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Isotope Specialist Report: Multi isotope analysis of three humans and four animals from Großmugl, Austria.

Summary

This report presents the results of an isotopic study into the diet and mobility of three individuals from the Hallstatt period site of Großmugl, Austria. Carbon isotope results indicate that these three individuals ate a predominantly terrestrial C₃ diet consistent with Austria and other regions of Western and Central Europe during the Hallstatt period. The nitrogen isotope ratios observed in the individuals are unusually high in comparison to contemporaneous populations, and may suggest the use of crop manuring, consumption of omnivorous animals (e.g., suckling pigs), and/or the inclusion of freshwater fish in their diet. Periods of nutritional or physiological stress have also been identified in each individuals' incremental dentine profile. The strontium and oxygen isotope data from the Großmugl individuals and the associated faunal remains are consistent with the site and the surrounding area, although these values are not unique and can be found across many other areas of Europe such as Germany.

Contents

1 Introduction	p. 3
2 Dietary Reconstruction	p. 3
2.1 Carbon and nitrogen isotope analysis	p. 3
2.2 Materials and methods	p. 4
2.3 Results and interpretation	p. 5
3 Assessing Geographical Origins	p. 13
3.1 Oxygen isotope analysis	p. 13
3.1.1 Materials and methods	p. 14
3.1.2 Results and interpretation	p. 15
3.2. Strontium isotope analysis	p. 18
3.2.1 Materials and methods	p. 18
3.2.2 Results and interpretation	p. 19
4 Discussions	p. 25
5 Conclusions	p. 26
 References	 p. 28
Appendix	p. 36

Note: The isotope background sections of this report are based on a template adjusted to suit the specifics of this particular project. Much of the wording is therefore reproduced for other projects. These sections are therefore suitable for unpublished and general reporting but may require omission or adjustment for full publication

1. Introduction

This report presents the results of the multi-isotope analysis of three individuals recovered from the Hallstatt period site of Großmugl, Austria. Teeth and rib fragments were analysed for carbon, nitrogen, strontium, and oxygen isotopes to reconstruct diet and childhood mobility. Complete permanent second molars (M2) representing life between 2.5 years and 15.5 years were analysed for SK1 and SK3 and a permanent lower incisor (I) with incomplete root apex formation was analysed from SK2 representing life between 7.5 months and approximately 8.5 years. Dentine from permanent second molars of three sheep (*Ovis aries*) and a permanent third molar of a pig (*Sus scrofa*) found at Großmugl were analysed for strontium isotopes. The results have been interpreted and discussed in context with previously published data from contemporaneous sites.

2. Dietary Reconstruction

2.1. Carbon and nitrogen isotope analysis

Isotopic analysis of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) is a well-established technique for the reconstruction of diets in past populations using archaeological teeth and bones. Within these two tissue types, collagen is the predominant protein present and provides a rich source of averaged $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. The composition of these $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values reflect the isotopic composition of the foods consumed during the tissue's formation. This is due to the metabolism and reuse of dietary proteins acquired from the plant and animal products consumed during collagen synthesis. Therefore, once metabolic fractionation has been accounted for, these $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values can be used to garner insights into not only the types and quantities of food resources utilised by a population, but also the socioeconomic and cultural influences surrounding the dietary practices of groups as well as individuals.

The variations that arise in $\delta^{13}\text{C}$ values result from differences in ecosystems (marine vs. terrestrial) and the photosynthetic pathways (C_3 and C_4) used by plants in their

manufacture of carbohydrates. As such, variations in $\delta^{13}\text{C}$ values allows differentiation between the relative contribution of C_3 or C_4 plants and the animal products based on these plants, to diet. C_4 plants tend to grow in warmer or more arid climates, where their photosynthetic pathway allows them to cope with less water availability.

Variability in $\delta^{15}\text{N}$ isotope values reflects the balance between biological nitrogen fixation, biosphere recycling and nitrogen release. This variability facilitates the visualisation of terrestrial and marine food source input into diet as marine based food sources tend to be more enriched in ^{15}N than land-based food sources. In addition to this, $\delta^{15}\text{N}$ values also vary with trophic level due to metabolic fractionation, creating a 2–6 ‰ enrichment with every trophic level shift. This shift is most noticeable in marine food consumers as aquatic food sources have high $\delta^{15}\text{N}$ values owing to the relatively long food chains compared to those observed in terrestrial food sources. Combining the analyses of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values can facilitate the interpretation of plant, animal and marine protein contributions to past diets. This allows distinctions to be made between broad categories of food groups, such as herbivore vs. carnivore or marine vs. terrestrial input and any potentially non-local influence on diet.

2.2. Materials and methods

Bone and dentine samples were collected from the three individuals and cleaned and abraded to a depth of $>100\text{ }\mu\text{m}$ using a tungsten carbide dental drill. Dentine increments were prepared and sampled following method 2 in Beaumont *et al.* (2013). Collagen extraction was carried out using O'Connell and Hedges (1999) method, briefly summarised here: bone and dentine samples (c. 200 mg) were demineralised in refrigerated 0.5M HCl for several weeks and then thoroughly washed in Millipore Alpha Q (MQ) water before gelatinisation in a pH 3 solution of HCl at 70°C for 48 hours. The samples were then filtered, frozen and freeze-dried before being weighed into tin capsules and measured in duplicate using a Thermo Scientific Delta V Advantage isotope ratio mass spectrometer in the Stable Isotope Biogeochemistry Laboratory (SIBL), Durham University. Calibration using internal reference samples (e.g., Glutamic Acid, Glycine, SPAR and Urea) and international reference standards (e.g., USGS 24, USGS 40, IAEA 600, IAEA N1, IAEA N2) determined a standard

deviation of $\pm 0.1\text{‰}$ (1σ) for collagen carbon and nitrogen isotopes. Replicate analysis of collagen samples averaged a standard deviation of $\pm 0.2\text{‰}$ (1 sd).

2.3. Results and interpretation

The mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ data from the rib and dentine samples are presented in Table 1 and Figure 1. Due to the fast rate of bone turnover in the ribs, the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values measured in these tissues reflect an average for the dietary protein consumed during approximately the last 5 years prior to death (Cox and Sealy 1997; Fahy *et al.* 2017). The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ data from the permanent second molars of SK1 and SK3 represent the dietary protein consumed between the ages of 2.5 years (± 0.5 years) to 15.5 years (± 0.5 years), while the data from the permanent incisor of SK2 represents dietary protein consumption between the ages of 7.5 months (± 1.5 months) to 8.5 years (± 0.5 years). For SK1 and SK3, the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ data from the incremental dentine samples represent childhood diet and can be compared to the rib samples, which reflect diet later in life during adolescence/adulthood, to identify any potential dietary changes. As SK2 likely died in later childhood, the development of the rib and the incisor would have overlapped, meaning that these two tissues can be used together to infer dietary behaviour across this individual's short lifetime.

The mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values from the Großmugl individuals are presented in Figure 1 along with the same data from contemporaneous Hallstatt burials found at nine different sites in Northern Austria (Le Huray and Schutowski 2005). The $\delta^{13}\text{C}$ values from the rib samples of SK1 and SK3 align with those expected for individuals consuming a diet consisting wholly or predominantly of terrestrial C_3 cereals (e.g., wheat, rye, and barley), fruit and vegetables and animals fed on these plants. These $\delta^{13}\text{C}$ values do not suggest the inclusion of C_4 plants or marine resources within the diet (Kellner and Schoeninger 2007). SK2 has a slightly higher $\delta^{13}\text{C}$ value, which is greater than the upper boundary expected for a purely C_3 terrestrial diet in studies of northwest European populations (Bonsall *et al.* 2009) but this boundary may be slightly higher in lower latitudes of mainland Europe due to climatic differences producing higher $\delta^{13}\text{C}$ values in plants (van Klinken *et al.* 1994), which may suggest a 100% terrestrial diet for SK2 is a strong possibility.

The $\delta^{15}\text{N}$ values observed in the rib samples from SK1 and SK3 are consistent with consumption of a predominantly mixed plant and animal protein diet, including such herbivorous mammals as sheep (*Ovis aries*) or cattle (*Bos taurus*). Both individuals' $\delta^{15}\text{N}$ values are slightly higher than the ~3-5‰ increase expected for a single trophic level shift (Bocherens and Drucker 2003) when compared to published mean $\delta^{15}\text{N}$ values for terrestrial herbivorous faunal data from the contemporaneous Hallstatt settlement of Altdorf, Bavaria (5.4‰ in Murray and Schoeninger 1988; Wells 1986). This may imply that SK1 and SK3 sourced some of their dietary protein from terrestrial omnivores, such as pigs (*Sus scrofa*), as well as herbivores during later adolescence/adulthood. As both sheep and pig remains have been found at Großmugl, it is possible that throughout their lives, SK1 and SK3 consumed a diet with protein derived from both herbivorous and omnivorous mammals and their products, as well as from plants.

The $\delta^{15}\text{N}$ value measured in the rib collagen of SK2 is much higher than the other two individuals and is unusually high for prehistoric farming populations in Europe. It is not typical for an individual exclusively sourcing their protein from terrestrial herbivores (Schoeninger and DeNiro 1984). The consumption of a significant amount of freshwater fish could explain this high $\delta^{15}\text{N}$ value, with aquatic food webs being typically more complex than terrestrial ones and having more trophic levels which each have a ^{15}N -enrichment between them. Freshwater fish, therefore, often have higher $\delta^{15}\text{N}$ values than terrestrial mammals, which are in turn incorporated into and reflected in human tissue upon consumption (Minigawa and Wada 1984; Schoeninger and DeNiro 1984; Dufour *et al.* 1999). Consumption of omnivorous and/or young suckling pigs is another potential explanation, as these animals would have higher $\delta^{15}\text{N}$ values than expected due to them either being fed animal protein such as dairy or meat as a form of animal husbandry, or still consuming their mothers' milk at the time they are culled (Jay and Richards 2006). Remains of piglets found within Iron Age sites and burials in Britain have previously been used to suggest that these young animals were an important component within the diet (Jay and Richards 2006). The corresponding $\delta^{13}\text{C}$ value would normally be considered too low for these elevated $\delta^{15}\text{N}$ values to be caused by marine resource contributions in the diet.

Alternatively, considering the estimated young age at death for SK2, this significantly higher $\delta^{15}\text{N}$ value observed in the rib collagen may be due to nutritional or

physiological stress experienced in the few years prior to death, rather than dietary behaviour. High $\delta^{15}\text{N}$ values have previously been linked to periods of stress caused by numerous diverse factors including illness, malnutrition, starvation, puberty, fasting, and metabolic disorders; whereby the body's tissues become enriched in ^{15}N due to the breakdown of stored protein to provide enough energy to supplement dietary protein intake (Beaumont and Montgomery 2016; Hatch *et al.* 2016; Mekota *et al.* 2006; Fuller *et al.* 2005). This stress may also manifest as skeletal abnormalities, although no remains were available to assess this. SK2's mean dentine $\delta^{15}\text{N}$ value (13.7‰) is also unusually high and the incremental profile (discussed below) supports the data from the rib in suggesting that this individual likely experienced significant stress throughout their life. The $\delta^{15}\text{N}$ data for SK2 ultimately suggests that although this individual's protein intake may have been supplemented by additional resources such as freshwater fish or omnivorous/suckling pigs alongside the expected terrestrial herbivores and plants, the noticeably high $\delta^{15}\text{N}$ values are more likely linked to periods of stress occurring before and leading up to this individual's death in childhood.

The $\delta^{15}\text{N}$ values from the Großmugl individuals are significantly higher than the contemporaneous Hallstatt burials from across Northern Austria (Figure 1), which range from 7.1‰ to 10.1‰ (LeHuray and Schutkowski 2005). This may result from the use of farming practices at Großmugl such as crop manuring, which can elevate $\delta^{15}\text{N}$ values by several per mille (Bogaard *et al.* 2007; Fraser *et al.* 2011). The data in Figure 1 also implies that the Hallstatt period in Austria, and possibly more broadly across Central Europe, was characterised by a largely terrestrial but still varied diet. Whilst the Großmugl individuals and the animals they ate were likely C_3 plant consumers, the individuals in Figure 1, and Stadlau in particular, appear to have been consuming a mixed C_3/C_4 diet, either from ingestion of C_4 plants (e.g., millet or sorghum) or the consumption of C_4 -plant-fed animals (LeHuray and Schutkowski 2005). A mixed C_3/C_4 also appears to be the norm for Austria in the later early medieval period (Figure A1).

[REDACTED] Table 1: Mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ data from incremental dentine and bulk rib collagen of the Großmugl individuals

Skeleton	Dentine* (mean)		Rib	
	$\delta^{13}\text{C}_{\text{VPDB}} \text{‰}$	$\delta^{15}\text{N}_{\text{AIR}} \text{‰}$	$\delta^{13}\text{C}_{\text{VPDB}} \text{‰}$	$\delta^{15}\text{N}_{\text{AIR}} \text{‰}$
SK1				
SK2				
SK3				

* The full dataset for the incremental analysis is included in the Appendix (Table A1-A3)

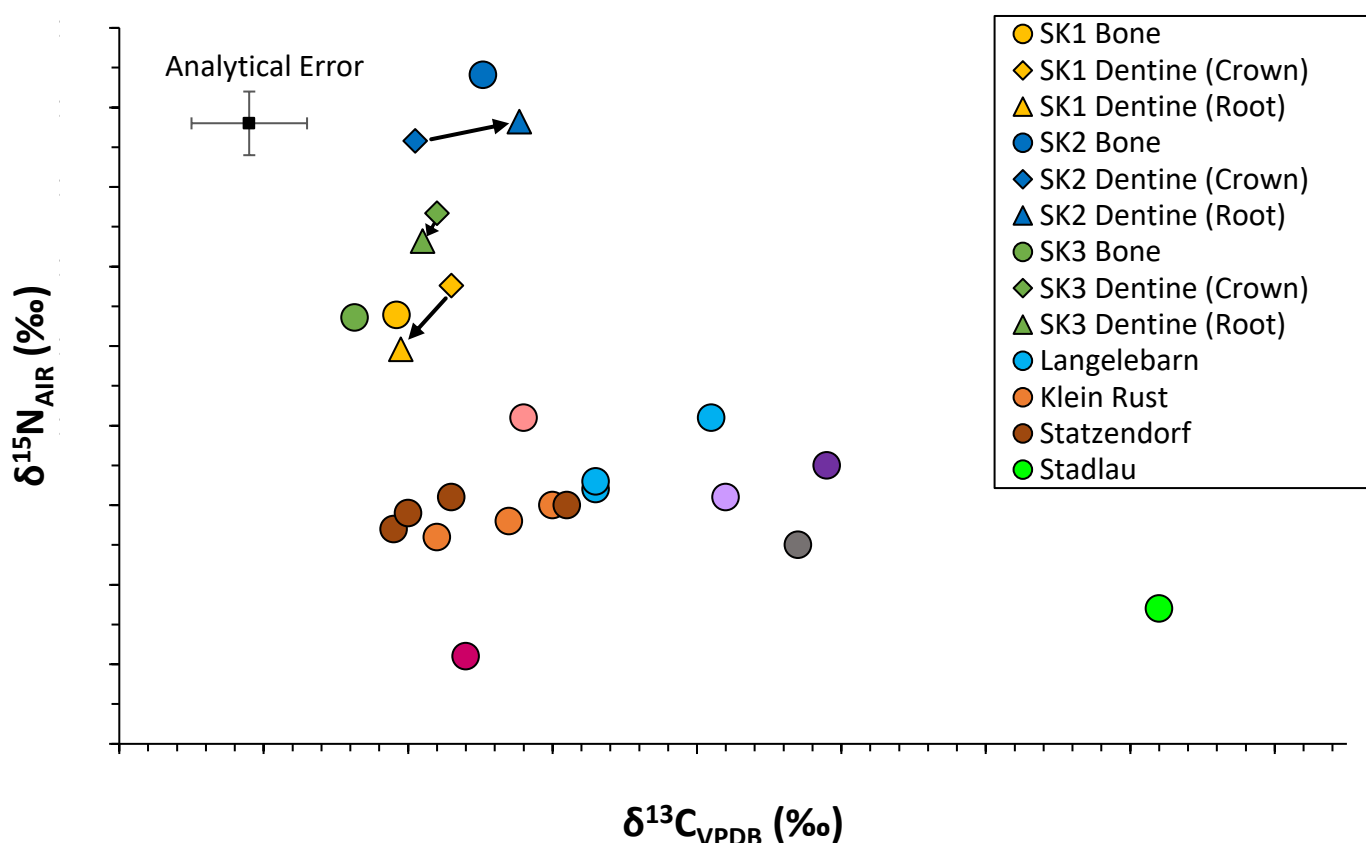


Figure 1: Großmugl $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ data shown alongside published comparative data from contemporaneous Hallstatt Culture humans from Northern Austria (Le Huray and Schutkowski 2005). The Großmugl dentine values are separated into crown and root and are paired by an arrow from the crown to the later-forming root. Analytical error is shown at 2σ .

Whilst the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values from bone and dentine collagen predominantly reflect the protein component of the diet, the $\delta^{13}\text{C}$ values from bone and enamel apatite represent the whole diet (carbohydrates, proteins, and fats) (Ambrose and Norr 1993; Jim *et al.* 2004 Froehle *et al.* 2010). By comparing the $\delta^{13}\text{C}$ values from collagen and apatite, a more comprehensive assessment of past diets can be made (Kellner and Schoeninger 2007). The $\delta^{13}\text{C}$ values from the enamel apatite of the Großmugl individuals are presented in Table 2 (see section 3.1 below) and are plotted against the $\delta^{13}\text{C}$ values from the individuals' crown dentine collagen in Figure 2 alongside the dietary regression lines adapted from Froehle *et al.* (2010). As both of these tissues form during the same period of life, the $\delta^{13}\text{C}$ values reflect the composition of the individuals' whole diet during the development of the crown of their sampled tooth throughout childhood. For the second permanent molars from SK1 and SK3, the $\delta^{13}\text{C}$ values represent whole diet between the ages of 2.5 years (± 0.5 years) and 8.5 years (± 0.5 years). Whereas, for the permanent incisor from SK2, the $\delta^{13}\text{C}$ values represent whole diet between the ages of 7.5 months (± 1.5 months) and 4.5 years (± 0.5 years) (AlQahtani 2009; Beaumont and Montgomery 2015). The regression lines in Figure 2 distinguish between C_3 and C_4 /marine diets. The left of these lines reflect consumption of entirely C_3 resources, and individuals move along the line towards the right of the plot with increasing amounts of C_4 or marine protein incorporated into their diet. As shown in Figure 2, the $\delta^{13}\text{C}$ values measured in the crown dentine collagen and the enamel apatite of the Großmugl individuals cluster tightly on the lower left of the plot, implying a predominantly terrestrial C_3 diet was consumed by all individuals during childhood with no or minimal contributions from C_4 and marine food sources. This dietary behaviour is also corroborated by the rib $\delta^{13}\text{C}$ values, implying that SK1 and SK3 continued to rely on C_3 plants and C_3 -plant fed animals in their later life, and SK2's diet likely did not change radically in the years leading up to death.

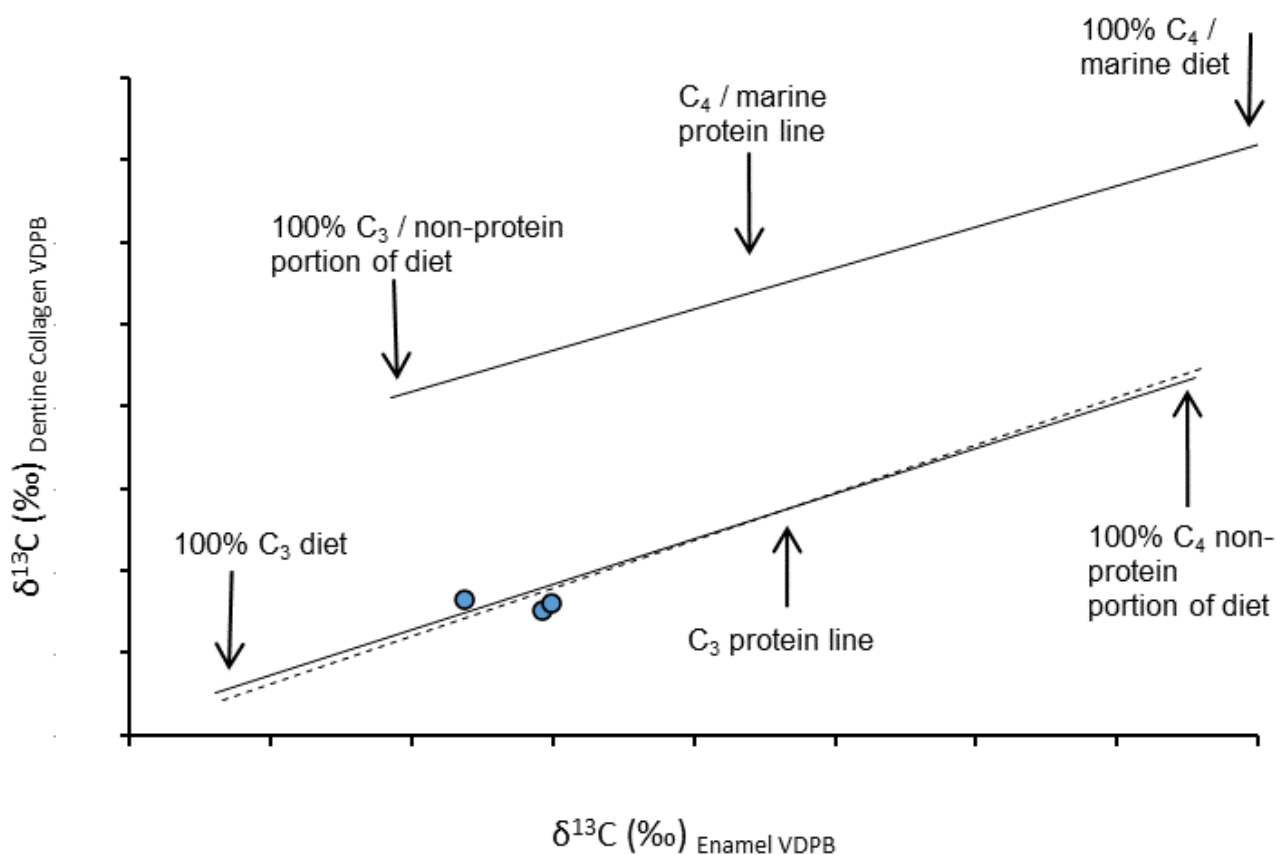


Figure 2: Crown dentine collagen and tooth enamel apatite $\delta^{13}\text{C}$ values from the Großmugl individuals. (Regression lines from Froehle *et al.* 2010, adapted by Jay, M.)

Incremental dentine profiles for the Großmugl individuals are presented in Figure 3, with the full incremental datasets available in the appendix (Tables A1 to A3). The incremental profiles for SK1 and SK3 represent the full developmental period of the permanent second molar, whilst the profile for SK2 covers the period between the start of the incisor's development up to the individual's death, at which point the root apex was not fully formed. All ages are approximate. All of the dentine increments produced C:N atomic ratios within the acceptable range for well-preserved collagen (DeNiro 1985; Ambrose 1990; van Klinken 1999), except for increments 9 and 10 from SK2's incisor (see Table A2). The C:N atomic ratios of these two increments are only just outside of DeNiro's (1985) accepted range of 3.1 – 3.5, so are still included within the incremental dentine profile discussed below. Due to the already small number of increments the incisor yielded, these two increments from the root are important to help infer the diet of SK2 up to the point of death when root development was arrested.

SK2's profile shows a gradual rise in $\delta^{13}\text{C}$ from the start of the incisor's development until around 5.5 years, which may indicate the consumption of small amounts of C_4 plants or marine protein during early childhood. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values from the SK2's dentine increments are relatively consistent with the values seen in the rib, for which the developmental period would have overlapped with that of the incisor. SK2's $\delta^{15}\text{N}$ values remain high throughout the individual's life, from the start of the incisor's development until the child's death at a young age and are significantly higher than those seen in the incremental profiles of SK1 and SK3. These consistently high values are either indicative of eating high-trophic level foods (such as breastmilk or omnivores) throughout childhood or they may support the inference that SK2 suffered through several periods of stress, with their body potentially being under such continuous prolonged stress from such a young age that the nitrogen isotope composition of their tissues never had a chance to return to normal dietary levels before death. Instead, the dentine $\delta^{15}\text{N}$ values which would usually primarily reflect the individual's protein consumption may have been considerably impacted by the ^{15}N -enrichment of tissues as a result of the body frequently being in a catabolic state due to the nutritional, physiological, and/or pathological stress it was under (Mora 2022; Beaumont and Montgomery 2016; Hatch *et al.* 2006; Mekota *et al.* 2006; Fuller *et al.* 2005). If these $\delta^{15}\text{N}$ values are indicative of diet rather than stress, the relatively consistent $\delta^{15}\text{N}$ profile could indicate a stable protein diet, with previous suggestions of the inclusion of omnivorous/suckling pig or marine protein within SK2's diet or the use of crop manuring potentially explaining the significantly high values.

SK3 has a relatively flat $\delta^{15}\text{N}$ profile from the age of 4.5 years onwards and shows minor variation in $\delta^{13}\text{C}$ values between 4.5 – 11.5 years, indicating a relatively stable terrestrial C_3 diet during childhood with minimal input from other food sources. There is a slight increase in $\delta^{13}\text{C}$ values between 11.5 and 13.5 years which may indicate very minor contributions from C_4 plants or marine protein to the diet over a limited time period, followed by a return to a predominantly C_3 terrestrial diet. The rib $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values are slightly lower than those measured in the dentine, suggesting that during adulthood this individual may have consumed less animal protein, although the difference between the values measured in the two tissues does not constitute a significant shift in dietary behaviour. The incremental profile of SK1 shows more variation. Between the ages of 5 – 8.5 years, the lower $\delta^{15}\text{N}$ values suggest this

individual consumed less animal protein, potentially due to limited availability of animal produce or a greater reliance on plant-based foods. A concurrent increase in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values then occurs between 12 and 14 years, which may indicate the reintroduction of greater amounts of animal protein to the diet or an increased consumption of terrestrial omnivores or marine fish. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values decrease after 14 years until the end of the incremental profile and become consistent with the rib values, implying that this individual maintained a predominantly terrestrial C_3 diet into adulthood.

The second permanent molars of SK1 and SK2 both show a co-occurring decrease in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ from the beginning of development until 4.5 – 5 years. This pattern is usually typical of weaning, but it is not expected to be visible in the second permanent molar at such a late age, suggesting that this decrease in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ could more likely be due to a dietary shift occurring for both of these individuals around the same age (Jay 2009; Chinique de Armas *et al.* 2022). The decreasing $\delta^{15}\text{N}$ values during this period indicate less animal protein, or lower-trophic level animal protein, was being consumed and are consistent with a trophic level shift down the food chain, which is especially evident in SK1's profile. This change in diet during early childhood may therefore represent a gradual transition away from consuming significant quantities of animal produce such as dairy products and milk (as perhaps seen in the profile of SK2). Perhaps this was a movement towards a more typical terrestrial "adult" diet, as suggested by the lower and more stable $\delta^{13}\text{C}$ values after this age which are more similar to those observed in the rib collagen. There are many social, cultural, economic, and/or environmental reasons why a dietary change may occur at a young age, and it is particularly interesting that SK1 and SK3 appear to experience it around the same age. These two individuals also seem to have experienced a period of stress around the same age, with an increase in $\delta^{15}\text{N}$ values and co-occurring decrease in $\delta^{13}\text{C}$ values visible in both incremental profiles. This opposing covariance occurs between the ages of 8.5 – 12 years for SK1 and 9.5 – 11.5 years for SK3 and is a potential indicator of physiological or nutritional stress affecting the body (Beaumont and Montgomery 2016).

Mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the crown and root increments of each tooth have also been plotted separately in Figure 1 alongside the data for the rib collagen and the comparative northern Austrian Hallstatt culture burials (Le Huray and Schutkowski

2005). For the second permanent molars of SK1 and SK3, crown development is represented by the first four dentine increments, and for SK2's incisor only the first three increments represent crown development. The separate plotting of these two stages of tooth development highlights that the mean crown $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of SK1 and SK3 are higher than those of the later-forming root, whilst the opposite is observed for SK2. This inverse trend emphasises the unusually high $\delta^{15}\text{N}$ values seen towards the end of SK2's profile and supports the inference that this individual suffered significant stress before their death, in contrast to SK1 and SK3 who survived into late adolescence/adulthood and experienced minor periods of stress and/or dietary changes before settling into a stable terrestrial C_3 diet.

In sum, taken together, the carbon and nitrogen isotope ratios from the Großmugl individuals are consistent with a predominantly terrestrial C_3 diet but appear to be quite different (lower $\delta^{13}\text{C}$ and/or higher $\delta^{15}\text{N}$) from both Iron Age and Early Medieval populations from Austria. This may imply the use of crop manuring, consumption of freshwater fish and/or the inclusion of higher trophic level foods such as omnivorous animals (e.g., suckling pigs) within the diet.

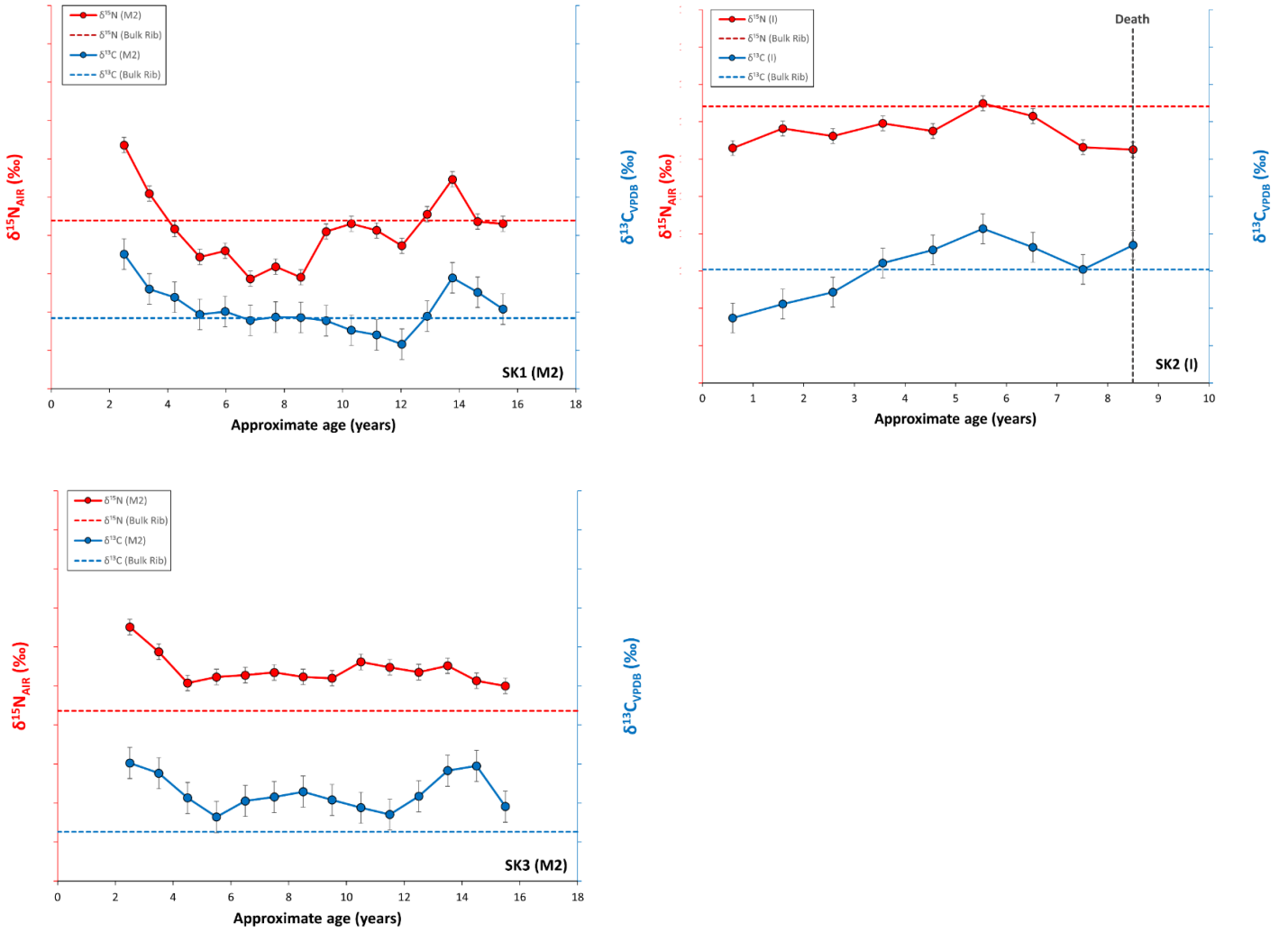


Figure 3: Incremental dentine $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ data for the Großmugl individuals plotted against age in years. The dashed red and blue straight lines across the plots indicate the average $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values obtained from the individuals rib collagen. For SK2, the vertical black dashed line represents the arrestation of tooth development for the incomplete incisor at the estimated age-at-death.

3. Assessing Geographical Origins

3.1 Oxygen isotope analysis

Oxygen incorporated into hydroxyapatite is predominantly derived from ingested fluids, the isotopic composition of which fluctuates due to climatic and environmental variables such as temperature, rainfall, altitude and latitude (Darling and Talbot, 2003). Therefore, oxygen isotopes ($\delta^{18}\text{O}$) measured in human tissues are an indirect reflection of the local meteoric water composition (Kohn, 1999). Oxygen undergoes

metabolic fractionation once ingested. Therefore, regression formulae must be applied to allow comparison with modern drinking water values in order to discern childhood geographical origins and palaeo-climate (Chenery et al., 2012; Fricke et al., 1995). In addition to this, $\delta^{18}\text{O}$ values can also be influenced by culturally mediated behaviour. The processing (boiling, brewing etc.) of a significant portion of an individual's drinking water before ingestion can result in higher than expected values, and as such, interpretation must be performed with caution (Brettell et al., 2012; Camin et al., 2008; Daux et al., 2008).

3.1.1 Materials and methods

Tooth enamel samples were cleaned and abraded to a depth of $>100\mu\text{m}$ using a diamond-tipped dental drill to remove dentine and surface contaminants. Powdered tooth enamel samples (c. 16 mg) were then collected on foil using a diamond-tipped burr and transferred to 1.5 mL microcentrifuge tubes. Samples were not pre-treated with an oxidising agent such as sodium hypochlorite or acetic acid, as they have been shown to cause isotopic shifts (Pellegrini and Snoeck 2016). Recent studies have deemed this step unnecessary for enamel pre-treatment (e.g., Balasse *et al.* 2020; Hodgkins *et al.* 2020). The samples were transferred to Iso Analytical for stable isotope analysis. Here, the samples were weighed into ExetainerTM tubes and flushed with 99.995% helium. Carbonate in the samples was converted to CO_2 by adding phosphoric acid and letting the sample sit overnight for the reaction to occur. Reference materials (IA-R022, NBS-18, and IA-R066) were prepared along the same methods. CO_2 from the samples was then analysed by Continuous Flow-Isotope Ratio Mass Spectrometry (CF-IRMS). The CO_2 was sampled from the ExetainerTM tubes into a continuously flowing He stream using a double holed needle. The CO_2 was resolved on a packed column gas chromatograph and the resultant chromatographic peak carried forward into the ion source of a Europa Scientific 20-20 IRMS where it was ionized and accelerated. Gas species of different mass were separated in a magnetic field then simultaneously measured using a Faraday cup collector array to measure the isotopomers of CO_2 at m/z 44, 45, and 46. The phosphoric acid used for digestion was prepared in accordance with Coplen et al. (1983) and was injected through the septum into the vials. All samples were run in duplicate. Analytical errors for the analysis were $\delta^{13}\text{C} \pm 0.2 \text{ ‰}$ (1 sd) and $\delta^{18}\text{O} \pm 0.2 \text{ ‰}$ (1 sd).

3.1.2 Results and interpretation

The Großmugl individuals' oxygen isotope data is presented in Table 2 and Figure 4, alongside comparative data from the early Iron Age western Hallstatt culture tumulus at Magdalenenberg, Germany (Oelze *et al.* 2012) and the Late Bronze Age hillfort at Stillfried an der March, Austria (Retzmann *et al.* 2020).

The Großmugl individuals' $\delta^{18}\text{O}$ values were measured in carbonate and converted to phosphate values (Chenery *et al.* 2012) and precipitation/drinking water values (Daux *et al.* 2008) to facilitate comparison with other published data. These $\delta^{18}\text{O}_{\text{phos}}$ values range from REDACTED to REDACTED with a median of REDACTED. SK1 and SK3's $\delta^{18}\text{O}_{\text{phos}}$ values differ by only 0.2‰ and are therefore within analytical error of each other, whilst SK2's $\delta^{18}\text{O}_{\text{phos}}$ value is 1.0‰ and 0.8‰ higher than SK1 and SK3, respectively. As oxygen isotope ratios can be influenced by factors such as temperature, altitude, rainfall levels, and latitude (Darling and Talbot 2003), the similar lower phosphate $\delta^{18}\text{O}$ values of SK1 and SK3 could suggest these individuals procured their water from a source with similar geographical conditions or lived during a period of similar climatic conditions. In contrast, SK2's higher phosphate $\delta^{18}\text{O}$ value may imply this individual obtained their water, or potentially originated, from an area with a different altitude, latitude, amount of rainfall, or temperature. Alternatively, SK2 may not be contemporaneous with the other two individuals and may instead have spent their childhood in the same area during a time of warmer climatic conditions (Darling and Talbot 2003). Regional and localised changes in climate are documented within the palaeo-environmental record across Europe during the Bronze Age and Iron Age (Molloy 2022). The higher $\delta^{18}\text{O}_{\text{phos}}$ value observed in SK2's enamel may also be influenced by the early developmental age of the permanent incisor in comparison to the permanent second molars. With development starting at around 7.5 months of age, the enamel of the permanent incisor may incorporate $\delta^{18}\text{O}$ from breast milk and yield a higher oxygen isotope ratio, as breast milk is often enriched in ^{18}O in comparison to the drinking water an individual consumes later in life (Chinique de Armas *et al.* 2022).

In the context of geographical origin, all of the Großmugl individuals' $\delta^{18}\text{O}$ values lie within the range expected for the site and are therefore consistent with having a local

childhood provenance. Lightfoot and O'Connell's (2016) map of regional modern precipitation oxygen isotopic values have been used to estimate the local drinking water value ($\delta^{18}\text{O}_{\text{dw}}$) range for Großmugl, which has been converted to phosphate values to be shown in Figure 4. The site and its wider region sit on a boundary between a zone of expected higher $\delta^{18}\text{O}_{\text{dw}}$ values (-10‰ to -8‰) and one of expected lower $\delta^{18}\text{O}_{\text{dw}}$ values (-12‰ to -10‰), making the estimated range of local $\delta^{18}\text{O}$ values quite large (-12‰ to -8‰). SK1 and SK3's $\delta^{18}\text{O}_{\text{dw}}$ values lie close to the lower end of this local range and both fall outside the 2 sd range for Großmugl when the analytical error and large uncertainties associated with oxygen conversion equations are considered. Therefore, whilst these two individuals have $\delta^{18}\text{O}$ values consistent with a local upbringing, their oxygen isotope data cannot rule out other potential residential origins in areas with lower $\delta^{18}\text{O}$ values such as the Alps to the west of Großmugl (Lightfoot and O'Connell 2016). SK2's $\delta^{18}\text{O}_{\text{dw}}$ value lies in the middle of the local range and remains within it at 2 sd, therefore suggesting this individual's oxygen isotope ratios are consistent with a childhood residence local to Großmugl.

However, as can be seen in Figure 4, the majority of the $\delta^{18}\text{O}$ values from the Magdalenenberg tumulus also lie within this estimated local range despite the individuals being inferred as having heterogeneous origins (Oelze *et al.* 2012). This comparison serves to emphasise that this estimated local range of $\delta^{18}\text{O}$ values is not exclusive to the site or Austria in general and can be found across vast areas of central and eastern Europe as well as Britain (Lightfoot and O'Connell). In the absence of other suitable comparative literature and oxygen isotope data from local samples, it is not possible to ascertain a more accurate local oxygen isotope range for Großmugl. Modern regional datasets such as Lightfoot and O'Connell (2016) are an appropriate alternative to exclude potential residential origins and infer regional locality. For instance, using this comparative dataset, it is possible to exclude potential residential origins for all three Großmugl individuals in most of western Europe, Italy and most of south-east Europe where higher $\delta^{18}\text{O}$ values are expected.

[REDACTED] Table 3: Measured carbonate ($\delta^{13}\text{C}_{\text{VPDB}}$ and $\delta^{18}\text{O}_{\text{VPDB}}$) and calculated $\delta^{18}\text{O}_{\text{VSMOW}}$ and drinking water values for the enamel samples from Großmugl

Skeleton	$\delta^{13}\text{C}_{\text{VPDB}}$ carbonate measured (‰)	$\delta^{18}\text{O}_{\text{VPDB}}$ carbonate measured (‰)	$\delta^{18}\text{O}_{\text{VSMOW}}$ carbonate calculated (‰)	$\delta^{18}\text{O}_{\text{VSMOW}}$ phosphate calculated (‰)	$\delta^{18}\text{O}_{\text{VSMOW}}$ precipitation calculated (‰)
SK1					
SK2					
SK3					

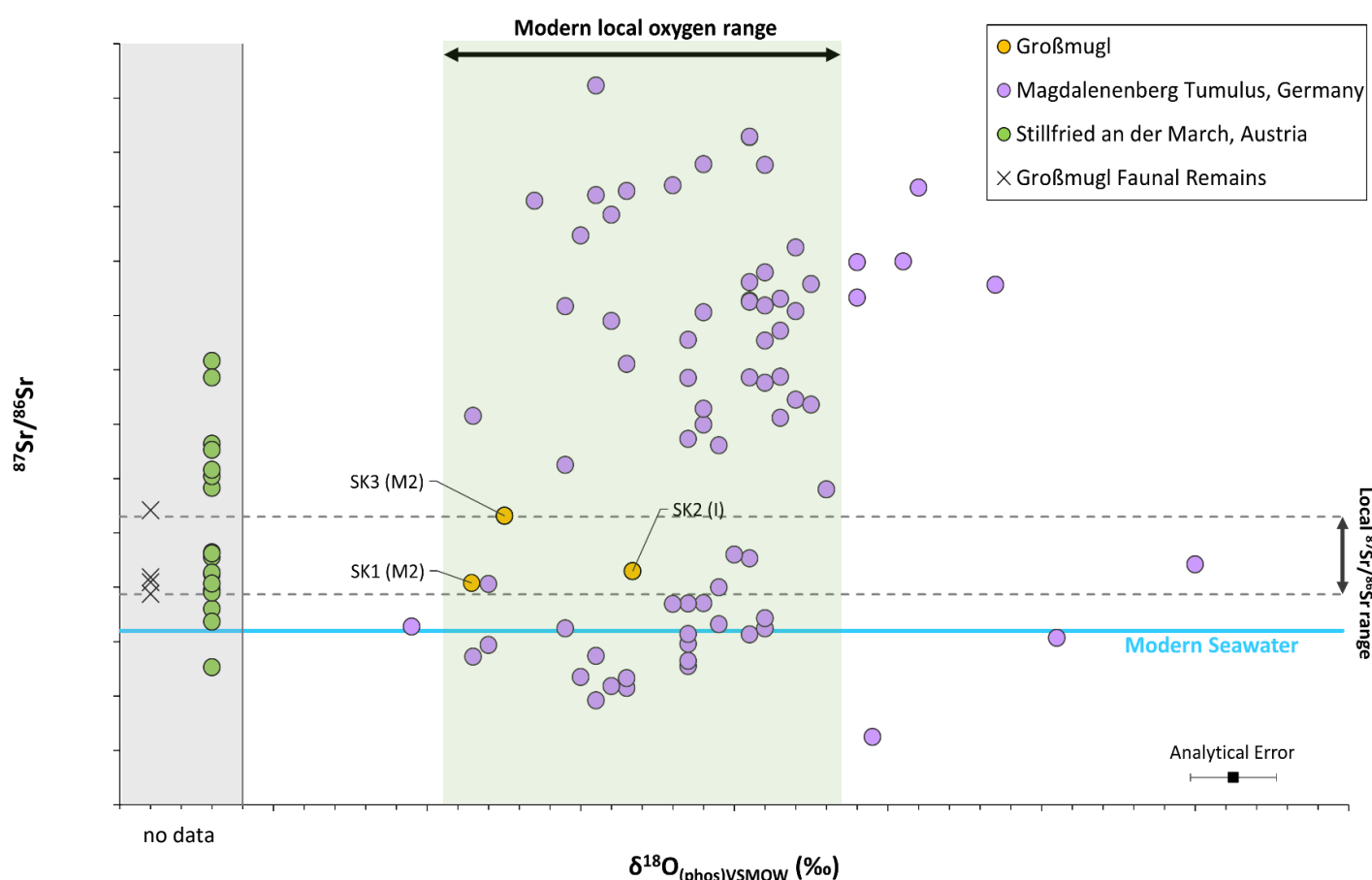


Figure 4: Großmugl $^{87}\text{Sr}/^{86}\text{Sr}$ and calculated $\delta^{18}\text{O}$ phosphate data alongside comparative data from Stillfried an der March, Austria (Retzmann *et al.* 2020) and the Magdalenenberg tumulus, Germany (Oelze *et al.* 2012). The horizontal dotted lines represent the bioavailable strontium isotope range for Großmugl (Retzmann *et al.* 2020). The shaded green box represents the oxygen isotope range expected for the site and its surrounding region (Lightfoot and O'Connell 2016). Oxygen error shown to 1σ , strontium errors are within the symbol.

3.2 Strontium isotope analysis

The isotope analyses of strontium ($^{87}\text{Sr}/^{86}\text{Sr}$) can provide information on the movements and mobility of past populations by identifying individuals who have different isotopic compositions to the geographic area in which they were found (Evans *et al.* 2012). Strontium isotopes offer a direct link between an individual and their geographic origin because strontium derives from bedrock and is transferred from the rock through soil, plants, and animals whilst still remaining indicative of the source rock (Bentley 2006). The strontium isotope ($^{87}\text{Sr}/^{86}\text{Sr}$) characteristics of plants and animals that eat these plants will vary in different regions depending on the relative contributions of strontium from different underlying rocks and the presence or absence of superficial drift deposits (Bentley 2006). Strontium becomes incorporated into the hydroxyapatite lattice of human bone through ingestion of food and water (Montgomery *et al.* 2010). Assuming that the majority of a population's food and drink is sourced locally, the strontium isotope composition in human skeletal material should then reflect the bioavailable strontium isotopes in their region of origin (Montgomery *et al.* 2007).

3.2.1 Materials and methods

Core enamel samples (c. 5 mg) from permanent second molars of SK1 and SK3 and a permanent incisor from SK2 were prepared for strontium isotope analysis using column chemistry methods outlined in Font *et al.* (2008) at the Arthur Holmes Isotope Geology Laboratory (AHIGL), Durham University. In brief, samples were digested overnight in 3M HNO_3 on a hotplate at 100°C before being loaded onto cleaned and preconditioned columns containing Eichrom strontium-specific resin. A purified Sr fraction was eluted from the column in 400 μl H_2O and acidified with 15.5M HNO_3 to yield a 3% HNO_3 solution.

Following Sr purification, the size of the ^{86}Sr beam was tested for each sample to derive a dilution factor so that each sample yielded a beam size of approximately 25V ^{88}Sr to match the intensity of the isotopic reference material NBS987. Samples were aspirated using an ESI PFA-50 nebuliser coupled to a Glass Expansion Cinnabar micro-cyclonic spraychamber. Sr isotopes were measured using a static multi-collection routine with each measurement comprising a single block of 50 cycles with

an integration time of 4s per cycle (total analysis time ~3.5 minutes). Instrumental mass bias was corrected for using an $^{88}\text{Sr}/^{86}\text{Sr}$ ratio of 8.375209 (the reciprocal of the more commonly used $^{86}\text{Sr}/^{88}\text{Sr}$ ratio of 0.1194) and an exponential law. Corrections for isobaric interferences from Rb and Kr on ^{87}Sr and ^{86}Sr were performed using ^{85}Rb and ^{83}Kr as the monitor masses but were insignificant. In all samples the ^{85}Rb intensity was $< 1\text{mV}$ with an $^{85}\text{Rb}/^{88}\text{Sr}$ ratio of < 0.0003 (average 0.0001). ^{83}Kr was between 0.12 and 0.14mV in all samples. Samples were analysed during a single analytical session during which the average $^{87}\text{Sr}/^{86}\text{Sr}$ ratio and reproducibility for the international reference material NBS987 was 0.710248 ± 0.000009 (2σ ; $n = 13$). Maximum error based on internal precision of individual analysis and analytical reproducibility of the reference material is considered to be 0.000009 (2σ). Sr isotope data for samples is normalised to an 'accepted value for NBS987 of 0.710240.

Dentine samples from faunal remains recovered at Großmugl, including one permanent third molar from a pig and permanent second molars from three sheep, were also analysed following this method. The faunal dentine samples were analysed in a separate single analytical session, during which the average $^{87}\text{Sr}/^{86}\text{Sr}$ ratio and reproducibility for the international reference material NBS987 was 0.710255 ± 0.000017 (2σ ; $n = 11$). The $^{87}\text{Sr}/^{86}\text{Sr}$ data for the Großmugl fauna are presented in the Appendix (Table A4).

3.2.2 Results and interpretation

The Großmugl individuals' strontium isotope data is presented in Table 4 and Figure 4 alongside comparative data from Magdalenenberg (Oelze *et al.* 2012) and Stillfried an der March (Retzmann *et al.* 2020). The $^{87}\text{Sr}/^{86}\text{Sr}$ values from the tooth enamel of the Großmugl individuals range from REDACTED, with a median of REDACTED, which is within the expected range for Austria (Voerkelius *et al.* 2010). Tooth enamel strontium concentrations from these individuals ranged from REDACTED to REDACTED, with a median of REDACTED, and are all typical of archaeological human populations (Evans *et al.* 2012).

Großmugl is situated in the Molasse Basin which consists of mainly Cenozoic sands, gravels and marls. The site also closely borders the Waschberg Zone; a narrow nappe of Cenozoic sandstone with some outcrops of Jurassic limestone (Granado *et al.* 2016;

Gebhardt 2020). No strontium isotope data from modern or archaeological samples was available for Großmugl or the site's immediate local area. However, environmental samples from the Late Bronze Age hillfort at Stillfried an der March, around 46km east of Großmugl, have been used as a proxy for the local bioavailable strontium isotope range due to the site's similar underlying Cenozoic sedimentary geology. From this, the local bioavailable strontium isotope range for Großmugl has been estimated to lie between 0.7098 and 0.7113 (Retzmann *et al.* 2020). It is expected that people who source most of their food and drink from within regions of Cenozoic sands and marls would have strontium isotope ratios close to this range. The $^{87}\text{Sr}/^{86}\text{Sr}$ values from the dentine of the Großmugl fauna range from REDACTED to REDACTED (with a mean of REDACTED) and all mostly lie within this expected local range, with only one sheep sitting just outside (See Figure 4). As dentine is susceptible to diagenetic alteration in the burial environment and can exchange mineral and organic elements with the surrounding soil, these faunal dentine $^{87}\text{Sr}/^{86}\text{Sr}$ values can be used as a proxy for the labile strontium isotope range for the burial environment at Großmugl (Budd *et al.* 2000; Dauphin and Williams 2004; Montgomery *et al.* 2007; Kendall *et al.* 2018). As shown in Figure 4 the $^{87}\text{Sr}/^{86}\text{Sr}$ values for the enamel samples of all three Großmugl individuals plot within the expected local strontium range (Retzmann *et al.* 2020), with SK3 sitting on the upper boundary, and all lie within the local faunal range. These individuals therefore have strontium isotope ratios consistent with a childhood spent in the Großmugl area.

However, it is important to note that these $^{87}\text{Sr}/^{86}\text{Sr}$ values are not unique to Großmugl and can also be found in other regions with similar underlying geology such as areas of central and south-west England, southern Scotland, Hungary, Germany and elsewhere in central and northern Europe (Voerkelius *et al.* 2010; Evans *et al.* 2010). Notably, the strontium isotope ratios for the tooth enamel of SK1 and SK2 sit within a largely undiagnostic range of values that occur throughout much of Europe due to shared characteristics of the geological terrain (Montgomery *et al.* 2014). Consequently, although the three individuals have $^{87}\text{Sr}/^{86}\text{Sr}$ values consistent with a local childhood residence, Großmugl cannot be established as their only possible provenance. Areas with older, more heterogeneous and granitic lithologies to the southwest such as the Alps and the Bohemian Massif to the north of the site, can however be excluded as likely residential origins for the Großmugl individuals, as they

would be expected to produce much higher $^{87}\text{Sr}/^{86}\text{Sr}$ values exceeding 0.7120 (Oelze *et al.* 2012).

[REDACTED] Table 4: Measured tooth enamel $^{87}\text{Sr}/^{86}\text{Sr}$ values from the Großmugl individuals

Skeleton	Tooth	Sr ppm	$^{87}\text{Sr}/^{86}\text{Sr}$	2SE
SK1	M2			
SK2	I			
SK3	M2			

4. Discussion

Diet and mobility of three individuals from Großmugl were investigated using a range of isotopes to explore diet and migration throughout life. Bulk carbon and nitrogen isotope results show that all three individuals had a diet predominantly based on terrestrial C_3 resources, the carbon isotopes in their tooth enamel, which represents whole diet supports this interpretation. Examining the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ spacing between these individuals and contemporaneous animals from Austria suggests that they all had long-term diets predominantly based on terrestrial C_3 resources, with the inclusion of higher trophic level animal protein such as suckling pig or freshwater fish. Incremental dentine analysis highlights how SK1 and SK3 had relatively stable terrestrial C_3 diets throughout childhood, but both experienced periods of physiological or nutritional stress between the ages of 8 to 12 years. Although all three individuals appear to have eaten a predominantly terrestrial C_3 diet, SK2 has consistently higher nitrogen isotope values in their rib an incremental dentine profile. While it may be possible that this individual incorporated some marine protein into their diet it is likely that this difference is due to chronic physiological stress.

Mobility isotopes (Sr, O) and trace element analyses indicate that SK1 and SK3 grew up in a similar, if not the same location. Both individuals have strontium and oxygen isotope ratios consistent with Großmugl, and low lead and arsenic concentrations typical for the period. Although SK2 also has strontium and oxygen isotope ratios consistent with Großmugl (the slightly higher oxygen value likely due to breastfeeding),

their higher strontium concentration suggest they did not grow up in the same environment as SK1 and SK3. It is also important to note that strontium isotope ratios seen in all three of these individuals are not unique to Großmugl and can also be found in other regions with similar underlying geology such as Hungary, Germany and elsewhere in central and northern Europe.

In summary, the isotopic characteristics of these three individuals suggest local origins for SK1 and SK3, who both have consistently similar data for both their diet and geographic origins. However, SK2 is different. If contemporary with SK1 and SK3 then the differences in SK2's carbon, nitrogen and oxygen isotopes could be due to breastfeeding and chronic physiological stress. Alternatively, SK2 displays many of the isotopic characteristics of an individual from the Roman period, and if secure dating has not been carried out, radiocarbon dating is strongly recommended for this individual.

5. Conclusions

- The carbon isotope ratios from the Großmugl individuals are consistent with a predominantly terrestrial C₃ diet.
- Notably, the nitrogen isotope ratios from the Großmugl individuals are significantly higher than those observed in contemporaneous and Early Medieval populations in Germany and Austria. This may imply the use of crop manuring, consumption of freshwater fish and/or the inclusion of higher trophic level foods such as omnivorous animals (e.g., suckling pigs) within the diet.
- The incremental dentine profiles of the three individuals support the consumption of a primarily terrestrial C₃ diet during tooth formation in childhood, with some minor dietary changes visible in the profiles of SK1 and SK3.
- Periods of nutritional or physiological stress were identified in the profiles of each Großmugl individual. For SK2 in particular, the sustained high $\delta^{15}\text{N}$ values across the profile suggest this individual experienced continuous stress during tooth development. This is consistent with their estimated age-at-death in later childhood and the high $\delta^{15}\text{N}$ values observed in SK2's rib bone collagen.

- The oxygen isotope data of all three Großmugl individuals is consistent with a local origin to the site. SK1 and SK3's $\delta^{18}\text{O}$ values are very similar and could also be consistent with residential origins in areas with lower $\delta^{18}\text{O}$ values such as the Alps to the southwest of the site or they may have resided at Großmugl during a time with differing climatic conditions to SK2.
- All the Großmugl individuals have strontium isotope ratios consistent with a childhood spent local to the site, although residential origins in other areas with similar underlying geology cannot be ruled out. The faunal data was also consistent with a local origin.
- The strontium concentrations for the Großmugl individuals were consistent with archaeological humans with childhood residential origins at Großmugl or an area with similar underlying geology.

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