

Supplementary Materials 4: The methods used for stable isotope analysis of specimens presented in this paper for the first time

From “New perspectives on human subsistence during the Magdalenian in the Swabian Jura, Germany” by G. L. Wong, B. M. Starkovich, D. G. Drucker, and N. J. Conard

For this paper, we have compiled isotopic data from published sources (Drucker et al., 2011; Wong et al., 2020; Bocherens et al., 2011; Immel et al., 2015; Stevens and Hedges, 2004; Drucker et al., 2015; Münzel et al., 2016; Fellows et al., 2017), as well as the results from eight bone samples from reindeer (n=4) and horses (n=4) from the sites of Geißenklösterle and Schussenquelle (Supplementary Table 4). Except for one horse sample from Geißenklösterle (GK20/OxA-21722), the extraction of collagen was conducted at the Department of Geosciences at the University of Tübingen (Germany) following a protocol based on Longin (1971) and modified by Bocherens et al. (1997). The extraction process includes a step of soaking in 0.125 M NaOH between the demineralization and solubilization steps to achieve the elimination of lipids and humic substances. The sample GK20/OxA-21722 was treated in Oxford following a protocol described in Bronk Ramsey et al. (2004) that includes a step of separation of lower molecular weight components from the gelatin extracted from bones using Longin’s (1971) method.

The elemental analysis (C_{coll} and N_{coll}) and isotopic analysis ($\delta^{13}C_{\text{coll}}$, $\delta^{15}N_{\text{coll}}$) were performed at the Geochemical unit of the Department of Geosciences at the University of Tübingen using an elemental analyzer NC 2500 connected to a Thermo Quest Delta+XL mass spectrometer for all collagen. The only exception is a horse tibia from Geißenklösterle (GK21) that was submitted to elemental analyses and isotopic measurements in duplicate at the Institute of Environmental Science and Technology of the Universitat Autònoma de Barcelona (ICTA-UAB) using a Thermo Flash 1112 (Thermo ScientificVC) elemental analyzer coupled to a Thermo Delta V Advantage mass spectrometer with a Conflo III interface. The isotopic ratios are expressed using the “ δ ” (delta) value as follows: ($\delta^{13}C = [(^{13}C/^{12}C)_{\text{sample}} / (^{13}C/^{12}C)_{\text{standard}} - 1] \times 1000\text{‰}$ and ($\delta^{15}N = [(^{15}N/^{14}N)_{\text{sample}} / (^{15}N/^{14}N)_{\text{standard}} - 1] \times 1000\text{‰}$). The measurements were calibrated in reference to the international standards (V-PDB for carbon and AIR for nitrogen) using USGS-24 ($\delta^{13}C = -16.0\text{‰}$), IAEA 305A ($\delta^{15}N = +39.8\text{‰}$) at Tübingen, IAEA 600 ($\delta^{13}C = -27.8\text{‰}$), ($\delta^{15}N = +1.0\text{‰}$) at Barcelona and in-house reference materials (modern collagen of camel and elk) at both places. Analytical precision, based on within-run replicate measurement of laboratory standards (egg albumin, keratin, alanine amino acid, modern collagen), was $\pm 0.1\text{‰}$ for $\delta^{13}C$, and $\pm 0.2\text{‰}$ for $\delta^{15}N$ at Tübingen and below $\pm 0.2\text{‰}$ at Barcelona. The reproducibility error for the amounts of C and N was lower than 5% at Tübingen and lower than 2% at Barcelona. The biochemical reliability of the

collagen was estimated by considering the chemical composition of collagen, with C/N atomic ratio ($C:N_{coll}$) ranging from 2.9 to 3.6 (DeNiro 1985) and contents of C_{coll} and N_{coll} above 8% and 3% respectively (Ambrose 1990).

See Supplementary Materials 6 for a summary of the locations where collagen extraction and stable isotope analyses of the previously unpublished data were performed.

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