# What Ancient Proteins Says reveals in Study about Real Medieval Meals

Researchers reconstructed historical <u>dietary habits</u> using proteomic and stable isotope analyses.

Paleodietary studies can reconstruct the historical lifeways of humans. Stable isotope analysis is used to study the human environment, diet, and mobility. Stable isotope values of bioelements (carbon, hydrogen, sulfur, nitrogen, and oxygen) in body tissues are linked to the diet composition of individuals. Using stable carbon isotope value ( $\delta$ 13C), the content of C3 vs. C4 plants and indications of terrestrial vs. marine sources in diets could be inferred.

Stable nitrogen isotope values ( $\delta$ 15N) are related to the origin and quantity of dietary animal protein. In addition, stable sulfur isotope values ( $\delta$ 34S) provide evidence of marine influences, such as sediments of marine origin, the sea-spray effect, and sea products. While stable isotope analysis can reconstruct historical and <u>ancient diets</u>, it has limitations when identifying specific food types and cannot reliably detect certain dietary components, such as legumes or moderate fish consumption. However, biomolecular analyses, such as proteomics, can provide a more detailed picture of ancient diets by identifying specific plant and animal species that were consumed.



### Study

In the present study, researchers aimed to reconstruct historical dietary habits using stable isotope analysis and ancient proteomics. They included <u>anthropological</u> collections from the Baar cemetery in the Canton of Zug, Switzerland, and the Dalheim monastic cemetery in North Rhine-Westphalia, Germany, dating to the 7th century CE and the 9th to 12th centuries CE, respectively. Stable isotope analysis was performed on bone samples from 11 individuals and tooth samples from eight individuals from Dalheim.

Dental calculus samples were also available for isotope analysis following sample preparation for proteomics. The resultant calculus and collagen samples were used to measure the stable isotopes of carbon, sulfur, and nitrogen using an isotope ratio <u>mass spectrometer</u>. In total, 52 dental calculus samples from the two sites were subject to ancient proteomic analysis. However, due to poor preservation, only 37 samples passed quality screening, with 15 samples excluded from the main analysis.

Proteins were extracted, and mass spectrometry analysis was performed. Dietary peptides were assessed, and <u>peptide spectrum matches</u> (PSMs) were searched against the translated

nucleotide database using the Basic Local Alignment Search Tool (BLAST). Positive peptide identification was achieved when 100% peptide coverage and 100% homology to the desired dietary protein were observed.

#### **Results**

The collagen yields of the bone and dentin samples from Dalheim ranged from 7.4% to 15.8% and 6.8% to 17.7%, respectively. The high collagen yield and molar C/N ratios indicated good collagen preservation with no evidence of microbiological or collagen degradation of native <u>protein</u>. The average bone collagen  $\delta$ 13C,  $\delta$ 15N, and  $\delta$ 34S values were -20‰, 10‰, and 9.4‰, respectively.

The mean dental collagen  $\delta$ 13C,  $\delta$ 15N, and  $\delta$ 34S values were -20.1‰, 10.1‰, and 8.6‰, respectively. Conversely, <u>dental calculus</u> samples differed from collagenous samples, with mean  $\delta$ 13C,  $\delta$ 15N, and  $\delta$ 34S values of -22.4‰, 10.6‰, and 6.8‰, respectively. The  $\delta$ 13C values of calculus, bone, and dentin were significantly different.

Importantly, the researchers concluded that dental calculus does not yield reliable isotopic signals for dietary reconstruction due to its inherent variability and complex composition. The elevated  $\delta$ 34S values observed likely reflect local geological marine sediments from the Paderborn plateau rather than consumption of marine fish.

In total, 16 dietary proteins (124 PSMs, 34 unique peptides) were identified from 15 individuals; two were of animal origin and 14 were from plant sources. Fabaceae was the most representative plant family, detected only in individuals from Dalheim. Five seed proteins were identified: legumin A, vicilin, convicilin, p54, and legumin J.

Seven unique peptides were specific to the Fabeae tribe, and 15 were specific to green peas (Pisum sativum). The identification of green pea proteins is particularly significant because plant proteins, especially from legumes, are rarely recovered from archaeological contexts. The preservation of these <u>legume proteins</u> may be enhanced by their globular structure, with compact quaternary arrangements and disulfide bonds that provide stability in archaeological environments.

Pseudo-cereals (C4 plants) and <u>cereals</u> (C3 plants) were also identified in samples from both sites based on one plant development protein and three uncharacterized proteins.

In Dalheim individuals, three unique peptides (14 PSMs) were identified as being specific to rice, millet, and common <u>wheat</u>. Evidence of spinach consumption was also detected, which aligns with historical records of its cultivation in medieval Europe.

In Baar individuals, two unique peptides (seven PSMs) were specific to barley and wheat. Five proteins belonged to other plants. Two animal proteins (fish and <u>milk proteins</u>) were detected. The fish protein (one unique peptide, 10 PSMs) was specific to the European perch and detected in one individual from Baar and four from Dalheim.

The identification of freshwater perch helps clarify that the elevated <u>sulfur isotope</u> values were of geological origin rather than dietary. The milk protein (one unique peptide, 2 PSMs) was  $\beta$ -lactoglobulin, found in one individual from Baar.

Notably, despite isotopic evidence suggesting the consumption of terrestrial animal protein, no proteins from terrestrial animal sources (muscle, <u>blood</u>, or milk) were identified in the Dalheim samples, highlighting the complementary nature of these analytical approaches.

## **Conclusion**

In summary, the study performed stable carbon, sulfur, and nitrogen isotope analyses on bone, tooth, and dental calculus samples from two medieval European populations. The combined analysis of ancient proteins and stable isotopes provided a complementary approach to reconstructing historical human diets. Each method addressed limitations of the other: stable isotope analysis provided broad <u>dietary patterns</u>, while proteomics enabled species-level identification.

The findings indicate that the Dalheim population primarily relied on plant intake and terrestrial animal proteins.

While stable isotope data could not provide clear evidence of legume or fish intake, proteomic data confirmed the presence of both in the diet. Identified taxa included European perch, common wheat, green peas, millet, and barley.

These findings align with historical knowledge of medieval diets, where legumes served as crucial protein sources for lower socioeconomic groups, and <u>freshwater fish</u>, such as perch, were commonly consumed, particularly during religious fasting periods when terrestrial meat was prohibited.

The study also revealed critical methodological considerations, including the unreliability of dental calculus for isotopic dietary reconstruction and the challenges of differential protein preservation in <u>archaeological samples</u>. The researchers noted that the absence of dietary proteins doesn't necessarily mean those foods weren't consumed, as protein recovery depends on preservation conditions and database limitations.

The study recommends that future research should implement stricter decontamination protocols, standardize preservation assessment procedures, and consider the role of protein secondary and tertiary structures in preservation to enhance the reliability and interpretability of ancient <u>proteomic data</u>.

Overall, the study lays the groundwork for further interdisciplinary analyses to construct a more accurate and detailed picture of the lifeways of ancient and <u>historical populations</u>.

#### Source:

https://www.news-medical.net/news/20250724/Digging-into-the-past-What-ancient-proteins-say-about-real-medieval-meals.aspx