Tracing intensive fish and meat consumption using Zn isotope ratios: evidence from a historical Breton population (Rennes, France)

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Here we report Sr and Zn isotope ratios of teeth of medieval to early modern Breton people a population whose diet is known from historical, archeological and collagen isotope data. Most of the population, buried in the Dominican convent of Rennes, France, consists of parliamentary nobles, wealthy commoners and ecclesiastics, who had a diet rich in animal products. Our aim is to assess how the Zn isotope ratios of their teeth compare to those of other French historical populations previously studied, which were characterized by cereal-based diets, and those of modern French individuals, who daily eat animal products. We describe a clear offset (∼0.35‰) between local and non-local human individuals in Zn isotope ratios. The δ66Zn values of local individuals overlap that of modern French people, and are lower than those of local carnivores. Non-local δ66Zn values are similar to those of historical individuals analyzed previously. We conclude the lower Zn isotope ratios of local humans relative to the associated fauna can be explained by the consumption of carnivorous fish and pork, in agreement with historical, zooarchaeological and collagen (C, N, S) isotope data. Zn isotopes could therefore be a tracer of fish and/or substantial meat consumption in ancient populations.

The origin of Zn isotopic variability in human tissues remained unknown until Van Heghe et al. (2012)1, reported the strong impact of meat and fish consumption on blood Zn isotope ratios (66Zn/64Zn expressed as δ66Zn values), a preliminary conclusion quickly confirmed by Costas-Rodriguez et al. (2014)2. A parallel study on African food webs did not quantify the exact relationship between diet and bone Zn isotope ratios3; however by focusing on a much smaller geographical area, the sensitivity of Zn isotopes to diet was demonstrated4: Zn isotope ratios of bones and teeth clearly differ between carnivores and herbivores, with carnivores exhibiting the lowest ratios. The dependence of Zn isotope ratios on trophic level has also been confirmed in a marine ecosystem5.

The isotopic composition of Zn in animal tissues is controlled by two dietary factors: the isotopic fractionation that occurs during intestinal absorption and the Zn isotope ratios of the food products. Dietary Zn mainly comes from animal products, notably because Zn – and preferentially its lighter isotopes - from plants tends to precipitate in the gastro intestinal tract6. This precipitation is likely to trigger isotopic fractionation inducing the preferential absorption of heavy Zn isotopes. Additionally, plant products usually have the most elevated δ66Zn values7. As a consequence, herbivore tissues exhibit higher Zn isotope ratios compared to carnivore or omnivore tissues8,9. Muscles are 66Zn depleted relative to the average isotopic composition of the body and no isotope fractionation of Zn is expected during meat consumption3. Carnivores therefore have lower δ66Zn values than their prey: the higher the trophic level of an animal is, the lower are the Zn isotope ratios of its body tissues9.

Zn isotope ratios of dental enamel from populations from different locations and historical periods were recently compared7. The study highlighted a very surprising trend: the δ66Zn dental values of preindustrial

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populations were much higher than those of modern individuals. Two explanations were then hypothesized to explain such a pattern. The observed trend could be due to:

1) an increase in fish and meat consumption in the 20th century. As mentioned above, elevated Zn isotope ratios are expected in tissues of individuals with plant-based diets. Conversely, consumption of high trophic level food products, such as carnivorous fish (e.g. tuna, salmon, cod, pike) is expected to generate low Zn isotope ratios of mammal tissues.

2) the release of anthropogenic Zn in modern environments by industries and/or the use of manuring products. The anthropogenic Zn can indeed exhibit low Zn isotope ratios and enter modern food webs.

In order to decide between these two hypotheses, we analyzed a preindustrial population (13th to 18th century) characterized by diets with significant meat and fish consumption. This allows us to test if historic human populations living before the release of anthropogenic Zn into modern environments exhibit δ66Zn values closer to other preindustrial populations characterized by a cereal-based diet, or are more like modern individuals who also had diets with intensive meat and fish consumption. We studied the wealthy/elite late medieval to early modern population of the former Dominican convent of Rennes (Brittany, France, Fig. 1), for which we already reconstructed the diet using C, N and S isotopes. The medieval and early modern diets of the aristocracy also included a very important amount of meat on non-fasting days. The C, N and S isotope ratios determined from the bones and teeth of the individuals buried in Rennes’s Dominican convent as well as the zooarchaeological study performed on this site and the nearby refuse midden, indicate a

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**Figure 1.** Location of Rennes and its Dominican convent. (A) Historical border of Brittany in the 15th century (blank map from Daniel Dalet, [http://www.histgeo.ac-aix-marseille.fr](http://www.histgeo.ac-aix-marseille.fr)). (B) Location of the convent and the refuse dump outside of the walls in the 16th century (after the city map drawn by Hévin in 1685, image free of rights, [http://www.wiki-rennes.fr/Fichier:Plan_Hevin.jpg](http://www.wiki-rennes.fr/Fichier:Plan_Hevin.jpg)). Both maps were created using the software Adobe Illustrator CS6.
substantial consumption of animal products: terrestrial herbivores and omnivores, including suckling pigs, eels and marine fish.

Since the environmental context can impact local Zn isotope ratios, the geographical origin of the individuals was assessed using Sr isotope ratios in human dental enamel as well as previously published S isotope data obtained on tooth collagen (Supplementary Information 1). Potential impacts of marine food consumption on S and Sr isotope ratios are assessed using previously published δ13C values by evaluating the absence or presence of a correlation between these isotope ratios.

In this paper, we present stable Zn and radiogenic Sr isotope data from the analysis of dental enamel of 54 individuals from the Dominican convent of Rennes. The associated fauna (6 terrestrial animals) was also analyzed to better interpret the diet of this population and compared the isotopic data of C, N and S previously conducted on the collagen from the same teeth.

Results
A socio-economic group was attributed to the different individuals depending on their burial location. The majority are elite and wealthy individuals who are buried in the church and its chapels (group Privileged, A), but three subgroups can be defined within this group. The first subgroup (A') includes the aristocrats identified by discriminatory funeral practices (embalming and/or lead coffins). A second subgroup is identified as slightly less favored as the others (B') and includes the individuals buried in the nave of the church. The rest of the individuals buried in the church choir and the chapels corresponds to the last subgroup (A-A'). The ‘non-privileged’ individuals are buried outside the walls of the convent in the cloister garden and in the immediate exteriors of the convent (group Non-privileged, B-B'). In addition, the individuals from the chapter house are likely to be Dominican ecclesiastics (group Ecclesiastics, C). Finally, men with blade injuries found in mass graves out of the convent’s walls were probably soldiers (group Soldiers, D). The distribution of the individuals sampled among socio-economic group, phase, age of death and sex are given in the Table.

Sr isotope analyses. The Sr isotope ratios of terrestrial mammal teeth (n = 6) range from 0.7115 and 0.7146. Terrestrial domestic animals (pig, cat, dog, cows and sheep) being raised in the city or in the nearby country, have local bioavailable 87Sr/86Sr values. This range also fits with the local values expected from the IRHUM database (Isotopic Reconstruction of Human Migration), ranging from 0.710 to 0.716 (Fig. 2A). The IRHUM database documents a really limited sea spray effect on the coasts of Brittany (Fig. 2B). The individuals exhibiting lower, “non-local” tooth values are supposed to have spent the end of their childhood or the beginning of their adolescence in a sedimentary or volcanic region. Seventeen percent of the individuals analyzed are exhibiting
δ^{66}Zn 2 SD n δ^15N 2 SD n
Animals
carnivore (cat) 0.66 — 1 0.7127 — 1
Omnivores (pig and dog) 0.58 0.14 2 0.7116 0.0002 2 11.9 2.4 2
Herbivores (cow and sheep) 0.96 0.24 3 0.7136 0.0022 3 8.7 1.8 3

Probable locals
Non-Privileged (B-B') 0.34 0.26 5 0.7119 0.0018 5 12.5 1.8 5
Ecclesiastics (C) 0.35 0.28 8 0.7115 0.0016 8 13.2 1.4 8
Privileged (A, B') 0.31 0.30 30 0.7116 0.0016 30 13.2 2.2 25
Soldiers (D) 0.51 — 1 0.7109 — 1 13.1 — 1
All 0.33 0.28 45 0.7116 0.0016 45 13.2 2.0 39

Non-locals
Non-Privileged (B-B') 0.69 0.26 4 0.7093 0.0010 4 12.0 1.0 3
Ecclesiastics (C) 0.72 — 1 0.7096 — 1 11.5 — 1
Privileged (A, B') 0.63 0.82 3 0.7094 0.0004 3 13.0 1.8 3
Soldiers (D) 0.70 — 1 0.7091 — 1 10.2 — 1
All 0.68 0.44 9 0.7093 0.0008 9 12.1 2.2 8

Table 2. Sr, Zn and N isotope ratios of human and animal teeth depending on the socio-economic group of the individuals buried in the Dominican convent of Rennes, Brittany.

Figure 2. Sr isotope ratios in Brittany (according to IRHUM database) and in dental enamel of Rennes individuals. J: juveniles. F: female. I: Indeterminate. PM: probable male. M: male. C+O=carnivorous and omnivorous animals. H: herbivores. The error bars are smaller than the symbol size. The map of Brittany and associated Sr isotope ratios was created using Adobe Illustrator CS6 based on the information available on the IRHUM database website (http://80.69.77.150/, blank map from Daniel Dalet, http://www.histgeo.ac-aix-marseille.fr).
non-local $^{87}\text{Sr}/^{86}\text{Sr}$ ($^{87}\text{Sr}/^{86}\text{Sr} < 0.710$ based on the subdivisions from the IRHUM database reproduced in Fig. 2), and are therefore likely to have spent their childhood outside of Brittany (9/54 teeth). By combining $S$ isotopes that provide information on the relative distance to the coast – or more exactly, the existence of a marine influence on local values\(^{17}\) – to $S$ isotopes, tracers of the local geology, 6 categories of geographical origins were defined/can be defined (Table 3). Two individuals exhibit inland $S$ isotope values (NC-non-coastal) - therefore are not compatible with the observed Breton range- but $^{87}\text{Sr}/^{86}\text{Sr} > 0.710$ (R-radiogenic) –compatible with the local range (R_NC). One individual is characterized by a coastal ($C$, $\delta^{34}S > 11\%$\(^{11,17}\) but non-local $S$ (NR-non-radiogenic, $^{87}\text{Sr}/^{86}\text{Sr} < 0.710$) isotope signature (NR_C). Four individuals have both non-local $S$ and $Sr$ isotope values (inland $\delta^{34}S$ value and $^{87}\text{Sr}/^{86}\text{Sr} < 0.710$. NR_NC). Finally, for the remaining four individuals with non-local $Sr$ isotope ratios, the $S$ isotope signature of their teeth was not available (NR_X). As previously observed with $S$ isotopes, all women and nobles tested (group 2 A' and 3 A', Table S1) fall into the “probable local” group ($n$women = 10, nnobles = 8). All soldiers showed non-local $S$ isotope values in teeth or bones ($n$soldiers = 3) but only one of the three teeth analyzed exhibit a $^{87}\text{Sr}/^{86}\text{Sr}$ value incompatible with the local range. One ecclesiast from the second phase, already spotted as non-local with its $\delta^{34}S$ value\(^{11}\), is also characterized by a non-local $^{87}\text{Sr}/^{86}\text{Sr}$, whereas other ecclesiastics exhibit values compatible with the local range (6/7 ecclesiastics). The other non-local individuals mostly consist of people from the less-privileged groups (1B, 2B, and 3B') (4 non-privileged for 9 non-local individuals).

**Zn isotope analyses.** As previously observed, Zn isotope ratios of dental enamel do not correlate with Zn concentrations\(^{7,18}\). This shows the absence of a mixing line between diagenetic and biogenic endmembers, and therefore argues for the absence of soil contamination\(^{75}\). Typical analytical uncertainties on $\delta^{66}\text{Zn}$ are 0.04‰. The cattle and sheep $^{66}\text{Zn}$ values range from 0.85 to 1.09‰ ($n = 3$). They are higher than the cat ($^{66}\text{Zn} = 0.67\%$) and the dog ($^{66}\text{Zn} = 0.50\%$) values, an observation which is in agreement with the trophic level effect observed in

| $\delta^{66}\text{Zn}$ | 2 SD | n |
|----------------------|------|---|
| NR_C 0.7091 | — | 1 |
| NR_NC 0.7099 | 0.0011 | 4 |
| NR_X 0.7094 | 0.0008 | 4 |
| R_C 0.7166 | 0.0011 | 21 |
| R_NC 0.7121 | 0.0002 | 2 |
| R_X 0.7115 | 0.0016 | 22 |

| $\delta^{15}\text{N}$ | 2 SD | n |
|---------------------|------|---|
| NR_C 12.4 | — | 1 |
| NR_NC 11.8 | 2.4 | 4 |
| NR_X 12.8 | 2.2 | 3 |
| R_C 13.3 | 2.0 | 21 |
| R_NC 12.2 | 1.8 | 2 |
| R_X 13.1 | 2.0 | 16 |

| $\delta^{13}\text{C}$ | 2 SD | n |
|---------------------|------|---|
| NR_C −19.5 | — | 1 |
| NR_NC −19.2 | 0.5 | 5 |
| NR_X −18.9 | 0.6 | 2 |
| R_C −19.3 | 0.6 | 21 |
| R_NC −18.7 | 0.1 | 2 |
| R_X −19.2 | 0.9 | 16 |

| $^{87}\text{Sr}/^{86}\text{Sr}$ | 2 SD | n |
|---------------------------|------|---|
| NR_C 0.7091 | — | 1 |
| NR_NC 0.7093 | 0.0011 | 4 |
| NR_X 0.7096 | 0.0008 | 4 |
| R_C 0.7116 | 0.0011 | 21 |
| R_NC 0.7121 | 0.0002 | 2 |
| R_X 0.7115 | 0.0016 | 22 |

Table 3. $Sr$, $Zn$, $C$ and $N$ isotope compositions of human teeth depending on the geographical origin of the individuals buried in the Dominican convent of Rennes, Brittany. Results are grouped according to the geographical origin of the humans, assessed from the $S$ and $Sr$ isotope compositions of their teeth. NR_C: coastal $\delta^{34}S$ and $^{87}\text{Sr}/^{86}\text{Sr} < 0.71$; NR_NC: non-coastal $\delta^{34}S$ and $^{87}\text{Sr}/^{86}\text{Sr} < 0.71$; NR_X: unknown $\delta^{34}S$ value and $^{87}\text{Sr}/^{86}\text{Sr} < 0.71$; R_C: coastal $\delta^{34}S$ and $^{87}\text{Sr}/^{86}\text{Sr} > 0.71$; R_NC: non-coastal $\delta^{34}S$ and $^{87}\text{Sr}/^{86}\text{Sr} > 0.71$; NR_X: unknown $\delta^{34}S$ value and $^{87}\text{Sr}/^{86}\text{Sr} > 0.71$. C stands for coastal, R for radiogenic, N for non-.
previous studies. The suckling pig has a $\delta^{66}$Zn value similar to that of carnivores ($\delta^{66}$Zn = 0.65‰). This observation is not surprising considering that (1) the written record of the hospital documents the feeding of the pigs with leftovers, which included fish and meat (2) we documented the high trophic level of those pigs in our previous study using C, N and S isotopes. The isotope ranges are consistent with previous observations in other terrestrial mammal food webs, as well as the trophic level offset between herbivores and omnivores/carnivores (0.35‰ in Rennes, 0.45‰ in terrestrial Kenyan mammal food web, 0.3‰ in Arctic marine mammal food web). Human $\delta^{66}$Zn values are generally lower than those for animals. Individuals showing $^{87}$Sr/$^{86}$Sr compatible with the local range have significantly lower Zn isotope ratios than those of non-local individuals (Table 2, normal distribution, two-tailed, t-test $p = 1 \times 10^{-7}$), whose $\delta^{66}$Zn values overlap with those of omnivorous/carnivorous mammals (Fig. 3). Contrary to what was previously observed for $\delta^{15}$N values, there are no differences between the Zn isotope ratios of men and women. Among the individuals who exhibit $^{87}$Sr/$^{86}$Sr compatible with the local range, we did not notice differences between socio-economic groups for both $\delta^{15}$N or $\delta^{66}$Zn values (Table 4). The $\delta^{66}$Zn values of the locals from Rennes overlap with values of modern humans from France, whereas non-local individuals have $\delta^{66}$Zn values similar to that observed in historical populations from South-Eastern France.

**Correlation between isotope tracers.** In order to investigate the influence of different food categories, including marine fish, on the isotope signatures of Sr and Zn, we explored the presence or absence of correlation between these isotope ratios and other dietary proxies (C, N and S stable isotope signature). Among the 54 human
overprint these original ratios. 66Zn values of the humans account for the peculiar isotope signature of the local Bretons from Rennes. The δ66Zn isotope signatures but non-coastal ecclesiastics, commoners and soldiers buried in mass graves (Fig. 4). Two individuals buried in the church have South-Eastern England or South-Eastern France. Strontium isotope data confirm a local origin of women and regions still had higher δ66Zn values coming from regions with igneous or sandstone bedrocks. Moreover, individuals coming from igneous inland bedrock indeed exhibited the highest Zn isotope ratios, but were not significantly different from the humans the associated bedrock (Supplementary Information 1). We found that humans coming from regions with chalky the group and the historical phase. Phase 1, 2 and 3 are described in the material section.

Zn and N isotope ratios of humans exhibiting local Sr isotope ratios depending on the socio-economic Table 4. Zn and N isotope ratios of humans exhibiting local Sr isotope ratios depending on the socio-economic group and the historical phase. Phase 1, 2 and 3 are described in the material section.

| Phase | Non-Privileged (B-B’) | Privileged (A, B’) | Nobles (A) | Non-Privileged (B-B’) | Ecclesiastics (C) | Soldiers (D) | Ecclesiastics (C) |
|-------|-----------------------|-------------------|-----------|-----------------------|------------------|--------------|------------------|
| Phase1| 0.39 0.08 2 13.0 — 1 | 0.41 0.02 2 13.5 0.6 2 | 0.35 0.30 4 12.7 1.8 4 | 0.13 0.10 2 12.4 2.0 2 | 0.41 0.28 2 12.5 1.8 2 | 0.13 0.10 2 12.4 2.0 2 | 0.41 0.10 4 13.6 0.4 4 |

Geographical origin of Rennes’ humans and animals. Two of the three herbivores analyzed exhibit 87Sr/86Sr higher than the expected local range according to the IRHUM database (Fig. 2), but such values exist in the surroundings of Rennes. In agreement with historical evidence, cattle and sheep were probably raised in the countryside, whereas dogs, cats and pigs were urban animals (Fig. 2). The local range defined by all domestic animal Sr isotope signatures therefore represents Rennes and the nearby countryside.

The local 87Sr/86Sr range defined here is compatible with the one predicted for the whole Armorican Massif, which is also the case for the local S isotope signatures. Individuals exhibiting S and Sr isotope signatures compatible with the local ranges are therefore likely to be originating from Brittany. Individuals showing lower 87Sr/86Sr Sr isotope signature are likely to come from sedimentary regions, such as the Parisian and Aquitaine Basins, South-Eastern England or South-Eastern France. Strontium isotope data confirm a local origin of women and nobles buried in the Dominican convent, as well as the presence of non-local individuals among the medieval ecclesiastics, commoners and soldiers buried in mass graves (Fig. 4). Two individuals buried in the church have high 87Sr/86Sr Sr isotope signatures but non-coastal δ34S values, suggesting they have spent their childhood out of Brittany.

Zn isotope ratios: a promising new dietary tracer?. The range of δ66Zn values measured in teeth showing Sr isotope ratios compatible with local signatures overlaps with that of modern (20th century) individuals but strongly differs from previous historical periods (17th to 19th centuries) (Fig. 5). However, individuals exhibiting Sr isotope signatures compatible with other rock types such as volcanic or sedimentary rocks - that is to say lower than the local range - have similar Zn isotope ratios to the abovementioned historical populations. A large part of the preindustrial teeth previously studied belonged to individuals coming from sedimentary regions (Supplementary Information 1). The Zn isotope composition of igneous rocks and clastic sediments is fairly constant (0.2 < δ66Zn < 0.4‰)13, but siliceous and calcareous sediments can show higher Zn isotope ratios13,21, especially in limestones, for which the δ66Zn values can reach up to 1.35‰22,23. To verify if the Zn isotope differences between individuals showing high 87Sr/86Sr and those having low 87Sr/86Sr ratios could be simply related to the Zn isotope composition of the bedrock, we compared the Zn isotope ratios of all human historical teeth from this study and a previous one on Zn isotope ratios in teeth of French individuals7, to the expected values of the associated bedrock (Supplementary Information 1). We found that humans coming from regions with chalky bedrock indeed exhibited the highest Zn isotope ratios, but were not significantly different from the humans coming from regions with igneous or sandstone bedrocks. Moreover, individuals coming from igneous inland regions still had higher δ66Zn values than the local Bretons from this study (Supplementary Information 1). If the geology can have an impact on local human teeth ratios3,4, it seems that biological processes in soils tend to overprint these original ratios.

Based on the absence of relationship between Zn isotope ratios of teeth and geology, as well as the established difference between δ66Zn values of herbivores and omnivores/carnivores1,2,4, diet is the most likely factor to account for the peculiar isotope signature of the local Bretons from Rennes. The δ66Zn values of the humans are clearly lower than that of the associated fauna, including the cat and the dog, and overlap those of modern
individuals (carnivores, Figs 3, 5). As mentioned above, this low Zn isotope ratio could be the signature of a substantial animal product consumption of high trophic level, which would explain the lower Zn isotope signatures than the local carnivores. The French historical populations, which have been previously studied, characterized by high Zn isotope ratios, were workers coming from inland regions (e.g. French Alps, Rhone Valley, Jura, Pyrenees,
Vosges), with limited access to fish consumption\(^7\),\(^25\). Whereas, Rennes’s individuals are likely to have had a frequent meat and fish consumption: nobles and middle class to display their wealth, ecclesiastics to respect fasting rules, but also urban workers who were complaining of too much salted fish consumption\(^15\). The Bretons were also known to have a substantial meat consumption relative to human populations from other French regions\(^12\). Therefore, high \(\delta^{66}Zn\) values of non-local individuals, mostly buried out of the convent walls and therefore likely to be commoners, could be explained by a cereal-based diet. Cereals, as most plant foods, have indeed much higher \(\delta^{66}Zn\) values than animal products\(^2\), and were the main dietary source of the non-privileged French population of that time\(^12\), especially outside of Brittany\(^15\). To explain the fact that Rennes humans exhibit lower Zn isotope ratios than dogs and cats, the existence of high trophic level food consumption, namely carnivorous fishes and/or suckling pigs, must be invoked. The pigs from the refuse dump of Rennes were bred in a hospital yard and fed with leftovers which daily included meat and fish\(^20\). Zinc isotope studies in archeological contexts being in their infancy, it is not possible to say at this stage if different signatures of freshwater or marine fish consumption can be expected. The absence of a correlation between carbon isotope ratios and \(87\text{Sr}/86\text{Sr}\) values documents a weak contribution - albeit existing, according to zooarcheological evidence - of marine fish in Rennes human diets. We documented previously very high \(\delta^{15}N\) values in tooth and bone collagen of humans and animals, and could not conclude if these high N isotope ratios were more due to the consumption of herbivorous mammals, suckling animals or eels\(^11\). Given the fact that eels are migratory aquatic organisms and constitute 45% of the fish remains found in the refuse midden close to the convent as well as the refectory soil\(^20,26\) and that suckling pigs were a sought-after food at that time\(^11\), the Zn isotope ratios could reflect the signature of a substantial eel and pork consumption. Animal product consumption in Rennes’ diets is in every instance more important than in those of the historical French populations previously studied\(^12,15,16,25,26\), which explains the low Zn isotope ratios.

**Absence of correlation between dietary tracers.** If both Zn isotope ratios and N isotope ratios are indicators of the trophic level effect, one should expect a correlation between these two tracers. This correlation exists when all species are considered together (Fig. 3B), but is absent among human values. This pattern has already been observed in marine mammals\(^3\): an interspecific correlation between \(\delta^{66}Zn\) and \(\delta^{15}N\) values was reported but did not exist between individuals from the same species. Zn isotope ratios were measured in tooth enamel, whereas N isotope ratios were measured in the dentine. These two dental hard tissues with different formation times could therefore record different diets. We however reject a change of diet between the time of formation of the dental enamel and dentine sampled as being the explanation of the absence of correlation between \(\delta^{66}Zn\) and \(\delta^{15}N\) values, since the lack of correlation has already been reported for bones and blood\(^18,27\).

The two isotope tracers are therefore both influenced by trophic level, but other dietary factors may influence one of the isotope ratios and not the other one. For example, N isotope ratios, measured in collagen, mostly provide information on the protein portion of the diet\(^28\), whereas Zn, measured in bioapatite, is likely to reflect the bulk diet – albeit mostly the animal product portion. A trophic level effect is also observed in breastfeeding individuals\(^29\) for N isotope signatures but is not expected for Zn isopotes: the M1 milk teeth - which form their enamel during the first year of life - that we measured in this study, and that has also been previously analyzed\(^7\) did not differ from M2 and M3 permanent teeth. Moreover, milk products do not show depleted Zn isotope signatures compared to meat or fish\(^7\). Finally, environmental factors are likely to differentially influence the local Zn and N isotope signatures of the soils and plants\(^4,13,20,31\), which could account for the lack of correlation between these two dietary indicators.
Diet, mobility and social status. Colleter et al.\textsuperscript{11} reported dietary differences related to social status for N isotopes, although they were more obvious among adults (bone values) than during childhood (dentine values). When the whole population is considered together, dietary and mobility differences can also be observed for Zn isotopes relative to the burial location: individuals buried in the church and the chapels (groups A and B) are, in general, more local and to also have a diet richer in animal products (according to δ\textsuperscript{66}Zn and δ\textsuperscript{15}N values, Figs 3 and 4) than individuals buried in the exteriors. However, when only individuals with local 87Sr/86Sr ratios are considered, the isotope differences between burial locations disappear for δ\textsuperscript{66}Zn and δ\textsuperscript{15}N values (Fig. 4, Table 2). In the present study, most of the non-local individuals were buried in the exteriors. It is therefore difficult to know whether social status isotope differences are due to dietary or environmental factors, or if it is related to a sample size bias. Nevertheless, as mentioned before, a substantial animal product consumption of the urban workers, likely to be buried in the exteriors, is consistent with historical writings\textsuperscript{15}. A gender difference is clearly existing for N isotopes in teeth\textsuperscript{11} but not for Zn isotopes. A female diet including a significant proportion of young animals but a similar amount of animal products relative to the male diet could explain such an isotopic pattern.

Conclusions

The Zn stable isotope compositions measured in the teeth of individuals buried in the Dominican convent of Rennes show a remarkable pattern: (1) local privileged individuals exhibit overlapping values with modern individuals, which could be explained by substantial meat and/or fish consumption (2) individuals identified as migrants using Sr isotopes have Zn isotope ratios similar to those of poor French individuals previously analyzed from the 17\textsuperscript{th} to the 19\textsuperscript{th} centuries. This is partially explained by a limited influence of the geology on the Zn isotope composition of food products eaten during the childhood combined with a reduced meat consumption of migrating individuals. Local Rennes humans exhibit lower Zn isotope ratios than the associated fauna, including carnivores, which can possibly be explained by carnivorous fish and pork consumption. Fasting rules indeed imposed the consumption of fish a day out of three in medieval and early modern Western Europe. Given the historical, zooarcheological and other isotope data obtained from Rennes Dominican’s convent, this carnivorous fish consumption could mostly consist of codfish and eels. Zn isotopes have therefore a strong potential to trace the consumption of high trophic level food, and potentially fish, in ancient human diets.

Methods

Material. Rescue excavations in the city center of Rennes recently permitted the study of the implantation and evolution of a mendicant convent which has been described by Le Cloirec (2016)\textsuperscript{35}. The Dominican convent was founded outside the walls of the city in 1368. Between the end of the 14\textsuperscript{th} and the 18\textsuperscript{th} century, the settlement was an important place of pilgrimage – because of the presence of a “miraculous painting” in the convent – and burial, especially for the parliamentary nobility\textsuperscript{14,15,32}. A multi-isotope study previously documented the diet of this population\textsuperscript{11}. Three phases of burial are differentiated on the site. The first phase (13\textsuperscript{th} c., phase 1) predates the construction of the convent. The second (phase 2) goes from the end of the 14\textsuperscript{th} century to the 16\textsuperscript{th} century. The last period (phase 3) covers the 17\textsuperscript{th} and 18\textsuperscript{th} centuries and therefore corresponds to the modern period. Additional information is available in the Table S1 of the Supplementary data. In total, 54 human molars (M2 and M3) were sampled. The faunal remains (16\textsuperscript{th} century, end of the phase 2, Fig. 1) consist in the most common species found in the refuse midden of the hospital concomitant and contemporaneous to the convent.

Methods. All the analyses were conducted in the laboratories of the Department of Human Evolution at the Max Planck Institute for Evolutionary Anthropology (MPI-EVA) in Leipzig, Germany, in accordance with approved guidelines and regulations.

The samples were mechanically cleaned using a dental drill equipped with a diamond tip. Two small pieces were sampled (5–20 mg). Dentine was removed with a diamond-tipped burr. For Sr isotope analyses, samples were digested in nitric acid and purified using the ion exchange method described in Maréchal et al. (1999)\textsuperscript{35}. Each batch of preparation included 13 samples. One blank and one external standard SRM 1486 (bone meal) were analyzed to verify that no contamination or purification problem happened during the column chromatography. For Zn isotope analyses, samples were digested in hydrochloric acid (HCl), evaporated and dissolved in hydrobromic acid (HBr, 1.5 M). Each batch of preparation included a blank and an external standard (in house standard AZE bone powder and/or the SRM 1400 bone ash, Table S4). Zinc was then purified using a protocol adapted from Moynier et al. (2006)\textsuperscript{34} previously described in Jaouen et al. (2016)\textsuperscript{35}. The Sr and Zn isotope analyses were conducted using a Thermo Fisher Neptune MC-ICP-MS at the Max Planck Institute for Evolutionary Anthropology (Leipzig, Germany). δ\textsuperscript{66}Zn values are expressed relative to the standard JMC-Lyon. External reproducibility is 0.04% for δ\textsuperscript{66}Zn values and 0.000024 for Sr ratios (1 SD). The protocol followed for isotopic and concentration analyses was already described for Sr\textsuperscript{35,36} and for Zn\textsuperscript{35}.

Data availability statement. All data generated or analyzed during this study are included in this published article and the Supporting Information file.

References

1. Van Heghe, L., Engström, E., Rodushkin, I., Cloquet, C. & Vanhaecke, F. Isotopic analysis of the metabolically relevant transition metals Cu, Fe and Zn in human blood from vegetarians and omnivores using multi-collector ICP-mass spectrometry. J. Anal. At. Spectrom. 27, 1327–1334 (2012).
2. Costas-Rodríguez, M., Van Heghe, L. & Vanhaecke, F. Evidence for a possible dietary effect on the isotopic composition of Zn in blood via isotopic analysis of food products by multi-collector ICP-mass spectrometry. Metallomics 6, 139–146 (2014).
3. Jaouen, K., Pons, M. -L. & Balter, V. Iron, copper and zinc isotopic fractionation up mammal trophic chains. Earth Planet. Sci. Lett. 374, 164–172 (2013).
4. Jaouen, K., Beasley, M., Schoeninger, M., Hublin, J.-J. & Richards, M. P. Zinc isotope ratios of bones and teeth as new dietary indicators: results from a modern food web (Koobi Fora, Kenya). Sci. Rep. 6 (2016).
5. Jaouen, K., Szpak, P. & Richards, M. P. Zinc isotope ratios as indicators of diet and trophic level in arctic marine mammals. PloS One 11, e0152299 (2016).
6. Lønnerdal, B. O. Dietary factors influencing zinc absorption. J. Nutr. 130, 1378–1383 (2000).
7. Jaouen, K., Hersch, E. & Balter, V. Copper and zinc isotope ratios in human bone and enamel. Am. J. Phys. Anthropol. 162, 491–500 (2017).
8. Aymard, M. Les pratiques de l'alimentation carnée en France. in Le mangeur et l'animal, Mutation de l'élevage et de la consommation. Autrement, Paris. 172p (Pallat M., 1997).
9. Cloquet, C., Carignan, J., Libourel, G. Isotopic composition of Zn and Pb atmospheric depositions in an urban/periurban area of northeastern France. Environ. Sci. Technol. 40, 6594–6600 (2006).
10. John, S. G., Park, J. G., Zhang, Z. & Boyle, E. A. The isotopic composition of some common forms of anthropogenic zinc. Chem. Geol. 245, 61–69 (2007).
11. Colleter, R. et al. Social status in late medieval and early modern Brittany: insights from stable isotope analysis. Anthropological and Archaeological Sciences (in press). https://doi.org/10.1007/s12520-017-0547-9.
12. Quellier, F. La table des Français: une histoire culturelle (XVe-début XIXe siècle). 274p. (PU Rennes, 2007).
13. Moynier, F., Vance, D., Fujii, T. & Savage, P. The isotope geochemistry of zinc and copper. Rev. Mineral. Geochem. 82, 543–600 (2017).
14. Colleter, R. et al. Procedures and Frequencies of Embalming and Heart Extractions in Modern Period in Brittany. Contribution to the Evolution of Ritual Funerary in Europe. PloS One 11, e0167988 (2016).
15. Croix, A. La Bretagne aux 16e et 17e siècles: la vie, la mort, la foi. 2, 571 p. (Maloine, 1981).
16. Williams, M. et al. The IRHUM (Isotopic Reconstruction of Human Migration) database. Earth Syst. Sci. Data 6, 117 (2014).
17. Nehlich, O. The application of sulphur isotope analyses in archaeological research: a review. Earth-Science Reviews 142, 1–17 (2015).
18. Jaouen, K. et al. Fe and Cu stable isotopes in archeological human bones and their relationship to sex. Am. J. Phys. Anthropol. 148, 334–340 (2012).
19. Reynard, B. & Balter, V. Trace elements and their isotopes in bones and teeth: Diet, environments, diagenesis, and dating of archeological and paleontological samples. Palaeogeogr. Palaeoclimatol. Palaeoecol. 416, 4–16 (2014).
20. Clavel, B. Données archéozoologiques et fouilles d’hôpitaux: l’exemple de l’hôpital sainte Anne (Rennes, Ille-et-Vilaine). In Les établissements hospitaliers en France du Moyen Âge au XIXe siècle. 393p. (Le Clech-Charlton S., 2010).
21. Cloquet, C., Carignan, J., Lehmann, M. F. & Vanhaecke, F. Variation in the isotopic composition of zinc in the natural environment and the use of zinc isotopes in biogeosciences: a review. Anal. Bioanal. Chem. 390, 451–663 (2008).
22. Luck, J. M., Ben, O. D., Albarede, F. & Telouk, P. Zn and Cu Isotopes Tracers of Metal Origin in the Dissolved and Particulate Loads of Rain. Geochim Cosmochim Acta 71(1999).
23. Pichat, S., Douchet, C. & Albarede, F. Zinc isotope variations in deep-sea carbonates from the eastern equatorial Pacific over the last 175 ka. Earth Planet. Sci. Lett. 210, 167–178 (2003).
24. Fekiaccova, Z., Cornu, S. & Pichat, S. Tracing contamination sources in soils with Cu and Zn isotopic ratios. Sci. Total Environ. 517, 96–105 (2015).
25. Herrsch, E., Bocherens, H., Valentin, F. & Colardelle, R. Comportements alimentaires au Moyen Âge à Grenoble: application de la biogéochimie isotopique à la nécropole Saint-Laurent (XIIe–XVe siècles, Isère, France). Comptes Rendus Académie Sci.-Ser. III-Sci. Vie 324, 479–487 (2001).
26. Le Cloirec, G. Étude archéologique du couvent des jacobins de Rennes (35), du quartier antique à l’établissement religieux: rapport final d’opérations archéologique. 3835 p (Excavation report) (2016).
27. Jaouen, K. et al. Is aging recorded in blood Cu and Zn isotopic compositions? Metallomics 5, 1016–1024 (2013).
28. Hedges, R. E. M. & Reynard, L. M. Zinc isotopes and the trophic level of humans in archaeology. J. Archaeol. Sci. 34, 1240–1251 (2007).
29. Fogel, M. L., Tuross, N. & Owsley, D. W. Nitrogen isotope tracers of human lactation in modern and archaeological populations. Carnegie Inst. Wash. Yearb. 88, 111–117 (1989).
30. Stevens, R. E. & Hedges, R. E. M. Carbon and nitrogen stable isotope analysis of northwest European horse bone and tooth collagen, 40,000BP–present: Palaeoecological interpretations. Quat. Sci. Rev. 23, 977–991 (2004).
31. Drucker, D. G., Bocherens, H. & Billiou, D. Evidence for shifting environmental conditions in Southwestern France from 33 000 to 15 000 years ago derived from carbon-13 and nitrogen-15 abundances in collagen of large herbivores. Earth Planet. Lett. 216, 163–173 (2003).
32. Bordeaux, C. Mois de Bordeaux: journal d’un bourgeois de Rennes au 17ème siècle. 253 p. (Apogee, 1992).
33. Deniel, C. & Pin, C. Single-stage method for the simultaneous isolation of lead and strontium from silicate samples for isotopic measurements. Anal. Chim. Acta 426, 95–103 (2001).
34. Moynier, F., Albarelle, F. & Herzog, G. F. Isotopic composition of zinc, copper, and iron in lunar samples. Geochim. Cosmochim. Acta 70, 6103–6117 (2006).
35. Britton, K., Grimes, V., Dau, J. & Richards, M. P. Reconstructing faunal migrations using intra-tooth sampling and strontium and oxygen isotope analyses: a case study of modern caribou (Rangifer tarandus granti). J. Archaeol. Sci. 36, 1163–1172 (2009).
36. Hartman, G. & Richards, M. P. Zinc isotope ratios of bones and teeth as new dietary indicators: results from a modern food web (Koobi Fora, Kenya). Sci. Rep. 6 (2016).
