PNAS
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Supplementary Information for

Relief Food Subsistence Revealed by Microparticle and Proteomic Analyses of Dental Calculus From Victims of the Great Irish Famine

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www.pnas.org/cgi/doi/10.1073/pnas.1908839116

Supplementary Text

Material and Methods

The mass burial ground at the Kilkenny Union Workhouse, Kilkenny City, Ireland (Figure S1), was excavated in 2006 (1). A minimum of 970 individuals, from 63 burial pits, were recovered. The skeletal remains (Figure S2) were analysed following standard osteological methods. Age-at-death was determined from stages of dental mineralisation and eruption (2, 3), epiphyseal fusion (4), the morphology of the auricular surfaces and pubic symphyses of the coxal bones (5, 6), the sternal ends of the ribs (7, 8), and ectocranial suture obliteration (9). Sex was determined from pelvic and cranial morphological features (10). Following full osteological and palaeopathological analysis, the human remains were repatriated and reinterred in Kilkenny City in 2010.

Dental calculus was sampled from individual teeth extracted from a subsample of 42 skeletons prior to the reburial (Figure S3). The individuals consist of two adolescents (13–17 years), four young adults (18–25 years), 12 early middle adults (26–35 years), 18 late middle adults (36–45 years) and six older adults (≥46 years). Twenty-two samples derive from male individuals and 19 samples from female individuals. Sampling was undertaken in a clean laboratory environment at the National Museum of Ireland, where modern plants/proteins/DNA are not analysed. Prior to removal, the extent of the calculus was described using the protocol established by Dobney and Brothwell 1987 (11). Calculus was gently removed directly into 1.5 ml centrifuge tubes using dental picks that were cleaned with isopropanol between each sampling. Where possible, subsamples of approximately equal size were transferred into a separate 1.5 ml sterile centrifuge tube to save for future analyses. Samples were not weighed initially as a microbalance was not available at the sampling or microparticle extraction locations. However, subsamples were weighed prior to protein extraction (Dataset S2).

Microparticle extraction. Microparticles were extracted from 42 dental calculus samples (on average approximately 3×3 mm) using the EDTA extraction method described in Tromp et al. 2017 (12) and briefly reviewed here. All calculus preparation and decalcification took place in a positive pressure/laminar flow hood located in the Department of Anatomy, University of Otago aDNA facility to prevent contamination. Large pieces of dental calculus were removed from the collection tubes using a 20 µl pipette tip and placed into a clean tube. Four blank samples were included to ensure any contamination would be detected (i.e. empty tubes treated in the same manner as the samples). Samples were rinsed with sterile, filtered, boiled water before heat treated 0.5M EDTA was added to the samples for 30 minutes to remove potential surface contamination. Samples were then vortexed and all loose debris and liquid were removed from the sample. In order to decalcify the samples, heat-treated 0.5M EDTA was added to each sample and put on a nutator tray for 48–144 hours. Some of the lager samples were gently broken further with sterile pipette tips throughout the decalcification process. Samples were rinsed three times with sterile, heat-treated water. Water traps, as well as spot tests of bodysuits, latex and nitrile gloves, were conducted prior to and during the decalcification of the samples in order to test for potential sources of contamination. Contamination tests are reported in Tromp et al. 2017 (12).

Protein extraction, digestion, and LC-MS/MS. Proteins were extracted from decalcified dental calculus samples in an ancient protein clean lab at the Max Planck Institute for the Science of Human History according to guidelines recommended by Hendy et al. (13). Samples were extracted and digested using a modified version of filter-aided sample preparation (FASP), previously described by Jeong et al. (14). Extracted peptides were analysed by LC-MS/MS using a Q-Exactive HF mass spectrometer (Thermo Scientific, Bremen, Germany) coupled to an ACQUITY UPLC M-Class system (Waters AG, Baden-Dättwil, Switzerland) at the Functional Genomics Center Zurich of the University/ETH Zurich according to previously described protocols (14). In order to monitor any potential contamination, two negative extraction blanks were processed in parallel with experimental samples. No dietary proteins were identified in the blanks, which contained only laboratory reagents (trypsin) and common laboratory contaminants (keratin, serum albumin, and uncharacterized bacterial proteins). Injection blanks were additionally performed between each sample to monitor and reduce any potential carryover among samples.

Protein database searching. Tandem mass spectra were converted to Mascot generic files by MSConvert version 3.0.11781 with the 100 most intense peaks in each spectrum. All MS/MS samples were analysed using Mascot (Matrix Science, London, UK; version 2.6.0). Mascot was set up to search the databases SwissProt_2017_07.fasta (555,100 entries, 198,754,198 residues) and uniprot_trembl_2017_07 database (88,032,926 entries, 29,627,301,199 residues) assuming the digestion enzyme trypsin and selecting the automatic decoy option. Mascot was searched with a fragment ion mass tolerance of 0.01 Da and a parent ion tolerance of 10 ppm. Because of treatment with IAA during protein extraction, carbamidomethyl of C was specified in Mascot as a fixed modification. Deamidation of N and Q and oxidation of M and P were specified in Mascot as variable modifications.

Criteria for protein identification. Scaffold (version Scaffold_4.8.9), Proteome Software Inc., Portland, OR) was used to validate MS/MS based peptide and protein identifications. Peptide identifications at a local FDR of less than 1.0% (Scaffold Local FDR algorithm) and protein identifications at an FDR of less than 5.0% and supported by at least three identified peptides were accepted. Protein probabilities were assigned by the Protein Prophet algorithm (15). The estimated peptide and protein FDR of the final dataset is 0.02% and 0.1%, respectively. Proteins that contained similar peptides and could not be differentiated based on MS/MS analysis alone were grouped to satisfy the principles of parsimony. Protein identifications are provided in Dataset S4, and the data files supporting these identifications are provided in the PRIDE repository under the identifier PXD015002. Putative dietary proteins assigned to Viridiplantae, Metazoa, or Fungi were manually validated by searching all peptides against the NCBI nr database using BLASTp to assess taxonomic specificity. Taxonomic variants defined by amino acids that are susceptible to age- and sample preparation-dependent deamidation (N, Q) were noted as ambiguous. A detailed summary of putative dietary peptides is provided in Dataset S2.

Fig. S1. Map of the Irish and British Isles, showing the location of Kilkenny City.

Fig. S2. In-situ photograph of three adult skeletons analysed in this study during the archaeological excavation at the Kilkenny Union Workhouse: (a) ≥46-year-old male (CCCXVIII); (b) 26–35-year-old female (CCXXXIV); (c) 36–45-year-old male (CXCV). Image courtesy of Margaret Gowen (Margaret Gowen & Co. Ltd).

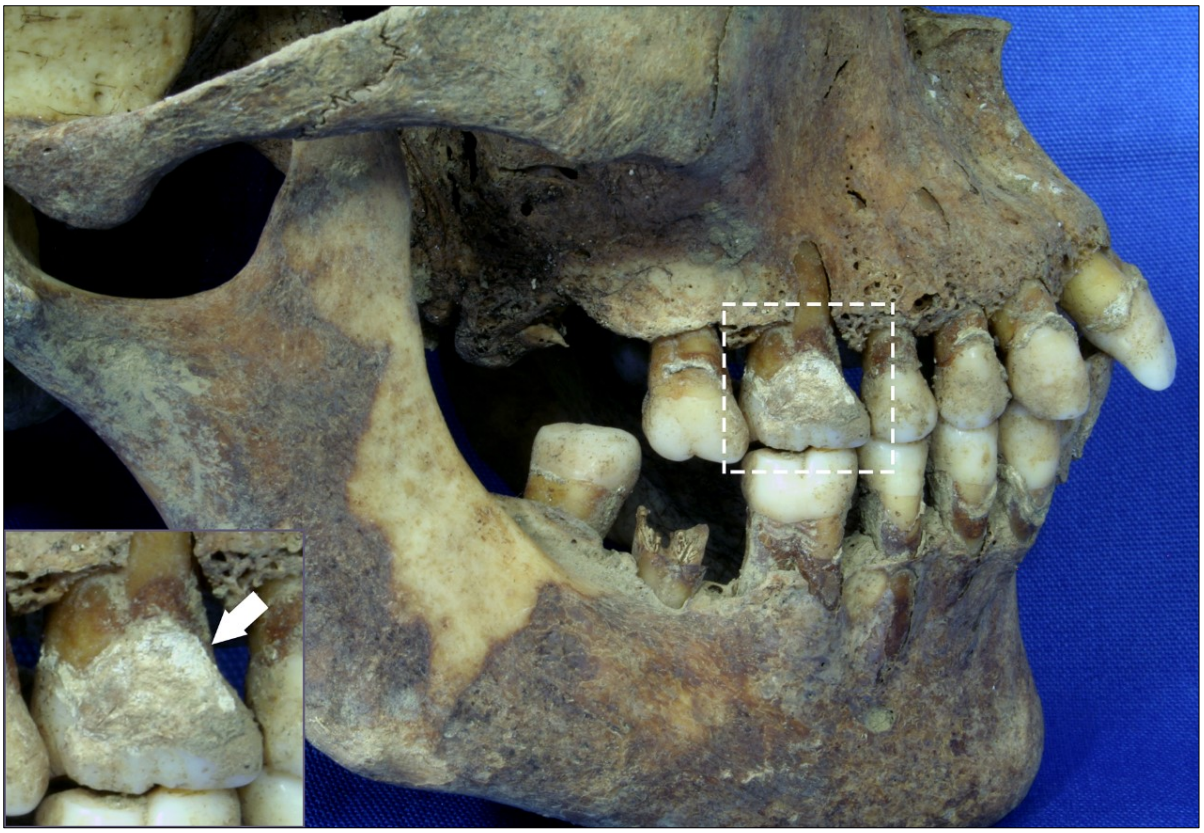

Fig. S3. The right side of the dentition of a 26–35-year-old female (CV), with an insert of the tooth (FDI 16) used for calculus deposit sampling (arrow).

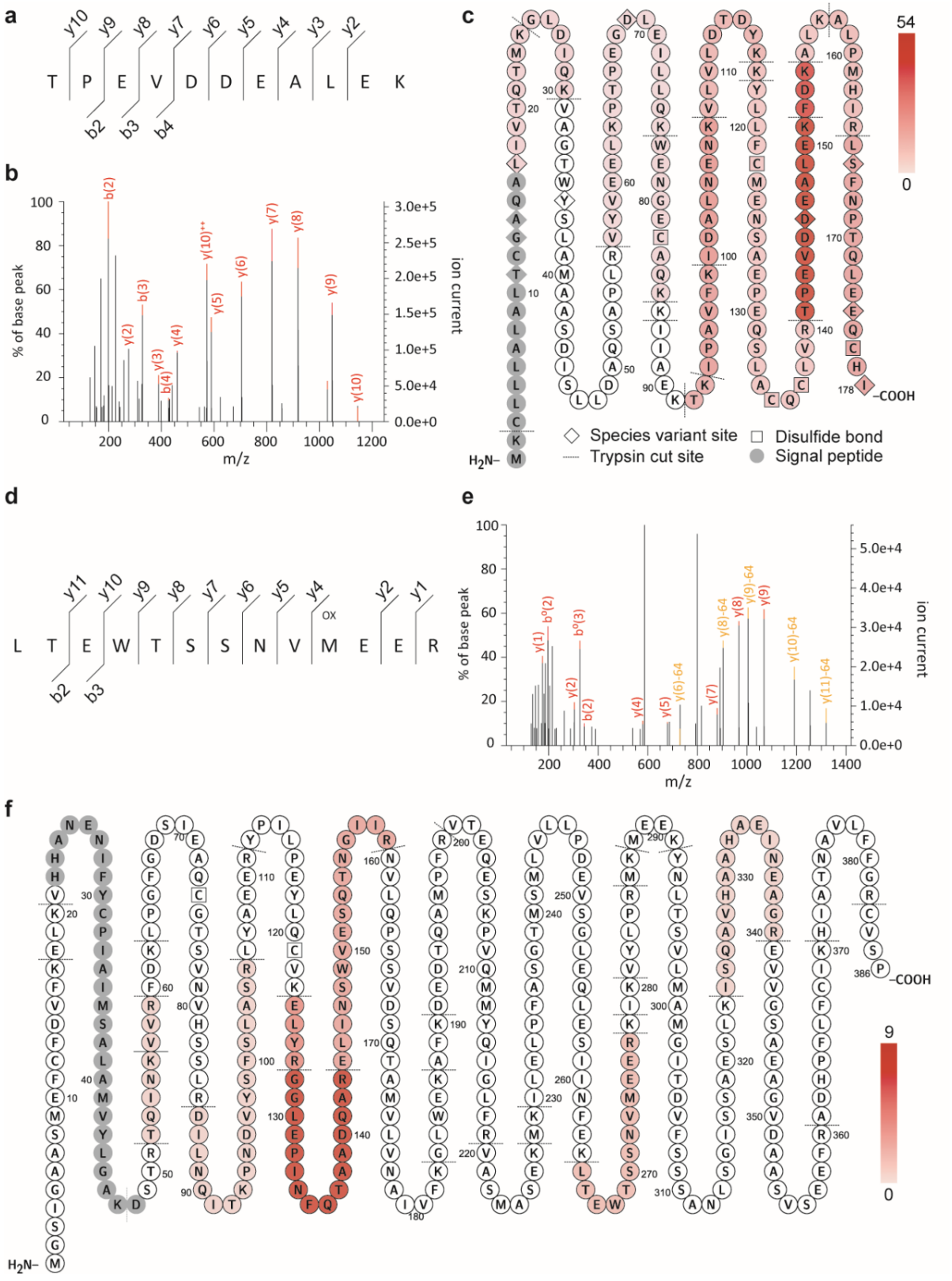

Fig. S4. Milk beta-lactoglobulin (a–c) and egg ovalbumin (d-f) proteins recovered from dental calculus: (a) An example b- and -y ion series and (b) spectrum for TPEVDDEALEK, the most frequently identified beta-lactoglobulin peptide. (c) Protein coverage map of beta-lactoglobulin. (d) An example b- and y- ion series and (e) spectrum for LTEWTSSNVMMEER, an ovalbumin peptide specific to the avain family Phasianidae, which includes chickens. (f) Protein coverage map of ovalbumin. Protein coverage maps are visualized using Protter (16); for beta-lactoglobulin, species variant sites distinguishing cattle, sheep, and goats are indicated by diamonds. The scale of the coverage heatmap ranges from 0 (white) to the maximum (dark red) amino acid coverage observed for each protein (beta-lactoglobulin, 54; ovalbumin 9).

Table S1. The dietary for adult inmates (≥ 15 years of age) in the Kilkenny Union Workhouse, in 1842, 1847 and 1848 (17-21).

Month and year	Breakfast	Dinner
October 1842	8 ounces of [oat]meal in stirabout, ½ pint of new milk	4 lbs of potatoes, 1 pint of skimmed milk
September 1847	8 ounces of oatmeal [in stirabout], ½ pint of new milk	10 ounces of Indian meal [in stirabout], 1 pint of sour milk
October 1847	8 ounces of Indian meal [in stirabout], 1 ounce of rice or 8 ounces of oatmeal [in stirabout], 1 ounce of rice	10 ounces of Indian meal [in stirabout], 1 pint of butter milk
July 1848	8 ounces of Indian meal or oatmeal with 1 ounce of rice [in stirabout], ½ pint of new milk	10 ounces of oatmeal [in stirabout], 1 pint of buttermilk
December 1848	8 ounces of Indian meal with 1 ounce of rice [in stirabout], ½ pint of new milk	¾ lbs brown bread, ½ pint of soup

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Additional data tables (separate files)

Dataset S1 (Dataset S1.xlsx). Microparticle counts for all examined dental calculus samples.

Dataset S2 (Dataset S2.xlsx). Dietary protein summary data.

Dataset S3 (Dataset S3.xlsx). Starch granule measurements and identifications.

Dataset S4 (Dataset S4.xlsx). Complete proteomic search parameters and data.

Dataset S5 (Dataset S5.pdf). Microparticle photographs.