits our knowledge of cultural practices thus limiting the specificity of our interpretations. In the Beemster, we propose that dietary and cultural practices such as poor weaning foods, prolonged swaddling, occlusive clothing, and a lack of time spent outdoors could have all played a part in causing a high frequency of rickets in the two- and three-year-old subadults. Also possible is the development of rickets secondarily to a different disease. The suggestions regarding causes of rickets are meant to provoke discussion and research about non-urban-based factors and contribute to our understanding of the aetiological intricacy of vitamin D in Western Europe.

CONCLUSION

In the post-Medieval Middenbeemster cemetery, rickets occurred predominately in two and three years olds. As a rural community, Beemster had ample access to sunlight for over half the year; cultural practices and possibly dietary factors were likely causes of most rickets cases. The interplay of factors limiting vitamin D synthesis, including poor weaning foods, prolonged swaddling, occlusive clothing, and a lack of time spent outdoors, is proposed to have been major causes of rickets in the Beemster infants. Future research will assess the prevalence of residual rickets and osteomalacia in adolescents and adults which will enhance our knowledge about the impact of vitamin D deficiency on the Beemster community as a whole and allow us to analyse other groups that may have been susceptible to developing a vitamin D deficiency, particular women, and how this may have affected their offspring. Compared to contemporaneous Dutch urban sites, Beemster had a higher crude rickets prevalence, highlighting the importance of examining not only industrialized, urban communities for rickets. Moreover, this research demonstrates that rickets is 'not only the English disease' (Belton, 1986: 68) as the overall Beemster prevalence, while lower than that of the comparative British urban sites, is very high in the two and three year olds and higher than that of the British rural site.

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3 Gender-related vitamin D deficiency in a Dutch 19th century farming community

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ABSTRACT

The most common cause of vitamin D deficiency is inadequate dermal exposure to sunlight. Residual rickets is nonadult vitamin D deficiency still evident in an adult individual, whereas osteomalacia occurs in adulthood. Previous research on the Beemster population, a 19th century rural community in the Netherlands, identified rickets in 30.4% of the nonadults between the ages of two and four years (n =7/23). Because the sex of these nonadults was not known it was not possible to determine if there were differences between boys and girls. To overcome this gap in our knowledge, the aim of this paper is to determine if there are gender related differences in vitamin D deficiency in the Beemster skeletal collection, based on adults with residual rickets and osteomalacia. Out of 200 adults (100 females; 100 males) no cases of osteomalacia were detected. However, there were 29 cases of residual rickets (14.5%), with 21 of those cases in females (21.0%; 21/100). A complex interplay of multiple factors is proposed to have affected vitamin D levels in nonadults, including sociocultural variables such as gender-based labour norms. This research highlights the importance of continuing to explore gender-based health differences in past populations.

INTRODUCTION

Limited access to sunlight due to narrow streets and tall buildings, air pollution, and overcrowding, as can be observed in historical populations, are commonly considered to be important contributing factors to the prevalence of vitamin D deficiency (Brickley et al., 2007; Maat et al., 2002; Mays et al., 2006). However, other aspects, such as habitual activities and sociocultural practices that influence sunlight exposure (i.e. time spent indoors or covering clothing), have a large impact on the amount of vitamin D that can be synthesized in the skin. Therefore, the frequency of vitamin D deficiency may provide information about sociocultural practices, as noted in modern (van der Meer et al., 2006; Miyako et al., 2005; Underwood and Margetts 1987; Yassin and Lubbad, 2010) and archaeological populations (Brickley et al., 2014; Giuffra et al., 2015; Palkovich 2012). Most Dutch studies focusing on vitamin D deficiency concentrate on urban populations (Baetsen 2001; Maat et al., 1998; Maat et al., 2002). Little research has been performed on vitamin D deficiency in rural populations from the Netherlands (Veselka et al., 2015) and research on the influence of sociocultural habits on the occurrence of vitamin D deficiency in either urban or rural settlements is lacking.

A recent study on a 19th century rural population from Beemster, the Netherlands, reported a high prevalence of vitamin D deficiency in individuals under the age of four years (Veselka et al., 2015). The high occurrence was partly attributed to sociocultural habits, such as prolonged swaddling, suboptimal weaning foods, and concealing clothing. In addition, a gender-based division in activities was postulated as a potential factor contributing to vitamin D deficiency in girls who may have been required to more often assist with indoor tasks (Veselka et al., 2015). This would predispose girls to developing rickets. However, due to the inaccuracy of current macroscopic methods for sex estimation in nonadults, this hypothesis could not be tested on the basis of the nonadult data alone.

Another way to assess the influence of sociocultural habits, especially the gendered division of labour, on the risk of developing rickets in the Beemster population, is to assess residual rickets, whereby remnant bending deformities of nonadult rickets are studied in adults. Commonly observed residual rickets features include bending deformities of the lower limbs. It is also useful to examine osteomalacia, which is vitamin D deficiency that occurs in adolescents and adults (Brickley et al., 2010), as it may reveal gender differences in vitamin D deficiency in the adult Beemster community. Osteomalacia is evident as pseudofractures in specific parts of the skeleton and, in severe cases, buckling and bending deformities of certain parts of the skeleton (Brickley and Ives, 2008: 127–129). Therefore, the aim of this paper is firstly to determine if there are gender differences in vitamin D deficiency in the Beemster skeletal collection. The two ways of assessing this are via residual rickets as a marker for the impact of vitamin D deficiency in nonadults and osteomalacia as a marker for adults. Secondly, with these data a life course approach is applied to assess the impact of sociocultural habits on disease levels in the sexes and various age groups within this rural population. This research will provide information on whether genderbased differences in activities increased the Beemster female risk of developing vitamin D deficiency.

MATERIALS AND METHODS

The sample for this research comes from Middenbeemster, a cemetery in Beemster which is situated in the province of North-Holland, the Netherlands, and dates mostly to the 19th century. The sample consisted of 200 adult individuals, 100 females and 100 males, with ages ranging from 18 to 50+ years. This rural population is comprised of individuals with various occupations mainly related to cattle farming (Falger et al., 2012).

Age-at-death was estimated by assessing changes to the pubic symphysis (Suchey and Brooks, 1990), auricular surface (Buckberry and Chamberlain, 2002), and sternal rib ends (Isçan et al., 1984), evaluating the degree of cranial suture closure (Meindl and Lovejoy, 1985), and dental attrition (Maat, 2001). Individuals were placed in the following age categories: early young adult (18–25 years), late young adult (26–35 years), middle adult (36–49 years), old adult (50+ years), or 18+ years if a more narrow age range could not be obtained. Sex was estimated using methods outlined in the Workshop of European Anthropologists (1980) and Buikstra and Ubelaker (1994), assessing the Phenice traits (Phenice, 1969), and a number of metric measurements (McCormick et al., 1991; Stewart, 1979; Steyn and İşcan, 1999). Individuals are categorised as Female (F), Probable Female (PF), Indeterminate (I), Probable Male (PM), and Male (M). In statistical tests, individuals sexed as PM are added to the M category, and the same is done for PF and F; this is done to enlarge the sample size to enable more robust statistical comparisons.

To identify residual rickets, macroscopic changes, as described by Brickley et al. (2010) and Brickley and Ives (2008: 110–111) are evaluated in all skeletons. They consist of remnant deformities of rickets, such as residual bending of the long bones. For a positive diagnosis of residual rickets, clear bending deformities of at least two of the lower limb bones needed to be present. Bowing of only one lower limb bone was diagnosed as possible residual rickets. This may result in a slight underrepresentation of the actual number of affected individuals, but this cautious approach is believed to produce more reliable results.

Although bending deformities of the upper limbs have been reported in cases of rickets (Brickley and Ives, 2008; Mays et al., 2006; Ortner, 2003; Ortner and Mays, 1998) they are rarely used as diagnostic for residual rickets (Baetsen, 2001; Brickley et al., 2010; Ellis, 2010;

Maat et al., 2002; Pinhasi et al., 2006). Bowing of the arms due to vitamin D deficiency will develop at an age when nonadults are not yet able to walk and the arms function as the weight bearing long bones during crawling (Mays et al., 2006; Ortner, 2003; Ortner and Mays, 1998). The most common age of rickets onset is between three and 18 months of age (Ortner, 2003; Wagner and Greer, 2008) and the mean age of walking lies between nine and 12 months of age (Clark et al., 1988; Hindley et al., 1966). This implies that the majority of individuals developing rickets can walk rather than crawl at the time of onset of the condition. Thus, bending deformities of the arms are expected to occur less frequently than those of the legs (Brickley et al., 2010; Mays et al., 2006; Ortner and Mays, 1998). Moreover, the arms are used for a large variety of activities (Pearson, 2000; Rhodes and Knüsel, 2005; Stock and Pfeiffer, 2004) which further impairs the distinction between pathological bowing and changes in shape due to activity. Therefore, individuals with bending deformities of the upper limbs were scored as having possible residual rickets. In order to enhance comparison with other skeletal assemblages and improve our knowledge of bending deformities in residual rickets, the location of all the deformities is noted. In addition, the location of the deformities in the long bones of nonadults from this population are added to observe possible differences between rickets and residual rickets deformities.

Macroscopic changes due to osteomalacia, as described by Brickley and Ives (2008: 127–129) and Brickley et al. (2005), are evaluated in all skeletons, and included diffuse porosity and fine pitting of the cortical surface of the cranium, vertebral bodies that show biconcave compression or are wedge shaped, scoliosis or kyphosis, lateral straightening of rib shafts, rib neck angulation, folding of ilia, obstruction of the pelvic inlet, extreme angulation of the sacrum, anterolateral bending of femoral shafts, coxa vara of femoral necks, knock-knee angulation of the femur, increased curvature of the scapular blade, and the presence of Looser's zones or pseudofractures, which are strongly diagnostic for osteomalacia (Ives and Brickley, 2014; Brickley and Ives, 2008: 127–129; Brickley et al., 2007). Looser’s zones can be present in specific parts of the skeleton, such as in the scapular spines, pubic rami, ribs and femoral necks, and are a frequently encountered clinical trait (Bhan et al., 2012; Mankin, 1974; Ortner, 2003: 399; Vigorita, 1999; Whyte and Thakker, 2005). Macroscopically, pseudofractures will be visible as minor fissures of the cortex while radiographically they will be present as a radiolucent area (Berry et al., 2002; Mays, 2008: 218; Parfitt, 1990: 345; Waldron, 2009: 130). However, pseudofractures do not always develop and their absence in vitaminh D deficient individuals is not uncommon (Berry et al., 2002; Brickley and Ives, 2008; Brickley et al., 2007; Whyte and Thakker, 2005). In addition, it is possible that some pseudofractures will only be visible radiographically (Berry et al., 2002) which may lead to a underrepresentation of the actual number of pseudofractures when only macroscopic analyses are applied. To minimize this possibility, radiographs were taken of the scapular spines and lateral borders of all individuals that presented other features which may be related to osteomalacia (n = 9). This area was chosen because pseudofractures in the scapulae are frequently encountered in archaeological cases of adult vitamin D deficiency (Brickley et al., 2007; Ives and Brickley, 2014). Radiographs were taken with a hand-held Nomad Pro (60 kV, 2.5 μA, exposure from 0.10–0.20 s).

It should be kept in mind that the manifestation of osteomalacia is highly variable between individuals, caused mainly by the age of onset of the vitamin D deficiency and its duration. The later in life the deficiency occurs, the more likely the manifestations will resemble lesions that can be attributed to age-related osteoporosis (Parfitt, 1990: 341). The previously reported relatively high rickets prevalence in the Beemster collection could be indicative of an elevated risk of developing more severe manifestations of vitamin D deficiency in adulthood, enabling its better recognition. However, osteomalacia needs to be chronic for diagnostic lesions to develop and recognition of, in particular, the earlier stages of this disease in an archaeological skeleton is difficult (Brickely and Ives, 2008; Brickley et al., 2007; Ortner, 2003; Waldron, 2009). In this study, a conclusive diagnosis of osteomalacia requires pseudofractures and/or multiple macroscopic changes to be present, such as multiple vertebral bodies showing biconcave compression, anterolateral bending of the femoral shafts, and obstruction of the pelvic inlet.

Since a number of macroscopic changes can be attributed to both residual rickets and osteomalacia, additional radiological analysis of cases with (possible) residual rickets and possible osteomalacia needed to be undertaken. The fifth lumbar vertebra is ideal for this because it has a high bone turnover rate (Brickley and Ives, 2008; Schamall et al., 2003) and will display the effects of vitamin D deficiency earlier than many other parts of the skeleton. Due to remodeling, radiological evidence of residual rickets will most likely be obliterated, whereas in osteomalacia the trabeculae will show a radial orientation, and the vertebrae will have decreased trabecular bone density (Schamall et al., 2003). Schamall et al. (2003) describe trabecular organisation in osteomalacic individuals as irregularly arranged with a heterogeneous appearance. Forty-one individuals were diagnosed with residual rickets, possible residual rickets, or showed non-diagnostic lesions that might be attributed to osteomalacia, of which 22 had the fifth lumbar vertebra available for CT-scanning. In addition, five lumbar vertebrae of individuals without any macroscopic lesions of the skeletons were used as a control sample. The individuals were divided into the aforementioned age categories to take the effect of age on trabecular bone structure into account. Ct scans were performed on a Philips Brilliance 64 scanner (Philips Medical Systems Best) set at 199 mAs and 120 kV with a slice thickness of 1 mm. Since some vertebrae contained a large amount of soil and/or were taphonomically damaged, the quantitative and qualitative information on trabecular bone density that can be calculated is limited. Therefore, trabecular bone density was not measured. Rather, the trabecular organisation and structure are evaluated and scored as 'regular' or 'disorganised'.

To assess whether differences in observed and expected results between sexes and age categories were statistically significant, a Pearson's χ^2-test and a Mann-Whitney U test, respectively, are performed. In this way, the null-hypothesis, that there is no difference between females and males nor age categories, is tested. The results of these analyses are assessed to determine whether there is a significant difference between men and women in the occurrence and age-of-onset of vitamin D diseases in the rural population of Beemster.

RESULTS

Residual rickets

A total of 29 individuals displayed evidence of residual rickets. Three additional individuals were diagnosed with having possible residual rickets due to bending deformities of only one lower limb bone or bowing of the upper limb bones. Table 1 presents an overview of the location of bending deformities per sex and the statistical significance of the observed differences in females and males. The data relating to the bending deformities of the nonadults from this population are added, including the results of the statistical analyses, to offer a more thorough life course approach.

The affected femora in this study display anterolateral bending of the proximal third of the shaft. The tibiae displayed either lateral bending of the proximal part, as observed in figure 1, or posterior bowing of the distal end. A number of individuals displayed bowing of both upper and lower limbs (12.5%; 4/32). No male displayed bending deformities of any of the upper limbs. However, the difference between females (n = 7) and males (n = 0) displaying

Table 1. Frequency of affected bones in adults individuals with residual rickets and possible residual rickets per sex, with nonadult rickets data for comparison.

Affected bones[a]	F (n = 23)	M (n = 9)	Mann-Whitney U	p-value	Total adults (n=32)	Nonadults (n=9)	χ^2	p-value
Femur and tibia	26.1% (6/23)	0.0%	72,000	0.086	18.8% (6/32)	22.2% (2/9)	2778	0.096
Femur, tibia, and fibula	21.7% (5/23)	11.1% (1/9)	92,000	0.133	8.8% (6/32)	25.0% (2/8)	2778	0.096
Femur	17.4% (4/23)	22.2% (2/9)	98,500	0.976	18.8% (6/32)	0.0%	-	-
Tibia	8.7% (2/23)	33.3% (3/9)	75,000	0.101	15.6% (5/32)	22.2% (2/9)	2778	0.096
Tibia and fibula	8.7% (2/23)	11.1% (1/9)	97,000	0.865	9.3% (3/32)	25.0% (2/8)	2778	0.096
Fibula	8.7% (2/23)	22.2% (2/9)	97,880	0.865	12.5% (4/32)	0.0%	-	-
Radius	17.4% (4/23)	0.0%	81,000	0.118	12.5% (4/32)	25.0% (2/8)	2000	0.157
Ulna	13.0% (3/23)	0.0%	90,000	0.358	9.3% (3/32)	25.0% (2/8)	2000	0.157
Humerus	13.0% (3/23)	0.0%	85,500	0.252	9.3% (3/32)	37.5% (3/8)	0,500	0.480

[a] Individuals were counted per affected bone not distinguishing between unilateral or bilateral. All long bones from the affected and possibly affected adult individuals were present for analysis. F = female, M= male.

Figure 1. Bending of tibiae and fibulae individual S307V0567 (Veselka, 2014).

bending deformities of any of the upper limbs did not reach statistical significance (Fisher's exact test $p = 0.077$).

In the nonadults, the most frequently bowed long bones are the humeri (37.5%; 3/8). The next most frequently observed bending deformities are in the rest of the combinations mentioned in Table 1, although no case of bending deformities of just the femora or just the fibulae are observed. The difference in the frequency of bending deformities per location is not statistically significant, partly due to the small sample size, as shown in Table 1.

The frequency of residual rickets is not affected by age since the difference between the number of affected and unaffected individuals within each age category is not statistically significant, although this is partly due to small sample size. Twenty-one females and eight males displayed bending deformities of two or more lower limb bones. The difference between the number of affected females and males is statistically significant ($\chi^2 = 6.989$, $p = 0.008$). There is no statistically significant difference in the distribution of affected and possibly affected individuals by age-at-death (Mann-Whitney $U = 89.000$, $p = 0.524$).

Osteomalacia

None of the individuals in the sample are diagnosed with definitive osteomalacia since pseudofractures were not macroscopically nor radiologically evident. Several individuals did display non-diagnostic lesions, such as biconcave compression of a single vertebra or noncongenital scoliosis. The organisation of the trabeculae did not appear irregular, heterogeneous or disorganised in any of the 22 L5 vertebrae.

DISCUSSION

Differential diagnosis

Several pathological conditions can cause bending deformities of the long bones in childhood, such as Blount's disease, congenital bowing, infantile cortical hyperostosis, metaphyseal chondrodysplasia, osteogenesis imperfecta, trauma, and treponemal diseases (Bleck, 1982; Brickley and Ives, 2008; Cheema et al., 2003; Silverman, 1985). In addition, some degree of physiological bowing is commonly observed in children and falls within the range of what would be considered ‘normal' bowing (Bleck, 1982). When interpreting bowing deformities of the long bones, other conditions needed to be ruled out for the diagnosis to be residual rickets. Most of the other causes of physiological bending deformities, as well as congenital bowing, would not persist throughout life and would occur at other locations, such as the femoral neck (Bleck, 1982; Cheema et al., 2003). All affected femora in this study displayed anterolateral bending of the proximal third of the shaft and differ from the other bending deformities commonly observed in children. The tibiae of the affected individuals either showed lateral bending of the proximal end or posterior bowing of the distal third portion, thus ruling out Blount's disease which produces a sharp lateral bend of the proximal tibia and includes the joint (Jain et al., 2002). Infantile cortical hyperostosis is rare and can cause bending deformities of the long bones in childhood, but is a disease which usually resolves itself within six to nine months (Brickley and Ives, 2008; Caffey, 1956). Metaphyseal chondrodysplasia, osteogenesis imperfecta, and treponemal diseases will display other lesions besides bowing of the long bones thus enabling the distinction between these pathological conditions and residual rickets (Brickley and Ives, 2008; Ortner, 2003; Waldron, 2009). Bending due to trauma can most of the time be distinguished quite easily from other causes of bending and is not the cause of the bending deformities observed in the Beemster individuals.

Therefore, the observed bowing of the lower limbs in the Beemster adults can be confidently interpreted as remnant bending due to childhood rickets. The frequency of bowing in the various lower limb bones is similar to the residual rickets bending deformities observed by Brickley et al. (2010) in the collection of St. Martin's from Birmingham, UK, but some Beemster adults also displayed bending deformities of the humerus, radius, and ulna. These deformities might also be attributed to residual rickets and could be indicative of cyclical or ongoing vitamin D deficiency. A nonadult would acquire vitamin D deficiency at a crawling age whereby the arms would be the weight bearing bones. If the disease endured or came back at an age when this individual was able to walk, the legs would bend as well. Bending of upper and lower limbs, as noted in Table 1, suggest multiple periods of or ongoing vitamin D deficiency. However, the shape of the adult humeri is more difficult to interpret as a marker of vitamin D deficiency as a greater variety of activities are performed with the arms and greater differences in upper arm musculature are expected. This will obscure recognition of residual upper limb bending deformities. However, although deformities of the arms as a result of vitamin D deficiency are less common and changes in shape are more difficult to recognize and interpret, they should be considered more often as a possible indication of residual rickets.

No macroscopically evident pseudofractures were present and radiographic Looser's zones were not observed in the scapular spines nor the lateral borders, hence no cases of osteomalacia were diagnosed. Several individuals did display non-diagnostic lesions that can be due to osteomalacia, such as scoliosis or concave compression of the vertebral bodies. However, as pointed out by Brickley and Ives (2008) and Brickley et al. (2007), other pathological conditions can cause loss of vertebral body height, biconcave compression of the vertebral bodies, and scoliosis/kyphosis, and these skeletal deformities need to be accompanied by other lesions for the diagnosis to be osteomalacia.

The CT-scans taken from the fifth lumbar vertebrae were inconclusive for osteomalacia. Unfortunately, several lumbar vertebrae contained soil particles and taphonomic damage could have caused a decrease in trabecular bone density. Therefore, only trabecular organisation was assessed but none of the individuals presented with an irregular or disorganised structure of the trabeculae. Since the macroscopic features could not provide a definitive osteomalacia diagnosis and the radiographs and CT-scans did not provide additional evidence of adult vitamin D deficiency, the observed skeletal lesions cannot be attributed to osteomalacia.

Sociocultural practices affecting vitamin D levels

Several factors can be responsible for the occurrence of a prolonged shortage of vitamin D. These include geographic and climatological characteristics, such as latitude and available solar hours, but also sociocultural habits related to sunlight exposure and diet (Brickley et al., 2014; Giuffra et al., 2015; Palkovich, 2012). A way to assess the influence of these sociocultural habits is by evaluating vitamin D deficiency prevalence. Previous research indicated that the high percentage of childhood rickets in the rural population of Beemster was most likely caused by a multitude of factors, including sociocultural habits related to sunlight exposure, such as type of clothing and time spent indoors (Veselka et al., 2015).

Dietary practices may also have contributed to infant vitamin D deficiency via maladaptive breastfeeding and weaning practices. Recent stable isotope research of the Beemster nonadults, showed relatively short periods of breastfeeding, on the order of a few weeks to months, and some infants appear not to have been breastfed at all (Waters-Rist and Hoogland, 2017). Rather, it is likely that these young infants were fed raw (i.e. unpasteurised) cow's milk. Cow's milk is very different from human breastmilk and predisposes infants to gastrointestinal bleeding, intestinal blockages, bacterial infection, reduced immune function, gastric symptoms (vomiting, diarrhea), breathing difficulties, and iron deficiency anemia (Butte et al., 2002; Haug et al., 2016; Sullivan, 1993; Thompkinson and Kharb, 2007). In regards to vitamin D, neither breastmilk nor cow's milk are very good sources of it, but the vitamin D in cow's milk becomes less bioavailable when the infant is suffering from the conditions listed above. Therefore, the infant feeding practices of this community may have been an important contributing factor in infants and children developing vitamin D deficiencies (Waters-Rist and Hoogland, 2017).

Clothing is commonly region specific and often the result of community norms and traditions (de Leeuw, 1920). Furthermore, the type of clothing worn can be influenced by socioeconomic status and sex. During the 19th century, Dutch nonadults and adults dressed similarly, exposing little to no skin, as shown in figure 2. Although this picture shows Beemster school children in their best clothes (Beemster inhabitant, Mr. T. Molenaar: pers. comm.), it is postulated that clothes worn everyday would resemble the ones worn on specials occasions in covering about the same amount of skin.

Figure 3. Early 20th century tree harness (Photograph: courtesy of Mrs. A.Vis-Best, inhabitant of Beemster).

Covering clothing would substantially decrease dermal synthesis of vitamin D. However, if adults exposed their face, hands, and arms every day during the late spring and summer months, vitamin D levels should have been adequate (Brickley and Ives, 2008: 77; Holick, 2005). Infants that were fully dressed (without a hat) would need two hours of sunlight exposure in these months (Pettifor and Daniels, 1997: 665). Yet, the Beemster is an area with many canals and houses would have been surrounded by water. Constant danger

Figure 2. Beemster school children from the 19th century (Photograph: courtesy of Mr. T. Molenaar, inhabitant of Beemster).

of drowning would have kept young children inside. On occasions, these children were allowed to play outside, but would have been tied to a tree for a short period of time to prevent them from drowning while their mother or grandmother performed activities in and around the house (Beemster inhabitants Mr. K.C. Visser and Mrs. A. Vis-Best, pers. comm.). figure 3 displays one of these 'tree harnesses'. Thus, just because ample sunlight was available in spring and summer months in this rural region, it does not mean the nonadults had access to it. Clearly many did not.

Indoor activities or activities in the shade combined with concealing clothing would further increase the risk of developing vitamin D deficiency. This would imply equal risk of developing rickets for boys and girls at least up to the age of five to six years, when children were expected to help with daily tasks. Most of the Beemster inhabitants performed activities involving cattle farming, such as herding, milking, churning butter, and making cheese (Falger et al., 2012). Typically, the men worked in the fields while women performed work in and around the house, such as spinning, sawing, laundry, churning butter, making cheese, and tending to the children (van Cruyningen, 2015; Falger et al., 2012; van Nederveen Meerkerk, 2015; Palmer et al., 2016). Up to the 19th century, this division of labour started early in life, whereby children from the age of six years, and sometimes even younger, were put to work (Brugmans, 1929: 93; Schenkeveld, 2008). Common activities for boys in cattle farming communities included herding and milking the cows, whereas girls would knit, sow, help their mothers or grandmothers with house chores, and tend to younger brothers and sisters (Schenkeveld, 2008: 38, 50, 53).

Residual rickets was significantly more frequently observed in the Beemster females than males, which suggests female nonadults received less sunlight exposure than male nonadults. Gendered division of labour wherein older boys (6+ years) spent more time in the fields is proposed to have enabled more of them to overcome a vitamin D deficiency they might have developed during their early years of life. Fewer females were able to overcome the deficiency, being involved in tasks that kept them indoors or closer to home and farm building that may have shaded their outdoor work. Some of these females may even have experienced additional bouts of vitamin D deficiency as they grew.

An alternative explanation for the finding that significantly more females than males had residual rickets must be considered. This pattern would result if more nonadult males with vitamin D deficiency died, causing more adult females with bending deformities to be part of the analyzed sample. If indeed nonadult males experienced a greater frailty and the risk of developing rickets was about the same for female and male nonadults up the age of six years, more male than female nonadults under the age of six years are expected to be buried in the Beemster cemetery. However, archival data of the Beemster cemetery have shown that the age-at-death distribution of male and female nonadults is not statistically significant ($U = 492{,}000$; $p = 0.821$).Therefore, nonadult males and females are postulated to have comparable frailty. In addition, vitamin D deficiency is rarely fatal (Brickley et al., 2010). Taking into account that the diet was similar for girls and boys (Waters-Rist and Hoogland, 2017) and that the properties of the skin needed to synthesize vitamin D do not differ substantially between males and females, this strongly suggests that gender related differences in activities were a key reason for the higher frequency of residual rickets in females.

Although the relatively high rickets and residual rickets prevalence in this population would suggest a high prevalence of osteomalacia (Brickley et al., 2014), no definitive case of adult vitamin D deficiency was observed. Several individuals displayed non-diagnostic lesions that failed to meet the criteria for a diagnosis of osteomalacia. Manifestations of adult vitamin D deficiency often are more subtle and severe manifestations of this disease are rarely found in the archaeological record (Brickley et al., 2007; Ives and Brickley, 2014; Ortner, 2003; Waldron, 2009). While the lack of definitive cases of adult vitamin D deficiency in the Beemster sample may suggest that gender related differences in activities were absent in adulthood, this is considered unlikely. Most likely, a large part of the reason for osteomalacia not being detected can be attributed to its subtle, non-diagnostic osseous changes.

Indeed, in most populations of this region and time, including the Beemster, a rigid gender- related labour division was the norm (van Cruyningen, 2015; van Nederveen Meerkerk, 2015; van Poppel et al., 2009). Importantly, however, it has been noted that at times, women were needed to work in the fields together with the men (van Cruyningen, 2015; Falger et al., 2012; Schenkeveld, 2008). In such scenarios, it was the girls that were tasked with tending to the home and younger children together with the grandmothers (Schenkeveld, 2008; Beemster inhabitants Mrs. M. de Reus-Kilsdonk, Mr. K.C. Visser and Mrs. A. Vis-Best, pers. comm.), thereby continuing their time spent mostly indoors. Maybe these events contributed to adult females having an adequate or only slightly low level of vitamin D.

Several researchers have studied gender-based differences in activity using musculoskeletal markers (e.g. Eshed et al., 2004; Palmer et al., 2016; Villotte et al., 2010) and disease (e.g. Agarwal, 2012; Sofaer Deverenski, 2000; Weiss and Jurmain, 2007). As this paper has shown, vitamin D deficiency prevalence can also be used to assess gender-based differences in activity. Although historical and contemporary sources contain information on various activities in the past, women and children are often invisible in these sources (van Cruyningen, 2015; Dekker, 2015; van Poppel et al., 2009; van Nederveen

Meerkerk, 2015). This research on vitamin D deficiency prevalence in the Beemster enhances our knowledge of gender related differences in activities in this population and the impact of this division on various stages in life, and provides more information on the past lives of women and children in rural areas.

CONCLUSION

Besides geographic and climatological characteristics, sociocultural habits, such as gender- related differences in activity that affect the amount of time spent outdoors, can influence vitamin D levels Therefore, vitamin D deficiency prevalence can be informative about the impact of these habits on various age groups. In addition, it can enhance our knowledge about the past lives of women and children, who are often invisible in historical records. The prevalence of residual rickets in the rural Beemster population showed that female nonadults were more frequently affected by vitamin D deficiency than male nonadults. Although the residual rickets prevalence strongly suggests a gender-related division in activities contributed to higher female vitamin D deficiency in childhood, the impact nor the existence of this division in adulthood is clear. Methods to better detect non-severe cases of osteomalacia in archaeological skeletal remains are needed.

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4 The influence of sociocultural practices on vitamin D deficiency in five post-Medieval communities from the Netherlands

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ABSTRACT

This paper aims to improve our understanding of factors influencing past vitamin D deficiency by comparing five 17th - 19th century communities of varying population size, location, and socioeconomic statuses (SES) from different parts of the Netherlands. Vitamin D deficiency was evaluated in: Rotterdam (n = 37), Gouda (n = 45), Roosendaal (n = 60), Hattem (n = 128), and Beemster (n = 295). Lesions for rickets, residual rickets, and osteomalacia are scored. At sites with more than 10 nonadults (< 18 years), rickets prevalence ranges from 15.3% (9/59) in Beemster to 23.8% (5/21) in Hattem. Across all sites, younger nonadults (< 4 years) were significantly more at risk of developing rickets than older nonadults (≥ 4 years). The prevalence of residual rickets ranges from 12.5% (4/32) in Gouda to 23.9% (21/88) in Hattem. All rural and small urban low-middle SES sites have more females (22.3%, 39/175) than males (12.0%, 24/200) with residual rickets. Only one individual (1.0%; 1/100), from Hattem, was diagnosed with osteomalacia. The high prevalence of rickets and residual rickets is interesting considering that common factors known to produce vitamin D deficiency, such as densely-packed tall buildings, are unlikely to have been involved. Instead, sociocultural practices are postulated to have

contributed to vitamin D deficiency, including child-rearing practices, and a gendered division of labour. Only post-Medieval sites were included in this research and future analysis of Medieval sites is needed to enhance our understanding of the influence of sociocultural practices on vitamin D deficiency development.

INTRODUCTION

Vitamin D is needed for several metabolic processes in the body and one of its functions is enabling mineralisation of newly formed bone to withstand muscular tension and gravity; with time mechanical deformity will occur in poorly mineralised bones (Brickley et al. 2018; Brickley and Ives, 2008). Nonadult vitamin D deficiency is referred to as rickets, with a distinction between active and healing lesions indicating the disease's stage at time of death. Residual rickets is used to describe remnant bending deformities due to nonadult vitamin D deficiency still visible in the adult skeleton. Adult vitamin D deficiency lesions are referred to as osteomalacia.

The most effective way of obtaining vitamin D is via dermal synthesis under the influence of ultraviolet B (UV-B) radiation in sunlight (Holick, 2005, 2006). A limited number of foods, such as cod-liver oil, oily fish, beef liver, and egg yolk, are natural sources of vitamin D, although the amount of it is small in most of these foods (Holick, 2006). Apart from environmental factors, such as latitude and available solar hours, many culturally related factors limiting sunlight exposure are attributed to industrialisation in large urban centres, such as densely packed tall buildings, and air pollution (Holick, 2003, 2006; Brickley et al. 2014; Prentice, 2008).

Recent research, showed that rickets and residual rickets prevalence were relatively high in a 19th century rural, non-industrialised Dutch community (Veselka et al. 2015, 2017). Other studies have also found vitamin D deficiency in past and recent non-urban populations (Joshi, 2008; Ortner and Mays, 1998; Palkovich, 2012; Sachan et al. 2005; Underwood and Margetts, 1987; Van der Meer et al. 2006). This suggests that all past populations should be assessed for vitamin D deficiency, not just those that lived or worked in large, urban, industrialised centres. This paper will do exactly that by providing the first overview of vitamin D deficiency prevalence in the post-Medieval period (circa AD 1600 – AD 1900) across a large area of the Netherlands in centres of different size, density, and socioeconomic status (SES). To research the prevalence of vitamin D deficiency in relation to the socioeconomic environment at different moments in time, this study provides a life course perspective, whereby biological characteristics are combined with cultural factors to assess their influence on the development of this disease (Hertzman, 1994, 1999; Power and Hertzman, 1997; Ben-Shlomo and Kuh, 2002). Employing a life course perspective improves our understanding of differences in morbidity and mortality across the lifespan and has been widely used in bioarchaeological and modern-day studies (Palkovich, 2012; Agarwal, Docio et al. 1998; Dufoue, 2006; Gowland, 2015, Meltzer, 2007).

The aims of this paper are threefold. Firstly, this paper will contribute vitamin D deficiency data on the full range of settlement types that existed in 17th - 19th century Netherlands increasing our knowledge of life in northern Europe during this period of significant social change. Most published data on vitamin D deficiency in past Dutch populations come from urban centres (Aten, 1992; Baetsen, 2001; Berk 2007; Maat et al. 1998, 2002; Baetsen and Weterings-Korthorst, 2013). Data on rural communities and small towns are lacking in both the Netherlands and other areas of Europe, hence this paper intends to decrease these lacunae in our knowledge. Secondly, this research proposes to improve our understanding of the range of factors affecting vitamin D levels, including clothing, aspects of diet, and the division and type of labour. Lastly, via a life course approach, the effect of vitamin D deficiency on various age groups, both sexes, different SES groups, and various regional locations will be assessed by linking biophysical and culturally related data. This paper strives to provide a biocultural perspective on vitamin D deficiency analysis by embedding biological variables of individuals from past communities in their cultural contexts enabling an in-depth understanding of the influence of this disease that would not have been obtained otherwise.

Figure 1. The distribution of sites in the Netherlands divided in provinces.

MATERIALS AND METHODS

The distribution of the five sites analysed for this paper are shown in Figure 1.

To remove or minimise the potential effect of differences in skeletal preservation and completeness between sites, these limits and criteria were applied. The preservation of the majority of the skeletons from all sites was 'excellent' to 'fair', corresponding to weathering stages 1-3 (Behrensmeyer, 1978); fewer than 5% of the skeletons had weathering stage 4. For the majority of the skeletons, completeness was between 50 – 100%. Only the nonadults with more than 50% of the skeleton and at least one intact femur or tibia were considered observable for rickets. Adult individuals needed to have two or more lower limb long bones present to be scored for residual rickets. Finally, to be scored for osteomalacia, adult skeletons needed the following bones present: one-

Table 1. Number of nonadults and adult females and males assessed for vitamin D deficiency with socioeconomic status and settlement type (rural vs. urban) and size (small vs. large) for each site.

Sites	Nonadults	Adults			Total Individuals	Average SES	Settlement type
		Females	Males	Total			
Beemster	95	100	100	200	295	M - L	Rural
Gouda	4	18	23	41	45	H	Small urban
Rotterdam	3	16	18	34	37	M - L	Large urban
Roosendaal	15	18	27	45	60	M - L	Small urban
Hattem	28	47	53	100	128	M - L	Small urban
Total	145	199	221	420	565		

SES = socioeconomic status, M - L = middle to low, H = high.

third of the 24 ribs, one scapula, and one-third of the vertebral column. Table 1 presents an overview of the age and sex distribution of analysed individuals.

SES can be inferred based on population-specific occupation types and education levels, provided by archival data (for Beemster and Gouda) and historic sources (for Rotterdam, Roosendaal and Hattem). Historians commonly make the following distinctions: (1) elite (e.g. professionals), (2) middle class (e.g. foremen), (3) skilled workers (e.g. craftsmen), and (4) low class (e.g. unskilled labourers) (Van Bavel and Kok, 2004). However, only the burial location (inside the church for the elite vs. outside the church for the rest of the community) is considered to be a reliable SES indicator when archival data on SES was not available. Therefore, burial location is used in this study as the basis for the distinction between high SES and middle to low SES for all the sites, which is combined with additional archival data on SES for the populations of Beemster and Gouda (Bastiaensen et al. 2010; Pik, 1979; Sypkens Smit, 1964).

The first collection comes from Beemster (19th century), a rural population in the province of North-Holland, whose main activity concerned cattle farming (Falger et al. 2012). Archival data show the individuals buried outside the church in the surrounding cemetery had a middle to low SES. The second collection consists of individuals from Gouda that come from inside the St. John's church (17th - 19th centuries). Information from burial records indicates these individuals had a high SES, some of them being regents, mayors and prefects (Van Dasselaar, 2015). Rotterdam, the third collection, was a large urban centre with an important seaport by the 17th century (Van der Bent et al. 2011). The individuals from this collection come from the cemetery surrounding the St. Laurence church (17th century) and their SES is middle to low (Ploegaert, 2017). The fourth skeletal collection, Roosendaal, comes from a cemetery surrounding the St. John's church (17th - 19th centuries). Historical sources report Roosendaal to have been a small urban centre, and its main activities were agriculture and a small-scale textile industry (Van Gastel, 1995). The population buried outside the church consisted of individuals with a middle to low SES, although the majority are considered to have been poor [Bastiaensen et al. 2010; Van Gastel, 1995). Hattem is the last collection. Historic sources report Hattem to have been a small, prosperous town up to the 18th century, but afterwards several periods of French occupation caused severe impoverishment (Kamp, 1984). The individuals buried outside the St. Andrew's church (17th - 19th centuries) are expected to have had a middle to low SES (Sypkens Smit, 1964).

Age-at-death in nonadults was estimated using a combination of several methods: dental development of deciduous teeth (Demirjian et al. 1973), dental development of permanent teeth (Moorrees et al. 1963), dental measurements (Liversidge et al. 1998), and dental eruption (Ubelaker, 1979). If the nonadult teeth were unobservable, age was estimated by assessing the stage of long-bone epiphyseal fusion (Schaefer et al. 2009), long-bone length (Maresh, 1970), and clavicle length (Black and Sheuer, 1996). Nonadults were assigned to one of the following age categories: < 0 years, 0 – 3 years, 4 – 6 years, 7 – 12 years, and 13 – 15 years. Nonadults with an age-at-death of 16 to 17 years were excluded from analysis of rickets, since most of their long-bone epiphyses are expected to be fused and thus, many manifestations of rickets will be unobservable.

Adult age-at-death was estimated by assessing changes to the pubic symphysis (Brooks and Suchey, 1990), auricular surface (Buckberry and Chamberlain, 2002) and sternal rib ends (İşcan et al. 1984), and evaluating the degree of cranial suture closure (Meindl and Lovejoy, 1985) and dental attrition (Maat, 2001). The following age categories were used: 18–25 years, 26–35 years, 36–49 years, and 50+ years. If a narrower age range could not be obtained, the category 18+ years was used.

Only the sex of adult individuals was estimated, since the current macroscopic methods for nonadult sex estimation do not provide sufficiently accurate results (Lewis, 2007). The sex of adult individuals was estimated using methods outlined in the Workshop of European Anthropologists (1980). In addition, the Phenice (1969) traits were assessed, and metric measurements were taken from the clavicle, scapula, humerus, and femur (McCormick et al. 1991; Stewart, 1979; Steyn and İşcan, 1999). Individuals were assigned to the following categories: Female (F), Probable Female (PF), Probable Male (PM), and Male (M). To enable more robust statistical comparisons, PF was added to the F category and the same was done for the PM and M.

Vitamin D deficiency was assessed in all age groups by scoring macroscopic lesions. Rickets in nonadults was assessed by scoring lesions that include thickening of the sternal rib ends, flaring and/or thickening of the metaphyses, and bending deformities of the long bones (Ortner and Mays, 1998; Mays et al. 2006, 2009; Brickley and Ives, 2008). For the diagnosis to be rickets, bending of one or more femur or tibia and one other lesion attributed to nonadult vitamin D deficiency needed to be present. A distinction will be made in disease stage, active vs. healing, whereby active rickets is characterised by porosity underlying the growth plate (Mays et al. 2006).

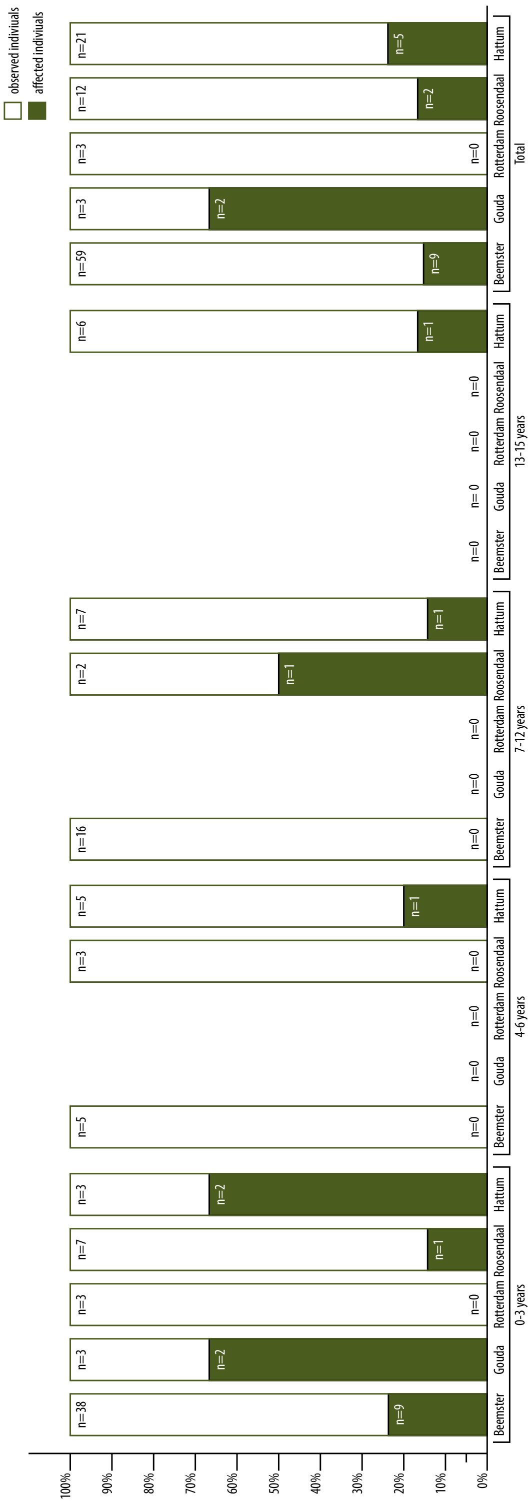

Figure 2. Rickets prevalence per age category per site. 3 years denotes 3.0–3.9 years and so on.

Bowing of two or more lower limb bones needed to be present for the diagnosis to be residual rickets (Brickley et al. 2010; Brickley and Ives, 2008). Pseudofractures needed to be present for osteomalacia to be diagnosed in adults (Brickley et al. 2005, 2007; Ives and Brickley, 2014; Brickley and Ives, 2008).

A Pearson's χ^2-test is performed to assess whether differences in vitamin D deficiency prevalence (rickets, residual rickets, and osteomalacia) between the sites are statistically significant. This test is also used to assess differences in residual rickets prevalence by sex. A Mann-Whitney U-test is used to assess statistical significance of rickets and residual rickets prevalence distribution among the various age categories.

RESULTS

Rickets

An overview of rickets prevalence per site per age category is provided in Figure 2. Since only Beemster (n = 18) and Gouda (n = 1) have individuals with an age-at-death ≤ 0 years, this age group is excluded from this figure.

The difference in rickets prevalence between the five sites is not statistically significant (χ^2 = 6.162, p = 0.187). However, the majority of all affected individuals are in the age category '0 – 3 years' (14/18 = 77.8%), and the difference in rickets prevalence between age categories across all sites is statistically significant (χ^2 =4.444, p = 0.035).

Table 2 presents an overview of affected nonadults with their mean age divided into active and healing cases of rickets. In Rotterdam, no rickets was observed and therefore this collection is left out. Figure 3 shows bending deformities of the tibiae and fibulae, and cupping of the distal metaphyses of individual S123V131, aged 2 ± 1 years, from Hattem. Across all sites it is only the 0-3-year-old category that contains individuals with active lesions while the older age categories contain only nonadults with healing lesions.

Residual rickets

Figure 4 shows residual rickets bending deformities of the left tibia and fibula in individual S066V072, a male aged 35+ years from Hattem.

An overview of the distribution of residual rickets per sex for each site is provided in figure 5.

The difference in residual rickets prevalence between the five sites is not statistically significant (χ^2 = 4.931, p = 0.294). As a whole, significantly more females are affected by residual rickets than males (χ^2 = 5.699, p = 0.017), which is driven by the difference in

Table 2. Overview of individuals and their mean age with active and healing rickets per site.

Sites	N Affected individuals	Active cases		Healing cases	
		N	Mean age of individual	N	Mean age of individual
Beemster*	9	5	3.0 months	4	3.5 months
			2.2 years		3.0 years
			2.5 years		3.5 years
			2.5 years		3.5 years
			3.0 years		
Gouda	2	2	3.0 months	n/a	n/a
			9.0 months		
Roosendaal	2	1	8.0 months	1	9.0 years
Hattem	5	2	2.0 years	3	6.5 years
			2.5 years		9.0 years
					15.0 years

* = data from Veselka et al. (2015).

Figure 3. Medial bending of the distal end of both tibiae. Flattening and bending midshaft of both fibulae. Proximal end is on the right. HT15S123V131, 2 ± 1 years with active rickets from Hattem. Scale is in cm.

prevalence levels between Beemster males and females ($\chi^2 = 6.816$, $p = 0.009$). Since the difference in the distribution of individuals affected by residual rickets over the various age categories does not meet statistical significance ($U = 8786.000$, $p = 0.105$), the data are not depicted in figure 5. Information on the distribution of affected individuals over the age categories is provided as supplementary data.

Figure 4. Residual bending of the left tibia and fibula of S066V072, a male adult (35+ years), from Hattem, the Netherlands. Note post-mortem damage to the proximal end of the fibula and proximolateral end of the tibia.

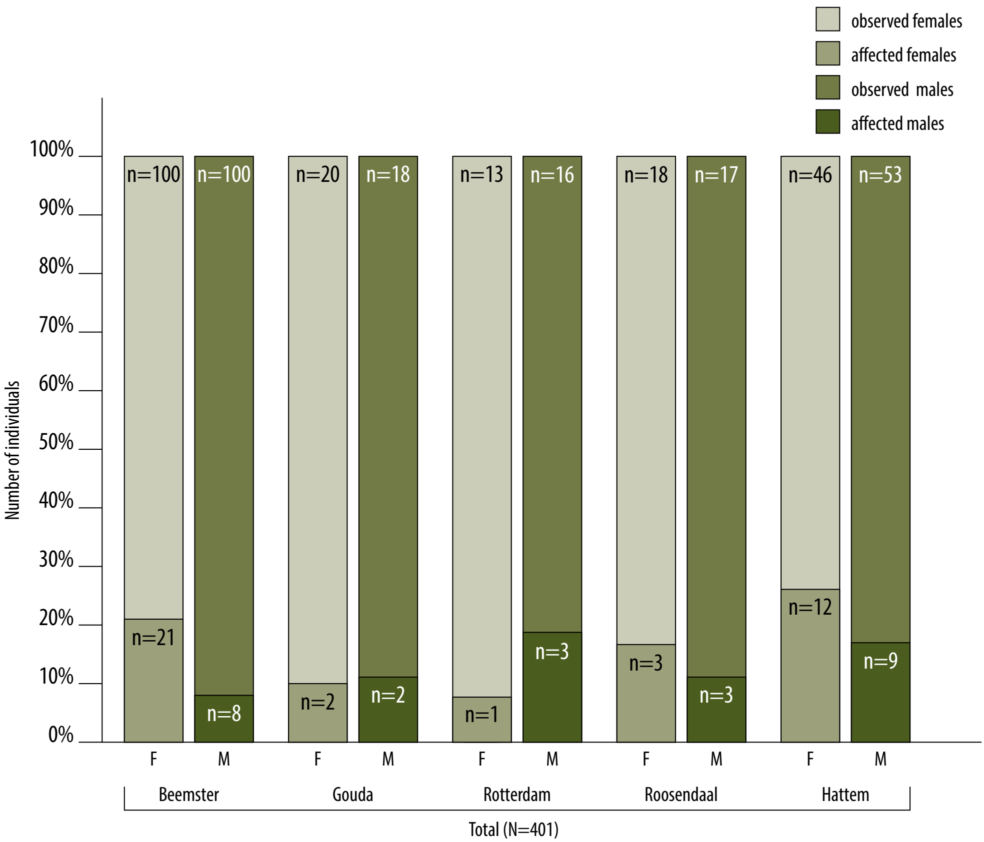

Figure 5. Residual rickets distribution per sex and age category for each site.

Osteomalacia

The adult form of vitamin D deficiency was observed only in Hattem. One male individual, age-at-death 36 – 49 years, was diagnosed with osteomalacia. Pseudofractures were observed in one rib fragment, the transverse processes of the 9th and 10th thoracic vertebrae,

Figure 6. Pseudofracture in the sacrum of individual S108V115 from Hattem. Ventral side is to the right and top. The location of both pseudofractures are depicted in the schematic overview of the sacrum (see black arrows), whereby A is shown in the photograph.

and in the sacrum. The sacrum has not previously been reported as a location where pseudofractures may occur. Figure 6 shows the sacrum with the two pseudofractures.

DISCUSSION

Although a wide range of acquired and congenital diseases can cause mineralisation defects, such as renal malfunctions and malabsorption, all of these conditions are very rare compared to nutritional vitamin D deficiency (Holick, 2007; Lee et al. 2008). Given the latitude of the Netherlands (53ºN) (Webb et al. 1988) and the known problems of vitamin D deficiency mentioned in the early medical literature (Holick, 1994), we argue that the vast majority of cases of rickets were due to nutritional deficiency rather than rare genetic conditions. Osteomalacia may have been present in low levels in past communities due to age related problems, such as a reduced kidney function (Halloran and Portale, 1997), but given the estimated age at death of the affected individual in this study a nutritional cause is more likely.

Vitamin D deficiency is a condition of equifinality, whereby a multitude of variables influence disease development and the interplay of all factors determine the final state (Von Bertalanffy, 1950). Therefore, skeletal lesions attributed to vitamin D deficiency can provide information on lived experiences of an individual and groups of individuals when connected to their historical, socioeconomic, and cultural contexts (Brickley et al. 2014; Agarwal, 2016; Goodman et al. 1988, Gilchrist, 2000, 2012; Redfern et al. 2015; Ellis, 2010; Gowland, 2015; Palkovich, 2012). This research of five 17th - 19th century populations from the Netherlands provides a unique opportunity to study the interplay of biological (e.g. age and sex) and cultural factors (e.g. settlement size and density, sociocultural practices and SES) on the prevalence of vitamin D deficiency by employing a life course perspective.

Settlement Size and Density

Vitamin D deficiency was common in the 19th century in large urban centres all over northwestern Europe and is often associated with the consequences of the Industrial Revolution (Holick, 2003; Larsen, 2015; Mays et al. 2006). Narrow alleys, smoke filled skies, and otherwise poor living conditions deprived individuals in industrialised urban centres from their much needed vitamin D obtained via skin exposure to UVB. The Netherlands did not experience the Industrial Revolution in the same way as England and many other European countries (Wintle, 2000; De Vries, 2000; Mokyr, 2000). Some cities developed significant industrial activities, such as Rotterdam, the area of Twente (in the eastern part of the Netherlands) with its textile industry (Wintle, 2000; Van Zanden, 1994), and the sugar-refining industry in Amsterdam (Wintle, 2000). However, most communities did not partake in the massive development of technology that increased the need for factories and workshops which resulted in rapid population growth and settlement expansion elsewhere in western Europe (Mokyr, 2000; Wintle, 2000). This may suggest vitamin D deficiency would be rare in the Netherlands. In particular, if vitamin D deficiency was typically only the result of large industrialised cities, small urban centres and rural populations would not be significantly affected. Our results clearly show this was not the case in the Netherlands.

In this study, Rotterdam is the largest urban centre analysed. It was an important player in the maritime economy during the Golden Age (17th century) and its population consisted of about 50,000 inhabitants living in a city that measured 140 hectares (357 individuals/hectare), which is clear overcrowding, with the population continuing to grow in the centuries thereafter (Van der Bent et al. 2011; Wintle, 2000). There are too few nonadults in the Rotterdam assemblage to assess the prevalence of rickets (n = 3), but there is a larger number of adults (n = 18) by which to evaluate the prevalence of residual rickets, at 22.2%. While this prevalence is amongst the highest of the five sites, it is not significantly higher than that of the smaller urban or rural centres suggesting the classic variables associated with vitamin D deficiency in large cities were perhaps not the main etiological agents at Rotterdam. Moreover, in the 17th to 19th centuries, many individuals migrated to Rotterdam for work (Van der Bent et al. 2011), so some of the affected individuals may have acquired a vitamin D deficiency elsewhere.

The rural farming community of Beemster presented with rickets prevalence of 15.3% and a residual rickets prevalence of 14.5%. The small urban centres with low to middle SES inhabitants, Roosendaal and Hattem, demonstrated comparable vitamin D prevalences (Roosendaal: rickets = 16.7%, residual rickets = 13.5%; Hattem: rickets = 23.8%, residual rickets = 23.9%). The small urban settlement with high SES inhabitants, Gouda, presented with a rickets prevalence of 66.7% (2/3) and residual rickets prevalence of 12.5% (4/32). The high rickets prevalence is due to the small number of nonadults. The individuals from Beemster, Roosendaal, Hattem, and Gouda did not live in settlements with densely-packed towering buildings and did not suffer from air pollution. Beemster was a rural cattle farming community (Falger et al. 2012), and the main activities of Hattem and Roosendaal concerned agriculture (Sypkens Smit, 1964; Van Gastel, 1995), whereby most activities would have taken place outdoors were sunlight is postulated to have been readily available. The main activities of the high SES Gouda individuals concerned politics, social events, and management (Van Dasselaar, 2015), which is suggested to have exposed them to enough sunlight.

Regardless of settlement type and density, our research show that the nonadults in all the five sites, were at risk of developing vitamin D deficiency and not just the individuals in large heavily industrialised urban centres. This suggests that a complex set of factors increased the risk of developing vitamin D deficiency in these communities beyond just settlement size, and it is fruitful to explore other variables, such as sociocultural practices, that may have contributed to rickets and residual rickets prevalence.

Gendered Division of Labour

From historic sources about rural Beemster, we know the division of labour was traditional (Falger et al. 2012), and it was postulated that the risk of developing rickets in girls was increased, visible as residual rickets in females (Veselka et al. 2015, 2017). More Hattem females (12/37 = 32.4%) than males (9/51 = 17.6%) displayed residual rickets, yet the difference is not statistically significant ($\chi^2 = 2.191$, $p = 0.139$). The same is true for Roosendaal: 8.3% of males had residual rickets (2/24) compared to 23.1% of females (3/13), but this difference does not meet statistical significance (Fisher's exact test: $p = 0.321$). It is the combined result of Beemster, Hattem and Roosendaal that results in the overall statistically significant difference in residual rickets between males and females for all five sites ($\chi^2 = 5.699$, $p = 0.017$). While differences in sample size have to be considered, this result suggests that a gendered division of labour was a minor or nonexistent risk factor for vitamin D deficiency in Hattem and Roosendaal. This does not mean a gender-related division in activities did not exist, merely that such a division was not a significant risk factor in developing rickets, as it was in the Beemster population. One of the main differences may be that in Hattem, women not only performed housework common for the Beemster women, but were also responsible for the piling of peat on ships for delivery to surrounding towns and cities (Scheper, 1984). This would have exposed Hattem females to more sunlight than those whose daily tasks kept them in and around the house and farm buildings. The same can be postulated for Roosendaal, where women performed various tasks besides those activities in and around the house, most of which were in agriculture, but other common occupations were laundress, seamstress, maid, and shop worker, whereas the majority of men worked in the fields (Van Gastel, 1995). It is possible that, as in Hattem, the traditional division in labour existed in Roosendaal, but did not significantly contribute to the development of vitamin D deficiency.

Infant Feeding, Child Rearing Practices, and Socioeconomic Status

The majority of individuals with active rickets were three years or younger, whereas the ones that showed healing rickets were older. This indicates that the onset of rickets occurred at a quite young age. Although the natural amount of vitamin D in most foods is low and the exposure to sunlight would still have been the determining factor for rickets development (Holick, 2006), it is important to consider if breastfeeding and weaning practices could have contributed to the development of a vitamin D deficiency. For the Beemster collection, Waters-Rist and Hoogland (2017) demonstrated that young infants were breastfed for relatively short periods of time (a few weeks to months) or not at all, and that weaning on cow's milk started relatively early in life. Furthermore, Waters-Rist and Hoogland (2018) have demonstrated lower stable nitrogen and carbon isotope ratios in rachitic 1-month to 3-year-olds who were no longer breastfed. This suggests these nonadults to have a weaning diet that contained less high trophic level foods that may have been an additional source of vitamin D, such as oily fish and eggs. More importantly, feeding young infants (< 1 year) cow's milk as an everyday weaning food may have caused gastrointestinal problems affecting calcium absorption, infections, and other ill effects which may have kept them inside thus decreasing sunlight exposure (Waters-Rist and Hoogland, 2018; Brickley et al. 2014; Henderson, 2005; Hollis and Wagner, 2004l Thandrayen and Pettifor, 2010). Stable isotope data for the other four sites considered in this paper is not yet available, but future research will investigate if the weaning diet was also a possible contributor to vitamin D deficiency in other Dutch post-Medieval sites.

Another factor to consider is the use of a wet-nurse, a practice known to have been commonly used in other European, post-Medieval populations (Campbell, 1989; Giuffra et al. 2015; Prühlen, 2007; Richter, 1996), but that is only reported in some Dutch high SES households (Clerkx, 1985; De Leeuw, 1991). Van Poppel and Mandemakers (2002) report that high SES mothers were well-informed about hygiene and nutrition and were given the advice that breastfeeding their own infants was preferred. Human milk contains low amounts of vitamin D (Henderson, 2005; Mulligan et al. 2010), and levels of vitamin D in breastmilk will decrease when lactation is prolonged (Mulligan et al. 2010). Moreover, wet nurses often were of low SES and would feed a number of young infants suggesting the quality of their milk and the levels of vitamin D to be even lower (De Leeuw, 1991; Van Poppel and Mandemakers, 2002). Thus, probably even in the high SES site of Gouda, most infants were breastfed by their own mother and the practice of wet-nursing is unlikely to have been an important factor for vitamin D deficiency in this collection.

Our data show a significant drop in the number of active cases of rickets after the age of about 3 years. Growth retardation as a result of vitamin D deficiency may have caused a slight underestimation of the age-at-death of the affected nonadults (Brickley and Ives, 2008; Pinhasi et al. 2006). However, the number of active and healing cases of rickets are similar in all the five sites and it is likely that an increase in vitamin D levels occurred in many nonadults after the age of about three years. Although growth in older nonadults will have slowed thereby decreasing the need for vitamin D (WHO 2006), it is still likely there was a shift in sunlight exposure. This may be related to child rearing practices.

De Leeuw (1991) reports that in high SES households the 0 – 3 year-olds were comparatively well-nurtured and attended to by the mother, nanny, and/or maid. Playing games and reading books in their rooms is said to have been important, but also going outside for a walk and visiting other families was part of their daily activities which may have exposed them to more sunlight (De Leeuw, 1991). In contrast, babies and toddlers in lower SES groups often received less attention because their mothers had responsibilities that prevented them from caring for them, who were thus tended to by grandparents, or older siblings (De Regt, 1978; Schenkeveld, 2008). They would have been kept inside to prevent them from harm, and some were kept silent by giving them a pacifier dipped in brandy or a spoon of laudanum with no room to play (De Regt, 1978). This would have significantly decreased their exposure to sunlight. Historic accounts note that as soon as middle to low SES children were old enough to help their parents, which was often from the age of about four years, they started learning various gender-specific chores thereby making them less house-bound and exposing many to more sunlight. This sociocultural habit likely contributed to the drop in active cases of rickets after the age of about 3 years in the low to middle SES households (De Regt, 1978; Schenkeveld, 2008).

Two out of three 0-3-year-olds from the high SES community of Gouda had active rickets. Unfortunately, this sample is too small to permit useful comparisons to the other sites. However, an ample number of adults (n = 32) from this high SES group were available for the assessment of residual rickets, which yielded a prevalence of 12.5%. While this prevalence is the lowest of all five sites, it is not statistically significantly different from the other sites. The prevalence of vitamin D deficiency in high SES populations is often expected to be small or even absent, as is the case in high SES Dutch populations from Alkmaar (Baetsen, 2001) and Zutphen (Berk, 2007). A higher SES may decrease the occurrence or severity of vitamin D deficiency through access to more and better food (Brickley and Ives, 2008; Holick, 2006). However, access to better foods does not imply the consumption thereof. More importantly, prior to the fortification of foods with vitamin D, diet seems to play a small role in preventing vitamin D deficiency (Holick, 2006; Jablonski and Chaplin, 2018). Indeed, vitamin D deficiency has been observed in high SES populations from Italy (Giuffra et al. 2015), Latvia (Pētersone-Gordina, 2013), and in the vault burials from Birmingham, UK (Brickley et al. 2010), so it is certainly known that any SES group is at risk. As four (out of 32) adults from Gouda had residual rickets, this high SES group was definitely also affected.

Via archival documents, three of the four affected individuals at Gouda have been identified as family members of Melchior Van Rietveld, one of the mayors from Gouda (Van Dasselaar, 2015). The mayor's wife, Catherine, displayed residual rickets, as did two of her sons. Sociocultural practices of the Van Rietveld family may have played a role in developing rickets, in particular physical markers of status couched in ideals of beauty. Especially in high SES groups, girls and women were encouraged to keep out of the sun to prevent their skin from getting tanned. Lighter skin was an overt sign of higher SES and set as a beauty ideal, whereby also the high SES males would avoid their skin from getting tanned (De Leeuw, 1991). This was possibly the case for Catharina van Rietveld and even Jacob and Melchior junior, who clearly did not receive enough sunlight to prevent rickets from developing.

According to historic information, all SES groups wore clothing that covered roughly the same amount of skin. The swaddling of infants up to six months of age was common in all SES classes, which left little skin uncovered (Clerkx, 1985; De Leeuw, 1991). After this period of swaddling, boys and girls wore the same clothing up to the age of six years: a wide (sometimes padded) dress and a padded bonnet to prevent damage to the head from falling (De Leeuw, 1991). This changed for high SES children in the 19th century, as they were no longer forced to wear the padded bonnet because their caretakers were meant to pay close enough attention to prevent serious falls and injuries (De Leeuw, 1994). This could have exposed some high SES infants and children to more sunlight, thereby preventing a vitamin D deficiency and resulting in lower prevalences. After the age of six years, children wore the same type of clothes as their parents. High SES girls would wear corsets and wide dresses, large hats, and gloves; and boys would wear hats and suits. Lower SES girls would wear bonnets and plain dresses, the boys plain jackets, shirts, and trousers (De Leeuw, 1991). Regardless, in all SES groups and all age groups, the clothing ideals were quite occlusive and certainly could have contributed to an insufficient amount of dermal vitamin D synthesis.

Adult vitamin D deficiency

Hattem is the first general population in the Netherlands where osteomalacia was observed. Recent research by Van der Merwe et al. (2018) observed four cases of osteomalacia in a skeletal collection from the psychiatric hospital Meerenberg in Bloemendaal (17th - 18th century) in the province of North-Holland. Depending on their condition, most of the patients were kept in bed, had limited to no access to sunlight, and diets low in vitamin D (Vijsselaar, 1997). The individual with osteomalacia from Hattem is a male with an age-at-death between 36 - 49 years. In clinical cases, vitamin D levels are often lower in older adults than in younger adults with the same exposure to sunlight and diet (Brickley and Ives, 2008; Holick, 2003). Several factors are responsible for this decline in vitamin D levels and include decreasing levels of 7-dehydrocholesterol in the skin, declining ability to absorb vitamin D via the intestines, and reduced skin thickness (Halloran and Portale, 1997; Chapuy and Meunier, 1997;

Holick and Adams, 1998). The age-at-death of this individuals does not suggest reduced kidney function nor other age related effects on vitamin D levels to have been important contributing factors to the development of osteomalacia. It is possible that another disease caused this individual to stay indoors which caused vitamin D deficiency exacerbating his poor condition. Since no other adult in Hattem displayed signs of osteomalacia, it is unlikely that sociocultural habits, diet and/or living conditions significantly increased the risk of developing osteomalacia for the Hattem adults. The possibility remains, however, that the same factors responsible for the high nonadult vitamin D deficiency may also have affected adult individuals and that the subtler and often nondiagnostic changes related to osteomalacia affected identification of osteomalacic adults.

CONCLUSION

This paper demonstrated the importance of employing a biocultural perspective to research on vitamin D deficiency in a large sample of individuals (n = 565) from urban and rural Dutch communities. Our findings demonstrate the equifinality of vitamin D deficiency as a multitude of factors can be at play. It is a disease which can and does affect any age, sex, and socioeconomic class, and most importantly, is common in all settlement types, not just large, industrialised cities. Embedding individual biological characteristics in the sociocultural contexts of the past Dutch communities enabled more profound insight into the likely factors that caused deficiency. In our study, infants under the age of four years were most at risk of developing rickets with factors such as child-rearing practices, clothing, and weaning foods being likely causes. A predominance of residual rickets in females suggests that at some sites occupational customs and the division of labour contributed to the development of the disease. Our research fills a lacuna in our understanding of vitamin D deficiency in rural and small, urban communities and improves our understanding of factors influencing past vitamin D deficiency.

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Supplementary Table 1. Overview of the distribution of affected individuals per age category. N/a = not applicable.

Site	18 – 25 years	26 – 35 years	36 – 49 years	50+ years	18+ years
Beemster	12.0% (3/25)	23.9% (11/46)	10.4% (8/77)	14.0% (7/50)	0.0% (0/2)
Gouda	50.0% (1/2)	0.0% (0/7)	5.6% (1/18)	0.0% (0/2)	22.2% (2/9)
Rotterdam	50.0% (1/2)	0.0% (0/5)	13.3% (2/15)	0.0% (0/2)	20.0% (1/5)
Roosendaal	50.0% (2/4)	36.3% (4/11)	0.0% (0/29)	0.0% (0/2)	N/a
Hattem	23.1% (3/13)	15.7% (3/19)	25.9% (14/54)	9.1% (1/11)	0.0% (0/2)
Total	21.7% (10/46)	20.5% (18/88)	6.2% (12/193)	12.1% (8/66)	16.7% (3/18)

5 Micro-CT assessment of dental mineralization defects indicative of vitamin D deficiency in two 17th - 19th century Dutch communities

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ABSTRACT

Objectives: This study investigates vitamin D deficiency patterns in individuals from birth to the beginning of adolescence. Microscopic computed tomography (micro-CT) evaluation of interglobular dentine (IGD) in the teeth provides information on the age of disease onset and the number of deficient periods per individual, which will increase our understanding of factors influencing vitamin D deficiency prevalence, including sociocultural practices and latitude.

Materials and Methods: Beemster and Hattem, two Dutch 17th to 19th century communities, yielded with relatively high prevalences of rickets (15 – 24%) and residual rickets (14 – 24%). From the affected individuals, a subsample of thirty teeth were selected for micro-CT scanning. Thin sections were made of 17 teeth, consisting of 11 teeth without observable IGD on micro-CT and six teeth that were included for method comparison.

Results: Nineteen out of 29 (65.5%) individuals (one tooth was deemed unobservable) presented with IGD. Eight of the 11 (72.7%) individuals without IGD on micro-CT demonstrated histologically visible IGD. In 40.7% (11/27) of the affected individuals, vitamin D deficiency was recurrent, and in four individuals, these episodes were

chronologically successive suggesting vitamin D deficiency was seasonal. In three individuals, IGD occurred in the dentine formed around birth, suggesting maternal vitamin D deficiency.
Discussion: Micro-CT analysis of IGD is found to be a valuable non-destructive method that can improve our understanding of the influence of sociocultural practices and latitude on disease development within age and sex groups in past communities.

INTRODUCTION

Vitamin D is important for calcium homeostasis needed for mineralization of osteoid, newly formed bone tissue (Holick, 2006). Mineralization ensures structural integrity of the skeleton enabling it to withstand gravity and muscular tension. Cutaneous production of vitamin D under the influence of ultraviolet B (UVB) radiation in sunlight is the most effective way of obtaining an adequate amount of vitamin D, with foods such as oily fish providing supplemental amounts (Brickley, Moffat & Watamaniuk, 2014; Holick, 2003, 2006). Insufficient sunlight exposure will lead to vitamin D deficiency and bending deformities of the skeleton may develop that are visible in archaeological human remains (Brickley, Mays & Ives, 2010; Brickley et al., 2014; Brickley, Mays, George & Prowse, 2018; Mays, Brickley & Ives 2006; Ortner & Mays, 1998).

Recent research has shown that skeletal evidence of vitamin D deficiency provides important information on sociocultural practices related to sunlight exposure and diet, and can aid in the reconstruction of past lifeways (Brickley et al., 2014; Giuffra et al., 2015; Palkovich, 2012; Veselka, Van der Merwe, Hoogland & Waters-Rist, 2017; Watts & Valme, 2018). Vitamin D deficiency in archaeological remains is traditionally assessed via macroscopic examination of various lesions. This approach does not offer information about the number of separate episodes of vitamin D deficiency nor the age at which deficiency occurred. A recently developed method by D'Ortenzio et al. (2016) demonstrated the use of histological examination of dental mineralization defects called interglobular dentine (IGD) as a marker of vitamin D deficiency. A recent clinical investigation has shown that IGD is visible on microscopic computed tomography (micro-CT) scans (Ribeiro, Costa, Soares, Williams Jr. & Fonteles, 2015) and Brickley et al. (2017a) have shown IGD can also be observed in archaeological teeth. IGD analysis via non-destructive micro-CT should enable the determination of the age of vitamin D deficiency onset and the assessment of the frequency of vitamin D deficient periods within an individual (Brickley et al., 2017a). This study set out to apply micro-CT analysis of IGD that can aid in the diagnosis of vitamin D deficiency. This paper will use micro-CT IGD analysis to investigate parameters of vitamin D deficiency in a subset (n = 30) of two previously studied 17^{th} - 19^{th} century

Dutch communities, Beemster (MB11) and Hattem (HT15) that were shown to have high levels of rickets (between 15% and 24%) and residual rickets (between 14% and 24%) (Veselka, Hoogland & Waters-Rist, 2015; Veselka et al., 2017; Veselka, Brickley, Hoogland & Waters-Rist, submitted). Although researchers have started to differentiate between cases of healing and active rickets (Brickley et al., 2018; Mays et al., 2006), the development of vitamin D deficiency in various age groups is still poorly understood, whereby the influence of sex and gender roles in nonadults is rarely investigated (Veselka et al., 2015). Although this can be examined in adults with residual rickets (Veselka et al., 2017), the information that can be obtained via macroscopic analysis is limited due to growth and the process of remodeling which may have obliterated certain features (Hess, 1930).

The objectives of this paper are, firstly, to investigate vitamin D deficiency patterns in individuals from birth to the beginning of adolescence via the assessment of the age of disease onset and the number of deficient periods per individual in two post-Medieval Dutch communities. Information on the age of disease onset improves our understanding of vitamin D deficiency development and may provide information on sociocultural practices that possibly increased the risk of deficiency or that exacerbated the condition. The influence of seasonality will be assessed by examining the age of the consecutive IGD formation periods. Furthermore, comparison of IGD severity and the number of IGD episodes between males and females may improve our understanding of the gendered division of activities that influenced sunlight exposure. Our second aim is to investigate the potential of micro-CT analysis of vitamin D deficiency by comparing macroscopic, micro-CT, and histological assessment of this condition. This study is the first to apply a wide-scale assessment of macroscopic, micro-CT, and histological methods of vitamin D deficiency diagnosis.

MATERIALS AND METHODS

Two communities were assessed for this paper: 1) Beemster (MB11), a rural settlement dating to the 19th century in the province of North-Holland with a skeletal sample consisting of 59 nonadults and 200 adults and 2) Hattem (HT15), a small urban center dating from 17th to 19th centuries in the province of Gelderland with a skeletal sample consisting of 21 nonadults and 88 adult individuals. Figure 1 shows the location of these sites.

Macroscopic analysis of both skeletal collections was undertaken prior to this study (Veselka et al., 2015, 2017, submitted). Rickets was assessed scoring various skeletal lesions, including flaring and/ or cupping of the long-bone metaphyses, porosity underlying the growth plate, and bending deformities of the long bones (Brickley & Ives, 2008; Mays et al., 2006; Ortner & Mays, 1998). Residual rickets was scored using the criteria described by Brickley and Ives (2008) and Brickley et al. (2010). Rickets

Figure 1. Location of Beemster and Hattem in the Netherlands.

prevalence in the Beemster community was 15.3% (9/59) and 23.8% (5/21) in Hattem. Residual rickets prevalence was observed to be 14.5% (29/200) in Beemster and 23.9% (21/88) in Hattem (Veselka et al., 2015, 2017, submitted). A subset of 30 affected individuals (out of 64) was selected for micro-CT IGD analysis, 15 from each site, including nonadults, and both sexes. For Beemster, none of the affected nonadults and only two males (out of the eight affected) had suitable teeth for micro-CT scanning. The selected individuals with their basic demographic information are presented in Table 1 in the Results section.

The first permanent molar was selected for micro-CT assessment if available and if dental wear did not exceed stage H (Lovejoy, 1985), indicating the dentine underlying the enamel was still intact. If the first permanent molar was not available (worn or absent), the permanent canine was sampled if available and dental wear did not exceed stage D (Lovejoy, 1985), ensuring the dentine would be observable. In the first permanent molar, dentine formation starts in utero and will enable IGD assessment up to the first 10 years of age, while in the permanent canine, dentine formation starts around 3 months of age and ends at about 14 years of age (Gustafson & Koch 1974; Massler, Schour, & Poncher, 1941; Moorrees, Fanning & Hunt, 1963).

Dentine is formed is two phases, whereby odontoblasts secrete predentine which then is mineralized (Beaumont, Gledhill, Lee Thorp & Montgommery, 2013; Bevelander & Nakahara, 1966; Hillson, 2002). If there are adequate nutritional conditions during the process of formation and calcification, the matrix will appear homogenous and fusion of calcospherites, spheres containing calcium salts, will be complete (D’Ortenzio et al. 2016; Hillson, 2002). When vitamin D levels are inadequate, some of the calcospherites fail to fuse, visible as poorly mineralized patches of dentine with a bubble-like appearance, referred to as IGD (Isokawa, Kosakai & Kajiyama, 1963; D'Ortenzio et al., 2016; Vital et al., 2012). The presence of IGD is clinically associated with conditions disrupting mineralization by affecting vitamin D, calcium or phosphate levels, and vitamin D deficiency is considered to be pathognomonic for this mineralization defect (Chaussain-Miller et al., 2003; McDonnell, Derkson, Zhang & Hlady, 1997; Souza, Soares, Alves dos Santos & Viasbisch, 2010; Vital et al., 2012).

D'Ortenzio et al. (2016) divide IGD into three grades of severity from 1 to 3. However, grading of IGD severity on micro-CT was not undertaken in this study since this grading method was based on histological assessment and features of IGD are less clear on a micro CT scan (Brickley et al., 2017a). Instead, in this study IGD is scored as 'present', 'absent', or 'unobservable'. If present, IGD will be visible as bands of dispersed micro-defects that can be distinguished from taphonomic degradation, because bands of IGD will follow the incremental lines found in dentine. Micro-CT detects differences in mineralization density and since the bubble-like spaces in grade 1 are relatively small, is it suggested that grade 1 is not visible on micro CT scan (Brickley et al., 2017a). Therefore, isolated micro-defects are more likely to be taphonomic or developmental defects. If patches of degradation or cracks are present and mimic bands, these are easily distinguished from IGD by switching to other views, for instance craniocaudal vs. transverse. Dentine grows in concentric cones (Eerkens, Berget & Bartelink, 2011; Hillson, 2002) at rate of about 4 – 6 µm a day in permanent teeth and the first mm of the dentine below the crown in the first permanent molar represents the period around birth to about 1.5 years of age (Beaumont et al., 2013; Massler et al., 1941; Moorrees et al., 1963). In this study, the age at which an episode of IGD occurred is estimated using the criteria of Massler et al. (1941) and Moorrees et al. (1963).

Micro CT scans were performed using a SkyScan 1272 (100 kV, 100 uA, Cu 0.11 mm, 10.0 µm). The scan was reconstructed using NRecon© software. The 3D reconstruction was assessed and when IGD was present, a section image from each plane (buccal – lingual, mesial – distal, and transverse) was made. The occurrence and number of IGD bands were assessed by all researchers independently and only cases which were separately classified as having IGD were included to limit individual bias.

Since IGD grade 1 is not expected to be detectable on micro-CT, a total of 17 thin sections for histological assessment were made from all individuals that did not display IGD on micro-CT (n = 10), in cases where observation for IGD was not possible (n = 1), and six individuals that display IGD on micro-CT for comparison (n = 6). The teeth were embedded in resin (EpoThin©) after which a thin section was made with an IsoMet® 1000 precision saw. After further grinding and polishing as described by De Boer, Aarents and Maat (2013), the thin section was mounted on a glass slide for histological analysis using a Leica® DM500 compound microscope with 40x magnification. The number and the severity of IGD periods were scored for each tooth, whereby grading of IGD was evaluated according to D'Ortenzio et al. (2016).

A chi-square test was performed to test the difference in the number of IGD periods per individual, the difference in the occurrence of the first episode of IGD, and to assess the statistical

Table 1. Overview of analyzed individuals with their sex, age, sampled tooth, macroscopic vitamin D deficiency, number of IGD periods, and the age of IGD formation.

Individual				Macro-scopy*	Micro-CT		Histology		
	Sex	Age-at-death (years)	Tooth sampled		N IGD periods	Age (period of deficiency)†	N IGD periods	Grade	Age (period of deficiency)†
HT15S020	U	15 ± 2	RM1	Possible	0	-	1	1	6 - 12 mths.
HT15S042	M	36 - 49	LM1	Present	1	2.5 yrs.	1	2	2.5 yrs.
HT15S062	F	36 - 49	LM1	Present	0	-	0	-	-
HT15S066	M	36 - 49	LM1	Present	1	6 - 12 mths.	NA	-	-
HT15S067	U	6.5 ± 0.5	LM1	Present	2	6 - 12 mths. 2.5 yrs.	NA	-	-
HT15S071	M	18 - 25	RM1	Present	0	-	0	-	-
HT15S075	F	36 - 49	LC1	Present	U	U	3	2 and 3	2.5 yrs. 5 yrs. 6 yrs.
HT15S080	M	26 - 35	RM1	Present	2	6 - 12 mths. 2.5 yrs.	NA	-	-
HT15S094	F	18 - 25	RM1	Present	2	6 - 12 mths. 3 yrs.	2	2	6 - 12 mths. 2.5 yrs.
HT15S099	F	18 - 25	LM1	Present	0	-	1	1 - 2	6 - 12 mths.
HT15S106	M	36 - 49	RM1	Present	1	6 - 12 mths.	NA	-	-
HT15S109	F	36 - 49	RM1	Present	0	-	1	1 - 2	2.5 yrs.
HT15S123	U	2 ± 0.5	RM1	Present	1	6 - 12 mths.	NA	-	-
HT15S127	U	9 ± 1	RM1	Present	2	Birth 6 - 12 mths.	NA	-	-
HT15S130	M	18 - 25	RC1	Present	4	12 mths. 2.5 yrs. 3 yrs. 5 yrs.	6	2 and 3	1.5 yrs. 2 yrs. 2.5 yrs. 3.5 yrs. 6.5 yrs. 7.5 yrs.
MB11S101	F	26 - 35	RM1	Present	2	6 -12 mths. 2.5 yrs.	3	2 and 3	6 - 12 mths. 2 yrs. 3 yrs.
MB11S126	F	36 - 49	RM1	Possible	0	-	1	1 - 2	Birth - 12 mths.
MB11S183	F	26 - 35	LM1	Present	0	-	1	1 - 2	6 - 12 mths.
MB11S234	F	18 - 25	RM1	Present	0	-	1	2 - 3	6 - 12 mths.
MB11S307	F	18 - 25	RM1	Present	4	Birth 6 - 12 mths. 2.5 yrs. 5 yrs.	4	2 and 3	1 yr. 2 yrs. 3 yrs. 5 yrs.
MB11S321	M	50+	RM1	Present	0	-	0	-	-
MB11S327	F	26 - 35	RM1	Present	2	6 - 12 mths. 2.5 yrs.	NA	-	-
MB11S401	F	26 - 35	LC1	Present	2	2.5 yrs. 3 yrs.	NA	-	-
MB11S413	F	36 - 49	LM1	Possible	1	6 - 12 mths.	NA	-	-
MB11S420	F	26 - 35	RM1	Present	1	2.5 yrs.	2	2 - 3	6 - 12 mths. 2.5 yrs.
MB11S422	F	36 - 49	RM1	Present	1	Birth - 12 mths.	NA	-	-
MB11S427	M	26 - 35	RM1	Present	1	6 - 12 mths.	NA	-	-
MB11S437	F	26 - 35	LM1	Present	2	6 - 12 mths. 2.5 yrs.	NA	-	-
MB11S488	F	36 - 49	RM1	Present	1	2.5 yrs.	NA	-	-
MB11S498	F	50+	LC1	Present	0	-	1	1 - 2	2.5 yrs.

* = data from Veselka et al. (2015; 2017; submitted). † = Age is based on Moorrees et al. (1963) and Massler et al. (1941). U = unobservable, M = male, F = female, RM1 = right first permanent maxillary molar, LM1 = left first permanent maxillary molar, RM1 = right first permanent mandibular molar, LM1 = left first permanent mandibular molar, RC1 = right permanent maxillary canine, LC1 = left permanent maxillary canine, LC1 = left permanent mandibular canine, mths. = months, yrs. = years, HT15 = Hattem, MB11 = Beemster, IGD = interglobular dentine, NA = not assessed.

significance of the difference in the occurrence of IGD bands in specific age periods per individual. A Mann-Whitney U test was performed to assess the statistical significance of the number of IGD episodes per site and the difference in the number of IGD episodes between males and females.

RESULTS

Table 1 provides an overview of all individuals whose teeth were selected for micro-CT scanning. For each individual basic demographic data is noted along with macroscopic vitamin D deficiency diagnosis, the number of IGD episodes, and the approximate age of formation of each IGD episode from both micro-CT and histological analyses. Grading of IGD is noted for the thin sections. Appendix A provides details of the macroscopic lesions attributed to vitamin D deficiency for each individual.

Nineteen individuals displayed bands of IGD on micro-CT, with the majority (89.5%; 17/19) displaying one or two periods of vitamin D deficiency. Figure 2 displays a first right permanent maxillary molar (RM1) of individual HT15S067 showing the location of two episodes of IGD.

As mentioned, the majority of individuals with observable IGD display one or two episodes and this difference is statistically significant (χ^2 = 13.586, p = 0.009). There is no statistically significant difference between the number of vitamin D deficient periods per individual between sites (Hattem: mean number of IGD periods = 1.20; Beemster: mean number of IGD periods = 1.21; U = 104.500, p = 0.983). In 63.2 % of the affected individuals (12/19), the first observable episode of vitamin D deficiency is present in the layer of dentine secreted between 6 and 12 months of age, which is statistically significant (χ^2 = 8.103, p = 0.044). The difference in IGD prevalence in the layer of dentine formed between 6 and 12

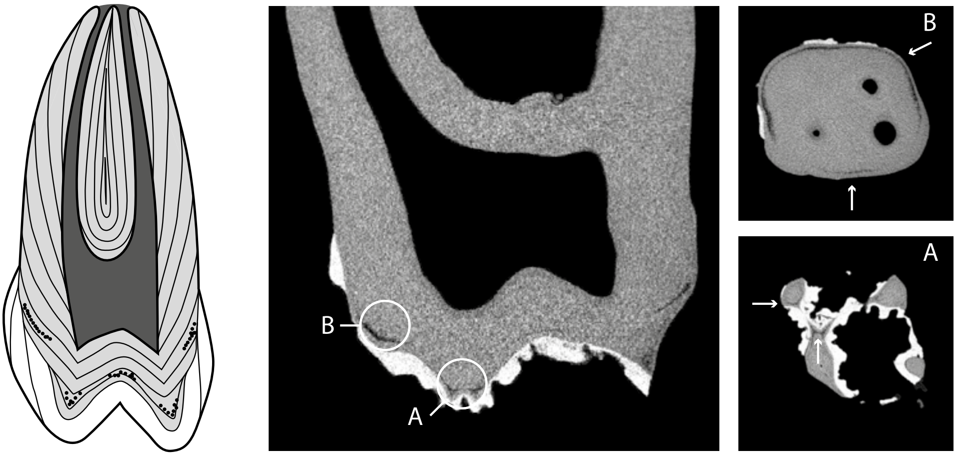

Figure 2. HT15S067 shows 2 episodes of IGD (A and B), visible in the schematic drawing, the micro-CT scan in craniocaudal view, and both episodes in transverse view marked by white arrows.

months of age is statistically significant (χ^2 = 10.862, p = 0.012, and most individuals demonstrated IGD in this layer of dentine (78.9%; 15/19). The majority of individuals (89.5%; 17/19) did not display IGD bands after about 2.5 – 3 years of age.

Eleven out of 17 individuals (64.7%; 11/17) displayed more IGD episodes on thin section than on micro-CT (including HT15S075 which was considered unobservable on micro-CT). Figure 3 shows a craniocaudal micro-CT image of the right permanent maxillary canine (RC1) of individual HT15S130 demonstrating 4 possibly 5 episodes of IGD (A to E). It was difficult to determine on micro-CT if episode A and B occurred in the same age period. Therefore, the fifth episode of IGD was considered to be possibly present. On the histological image of the same tooth, the same episodes are visible (A to E) and one additional period (F) that is not visible on micro-CT. In episode A of the histological image, two bands of IGD are clearly visible within the same layer of dentin, which could be the result of differences in appositional growth of the dentin throughout the layer (D'Ortenzio, Kahlon, Peacock, Salahuddin & Brickley, 2018).

Three out of the 30 individuals (10%) that displayed macroscopic lesions that were (possibly) attributed to vitamin D deficiency, did not present with IGD episodes after micro-CT and histological assessment. As described in the Appendix, these individuals (HT15S062, HT15S071, and MB11S321) have bending deformities of both tibiae. Individual HT15S062 also displayed bowing of the left femur, and HT15S071 presented with coxa vara of both femora.

Three different individuals were previously macroscopically diagnosed as having possible residual rickets due to only partially meeting the criteria described by Veselka et al. (2017). HT15S020 displayed anterolateral bending of both femora, MB11S126

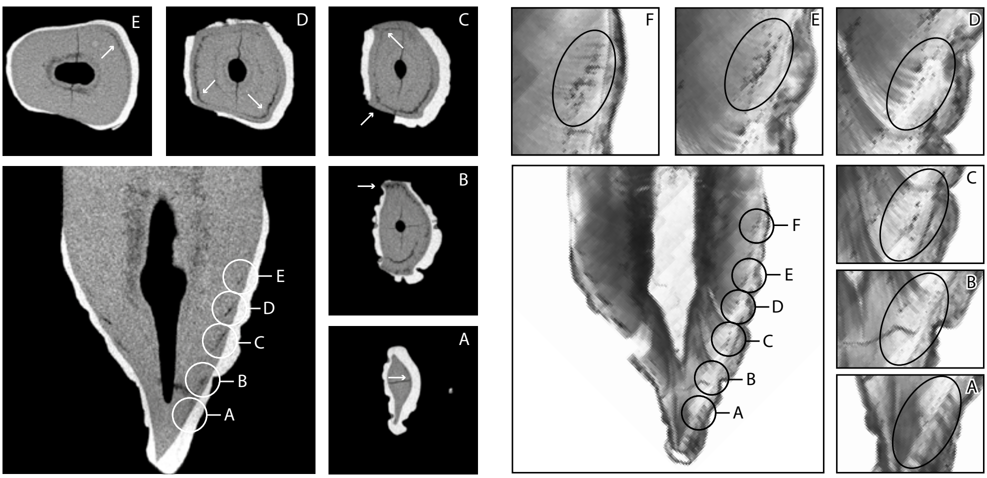

Figure 3. Craniocaudal micro-CT image of the right permanent maxillary canine (RC1) of individual HT15S130 with corresponding transverse image of each IGD episode marked by white arrows, and a thin section image at 40x magnification of the same tooth with each of the IGD episodes shown separately.

showed bending deformities of both radii and ulnae, and MB11S413 presented with bending deformities of the left femur and the left radius. All three presented with episodes of IGD; dentine defects were visible after micro-CT in MB11S413 and histological analysis in HT15S020 and MB11S126.

The difference in IGD prevalence on micro-CT between Hattem females (1/4) and males (5/6) is not statistically significant (Fisher's exact test: $p = 0.190$), which may partially be attributed to small sample size. It is worth noting that the majority of Hattem females (75.0%; 3/4) did not display visible bands of IGD on micro CT, whereas the majority of the males displayed one or more IGD episodes (83.3%; 5/6). It was not possible to assess differences between the prevalence of IGD in Beemster females and males, because only two male individuals had observable teeth.

DISCUSSION

Macroscopy, micro-CT and histology

Dentine and bone are mineralized tissues that display similarities in their formation and composition, and will react similarly to pathological conditions affecting mineralization (D'Ortenzio et al., 2016; Foster, Nociti & Somerman, 2014; Vital et al., 2012). Vitamin D plays a vital role in the mineralization process of both teeth and bone, and a vitamin D deficiency will disrupt this process. Vitamin D deficiency may lead to bending deformities of the long bones, changes to the metaphyses (flaring, cupping, and/or thickening) and sternal rib ends (thickening, and/or increased porosity), and other skeletal deformities in the growing skeleton (Brickley et al., 2014, 2018; Mays et al., 2006; Ortner & Mays, 1998). However, bone is a dynamic tissue that undergoes continuous remodeling, and if vitamin D deficiency is overcome, less severe bending deformities and skeletal lesions will remodel and may be completely obliterated (Brickley et al., 2017b; Hess, 1930), whereas dentine shows no turnover and poorly mineralized dentine will remain visible after vitamin D deficiency was overcome (D'Ortenzio et al. 2016; Foster et al., 2014; Vital et al., 2012).

Most individuals with macroscopic lesions due to rickets and residual rickets, with teeth available for scanning, presented with one or more vitamin D deficient periods visible as episodes of IGD on micro-CT (65.5%; 19/29). Histological assessment of 17 individuals demonstrated additional bands of IGD in 64.7% (11/17) of them. The combined results of micro-CT and histological analysis demonstrated that 90.0% (27/30) of the individuals with macroscopic lesions attributed to past episodes of vitamin D deficiency, displayed one or more episodes of IGD. This suggests that macroscopic assessment of vitamin D deficiency is relatively reliable. However, three individuals with macroscopic lesions attributed to vitamin D deficiency, did not display IGD on micro-CT or thin section. Bowing deformities can be produced by a wide range of pathological conditions (Brickley and Ives 2008: Table 5.8) and as discussed by Brickley and co-workers (2018), lesion development is complex and depends on several factors including nutrition, co-occurrence, and aspects of lifeways. It is possible that these individuals did suffer from vitamin D deficiency but that the condition may have developed after apical closure of the root of the permanent first molars and canines. The rate of appositional dentine growth in the roots of the teeth differs from the growth rate of the dentine formed near the crown which may result in the absence of IGD formation in the roots of the first permanent molar and permanent canine despite vitamin D deficiency (D'Ortenzio et al., 2018). However, the bending deformities observed in these individuals may simply represent more pronounced expression of human variation, or relate to an alternate cause; differential diagnosis undertaken produced no clear alternate diagnosis (Veselka et al. 2017; submitted), but this remains a possibility.

The use of the non-destructive micro-CT method to detect vitamin D deficiency can aid in the identification of the disease in individuals who lack clear lesions, as was the case with MB11S413. This individual was diagnosed with possible residual rickets due to partially meeting the criteria as described by Veselka et al. (2017), but displayed IGD on micro-CT supporting residual rickets diagnosis.

Previous research found that not all individuals that experience an episode of vitamin D deficiency develop lesions that could be identified via macroscopic examination (D'Ortenzio et al., 2018), and it is possible that some of the episodes of deficiency identified in the current investigation were not linked to skeletal lesions identified. Of those that do develop visible bending deformities of the long bones during childhood, only 10 – 15% are estimated to remain visible in the adult skeleton due to growth and the process of remodeling (Brickley et al., 2017b; Hess, 1930). Micro-CT assessment of IGD can also be applied to individuals with missing skeletal elements. Poor skeletal preservation and incompleteness of the skeleton have been shown to affect diagnosis in nonadults, and assessment of past vitamin D deficiency in adults may be hindered when long bones and ribs are unobservable since bending deformities of these skeletal elements are the most frequently observed (Brickley et al., 2018).

Extensive diagenetic change prevented assessment using micro-CT of one individual (HT15S075). In this case, assessment via thin section revealed that IGD was present and relatively easy to assess, demonstrating three episodes of IGD with grade 2 and 3 that should have been visible on micro-CT.

It was previously reported by Brickley et al. (2017a) that grade 1 IGD would not be detected using micro-CT assessment, but the current study analyzed a much larger number of individuals and results from this investigation demonstrate that depending on the micro-CT set-up, some individuals at the lower end of grade 2 IGD may also be missed. Most individuals without IGD on micro-CT and the individuals that displayed additional bands of IGD on thin section, presented with IGD grade 1 and a lower grade 2 on histological examination. Our results seem to support the limitation of IGD observation on micro-CT scan to grade 2 (excluding the lower end of the range) and 3 as noted by Brickley et al. 2017a).

Although histological analysis appears to provide the most accurate results and the process of producing thin sections is relatively fast and inexpensive, it requires the destruction of archaeological human remains that may not be desirable or possible in all skeletal collections. Furthermore, various other features not attributed to vitamin D deficiency are visible on thin sections, such as developmental IGD (a possible result of difference in the dentine formation rate in various parts of the tooth) and marbling or dappling (variation in normal dentine) (D'Ortenzio et al. 2018) that may hinder pathological IGD identification. If histological examination is not possible, micro-CT analysis of more severe IGD may aid in the identification of vitamin D deficiency in affected individuals without clear macroscopic lesions. More importantly, the 3D nature of a micro CT scan enables better comparison of various IGD periods than on thin section, whereby IGD can be evaluated in different planes of the tooth.

Number of deficient periods and age of onset

The results of micro-CT assessment demonstrated that three individuals (10.3%; 3/29) displayed their first period of vitamin D deficiency around birth. During pregnancy, the fetus is dependent on vitamin D levels of the mother (Dror & Allen, 2010; Mulligan, Felton, Riek & Bernal-Mizrachi, 2010; Thandrayen & Pettifor, 2010). If the mother is vitamin D deficient, the fetus will not be able to obtain sufficient vitamin D and may present with IGD in the first layers of dentine deposited around birth. It is possible that the mothers of HT15S127, MB11S307, and MB11S422, who show IGD in the first layers of dentine, may have suffered from adult vitamin D deficiency. The prevalence of IGD in the dentine just below the enamel may be used as an indication of adult vitamin D deficiency within a population.

The majority of individuals display the first vitamin D deficient episode before the age of about 2.5 years (78.9%; 15/19) whereby most affected individuals (89.5%; 17/19) do not present with IGD after the age of about 3 years. However, the possibility exists that individuals experienced periods of vitamin D deficiency during formation of the dentine in the roots (D'Ortenzio et al., 2018) or in adolescence which would be unobservable in the first permanent molar and permanent canine. Future analysis of the second and third permanent molars of the affected individuals will provide more information on periods of vitamin D deficiency in adolescence.

Recent macroscopic analysis of Beemster and Hattem nonadults yielded active and healing cases of rickets (Veselka et al., 2015, submitted), whereby active cases presented with underlying porosity of the growth plates (Brickley et al., 2018; Mays et al., 2006). Almost all of the active cases of rickets were observed in individuals younger than 3 years of age, whereas most healing cases (in which porosity of the growth plates was absent) were observed in older individuals. It was postulated that an increase of sunlight exposure occurred in individuals older than 3 years of age thereby decreasing the number of active cases after this age (Veselka et al., submitted). Although additional periods of vitamin D deficiency may be evident in the second and third molars, the results of micro-CT analysis appear to indicate an increase in vitamin D levels after the age of about 3 years, since the majority of affected individuals (89.5%; 17/19) do not present with periods of IGD after that age. However, this does not seem to have been the case for HT15S075, HT15S130, and MB11S307. These individuals experienced their first episode of vitamin D deficiency in the first year of their lives and on thin section presented with another 2, 3 and 5 consecutive periods respectively. The combined results of micro-CT and histological analysis demonstrate that 40.7% (11/27) of the individuals with IGD display two or more bands of IGD indicating vitamin D deficiency to have been recurrent in both communities. Since the Netherlands has a latitude of 35°N, no dermal synthesis of vitamin D takes place in the winter months (Jablonski & Chaplin, 2013; Webb, Kline & Holick, 1988), and vitamin D deficiency may have been seasonal, as suggested by the number of chronologically successive IGD periods in HT15S075, MB11S101, MB11S307, and HT15S130. Seasonal vitamin D deficiency, especially in more northern latitudes, has previously been proposed to be observable in the archaeological collection from St. Martin's Birmingham from the UK (Mays, Brickley & Ives, 2009), and the recurrent episodes of vitamin D deficiency that were observed in individuals from 15 Roman settlements across Western Europe may have been indicative of seasonality (Brickley et al., 2018). Our results provide the first clear evidence of vitamin D deficiency occurring in recurrent episodes that would fit seasonal vitamin D deficiency.

Differences in macroscopically visible rickets and residual rickets prevalence between Beemster and Hattem were not statistically significant (Veselka et al., submitted), and the prevalence of IGD (first episode and the number of episodes) seems to support the notion that both communities experienced a similar risk of developing vitamin D deficiency. Recent research suggested a gendered risk for developing vitamin D deficiency in the Beemster community, whereby significantly more females (21/100) than males (8/100) were affected (Veselka et al., 2017). Due to the small number of males with suitable teeth for micro-CT assessment, comparison of IGD between Beemster females and males was not undertaken. For Hattem, no significant difference ($\chi^2 = 2.191$, $p = 0.139$) between affected males and females was observed (Veselka et al., submitted) and the difference in IGD prevalence between Hattem males and females was not statistically significant (Fisher's exact test: p = 0.190), partially due to small sample size. It is, however, worth noting that the majority of males (83.3%; 5/6) displayed one or more IGD episode on micro-CT, whereas the majority of females (60.0%; 3/5) did not display IGD on micro-CT but did so on thin section. This suggests that males experienced more severe mineralization defects than females, which may be indicative of more severe periods of vitamin D deficiency in males. However, the linkages between the severity of IGD, the degree and presence of macroscopic bending deformities, and the severity of vitamin D deficiency, are complex and need further study.

CONCLUSION

The combined results of macroscopic, radiographic, and histological assessment of vitamin D deficiency suggested histological analysis of IGD provides the most accurate results. However, this study demonstrates that non-destructive micro-CT analysis of IGD is a valuable method that may aid in the identification of vitamin D deficient individuals without no observable, or subtle, macroscopic bending deformities. This method provides information on the age of onset and the number of deficient periods, allowing a more nuanced understanding of vitamin D deficiency development in both males and females during growth and development.

Results of micro-CT analysis support previous findings, that nonadults after the age of 3 years were likely to have experienced an increase in sunlight exposure. In three individuals, episodes of IGD were observed in the dentine formed around birth that suggests maternal vitamin D deficiency. Further examination of the number of IGD episodes and the attributed age periods showed vitamin D deficiency to be a recurrent condition in 40.7% (11/27) of the affected individuals and seasonal deficiency appears to have occurred in at least four individuals. This is an important finding that supports the notion that vitamin D deficiency is seasonal in more northern latitudes.

Micro-CT and histological analysis of IGD enables comparison of IGD severity between males and females. Although Hattem males and females are suggested to have experienced similar risks of developing vitamin D deficiency, the males display more severe IGD than females suggesting they experienced more severe periods of vitamin D deficiency. The comparison of IGD severity between males and females may provide valuable information on gendered differences. Moreover, it aids in better understanding the linkage between macroscopic lesions and IGD prevalence, and improves our knowledge of the influence of vitamin D deficiency on certain groups in past communities. Future research on the influence of the interplay of various biophysical variables (e.g. latitude, season), sociocultural factors (e.g. clothing, socioeconomic status, gendered division of labor), and aspects of diet on vitamin D deficiency prevalence using macroscopic examination, micro-CT and histological analysis of IGD will enhance our understanding of this relationship.

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APPENDIX.

Overview of macroscopic lesions per individual that may be attributed to vitamin D deficiency.

Individual			Bending deformities*										
	Age	Sex	DCP	ORP	EH	Femur	Tibia	Fibula	Humerus	Radius	Ulna	Clavicle	Ribs
HT15S020	15 ± 2 years	U				X							
HT15S042	36 - 49 years	M	X			X (L)	X						
HT15S062	36 - 49 years	F				X (L)	X						
HT15S066	36 - 49 years	M		X			X	X (L)					
HT15S067	6.5 ± 0.5 years	U					X	X					
HT15S071	18 - 25 years	M				X	X						
HT15S075	36 - 49 years	F			X	X			X			X	X
HT15S080	26 - 35 years	M	X			X		X					
HT15S094	18 - 25 years	F				X	X		X	X	X		
HT15S099	18 - 25 years	F		X		X	X						
HT15S106	36 - 49 years	M		X	X		X						
HT15S109	36 - 49 years	F				X	X						
HT15S123	2 ± 0.5 years	U		X		X	X	X	X	X	X		X
HT15S127	9 ± 1 years	U			X	X	X			X			
HT15S130	18 - 25 years	M	X		X	X	X	X		X	X		
MB11S101	26 - 35 years	F				X	X						
MB11S126	36 - 49 years	F								X	X		
MB11S183	26 - 35 years	F						X	X				
MB11S234	18 - 25 years	F				X							
MB11S307	18 - 25 years	F				X	X	X					
MB11S321	50+ years	M					X						
MB11S327	26 - 35 years	F				X	X		X				
MB11S401	26 - 35 years	F				X	X	X					
MB11S413	36 - 49 years	F				X (L)				X (L)			
MB11S420	26 - 35 years	F				X	X						
MB11S422	36 - 49 years	F					X			X			
MB11S427	26 - 35 years	M				X	X	X					
MB11S437	26 - 35 years	F				X							
MB11S488	36 - 49 years	F				X	X	X					
MB11S498	50+ years	F				X	X						

* = bending deformities are bilateral unless indicated otherwise, HT = Hattem, MB = Beemster, DCP = diffuse cranial vault porosity, ORP = orbital roof porosity, EH = enamel hypoplasia, L = left.

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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.

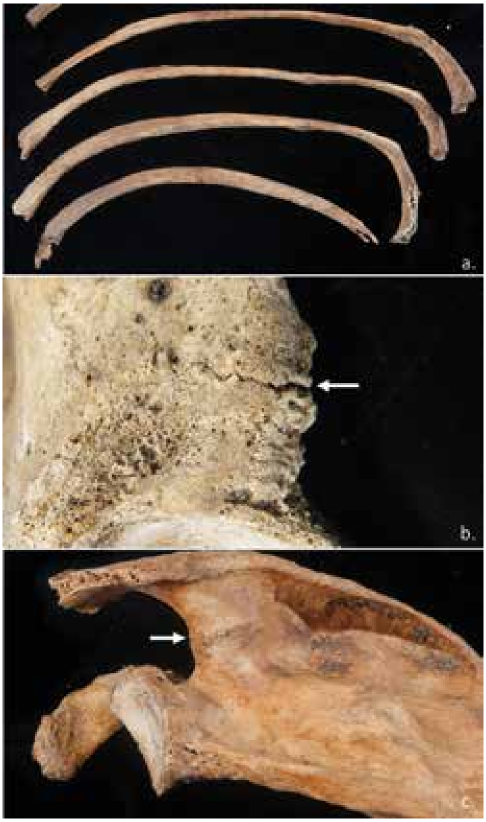

Figure 1. A composite figure illustrating some of the possible osteomalacia related lesions observed in Individual 1 (MeB S2039 V084). a. Lateral straightening of the ribs and several healed rib fractures. b. A possible pseudofracture of the left anterior inferior iliac spine (arrow). c. A pseudofracture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

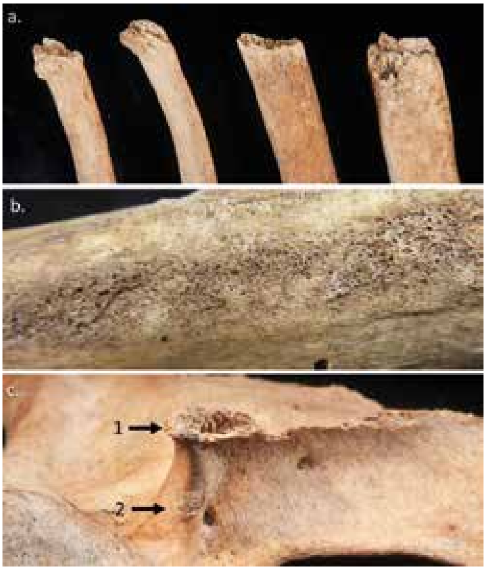

Figure 2. A composite figure illustrating some of the observed lesions possibly related to osteomalacia. a. Complete rib fractures presenting with signs of healing at the fractured ends in Individual 3 (MeB S2037 V148). b. Bone accumulations as observed bilaterally on the proximal femora, and (c.) postmortem 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

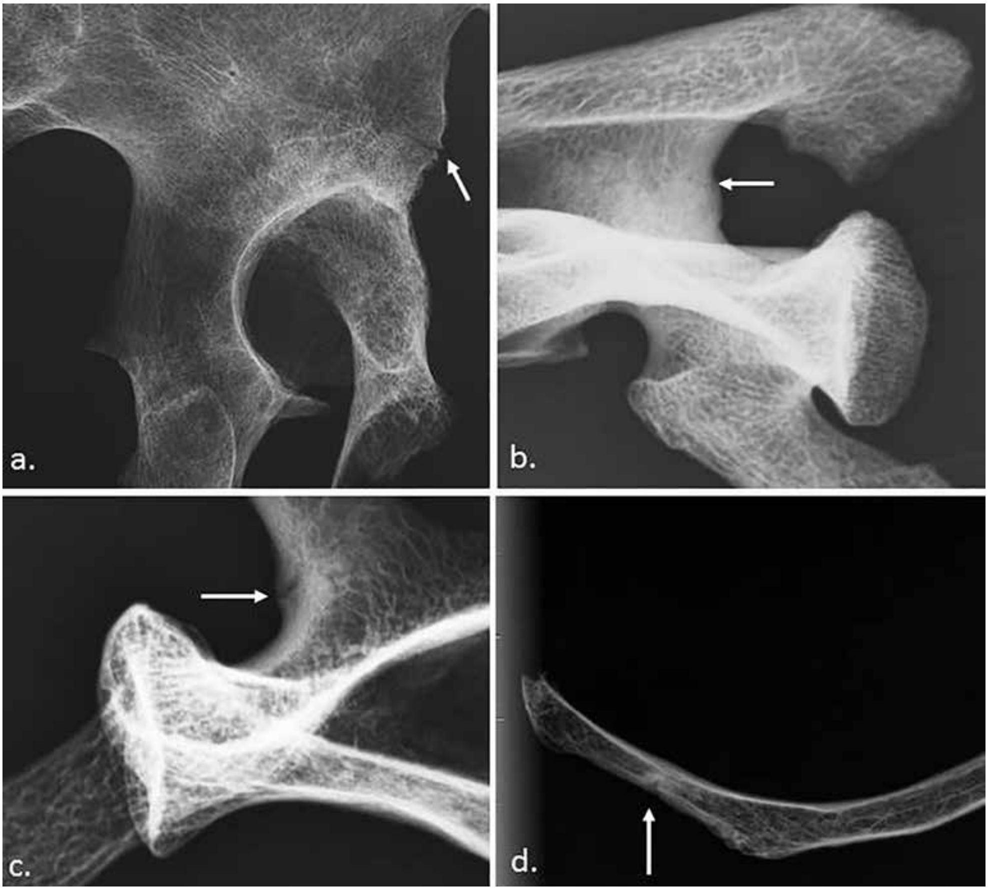

Figure 3. A composite figure presenting a selection of the possible osteomalacia related lesions observed in conventional radiography. a. A fracture of the left anterior inferior iliac spine in Individual 1 (MeB S2039 V084). Pseudofractures of the inferior lateral margins of the scapular spine as observed 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

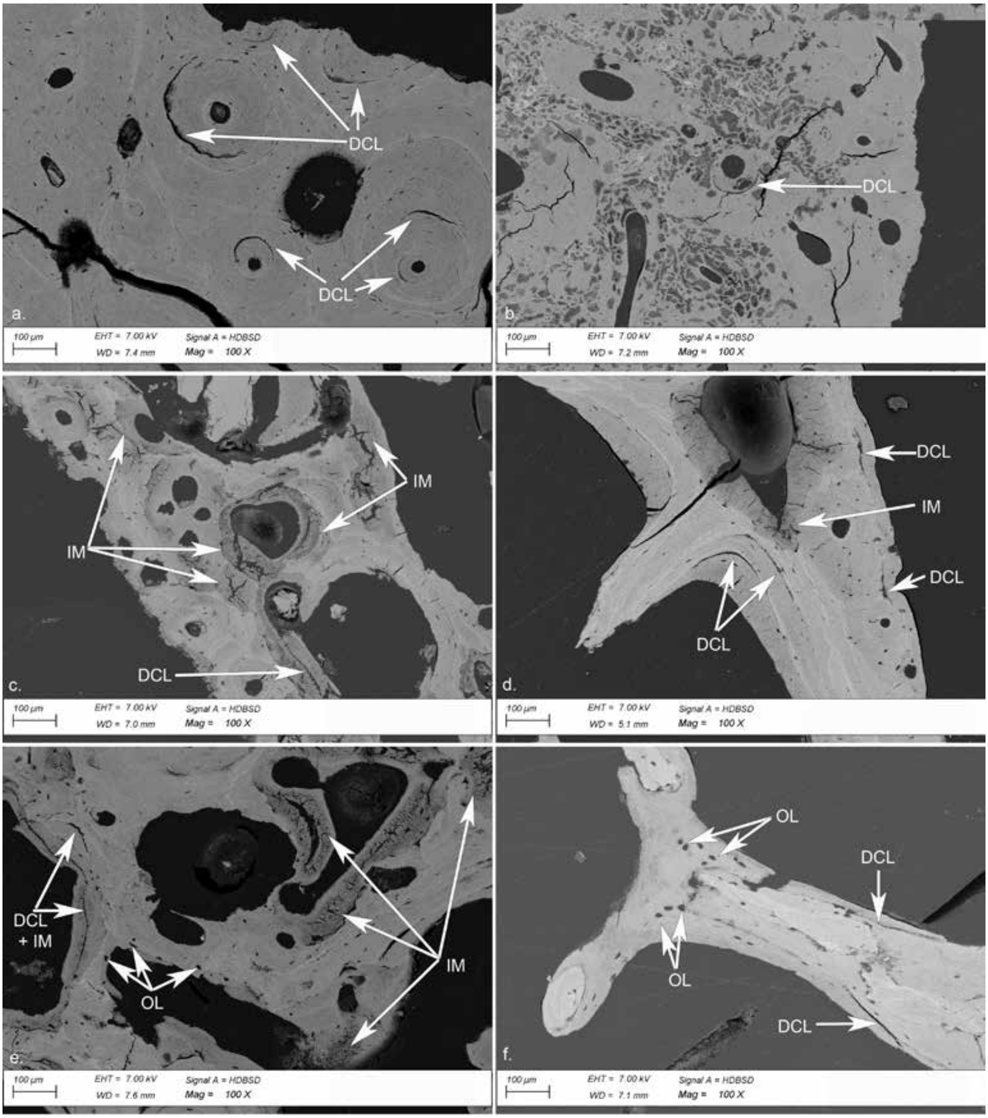

Figure 4. Composite figure of BSE-SEM images of the femoral, costal and vertebral bone samples taken from Individual 2 (MeB S2034 V138) (a., c. and e.) and Individual 3 (MeB S2037 V148) (b., d. and f.). Defective cement lines (DCL) are visible in all the sections. Note the difference in abundancy of 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.

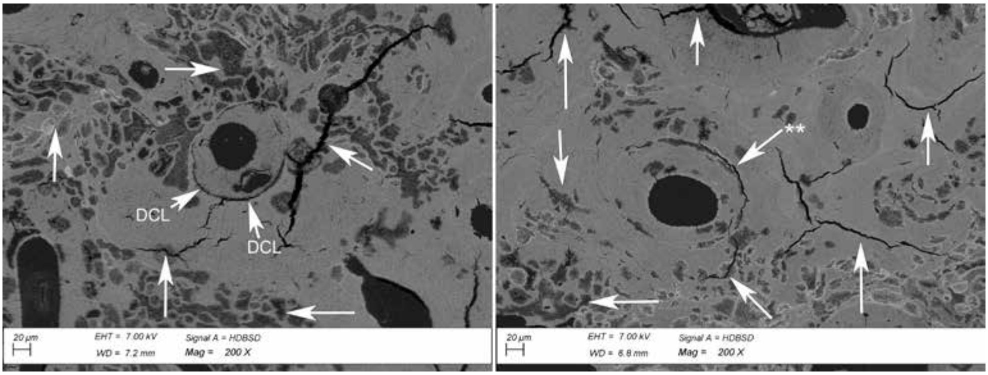

Figure 5. A composite figure illustrating the difference between mineralization defects and post-mortem changes (diagenetic changes) as observed during SEM investigation. Mineralization defects (DCL) were characterized by a fine granular, delicate appearance and were 'anatomically' distributed. In contrast, 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

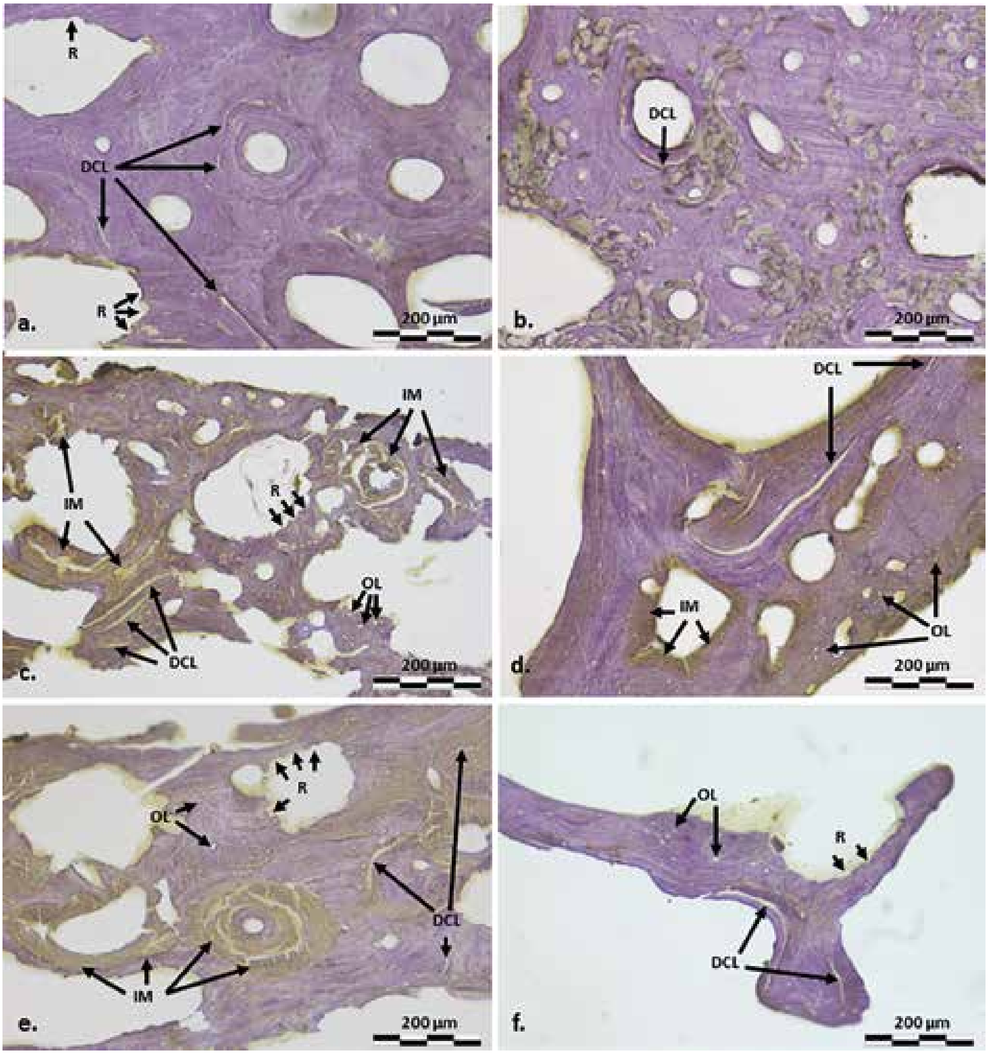

Figure 6. Composite figure of micrographs of undecalcified, haematoxylin stained, hand-ground sections of femoral, costal and vertebral bone samples from individual 2 (MeB S2034 V138) (a., c. and e.) and individual 3 (MeB S2037 V148) (b., d. and f.). All sections show defective cement lines (DCL). Furthermore, 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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7 Conclusion

This study of vitamin D deficiency in Dutch post-Medieval human remains provides a unique perspective on the influence of the industrialisation process in past communities. Some parts of the Netherlands developed into industrialised centres, such as Amsterdam with its sugar-refining industry, and the area of Twente with its textile industry, but the Netherlands did not experience the Industrial Revolution as the UK and many other European countries did, but rather followed a different path (De Vries 2000; Drukker and Tassenaar 1997; Moky 2000; Wintle 2000). Researchers argue that the Netherlands already had a relatively modern economy before the Industrial Revolution started in the UK, and technological innovation was part of the glory of the Golden Age (Drukker and Tassenaar 1997; Mokyr 1976; 2000; Wintle 2000), whereby this legacy and the cultural traditions and norms from that time period held back technological innovation in the beginning of the 19th century (Jacob 1997). Furthermore, by the end of the 18th and the beginning of the 19th century, the Netherlands experienced difficult times caused by many wars, and several periods of French occupation severely impoverished many provinces (De Vries 2000; Drukker and Tassenaar 1987; Mokyr 2000; Wintle 2000). Yet, economic, political, and social change occurred in the 17th to 19th centuries that likely affected all populations, both sexes, and all age groups in various degrees. This study is the first that evaluates vitamin D deficiency prevalence in 17th to 19th century communities from a large area of the Netherlands, to reconstruct changes in sociocultural practices, information that cannot be obtained from historic sources, to improve our understanding of this significant period in Dutch history.

The main research question of this study was: How does the occurrence of vitamin D deficiency in 17th to 19th century Dutch communities enhance our knowledge of changing sociocultural practices as a result of industrialisation?

This question was divided into three sub-questions:

Sub-question 1: How did differences in population aggregation affect vitamin D deficiency prevalence in 17th to 19th century Netherlands?

Sub-question 2: How is vitamin D deficiency prevalence influenced by age, sex, and gender in 17th to 19th century Netherlands?

Sub-question 3: How did socioeconomic status influence vitamin D deficiency prevalence in 17th to 19th century Netherlands?

Chapters 2, 3, 4, and 6 present data on vitamin D deficiency prevalence in all age and sex groups from rural and urban settlements, and a skeletal collection consisting of adult individuals from a psychiatric hospital. Although clear differences in economic organisation existed between the various Dutch regions, thereby distinguishing the more urbanised area of the coast vs. more rural communities from the southeastern part of the Netherlands (Drukker and Tassenaar 1987), in answer of sub-question 1, this research demonstrates that all the populations experienced similar risks of developing vitamin D deficiency, regardless of population size, their main occupations, and geographic location. Thus, it is argued that various sociocultural practices influenced vitamin D deficiency prevalence more so than the classic factors attributed to industrialisation, such as tall densely-packed buildings and air pollution.

Sub-question 2 is answered in Chapters 2, 3, 4, and 5. The impact of sociocultural practices on vitamin D deficiency prevalence in various age groups was assessed in these chapters, wherein Chapter 5 applied recently developed methods that evaluate interglobular dentine (IGD) via micro-CT and histology to provide information on the age of rickets onset and the number of vitamin D deficient periods. Macroscopic examination of rickets revealed the majority of healing cases appear in nonadults older than 3 years of age, whereas most active cases appear in the younger nonadults. Results of IGD assessment showed that in most individuals, the first band of observable IGD was present in the layer of dentine deposited between 6 and 12 months of age, and that the majority of individuals did not display IGD after the age of about 3 years. Thus, both macroscopic and micro-CT analyses suggest nonadults older than 3 years of age experienced an improvement in sunlight exposure. Three individuals displayed IGD in the dentine formed around birth, which was postulated to be indicative of maternal vitamin D deficiency, even though osteomalacia was not observed in any of the female individuals from the communities under study (not including the psychiatric hospital). This speaks to the more subtle nature of osseous lesions of osteomalacia and the difficulty of detecting and diagnosing anything but the most severe cases. It is possible that the detection of IGD in dentine formed before or around the time of birth could provide a more accurate indication of reproductively-aged adult female vitamin D deficiency than macroscopic analyses, an area for future research.

The difference in macroscopically diagnosed residual rickets prevalence between males and females shows that overall females were more affected than males, suggesting gender related sociocultural practices were at play in all communities under study, although this is most clearly observable in the collection of Beemster. Analysis of the number of IGD periods in Hattem males and females suggested a similar risk of developing rickets, but comparison of IGD severity revealed males had more severe IGD, which may suggest that vitamin D deficiency in females was less severe. In answer of sub-question 3, Chapters 4 and 6 demonstrate that SES was not found to be a main determining factor in the development of vitamin D deficiency. It is generally assumed that individuals with a higher SES have a decreased risk of developing this deficiency, since their living conditions and diets are suggested to be better than individuals with a lower SES. The high SES population of Gouda did present with a lower prevalence of vitamin D deficiency. However, the difference in vitamin D deficiency prevalence between the five sites (excluding the collection of Bloemendaal) is not significantly different, and still 12.5% (4/32) of the adult individuals from Gouda presented with bending deformities attributed to residual rickets. Furthermore, archival data enabled the identification of three of the four affected individuals, demonstrating them to belong to the same high SES family, suggesting that perhaps the particular habits of this family rather than SES in general contributed to vitamin D deficiency development. Chapter 6 described the majority of individuals from the Meerenberg Institute of Bloemendaal to be poor and of lower SES. However, most patients were bedridden and would have been kept inside. This would have deprived them from sunlight and inhibit vitamin D production in all individuals, regardless of SES.

The research presented in Chapter 5 is the first large-scale investigation of vitamin D deficiency that enabled comparison of macroscopic, micro-CT and histological assessment and provides information on the merits of each method of evaluation. More importantly, this study is the first to present clear evidence of vitamin D deficiency being recurrent which fits the notion of vitamin D being seasonal. This finding enhances our understanding of the influence of seasonality on vitamin D deficiency prevalence in communities from more northern latitudes.

As mentioned in the Introduction, the Netherlands experienced population growth, and urbanisation during the 17th century, factors that are suggested to have contributed to the rise of vitamin D deficiency prevalence (Hess 1930). Hess (1930) reports that rickets was already present in the Netherlands the 15th century, but that the development of larger cities together with a deficient amount of UVB in sunlight at this latitude (52°N) in the winter months increased vitamin D deficiency prevalence in the centuries thereafter. Early medical literature reports a rickets prevalence of 15% in Amsterdam at the end of the 19th century (Mays 2018), and Drukker and Tassenaar (1987) suggest life in urban areas (provinces of Noord - and Zuid - Holland) to be worse than life in the more rural provinces (Utrecht, Noord-Brabant, Overijssel, Gelderland, and Limburg). Our data show that Hattem and Roosendaal, collections that come from the 'rural traditional' part of the Netherlands (Drukker and Tassenaar 1987), exhibit relatively high prevalences of vitamin D deficiency, that were not significantly different from the prevalences observed in the rural community of Beemster, or the larger urban centre of Rotterdam. The collection of Rotterdam dates to the 17th century (Ploegaert 2017), and the individuals from this collection did not experience the classic consequences of industrialisation. Yet, this study demonstrated the difference in vitamin D deficiency prevalence between the five sites (excluding the collection of Bloemendaal) not to be statistically significant, suggesting other factors, such as sociocultural practices related to sunlight exposure, were at play.

Most sociocultural practices, such as a traditional division in labour and type of clothing, must have been present well before the rise of industrialisation, whereby economic and political pressure, increasing poverty, periods of war, cattle pests, floods, and other disastrous events that occurred during the 17th - 19th centuries, gradually may have changed these practices in the communities under study. A possible explanation for the risk of developing rickets in the nonadults younger than about 3 years of age, might be that their parents needed to put in many hours of work to provide for the family and that they were kept inside for long periods of time to prevent them from harm. This would have deprived them from their much needed vitamin D, exacerbated by the impaired production of vitamin D during the winter months. In the Beemster community, a gendered division in activities further increased the risk of developing rickets in girls (Chapter 2 and 3). Nonadults older than about three years may have been permitted more freedom to go outside and play with children as young as six years already sometimes required to assist their parents with various chores around the farmstead (Schenkeveld 2008).

This study demonstrated that the 17th - 19th century economic and political changes, including industrialisation, influenced sociocultural practices. Some of these practices likely contributed to the development of vitamin D deficiency prior to industrialisation, such as a gendered division of labour and type of clothing, whereby latitude, visible as seasonal vitamin D deficiency, must have played an important role. The economic and political changes that occurred in the 17th to 19th centuries are suggested to have accentuated the existing sociocultural practices. This study decreases the lacunae in our knowledge of Dutch vitamin D deficiency prevalence in rural and urban centres from the 17th to 19th century, aids in better understanding the social changes that occurred in this time period, and improves our insight in vitamin D deficiency development by providing data on the influence of sociocultural practices and seasonality.

Future analysis of IGD will be undertaken in the 1st, 2nd and 3rd permanent molar of each adult individual to enable vitamin D deficiency assessment from birth to adolescence. This will provide more accurate information on the number of recurrent episodes and the ages of IGD occurrence, which will enhance our knowledge of disease development in various age and sex groups. Furthermore, IGD in individuals with and without macroscopic lesions will be investigated to improve our understanding of the linkages between macroscopic lesions, the severity of vitamin D deficiency and IGD occurrence. Since some sociocultural practices that influenced vitamin D deficiency prevalence are postulated to have been present before industrialisation and rickets is reported to have been present already in the 15th century (Hess 1930), it is important to investigate vitamin D deficiency prevalence in communities prior to the 17th century. This will improve our understanding of the influence of sociocultural practices on vitamin D deficiency development before the economic and political changes of the 17th to 19th centuries affected these practices.

Sites from modern agriculture areas (Drukker and Tassenaar 1987) were not included in this study, but should be considered in the future and additional sites from the urban and rural traditional areas should also be investigated to improve our understanding of regional variation in vitamin D deficiency prevalence in the Netherlands. A study from a post-Medieval community from Vlissingen in the province of Zeeland, one of the modern agriculture areas, revealed relatively small vitamin D deficiency prevalence (4%; Maes 2010), whereas studies of large urban centres, such as ‘s-Hertogenbosch (Maat et al. 2002), Alkmaar (Baetsen 2001), Zutphen (Berk 2007), and Eindhoven (Baetsen and Weterings-Korthorst 2013), the prevalence of vitamin D deficiency varied from absent in Alkmaar to 7% in ‘s-Hertogenbosch. These sites and additional large urban centres should be analysed by applying the recently developed methods of IGD detection via micro-CT or histology to provide more accurate vitamin D deficiency prevalences which will improve our understanding of the interplay of sociocultural practices and industrialisation on vitamin D deficiency development. Furthermore, this study demonstrated the influence that seasonality had on the occurrence of vitamin D deficiency in Dutch communities. The analysis of communities in surrounding countries, such as Belgium, Germany, Denmark and the UK, should be undertaken to assess the influence of seasonality on vitamin D deficiency development, as suggested by Mays et al. (2009; 2018), in a wider area with comparable or more northern latitudes as the Netherlands.

Analyses of vitamin D deficiency in 17th to 19th century Dutch communities markedly enhanced our understanding of changing sociocultural practices related to economic and political changes that occurred in this period. These findings are important beyond this temporospatial context because most of these sociocultural practices are postulated to have been present before this period and are likely to have affected vitamin D deficiency prevalence well before the 17th century. This study demonstrates the importance of sociocultural practices in the development of vitamin D deficiency in all communities, regardless of main occupation, settlement size, geographical location, and average SES and stresses the need for vitamin D deficiency evaluation of all past communities. Furthermore, this study presented evidence of the influence of seasonality on vitamin D deficiency development that also may have played a role in other areas with more northern latitudes. The study of vitamin D deficiency prevalence enhances our knowledge of daily life in past communities that cannot be obtained from historic sources alone.

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Summary

This study investigates how vitamin D deficiency assessment can improve our understanding of changes that occurred in sociocultural practices due to industrialisation. The most efficient way of obtaining vitamin D is via dermal synthesis under the influence of ultra-violet B (UVB) radiation in sunlight. The consumption of foods such as oily fish, beef liver, and egg yolk, may provide supplemental amounts of vitamin D. A deficiency in vitamin D will arise if sunlight exposure is inadequate and bending deformities of the skeleton may develop. Vitamin D deficiency is commonly associated with a decrease in sunlight exposure due to the consequences of the Industrial Revolution, such as tall densely-packed buildings and air pollution blocking the sun. Yet, the Netherlands did not experience the Industrial Revolution in the same way as the UK and other western European countries. Therefore, vitamin D deficiency prevalence was not expected to be high in most Dutch communities. However, the 17^{th} to 19^{th} centuries in the Netherlands are characterised by significant economic and political changes, that must have affected all communities and may have had repercussions on health. To assess the influence of these changes on sociocultural practices, this study performed analysis of vitamin D deficiency in six 17^{th} to 19^{th} century Dutch communities, containing a total of 632 individuals, from various settlement types, different geographic locations, all age and sex groups, and socioeconomic statuses (SES).

The first collection is Beemster, which consists of predominantly 19^{th} century skeletal remains from a rural community whose main activities concerned cattle

farming. Archival data reports these individuals to have a middle to low SES. The skeletal collection from this community consists of 95 nonadults (< 18 years) and 200 adults (18+ years). Bloemendaal is the second collection and it consists of 67 adult individuals who were buried in the cemetery next to the psychiatric hospital of Meerenberg, which included patients and staff. This collections dates to the 19th century and historic sources report the majority of these individuals to have had a low SES. The third collection comes from Gouda and consists of 4 nonadults and 45 adults that were excavated inside the Sint-Janskerk dating to the 17th to 19th centuries. Archival data show these individuals had a high SES. The fourth collection is Rotterdam, a large urban centre, which already in the 17th century was an important seaport. The individuals from this collection were buried in the cemetery of the Laurenskerk and date to the 17th century. This collection consists of 3 nonadults and 34 adults. Roosendaal is the fifth collection and comes from the cemetery of the Sint-Janskerk. The skeletal remains date to the 17th to 19th centuries and include 15 nonadults and 45 adults. The main activity of the Roosendaal inhabitants was agriculture and the community had a small-scale textile industry. The last collection, Hattem consists of 28 nonadults and 100 adults dating to the 17th to 19th centuries. The main activity of Hattem was agriculture and the transportation of goods per ship to neighbouring towns and villages.

Relatively high prevalences of rickets were observed in all the communities under study and ranged from 15.3% (9/59) in Beemster to 23.8% (5/21) in Hattem. Results show that in all the communities, the majority of nonadults with active rickets were younger than about 3 years of age, whereas most healing cases were observed in older nonadults. It was postulated that regardless of settlement type, geographic location, occupation, and SES, all nonadults younger than 3 years of age were at risk of developing vitamin D deficiency and not just those from large, industrialised urban centres. After the age of about 3 years, an improvement in sunlight exposure is suggested to have occurred, since most healing cases of rickets were observed after this age. Residual rickets ranged from 12.5% (4/32) in Gouda to 23.9% (21/88) in Hattem, whereby in general, more females were affected than males, which was most clearly visible in Beemster. In this collection, significantly more females (21/100) than males (8/100) were affected. It was postulated that a gendered division of labour increased the risk of developing vitamin D deficiency in Beemster females. Although a gendered division of labour is likely to have existed in the other communities, it may have been less strict and differences between affected females and males per settlement were not statistically significant. Osteomalacia was observed in 1.1% (1/88) of the adult individuals in Hattem, the first general collection to present with adult vitamin D deficiency, excluding Bloemendaal as a psychiatric hospital collection where the risk of osteomalacia development (adult vitamin D deficiency) was expected to be elevated. In Bloemendaal, osteomalacia was observed in 6.0% (4/67) of the adult individuals. In Hattem, however, the risk of developing osteomalacia was expected to be low, yet one individual displayed adult vitamin D deficiency. Although it is unlikely that osteomalacia was common in Hattem and potentially this individual developed osteomalacia as a secondary condition, changes due to adult vitamin D deficiency are subtler than those of rickets and residual rickets and cases of osteomalacia may have been missed.

A subset of individuals (n = 30) from the collection of Beemster and Hattem that displayed macroscopic lesions likely due to rickets and residual rickets were analysed via micro-CT assessment of interglobular dentine (IGD) in the teeth, a microscopic dental mineralisation defect attributed to vitamin D deficiency. This enabled evaluation of the age of vitamin D deficiency onset and provided information on the number of deficient episodes per individual. In addition, thin sections were made from 17 individuals to facilitate comparison between micro-CT and histological analysis of IGD. One tooth was deemed unobservable via micro-CT assessment, and 65.5% (19/29) individuals displayed one or more episodes of IGD. The combined results of micro-CT and histological assessment of IGD show one or more deficient periods in 90.0% (27/30) of the individuals that display macroscopic lesions attributed to vitamin D deficiency. This suggests macroscopic investigation of vitamin D deficiency to be relatively reliable. However, three individuals with macroscopic bending deformities attributed to vitamin D deficiency did not present with IGD and most likely the observed deformities simply represent a more pronounced form of human variation. This study demonstrates micro-CT assessment to be a valuable method that aids in the diagnosis of vitamin D deficiency, whereby individuals with and without clear macroscopic lesions can be assessed for this condition to provide a more accurate overview of vitamin D deficiency prevalence.

In 63.2% (12/19) of the affected individuals, the first visible episode of IGD is present in the layer of dentine formed between 6 and 12 months of age. Furthermore, if IGD is present, it is most frequently observed in this layer of dentine (78.9%; 15/19). The majority of individuals (89.5%; 17/19) did not present with visible IGD after the age of 2.5 to 3 years, which supports the notion that nonadults older than 3 years of age experienced an improvement in sunlight exposure. In three individuals (10.3%; 3/29), the first episode of vitamin D deficiency is observed in the dentine formed around birth, which may be an indication of maternal vitamin D deficiency. No osteomalacia was observed in the females of Beemster or Hattem, yet IGD assessment suggests at least three mothers to have experienced adult vitamin D deficiency. Assessment of IGD can provide information on adult vitamin D deficiency even though macroscopic lesions are not observable. The combined results of micro-CT and histological assessment showed 40.7% (11/27) of the individuals with IGD presented with two or more bands of IGD suggesting vitamin D deficiency was recurrent in both communities. In at least four of them, the periods were chronologically successive suggesting vitamin D deficiency was seasonal. This is an important finding as clear evidence is presented on recurrent vitamin D deficiency which improves our understanding of the influence seasonality had on communities in more northern latitudes.

Comparison of IGD (number and severity) in Hattem females and males enabled assessment of the influence of sociocultural practices, such as a gendered division in labour. Although Hattem females and males are postulated to have experienced a similar risk in developing vitamin D deficiency, males presented with more severe periods of IGD than females, which may suggest vitamin D deficiency to have been more severe in males. However, the linkages between severity of IGD, macroscopic lesion development, and severity of vitamin D deficiency need further study.

This study demonstrates that the economic and political changes that occurred in the 17th - 19th centuries in the Netherlands, influenced sociocultural practices. Most of these practices are postulated to have been present before the rise of industrialisation and that many wars, general impoverishment, several cattle pests, and crop failures increased pressure on Dutch households to provide for their family's income. This may explain why nonadults aged 3 years and younger are suggested to have been more at risk of developing vitamin D deficiency than the older nonadults. The parents needed to work more and the younger nonadults needed to be kept inside to prevent them from harm, while it is possible that older nonadults could venture outside or even help their parents with various chores. The study of vitamin D deficiency presents information on the influence of sociocultural practices on the prevalence of this condition in past communities, including rural and urban centres thus decreases this paucity in our knowledge. This research stresses the importance of vitamin D deficiency investigation of all past communities regardless settlement size, main occupation, SES, and geographic location.

Samenvatting

Deze studie gaat na hoe het onderzoek naar het voorkomen van vitamine D-tekort ons begrip van veranderingen in socioculturele gebruiken ten gevolge van industrialisatie kan verbeteren. De meest effectieve manier om vitamine D te verkrijgen is middels de productie ervan in de huid onder invloed van ultraviolette (uv) straling in zonlicht. De consumptie van voedingsmiddelen, zoals vette vis, lever en eigeel, kunnen zorgen voor aanvullende hoeveelheden vitamine D. Een tekort aan vitamine D ontstaat wanneer de huid onvoldoende aan zonlicht wordt blootgesteld en kromming van het skelet kan het gevolg zijn. Vitamine D-tekort wordt doorgaans geassocieerd met een vermindering in de blootstelling aan zonlicht als gevolg van de consequenties van de Industriële Revolutie, zoals hoge, opeengepakte gebouwen en luchtvervuiling die het zonlicht blokkeren. Echter, in Nederland heeft de Industriële Revolutie nooit in die mate plaats gevonden zoals te zien was in Engeland en andere West-Europese landen. Zodoende werd niet verwacht dat vitamine D-tekort in grote mate in Nederland voorkwam. Hoewel er van een Industriële Revolutie in Nederland geen sprake was, wordt de 17e tot en met de 19e eeuw wel gekenmerkt door substantiële economische en politieke veranderingen die van invloed moeten zijn geweest op alle gemeenschappen en die een negatieve uitwerking moeten hebben gehad op de gezondheid. Om de invloed die deze veranderingen hadden op de socioculturele gebruiken te onderzoeken, werden voor deze studie de menselijke skeletten uit een zestal 17e- tot 19e-eeuwse gemeenschappen onderzocht met een totaal van 632 individuen. De gemeenschappen

hebben verschillende nederzettingsgroottes, zijn afkomstig uit verschillende delen van Nederland, bevatten individuen uit alle leeftijd- en geslachtsgroepen en alle sociaaleconomische statussen (SES) zijn vertegenwoordigd.

De eerste collectie is Beemster dat voor het grootste gedeelte bestaat uit 19e-eeuwse menselijke skeletten die afkomstig zijn uit een plattelandsgemeenschap die zich voornamelijk bezighield met veehouderij. Informatie uit het archief vermeldt dat de individuen een gemiddelde tot lage SES hadden. De collectie bestaat uit 95 onvolwassenen (< 18 jaar) en 200 volwassenen (18+ jaar). Bloemendaal is de tweede collectie en bestaat uit 67 volwassen individuen die begraven waren op de begraafplaats naast het psychiatrische ziekenhuis te Meerenberg, waar zowel staf als patiënten begraven werden. Deze collectie dateert uit de 19e eeuw en historische bronnen vermelden een doorgaans lage SES voor deze individuen. De derde collectie is afkomstig uit Gouda en bestaat uit 4 onvolwassenen en 45 volwassenen die zijn opgegraven in de Sint Janskerk en dateren uit de 17e tot en met 19e eeuw. Archiefdata vermeldt een hoge status voor deze individuen. De vierde collectie is afkomstig uit Rotterdam, een groot urbaan centrum dat al in de 17e eeuw een belangrijke havenstad was. De onderzochte individuen waren begraven in het kerkhof van de Sint Laurenskerk en dateren uit de 17e eeuw. De collectie bevat 3 onvolwassenen en 34 volwassenen. Roosendaal is de vijfde collectie en komt van het kerkhof van de Sint Janskerk. De skeletcollectie dateert uit de 17e tot en met de 19e eeuw en bevat 15 onvolwassenen en 45 volwassenen. De belangrijkste dagelijkse bezigheid van de inwoners van Roosendaal was landbouw en kleinschalige textielnijverheid. De laatste collectie betreft de menselijke resten uit Hattem dat 28 onvolwassenen en 100 volwassenen bevat die gedateerd worden tot de 17e tot en met de 19e eeuw. De voornamelijk activiteiten van de inwoners van Hattem was landbouw en goederenvervoer per schip naar omringende dorpen en steden.

Relatief hoge rachitis prevalenties werden in alle onderzochte gemeenschappen waargenomen en varieerden van 15,3% (9/59) in Beemster tot 23,8% (5/21) in Hattem. De meerderheid van de onvolwassenen met vitamine D-tekort van 3 jaar en jonger vertoonde rachitis in actieve staat, terwijl onvolwassenen ouder dan 3 jaar de ziekte in helende staat lieten zien. Dit gold voor alle onderzochte gemeenschappen en de veronderstelling is dat ongeacht de grootte van de nederzetting, geografische locatie, dagelijkse bezigheid en SES, alle onvolwassenen van 3 jaar en jonger het risico liepen een vitamine D-tekort te ontwikkelen en niet alleen de onvolwassenen uit grote, geïndustrialiseerde steden. Na het derde levensjaar zou gesteld kunnen worden dat er een verbetering in de blootstelling aan zonlicht optrad, omdat de meeste gevallen van helende rachitis te zien zijn na deze leeftijd. Het percentage residuaal rachitis varieerde van 12,5% (4/32) in Gouda tot 23,9% (21/88) in Hattem, waarbij over het algemeen meer vrouwen dan mannen vitamine D-tekort in de kinderjaren vertoonden. Dit was met name in Beemster zichtbaar, waarbij significant meer vrouwen (21/100) dan mannen (8/100) residuaal rachitis vertoonden. Gesuggereerd wordt dat een geslachtsgebonden verdeling in activiteiten het risico op vitamine D-tekort in de Beemster vrouwen vergrootte. Hoewel deze geslachtsgebonden verdeling in dagelijkse activiteiten ook in andere gemeenschappen aanwezig moet zijn geweest, lijkt het minder strikt te zijn geweest omdat het verschil in mannen en vrouwen met residuaal rachitis niet statistisch significant is. Osteomalacie kwam voor in 1,1% (1/88) van de volwassen individuen in Hattem, de eerste algemene skeletcollectie waar volwassen vitamine D-tekort is aangetoond. De collectie van Bloemendaal wordt niet als algemene skeletcollectie geclassificeerd omdat het individuen uit een psychiatrisch ziekenhuis betreft waarvan verwacht wordt dat het risico op osteomalacie hoger is dan bij andere collecties. In 6,0% (4/67) van de volwassenen uit Bloemendaal werd osteomalacie geconstateerd. In Hattem was de verwachting dat osteomalacie niet zou voorkomen en toch vertoonde een volwassene vitamine D-tekort. Het is niet aannemelijk dat osteomalacie veel voorkwam in Hattem en mogelijk heeft dit individu osteomalacie ontwikkeld ten gevolge van een andere ziekte. Echter, de veranderingen als gevolg van volwassen vitamine D-tekort zijn subtieler dan die van rachitis en residuaal rachitis waardoor de mogelijkheid bestaat dat andere gevallen van osteomalacie niet worden opgemerkt.

Een deel van de individuen (n = 30) met macroscopische laesies ten gevolge van vitamine D-tekort in de kinderjaren uit Beemster en Hattem, werd onderworpen aan een micro-CT-analyse van interglobulaire dentine (IGD) in de tanden, een microscopisch mineralisatie defect als gevolg van vitamine D-tekort. Hiermee kon de leeftijd waarop vitamine D-tekort ontstond worden geschat en kon het aantal deficiënte periodes per individu worden bepaald. Aanvullend hierop zijn van 17 tanden histologische secties gemaakt om analyse van IGD middels micro-CT en microscopie te vergelijken. Een tand kon niet worden bekeken via micro-CT door vergevorderde degradatie en 65,5% (19/29) van de individuen vertoonde een of meer banden IGD. Een combinatie van de resultaten van de micro-CT en histologische analyse van IGD laat zien dat 90,0% (27/30) van de individuen met macroscopische laesies ten gevolge van vitamine D-tekort een of meer banden met IGD vertonen. Dit suggereert dat macroscopische analyse van vitamine D-tekort relatief betrouwbaar is. Echter, drie individuen met macroscopische krommingen geassocieerd met vitamine D-tekort hadden geen IGD en het is aannemelijk dat de geobserveerde krommingen het gevolg zijn van een meer geprononceerde natuurlijke menselijke variatie. Dit onderzoek laat zien dat de analyse van IGD middels micro-CT een waardevolle methode is die bijdraagt aan de diagnose van vitamine D-tekort en kan worden ingezet om te testen of individuen die macroscopische laesies vertonen die over het algemeen aan vitamine D-tekort kunnen worden toegeschreven, ook daadwerkelijk aan deze ziekte geleden hebben.

In 63,2% (12/19) van de gevallen is de eerste band IGD aanwezig in de laag dentine die in de leeftijd van tussen de 6 en 12 maanden gevormd is. Daarnaast is dit de laag waarin IGD het vaakst geobserveerd wordt (78,9%; 15/19). Het merendeel van de individuen (89,5%; 17/19) vertoonde geen IGD meer na de leeftijd van 2,5 tot 3 jaar. Dit komt overeen met de resultaten van de macroscopische analyse, waarbij het merendeel van de onvolwassenen ouder dan 3 jaar rachitis in helende staat vertoont. In drie individuen (10,3%; 3/29) vertoonde de dentine die rond de geboorte gevormd wordt IGD dat kan duiden op een vitamine D-tekort van de moeder. Echter, bij geen enkele vrouw uit de Beemster en Hattem collectie is osteomalacie vastgesteld. Onderzoek van IGD kan informatie opleveren over volwassen vitamine D-tekort ondanks dat macroscopische laesies niet zichtbaar zijn. De gecombineerde resultaten van de micro-CT en histologische analyse van IGD laten zien dat 40,7% (11/27) van de individuen met IGD twee of meer episoden had. Gesteld kan worden dat vitamine D-tekort voor een groot deel van de individuen uit beide gemeenschappen een terugkerende ziekte was. Bovendien kon worden vastgesteld dat in vier van hen de banden IGD dusdanig op elkaar volgden dat gesteld kan worden dat vitamine D-tekort seizoensgebonden was. Dit is een belangrijk resultaat dat niet alleen duidelijk aantoont dat vitamine D-tekort een terugkerende ziekte was, maar dat ook het seizoen een grote rol moet hebben gespeeld bij de ontwikkeling van vitamine D-tekort in gemeenschappen met een meer noordelijke breedtegraad.

De vergelijking van IGD (het aantal banden en de ernst ervan) in Hattem vrouwen en mannen maakt het mogelijk om de invloed van socioculturele gebruiken, zoals geslachtsgebonden verdeling van werk, te evalueren. Hoewel de mannen en vrouwen in Hattem een overeenkomend risico op het ontwikkelen van een vitamine D-tekort in de kinderjaren lijken te hebben gehad, is de IGD bij mannen ernstiger dan bij vrouwen. Dit lijkt te suggereren dat vitamine D-tekort ernstiger was in mannen dan in vrouwen. Echter, de relatie tussen de ernst van IGD, het ontwikkelen van macroscopische laesies en de ernst van het vitamine D-tekort is complex en moet verder onderzocht worden.

Dit onderzoek laat zien dat economische en politieke veranderingen die in de 17e tot en met de 19e eeuw in Nederlands voorkwamen, van invloed zijn geweest op de socioculturele gebruiken. Het is aannemelijk dat de meeste van deze gebruiken al aanwezig waren voor de opkomst van de industrialisatie en dat de vele oorlogen, de algemene armoede, periodes van veepest en mislukte oogsten de druk op de Nederlandse huishoudens om te voorzien in het inkomen heeft doen toenemen. Dit zou kunnen verklaren waarom onvolwassenen van 3 jaar en jonger een groter risico liepen op het ontwikkelen van een vitamine D-tekort dan de oudere onvolwassenen. De ouders moesten meer werken en de jongere onvolwassenen moesten binnen blijven om te voorkomen dat ze zich zouden bezeren, terwijl de oudere onvolwassenen mogelijk meer naar buiten mochten of al moesten helpen met bepaalde huishoudelijke taken. Het onderzoek naar vitamine D-tekort levert informatie op over de invloed van socioculturele gebruiken op het ontwikkelen van deze ziekte in vroegere gemeenschappen, waaronder plattelandssamenlevingen en kleine urbane centra, waarbij dit gat in onze kennis wordt verkleind. Deze studie benadrukt de waarde van het onderzoeken van vitamine D-tekort in alle gemeenschappen, ongeacht de grootte, de dagelijkse bezigheid, SES en geografische locatie.

Curriculum vitae

Barbara Veselka was born on the 15th of January 1976 in Amsterdam, the Netherlands. In 1995, she finished her VWO at the Bonaventura College in Leiden, after which she did her first master. She studied Archaeology of Meso – America, at Leiden University in the years 1995-2000, and excavated in the Caribbean an Mexico, where she did her master's research. After working 12 years at the Chamber of Commerce, she undertook another master, Human Osteoarchaeology, at Leiden University (2011-2012). After finishing, she started her own company as a physical anthropologist, and after one year she was employed by Stichting LAB. She has partaken in many excavations in the Netherlands, Belgium, Guadeloup, Mexico, and Oman, and became specialised in the analysis of inhumated and cremated human remains. She started her PhD in 2013.

Currently, she is a board member of the Dutch Association for Physical Anthropology (Nederlandse Verenging voor Fysische Antropologie) and still works as a physical anthropologist for Stichting LAB. She will start her post-doctoral research at the Vrije Universiteit in Brussels at the 1st of January 2019.