Introduction

Bioarchaeology of dental caries and antemortem tooth loss

Dental caries result from a disease process in which acid produced by the bacterial fermentation of carbohydrates causes focal demineralization of hard dental tissues (Larsen, 2015). The pathogenesis of caries involves the tooth surface being exposed to, among other things, the oral environment, oral bacterial flora, salivary glycoprotein, dental plaques, diet, crown size and morphology, enamel defects, occlusal surface wear, and oral pH (Larsen, 2015). In bioarchaeological contexts, dental caries has been linked to carbohydrate intake quantity; notably, agriculturists who ingested soft, sticky foods and sugars have exhibited a high prevalence of caries (Hillson, 1990, 2008). Differences in the prevalence of carious lesions, and thus carbohydrate intake, have also been found between social classes (Sakura, 1964; White, 1994; Sakashita et al., 1997; Whittington, 1999; Cucina and Tiesler, 2003; Oyamada et al., 2004; Klaus et al., 2010).

Periodontal disease is associated with alveolar bone loss, such as horizontal bone loss relative to the cementoenamel junction and vertical bone loss around an individual tooth (Hillson, 1996). It impairs the skeletal support of teeth and eventually results in antemortem tooth loss (AMTL). Once alveolar bone loss occurs, the tooth socket is destined to be obliterated through remodeling. The causes of periodontal disease are multifactorial and include bacteria, poor oral hygiene, cariogenesis, malocclusion, nutritional stress, pregnancy, and teeth being subject to extreme mechanical loads (Larsen, 2015). In particular, bioarchaeologists have associated the shift from hunting-gathering to farming with increased periodontal disease and AMTL in North America’s Western Woodlands (Patterson, 1984) and Egypt’s Nile Valley (Rose et al., 1993). While some scholars also found that the prevalence of AMTL varies by social class and sex due to differences in diet and food consumption practices (Costa, 1980; Frayer, 1984), only a handful of studies have been done on this topic to date.

Both carious and AMTL rates vary widely according to...
substance, social class, sex, and age. However, it remains unclear how developments in sociality and subsistence, especially as related to the emergence of stratified societies, may have affected variation in dental caries and AMTL. Using Andean civilization skeletal remains from the initial stage of its social stratification and subsistence development, we compared the prevalence of carious lesions and AMTL within and between groups and assessed the causative factors contributing to any variations.

Archaeological settings

The Formative period (3000–50 BCE) in the Central Andes is characterized by the rise and development of the Andean civilization (Burger, 1992; Onuki, 1998; Onuki et al., 2010). Large monumental complexes first appeared in the Supe Valley, on the central coast of Peru, around 3000 BCE (Shady et al., 2001). In recent years, there have been reports of cases dating back to 3000 BCE and cases of contemporaneous monumental complexes in the central and north-central coast (Haas and Creamer, 2006). During the Middle and Late Formative periods (700–250 BCE), the construction and renovation of public architecture occurred; these activities promoted population growth and social integration in the Central Andes (Onuki, 1998). These innovations promoted social integration during the Formative period at Huacacloma, located in the northern highlands; however, no evidence of social hierarchy has been found at this site (Terada and Onuki, 1982). This contrasts with evidence from investigations into other sites in the northern highlands, such as Kuntur Wasi and Ingatambo, as well as in the southern highlands at Campanayuq Rumi. At Kuntur Wasi, social differentiation was evident in the more than seven tombs found comprising complex, boot-shaped structures that contained gold objects, Ecuadorian shell ornaments, and Bolivian sodalite beads (Seki, 2014). Moreover, the individuals buried in these tombs showed evidence of being subject to artificial cranial deformation (Seki, 2014): their crania were partially covered with cinnabar, which would have been imported from Peru’s central highlands—these findings suggest that the individuals buried in these tombs at Kuntur Wasi were members of an elite group (Seki, 2014). Meanwhile, recent excavations at Ingatambo in the Huancabamba Valley have also yielded evidence social stratification; more specifically, excavators found large and complex ceremonial architectures and indications of long-distance exchanges of precious goods during the Ingatambo phase (800–400 BCE) (Yamamoto, 2012). Last, social stratification is also evident in the gold metallurgy recovered from a Campanayuq Rumi site from the Late Formative period (800–200 BCE) (Matsumoto and Cavero Palomino, 2012): a gold artifact and ceramic casting mold indicate technological innovations from simple hammering techniques common during the Early and Middle Formative periods (1800–800 BCE) metallurgy (Matsumoto and Cavero Palomino, 2012). Although the large monumental complexes in the Supe Valley have been dated back to around 3000 BCE, the above recent archaeological data from Kuntur Wasi, Ingatambo, and Campanayuq Rumi demonstrates that social stratification likely appeared in the Central Andes during the Late Formative period (800–200 BCE).

We began our archaeological project at Pacopampa in 2005. Pacopampa is a Formative period site along the eastern slope of Peru’s northern highlands with an altitude of 2500 m above sea level (Figure 1). It is set back roughly 70 km from the Pacific coast and far from Peru’s tropical riverbanks. The Pacopampa site was composed of three large, low platforms, which covered a total area of approximately 4 hectares (Figure 2). The platforms surrounded a sunken plaza (Figure 2). Elaborate subterranean canal systems seem to have played an important role in rituals related to water and agricultural production (Seki et al., 2008). The presence of large-scale public architecture, the plaza, decorated stone sculptures, and archaeological remains related to rituals suggest that the site functioned as a ceremonial center (Seki et al., 2008). Reconstructions of means of subsistence from zooarchaeological and stable isotope data revealed that the site demonstrated an increased use of domesticated plants and animals (Uzawa, 2008; Takigami and Yoneda, 2017).

Our Pacopampa archaeological project led to important findings concerning socioeconomic inequality during the Formative period. Notably, the westernmost platform may have been the most important religious space in the complex. From that platform, an adult female (Individual No. 09PC-C-Ent 09-2-H2) was recovered from an elite tomb with a boot-shaped structure (the ‘Lady of Pacopampa’ tomb) dated to the beginning of the Pacopampa II phase (700–400 BCE). Cinnabar and azurite were found on the skull along with a pair of gold earplugs, a pair of gold earrings, and shell-based objects (Figure 3b; Table 1) (Nagaoka et al., 2012b). The bright red cinnabar was sourced from cinnabar ore mined in Huancavelica, over 850 km to the

Figure 1. Map of Peru showing the location of Pacopampa.
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A detailed examination of the neurocranium revealed the presence of a frontal-occipital type of artificial cranial deformation, which had previously only been found with human remains that, based on the precious burial goods found with them, appeared to be from the highest social class (Nagaoka et al., 2012b).

The project further detected another elite tomb with a boot-shaped structure (the ‘Serpent-Jaguar Priests’ tomb) from which we recovered two individuals (Individual No. 15PC-B2-Ent 541-H1 and -H2) from the Pacopampa II phase (Figure 3g, h; Table 1) (Nagaoka et al., 2020). At the bottom of the tomb, a middle-aged female was found lying over the remains of a 15-year-old male (Nagaoka et al., 2020). The central position of the tomb and a rich repertoire of grave goods, including gold ornaments and pigments, suggest that these individuals had symbolic importance and belonged to an elite social group (Nagaoka et al., 2020). The possible presence of artificial cranial deformation in the female suggests that these buried individuals were socially different from the other individuals found buried at this site (Nagaoka et al., 2020).

Figure 3 shows individuals from elaborate burials associated with gold and silver ornaments, pigments, and artificial cranial deformation. In total, our 11-year excavations yielded one fetal individual associated with pigments (Figure 3f) in the Pacopampa I phase (1200–700 BCE) and nine adult individuals associated with gold and silver ornaments, pigments, and artificial cranial deformation (Figure 3a–e, g, h) from the Pacopampa II phase (700–400 BCE) (Table 1). The precious goods found in their graves and their special burial treatment suggest that social inequality at Pacopampa may date back at least to the Pacopampa II phase. A variety of exotic artifacts from the northern coast allow us to trace the wide-ranging connections kept by the ceremonial center and the patterns of exchange that were essential to maintaining the prestige of Pacopampa and its elites (Seki et al., 2008). The Pacopampa site offers some of the earliest evidence of a highly stratified society in the Central Andes and our data from Pacopampa, detailed below, offer new insights into the relationship between social development and pathology.

The purpose of this study

Human remains from well-contextualized burials are ideal for clarifying the impact of social stratification on the prevalence of caries and AMTL. In order to clarify the relationship between social stratification and dental pathology, this study aimed to examine the occurrence of carious lesions and AMTL from Pacopampa and compare observed variations within and between groups to uncover causative factors.

Materials

We conducted our study over 11 field seasons from 2005 to 2015. This work yielded the remains of 103 individuals from the Formative period, comprising 38 non-adults (under 15 years) and 65 adults (15 years or older) (Nagaoka et al., 2017, 2019). Archaeological artifacts, architecture, stratigraphy, and radiocarbon dating revealed that the chronological ages of the human remains belonged to the Middle Formative period (Pacopampa I phase; 1200–700 BCE) and the earlier half of the Late Formative period (Pacopampa II phase; 700–400 BCE). Beta Analytic, a laboratory in Miami, FL, USA, conducted radiocarbon dating of the bones.

Because the materials were excavated from the ceremoni-
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al center, they may all have played special roles in ritual activities. Assuming that the found precious objects, pigments, and artificial cranial deformations serve as proxies of social class, we wagered that our materials comprised remains from different social classes. The target of this study was the permanent teeth and alveoli from the remains of 65 adult human (3 individuals from the Pacopapma I phase and 62 individuals from the Pacopapma II phase). All individuals represent individuals with well-contextualized stratigraphic origins.

The materials were curated by the Pacopampa Archaeological Project and temporarily housed at the Center for Pacopampa Archaeological Project (Jr. Bolognesi, Centro Poblado de Pacopampa, Distrito de Querocoto, Provincia de Chota, Region Cajamarca, Peru) under the permission of the Peruvian Ministry of Culture.

Methods

Age-at-death estimation and sex determination

We estimated the ages of non-adult skeletons based on dental development (Ubelaker, 1989), measurements of temporal and occipital bones (Nagaoka et al., 2012a; Nagaoka and Kawakubo, 2015), ilia (Ubelaker, 1989), and limb bones (Scheuer et al., 1980), as well as closure of the occipital synchondrosis (Wakebe, 1990) and the epiphyseal union of the ilia and long bones (Buikstra and Ubelaker, 1994). We classified the ages of adults aged 15 years or older into two categories due to the small sample size: younger (15–54 years) and older (≥55 years), based on the chronological metamorphosis of the auricular surface of the ilium (Lovejoy et al., 1985; Buckberry and Chamberlain, 2002), pubic symphysis (Todd, 1920, 1921; Brooks and Suchey, 1990), first ribs (Kunos et al., 1999), and dental wear (Lovejoy, 1985).

We determined the sex of individuals aged ≥15 years based on a macroscopic assessment of pelvic and cranial traits (Phenice, 1969; Bruzek, 2002; Walker, 2008).

Classification of burial types

Several individuals were excavated from elaborate burials associated with gold and silver ornaments, pigments, and artificial cranial deformation. In this study, these individuals and those who were buried with them were classified as a higher-class burial type; meanwhile, those lacking these elements were classified as a lower-class burial type. The terms ‘higher’ and ‘lower’ here are relative and, because they were all derived from ceremonial centers at Pacopampa, are used to signify ‘elaborate’ and ‘unelaborated’ burial contexts rather than ‘elite’ and ‘non-elite’ subjects.

It is highly likely that the subterranean construction of the tombs of the ‘Lady of Pacopampa’ and ‘Serpent-Jaguar Priests’ played an important role in maintaining the prestige of Pacopampa and its elites. In contrast, it is difficult to estimate the social classes of the remaining skeletons because little is known about their roles in the society. Although the
Table 1. Individuals from elaborate burials associated with gold and silver ornaments, pigments, and artificial cranial deformation

| Individual No. | Age       | Sex     | Cultural phase | Metal ornaments | Pigments | Artificial cranial deformation | Associated burial goods                                                                 | Remarks                                                                                                                                                                                                 | Photos | References                        |
|----------------|-----------|---------|----------------|-----------------|----------|-------------------------------|-----------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------|-------------------------------|
| 09PC-B2-Ent 509 | Younger   | Male    | Pacopampa II   | Absent          | Present  | Absent                        | A stirrup jar, a jug, a small bowl, a morning glory bowl, a bone tool, a stone point, and stone ornament |                                                                                                                                                                                                          | Figure 3a |                                |
| 09PC-C-Ent 09-2-H2 | Younger  | Female  | Pacopampa II   | Present         | Present  | Present                       | A pair of gold earplugs, a pair of gold earrings, shell objects, a legged tray, a small bowl, a bowl, and mineral pigments |                                                                                                                                                                                                          | Figure 3b | Nagaoka et al. (2012b)         |
| 12PC-B2-Ent 527-H1 | Younger  | Male    | Pacopampa II   | Absent          | Absent   | Present                       | None                                                                                     | Buried with 12PC-B2-Ent 532-H1. The context of the burials of both individuals seems likely to be the same.                                                                                                      | Figure 3c |                                |
| 12PC-B2-Ent 530  | Younger   | Female  | Pacopampa II   | Present         | Absent   | NA                            | A silver needle, a chrysocolla spindle wheel, calciet balls, chrysocolla balls, a cactus pattern pottery, and mineral pigments |                                                                                                                                                                                                          | Figure 3d |                                |
| 12PC-B2-Ent 532-H1 | Older    | Female  | Pacopampa II   | Present         | Present  | Absent                        | Chrysocolla balls and a bowl                                                             |                                                                                                                                                                                                          | Figure 3e |                                |
| 12PC-C-Ent 12-03 | Fetal 9th months | Unknown | Pacopampa I    | Absent          | Present  | NA                            | Chrysocolla balls, malachite, and mineral pigments                                      |                                                                                                                                                                                                          | Figure 3f |                                |
| 15PC-A-Ent 10-H1 | Younger   | Male    | Pacopampa II   | Absent          | Absent   | Present                       | None                                                                                     | Buried with 15PC-B2-Ent 541-H2.                                                                                                             | Figure 3g | Nagaoka et al. (2020)         |
| 15PC-B2-Ent 541-H1 | Younger  | Female  | Pacopampa II   | Absent          | Absent   | Present                       | A ceramic stirrup bottle                                                                | Buried with 15PC-B2-Ent 541-H1.                                                                                                             | Figure 3h | Nagaoka et al. (2020)         |
| 15PC-B2-Ent 541-H2 | Younger   | Male    | Pacopampa II   | Present         | Present  | NA                            | A gold necklace made of ovoid gold beads and an oval pendant, and mineral pigments       |                                                                                                                                                                                                          |                                |                                |

NA, not available.
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presence of precious grave goods and pigments as well as artificial cranial deformation may be proxies of an elite class, this reading cannot be taken as a verified truth. Using the prevalence of dental caries and AMTL as a bioarchaeological lifestyle indicator, we tested whether variations existed in the prevalence of these dental diseases according to burial context to explore the relationship between pathology and social stratification.

Dental caries (Figure 4)

Teeth were observed by the naked eye using a dental explorer. When carious lesions were detected, a ×10 magnifying glass was used for further observations. Radiographic analyses were not possible because permission was not obtained to transport the skeletal remains to our institutions.

Carious rates

Carious rates were calculated by total tooth units (i.e., number of affected teeth/total number of teeth observed) (Lukacs, 1995). Carious rates were analyzed based on cultural phase, age, sex, and burial type (as categorized above), to enable examination of their contribution to the carious variability in the initial stage of social stratification in the Central Andes.

Carious depth

The degree of carious depth was classified into four stages according to the diagnostic criteria by Sakashiita et al. (1997) and the modified version by Lanfranco and Eggers (2010).

Stage 1: The extent of caries is limited to the enamel layer. The lack of decalcification of the enamel in which a dental probe cannot be inserted was excluded.
Stage 2: The carious cavity does not reach the dental pulp and is limited to the dentin. The apex of a dental probe is buried more than 2 mm into a cavity in the dentin.
Stage 3: The carious cavity reaches the dental pulp and is often associated with abscesses and granuloma.
Stage 4: Tooth crowns are destroyed by dental decay, severe dental wear, and trauma.

Carious location

The carious location was classified into eight categories according to the diagnostic criteria of Hillson (1996, 2001) and the modified version of Lanfranco and Eggers (2010). Lanfranco and Eggers (2010) defined eight scores for the carious location as follows.

Score 1: Occlusal caries in the fissures, grooves, and fossae of molars and premolars. Occlusal caries of molars often occurs in deep fissures without pathological changes in surfaces and are difficult to diagnose by macroscopy alone (Hillson 2001). We identified occlusal caries from the presence of both staining and cavities on the occlusal surface.
Score 2: Pit caries in the buccal pits of molars and lingual pits of incisors and canines.
Score 3: Smooth surface caries in the buccal/labial and lingual enamel smooth surfaces.
Score 4: Contact area caries appearing in the approximal area between two teeth.
Score 5: Smooth surface caries in the approximal surface below the contact area between two teeth in the coronal cervical third, not involving the cementum–enamel junction (CEJ).
Score 6: Root caries in the root surface and CEJ.
Score 7: Occlusal attrition facet dentin caries and occlusal attrition facet enamel edge chipping and caries associated with intense dental wear.
Score 8. Gross–gross caries caused by intense and rapid dental wear is sufficient to surpass the capacity of secondary dentin production.

When a tooth was affected by multiple caries with different depths on different surfaces, the highest score of location and depth was adopted to express cariogenicity and carious severity (Lanfranco and Eggers, 2010). In order to provide comparative data of carious location of the Andes, this study employed the eight categories established by Lanfranco and Eggers (2010).
AMTL (Figure 4)

We observed missing teeth according to the criteria by Hillson (1996, 2001), who referred to a missing tooth that left an empty socket with signs of remodeling or a missing tooth with full remodeling that left no contour of the socket. AMTL rates were calculated by the number of missing teeth per the total number of alveoli observed.

Dental wear

In general, dental wear is negatively correlated with caries (Hillson 1990, 2008). This study recorded dental wear on a scale ranging from stage 1 to stage 8, according to the diagnostic criteria of Molnar (1971).

Stage 1: No observable wear.
Stage 2: Minimal evidence of wear observable in incisors and canines; no dentin observable in premolars and molars.
Stage 3: Observable obliterated cusp pattern and small dentin patches.
Stage 4: Minimal dentin patches observable in incisors and canines, two or more dentin patches observable in premolars, and three or more small dentin patches observable in molars.
Stage 5: Extensive dentin patches were observed in the incisors and canines; two or more dentin patches and slight secondary dentin observable in premolars; and three or more small dentin patches and none to slight secondary dentin observable in molars.
Stage 6: Secondary dentin observable in incisors and canines; moderate to heavy secondary dentin and entire tooth surrounded by enamel and observable in premolars and molars.
Stage 7: Crown is worn away on at least one side and extensive secondary dentin is observable.
Stage 8: Roots functioning in occlusal surfaces.

Statistical analyses

Fisher’s exact test with Bonferroni correction was used to analyze contingency tables in the software package R 3.5.2 (R Core Team, 2018). The significance levels of $P < 0.01$ and $P < 0.05$ were respectively corrected into $P < 0.0009$ (0.01/11) and $P < 0.0045$ (0.05/11) because we performed Fisher’s exact test for 11 dentition groups.

Results

Caries rates

The proportion of carious teeth was 17.2% of 1760 teeth (303/1760) (Supplementary Table 1). The proportion of carious teeth was 10.7% (3/28) in younger females in the Pacopampa I phase (Supplementary Table 1). In the Pacopampa II phase, the proportion of affected teeth was 3.7% (4/107) for higher-class younger females, 26.9% (7/26) for higher-class older females, and 8.3% (11/133) for higher-class younger and older females; meanwhile, these proportions were 16.6% (51/308) for lower-class younger females, 49.1% (28/57) for lower-class older females, and 21.6% (79/365) for lower-class younger and older females (Supplementary Table 1). The proportion was 0.0% (0/73) for higher-class younger males, whereas these proportions were 18.0% (58/322) for lower-class younger males, 21.1% (4/19) for lower-class older males, and 18.2% (62/341) for lower-class younger and older males (Supplementary Table 1).

The results of Fisher’s exact tests indicated significant differences by age and burial type: carious rates increased from younger to older females both in higher-class ($P = 0.0009$) and lower-class ($P < 0.0001$) burials (Figure 5a; Table 2); lower-class individuals had higher carious rates than higher-class individuals in younger females ($P = 0.0004$), younger and older females ($P < 0.0001$), and younger males ($P = 0.0004$) (Figure 5c; Table 2). In contrast, there was no significant difference in carious rates between cultural phases ($P > 0.0045$) (Table 2).

Caries depth

In all groups, the most frequent carious depth was stage 2, in which the carious cavity is limited to dentin (Supplementary Table 2). The rates of carious depth stages did not show any significant differences according to cultural phase, age, sex, and burial type (Table 3).

Caries location

The most frequent carious location was root caries in all groups (Supplementary Table 3). Within the Pacopampa II phase, younger males from lower-class burials had more frequent occlusal caries (score 1), smooth surface caries in the buccal/labial and lingual enamel smooth surface (score 3), contact area caries in the approximal area (score 4), smooth surface caries on the approximal surface (score 5), and gross–gross caries (score 8) than younger females from lower-class burials ($P = 0.0003$) (Table 4).

Number of carious lesions per tooth

The proportion of carious teeth with one carious lesion was greater than those with more than one carious lesion in all age groups (Supplementary Table 4). Rates of carious depth stages did not show any significant differences according to cultural phase, age, sex, and burial type (Table 5).

AMTL rates

The proportion of AMTL was 12.6% of 2014 alveoli (254/2014) (Supplementary Table 1). The proportion of AMTL was 0.0% (0/28) in younger females in the Pacopampa I phase (Table 2). In the Pacopampa II phase, the proportions of affected teeth were 0.9% (1/108) for higher-class younger females, 0.0% (0/26) for higher-class older females, and 0.7% (1/134) for higher-class younger and older females; meanwhile, these proportions were 14.2% (51/359) for lower-class younger females, 41.8% (41/98) for lower-class older females, and 20.1% (92/457) for lower-class younger and older females (Supplementary Table 1). The proportion of AMTL was 0.0% (0/73) for higher-class younger males; meanwhile, these proportions were 9.0% (32/354) for lower-class younger males, 17.4% (4/23) for lower-class older males, and 9.5% (36/377) for lower-class younger and older males (Supplementary Table 1).

The results of Fisher’s exact tests showed the significant differences by age, sex, and burial type. The proportion of
Figure 5. Comparison of carious rates and antemortem tooth loss (AMTL) rates according to ages (a), sexes (b), and burial types (c). The significance levels of **$P < 0.01$ and *$P < 0.05$ were respectively corrected to $P < 0.0009 \ (0.01/11)$ and $P < 0.0045 \ (0.05/11)$. 
Sex differences

**Caries**

- **Age: Younger**
  - Burial type: Higher-class
  - Frequency distribution for maxilla, mandible, and total.

- **Age: Younger**
  - Burial type: Lower-class
  - Frequency distribution for maxilla, mandible, and total.

- **Age: Older**
  - Burial type: Lower-class
  - Frequency distribution for maxilla, mandible, and total.

- **Age: Younger and older**
  - Burial type: Lower-class
  - Frequency distribution for maxilla, mandible, and total.

**AMTL**

- **Age: Younger**
  - Burial type: Higher-class
  - Frequency distribution for maxilla, mandible, and total.

- **Age: Younger**
  - Burial type: Lower-class
  - Frequency distribution for maxilla, mandible, and total.

- **Age: Older**
  - Burial type: Lower-class
  - Frequency distribution for maxilla, mandible, and total.

- **Age: Younger and older**
  - Burial type: Lower-class
  - Frequency distribution for maxilla, mandible, and total.

Legend:
- **Female**
- **Male**

Figure 5. (continued)
Figure 5. (continued)
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Table 2. Fisher’s exact test of carious rates and AMTL rates according to cultural phases, ages, sexes, and burial types

Comparison between cultural phases (Pacopampa I vs. II)

| Ages    | Sexes    | Rates      | Maxilla | Mandible | Total |
|---------|----------|------------|---------|----------|-------|
|         |          |            | I        | C        | P     | M     | Total | I        | C        | P     | M     | Total |
| Younger | Females  | Carious rates | ns       | ns       | ns     | ns     | ns     | ns       | ns       | ns     | ns     | ns     |
|         |          | AMTL rates | ns       | ns       | ns     | ns     | ns     | ns       | ns       | ns     | ns     | ns     |
| Comparison between ages in the Pacopampa II phase (younger vs. older)

| Sexes    | Burial types | Rates      | Maxilla | Mandible | Total |
|----------|--------------|------------|---------|----------|-------|
| Females  | Higher-class | Carious rates | ns       | ns       | ns     | ns     | O>Y     | ns       | ns       | ns     | ns     | O>Y     |
|          |              | AMTL rates | ns       | ns       | ns     | ns     | ns     | ns       | ns       | ns     | ns     | ns     |
| Females  | Lower-class  | Carious rates | ns       | ns       | ns     | ns     | ns     | O>Y     | ns       | ns       | O>Y     | O>Y     |
|          |              | AMTL rates | O>Y      | ns       | O>Y    | ns     | O>Y    | ns       | O>Y     | ns     | O>Y     |
| Males    | Lower-class  | Carious rates | ns       | ns       | ns     | ns     | ns     | ns       | ns       | ns     | ns     | ns     |
|          |              | AMTL rates | ns       | ns       | ns     | ns     | ns     | ns       | ns       | ns     | ns     | ns     |

Comparison between sexes in the Pacopampa II phase (female vs. male)

| Ages    | Burial types | Rates      | Maxilla | Mandible | Total |
|---------|--------------|------------|---------|----------|-------|
| Younger | Higher-class | Carious rates | ns       | ns       | ns     | ns     | ns     | ns       | ns       | ns     | ns     | ns     |
|         |              | AMTL rates | ns       | ns       | ns     | ns     | ns     | ns       | ns       | ns     | ns     | ns     |
| Younger | Lower-class  | Carious rates | ns       | ns       | ns     | ns     | ns     | ns       | ns       | ns     | ns     | ns     |
|         |              | AMTL rates | ns       | ns       | ns     | ns     | ns     | ns       | ns       | ns     | ns     | ns     |
| Older   | Lower-class  | Carious rates | ns       | ns       | ns     | ns     | ns     | ns       | ns       | ns     | ns     | ns     |
|         |              | AMTL rates | ns       | ns       | ns     | ns     | ns     | ns       | ns       | ns     | ns     | ns     |
| Younger and older | Lower-class | Carious rates | ns       | ns       | ns     | ns     | ns     | ns       | ns       | ns     | ns     | ns     |
|          |              | AMTL rates | F>>M     | ns       | F>>M   | F>>M   | ns       | ns       | ns       | F>>M   |

Comparison between burial types in the Pacopampa II phase (higher-class vs. lower-class)

| Ages    | Sexes    | Rates      | Maxilla | Mandible | Total |
|---------|----------|------------|---------|----------|-------|
| Younger | Females  | Carious rates | ns       | ns       | ns     | ns     | ns     | ns       | ns       | ns     | ns     | ns     |
|         |          | AMTL rates | ns       | ns       | ns     | ns     | ns     | ns       | ns       | ns     | ns     | ns     |
| Older   | Females  | Carious rates | ns       | ns       | ns     | L>H    | ns     | ns       | ns       | ns     | ns     | L>H    |
|         |          | AMTL rates | ns       | ns       | L>H    | ns     | L>H    | ns       | ns       | ns     | L>H    |
| Younger and older | Females | Carious rates | ns       | ns       | ns     | L>H    | ns     | ns       | ns       | ns     | L>H    | L>H    |
|         |          | AMTL rates | ns       | ns       | L>H    | ns     | L>H    | ns       | ns       | ns     | L>H    |
| Younger | Males    | Carious rates | ns       | ns       | ns     | ns     | ns     | ns       | ns       | ns     | ns     | ns     |
|         |          | AMTL rates | ns       | ns       | ns     | ns     | ns     | ns       | ns       | ns     | ns     | ns     |

AMTL, antemortem tooth loss; Y, younger; O, older; F, female, M, male; H, higher-class burial; L, lower class burial; ns, not significant.
The significance levels of $P < 0.01$ and $P < 0.05$ were respectively corrected to $P < 0.0009 (0.01/11)$ and $P < 0.0045 (0.05/11)$. They are respectively shown by $>>$ and $>$.

Dental wear rate

The proportions of teeth with the eight different types of dental wear were compared according to cultural phase, age, sex, and burial type (Table 6). Dental wear at stages 2–3 was most frequent in younger age groups, and the severity of dental wear increased with age (Supplementary Table 5). The results of Fisher’s exact tests indicated that there was no significant difference in dental wear between cultural phases ($P > 0.0045$) (Table 6). Within the Pacopampa II phase, significant differences in dental wear stages were observed across age, sex, and burial type. Dental wear severity increased with age in higher-class females ($P < 0.0001$), lower-class females ($P < 0.0001$), and lower-class males ($P < 0.0001$) (Table 6). Females exhibited more severe dental wear than males in higher-class younger ($P < 0.0001$), lower-class younger ($P < 0.0001$), and lower-
class younger and older \((P < 0.0001)\) groups (Figure 6b; Table 6). Furthermore, individuals from lower-class burials exhibited more severe dental wear than those from higher-class burials in younger females \((P < 0.0001)\), younger and older females \((P < 0.0001)\), and younger males \((P < 0.0001)\) (Figure 6c; Table 6). Incisors also exhibited significant differences in dental wear stages across age, sex, and burial type \((\text{at least } P < 0.0045)\) (Table 6). In summary, those who were ‘older,’ ‘female,’ and ‘lower-class’ demonstrated more severe dental wear than those who were ‘younger,’ ‘male,’ and ‘higher-class.’

**Discussion**

**Dental wear and its influences on caries and AMTL at Pacopampa**

This study examined the severity of dental wear at Pacopampa and compared it across cultural phase, age, sex, and burial type. The results showed variations according to age \((\text{the severity of dental wear increased with age}),\) sex \((\text{females had more severe dental wear than males}),\) and burial type \((\text{individuals from lower-class burials had more severe dental wear than those from higher-class burials})\) (Table 6).

Typically, there is a negative correlation between dental wear and carious rates because severe dental wear often eliminates occlusal caries \((\text{Maat and Van der Velde, 1987})\). At Pacopampa, younger males from lower-class burials had frequent occlusal caries \((\text{score 1}),\) smooth surface caries in the buccal/labial and lingual enamel smooth surface \((\text{score 3}),\) contact area caries in the approximal area \((\text{score 4}),\) smooth surface caries in the approximal surface \((\text{score 5}),\) and gross–gross caries \((\text{score 8});\) meanwhile, younger females from lower-class burials predominantly demonstrated root caries \((\text{score 6})\) (Table 6). This difference suggests that occlusal caries in females with more severe occlusal wear than males decreased with the progress of dental wear on occlusal surfaces. Therefore, we inferred that the negative relationship between dental wear and carious rates may be reflected in sexual dimorphism in carious location.

However, rapid and intense dental wear resulting in pulp exposure may increase caries and AMTL, yielding a positive relationship between dental wear and caries. This study further indicated a positive relationship between dental wear and caries in teeth inconsistent with this general trend. Indeed, older females from lower-class burials with a higher proportion of severe dental wear \((\text{4.2% with stage 8 dental wear})\) than any other group demonstrated a high proportion of AMTL \((\text{41.8%})\) (Table 2). In addition, the proportion of carious lesions resulting from severe dental wear \((\text{gross–gross caries in stage 8 carious location})\) was \(42.9\)% in older females from higher-class burials, which was the highest across all groups \((\text{Supplementary Table 5})\). Those with severe dental wear at Pacopampa were likely at high risk of caries and AMTL. Dental wear is related to a decrease in
occlusal caries in an early stage of wear, whereas excessive dental wear that surpasses the capacity of secondary dentin production could result in pulp exposure, pulp death, gross–gross caries, and, finally, tooth loss.

In bioarchaeological contexts, excessive extramasticatory loads (such as the processing of animal hides and twisting of fibers on teeth and jaws, the use of stone grinding implements, and the incorporation of grit into foods) led to severe tooth wear and pulp exposure (Larsen, 2015). Unfortunately, little is known about the causes of dental wear that may explain the variability across age, sex, and burial type. Current data does not offer evidence of an increase in grinding stone implements during the Pacopampa I and II phases (Megumi Arata, personal communication) and no ethnological example yet reports the twisting of fibers with teeth in the Central Andes.

The wide variation in the prevalence of dental caries and AMTL at Pacopampa

This study examined the prevalence of caries and AMTL at Pacopampa and compared them across cultural phase, age, sex, and burial type. Results revealed variation according to age (rates increased with age), sex (females exhibited higher carious and AMTL rates than males), and burial type (lower-class individuals had higher carious and AMTL rates than higher-class individuals) (Table 3). These tendencies are consistent with the age, sex, and burial differences detected in dental wear (Table 6).

Population differences in the prevalence of caries and AMTL between Pacopampa and other sites

Turner (1979) showed an increasing trend in carious rates from hunting-gathering to agriculture, using a sample of populations cited globally (hunting-gathering, 1.7%; agriculture, 8.6%; mixed economy, 4.4%). The carious rate of Pacopampa (17.2%) was higher than the average value of agriculturists, 8.6% (Turner, 1979) except for individuals from higher-class burials (Supplementary Table 1). Data from zooarchaeological and stable isotope analyses from Pacopampa revealed the increased use of domesticated plants and animals in the Pacopampa II phase (Uzawa, 2008; Takigami and Yoneda, 2017), which is consistent with the carious rates detected at Pacopampa.

Lanfranco and Eggers (2010) examined carious and AMTL rates of the populations of the Early Formative period (4400 ± 110 BP), Middle Formative period (3920 ± 110 BP), Late Formative period (Salinar) (2340 ± 90 BP), and Late Intermediate period (1000–1470 CE) from Peru’s northern coast. This chronology, which differs from this study, was directly quoted from Lanfranco and Eggers (2010). The chronological terms were different according to archaeological sites and archaeological cultures. The subsistence of the former was based on fishing, whereas that of the latter three was based on agriculture. The results showed that the carious rates accounted for 21.6%, 21.7%, 20.7%, and 22.1% in the Early Formative, Middle Formative, Sal-
which were all greater than the average value of agriculturists of Turner (1979). As already mentioned by Lanfranco and Eggers (2010), this is inconsistent with Turner’s (1979) expectation of a significant difference in carious rates between fishers and agriculturists. However, the difference in subsistence strategy was not reflected by carious rates, but carious location and AMTL rates (Lanfranco and Eggers, 2010). Occlusal caries was the most frequent occurrence in fishers during the Early Formative period, whereas extraocclusal caries, such as caries of roots and CEJs, increased in agriculturists later on (Lanfranco and Eggers, 2010). The higher prevalence of root caries associated with agricultural development can be linked to periodontal disease, gingival retraction, and dental extrusion, which are caused by the consumption of sucrose and starch, cervical bacterial plaque accumulation, and xerostomia (Lanfranco and Eggers, 2010). Furthermore, the subsistence shift was also accompanied by an increase in AMTL rates (Lanfranco and Eggers, 2010). Thus, it is clear that the development of agriculture in the northern coast was associated with an increase in extraocclusal caries in the latter. Secular trends in caries and AMTL across the northern highlands and the northern coast demonstrate some similarities.

We compared the carious and AMTL rates of Pacopampa with Salinar (400–1 BCE) and Gallinazo (1–400 CE) (Gagnon and Wiesen, 2013) (Table 7). This chronology, which differs from that of Lanfranco and Eggers (2010), was directly quoted from Gagnon and Wiesen (2013). The chronological terms were different according to archaeological sites and archaeological cultures. The Pacopampa population exhibited lower frequencies of both dental caries and AMTL than subsequent populations (Table 7). Seki and Yoneda (2005) reconstructed Kuntur Wasi using a carbon and nitrogen isotope and demonstrated that Formative period people consumed fewer C4 foods than people of subsequent eras. If this trend is applied to Formative period societies in the northern highlands, the prevalence of dental caries and AMTL at Pacopampa would be consistent with a lower consumption of C4 foods indicated by isotopic data.

Age and sex differences in the prevalence of caries and AMTL

It is not surprising that the age-related change in the prevalence of caries and AMTL monotonically increases with age (Table 2). This is because caries and AMTL do not heal after they appear, whereas severe dental wear eliminates

| Table 5. Fisher’s exact test of rates of carious teeth with one carious lesion and those with more than one carious lesions according to cultural phases, ages, sexes, and burial types |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| **Comparison between cultural phases (Pacopampa I vs. II)** |
| **Ages** | **Sexes** | **Maxilla** | **C** | **P** | **M** | **Total** | **I** | **C** | **P** | **M** | **Total** |
| Younger | Females | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| **Comparison between ages in the Pacopampa II phase (younger vs. older)** |
| **Sexes** | **Burial types** | **Maxilla** | **C** | **P** | **M** | **Total** | **I** | **C** | **P** | **M** | **Total** |
| Females | Higher-class | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| Females | Lower-class | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| Males | Lower-class | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| **Comparison between sexes in the Pacopampa II phase (female vs. male)** |
| **Ages** | **Burial types** | **Maxilla** | **C** | **P** | **M** | **Total** | **I** | **C** | **P** | **M** | **Total** |
| Younger | Higher-class | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| Younger | Lower-class | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| Older | Lower-class | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| Younger and older | Lower-class | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| **Comparison between burial types in the Pacopampa II phase (higher-class vs. lower-class)** |
| **Ages** | **Sexes** | **Maxilla** | **C** | **P** | **M** | **Total** | **I** | **C** | **P** | **M** | **Total** |
| Younger | Females | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| Older | Females | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| Younger and older | Females | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| Younger | Males | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |

NA, not available; ns, not significant. The significance levels of **P < 0.01 and *P < 0.05 were respectively corrected to P < 0.0009 (0.01/11) and P < 0.0045 (0.05/11).
occlusal caries (Maat and Van der Velde, 1987).

The higher prevalence of caries and AMTL in females than in males at Pacopampa (Figure 5b; Table 2) is related to cultural and biological factors concerning sex dimorphism in oral health. Higher carious prevalence in females has been reported in modern and ancient human populations (Larsen, 2015) and has been explained by easy access to food during its preparation and early eruption of permanent teeth in females (Lukacs and Largaespada, 2006). Moreover, pregnancy is likely related to sex differences in caries and AMTL: increases in estrogen and progesterone are related to increases in cariogenesis and decreases in mouth pH (Laine, 2002).

We also observed a higher proportion of root caries in females than in males (Table 4; Supplementary Table 3). Root caries, which is the most frequent carious location at Pacopampa, is generally related to periodontal disease, gingival retraction, and dental extraction due to the consumption of starchy foods in combination with cervical plaque accumulation, and low saliva secretion (Lanfranco and Eggers, 2010). Together with cultural and biological factors, the use of chicha for rituals of reciprocal exchanges between humans and divine entities (Hastorf and Johannessen, 1993) increased the risk of caries and AMTL in females. The maize was chewed and mixed with saliva in the mouth by females, and then fermented in bottles (Hastorf and Johannessen, 1993). Regarding the production of chicha during the Formative period, Burger and Van der Merwe (1990) advise, based on the results of an isotope ratio analysis of human bones excavated around the Chavin de Huántar site, that it may be traced back to the Late Formative period. Meanwhile, Seki (1998) inferred that chicha production in the Cajamarca basin of Peru’s northern highlands could be traced back to the Late Formative period, and archaeological evidence from Pacopampa suggests that the wide-mouthed vessels for chicha boiling and short-necked jars for chicha storage appeared and increased during the Pacopampa II phase (Nagisa Nakagawa, personal communication).

Chicha usually worsened oral environments, particularly in females, due to the acidification that resulted in periodontal disease and an increase of cariogenic colonies in the mouth (Lanfranco and Eggers, 2010). The use of chicha at Pacopampa as an explanation of sex differences in caries and AMTL is a fascinating theory that is consistent with the higher prevalence of caries and AMTL in females.

Thus, we concluded that the age and sex differences in the prevalence of caries and AMTL detected in this study could reflect cultural and biological factors.

### Burial differences in the prevalence of caries and AMTL

Our results show that the division of burial types at Pacopampa was in accord with the differences in frequencies of caries and AMTL. Individuals from higher-class burials had lower frequencies of caries and AMTL than those from lower-class ones. They also possessed lower frequencies of
dental caries and AMTL than other populations from the Early Formative, Middle Formative, Salinar, and the Late Intermediate periods (Lanfranco and Eggers, 2010) and the Salinar and Gallinazo cultures (Gagnon and Wiesen, 2013) (Table 7). The chronological terms were different according to archaeological sites and archaeological cultures.

It is highly likely that observed differences in carious and AMTL rates between burial types reflected social backgrounds consistent with the findings of White (1994), Cucina and Tiesler (2003), and Klaus et al. (2010). Carious rates were higher in low-class individuals than in those who occupied higher social positions in the Middle Sicán period (900–1100 CE) in Peru (Klaus et al., 2010) as well as the Classic period (250–900 CE) in Mayan sites like Copan in Honduras (White, 1994), Lamanai in Belize (Whittington, 1999), and Peten at Calakmul (Cucina and Tiesler, 2003), which implies that elite individuals consumed less maize within these societies—all along these lines, these studies suggested that lower rates of maize consumption were common for higher-class individuals within these societies.

Based on our findings, we reason that the relationship between burial type and dental pathology at Pacopampa can be

![Figure 6](image_url)
attributed to social stratification and subsistence during the Formative period. Stable isotopic data from Kuntur Wasi, a neighboring contemporary site along the western slopes of the Andes mountains demonstrated that individuals from higher-class burials consumed fewer $C_4$ foods than those from lower-class ones (Seki and Yoneda, 2005). Zooarchaeological and stable isotopic data also suggest domesticated animals and plants were prevalent at Pacopampa during the Pacopampa II phase (Uzawa, 2008; Takigami and Yoneda, 2017). Social differences in carious and AMTL rates at Pacopampa could be explained by the idea that individuals from higher-class burials were less exposed to cariogenic foods, under the assumption that the dietary patterns detected at Kuntur Wasi were common in the northern highlands.

At Pacopampa, quantitative differences in maize consumption and dental wear between burial types are similar. Individuals with more severe dental wear would have exhibited higher rates of caries and AMTL because severe dental wear that surpasses the capacity of secondary dentin production can result in pulp exposure, caries, and periodontal diseases. Biological and cultural factors causing wide variations in dental wear accord with social differences across frequencies of caries and AMTL. Although no archaeological evidence yet affirms an increase in grinding implements

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**Figure 6.** (continued)
during the Pacopampa II phase, we believe that the incorporation of grit into foods cannot be ruled out as a cause of severe dental wear and its inducement of caries and AMTL. This study demonstrated possible social differences in carious rates between higher- and lower-status individuals during the Formative period when social stratification first appeared in ancient Peru; this may be because higher-class individuals may have been free from excessive masticatory labor. We tentatively conclude that social differences in diet and cultural practices may have resulted in a wide variability in carious and AMTL rates in our sample population. Future studies are needed to explore diets using stable isotopic data to test this assumption.

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Author contributions

T.N. designed the research, cleaned and reconstructed the human skeletons, collected and analyzed the data, and wrote the paper. Y.S. conducted the fieldwork and revised the paper. K.U. revised the paper. W.M. cleaned and reconstructed the human skeletons and revised the paper. D.M.C. conducted the fieldwork.
Ethical considerations

The materials and methods used in this study did not include anything that required approval by the ethical committee of our institute. All necessary permits were obtained for the described study, which complied with all relevant regulations: the permissions of the National Institute of Culture of Peru from 2005 to 2010 (Permission Nos. 1108/INC, 1868/INC, 979/INC, 1061/INC, 815/INC, and 1403/INC) and those of the Peruvian Ministry of Culture from 2011 to 2015 (Permission Nos. 265-2011-DGPC-VMPCIC/MC, 593-2012-DGPC-VMPCIC/MC, 006-2013-DGPA-VMPCIC/MC, 363-2014-DGPA-VMPCIC/MC, and 270-2015-DGPA-VMPCIC/MC).

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Conflict of Interest

The authors declare that they have no conflict of interest.

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