



## Objectives

Dairying practices in prehistoric Italy may have differed between time periods and regions. Here we aim at:

- **Tracing milk consumption** in two Middle Bronze Age Italian populations
- **Detecting** any difference in the **animal species** raised

## Introduction

It has always been hard to define a relative chronology during the Early History in Italy. The reason might lie on its extremely complex and irregular territory, which implies lack of contacts and cultural exchange among populations established in the Peninsula [1]. Proxies on food practices, such as stable carbon and nitrogen isotopes, suggest an interesting complexity in Italy, with coeval sites often showing significant differences [2][3]. With this perspective, it is not unlikely that dairy might have been practised or not in the same period in different regions.

In order to identify milk proteins and trace dairy production and consumption, here we present the results obtained from the analysis of human dental calculus from two Middle Bronze Age populations which have nothing in common but the time frame.



## Materials and Methods

Dental calculus is used as a powerful tool in bioarchaeology to detect direct evidence of the health status, the diet and possibly the occupation of an individual and/or of a population[4][5][6]. We performed a shotgun metaproteomic analysis of 9 samples of dental calculus; 6 samples from Bovolone, and 3 from Sant'Abbondio. Only supragingival calculus has been sampled, predominately from premolars and molars, and collected in 2.0 mL tubes. We used between 1.9 and 26.7 mg of dental calculus for protein extraction.

Shotgun metaproteomics consists of the proteolytic digestion of proteins belonging to a mixture extremely increased in complexity. We applied a GASP (Gel-Aided Sample Preparation) protocol, modified for ancient mineralized samples [7]. Extractions were performed at BioArCh, Department of Archaeology at the University of York. Extracted peptides were analysed using liquid chromatography tandem mass spectrometry (Q-Exactive) at the Mass Spectrometry laboratory of the Target Discovery Institute at the University of Oxford. Spectra were searched using Mascot (Matrix Science™) against Uniprot database.



Picture from C. Warinner's personal protocol.



## Conclusions

- Caseins peptides were detected suggesting the use and consumption of **milk** by both populations examined. Since their detection was possible only through one peptide per sample, milk consumption is advanced with caution.
- This is the **first time wheat peptides have been detected in ancient dental calculus**, besides matching with protein uniquely found in the fruit of the plant, which is of great methodological importance as well as in relation with the present study itself.
- We also found abundance of **human** and **bacterial peptides**, opening a window on the health status of these two populations. In the near future, we all the sample will be run against a more specific database looking for the complete oral microbiome scenery.

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## Important results

Looking at the number of spectral queries produced for each sample, is evident that using more sample weight for protein extraction does not necessarily yield more peptide spectra. This variation in protein preservation means that making inter-population comparisons is challenging. However, although these sites may not be able to be suitable compared, several results are worth to be presented.

- **Milk peptides:** several casein proteins were identified in both the necropolis although with only one peptide each. However, their presence might suggest milk consumption. One of the peptides is unique for *Bos* genus (bovine) and it belongs to the Sant'Abbondio assemblage.
- **Other dietary peptides:** In one Sant'Abbondio sample wheat consumption might have been detected through the presence of seven peptides, belonging to three proteins existing only in the edible part of the plant, the caryopsis. All the peptides are unique for the *Triticaceae* tribe and one of them only for *Triticum aestivum*. In one Bovolone sample has been detected the presence of peptides related to some *Brassicaceae* species, suggesting the consumption of these plants.
- **Human proteins:** Human proteins make a large percentage of the total number of identified proteins in Bovolone. Many are involved in antimicrobial, responses, some in anti-inflammatory activities and other have structural roles.
- **Bacterial and fungi peptides:** In Bovolone it has been detected the presence of *Porphyromonas gingivalis*, *Porphyromonas gulae* and *Bilophila wadsworthia*, bacteria associated with oral diseases, and some commensal bacteria and *fungi* typical of the human oral cavity as *Candida albicans*, *Propionibacterium acnes* and other species.



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