Supplementary Information

Isotopic insights into the Early Acheulean (1.95 Ma - 1.66 Ma) high-elevation paleoenvironments at Melka Kunture (Upper Awash Valley, Ethiopia)

Authors: Giuseppe Briatico*, Hervé Bocherens, Raymonde Bonnefille, Denis Geraads, Margherita Mussi

*Corresponding author. E-mail: giuseppe.bria@gmail.com (G. Briatico)

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Supplementary Text

Supplementary A. Laboratory procedures

All laboratory activities were carried out following the internal protocol for the preparation and isotopic analysis of archaeological teeth provided by the Biogeology Research Group (Department of Geosciences - University of Tübingen, Germany).

Sampling. The enamel samples were collected in November 2019 at the National Museum of Ethiopia (Addis Ababa) in agreement with the Authority for Research and Conservation of Cultural Heritage (ARCCH). The uppermost surface of the tooth was first cleaned with a diamond drill bit (>2.0 mm) to remove exterior and potential contaminants. Enamel samples were obtained using a drilling device equipped with a diamond-tipped bit (<2.0 mm) to obtain 12-15 mg powder. After each sample, the tools were cleaned with an ethanol solution to avoid contamination. When possible, enamel samples were collected along the length of the tooth (bulk sampling) to obtain an average diet and ecological setting during the period of tooth formation (Fricke and O'Neil, 1996). However, due to the fragmented status of the tooth remains, the internal fractures of enamel, and the concretions on the tooth surface, it was not always possible to encompass the full crown height of the archaeological teeth. As a result, the samples do not represent the full mineralization time of the tooth but rather a short time.

Pretreatment. This step was conducted at the Institut für Geowissenschaften (Hölderlinstrasse 12 - Biogeology Research Group, University of Tübingen). To remove the organic residues, the powdered tooth enamel was soaked in 1.35 ml sodium hypochlorite (NaOCl) at 2–3% concentration. The NaOCl was replaced twice, and the samples were rinsed three times with Millipore water (Milli-Q H_2O) to remove all the NaOCl. The remaining samples were treated with 1.35 ml of 0.1 M buffer acetic acid-calcium acetate (CH_3COOH) (pH = 4.66) for 24 hours at 20°C to remove exogenous carbonate. Subsequently, the samples were rinsed three times with Milli-Q H_2O and placed in an oven to dry at 40°C for 72 hours (Bocherens et al., 1996). This pretreatment method is the most used for cleaning bioapatite samples and effectively removes organic matter and exogenous carbonate (Snoeck and Pellegrini, 2015; Pellegrini and Snoeck, 2016). Approximately 2.5-3 mg of the structural carbonate proceeded into the IRMS analysis (Koch et al., 1997).

Isotopic analyses. The pretreated samples were reacted with 99% H_3PO_4 for 4 hours at 70°C, using continuous-flow isotope ratio mass spectrometry (IRMS) at the Biogeology Research Group (Department of Geosciences - University of Tübingen, Germany). IonOS software (Version 4.3) by Elementar was used to carry out multi-point standard isotope calibration by generating a trend line ($y = mx + c$) that maps measured versus expected isotopic results of standards, which was then used to calibrate sample results. Two internal enamel samples used as secondary reference material (SRM) (Elephant and Hippo) were processed with every set of samples following the same protocol. Two international standards (IAEA-603, NBS-18) and one internal standard (LM = Laaser Marmor) were used. The international reference standards are Vienna PeeDee Belemnite (VPDB) for carbon and oxygen and Vienna Standard Mean Ocean Water (VSMOW) for oxygen (Wright and Schwarcz, 1999).

Supplementary B. Statistical analysis

Statistical analyses were performed using JMP 16, with the significance level set at $p = 0.05$. The Shapiro-Wilk test was used to verify whether the data followed a normal distribution. When the hypothesis of the normal distribution is accepted, parametric tests such as the t-test and the analysis of variance (ANOVA) test are used; when the hypothesis of the normal distribution is rejected, non-parametric tests such as the Mann-Whitney U-test (also known as Wilcoxon Rank Sum Test) or the Kruskal-Wallis test are used. The t-test and Mann-Whitney U-test were used with two groups or levels, whereas ANOVA and Kruskal-Wallis tests were used with three or more groups (see Table S2). We noted that some sample sizes (Bovidae, Equidae, Suidae) were very small, limiting the power of the statistical analysis.

References

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