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Publication Date

2024-09-01

DOI

10.1016/j.jas.2024.106069

Journal of Archaeological Science xxx (xxxx) xxx

Contents lists available at ScienceDirect

Journal of Archaeological Science

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

Immune proteins recovered in tooth enamel as a biochemical record of health in past populations: Paleoproteomic analysis of Mission Period Native Californians

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ARTICLE INFO

Keywords:
Paleoproteomics
Archaeoproteomics
Tooth enamel
Immune proteins
IgG
C-Reactive protein
Serum albumin
Mission Period California

ABSTRACT

The enamel proteome includes a range of proteins that are well-preserved in archaeological settings but have so far received less study than those associated with sex-estimation of enamel. We look beyond sex-specific sequencing of amelogenin to investigate the potential of several serum proteins, including immunoglobulin gamma (IgG), the major immunoglobulin found in blood serum, and C-reactive protein (CRP), which is associated with inflammatory response, to provide insight into the health and stresses experienced by individuals in the past. We apply this approach to enamel samples from Mission-Period ancestral Ohlone interred at Asistencia San Pedro y San Pablo (CA-SMA-71/H; n = 11). For comparison, we also examine enamel from historic-period European-Americans interred in the City Cemetery in San Francisco (n = 12), and extracted third molars from present-day military cadets (n = 8). Results indicate that IgG is elevated among individuals at the asistencia relative to samples from present-day military cadets, and historic City Cemetery individuals (ANOVA with post-hoc Tukey Kramer tests, $p < .02$). Further, the inflammatory protein CRP, normally expressed at much lower levels than IgG, was present in 55% (6 of 11) of the asistencia samples, and in 17% (2 of 12) of the historic City Cemetery samples, but was not detected in enamel samples from present-day military cadets. While more studies are needed, we argue that the difference in IgG could reflect higher levels of chronic diseases such as tuberculosis among Ohlone living within the Mission system, and the presence of measurable amounts of CRP could relate to higher degrees of physical, social, and emotional stresses. To our knowledge, this is the first paleoproteomic study of immune proteins in tooth enamel. The ability to track immune responses during tooth formation could provide valuable and high-resolution information on ancient health and disease at the level of the individual over archaeological time-scales.

1. Introduction

Paleoproteomic analysis of ancient biological tissues is growing rapidly in archaeology (Hendy, 2021; Warinner et al., 2022). To date, the main applications of paleoproteomics have been to aid species identification of unidentified bone (AKA ZooMS; Buckley, 2018; Richter et al., 2022), sex estimation (Parker et al., 2019; Stewart et al., 2017), and characterization of proteins preserved in dental calculus to aid

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https://doi.org/10.1016/j.jas.2024.106069
Received 16 March 2024; Received in revised form 25 August 2024; Accepted 29 August 2024

paleodietary studies (Charlton et al., 2019; Hendy et al., 2018a; Jersie-Christensen et al., 2018).

Another exciting avenue of paleoproteomic research in bioarchaeology is exploring ways in which protein expression can provide novel information on individual life experiences or population-level environmental effects on human health. Many human-produced proteins vary in their level of expression depending on the environment experienced by an individual. In particular, certain immune proteins are expressed in higher amounts in response to disease or stress (Cundiff et al., 2024; Gonzalez-Quintela et al., 2008; Meca et al., 2022; Segerstrom and Miller, 2004; Shenhar-Tsarfaty et al., 2015; Yoon et al., 2017). To the extent that such proteins accumulate and are preserved in ancient tissues such as tooth enamel, dentine, or bone, they could shed light on the health and well-being of individuals during development and among ancient populations across archaeological time. Here we use data obtained during proteomic sex estimation to assess the potential of two immunological proteins to inform on past exposure to chronic disease and emotional stress.

Proteomic analyses of dental enamel from 12 individuals buried at the Asistencia San Pedro y San Pablo (referred to below simply as the asistencia; CA-SMA-71/H) were conducted at the request of the state-assigned Most Likely Descendant (CZ) to assist in life history reconstruction. Part of that work included sex estimation (reported in Buonasera et al., 2025) which was particularly important for younger individuals lacking diagnostic osteological markers for sex (see Buonasera et al., 2020, 2022; Parker et al., 2019, 2021). However, the shotgun approach we employ in our laboratory also provides a rich source of data on the wider enamel proteome. Given the stability of the enamel proteome (Buonasera et al., 2020; Gil-Bona and Bidlack, 2020) these proteins have the potential to provide a record of the health status of individuals when enamel biosynthesis occurred in developing permanent dentition.

We explore health-related serum proteins, immunoglobulin gamma (IgG) and C-reactive protein (CRP), that are simultaneously observed in enamel extracts. Because IgG and CRP are found in blood serum, we compare their abundance to the most abundant protein found in serum, namely the highly ubiquitous serum albumin. All three proteins are soluble and globular. We argue that the level of degradation therefore should occur with similar kinetics, and the relative densities of these proteins could provide a record of stress and disease during the period of tooth formation. These protein profiles are compared to those from enamel of similarly sized groups of historic-era European Americans interred in the French Mutual Benevolent Society (FMBS) section of City Cemetery in San Francisco (Eerkens et al., 2023) and to extracted third molars from healthy modern United States Air Force Cadets (AFA) (Parker et al., 2019; Regan, 2006).

2. Background

Enamel is deposited incrementally in layers of protein-mineral matrix. Nutrition, injury, and disease can affect the structural and chemical composition of these layers (Hillson, 2024; Smith, 2018). Serum albumin is known to occur in the enamel proteome (Gil-Bona and Bidlack, 2020; Limeback et al., 1989; Robinson et al., 1995). For example, proteomic analyses show the developmental inclusion of serum albumin in animal models (Gil-Bona et al., 2023) and serum albumin has been detected in both dentine and enamel (Jágr et al., 2019). Likewise, a recent study of human enamel has extracted whole antibodies in teeth from a medieval (13th −15th century) cemetery in England but did not separate dentine or the pulp chamber from enamel (Shaw et al., 2023). Most recently, Wilkin et al. (2024) report the presence of various immune proteins in human bone and dentine.

Currently, the mechanism whereby serum proteins become incorporated into human tooth enamel is incompletely understood (Gil-Bona and Bidlack, 2020; Jágr et al., 2019; Limeback et al., 1989; Robinson et al., 1995). However, serum albumin is known to be present in the secretory and maturation stages of human enamel (Limeback et al., 1989; Robinson et al., 1994, 1995) and more recent animal models have further confirmed that albumin is deposited during odontogenesis (Robinson et al., 1994; Salido et al., 1992; Simmer, 1995; Stewart et al., 2017; Welker et al., 2020; Wilson et al., 2012; Zhang et al., 2012) (Gil-Bona et al., 2023). During odontogenesis, the enamel organ is surrounded by the dental follicle which also encapsulates blood vessels (Kwon and Jiang, 2018). Robinson et al. (1995) suggest that albumin might have a role in regulating enamel formation because it binds strongly to apatite and inhibits crystal growth. Albumin, along with other proteins, is later excluded from the enamel as it matures and hardens. Fully mineralized human enamel contains approximately 1% protein by weight, while higher levels of albumin have been associated with hypomineralized defects in both animal models and human teeth (Gil-Bona et al., 2023).

Because layers of enamel and dentine begin forming *in utero* and continue to develop through early adulthood and do not remodel, they provide a wealth of information on nutrition, weaning, season of birth, and mobility during the early life histories of individuals (Bartelink and Chesson, 2019; Eerkens et al., 2011; Eerkens et al., 2022; Sandberg et al., 2014; Smith, 2018; Smith et al., 2018). Similarly, the ability to track immune responses in dental tissues could reveal higher resolution information on health and disease during the early life of individuals, and allow for comparisons over archaeological time-scales.

While morphological alterations to bones and teeth can inform osteologists about past diseases, injuries, and nutritional deficiencies, many illnesses do not leave a visible trace on the skeleton. Even when illnesses do leave such markers, it still may not be possible to pinpoint the period of life in which the health crisis occurred. Using immune proteins entrapped within a robust tissue such as enamel or dentine, could provide a biochemical means of tracing health and disease in archaeological populations. In addition to enhanced preservation potential, tracing immune proteins in dental tissues that form at different stages of development and do not remodel could provide individual records of health and disease from birth to early adulthood.

In this study, we compare levels of immunoglobulin gamma (IgG) and C-reactive protein (CRP), two serum proteins associated with immune function, with levels of serum albumin (the most abundant protein in blood serum). We assume that the three proteins were transported together and deposited during tooth formation and argue that the relative intensity of these immune proteins could provide a record of stress and disease during the period of tooth formation. This is an area that deserves much additional research. To our knowledge, this is the first comparison of serum immune proteins IgG and CRP extracted from archaeological tooth enamel.

2.1. Immune proteins in serum

Serum is the fluid that remains after red blood cells and clotting factors have been removed from blood (Busher, 1990). Albumin comprises approximately 60 percent of the protein content of serum, while most of the remainder is composed of various immunoglobulins, of which, gamma immunoglobulin (IgG) is most prevalent (Busher, 1990; Calonga-Solís et al., 2019).

Immunoglobulins, also known as antibodies, are part of the adaptive immune system (also called the humoral or specific immune system). Immunoglobulins exist as either membrane-bound proteins or are secreted into the blood. They are composed of two heavy and two light chains, each with variable domains that bind to specific antigens and constant domains that define the overall functions of the molecules. There are five main types of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, which differ in their functional location, structure and specificity for different types of antigens (Schroeder and Cavacini, 2010). IgG is the primary immunoglobulin circulating in the bloodstream and can be divided into four subtypes: IgG1, IgG2, IgG3, and IgG4, which are present from highest to lowest concentrations in respective order

(Calonga-Solís et al., 2019).

Antibodies are crucial for health and survival, and concentrations of serum immunoglobulins are often used in the clinical detection of immunologic or inflammatory diseases (Bayram et al., 2019; Cassidy et al., 1974; Sela et al., 1987; Gonzalez-Quintela et al., 2008). IgG concentrations in serum are elevated in chronic inflammatory diseases, infections and cancers, while lower concentrations are associated with immune disorders, fasting (Gonzalez-Quintela et al., 2008; Khan et al., 2021), and poor nutrition (Govers et al., 2022). Psychosocial stress is also known to affect IgG levels (Segerstrom and Miller, 2004). Although numerous studies associate psychological stress with increased IgG levels, most focus on acute stressors. Kahn et al. (2021:10) propose that chronic stress could, in fact, result in lower levels of IgG in humans as lower amounts of IgG have been observed in rodent models exposed to chronic stress. Additionally, several studies note that serum IgG tends to be slightly higher in females than males and tends to increase slightly with age (Bayram et al., 2019; Gonzalez-Quintela et al., 2008). However, other studies have found no difference in IgG levels by age or sex (Khan et al., 2021).

Whereas IgG is part of the specific immune response and the main immunoglobulin in serum, CRP is associated with inflammation as part of the non-specific (or innate) immune response and is typically present at much lower concentrations. Routine analyses of plasma indicate the normal content of CRP is about two orders of magnitude lower than IgG and approximately four or more orders of magnitude lower than serum albumin (Anderson and Anderson, 2002). Similar to trends in IgG, elevated amounts of CRP are associated with chronic inflammation as well as psychosocial stress (Gouin et al., 2012; Meca et al., 2022; Shenhar-Tsarfaty et al., 2015). It is possible that chronic stress (as opposed to acute stress, see Khan et al., 2021) could decrease IgG while CRP remains elevated. CRP is a non-specific biomarker in chronic inflammatory diseases and researchers note elevated levels associated with other bacterial infections, burns, traumas, and cancers (Meca et al., 2022). Two recent meta-analyses of studies in medical journals find that CRP has a high sensitivity in screening for pulmonary tuberculosis, particularly in immune compromised individuals (Meca et al., 2022; Yoon et al., 2017). Beyond this, other studies have connected elevated levels of CRP to emotional stress (Johnson et al., 2013) or persistent fear in conflict zones (Shenhar-Tsarfaty et al., 2015). Indeed, there has been much recent interest in investigating connections between psychosocial stress, inflammation, and elevated CRP levels (Chiang et al., 2019; Cundiff et al., 2024; Kennedy and Niedzwiedz, 2022; Ziomkiewicz et al., 2021).

Based on a summary of the previous studies, it is possible that persistent, chronic emotional stress (in the absence of chronic disease) would lead to decreased IgG and elevated CRP. In the current study, we know that Native Americans living through contact and missionization events in California were exposed to both chronic infectious diseases such as tuberculosis, and chronic social and emotional stressors. We assume that many of the asistencia individuals would have experienced profound social ruptures and declines in health that accompanied missionization in central California.

Although several studies document serum albumin in tooth enamel and IgG in whole teeth (Gil-Bona and Bidlack, 2020; Limeback et al., 1989; Robinson et al., 1995; Shaw et al., 2023), precautions against laboratory contamination must be taken into account since serum albumin is the most common protein in blood serum and can be introduced in the laboratory. In the current study, we compared proteins recovered from sample blanks that were prepared alongside each batch of samples. To further validate the antiquity of serum proteins, we also compared deamidation in serum albumin across older and younger samples (Hendy et al., 2018b; Parker et al., 2019; Procopio et al., 2018). To assess the possibility of a salivary origin for serum proteins observed in enamel, we further screened all extracts for alpha-amylase, which is abundant in saliva and is reported to have an affinity for hydroxyapatite similar to that of albumin (Gil-Bona et al., 2023; Johnsson et al., 1993).

2.2. Individuals buried at San Pedro y San Pablo asistencia

This research involves individuals recovered during archaeological mitigation activities in 2017–2018 at Sanchez Adobe Park in Pacifica, California (site number CA-SMA-71/H) (Eerkens et al., 2025.). Mitigation followed construction work at the park in 2016, which resulted in the inadvertent discovery of significant archaeological resources. In partnership with the state-assigned Most Likely Descendant (CZ), and the Amah Mutsun Tribal Band of Mission San Juan Bautista, a range of additional archaeometric work was requested.

While there is an 1840s adobe at Sanchez Adobe Park, it is also the location of the older Asistencia San Pedro y San Pedro (Fig. 1). The asistencia was an agricultural outstation associated with the larger Mission San Francisco de Asís, also known as Mission Dolores. Founded in 1776, the location of the Mission San Francisco was not conducive to agriculture and could not produce enough food for the growing congregation. To alleviate this, the Franciscan missionaries, relying on indigenous labor, developed an outpost, or asistencia, in 1786. Food supply issues were quickly remedied, and by 1787 one of the Franciscans began performing mass at the asistencia every Sunday. The asistencia expanded over time and included its own quadrangle, agricultural activities, and cemetery (Milliken 1979).

Death records from Mission San Francisco de Asís list approximately 150 Native individuals who were associated with the mission but buried at the asistencia. The vast majority of these individuals were buried between 1787 and 1792, though burials at the site continued until 1800. Archaeological efforts in 2017–2018 resulted in the recovery of 15 Ohlone ancestors. Based on analyses matching some burials to historical records (Panich et al., 2025), and location within the cemetery, we are able to estimate a date of death for each individual (see Table 1). Hillson (2024) provides estimated ages for tooth formation, including enamel. For permanent first molars this is between 0 and 3 years, for permanent third molars this is between 7 and 16 years, and for deciduous second molars this is between in-utero and 2.5 years. Then, using the range for the estimated age at death, we can calculate approximate minimum and maximum years for the formation of the enamel analyzed for each individual. This information is given in Table 1. As shown, seven of the burials had enamel that likely fully formed before establishment of Mission San Francisco de Asís in 1776, though three individuals, Burials 6, 12, and 13 have enamel that could have formed within the 6 years before establishment of the mission. Five individuals, Burials 2, 9, 11, 14, and 15 had enamel that formed after 1776, well within the mission period.

Baptismal and death records indicate that the majority of individuals buried within the asistencia cemetery were from the Ohlone village of Pruristac, which was located near the site (Milliken 1979). However, records indicate that people from the south—near the present-day coastal communities of Half Moon Bay and San Gregorio—were also buried here (individuals who were originally part of Chiguan, Cotegen, Oljon, and Quiroste tribal groups). Milliken et al. (2009:101) suggest that many of these people may have preferred to be baptized at the coastal asistencia, rather than the geographically closer but more interior Mission Santa Clara.

More than 60 percent of the Native people buried at the asistencia were children (Caine et al., 2025). This high mortality rate was likely due to synergistic effects of disease, cultural suppression, and poor nutrition. Proteomic investigations were applied to help provide a deeper insight into the lives of those Ohlone ancestors who lived, worked, and died at this site, and to keep their memories alive in the present.

3. Methods

3.1. Sample selection

Analysis of immune proteins in enamel accompanied the proteomic

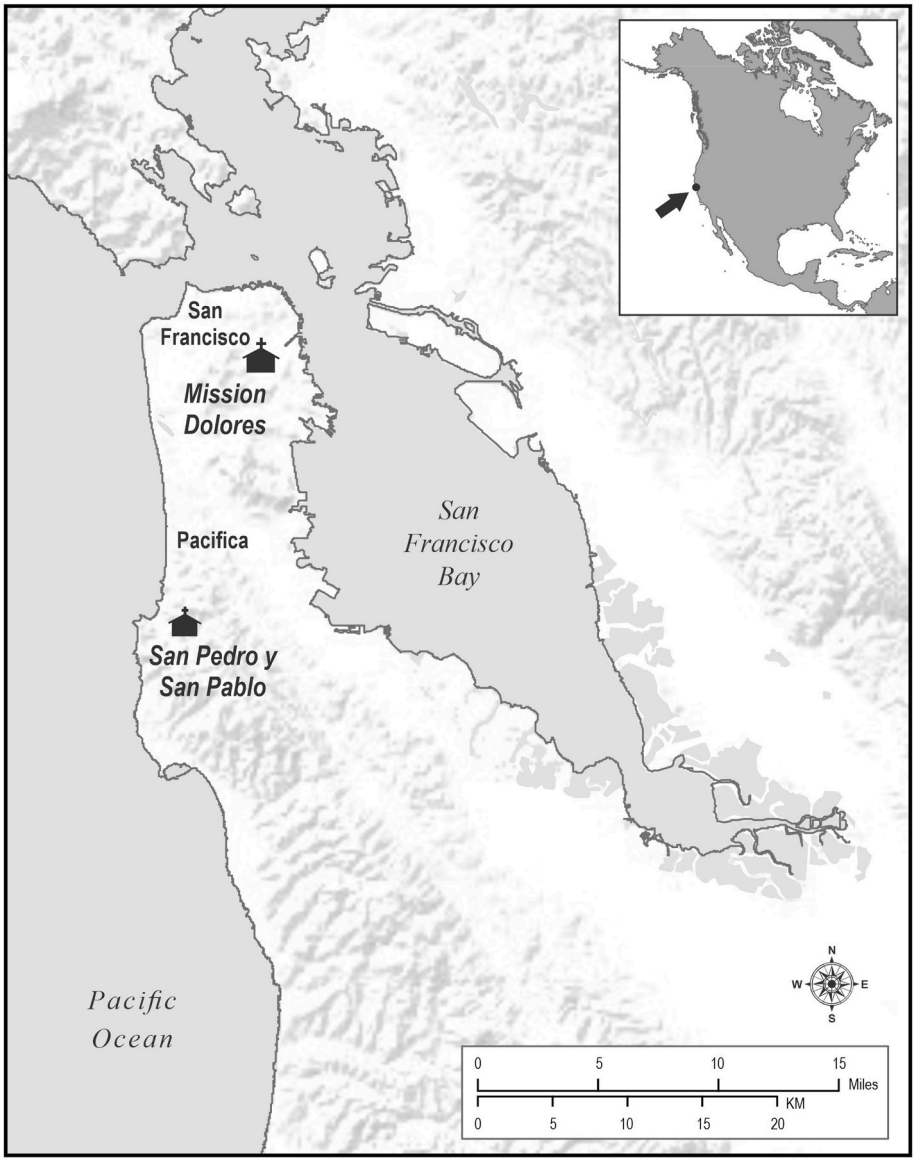

Fig. 1. Location of Asistencia San Pedro y San Pablo (SMA-71/H).

Table 1
Estimated ages at death and during enamel formation for individuals at Asistencia San Pedro y San Pablo (SMA-71/H). Burials where analyzed tooth enamel formed before establishment of Mission San Francisco de Asís in 1776 include: 3, 4, 5, 6, 8, 12, 13. Note that burials 6, 12, and 13 are close to the mission period, while tooth enamel analyzed from burials 2, 9, 11, 14, and 15 formed during the mission period.

Burial	Age	Tooth	Sex	Estimated Year of Death	Range of Enamel Formation (Years CE)
2	13–17	LLM3	M	1789	1779–1789
3	45–50	URM3	F	1787	1744–1758
4	50–60	LRM3	F	1792	1739–1758
5	45–50	LLM1	F	1787	1737–1745
6	18–25	LRM1	M	1788	1763–1773
8	46–60	LLM1	M	1787	1727–1744
9	2–2.5	dLLM2	M	1789	1786–1789
11	4–8	M1	M	1789	1781–1788
12	20–25	URM1	F	1787	1762–1770
13	20–24	LRM1	F	1788	1764–1771
14	5–7	ULM3	F	1792	1791–1792
15	12–13	URM3	M	1792	1786–1792

sex estimation of tooth enamel for skeletal remains of 12 of 15 individuals inadvertently discovered at CA-SMA-71/H during mitigation of construction work (Eerkens et al., 2025). Three individuals from this site either did not have teeth, in the case of two infants, or had significant dental wear that limited enamel availability, in the case of one mature adult. A range of archaeometric work was requested by, and conducted in close collaboration with, the state-assigned Most Likely Descendant (CZ) and the Amah Mutsun Tribal Band of Mission San Juan Bautista. One aim of this work was to better identify individuals and connect them with Mission records.

For comparison, we used proteomic data from two additional populations. First, we sampled enamel from historic-era (late 19th century) individuals buried in San Francisco Bay Area (Eerkens et al., 2023; Baker Beach Green Street Project, Case No. 2015-0070901ENV). These represent European Americans who did not live in mission settings, but in a period where a more modern understanding of disease was just emerging (i.e., germ theory), sanitation was poor, and life expectancy was significantly shorter than today. Second, present-day enamel was sampled from third molars removed from eight healthy male and female military cadets from the United States Airforce Academy in Colorado Springs. The extracted third molars were collected as part of a study that

sought to document carbon, oxygen, strontium and lead isotopes across the continental United States in order to provenance human remains (Regan, 2006). The subjects were all born between 1964 and 1987 and third molars were extracted between 2005 and 2006. Immediately following extraction, teeth were submerged in a 3% hydrogen peroxide solution for two days to clean and remove any adhering pieces of tissue or blood, before they were air dried and placed in plastic containers (Regan, 2006). This initial study was determined to be exempt by the overseeing institutional review board (HQ USAFA IRB FAC2005026H) and found to be research, not human subject research, by the University of California, Davis institutional review board (IRB 97955-2).

Archaeological comparisons were made to Historic-era European Americans (n = 12) interred in the French Mutual Benevolent Society (FMBS) section of City Cemetery in San Francisco, California between 1878 and 1899 (Eerkens et al., 2023). Strontium isotope values as well as historic documentation indicates that many of these individuals immigrated to the San Francisco Bay Area from northern and central France (Eerkens et al., 2023). While these were not wealthy individuals, their burial in the FMBS section of City Cemetery indicates they had sufficient capital to purchase membership in the society, which provided some access to medical care, and burial plots (Eerkens et al., 2023).

We assume that present-day military cadets experienced better nutrition and health than either set of archaeological samples. Additionally, while historic-era European American individuals interred in San Fransico may have had similar disease exposures as the asistencia individuals, we assume that, as a group, they experienced less social and nutritional stress during tooth development.

3.2. Laboratory analysis

All samples were analyzed in the same lab and were extracted over several different batches using the same protocol. A sample blank was analyzed along with each batch of samples and methods followed those established in Parker et al. (2019) with slight modifications in Buonasera et al. (2020). Powdered enamel samples (~20 mg) were demineralized by adding 200 μL of 1.2M hydrochloric acid to 2 mL sample vial with seven 2.8 mm ceramic beads (Omni-International Inc.). Samples were milled in acid for 3 min at 7000 rpm in a MagnaLyzer (Roche Inc.), then centrifuged for 5 min at 16000 g. After incubation in the same acid solution at 56 °C, 1200 rpm for 60 min, samples were neutralized with 2.0 M ammonium bicarbonate and 0.01% ProteaseMAX (6 μL of 0.5% w/v, Promega Inc.) was added to the sample vials along with mass spectrometry grade trypsin (1 μL of 0.5 μg/μL, Thermo Pierce, Inc). Each sample was then incubated at 37 °C for 20 h at 600 rpm. After incubation, the sample vials were centrifuged for 5 min and the supernatant was transferred to 0.22 μm centrifugal filters and centrifuged for 30 min. The filtrate was then transferred to clean Eppendorf® Protein LoBind tubes for ZipTip (Millipore Inc.) sample clean up to prepare for mass spectrometric analysis. Organic contaminants in aqueous stocks and solutions were removed by prior passage over solid phase extraction (SepPak, C18, Waters Inc.).

Digested peptides were desalted and concentrated using ZipTip C18 pipette tips (Millipore Inc.) with the eluted material lyophilized and resuspended in 2% (v/v) acetonitrile and 0.1% (v/v) TFA.

The peptide concentration was measured using the Pierce™ Quantitative Fluorometric Peptide Assay (Thermo Pierce™). Nano LC-MS/MS was performed on either a Thermo Scientific Exploris 480 Orbitrap Mass spectrometer in conjunction with a Dionex UltiMate 3000 RSLC system (Thermo Scientific) or a Thermo Scientific Q Exactive Plus Orbitrap Mass spectrometer in conjunction with a Proxeon Easy-nLC II HPLC (Thermo Scientific) and Proxeon nanospray source. For the Exploris system, digested peptides (generally less than 1.0 μg) were loaded using a PepSep analytical column (PepSep, Denmark): 150um x 8 cm C18 column with 1.5 μm particle size (100 Å pores), preceded by a PepSep C18 guard column, and heated to 40 °C. Separation was performed in a total run time of 90 min with a flow rate of 500 μL/min with mobile phases A: water/0.1% formic acid and B: 80% ACN/0.1% formic acid. Gradient elution was performed from 4% to 8% B over 3 min, from 8% to 46% B over 66 min, and from 46 to 99% B over 3 min, and after holding at 99% B for 2 min, down to 2% B in 0.5 min followed by equilibration for 15min. Spray voltage was set to 1.8 kV, funnel RF level at 45, and heated capillary temperature at 275 °C. The full MS resolution was set to 60,000 at m/z 200 and full MS AGC target was 300% with the injection time set to Auto. Mass range was set to 350–1500 for fragmentation spectra, and resolution at 15,000. Isolation width was set at 1.6 m/z, and normalized collision energy was set at 30%. The AGC target value was set to Standard, with a maximum injection time of 40 msec and we did TopN scans (30 scans for 60 min or 35 scans for 90 min). An isolation mass window of 1.6 m/z was used for the precursor ion selection, and normalized collision energy of 27% was used for fragmentation. A 5 s duration was used for the dynamic exclusion. Reagent blank samples were applied every 10 to 12 samples. For the Q-Exactive system, digested peptides (generally less than 1 μg) were loaded on a 100 μm x 25 mm Magic C18 100 Å 5U reverse phase trap where they were desalted online before being separated using a 75 μm x 150 mm Magic C18 200 Å 3U reverse phase column. Peptides were eluted using a 65-min gradient with a flow rate of 300 nl/min. An MS survey scan was obtained for the m/z range 300–1600, MS/MS spectra were acquired using an inclusion list of 28 ions (Supplemental Table S1) that were subjected to HCD (High Energy Collisional Dissociation). When inclusion list ions were not found, MS/MS was done on other ions in the MS survey scan. An isolation mass window of 1.6 m/z was used for precursor ion selection, and normalized collision energy of 27% for fragmentation. A 5 s duration was used for the dynamic exclusion. Washes were applied between each sample. After 10 samples, a blank run of BSA standards was applied to test for sample-to-sample contamination. The same inclusion list was used for both instruments.

Mass spectrometry datasets (RAW format) were processed using PEAKS (version 10 Pro) peptide matching software (Bioinformatics Solutions Inc., Waterloo, ON). The FASTA formatted UNIPROT *Homo sapiens* reference protein database (http://www.uniprot.org/proteomes/UP000005640) was modified to include additional FASTA protein entries of peptide sequences from all splice variants associated with AMELX_HUMAN (Q99217-1, −2, −3) and AMELY_HUMAN (Q99218-1, −2) proteins gene products (Salido et al., 1992; Simmer, 1995). The reference database was further modified to incorporate a decoy database and was validated in PEAKs™ Software (Zhang et al., 2012). Additionally, a contaminant database search was performed by adding the Common Repository of Adventitious Proteins (cRAP) database http://ftp.thegpm.org/fasta/cRAP to the larger reference database described above, and no relevant contaminants were found in the samples. Peptide matching spectral assignment was conducted using default conditions with the following exceptions: error tolerance, precursor mass, 10 ppm, Fragment ion, 0.04 Da, cleavage with trypsin with up to 2 missed cleavages, and up to one two non-specific cleavages. The algorithm searched for peptides partially modified by deamidation (NQ), oxidation (MHW), pyroglutamate conversion from glutamate and glutamine, and methionine dioxidation. All peptide assignments were filtered by a 1% false discovery rate. Each peptide was quantified by summing the intensity of each signal for the peptide-specific primary precursor mass over charge ratio (m/z). Positive protein identifications required a minimum of two unique peptides. Proteins with only one unique peptide were excluded from analysis.

Mass spectrometry proteomics data have been uploaded to the ProteomeXchange Consortium via the PRIDE partner repository (Perez-Riverol et al., 2022) with the dataset identifier PXD054717 (http://www.proteomexchange.org).

3.3. Serum albumin, IgG, and C-reactive protein

As described in the previous section, immunoglobulins are complex macromolecules composed of both variable and constant regions, as well

as heavy and light chains. The presence of the light and variable chains was not consistent across samples; some samples had several of these while others had only one, or none. However, all enamel samples from contemporary military cadets, the FMBS individuals, and the asistencia individuals (except Burial 11), contained peptides from the heavy constant region for IgG and these were present in the highest intensity across all immunoglobulin peptides (see supplemental material). To maintain consistency, only the total ion counts for the heavy constant region of IgG constant region (summed for subtypes 1–4) was compared across samples. The total ion counts for serum albumin and CRP were also compared across samples. Sequences for analyzed proteins were included in the human proteome database referenced in the prior section on the analysis of amelogenin in tooth enamel.

Uniprot sequences and accession numbers used for identifying and comparing intensities of serum proteins in enamel samples were limited to the following: P02768-1, −2, −3, Serum albumin, *Homo sapiens*; P02741 C-reactive protein, *Homo sapiens*; P01857 Immunoglobulin heavy constant gamma, *Homo sapiens*; P01859 Immunoglobulin heavy constant gamma 2, *Homo sapiens*; P01860 Immunoglobulin heavy constant gamma 3, *Homo sapiens*; P01861 Immunoglobulin heavy constant gamma 4, *Homo sapiens*; P01876 Immunoglobulin heavy constant alpha, *Homo sapiens*. Other sequences with different accession numbers for these same proteins were present in the database and were associated with fragmented or poorly annotated sequences. These were excluded from analysis.

The summed total ion intensities for IgG constant heavy chains (hereafter referred to as summed IgG) were compared among samples in two ways: 1) as a ratio to serum albumin intensity within the sample, and 2) normalized by sample weight. Serum albumin is the major protein in blood serum and remains fairly constant as a fraction of total blood protein content (Busher 1990). Assuming that serum albumin is a measure of how much blood protein was incorporated in enamel and/or dentine, the first measure (IgG/SA) should help to level potential sampling differences in the overall density of serum proteins. The second measure compares the amount of immune signal per mass of original sample, independent of serum albumin. Normalizing IgG and CRP by the intensity of serum albumin has the advantage of evening-out potential variability that could arise if certain samples inadvertently contained small amounts of dentine adhering to enamel, or if samples have been subject to different degrees of degradation. As stated previously, we assume that albumin, IgG and CRP were introduced together and degrade at similar rates as they are each globular proteins. Dentine contains a higher proportion of proteins in general and if some dentine powder inadvertently made its way into the enamel powder during sampling, this could bias IgG comparisons that were normalized by starting sample weight, but not by serum albumin. We want to stress, however, that enamel was dissected carefully under magnification and the possibility of small, infrequent contamination events cannot account for the systematic observation of serum albumin and IgG in our enamel samples. The intensity of CRP was compared in the same way as IgG, both as a ratio to serum albumin and normalized by sample weight.

3.4. Deamidation

Deamidation refers to a chemical conversion where an amide group on glutamine (Q) or asparagine (N), the two amino acids with amide-containing side chains, is replaced with a carboxylic acid (Jin et al., 2022). This shift results in a mass increase of +0.984 Da (Q+0.98, or N+0.98) and produces glutamic acid (E) from Q or aspartic acid (D) from N. Deamidation occurs spontaneously over time but rates can be further influenced by temperature, pH, and the relative mobility and accessibility of the amino acid residue (Demarchi et al., 2016; Mackie et al., 2018; Wilson, 2012). Deamidation is known to accumulate in older samples of enamel (Parker et al., 2019; Welker et al., 2020) and was compared here in serum albumin as an additional way to validate the antiquity of serum peptides. The relative antiquity of serum peptides should be reflected as a higher proportion of deamidation in serum albumin peptides from enamel of asistencia individuals versus serum albumin in enamel from present-day military cadets. Peptides from modern sources (i.e., laboratory contamination or modern enamel) are expected to have lower amounts of deamidation than those in enamel from Mission Period or FMBS individuals. Percent deamidation was calculated by comparing the total ion intensities for the most abundant glutamine-containing peptide and its glutamic acid analog in serum albumin, both of which were present in all samples: KVPQVSTPTLVEVSR and KVPQ(+0.98)VSTPTLVEVSR, where Q or Q+0.98 is residue number 441 in the human serum albumin amino acid sequence. We compared deamidation in IgG in a similar manner, selecting the most abundant and common peptide among samples: VVSVLTVLHQDWLNGK with Q (at position 94) and in this case, also N (at 98), although this peptide was not always present, and many samples had no peptides with either a Q or an N. It was not possible to compare deamidation in CRP as most samples with CRP did not contain peptides with either a Q or an N.

3.5. Statistical tests

One-way ANOVA with post-hoc Tukey-Kramer tests were used to compare mean percent SA deamidation and mean normalized peptide intensities for IgG among the three groups: asistencia enamel samples from 11 individuals, Air Force Academy cadet enamel samples from eight individuals, and enamel samples from 12 FMBS individuals. Mean normalized AMELX peptide intensities were compared in the same way among the three groups. We did not compare normalized mean CRP intensities, however, because this protein, typically expressed several orders of magnitude lower than IgG (Anderson and Anderson, 2002), was not detected in enough individuals. All statistical tests were performed with JMP Pro 17 software and differences were considered statistically significant at alphas below 0.05.

4. Results

Proteomic sex estimates for asistencia and FMBS individuals are reported comprehensively elsewhere (Buonasera et al., 2025; Eerkens et al., 2023). Sampling and amelogenin data for all teeth included in this study are provided in supplemental information (Table S2). For enamel samples lacking AMELY_HUMAN we assigned a probability of female sex based on the normalized cumulative AMELX_HUMAN signal following methods first described in Parker et al. (2019) and slightly updated in Buonasera et al. (2020). Seven of the asistencia individuals were male (n = 7) and five were female (n = 5). Five individuals were non-adults (four males and one female) and the youngest was a male infant approximately 2.5 years of age. Seven of the individuals were adults, including three young adults (two males and one female) and four adults that were middle-aged or older (one male and three females). The proteomic sex estimates for the seven adults were in complete agreement with prior osteological assessments based on pelvic and cranial morphology.

4.1. Immune proteins and serum albumin in tooth enamel

Demographic information on individuals and summed intensities (total ion count) for specific peptides from the conserved portion of IgG heavy chain (subunits 1–4), CRP, and serum albumin detected in associated enamel samples are provided in Table 2.

Enamel from asistencia Burial 11 (Table S2) was excluded from the immune protein analysis due to an unusually low overall amount of all peptides and the absence of IgG peptides. The enamel sample for this individual had much lower overall amounts of protein, including amelogenin values more than two orders of magnitude lower than other samples. Indeed, without the presence of AMELY peptides, this sample would have been indeterminate for sex estimation based only on the combined intensity of AMELX specific peptides.

Table 2
Relative intensity of serum albumin, IgG, and C-reactive protein in enamel from individuals interred at Asistencia San Pedro y San Pablo (SMA-71/H) and the FMBS section at City Cemetery in San Francisco (FMBS), and enamel from contemporary military cadets (AFA).

Association	Individual	Tooth	Med age	Sex	SA/mg	IgG/mg	IgG/SA	CRP/SA	Pathologies
CA-SMA-71/H	2	LLM3	11.0	M	2.06E+08	4.86E+07	0.235	5.93E-03	Dental hypoplasias, vertebral TB possible
CA-SMA-71/H	3	URM3	47.5	F	1.21E+08	1.32E+07	0.109	0.00E+00	Periodontal disease, healed ilium fracture
CA-SMA-71/H	4	LRM3	55.0	F	2.79E+08	2.64E+07	0.095	0.00E+00	Dental abscess, periodontal disease, healed vertebral fractures
CA-SMA-71/H	5	LLM1	47.5	F	3.79E+08	4.03E+07	0.106	1.57E-03	Dental abscess and periodontal disease
CA-SMA-71/H	6	LRM1	21.5	M	2.16E+08	3.14E+07	0.145	0.00E+00	Moderate periodontal disease
CA-SMA-71/H	8	LLM1	53.0	M	1.30E+08	1.33E+07	0.102	0.00E+00	Dental abscesses, degenerative disc disease
CA-SMA-71/H	9	dLLM2	2.3	M	8.74E+07	2.07E+07	0.237	1.25E-02	None observed
CA-SMA-71/H	12	URM1	22.5	F	1.28E+07	8.62E+06	0.676	3.90E-02	Dental hypoplasias
CA-SMA-71/H	13	LRM1	22.0	F	3.52E+08	4.89E+07	0.139	2.20E-03	Slight periodontal disease
CA-SMA-71/H	14	ULM3	6.0	F	2.10E+08	2.51E+07	0.120	1.82E-03	Dental defects, pit in LRM1
CA-SMA-71/H	15	URM3	12.5	M	2.20E+07	1.07E+06	0.049	0.00E+00	None observed
FMBS	B4Ind1	ULM1	35.0	M	7.43E+07	6.44E+06	0.087	0.00E+00	None observed (only 15% of skeleton)
FMBS	B4Ind2	URM1	35.0	M	7.47E+07	5.66E+06	0.076	0.00E+00	None observed
FMBS	B4Ind4	ULM1	35.0	M	1.69E+07	2.85E+05	0.017	0.00E+00	None observed
FMBS	B4Dis	URI2	Adult	M	6.39E+07	5.00E+06	0.078	0.00E+00	None observed
FMBS	B5Ind1	LLC	52.5	M	3.62E+07	7.47E+05	0.021	0.00E+00	Majority of dentition missing
FMBS	B6Ind2	LRM1	26.5	F	3.50E+07	5.48E+05	0.016	4.83E-03	Dental hypoplasias
FMBS	Cranium	ULI1	47.5	M	4.82E+07	1.68E+06	0.035	0.00E+00	Dental resorption and wear
FMBS	B6aInd1	URMI	52.5	M	7.16E+07	1.02E+06	0.014	0.00E+00	Dental hypoplasias
FMBS	B7Ind1	LRM1	42.0	M	7.63E+07	2.35E+06	0.031	0.00E+00	Poor dental health, multiple abscesses and carries
FMBS	B7Ind2	ULM1	35.0	M	2.38E+09	3.33E+07	0.014	0.00E+00	Lower teeth highly worn, antemortem tooth loss
FMBS	B8Ind1	LRC	50.0	M	1.01E+08	4.71E+06	0.046	2.44E-03	Dental hypoplasias
FMBS	B8Ind4	ULM1	Adult	M	8.14E+06	3.12E+05	0.038	0.00E+00	Unknown
AFA	11	M3	19.5	M	3.48E+08	7.44E+06	0.021	0.00E+00	Unknown
AFA	27	M3	19.5	M	1.50E+07	5.60E+05	0.037	0.00E+00	Unknown
AFA	39	M3	19.5	M	1.20E+07	2.92E+05	0.024	0.00E+00	Unknown
AFA	87	M3	19.5	F	8.11E+07	1.00E+06	0.012	0.00E+00	Unknown
AFA	103	M3	19.5	M	2.48E+08	2.01E+07	0.081	0.00E+00	Unknown
AFA	130	M3	19.5	F	1.98E+08	8.13E+06	0.041	0.00E+00	Unknown
AFA	133	M3	19.5	M	3.94E+06	2.54E+05	0.065	0.00E+00	Unknown
AFA	234	M3	19.5	F	2.73E+07	6.51E+05	0.024	0.00E+00	Unknown

Fig. 2 shows a strong positive linear relationship between serum albumin and IgG for each group (R^2 AFA = 0.87, R^2 FMBS = 0.78, R^2 SMA-71/H = 0.66) and is in general agreement with relative proportions of these proteins in plasma, which typically differ in concentration by approximately two orders of magnitude (Anderson and Anderson, 2002). This relationship tends to support an inference that proportions of these proteins reflect those circulating in the blood of individuals during tooth formation.

Sample blanks (n = 9) were prepared and analyzed along with each batch (Table S3). No CRP peptides were detected in any blanks. One blank contained a single peptide of IgG, and four of eight blanks contained one or more peptides of serum albumin, which can occur as column carryover. In each case, the intensity of serum albumin was two to three orders of magnitude lower than the lowest signal in the samples from the corresponding batch (Tables S2 and S3). Potential contributions at this scale would be far too low to affect any of the comparisons made in this study. Nevertheless, peptide intensities in blanks were subtracted from the intensities of corresponding samples. Further, most of the sample blanks contained zero peptides of interest.

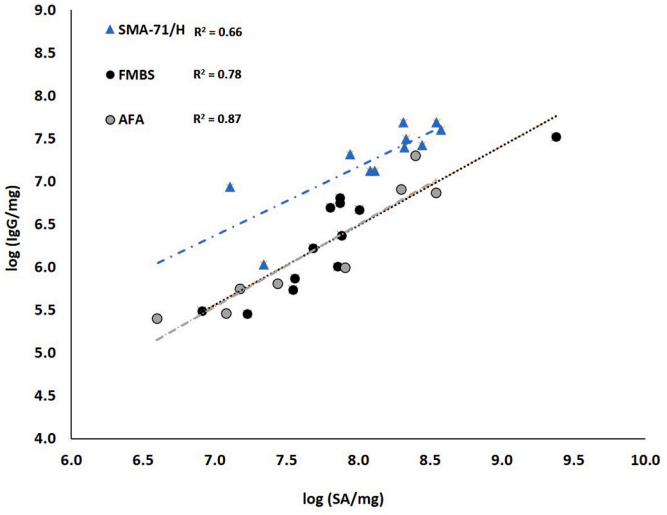

Fig. 2. Relationship of log transformed total IgG peptide signal intensity to log transformed SA peptide intensity for tooth enamel from Asistencia San Pedro y San Pablo (SMA-71/H individuals, n = 11), Historic-era European Americans interred in the FMBS section of City Cemetery in San Francisco (FMBS, n = 12), and third molars from present-day military cadets (AFA, n = 8).

Paleoproteomic analyses are typically run in proteomic facilities on nanoLC-MS/MS instruments that also run many other types of samples. These include digests of human cells and secretory products with dense arrays of peptides. One way that we guard against carryover is to run a sample blank, or negative control, prior to the remainder of the batch samples. This helps to both detect and prevent carryover to additional samples. A recent paper in Nature Protocols (Taurozzi et al., 2024) discusses this issue and suggests running two or more washes prior to paleoproteomic runs.

One way of assessing the relative antiquity of serum peptides in enamel, is by comparing percent deamidation (Hendy et al., 2018b; Parker et al., 2019; Procopio et al., 2018). Peptides from older samples are expected to have a higher degree of deamidation than those from very recent samples. Deamidation was compared as a percentage for the serum albumin amino acid sequence KVPQVSTPTLVEVSR (Q to Q+0.98) at amino acid number 441. This sequence was selected because it corresponds to the most abundant peptide with a Q residue (glutamine) that was present in all samples, thus providing a consistent measure among samples. Although glutamine is present in other serum peptides, those peptides were less abundant and/or were not present in all samples. An ANOVA yielded significant differences among the groups, $F(2,28) = 8.79$, $p = .001$. As expected, a greater degree of deamidation was present in serum albumin from archaeological enamel samples (mean SMA-71/H = 30% and FMBS = 36%) compared to the

present-day military cadet enamel (mean = 12%) (Fig. 3, Table S4A). A post-hoc Tukey test showed that deamidation in serum albumin for contemporary AFA samples was significantly lower than either group of archaeological samples ($p < .02$), while mean deamidation was not significantly different between the two archaeological groups (Table 3A). Deamidation of serum albumin was further compared as the percentage intensity of all Q441 and/or N553 residues that were deamidated (Q441((+0.98) and/or N553 (+0.98)). An ANOVA of this comparison showed the same relationship with a high significance, $F(2, 28) = 17.33$, $p < .001$ (see Tables S4B–C).

We also compared deamidation in IgG, but peptides containing Q or N were variable and not all samples contained peptides with a Q or a N. Where present, we compared intensities for the following peptides: VVSVLTVLHQDWLNGK with VVSVLTVLHQDWLN(+0.98)GK, VVSVLTVLHQ(+0.98)DWLNGK, and/or VVSVLTVLHQ(+0.98)DWLN (+0.98)GK, which were the most common among all samples. Deamidation was greater among archaeological samples (69%, n = 8) versus modern enamel (28%, n = 3).

It is of further note that no peptides for alpha amylase were detected in any sample extracts. Alpha-amylase is a major protein in saliva and has an affinity for hydroxyapatite similar to that of albumin (Gil-Bona et al., 2023; Johnsson et al., 1993). This argues against a salivary origin for the serum proteins, either absorbed from the oral cavity or through dental calculus.

4.2. Comparison of IgG and CRP

Individuals at the asistencia had higher amounts of IgG and CRP peptides than enamel from present-day military cadets or the historic-era FMBS individuals, both as a ratio of serum albumin (Figs. 4A and 4B), and as intensity normalized by sample weight. The differences in IgG/mg were significant according to ANOVA, $F(2,28) = 9.88$, $p < .001$. Importantly, ANOVA of IgG as a ratio of SA (IgG/SA) also yielded significant variation among these groups, $F(2,28) = 6.62$, $p = .004$. In both cases, post-hoc Tukey tests indicate significantly lower relative amounts of IgG among FMBS and present-day military cadets relative to asistencia individuals (Tables 3B and 3C). In contrast, amounts of serum albumin normalized by sample weight, were not significantly different among the three groups, $F(2,28) = 1.36$, $p = .273$. Similarly, the combined intensity of AMELX, a common enamel protein, normalized by sample weight, was not statistically different among the three groups, $F(2,28) = 2.48$, $p = .102$.

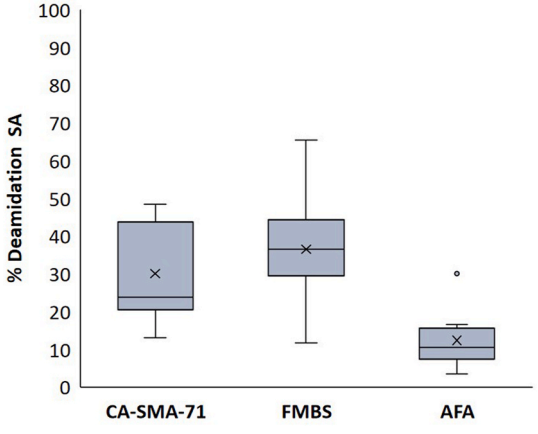

Fig. 3. Percent deamidation of serum albumin peptides from the asistencia (SMA-71/H), Historic-era European Americans interred in the FMBS section of City Cemetery in San Francisco, and military cadet (AFA) enamel samples. Deamidation was compared as a percentage for the serum albumin amino acid sequence KVPQVSTPTLVEVSR versus KVPQ(+0.98)VSTPTLVEVSR (Q to Q+0.98) with Q at amino acid residue number 441. This sequence was the most abundant peptide with a Q residue, present in all samples.

Although they were not detected in all samples, when they were detected, CRP peptides were about two to three orders of magnitude lower than serum albumin intensities. In three additional samples, only a single peptide was detected and was not considered a positive identification for the protein as the standard acceptance requires a minimum of two unique peptides (Cottrell, 2011; Hendy et al., 2018b). This large difference in abundance is consistent with the relative concentrations for these proteins in plasma (Anderson and Anderson, 2002). Nonetheless, CRP was detected in about 55% (6 of 11) of the asistencia samples, and 17% (2 of 12) of the FMBS samples. No CRP peptides were detected in enamel samples from contemporary military cadets (Table 2).

Relative amounts of IgG and CRP in individuals at the asistencia are plotted by median age-at-death in Fig. 5. The highest relative amounts were observed in enamel from younger individuals, particularly Burials 2, 9, and 12. These individuals are likely to have lived at the asistencia during the period in which their teeth were forming. By contrast, individuals who died at older ages have lower relative amounts of IgG and CRP. Given their ages at death and the dates the asistencia was in operation, the older individuals are believed to have lived outside the mission system, potentially in nearby Indigenous villages, during the time in which their enamel was forming (Eerkens et al., 2025.).

Asistencia Burials 2 and 12 each had dental hypoplasias, and Burial 2 had lesions that were consistent with vertebral tuberculosis (Caine et al., 2025). Burial 9 was the youngest individual in this analysis (a male infant, approximately 2.5 years of age) and no skeletal pathologies were observed on his skeleton. The enamel from this infant was the only deciduous tooth sampled in the larger set (Table 2). Two FMBS individuals had multiple teeth with hypoplasias. In this study, 4 of 5 individuals with visible dental hypoplasias also had measurable amounts of CRP, though not all individuals with CRP had visible hypoplasias (Table 2).

In Fig. 6, relative intensities of IgG/SA and CRP/SA are plotted by the average age of crown completion for each tooth, rather than age of death. Based on this comparison, it does not appear that the high amounts of serum immune proteins observed among asistencia individuals who died at younger ages can be explained by the age of tooth formation. Instead, this lends support to the idea that greater exposure and susceptibility to disease, poor nutrition, and other stresses leave a record in developing enamel.

5. Discussion

Although additional studies are needed to better understand the mechanism and timing of deposition, we believe that comparison of immune proteins preserved in enamel and dentin could provide a record of relative health and stresses experienced over the early life of individuals. To our knowledge, this is the first paleoproteomic (or archaeoproteomic) comparison of immune proteins (IgG and CRP) and serum albumin in extracts of human tooth enamel. This approach to recovering and analyzing relative amounts of peptides associated with immune proteins preserved in enamel, in addition to more routine proteomic sex estimation, could provide an important window into health and disease in the past. Because enamel is the human tissue most resistant to degradation, this could extend comparisons of health and disease by sex and developmental age to samples of much greater antiquity.

The relative concentrations of serum albumin and IgG are consistent across samples prepared by different lab analysts and were either not present in blank samples, or were present as one or more peptides several orders of magnitude lower than samples from the corresponding batch. We also note that significantly higher rates of deamidation were measured in serum albumin peptides from archaeological enamel samples compared to enamel from present-day individuals. Although deamidation is not a perfect proxy for geological age, as it is affected by preservation conditions such as pH, temperature, moisture and mineral interactions (Schroeter and Cleland, 2016), this supports our conclusions that laboratory contamination does not explain the persistent and

Table 3A
Difference in Percent deamidation for KVPQVSTPTLVEVSR, (Q441); the most abundant serum albumin peptide with glutamine (Q) in all samples. Tukey-Kramer pairwise tests for each of the three groups, AFA, SMA-71/H, and FMBS.

Group	Group	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
FMBS	AFA	24.16395	5.840603	9.71260	38.61530	0.0008*
SMA-71/H	AFA	17.74067	5.945848	3.02891	32.45242	0.0156*
FMBS	SMA-71/H	6.42328	5.341402	−6.79290	19.63946	0.4616

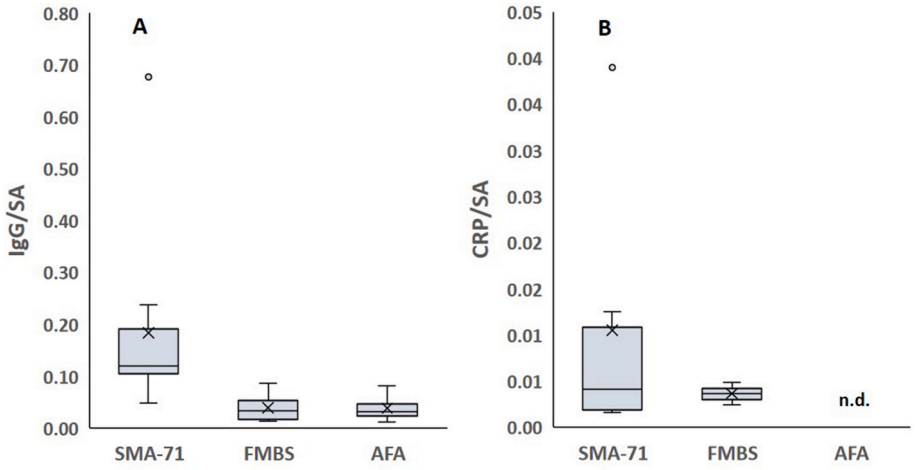

Fig. 4. **(A)** Box plot of total IgG peptide signal intensity relative to SA peptide signal intensity in tooth enamel from Asistencia San Pedro y San Pablo individuals (CA-SMA-71/H, n = 11), Historic-era European Americans (FMBS, n = 12), and present-day military cadets (AFA, n = 8), and **(B)** box plot of CRP peptide signal intensity relative to SA peptide signal intensity for the same three groups. CRP was not detected (n.d.) in any AFA individuals.

Table 3B
Difference in (Log10) IgG/mg.Tukey-Kramer pairwise tests for each of the three groups, AFA, SMA-71/H, and FMBS.

Group	Group	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
SMA-71/H	AFA	1.068182	0.2800232	0.375323	1.761041	0.0019*
SMA-71/H	FMBS	0.956515	0.2515565	0.334091	1.578939	0.0020*
FMBS	AFA	0.111667	0.2750667	−0.568928	0.792262	0.9134

relatively high intensity presence of these serum proteins in archaeological enamel.

We propose that serum proteins, including IgG, are incorporated into the enamel during tooth formation. As such, they may provide important data related to nutritional or environmental stress, including disease, encountered by individuals during the period of enamel formation. An assumption of this study is that the level of degradation of serum albumin and the IgG and CRP proteins would be equivalent, and that normalizing by serum albumin would control for potential contamination from dentine which is more vascular and likely to contain higher levels of both IgG and SA (at least under more favorable conditions of preservation). While enamel was intentionally sampled to exclude dentine, it is possible that very small amounts of dentine were present in one or a few samples. Any small amount of contamination would be equivalent for the three serum proteins IgG, SA, and CRP. Hence, the resulting ratios of serum proteins, and our conclusions, would be unaffected.

Due to the slow and constant accretion of enamel during development (Hillson, 2024; Smith, 2018), we believe that a measurable increase in serum immune proteins such as IgG and CRP is more likely to reflect accumulations over the course of chronic infections or chronic emotional stress, rather than from acute infections or brief episodes of heightened emotional stress. We assume that acute stressors are not present long enough to make a significant difference in our samples of enamel which would represent accumulated tissue that formed over several years. On the other hand, it may be possible to obtain finer-grained information across shorter timespans by serial sampling dentine and examining proteins within each section (see Eerkens et al., 2011 for a similar method examining dentinal collagen). Dentine provides a longer developmental record than enamel and contains a higher proportion of proteins (Hillson, 2024; Smith, 2018). It may also be possible to compare IgG in serial samples of enamel, or across teeth that formed during different developmental windows, though CRP is likely to remain below the limit of detection in some samples. However, neither possibility has yet been tested. As well, other immune proteins present in serum may also prove useful.

IgG and CRP were highest in enamel from three young individuals at the asistencia: Burials 2, 9, and 12. Burials 2 and 12 each had dental

Table 3C
Difference in IgG/SA. Tukey-Kramer pairwise tests for each of the three groups, AFA, SMA-71/H, and FMBS.

Group	Group	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
SMA-71/H	AFA	0.1448750	0.0490131	0.023602	0.2661475	0.0167*
SMA-71/H	FMBS	0.1435833	0.0440305	0.034639	0.2525275	0.0080*
FMBS	AFA	0.0012917	0.0481455	−0.117834	0.1204176	0.9996

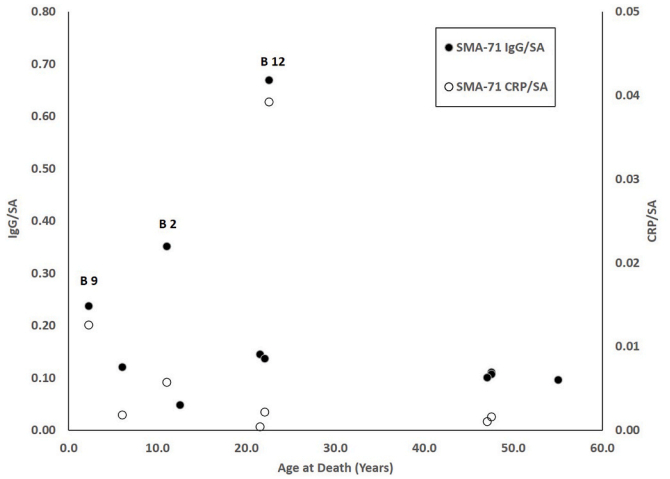

Fig. 5. Relative IgG levels and relative CRP levels by age at death for tooth enamel from Asistencia San Pedro y San Pablo individuals. We assume that older adults would have spent their early life, during the time of enamel formation, outside of the asistencia, before it was established.

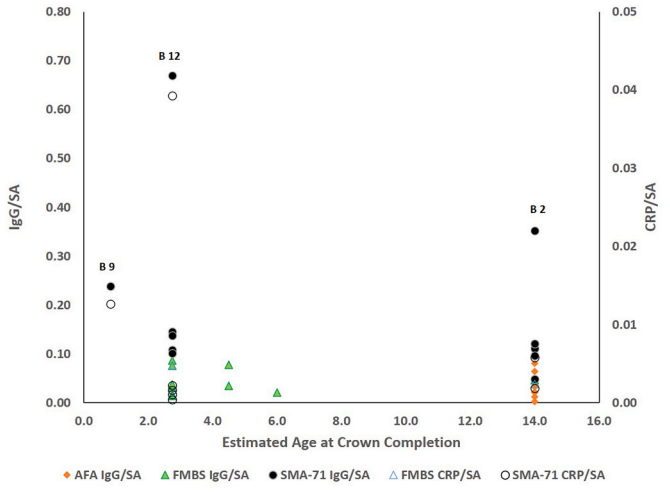

Fig. 6. Relative IgG levels and relative CRP levels by average estimated age of crown completion (Hillson, 2024) for Asistencia San Pedro y San Pablo individuals (CA-SMA-71/H, n = 11), Historic-era European Americans (FMBS, n = 12), and present-day military cadets (AFA, n = 8).

hypoplasias and likely experienced significant health stress during childhood when their sampled teeth would have been forming. The hypoplasia in asistencia Burial 12 was observed in her permanent canines, which would have been forming during the first year of life through about age six or seven years. A permanent first molar from this individual was sampled in our analysis, which would have formed between birth and three years of age, potentially overlapping with the hypoplasias observed in her canines. The tooth sampled for Burial 2 was a third molar which formed later in life than the teeth with hypoplasias (also permanent canines). However Burial 2 also had signs of severe vertebral tuberculosis and is estimated to have died between the ages of 9–13 years of age, around the time that the enamel on his third molars would have been forming. High levels of both IgG and CRP have been associated with tuberculosis and other chronic diseases in modern studies (Kashyap et al., 2020; Lee et al., 2020; Meca et al., 2022; Sela et al., 1987; Yoon et al., 2017). Burial 9, a 2.5-year-old male, also had high relative amounts of both CRP and IgG, but had no observable osteological pathologies. Enamel from this individual came from the only deciduous tooth in the sample set. It is possible the higher levels of CRP peptides are related to the earlier age of formation for this tooth as very young individuals typically have slightly higher expression of CRP and enamel (Kashyap et al., 2020). On the other hand, this individual also had very high levels of IgG, which is usually lower among individuals less than 3 years of age as their adaptive immune system is still developing (Bayram et al., 2019). Moreover, a plot of IgG/SA and CRP/SA by age of crown completion did not reveal an increasing trend in teeth that completed crown formation within the first few years after birth and those that formed later in childhood or adolescence (Fig. 6).

Overall, resulting comparisons of immune proteins, IgG and CRP, detected in enamel are consistent with the expectation that asistencia individuals had higher exposures to chronic diseases and/or endured higher levels of social and emotional stress than contemporary individuals who became cadets in the US Air Force, and to a lesser degree, historic-era European Americans. The displacement, disease, and high mortality endured by Native American communities during the Mission Period in California was profound (Jones et al., 2021; Milliken, 1995; Panich, 2020) and both explanations are consistent with the higher levels of IgG and CRP. Additional support for this hypothesis can be found by comparing elder residents at the asistencia with younger individuals. Higher concentrations of IgG and CRP are found among those under 25 years of age (Fig. 5). These younger individuals were more likely to have been living at the asistencia when their teeth were forming (Table 1). As well, younger individuals may have been exposed to higher levels of introduced diseases that spread ahead of direct contact with Euroamerican settlers and before their families entered the mission system (e.g., Cook, 1935). Older individuals, on the other hand, may have lived outside of the mission system during the time that their teeth were forming and been exposed to lower rates of disease and/or social stress.

We note that there could also be other life history or heritable differences that account for some of the differences between the asistencia and modern cadet groups. Non-random associations between serum IgG levels and lifestyle and ethnicity have been noted in a recent medical literature review (Khan et al., 2021). That study could not determine whether differences in IgG levels were linked to heritable factors or to potential environmental differences such as early life exposures to microbes. However, at least one study has found that ancestry-specific single nucleotide polymorphisms affected both innate (non-specific) and adaptive (specific) immune responses (Norris et al., 2018). We intend to explore this possibility among different populations in future studies.

6. Conclusions

Proteomic analysis of human tooth enamel was applied to the skeletal remains of 12 individuals interred at the Asistencia San Pedro y San Pablo (CA-SMA-71). Results reveal abundant serum proteins, primarily serum albumin and several proteins involved in immune function. Levels of immune proteins embedded in enamel could provide information on health statuses and disease exposures in past populations. This study compared relative intensities of peptides for IgG (a major part of the adaptive immune system) and CRP (a non-specific biomarker of inflammation) embedded in the enamel of individuals at the asistencia to enamel from eight present-day United States Airforce military cadets and 12 historic-era European American individuals interred in San Francisco, California. Prior to the study, we assumed that individuals living at the Mission Period asistencia experienced higher exposures to both acute and chronic diseases than individuals living in the present-day United States. We also assumed that individuals at the asistencia experienced high levels of emotional and physical stress as they endured poor nutrition, hard labor, loss of loved ones, and displacement from natal communities under the California mission system. Results from the study are consistent with both assumptions.

Results indicate that IgG is elevated in enamel from individuals at the asistencia relative to samples from third molars of present-day military

cadets or Historic-era European Americans. Additionally, while CRP was not detected in all samples, the highest levels of CRP were detected in enamel from individuals at the asistencia. Two individuals from the asistencia with dental hypoplasias, one of whom also had osteological indications of chronic tuberculosis infection, were among those with the highest levels of both IgG and CRP peptides. Our results are in agreement with expectations for higher levels of IgG and CRP under conditions of both chronic disease and chronic emotional stress. Additional studies of immune proteins present in enamel and dentine along with their mechanism of deposition are merited. The ability to track immune responses during tooth formation could provide valuable information on health and disease during the early life of individuals over archaeological time-scales. Additionally, it may be possible to make finer distinctions of specific immune responses and non-specific immune (inflammatory) responses in the future by systematically sampling enamel from different teeth or serial sampling enamel or dentine from the same tooth.

CRediT authorship contribution statement

Tammy Buonasera: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Jelmer Eerkens:** Writing – review & editing, Writing – original draft, Resources, Methodology, Funding acquisition. **Diana Malarchik:** Writing – review & editing, Investigation. **Lee M. Panich:** Writing – review & editing, Investigation. **Christopher Canzonieri:** Writing – review & editing, Investigation. **Christopher Zimmer:** Writing – review & editing. **Courtney Clough:** Writing – review & editing. **Thomas Ostrander:** Writing – review & editing, Investigation. **Aja Sutton:** Investigation. **Michelle Salemi:** Investigation. **Glendon Parker:** Writing – review & editing, Resources, Methodology, Funding acquisition, Formal analysis.

Declaration of competing interest

None.

Acknowledgments

We thank the Amah Mutsun Tribal Band of Mission San Juan Bautista, including Chairwoman Irenne Zwierlein and Michelle Zimmer, for the opportunity to work with them in better understanding the lives of their ancestors. We also thank Andrew Galvan of the Ohlone Indian Tribe, Beth Armstrong, and Desiree Vigil who served as tribal monitors, Colin Busby and Melody Tannam of Basin Research Inc., and San Mateo County Parks. We appreciate Alison Vanderslice and Kari Hervey-Lentz of San Francisco Planning Department for supporting the proteomic analyses of the historic California population. The authors thank Laura Regan for providing modern enamel samples. Comments from three anonymous reviewers helped to strengthen this paper. Funding for proteomic analysis was provided by a grant to GJP (PI) and JWE from the National Science Foundation (#BCS-1825022).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jas.2024.106069.

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