The Iceman’s Last Meal Consisted of Fat, Wild Meat, and Cereals

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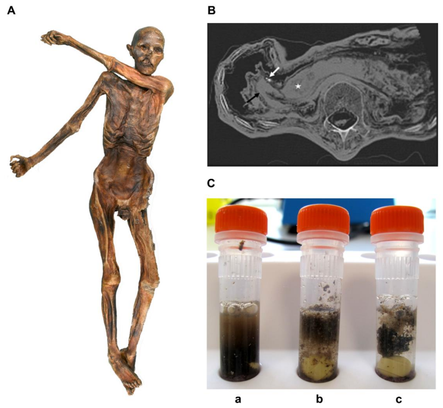
Abstract

Human evolution and subsistence strategies are closely linked to dietary changes and food processing. This is clearly observed with the transition from hunter-gatherer lifestyle to agriculture, which gave rise to cultivation of crops, animal husbandry and permanent settlements. The more stable availability of food boosted ancient population growth. However, changes in diet had drawbacks for health such as increased rates of caries. Added to this, permanent large settlements with adoption of agriculture promoted the spread of density-dependent infectious diseases. This study aims to reconstruct the dietary choices of ancient populations and the consequences these may have had for their health.

The detection of the Iceman´s stomach content provided the unique opportunity to fully reconstruct the main components of an Alpine Copper Age meal. Initial macro- and microscopic analysis revealed that the material is extraordinarily well preserved and contains large amounts of fat residues. By using a combined multi-omics approach targeting biomolecules (ancient DNA, proteins, metabolites, and lipids), we obtained a molecular “fingerprint” of the Iceman’s diet preceding his death.

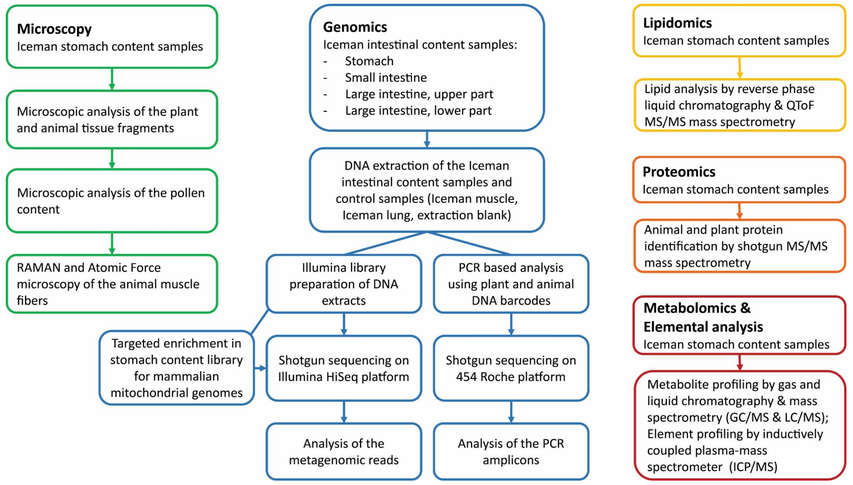
The molecular data we present shows the presence of four components in the Iceman´s last meal: ibex and red deer meat supplemented with cereals and traces of bracken. In addition, the distribution of triglycerides and their constituting fatty acids is consistent with the consumption of animal muscle and adipose tissue. Our multi-omics study provides important insights into the general life and nutritional habits of a Copper Age individual in the Alpine area.

Iceman samples



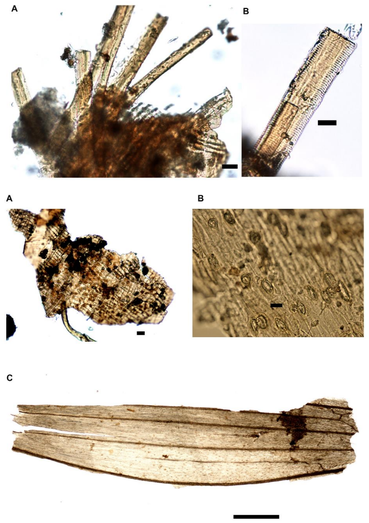
(A) The Tyrolean Iceman, Ötzi, is one of the oldest human mummies discovered. His body was preserved for more than 5,300 years in an Italian Alpine glacier before he was discovered by two mountaineers at an altitude of 3,210 m above sea level in September 1991. (B) The Iceman’s stomach was discovered in a reappraisal of radiological data and contains the food he ingested shortly before his death (asterisk, C).The study material included the hydrophobic stomach content (c), and content of the small (b) and large intestines (a).

Workflow



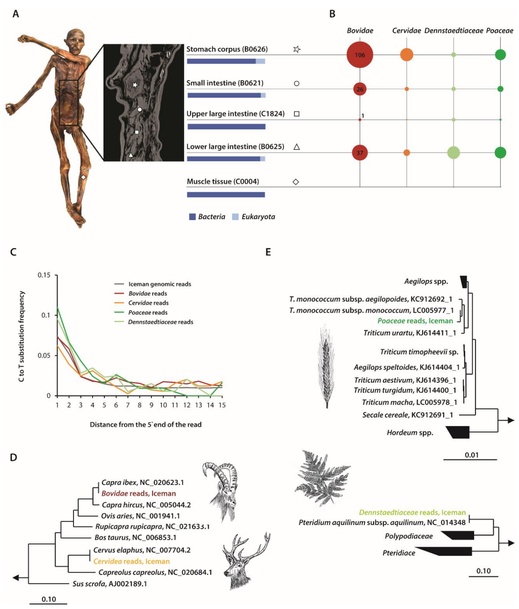
To analyze the exact composition of the stomach/intestine contents we combined classical microscopy with modern molecular analysis using the whole spectrum of available biomolecules (ancient DNA, proteins, metabolites, lipids). The combined multi-omics approach using cutting-edge metagenomics, proteomics, lipidomics and metabolomics analyses should compensate for the varying degree and states of preservation of the different biomolecules to finally obtain a molecular “fingerprint” of the Iceman’s diet preceding his death.

Light microscopy



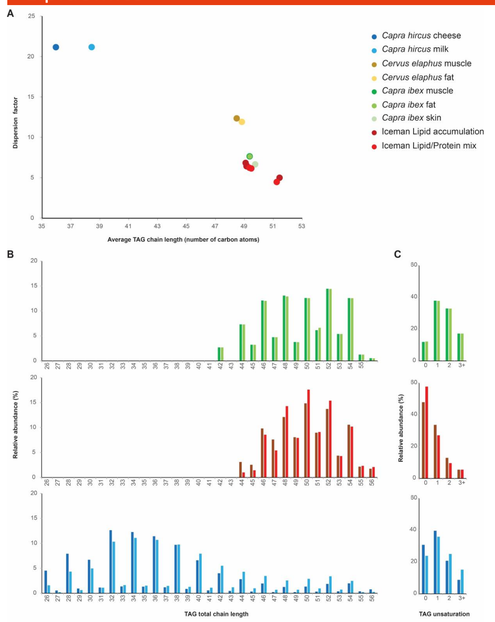
Microscopy revealed both animal (A, B) and plant tissue remains (C, D, E). Most prevalent tissue types were, animal muscle fibres with still visible striations, and plant fragments identified as bran and glumes belonging to the Triticum/Secale type.

Genomics



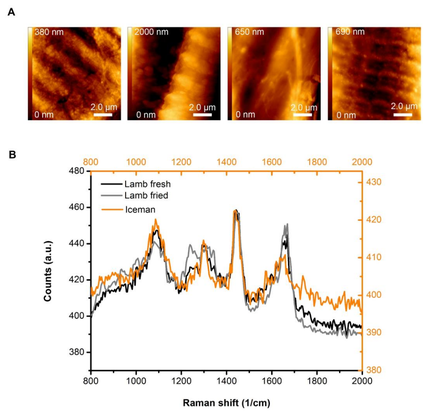
Metagenomic analysis of the Iceman´s biopsy samples showed a high background of bacterial reads in all gastrointestinal tract contents (A). Alignment against animal mitochondrial and plant chloroplast genomes assigned the majority of the non-human *Metazoa* and *Viridiplantae* reads to the families Bovidae, Cervidae, *Dennstaedtiaceae* and *Pocaeae* (B). Reads display aDNA damage pattern (C) and can be phylogenetically assigned to the ibex, red deer (D), Einkorn and bracken (E).

Lipidomics



Liquid chromatography MS/MS analysis revealed that the best preserved lipid species are triglycerides, mostly un- or mono-unsaturated (B, C). Distribution of triglycerides and their constituting fatty acids in comparison with contemporary samples (A, B, C) indicates the consumption of animal muscle and adipose tissue of ruminants.

AFM and RAMAN microscopy



When meat is heated i.e. cooked or fried the regular striations disappear (A). Raman spectroscopy reveals that the heat induced denaturation of the meat results in an increase in non-helical structures (B). The data indicates that the analysed animal fibres from the stomach content did not undergo a thermal treatment before they were eaten.

Conclusions

The molecular data we present shows the presence of four components in the Iceman´s last meal: ibex and red deer meat supplemented with cereals and traces of bracken. In addition, the distribution of triglycerides and their constituting fatty acids is consistent with the consumption of animal muscle and adipose tissue. He ate the meat either fresh or air dried. Our multi-omics study provides important insights into the general life and nutritional habits of a Copper Age individual in the Alpine area