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Quantifying the evolution of animal dairy intake in humans using calcium isotopes

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ABSTRACT

The contribution of dairy products to modern human diets has a debated role in the expansion of Neolithic economies and the dynamics of demographic transitions. While current methods allow discussing dairy production and processing, no approach allows reconstructing quantitatively its effective consumption. Calcium isotopes ($\delta^{44/42}$Ca) potentially represent such a marker due to the abundance of isotopically fractionated Ca in dairy products. Here, we test Ca isotope sensitivity to dietary intake of dairy product: we first used a dietary model based on a compilation of available data of dietary Ca sources; we then compared the modelled outputs to available and newly acquired skeletal $\delta^{44/42}$Ca values of individuals from populations with documented and markedly distinct dairy consumption habits. Our model predicts a marked decrease of skeletal $\delta^{44/42}$Ca values with dairy Ca intake. We measure a significant difference in $\delta^{44/42}$Ca values between populations eating no dairy products and those with high proportions of dairy Ca in their diet. The average dairy Ca intakes inferred by the model agree well with the documented dietary habits of these populations. Finally, we observe a significant drift across Neolithic and Iron Age periods up to modern times in societies producing dairy, contrasting with the populations consuming no dairy products. This trend marks a dramatic change in the utilization of dietary Ca. Given the available data, our model supports a marked increase in dairy intake starting from the late Neolithic period in Western Europe. Calcium isotopes could yield significant insight into the evolutions of dairy product intakes and the biocultural revolutions experienced by Neolithic populations.

1. Introduction

The neolithization processes cover the progressive expansion of agriculture and livestock farming over previously dominating hunting-gathering subsistence modes. These came with major dietary changes as well as radical modifications of the relationships the societies had with their environment (e.g., Vigne, 2011). The development of the production, the processing and the consumption of domesticated animal milk played a major yet debated role in the expansion of Neolithic lifestyles and subsequent economies (Vigne, 2011; Vigne and Helmer, 2007; Gerbault et al., 2011; Leonardi et al., 2012; Cramp et al., 2014; Debono Spiteri et al., 2016; Ethier et al., 2017; Cook, 2014; Gillis et al., 2017).

The benefits of consuming dairy products are potentially numerous and include: the provision of energy and amino acids

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through carbohydrates, lipids or proteins; the intake of electrolytes, vitamin D and uncontaminated water through consumption of fresh milk; or the year-long availability of transportable high-energy foodstuffs such as cheese (e.g., Ségurel and Bon, 2017). However, the consumption of dairy products is investigated for its role in the expansion of Neolithic lifestyles and populations (e.g., Debono Spiteri et al., 2016; Ethier et al., 2017; Gillis et al., 2017), the changing human ecology and demographic transitions experienced by Neolithic populations (e.g., Vigne, 2011; Bocquet-Appel, 2011; O'Brien and Laland, 2012) or through genetic studies examining the spread of mutations responsible for lactase persistence, enabling adults to digest lactose from milk (e.g., Leonardi et al., 2012; Ségurel and Bon, 2017).

The existing approaches for studying the evolution of dairying mainly focus on the production and processing of animal milk. Archaeozoological assemblages, and in particular slaughter age profiles of domesticated ungulates, together with traditional stable isotopes, allow to document the management of herds, dedicated for instance to meat and/or milk production (Vigne and Helmer, 2007; Balasse and Tresset, 2002; Balasse et al., 2017). The identification and stable isotope compositions of dairy lipid and protein residues permit tracking dairy production when recovered on ceramic shards (Copley et al., 2003; Copley et al., 2005a, 2005b; Evershed et al., 2008; Salque et al., 2013; Cubas et al., 2020) and sometimes its consumption when found in dental calculus (Warinner et al., 2014).

These approaches help characterize the production and use of animal milk, in a rather qualitative manner, but do not allow to quantify the actual proportion of dairy in the total diet of ancient populations. The stable isotopes of Ca have this potential for several reasons.

i. The Ca isotope compositions of mammal bones are primarily controlled by dietary Ca, with an average offset of $\delta^{44/42}$Ca values from diet to bone of -0.54 ± 0.08‰ (95% confidence interval of Student's *t*-test, noted 95% c.i. thereafter) as measured in 6 different species of mammals (Skulan and DePaolo, 1999; Chu et al., 2006; Hirata et al., 2008; Tacail et al., 2014; Heuser et al., 2016). As a result, human bone $\delta^{44/42}$Ca values are expected to reflect the average isotope compositions of their diet.

ii Animal milk is a major source of dietary Ca. For instance, the Ca concentration in the milk of ungulates lies between 1000 and 2000 mg/L (1220, 1930 and 1340 mg/L for cow, sheep and goat milk respectively, c.f. Pereira, 2014) whereas the production of cheese leads to even higher Ca contents, typically ranging from 3000 to 12000 mg/kg depending on the type of processing (Zamberlin et al., 2012). Moreover, the Ca from dairy products is particularly easily assimilated by consumers when compared with other types of food (Guéguen and Pointillart, 2000; Rozenberg et al., 2016). Therefore, even minor dairy consumption contributes significantly to the total dietary Ca.

iii The Ca isotope composition of mammal milk and derived dairy products are systematically depleted in heavy isotopes by about -0.60‰ when compared to dietary sources of lactating females, as described in humans, cows and ewes (Chu et al., 2006; Tacail et al., 2017; Tacail et al., 2019 and Table S1). When compared to plants, which display the second lowest $\delta^{44/42}$Ca average value among main dietary Ca sources (Figure S1), the consumption of dairy products is thus expected to induce a significant shift of the consumer's bone $\delta^{44/42}$Ca values of up to -0.60‰.

iv Calcium constitutes around 40% of the mineral phase of the skeleton (LeGeros and LeGeros, 1984), making it poorly sensitive to diagenesis (Tacail et al., 2019; Melin et al., 2014; Martin et al., 2017; Hassler et al., 2018) and especially adapted to sampling of minute amounts of potentially precious material (less than 100 μg hydroxyapatite required, see for instance Tacail et al., 2017; Tacail et al., 2019; Tacail et al., 2016; Li et al., 2016).

For these reasons, Chu et al. (2006) proposed that Ca isotopes could be of use to either track the consumption of maternal milk by infants or the consumption of animal dairy products by human populations. For example, breastfeeding practices could be inferred using tooth enamel in modern humans and ancient hominins (Tacail et al., 2017, 2019). However, the effect of the consumption of animal dairy products was inconclusively tested in a previous publication (Reynard et al., 2011), where the focus was the difference in Ca isotope composition between archaeological human populations and associated fauna remains from Near East Mesolithic, early Neolithic and British Iron Age sites. Despite possible but non-significant trends, the observations led the authors to the conclusion that Ca isotopes were unlikely to help study the rise in dairy Ca consumption during the Neolithic.

In this study, in order to assess the sensitivity of Ca isotopes to the consumption of dairy products on a broader scale, we adopt a complementary population approach combined with modelling. Using an updated compilation of literature data, we present a dietary mixing model to assess the expected distributions of bone isotope compositions as a function of the proportion of Ca from dairy products in total diet. The outputs of these simulations are compared to the skeletal Ca isotope compositions from the literature ($n = 130$) as well as newly acquired data ($n = 85$) from a wide range of archaeological and modern human populations that exhibit contrasting dairy consumption habits according to archaeological and ethnographic data (see Table 1 and details in SI). We first compile and measure Ca isotope compositions from prehistoric, protohistoric and modern populations known to consume no dairy products (Group 1, $n = 74$, listed and described in Table 1), as well as from modern populations known to consume significant amounts of dairy products (Group 2, $n = 56$). This dataset is used to test the dietary mixing model presented in this study. Finally, we compare the modelled outputs to the Ca isotope data from Near East and European Neolithic and Iron age populations for which dairying is possible or documented, but effective consumption is not quantified (Group 3, $n = 85$). This allows us to assess the sensitivity of Ca isotopes to a potential time-dependent evolution of the dairy products intake in populations practising livestock farming.

2. Material & methods

2.1. Modelling Ca isotope compositions of diet and consumer's bone

We developed a simple broad model to estimate the distribution of diet $\delta^{44/42}$Ca values (5, 25, 50, 75 and 95% quantiles) as a function of the proportion of Ca taken from dairy products.

First, we compiled literature data in order to estimate the isotope compositions of main Ca dietary sources, namely drinking water, marine resources (crustaceans and molluscs, fish), meat (herbivores blood and soft tissues), eggs, plants (vegetables and fruits, cereals) and dairy products (Figure S1, Table S2). We choose to make no assumption as to what is the predominant type of plant foodstuffs in the non-dairy Ca budget and we thus consider a distribution of $\delta^{44/42}$Ca values representative of a balanced mix between dicotyledon roots, stems, leaves and fruits as well as monocotyledons such as cereals. This compilation also regroups Ca isotope compositions from a broad range of geological contexts in order to account for the variability of mineral Ca isotope

Table 1
Description of the three groups of populations with contrasted dairy product consumption habits (details in methods and SI text) with the following codes: *n* is the number of individuals with measured skeletal Ca isotope composition (together with the literature reference of the dataset, * referring to this study); Tissue is either bone (B) or tooth enamel (E); Period abbreviations stand for Mesolithic (Meso.), Epipalaeolithic (Epipal.), Neolithic (Neo., with PPNB standing for Pre-pottery Neolithic B, LBK standing for Linearbandkeramik or Linear Pottery Culture); Geology column presents the main represented regional surface bedrock types and lithologies: S: Sedimentary, V: Volcanic, M: Metamorphic, P: Plutonic, C: Carbonates, D: Detrital. Literature reference are given in brackets.

Site	*n indiv.*	Tissue	Period	Date range	Lifestyle	Geology
Group 1: No dairy consumption *(No domesticated dairy producers)*						
Abu Hureyra 1, Syria	12 (Reynard et al., 2010)	B	Meso.	13.9–12.7 kyr cal BP	Hunting-Gathering (Moore and Hillman, 1992; Moore et al., 2000; Hillman et al., 2001)	S (C) (Moore et al., 2000)
Aveline's Hole, UK	1 (Reynard et al., 2010)	B	Meso.	10.5–9.5 kyr cal BP	Hunting-Gathering (Schulting and Richards, 2000; Schulting and Wysocki, 2002; Meiklejohn et al., 2011)	S (C, D) (British Geological Survey, 1999)
Ogof yr Ychen, UK	1 (Reynard et al., 2010)	B	Meso.	9.6–7.5 kyr cal BP	Hunting-Gathering (Meiklejohn et al., 2011; Schulting and Richards, 2002)	S (C, D) (British Geological Survey, 1983)
Taforalt, Morrocco	6 (Reynard et al., 2010)	B	Epipal.	>12.9 kyr cal BP	Hunting-Gathering (Reynard et al., 2011; Barton et al., 2007; Bouzouggar et al., 2007; d'Errico et al., 2009; Mariotti et al., 2009)	S (C) (Bouzouggar et al., 2007)
Téviec, France	3 (Reynard et al., 2010)	B	Meso. (Late)	7.5–4.25 kyr cal BP	Hunting-Gathering (Schulting, 1996; Schulting and Richards, 2001; Dupont et al., 2009)	P and M (Cagnard, 2009)
Totty Pot, UK	1 (Reynard et al., 2010)	B	Meso.	9.3–9 kyr cal BP	Hunting-Gathering (Schulting et al., 2010)	S (C, D) (British Geological Survey, 1999)
Worm's Head, UK	1 (Reynard et al., 2010)	B	Meso.	11.2–9.6 kyr cal BP	Hunting-Gathering (Meiklejohn et al., 2011)	S (C, D) (British Geological Survey, 2002)
Talasiu, Kingdom of Tonga	20*	B	Late Lapita	2.65 kyr cal BP	Fishing-Gathering-Horticulture (Valentin et al., 2020; Herrscher et al., 2018; Clark et al., 2015)	S (C), V (Herrscher et al., 2018)
Melanesians, New Caledonia	23*	B	Modern	ca. 0.1 kyr BP	Horticulture, Chicken (Dubois, 1984; Murdock, 1967; Djama, 1999)	V, M, S (Paris, 1981)
Baka, Cameroon	6*	E	Modern	- 0.5 kyr BP	Hunting-Gathering (Reyes-García et al., 2018; Duda, 2017)	M, P (Van den Hende and Van den Hende, 1970)
Group 2: High dairy consumption *(Significant intake of Ca from dairy)*						
Spitalfields Crypt, London, UK	11 (Reynard et al., 2013)	B	Modern	ca. 0.15 kyr BP	Agriculture (Industrializing) (Reynard et al., 2013; Nitsch et al., 2010, 2011)	S (D, C) (British Geological Survey, 2006)
Hôtel Dieu Coll., Lyon, France	36*	B	Modern	ca. 0.1 kyr BP	Agriculture (Industrializing) (Toutain, 1971; Flandrin and Montanari, 1996; Heyberger, 2009, 2014)	S (C, D), M, P (Saint Martin, 2007)
France	9 (Tacail et al., 2017)	E	Modern	- 0.5 kyr BP	Agriculture (Industrialized) (Tacail et al., 2017)	S (C, D), M, P (Chantraine et al., 2003)
Group 3: Unknown or documented but unquantified dairy consumption *(Presence of domesticated dairy producers and/or evidence of dairy production)*						
Abu Hureyra 2, Syria	28 (Reynard et al., 2010)	B	Neo. (PPNB)	10.8–7.9 kyr cal BP	Farming onsets (Moore and Hillman, 1992; Moore et al., 2000; Hillman et al., 2001)	S (C) (Moore et al., 2000)
Asikli Hoyuk, Turkey	12 (Reynard et al., 2013)	B	Neo. (PPNB)	10.5–9.5 kyr cal BP	Farming onsets (Reynard et al., 2013; Pearson et al., 2010)	V (Stiner et al., 2014)
Balatonszarszo, Hungary	12 (Reynard et al., 2010)	B	Neo. (LBK)	7.3–6.9 kyr cal BP	Farming (Oross et al., 2008; Oross and Bánffy, 2009; Reynard and Hedges, 2008; Whittle et al., 2013)	S (D, C) (Whittle et al., 2013)
Bouqras, Syria	1 (Reynard et al., 2010)	B	Neo. (Late PPNB)	9.5–8.5 kyr cal BP	Farming onsets (Merrett and Meiklejohn, 2007)	S (Merrett and Meiklejohn, 2007)
Danebury, UK	9 (Reynard et al., 2010)	B	Iron Age	2.5–2 kyr cal BP	Farming (Copley et al., 2005a; Copley et al., 2005b; Cunliffe et al., 1984)	S (C) (Cunliffe and Poole, 1991)
Magura, Romania	8 (Reynard et al., 2010)	B	Neo. (Balkanic)	8-7 kyr cal BP	Farming (Evershed et al., 2008; Howard et al., 2004; Bailey et al., 2002)	S (D, C) (Howard et al., 2004; Burchfiel and Bleahu, 1976)
Ensisheim (Octrois, Ratfeld), France	15 (Hamilton et al., 2013)	B	Neo. (LBK)	uncert. 7-6 kyr cal BP	Farming (Hamilton et al., 2013; Bickle et al., 2013)	S (C, D) (Skrzypek et al., 2008)

compositions that could explain differences in local baseline $\delta^{44/42}$Ca values. This is notably the case for the two main non-dairy Ca sources that are drinking water and plants. Furthermore, the variability of $\delta^{44/42}$Ca values induced by local catchment lithology is expected to be rather moderate, previous compilations showing that the main rock types display close average $\delta^{44/42}$Ca values, notably −0.21 ± 0.18‰ (1SD, n = 1301) for carbonates, −0.04 ± 0.13‰ (1SD, n = 153) for silicates, as well as −0.03 ± 0.10‰ (1SD, n = 33) in soil porewater (Fantle and Tipper, 2014). We thus can expect geologically induced variations of dietary sources $\delta^{44/42}$Ca average baselines of the order of 0.20‰.

Second, we estimated the average relative contributions of these different Ca dietary sources to the non-dairy Ca budget. To this end, we considered the data from a thorough survey of total French modern diet (Leblanc et al., 2004) as an actualistic approach (Table S2). Using energy budgets and average Ca source contributions as estimated for Palaeolithic and Neolithic populations in Eaton and Nelson, 1991, we also estimated 3 different settings of Ca source contributions (in % of total non-dairy Ca, Table S2). We calculated the absolute amount and relative contribution of daily Ca intake considering three different plant:animal subsistence ratios (80:20, 35:65 and 50:50 in wt. %) and considering an average Ca concentration in plant and animal foodstuffs (Eaton and Nelson, 1991). We also considered a baseline intake of Ca from drinking water of 100 mg/d (1L of water per day with average 100 mg/L Ca concentration or 2L for 50 mg/L).

Third, we calculated the distributions of $\delta^{44/42}$Ca values as a function of the fraction of Ca from dairy products in the total dietary Ca budget. This calculation is based on a mixing model equation (as described for instance in Albarède, 1995) which gives:

$$\delta^{44/42}Ca \simeq \sum_{j=1}^{n} \phi_j \times \delta^{44/42}Ca_j$$

where ϕ_j is the mass fraction of Ca brought into dietary Ca budget by foodstuff j.

Finally, considering an average diet to bone offset of −0.54‰ (−0.54 ± 0.08, 95% c.i., average as calculated for 6 different mammal species from Skulan and DePaolo, 1999; Chu et al., 2006; Herrscher et al., 2018; Tacail et al., 2014; Heuser et al., 2016), we presented the output of the diet model in $\delta^{44/42}$Ca as expected in bone of the consumer.

2.2. Material

In addition to the compilation of available bone Ca isotope data (130 individuals from 15 sites, Tacail et al., 2017a; Reynard et al., 2013; Reynard et al., 2010; Hamilton et al., 2013), we sampled bone and late forming enamel of 85 individuals from 4 different populations with various documented lifestyles and dairy products consumption habits. We consider here the mineralized tissues of individuals that are old enough in order to lie beyond the possible influence of breastfeeding (e.g., Reynard et al., 2013; Tacail et al., 2017a).

All the populations of the dataset were divided into three main groups with respect to their documented dairy product consumption (Table 1): a first group (Group 1) of Mesolithic, Neolithic, historic and modern populations known to consume no dairy products, a second group (Group 2) of historical and modern populations known to consume a significant amount of dairy products and finally a third group (Group 3) of Near East and European Neolithic and Iron age populations for which dairying is possible or documented but not quantified.

2.2.1. Group 1 of populations consuming no dairy products

The group 1 consists of individuals consuming no dairy products, whose lifestyles do not imply the use of domesticated animal milk.

We analysed the bone isotope compositions of 20 individuals from the Talasiu site (Tongatapu, Kingdom of Tonga, described in Valentin et al., 2020 and Table S3). This site, which dates back to 2650 cal BP, corresponds to a Late Lapita settlement of Polynesia colonizers known to rely on a mix of marine and terrestrial resources, to practice horticulture and making use of domesticated chicken (Herrscher et al., 2018; Clark et al., 2015). This group made no use of dairy producing animals since these animals are not present under wild nor domesticated form in Polynesia prior to European arrivals in the region.

We took bone samples from 23 adult Melanesian individuals (New Caledonia natives) from the historical Guépy Collection (1881) curated at the Centre de Conservation et d'Etude des Collections (CCEC), Musée des Confluences of Lyon, France (Table S4). These New Caledonian individuals died before 1881. Cattle began to be raised, for meat mainly, by colonist communities who settled in the archipelago after the French colonization in 1853. Melanesian communities did not make significant use of cattle before the 1950's (Dubois, 1984). Given the political and historical context of this period, the consumption of dairy products by 19th century New Caledonia Melanesians was certainly anecdotal if not nonexistent. Melanesian native individuals of 19th century New Caledonia relied mainly on intensive and irrigated horticulture of yam (genus *Dioscorea*) and taro (genus *Colocasia*). They also cultivated sugar cane, bananas and coconuts, consumed to a lesser extent meat (pig, chicken), marine resources and did not make use of animal milk (Dubois, 1984; Murdock, 1967; Djama, 1999).

We finally analysed the late forming enamel of permanent teeth from 6 individuals of the extant Baka population of Moange-le-Bosquet, Cameroon (e.g., Ramirez Rozzi et al., 2015 and Table S5). All teeth were naturally shed or extracted for surgical purposes in accordance with the World Medical Association's Declaration of Helsinki. In each case, the informed consent of the patients or their parents was collected, as part of the agreement between the *Institut de Recherche pour le Développement* of France and the *Ministère de la recherche scientifique et technologique* of Cameroon. The sampling was approved by the *Comité National d'Ethique de la Recherche pour la Santé Humaine* of Cameroon (2018/06/1049/CE/CNERSH/SP). The enamel was sampled by micro-drilling following a method described elsewhere (Tacail et al., 2017). The semi-settled Baka people do not consume dairy products (Reyes-García et al., 2018) and rely on gathering and hunting of forest vegetal and animal resources (e.g., plants and fruits, honey, mushrooms, bushmeat, fish) as well as on horticulture to a certain extent, including some crops such as plantain (*Musa* spp.), cassava (*Manihot esculenta*) and yam (*Dioscorea* spp.) (Reyes-García et al., 2018; Duda, 2017).

Group 1 also includes a compilation of data from bone samples of 25 adult individuals from 7 different pre-domestication Epipaleolithic and Mesolithic hunter-gatherer sites listed and described in Table 1. These individuals are issued from predomestication cultures for which we assume an absence of animal dairy products consumption (see references in Table 1).

2.2.2. Group 2 of populations consuming dairy products

The group 2 consists of bone and enamel samples of individuals from urban 18th to 20th century Northern and Western European societies producing and consuming dairy products.

We analysed a set of bone samples from 36 adult individuals from the Lyon Hôtel Dieu hospital collection hosted by the CCEC

Musée des Confluences of Lyon, France (Table S6). This group of individuals, whose death dates back to 1881, includes 19 females and 17 males aged between 18 and 77 years, issued from various occupations and living standards. The consumption of Ca from dairy products of 19th century French population is expected to vary between around 250 and 500 mg/d in average according to historical estimations based on various historical records for France over the 19th century (Toutain, 1971; Flandrin and Montanari, 1996; Heyberger, 2009; Heyberger, 2014 and Table S7). These historical records allow to calculate estimates of average dairy Ca fraction in total diet, ranging between about 40 and 70% of Ca from dairy products (Table S6).

We also included a compilation of data from 11 adult female individuals from the 18th to 19th century Christ Church Spitalfields crypt (London, UK), as previously described (Nitsch et al., 2010, 2011) and analysed for Ca isotopes (Reynard et al., 2013). English poor workers of late 18th and 19th century consumed dairy products, with estimations of daily consumption of Ca from dairy products ranging between 130 and 240 mg/d (Clark et al., 1995; Oddy, 1970, and Table S8 and SI text section 1.1.). These figures likely underestimate the daily Ca intake from dairy products in the Spitalfields individuals. Indeed, dairy consumption increases with income and living standard and the individuals of this Spitalfields group were rather affluent (see SI text section 1.1. for further details). The well-off status of this group likely allowed for the consumption of more animal protein intake (Nitsch et al., 2010; Nitsch et al., 2011 and references therein), including higher intake of dairy products, as is suggested by historical studies (Oddy, 1970).

Finally, group 2 includes wisdom tooth enamel samples from 9 individuals living in France during formation of enamel (7–15 years, born between 1948 and 1990 A.D.) and previously analysed for Ca isotope compositions (Tacail et al., 2017). The average daily total Ca intake in late 20th century French population is estimated to lie between 600 and 750 mg/d of which 60–72% comes from dairy products on average, as reviewed from various estimations (Guéguen and Pointillart, 2000; Leblanc et al., 2004; Leblanc and Sirot, 2011).

More generally, modern North-Western European populations are known to take a high proportion of their dietary Ca from dairy products, with estimated means ranging from 50 to 70% of Ca from dairy products (Guéguen and Pointillart, 2000; Rozenberg et al., 2016; Leblanc et al., 2004; Leblanc and Sirot, 2011;Vissers et al., 2011; Bonjour, 2011). Furthermore, estimates of yearly dairy product consumption per individual by the Food and Agriculture Organization (Food and Agriculture Organization of the United Nations, 2016) report the average intake of 630 and 700 kg/capita/y of milk equivalent in UK and France, respectively (excluding butter and between 1963 and 2013). This corresponds to an average daily intake of Ca from dairy products of the order of 750 and 840 mg/d (assuming a cow milk Ca concentration of 1200 mg/L, c.f. Pereira, 2014). Although possibly overestimated, these values of dairy Ca intake would easily account for more than 50% of Ca intake from dairy products, considering an estimated intake of total Ca of the order of 600–1000 mg/d per individual in Western populations (Balk et al., 2017).

2.2.3. Group 3 of populations consuming unknown Ca from dairy products

The group 3 includes a compilation of bone samples of 73 individuals from 7 different sites ranging from Middle East Early Neolithic to Iron Age of Britain, roughly following the Danubian route of Neolithization (Reynard et al., 2013; Reynard et al., 2010; Hamilton et al., 2013). All these populations possibly consumed dairy products because human remains are associated with evidence of cattle, goat or sheep domestication, of dairy dedicated herd managements or with lipid and isotopic evidence of animal milk production or processing (Table 1 and references therein).

2.3. Sampling and isotope ratios measurement

2.3.1. Sampling

We considered Ca isotope compositions of skeletal tissues formed when the individuals were expected to follow an “adult diet” in order to avoid the possible influence of breastfeeding on the isotopic signals. This corresponds to bones of adult or adolescent individuals although the effects of breastfeeding are rapidly attenuated in bone of young individuals because of its rapid remodelling (Reynard et al., 2013). In the case of tooth enamel, only permanent tooth enamel formed later than 5.5 years of age was considered (see details thereafter).

The sampling of bone from the Talasiu site individuals was carried out previously (Herrscher et al., 2018) on long bones mainly (femur, tibia, ulna and humerus) as well as a mandible and a coxal bone (Table S3).

The bones of New Caledonia and Lyon adult individuals were sampled at the CCEC Musée des Confluences of Lyon, France, by removing chips (of around 1–10 mg) of the thin nasal cavity bones (lacrimal, nasal septum or concha bones) using metal tweezers.

The tooth enamel samples from the Baka individuals were taken on halved teeth, by performing a drilling with a MicroMill device, as previously described (Tacail et al., 2017; Tacail et al., 2016). Enamel samples weighed about 100 μg hydroxyapatite, representing 40 μg Ca. The enamel of the 6 permanent teeth (three lower M2, two upper M3 and one lower P4) was sampled in the cervix area in order to analyse enamel formed in the latest possible period. These areas corresponding to the enamel formed between three quarters and total crown completion are formed after 5.5 years of age and before 17.5 years (AlQahtani et al., 2010). We thus estimate that the isotope compositions of these enamel samples reflect adult-like diets and are beyond the possible influence of breastfeeding (e.g., Tacail et al., 2017).

2.3.2. Sample preparation

All sample preparation and chemical procedures were performed in a clean lab and involved trace-level reagents, including subboiled distilled acids and MilliQ water.

After weighing of powder aliquots (7–24 mg), the 20 Talasiu samples were leached according to previously described methods in order to dispose of any possible secondary deposited carbonates (Martin et al., 2017, 2018). Briefly, samples were leached in trace metal clean 2 ml Eppendorf tubes by sonication in 2 ml of 0.1N (C*) acetic acid during 10 min. Following subsequent centrifugation and discard of supernatant, 1 ml of MilliQ water was added to each sample before vortex agitation. These steps were repeated three times before evaporation of remaining moist in autoclave at 50 °C overnight.

In order to desorb potentially attached impurities, the 59 New Caledonia and Lyon 19th century samples were gently cleaned in trace metal clean 2 ml Eppendorf tubes by sonication in about 1.5 ml 1N HCl. Supernatant was then removed after centrifugation and samples were rinsed two times using about 2 ml MilliQ, before being dried in an autoclave for 2 h at 60 °C.

All samples were then chemically processed according to a method described previously (Tacail et al., 2014; Martin et al., 2017; Hassler et al., 2018; Tacail et al., 2016; Martin et al., 2018; Martin et al., 2015; Martin et al., 2017). Briefly, they were transferred to Savillex trace metal clean Teflon beakers together with two powder aliquots of SRM1486 international bone meal standard. Samples were digested by heating of sealed beakers on hotplate at 130 °C during 72 h in 2 ml concentrated HNO_3, followed by addition of 0.6 ml of 30% Suprapur H_2O_2 and further heating of sealed beakers overnight at 130 °C. Finally, samples were evaporated to dryness and taken up in 0.2 ml 6N HCl before the first step of the ion chromatography procedure. Chemical purification consisted in loading samples onto AG50WX12 cationic resin in dilute HCl medium in order to dispose of matrix elements, before elution and collection of Ca and Sr in 6N HCl. The remaining Sr was then removed by processing samples through Sr-Spec resin (Eichrom) in 3N HNO_3 medium. Calcium blanks are of the order of 100 ng Ca, which is negligible when compared to smallest processed samples (minimum of 40 μg processed Ca).

2.3.3. *Elemental and isotopic analyses*

In order to assess the preservation of bone material in archaeological bone, we measured trace and major element concentrations in bone samples by means of inductively coupled plasma (ICP) quadrupole mass spectrometer (iCAP Q, Thermo Scientific) and ICP atomic emission spectrometer (iCAP 6000) at the Laboratoire de Géologie de Lyon, France.

We measured Ca isotope compositions with a Neptune *plus* (Thermo-Scientific) Multi-Collector Inductively Coupled Plasma Mass Spectrometer (MC-ICP-MS) at the Laboratoire de Géologie de Lyon, France, following a previously described method (e.g., Tacail et al., 2014; Tacail et al., 2016; Martin et al., 2015). We used a standard-sample bracketing method with *ICP Ca Lyon* bracketing standard. All Ca isotope compositions are expressed using the “delta” notation, in permille unit, for $^{44}Ca/^{42}Ca$ and $^{43}Ca/^{42}Ca$ ratios, defined for the former as follows: $\delta^{44/42}Ca = [((^{44}Ca/^{42}Ca)_{sample}/(^{44}Ca/^{42}Ca)_{ICP\ Ca\ Lyon}) - 1]*1000$, where $(^{44}Ca/^{42}Ca)_{sample}$ and $(^{44}Ca/^{42}Ca)_{ICP\ Ca\ Lyon}$ are Ca isotope abundance ratios as measured in sample and ICP Ca Lyon bracketing standard, respectively. The accuracy of Ca isotope ratio measurements was assessed by regularly analysing the previously described cow bone meal SRM1486 NIST standard.

All other literature Ca isotope compositions presented in this study were converted to ICP Ca Lyon reference material when necessary following the same procedure as described previously (Tacail et al., 2017; Martin et al., 2018). All figures presenting $\delta^{44/42}Ca$ values are shown on primary axis with ICP Ca Lyon as reference material. All compiled and newly measured skeletal $\delta^{44/42}Ca$ values are given relative to both ICP Ca Lyon and SRM915a standards in SI dataset. All data analyses and figures were made using R version 3.5 (R core team, 2013).

3. Results

3.1. *Modelling bone Ca isotope composition as a function of dairy Ca*

The dietary model predicts a global decrease of the bone $\delta^{44/42}Ca$ values distributions with increasing proportions of dairy Ca, irrespective of the different hypotheses made on the contributions of the non-dairy Ca sources (see Figure S2).

Indeed, the four non-dairy Ca budgets that were tested yield very similar distributions (Figure S2): we observe a global decrease of the diet $\delta^{44/42}Ca$ values with the increase in the fraction of Ca from dairy products in the total diet. This reflects the fact that most Ca from dietary sources other than dairy products is taken from plants and, to a lesser extent from water. Other sources that could account for a high intake of energy, such as meat, do not represent an important source of Ca in an omnivorous diet because the Ca content is relatively low when compared to plants or dairy products (e.g., Leblanc et al., 2004; Eaton and Nelson, 1991; Leblanc and Sirot, 2011). Furthermore, excluding marine resources and eggs, animal inner Ca stores display $\delta^{44/42}Ca$ values that are close to those observed for plant sources. This explains why the different simulations considering various plant to animal dietary intake ratios, yield comparable results.

According to these first order model outputs, bones of individuals consuming no dairy products are thus expected to display distributions with a $\delta^{44/42}Ca$ median of −1.1 to −1.0‰. An increase of 10% in the fraction of dairy Ca is expected to induce a decrease of bone $\delta^{44/42}Ca$ median values by about −0.07‰; dairy Ca intake higher than 50% is expected to correspond to Ca isotope median values equal to about −1.35 to −1.40‰ or lower. Although this mixing model will gain in accuracy and precision in the future with the increasing datasets of dietary Ca sources isotope compositions, such a model could thus allow to approximate the range of dairy Ca proportion in total diet of these populations by comparing it with the actual distributions of skeletal $\delta^{44/42}Ca$ values.

3.2. *Ca isotope analyses*

3.2.1. *Quality of isotope analyses*

Accuracy and precision of Ca isotope ratio measurements was assessed for the whole dataset. When plotted against $\delta^{43/42}Ca$, $\delta^{44/42}Ca$ values measured in all samples and standards fall on a line (Figure S3), with a significant correlation ($R^2 = 0.972$, p-value $< 10^{-4}$, n = 89), a y-intercept indistinguishable from 0‰ (0.001 ± 0.023‰, 2SE) and a slope in very good agreement with the 0.507 slope predicted by the exponential law mass-dependent fractionation line (0.511 ± 0.018, 2SE). Furthermore, the 6 samples of chemically processed and repeatedly measured SRM1486 cow bone meal standard yield an average $\delta^{44/42}Ca$ value of −1.01 ± 0.001‰ (2SE, n = 59 measurements), in very good agreement with previously published values of −1.02 ± 0.01‰ (2SE, n = 404 (Martin et al., 2018)). The long-term precision yields a value of 0.09‰ (2SD on SRM1486, n = 59).

3.2.2. *Bone Ca isotope compositions of newly described populations*

The newly measured Ca isotope compositions presented in this study range from −2.10 to −0.73‰ and average at −1.24 ± 0.60‰ (2SD, n = 85) (Fig. 1, Table 2).

The bone Ca isotope compositions of the Talasiu archaeological remains (n = 20, Table S3) yield an average $\delta^{44/42}Ca$ of −0.99 ± 0.09‰ (2SD) with an interquartile range of 0.08‰. We observe homogeneous Ca/P mass ratios averaging at 2.05 ± 0.06 (2SD), comparable to modern SRM1486 measured in the same conditions (2.05) and to the expected range of modern bone ratios between 1.99 and 2.33 (e.g., Sillen, 1986; Balter et al., 2002). There are also no significant correlations of $\delta^{44/42}Ca$ values with concentrations in trace elements that are incorporated *post-mortem* in bone, such as Ti, Al, Mn or Pb (see Figure S9). Finally, their collagen is well preserved and allowed for the study of their C and N isotope compositions (Herrscher et al., 2018). Their Ca isotope compositions are thus not affected by diagenetic alterations. No significant differences between age categories, nor sex when determined, is observed in this group. The three youngest individuals of this group, aged 7 years old and between 1.5 and 2.5 years old at death, notably appear to have $\delta^{44/42}Ca$ values that are undistinguishable when compared to the adults (Wilcoxon−Mann−Whitney, p-value = 0.17). This is in agreement with the limited influence of breastfeeding over bone likely because of its rapid remodelling when compared to incrementally growing tooth enamel (Reynard et al., 2013; Tacail et al., 2017).

The bone $\delta^{44/42}Ca$ values of the 19th century Melanesians group (n = 23, Table S4) display an average of −1.08 ± 0.19‰ (2SD) and an

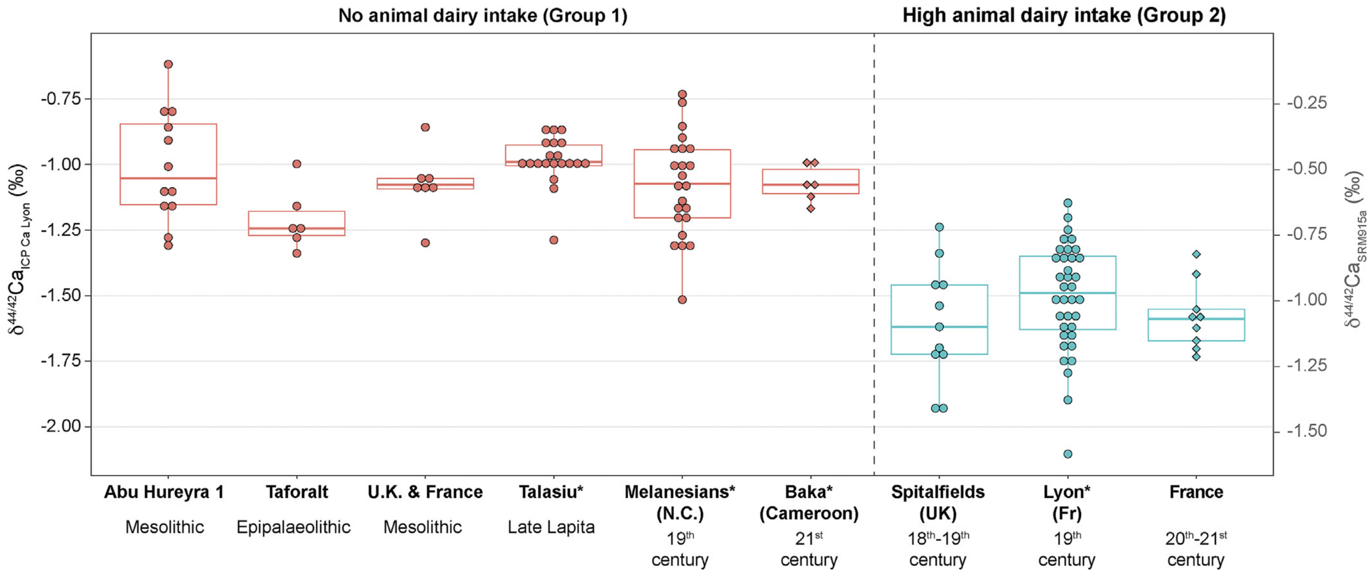

Fig. 1. **$\delta^{44/42}$Ca distributions and boxplots (‰, relative to ICP Ca Lyon on left axis, relative to SRM915a on right axis) of bone (circles) and enamel (diamonds) of populations consuming no dairy products (group 1, in red) and populations consuming significant amounts of Ca from dairy products (group 2, in blue).** Welch's ANOVA and Kruskal-Wallis tests performed on all groups show significant differences of average means (Welch's ANOVA p-value $< 10^{-4}$, F = 36.4, df = 8; Kruskal-Wallis p-value $< 10^{-4}$, df = 8; n = 130). Asterisks refer to data from this study, others are compiled from Tacail et al., 2017; Reynard et al., 2013; Reynard et al., 2010; Hamilton et al., 2013.

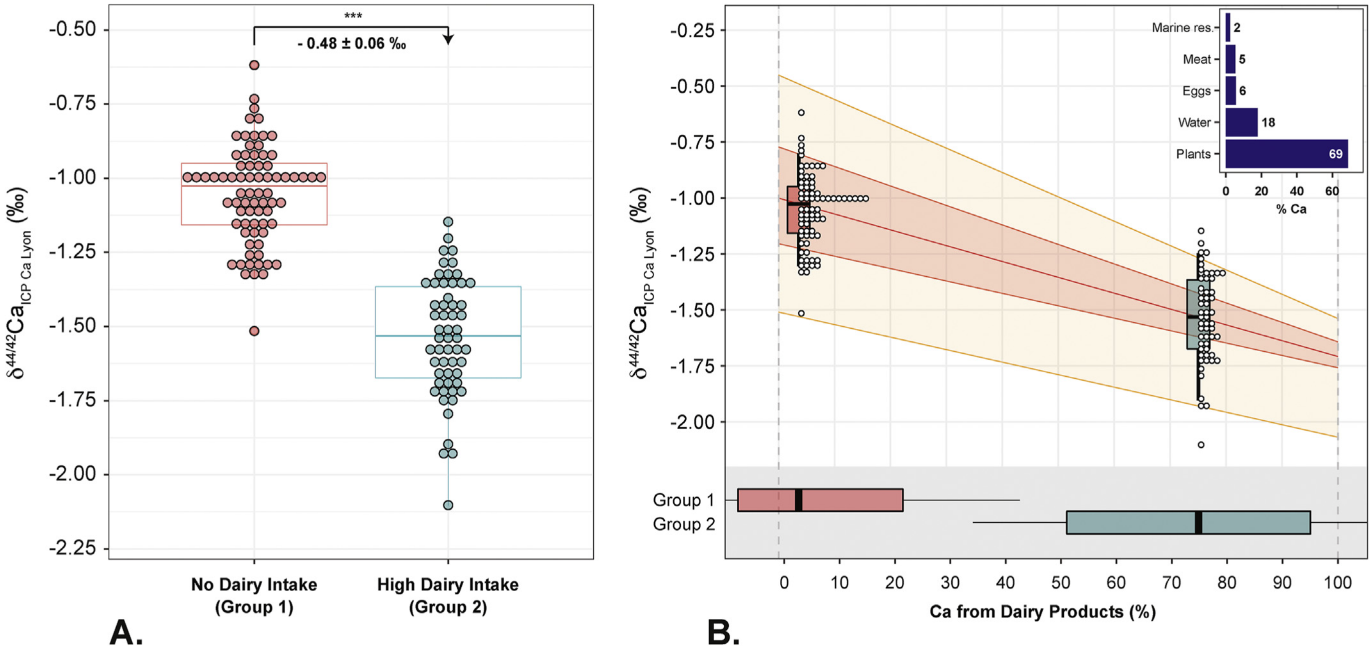

Fig. 2. **Distributions and boxplots of bone and enamel $\delta^{44/42}$Ca values of group 1 and group 2 populations (2.A.), compared with modelled $\delta^{44/42}$Ca distributions as expected in bone as a function of the fraction of Ca taken from dairy in total diet (2.B.).** Fig. 2A boxplots are presented with the *t*-test p-value (NS, nonsignificant p-value; *p = 0.01–0.05; **p = 0.001–0.01; and ***p < 0.001) and average difference between the two groups (±95% c.i.). In Fig. 2B., the central dark red line is the modelled median of these distributions, the two outer lines are the 5% and 95% percentiles and the two inner lines correspond to the 25% and 75% percentiles. The top-right blue bar graph corresponds to the non-dairy dietary Ca budget used to calculate these distributions (detail in methods and SI). The horizontal boxplots are the direct projections of group 1 and group 2 boxplots onto the dairy Ca fraction axis using the median line of the mixing model.

interquartile range of 0.26‰. No diagenetic effects are expected nor observed for these 19th century samples and the measured Ca/P ratios average at 2.00 ± 0.14 (2SD).

The modern permanent tooth enamel samples of the Baka individuals display a comparable distribution with an average $\delta^{44/42}$Ca value of -1.07 ± 0.07‰ (2SD, n = 6, Table S5) and an interquartile range of 0.09‰.

Contrastingly, the bone $\delta^{44/42}$Ca values of the 19th century Lyon group are lower, averaging at -1.51 ± 0.21‰ (2SD, n = 36) with an interquartile range of 0.28‰ (Table S6). Their Ca/P ratios yield a mean value of 2.05 ± 0.10 (2SD). No significant difference in $\delta^{44/42}$Ca values is observed according to sex in the Lyon group

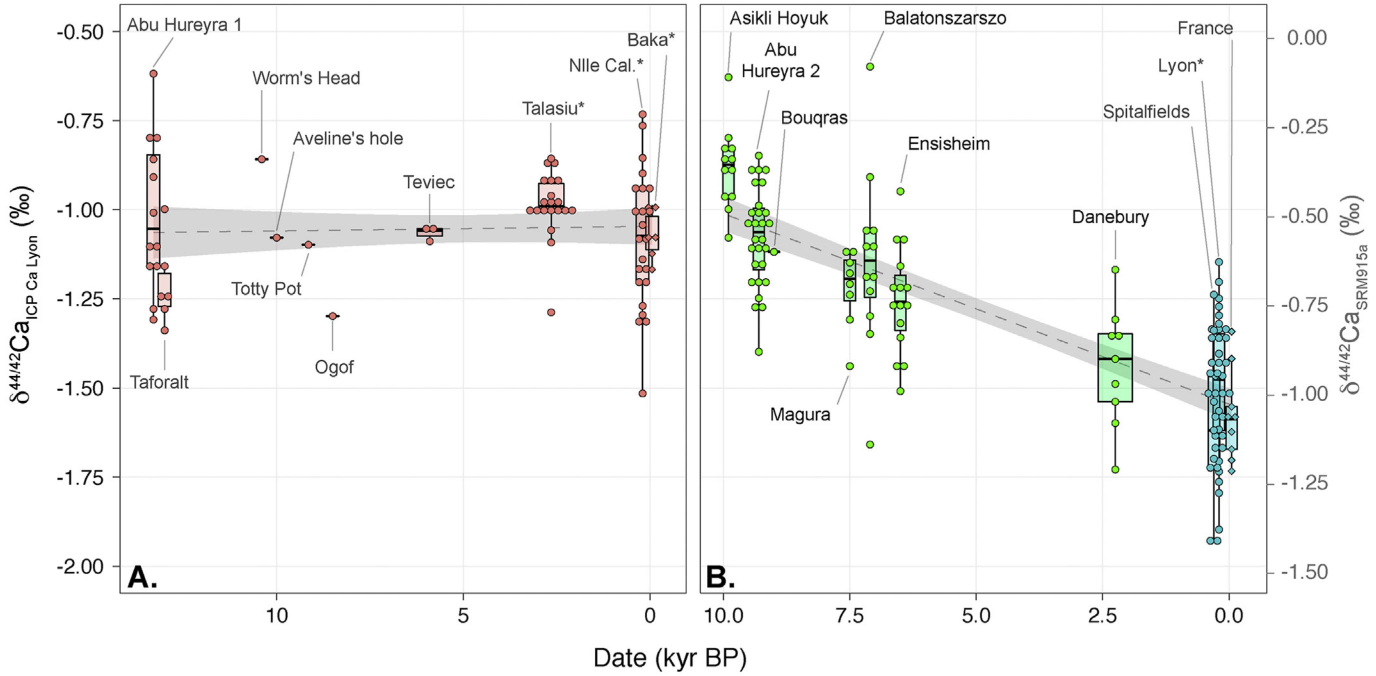

Fig. 3. Temporal evolution with time of $\delta^{44/42}$Ca values (‰, relative to ICP Ca Lyon on left axis, relative to SRM915a on right axis) of bone (circles) and enamel (diamonds) from (A.) populations consuming no dairy products (group 1, in red) and (B.) possible or confirmed but unquantified dairy consumption (group 3, in green) together with modern populations consuming significant proportions of dairy Ca (group 2, in blue). Data is from this study (marked with an asterisk) and compiled from literature (Tacail et al., 2017; Reynard et al., 2013; Reynard et al., 2010; Hamilton et al., 2013). The dashed grey lines correspond to the regression line together with its 95% confidence interval displayed as the grey shaded area, in both Fig. 3A. ($n = 74$, regression slope of 0.001 ± 0.007 with uncertainty being 95% c.i., $R^2 = 0.002$, p-value = 0.73) and Fig. 3B. ($n = 141$, regression slope of -0.054 ± 0.007, $R^2 = 0.603$, p-value = $1*10^{-29}$).

(*t*-test, p-value = 0.36; Wilcoxon–Mann–Whitney, p-value = 0.33, $n = 36$) nor in the Baka group (Wilcoxon–Mann–Whitney, p-value = 0.2, $n = 6$).

3.2.3. Distributions of bone Ca isotope compositions in groups 1 and 2

The whole dataset of skeletal $\delta^{44/42}$Ca values (compiled from Tacail et al., 2017; Reynard et al., 2013; Reynard et al., 2010; Hamilton et al., 2013 and newly acquired) range from −2.10 to −0.60‰ and average at -1.21 ± 0.55‰ (2SD, $n = 215$, Fig. 1, Table 2).

Globally, the $\delta^{44/42}$Ca values of bone and enamel of individuals from group 1 (populations eating no dairy products) and 2 (populations taking in significant proportions of dairy products) display highly significant differences of their mean values (Fig. 1; Welch's ANOVA p-value $< 10^{-4}$, $F = 36.4$, $df = 8$; Kruskal-Wallis p-value $< 10^{-4}$, $df = 8$; $n = 130$).

Considering the group 1 only, we observe potentially significant differences between means of their $\delta^{44/42}$Ca values (Welch's ANOVA p-value = 0.012, $F = 3.9$, $df = 5$; Kruskal-Wallis p-value = 0.03, $df = 5$; $n = 74$). On the other hand, no significant difference between the three sampled populations of group 2 is observed (Welch's ANOVA p-value = 0.29, $F = 1.3$, $df = 2$; Kruskal-Wallis p-value = 0.22, $df = 2$; $n = 56$).

Taken as a whole (Fig. 2A.), both bone and enamel from populations consuming no Ca from dairy products (Group 1) display globally homogeneous and ^{44}Ca-enriched isotope compositions, with an average $\delta^{44/42}$Ca value of -1.05 ± 0.04‰ (95% c.i. of *t*-test, $n = 74$). On the other hand, populations with high dairy Ca intake from group 2 (-1.54 ± 0.05‰, 95% c.i., $n = 56$) display significantly lower $\delta^{44/42}$Ca values (*t*-test p-value $< 10^{-4}$, t-value = 15.13, $df = 128$), the average offset being $\Delta^{44/42}Ca_{2\text{-}1} = -0.48 \pm 0.06$‰ from group 1 to 2 (95% c.i.).

4. Discussion

4.1. Comparison of bone and enamel $\delta^{44/42}$Ca values

Previous studies suggest that late enamel and bone of the same mammal individuals might display systematic differences in Ca isotope compositions (Martin et al., 2017; Heuser et al., 2011). This aspect likely relates to the different time periods recorded by these two tissues: enamel forms in several weeks and maintains a time-resolved structure while almost all bone types remodel permanently during life (e.g., Tacail et al., 2017; Tacail et al., 2019). As a result, both tissues do not integrate the same time duration, and enamel can be representative of a transient and temporary diet, such as breast milk, while bone remodelling is responsible for the buffering of possible transient dietary signals over multiple years. Here, the Ca isotope compositions of enamel samples (formed at ages for which influence of breastfeeding is very unlikely) parallel remarkably well the patterns observed in bones. Indeed, the difference between compositions of permanent tooth cervix enamel of hunter-gatherer Baka individuals and modern French individuals is significant (*t*-test p-value $< 10^{-4}$) and yields an average offset of -0.51 ± 0.13‰ from Baka to modern French individuals (95% c.i., $n = 15$, see Fig. 1). This is very close to the difference between bone-only $\delta^{44/42}$Ca values of populations from group 1 and group 2 ($\Delta^{44/42}Ca_{2\text{-}1} = -0.48 \pm 0.07$‰, 95% c.i., p-value $< 10^{-4}$, $n = 115$). Furthermore, the $\delta^{44/42}$Ca values of Baka enamel are indistinguishable from those of bone from group 1, and the same holds for modern French enamel when compared to bone from London and Lyon individuals. We thus discuss thereafter the results from enamel and bone together.

Table 2

Summary of $\delta^{44/42}$Ca values distributions as measured and compiled in bone and enamel of all populations. The tables show the average and 2SD values of each population $\delta^{44/42}$Ca values together with the values of the minimum (Min), maximum (Max), first quartile (Q1), median (Q2) and third quartile (Q3).

	Site	Date	$\delta^{44/42}Ca_{ICP\ Ca\ Lyon}$ (‰)		$\delta^{44/42}Ca_{ICP\ Ca\ Lyon}$ (‰)				
		(ky cal BP)	Av. ± 2SD	*(n)*	Min	Q1	Q2	Q3	Max
Group 1: No dairy consumption	Abu Hureyra 1, Euphrates Valley, Syria	13.3	−1.01 ± 0.21	*(12)*	−1.31	−1.15	−1.05	−0.85	−0.62
(No domesticated dairy producers)	Aveline's Hole, UK	10	−1.08 ± NA	*(1)*	–	–	−1.08	–	–
	Ogof yr Ychen, Caldey Island, UK	8.5	−1.30 ± NA	*(1)*	–	–	−1.30	–	–
	Taforalt, Morrocco	13	−1.21 ± 0.12	*(6)*	−1.34	−1.27	−1.24	−1.18	−1.00
	Téviec, France	5.9	−1.07 ± 0.02	*(3)*	−1.09	–	−1.06	–	−1.05
	Totty Pot, UK	9.15	−1.10 ± NA	*(1)*	–	–	−1.10	–	–
	Worm's Head, UK	10.4	−0.86 ± NA	*(1)*	–	–	−0.86	–	–
	Talasiu, Tongatapu, Kingdom of Tonga	2.65	−0.99 ± 0.09	*(20)*	−1.29	−1.00	−0.99	−0.93	−0.86
	Melanesians, New Caledonia, Guépy Coll.	0.2	−1.08 ± 0.19	*(23)*	−1.52	−1.20	−1.07	−0.94	−0.73
	Baka, Cameroon	−0.05	−1.07 ± 0.07	*(6)*	−1.17	−1.11	−1.08	−1.02	−0.99
Group 2: High dairy consumption	Spitalfields Christ Church crypt, London, UK	0.3	−1.60 ± 0.22	*(11)*	−1.93	−1.72	−1.62	−1.46	−1.24
(significant intake of Ca from dairy)	Hôtel Dieu Coll., Lyon, France	0.2	−1.51 ± 0.21	*(36)*	−2.10	−1.63	−1.49	−1.35	−1.15
	France	−0.05	−1.58 ± 0.13	*(9)*	−1.73	−1.67	−1.59	−1.55	−1.34
Group 3: Unknown Dairy Consumption	Abu Hureyra 2, Euphrates Valley, Syria	9.3	−1.08 ± 0.14	*(28)*	−1.40	−1.17	−1.06	−1.00	−0.85
(Presence of domesticated dairy producers and/or evidence of dairy production)	Asikli Hoyuk, Turkey	9.9	−0.88 ± 0.11	*(12)*	−1.08	−0.96	−0.87	−0.83	−0.63
	Balatonszarszo, Hungary	7.1	−1.15 ± 0.25	*(12)*	−1.66	−1.25	−1.14	−1.06	−0.60
	Bouqras, Syria	9	−1.12 ± NA	*(1)*	–	–	−1.12	–	–
	Danebury, UK	2.25	−1.44 ± 0.17	*(9)*	−1.73	−1.54	−1.42	−1.35	−1.17
	Magura, Romania	7.5	−1.22 ± 0.11	*(8)*	−1.44	−1.26	−1.19	−1.14	−1.12
	Ensisheim (les Octrois, Ratfeld), France	6.5	−1.25 ± 0.15	*(15)*	−1.51	−1.34	−1.26	−1.18	−0.95

4.2. Distributions of $\delta^{44/42}$Ca values and dietary practices

The distributions of bone and enamel $\delta^{44/42}$Ca values in populations consuming no dairy products (Group 1, Fig. 1) display rather narrow ranges, between about −1‰ (e.g., Talasiu group, n = 20) and −1.2‰ (e.g., Taforalt group, n = 6), despite the disparity of geological and cultural contexts (summarized in Table 1). These groups are indeed issued from areas with distinct surface lithologies, ranging from silicate volcanic, plutonic and metamorphic regions to sedimentary bedrock catchments including carbonates. The range of average $\delta^{44/42}$Ca values (~0.2‰) exhibited by these populations is similar to that of Earth surface geological reservoirs, from −0.04 to −0.21‰ for silicates and carbonates respectively (Fantle and Tipper, 2014). This suggests a limited influence of geological context over the average compositions of bone and enamel in human populations. The group 1 also includes various subsistence modes, namely hunting and gathering (Near-East and Europe Mesolithic, North-Africa Epipaleolithic and modern Baka groups) as well as horticulture, gathering, fishing and hunting (Talasiu group, New-Caledonia group, Table 1); and is spread over various habitats and environments (e.g., coastal in Talasiu group *vs.* equatorial forest in Baka communities). Part of the limited variability in group 1 could thus also arise from differences in non-dairy Ca dietary sources, such as marine resources or different plant types or organs (Figure S1 and Martin et al., 2018). Although such differences in dietary practices appear to have limited effects on their Ca isotope compositions, the relatively high $\delta^{44/42}$Ca values observed in the Talasiu group could for instance be partly due to a significant intake of Ca from marine resources (Herrscher et al., 2018), expected to display relatively ^{44}Ca-enriched compositions.

However, the most striking feature is the difference between groups 1 and 2 (Figs. 1 and 2.A.). When compared to group 1, the significantly ^{44}Ca-depleted distribution of group 2 values ($\Delta^{44/42}$Ca = −0.48 ± 0.06‰, 95% c.i. of Student *t*-test) suggests that lifestyles involving agriculture and livestock farming significantly affect the skeletal Ca isotope compositions of human groups. More precisely, such an offset in Ca isotope compositions likely primarily relates to the contrasted habits of dairy products consumption, as discussed below.

4.3. Quantifying dairy Ca intake in reference populations

The comparison of the model outputs with the observed distributions of skeletal $\delta^{44/42}$Ca values in groups 1 and 2 shows a good agreement with their documented habits of dairy products consumption (Fig. 2B., Figures S4-6 and SI text).

First, the observed distribution of bone and enamel isotope compositions of group 1 is in very good agreement with the distributions predicted by the model for populations consuming no dairy products (Fig. 2B and S4-6). For instance, the median of group 1 yields a $\delta^{44/42}$Ca value of −1.03‰ while the mixing models predict medians ranging from −1.10‰ to −1.00‰. In other words, the projection of this $\delta^{44/42}$Ca distribution onto the x-axis of dairy Ca fraction is in very good agreement with the null contribution of dairy products to the total diet expected for this group of populations. These results suggest that despite the various geographical and cultural contexts represented in group 1, the distribution of their $\delta^{44/42}$Ca values is compatible with a predominant intake of Ca from plants and to a lesser extent water and does not include consumption of dairy products.

Second, the predictions of the model for group 2 as a whole suggest a median dairy Ca intake of 70–75% (Fig. 2B and S4-6) and an interquartile range spanning from 45 to 95% dairy Ca. These results are in global agreement with the historical and ethnographic data available for these populations known to consume significant amounts of Ca from dairy products (details in SI text section 1). Briefly, the individuals from the 18th-19th century Spitalfields group display a distribution of their bone $\delta^{44/42}$Ca values compatible with a high intake of dairy Ca, which is rather well supported by the available historical estimates of dairy consumption and could relate to the higher socio-economic status of these individuals (SI text section 1.1). The distribution of $\delta^{44/42}$Ca values of bone from the individuals of the Lyon group suggests, according to the model, a median intake of Ca from dairy products of 65–70% in good agreement with the 40–70% dairy Ca intake inferred for 19th century France. Moreover, the distribution of bone $\delta^{44/42}$Ca values in this group appears to depend on individuals' occupations. The model notably suggests that the group of day laborers consumed around 30% dairy Ca in average, against about 70% dairy Ca for skilled workers from services and industry sectors, which is in agreement with the documented differences in dietary habits of these socio-economic categories (SI text section 1.2. and Figure S7). Finally, the distribution of enamel $\delta^{44/42}$Ca values within the group of modern French individuals (suggesting a median of about 80% Ca from dairy products) is also in good agreement with the documented dairy consumption habits of late 20th century French (accounting for 60–72% dairy Ca in total diet, c.f. Guéguen and Pointillart, 2000; Leblanc et al., 2004 and SI text section 1.3.).

As a result, the consumption of dairy products is the main factor explaining the contrasting distributions of $\delta^{44/42}$Ca values observed in the two groups of populations. Nevertheless, the quality and detailed interpretation of the modelled outputs primarily depend on the construction of an extensive dataset of human Ca dietary sources and their isotope compositions. The accuracy of the predicted dairy Ca fractions in total diet should thus improve with future work (e.g., allowing Bayesian statistical approaches, c.f. Fernandes et al., 2014) and the growing knowledge of isotope compositions of the main dietary sources. The high bioavailability of Ca from dairy products could also contribute to the significantly low $\delta^{44/42}$Ca values of dairy-consuming populations and its influence will have to be assessed in the future.

Beyond the apparent predominant influence of dairy products in such a broad approach, the remaining variability between and within groups could result from the interplay of other factors that will need to be further assessed in the future. Indeed, other environmental, dietary or physiological populational and individual features probably play a role in the observed variability.

First, although little direct data suggests it so far, it will be necessary to refine the relationship between the geological baseline and the isotope composition of the total diet and contributing dairy and non-dairy Ca sources.

Second, the nature of the non-dairy Ca sources and their weighted contribution to the total diet will have to be explored for their collateral effects on the effectively assimilated Ca (for instance the intrinsic or extrinsic bioavailability of Ca e.g., Guéguen and Pointillart, 2000; Rozenberg et al., 2016) or their potentially variable Ca isotope compositions (e.g., growing influence of cereals and pulses with the rise of agriculture).

Finally, the intra-population spread of the $\delta^{44/42}$Ca values is likely to bear further information and should be the focus of future work. First, as shown in the Lyon group (Figure S7 and SI text section 1.2.), part of this variability is likely reflecting differences in dairy intake in relation with social status and living standards. This part of the variability could also relate to other Ca sources, such as differences in specific consumption of plant types or plant organs from one individual to another. Second, some outliers present extreme values hardly explained by differences in dairy product consumption only. We consider an average physiologically induced isotopic trophic effect, but the amplitude of this effect could vary from one individual to another with respect to variations in the individuals Ca metabolism (e.g. bone or kidney functions e.g., Reynard et al., 2010; Morgan et al., 2012; Tacail, 2017). Moreover, chronic disorders of the Ca homeostasis, namely involving bone or kidney diseases (e.g., osteoporosis), could affect the individuals bone Ca isotope compositions on the long term (e.g., Skulan and DePaolo, 1999; Morgan et al., 2012; Heuser et al., 2019; Eisenhauer et al., 2019). As a result, such a variability in the physiopathology of Ca cycling possibly contributes to the variability of average bone $\delta^{44/42}$Ca values within a given socio-culturally homogeneous group.

To conclude, based on archaeological and ethnographic information, the dietary models predict the observed distributions of Ca isotope compositions of bone and enamel from groups 1 and 2 populations and support the interpretation of variations primarily induced by contrasting practices of dairy consumption. This suggests a strong sensitivity of Ca isotopes to the extent of Ca intake from dairy products.

4.4. Temporal evolution of $\delta^{44/42}$Ca values and neolithization

Considering all the available data from archaeological populations with either possible or documented dairy production (group 3), we observe a significant and drastic decrease in $\delta^{44/42}$Ca values over time, evolving from a dairy-free diet (similar to group 1) to modern dairy consumption (similar to group 2, Fig. 3). Indeed, while no significant change of isotope compositions is observed in groups from populations consuming no dairy Ca over a period spanning the upper Palaeolithic (about 13 000 yrs cal BP) to modern times range (regression $R^2 = 0.002$, p-value = 0.73, n = 74, group 1), we observe a significant time-dependent decrease of $\delta^{44/42}$Ca values as measured in bones across Neolithic, Iron age and up to modern times (regression $R^2 = 0.60$, p-value $< 10^{-4}$, n = 141, groups 2 and 3).

We pooled these populations in three different chrono-cultural sub-groups, namely preceramic Neolithic (Asikli Hoyuk, Abu Hureyra 2, Bouqras), Balkan and LBK Neolithic (Balatonszarszo, Magura, Ensisheim) and Iron age (Danebury), and observe a significant decrease over time that can be related to an increase of dairy Ca intake (Welch's ANOVA p-value $< 10^{-4}$, F = 68.8, df = 4; Kruskal-Wallis p-value $< 10^{-4}$, df = 4, see Figure S8). The preceramic Neolithic group displays $\delta^{44/42}$Ca values that are indistinguishable from the distributions observed in group 1 of populations consuming no dairy products (Student *t*-test p-value = 0.3, t-value = −1.04, df = 113; n = 115), suggesting a limited consumption of Ca from dairy products. The Balkan and LBK Neolithic group present $\delta^{44/42}$Ca values with an average of −1.21 ± 0.06‰ (*t*-test 95% c.i., n = 35) and are significantly lower than group 1 by −0.16 ± 0.07‰ in average (*t*-test 95% c.i., p-value $< 10^{-4}$, t-value = 4.4, df = 107, n = 109). This suggests that these populations present significant but limited consumption of dairy products, with medians of the order of 25% dairy Ca in total diet according to model predictions. Finally, the Iron age Danebury group displays an even more ^{44}Ca-depleted distribution significantly different from distributions of group 1, from the preceramic Neolithic group as well as the Balkan and LBK group (lower than group 1 by −0.39 ± 0.11‰, 95% c.i., p-value $< 10^{-4}$, t-value = 6.7, df = 81, n = 81, n = 83). This suggests a further increase in dairy product consumption, with a dairy Ca fraction predicted median of the order of 55%.

Albeit arguably offering a partial glimpse of the diversity of chrono-cultural complexes through time and space, this dataset suggests that the importance of dairy products consumption in the Ca dietary budget significantly rose only after the preceramic Neolithic, although some pieces of evidence suggest the possible early practice of dairy production in PPN Levant (e.g., Vigne and Helmer, 2007; Helmer et al., 2007). The dairy Ca consumption reached a significant proportion in the Balkan and LBK populations, which is consistent with the archaeozoological and dairy fat evidence of an increased importance of dairy production along the Danubian Neolithization route especially North of the Balkan Mountain range (e.g., Ethier et al., 2017; Salque et al., 2013). The Magura Balkan Neolithic group was notably associated with lipid evidence of animal dairy processing in a previous publication (Evershed et al., 2008). Finally, the present dataset suggests that dairy products had become a predominant Ca source by the onset of Iron Age, reaching comparable importance when compared to modern societies. This interpretation tends to be supported by the evidence from dairy fat retrieved from ceramic shards in Britain and especially in the Danebury Iron age site (Copley et al., 2005a; Copley et al., 2005b).

5. Generalizing the use of Ca isotopes in bioarchaeology

Calcium isotopes of bone and tooth enamel carry valuable information primarily related to the dietary habits of populations. Future research on Neolithic and post-Neolithic populations would thus greatly benefit from the inclusion of Ca isotopes in the bioarchaeological toolbox. Here, we identify several strategic milestones involving Ca isotopes in Neolithic research.

First, as mentioned earlier, the development of Ca isotopes for the quantification of dairy consumption in past populations will benefit from a comprehensive characterization of the variability of the dietary sources $\delta^{44/42}$Ca values. The quality of the outputs and interpretation of the model of dietary Ca isotope compositions indeed depend on the representativeness of such actualistic datasets, which sizes remain rather limited and depend on the implementation of high throughput analytical routines (e.g., Tacail et al., 2020).

Second, in the near future, the population scale approach that we develop in this proof-of-concept study should be combined with a single population comparison of human bone isotope compositions with those of associated fauna. Previous attempts were encouraging but in want of more data (Reynard et al., 2010, 2011). The representativeness of the fauna sample as an indicator of the potential dairy Ca source is questioned and should be further investigated to strengthen the interpretation of human bone isotope compositions in relation to associated fauna.

Third, the study of human skeletal Ca isotopes should be led in conjunction with well-established markers for food production and dietary practices. For instance, Ca isotopes could be used on human populations for which the dairy production was characterized by the analysis of dairy lipids retrieved in the pot shards (Copley et al., 2003; Copleyet al., 2005a; Copleyet al., 2005b; Evershed et al., 2008; Salque et al., 2013; Cubas et al., 2020). Such a combined approach will allow to confront insights on production intensity, brought by lipid biomarkers, with the actual dairy consumption habits of individuals at the scale of the population and even between distinct groups within such a population (social or health status, gender …).

More generally, Ca isotopes should be combined to other human skeletal biomarkers of protein intake (e.g., collagen δ^{15}N) and plant consumption (e.g., collagen δ^{13}C). Such a multi-proxy approach would allow to refine the estimation of dairy intake proportion by using this 3D space of biomarkers (C, N and Ca isotopes) and by rolling out statistical inversion approaches (such as Bayesian inference). On a more practical level, such a strategy would mesh very well with the current designs of the traditional stable isotope studies of bone (whole and intra-population scale, comparison with fauna). Indeed, in addition to the common sampling strategies of skeletal remains, developing the analysis of Ca isotopes on the very same samples would require almost no changes in the collagen sample preparation. Because Ca is the major element of the mineral phase (~40 wt % of hydroxyapatite, c.f. LeGeros and LeGeros, 1984), the samples of skeletal remains taken for analyses of traditional stable isotopes of the organic phase would very easily allow for the joint analysis of the Ca isotopes of the mineral phase. For instance, the analysis of collagen stable isotopes in archaeological remains typically requires 100–200 mg of bone. Such a sample size would correspond to at least 20′000 replicated analyses of its Ca isotope composition. As a by-product of the bone collagen extraction, the dissolved mineral phase can be easily treated as a Ca sample or archived for later analysis, provided that Ca cross contamination risks are minimal due to its abundance. A great deal of valuable samples of the mineral phase from human populations are otherwise regularly flushed down the sink and lost. The same applies to the potential combination of Ca isotopes studies with the recovery of ancient DNA in skeletal remains.

6. Conclusion

The distributions of bone and enamel $\delta^{44/42}$Ca values in human populations vary with the proportion of dairy Ca and reflect the culturally driven differences of dietary habits depending on lifestyles as well as socially driven dietary patterns within a population. Calcium isotopes thus bear the potential to study the evolving dietary practices of human populations with respect to animal dairy consumption. We monitor a dramatic change of dietary Ca utilization in the course of the European Neolithization process. From an ecological point of view, this pattern can be seen as the mark of a deep modification of human niche (O'Brien and Laland, 2012), and could be related to the major biological and cultural revolutions experienced by Neolithic and subsequent populations, such as the rise of livestock farming and its impact on ecosystems, as well as the spread of lactase persistence in European populations. These observations stress the need to explore the refined dynamics of the rise of dairy consumption using Ca isotopes, across chrono-cultural periods following preceramic Neolithic from the Balkans to North-Western Europe.

The results of this study should motivate the future development of a method combining the cross-population and the human-fauna comparison approaches as previously discussed (Reynard et al., 2010, 2011), including a further refined and broadened actualistic study of isotope compositions of dietary Ca sources. The use of Ca isotopes for quantification of dairy contribution to diet will greatly benefit from the complementary utilization of other isotope biomarkers of animal, plant or marine resources consumption (such as δ^{15}N, δ^{13}C, δ^{26}Mg and δ^{66}Zn), combined with Bayesian statistical approaches.

Author contributions

T.T., J.E.M. and V.B. designed research; T.T., J.E.M. and E.A. performed research; T.T. analysed data; J.E.M., E.H., C.V., F. R.-R., G.C., F.V, V.B. contributed reagents/materials/analysis tools; T.T., J.E.M and V.B. prepared the manuscript which was edited by all co-authors.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.quascirev.2021.106843.

References

Albarède, F., 1995. Introduction to Geochemical Modeling. Cambridge University Press https:/doi.org/10.1017/CBO9780511622960. (Accessed 16 October 2014).

AlQahtani, S.J., Hector, M.P., Liversidge, H.M., 2010. Brief communication: the London atlas of human tooth development and eruption. Am. J. Phys. Anthropol. 142, 481–490. https://doi.org/10.1002/ajpa.21258.

Bailey, D.W., Andreescu, R., Howard, A.J., Macklin, M.G., Mills, S., 2002. Alluvial landscapes in the temperate balkan neolithic: transitions to tells. Antiquity 76, 349–355. https://doi.org/10.1017/S0003598X00090438.

Balasse, M., Tresset, A., 2002. Early weaning of Neolithic domestic cattle (Bercy, France) revealed by intra-tooth variation in nitrogen isotope ratios. J. Archaeol. Sci. 29, 853–859. https://doi.org/10.1006/jasc.2001.0725.

Balasse, M., Tresset, A., Balasescu, A., Blaise, E., Tornero, C., Gandois, H., Fiorillo, D., Nyerges, E.A., Frémondeau, D., Banffy, E., Ivanova, M., 2017. Animal Board Invited Review: sheep birth distribution in past herds: a review for prehistoric Europe (6th to 3rd millennia BC). Animal 11, 2229–2236. https://doi.org/10.1017/S1751731117001045.

Balk, E.M., Adam, G.P., Langberg, V.N., Earley, A., Clark, P., Ebeling, P.R., Mithal, A., Rizzoli, R., Zerbini, C.A.F., Pierroz, D.D., Dawson-Hughes, B., 2017. Global dietary calcium intake among adults: a systematic review. Osteoporos. Int. 28, 3315–3324. https://doi.org/10.1007/s00198-017-4230-x.

Balter, V., Bocherens, H., Person, A., Labourdette, N., Renard, M., Vandermeersch, B., 2002. Ecological and physiological variability of Sr/Ca and Ba/Ca in mammals of West European mid-Würmian food webs. Palaeogeogr. Palaeoclimatol. Palaeoecol. 186, 127–143. https://doi.org/10.1016/S0031-0182(02)00448-0.

Barton, R.N.E., Bouzouggar, A., Ramsey, C.B., Collcutt, S.N., Higham, T.F.G., Humphrey, L.T., Parfitt, S.A., Rhodes, E.J., Schwenninger, J.-L., Stringer, C.B., Turner, E., Ward, S.C., 2007. Abrupt climatic change and chronology of the upper palaeolithic in northern and eastern Morocco. Rethink. Hum. Revolut. New Behav. Biol. Perspect. Orig. Dispersal Mod. Humans 177–186.

Bickle, P., Arbogast, R.-M., Bentley, R.A., Fibiger, L., Hamilton, J., Hedges, R., Whittle, A., 2013. Alsace. In: Bickle, P., Whittle, A. (Eds.), The First Farmers of Central Europe: Diversity in LBK Lifeways. Oxbow books.

Bocquet-Appel, J.-P., 2011. The agricultural demographic transition during and after the agriculture inventions. Curr. Anthropol. 52, S497–S510. https://doi.org/10.1086/659243.

Bonjour, J.-P., 2011. Calcium and phosphate: a duet of ions playing for bone health. J. Am. Coll. Nutr. 30, 438S–448S. https://doi.org/10.1080/07315724.2011.10719988.

Bouzouggar, A., Barton, N., Vanhaeren, M., d'Errico, F., Collcutt, S., Higham, T., Hodge, E., Parfitt, S., Rhodes, E., Schwenninger, J.-L., Stringer, C., Turner, E., Ward, S., Moutmir, A., Stambouli, A., 2007. 82,000-year-old shell beads from North Africa and implications for the origins of modern human behavior. Proc. Natl. Acad. Sci. Unit. States Am. 104, 9964–9969. https://doi.org/10.1073/pnas.0703877104.

British Geological Survey, 1983. Pembroke and Linney Head, 1:50 000. Solid and Drift Geology. New Series, Sheets 244 & 245. British Geological Survey.

British Geological Survey, 1999. Bristol District, 1:63 360. Solid and Drift Geology. New series, Special sheet. British Geological Survey.

British Geological Survey, 2002. Worms Head, 1:50 000. Solid and Drift Geology. New Series, Sheet 246. British Geological Survey.

British Geological Survey, 2006. North London, 1:50 000. Solid and Drift Geology. New Series, Sheet 256. British Geological Survey.

Burchfiel, B.C., Bleahu, M., 1976. Geology of Romania, Vol. 158. Geological Society of America, pp. 1–82.

Cagnard, F., 2009. Carte géologique harmonisée du département du Morbihan. BRGM/RP-56656-FR, pp. 1–297.

Chantraine, J., Autran, A., Cavelier, C., 2003. Carte géologique de la France à 1/1 000 000 6ème édition révisée. BRGM, Orléans.

Chu, N.-C., Henderson, G.M., Belshaw, N.S., Hedges, R.E.M., 2006. Establishing the potential of Ca isotopes as proxy for consumption of dairy products. Appl. Geochem. 21, 1656–1667. https://doi.org/10.1016/j.apgeochem.2006.07.003.

Clark, G., Huberman, M., Lindert, P.H., 1995. A British food puzzle, 1770–1850. Econ. Hist. Rev. 48, 215–237. https://doi.org/10.1111/j.1468-0289.1995.tb01416.x.

Clark, G., Grono, E., Ussher, E., Reepmeyer, C., 2015. Early settlement and subsistence on Tongatapu, Kingdom of Tonga: insights from a 2700-2650calBP midden deposit. J. Archaeol. Sci. Reports 3, 513–524. https://doi.org/10.1016/j.jasrep.2015.08.005.

Cook, C.J., 2014. The role of lactase persistence in precolonial development. J. Econ. Growth 19, 369–406. https://doi.org/10.1007/s10887-014-9109-5.

Copley, M.S., Berstan, R., Dudd, S.N., Straker, V., Payne, S., Evershed, R.P., 2005b. Dairying in antiquity. I. Evidence from absorbed lipid residues dating to the British Iron Age. J. Archaeol. Sci. 32, 485–503. https://doi.org/10.1016/j.jas.2004.07.004.

Copley, M.S., Berstan, R., Dudd, S.N., Aillaud, S., Mukherjee, A.J., Straker, V., Payne, S., Evershed, R.P., 2005a. Processing of milk products in pottery vessels through British prehistory. Antiquity 79, 895–908. https://doi.org/10.1017/S0003598X00115029.

Copley, M.S., Berstan, R., Dudd, S.N., Docherty, G., Mukherjee, A.J., Straker, V., Payne, S., Evershed, R.P., 2003. Direct chemical evidence for widespread dairying in prehistoric Britain. Proc. Natl. Acad. Sci. Unit. States Am. 100, 1524–1529. https://doi.org/10.1073/pnas.0335955100.

Cramp, L.J.E., Evershed, R.P., Lavento, M., Halinen, P., Mannermaa, K., Oinonen, M., Kettunen, J., Perola, M., Onkamo, P., Heyd, V., 2014. Neolithic dairy farming at the extreme of agriculture in northern Europe. Proc. R. Soc. B Biol. Sci. 281, 20140819. https://doi.org/10.1098/rspb.2014.0819.

Cunliffe, B., Brown, L., Coy, J., Evans, J., Everard, C., Grant, A., Henderson, J., Hooper, B., Howard, H., Jones, M., Jope, M., Lock, G., 1984. The excavations, 1969 – 1978 : the finds. Danebury: an Iron Age Hillfort in Hampshire, vol. 2. Council for British Archaeology.

Cunliffe, B., Poole, C., 1991. The excavations 1979-1988: the finds. Danebury: an Iron Age Hillfort in Hampshire, vol. 5. Council for British Archaeology.

Cubas, M., Lucquin, A., Robson, H.K., Colonese, A.C., Arias, P., Aubry, B., Billard, C., Jan, D., Diniz, M., Fernandes, R., Valcarce, R.F., Germain-Vallée, C., Juhel, L., de Lombera-Hermida, A., Marcigny, C., Mazet, S., Marchand, G., Neves, C., Ontañón-Peredo, R., Rodríguez-Álvarez, X.P., Simões, T., Zilhão, J., Craig, O.E., 2020. Latitudinal gradient in dairy production with the introduction of farming in Atlantic Europe. Nat. Commun. 2020 111 (11), 1–9. https://doi.org/10.1038/s41467-020-15907-4.

d'Errico, F., Vanhaeren, M., Barton, N., Bouzouggar, A., Mienis, H., Richter, D., Hublin, J.-J., McPherron, S.P., Lozouet, P., 2009. Additional evidence on the use of personal ornaments in the Middle paleolithic of North africa. Proc. Natl. Acad. Sci. Unit. States Am. 106, 16051–16056. https://doi.org/10.1073/pnas.0903532106.

Debono Spiteri, C., Gillis, R.E., Roffet-Salque, M., Castells Navarro, L., Guilaine, J., Manen, C., Muntoni, I.M., Saña Segui, M., Urem-Kotsou, D., Whelton, H.L., Craig, O.E., Vigne, J.-D., Evershed, R.P., 2016. Regional asynchronicity in dairy production and processing in early farming communities of the northern Mediterranean. Proc. Natl. Acad. Sci. Unit. States Am. 113, 13594–13599. https://doi.org/10.1073/pnas.1607810113.

Djama, M., 1999. Transformations agraires et systèmes ruraux mélanésiens en Grande Terre de Nouvelle-Calédonie. J. d'agriculture Tradit. Bot. appliquée 41, 201–224. https://doi.org/10.3406/jatba.1999.3708.

Dubois, J.P., 1984. L'élevage bovin en Nouvelle-Calédonie. Rev. El. Méd. Vét. N.C. 43–58.

Duda, R., 2017. Ethnoecology of Hunting in an Empty Forest. Practices, Local Perceptions and Social Change Among the Baka (Cameroon). Universitat Autonoma de Barcelona.

Dupont, C., Tresset, A., Desse-Berset, N., Gruet, Y., Marchand, G., Schulting, R., 2009. Harvesting the seashores in the late mesolithic of Northwestern Europe: a view from Brittany. J. World Prehistory 22, 93–111. https://doi.org/10.1007/s10963-009-9017-3.

Eaton, S.B., Nelson, D.A., 1991. Calcium in evolutionary perspective. Am. J. Clin. Nutr. 54, 281S–287S. https://doi.org/10.1093/ajcn/54.1.281S.

Eisenhauer, A., Müller, M., Heuser, A., Kolevica, A., Glüer, C.-C., Both, M., Laue, C., Hehn, U.v., Kloth, S., Shroff, R., Schrezenmeir, J., 2019. Calcium isotope ratios in blood and urine: a new biomarker for the diagnosis of osteoporosis. BoneKEy Rep. 10, 100200. https://doi.org/10.1016/j.bonr.2019.100200.

Ethier, J., Bánffy, E., Vuković, J., Leshtakov, K., Bacvarov, K., Roffet-Salque, M., Evershed, R.P., Ivanova, M., 2017. Earliest expansion of animal husbandry beyond the Mediterranean zone in the sixth millennium BC. Sci. Rep. 7 https://doi.org/10.1038/s41598-017-07427-x.

Evershed, R.P., Payne, S., Sherratt, A.G., Copley, M.S., Coolidge, J., Urem-Kotsu, D., Kotsakis, K., Özdoğan, M., Özdoğan, A.E., Nieuwenhuyse, O., Akkermans, P.M.M.G., Bailey, D., Andeescu, R.-R., Campbell, S., Farid, S., Hodder, I., Yalman, N., Özbaşaran, M., Bıçakcı, E., Garfinkel, Y., Levy, T., Burton, M.M., 2008. Earliest date for milk use in the Near East and southeastern Europe linked to cattle herding. Nature 455, 528–531. https://doi.org/10.1038/nature07180.

Fantle, M.S., Tipper, E.T., 2014. Calcium isotopes in the global biogeochemical Ca cycle: implications for development of a Ca isotope proxy. Earth Sci. Rev. 129, 148–177. https://doi.org/10.1016/j.earscirev.2013.10.004.

Fernandes, R., Millard, A.R., Brabec, M., Nadeau, M.J., Grootes, P., 2014. Food reconstruction using isotopic transferred signals (FRUITS): a bayesian model for diet reconstruction. PloS One 9, 1–9. https://doi.org/10.1371/journal.pone.0087436.

Flandrin, J.-L., Montanari, M., 1996. Histoire de l'alimentation. Fayard.

Food and Agriculture Organization of the United Nations, 2016. FAOSTAT Food Supply Database. FAO, Rome, Italy.. Retrieved August 4, 2018 from http://www.fao.org/faostat/en/#home. 2016.

Gerbault, P., Liebert, A., Itan, Y., Powell, A., Currat, M., Burger, J., Swallow, D.M., Thomas, M.G., 2011. Evolution of lactase persistence: an example of human niche construction. Philos. Trans. R. Soc. B Biol. Sci. 366, 863–877. https://doi.org/10.1098/rstb.2010.0268.

Gillis, R.E., Kovačiková, L., Bréhard, S., Guthmann, E., Vostrovská, I., Nohálová, H., Arbogast, R.-M., Domboróczki, L., Pechtl, J., Anders, A., Marciniak, A., Tresset, A., Vigne, J.-D., 2017. The evolution of dual meat and milk cattle husbandry in Linearbandkeramik societies. Proc. R. Soc. B Biol. Sci. 284, 20170905. https://doi.org/10.1098/rspb.2017.0905.

Guéguen, L., Pointillart, a, 2000. The bioavailability of dietary calcium. J. Am. Coll. Nutr. 19, 119S–136S. https://doi.org/10.1080/07315724.2000.10718083.

Hamilton, J., Bentley, R.A., Bickle, P., Fibiger, L., Hedges, R., Reynard, L., Wright, C., Cullen, P., Dale, C., Nowell, G., Whittle, A., 2013. Seeking diversity: methodology. In: Bickle, P., Whittle, A. (Eds.), The First Farmers of Central Europe: Diversity in LBK Lifeways. Oxbow books, pp. 29–48.

Hassler, A., Martin, J.E., Amiot, R., Tacail, T., Arnaud-Godet, F., Allain, R., Balter, V., 2018. Calcium isotopes offer clues on resource partitioning among Cretaceous predatory dinosaurs. Proc. R. Soc. B Biol. Sci. 285, 20180197. https://doi.org/10.1098/rspb.2018.0197.

Helmer, D., Gourichon, L., Vila, E., 2007. Development of the exploitation of products from Capra and Ovis (meat, milk and fleece) from the PPNB to the Early Bronze in the northern Near East (8700 to 2000 BC cal.). Anthropozoologica 2, 41–69.

Heuser, A., Tütken, T., Gussone, N., Galer, S.J.G., 2011. Calcium isotopes in fossil bones and teeth — diagenetic versus biogenic origin. Geochem. Cosmochim. Acta 75, 3419–3433. https://doi.org/10.1016/j.gca.2011.03.032.

Herrscher, E., Fenner, J.N., Valentin, F., Clark, G., Reepmeyer, C., Bouffandeau, L., André, G., 2018. Multi-isotopic analysis of first polynesian diet (Talasiu, tongatapu, kingdom of Tonga). J. Archaeol. Sci. Reports 18, 308–317. https://doi.org/10.1016/j.jasrep.2018.01.012.

Heuser, A., Eisenhauer, A., Scholz-Ahrens, K.E., Schrezenmeir, J., 2016. Biological fractionation of stable Ca isotopes in Göttingen minipigs as a physiological model for Ca homeostasis in humans. Isot. Environ. Health Stud. 52, 633–648. https://doi.org/10.1080/10256016.2016.1151017.

Heuser, A., Frings-Meuthen, P., Rittweger, J., Galer, S.J.G., 2019. Calcium isotopes in human urine as a diagnostic tool for bone loss: additional evidence for time delays in bone response to experimental bed rest. Front. Physiol. 10, 1–11. https://doi.org/10.3389/fphys.2019.00012.

Heyberger, L., 2009. Niveaux de vie biologiques, disponibilités alimentaires et consommations populaires en France au milieu DU XIXe siècle. Ann. Demogr. Hist. (Paris). 118, 167–191. https://doi.org/10.3917/adh.118.0167.

Heyberger, L., 2014. Received wisdom versus reality: height, nutrition, and urbanization in mid-nineteenth-century France. Cliometrica 8, 115–140. https://doi.org/10.1007/s11698-013-0095-1.

Hillman, G., Hedges, R., Moore, A., Colledge, S., Pettitt, P., 2001. New evidence of lateglacial cereal cultivation at Abu Hureyra on the euphrates. Holocene 11, 383–393. https://doi.org/10.1191/095968301678302823.

Hirata, T., Tanoshima, M., Suga, A., Tanaka, Y., Nagata, Y., Shinohara, A., Chiba, M., 2008. Isotopic analysis of calcium in blood plasma and bone from mouse samples by multiple collector-ICP-mass spectrometry. Anal. Sci. 24, 1501–1507.

Howard, A.J., Macklin, M.G., Bailey, D.W., Mills, S., Andreescu, R., 2004. Late-glacial and holocene river development in the teleorman valley on the southern Romanian plain. J. Quat. Sci. 19, 271–280. https://doi.org/10.1002/jqs.805.

Leblanc, J., Sirot, V., 2011. Étude de l'alimentation totale française 2 (EAT 2) Tome 1 - contaminants inorganiques, minéraux, polluants organiques persistants, mycotoxines et phyto-estrogènes. Anses, Agence Natl. sécurité Sanit. l'alimentation, l'environnement du Trav. 346.

Leblanc, J.C., Verger, P., Uérin, T., Volatier, J.L., 2004. Etude de l'alimentation totale française https:/doi.org/10.3166/sda.25.163-174.

LeGeros, R.Z., LeGeros, J.P., 1984. Phosphate minerals in human tissues. In: Phosphate Minerals. Springer Berlin Heidelberg, pp. 351–385.

Leonardi, M., Gerbault, P., Thomas, M.G., Burger, J., 2012. The evolution of lactase persistence in Europe. A synthesis of archaeological and genetic evidence. Int. Dairy J. 22, 88–97. https://doi.org/10.1016/j.idairyj.2011.10.010.

Li, Q., Thirlwall, M., Müller, W., 2016. Ca isotopic analysis of laser-cut microsamples of (bio)apatite without chemical purification. Chem. Geol. 422, 1–12. https://doi.org/10.1016/j.chemgeo.2015.12.007.

Mariotti, V., Bonfiglioli, B., Facchini, F., Condemi, S., Belcastro, M.G., 2009. Funerary practices of the Iberomaurusian population of Taforalt (Tafoughalt; Morocco, 11-12,000 BP): new hypotheses based on a grave by grave skeletal inventory and evidence of deliberate human modification of the remains. J. Hum. Evol. 56, 340–354. https://doi.org/10.1016/j.jhevol.2009.01.001.

Martin, J.E., Tacail, T., Adnet, S., Girard, C., Balter, V., 2015. Calcium isotopes reveal the trophic position of extant and fossil elasmobranchs. Chem. Geol. 415, 118–125. https://doi.org/10.1016/j.chemgeo.2015.09.011.

Martin, J.E., Tacail, T., Balter, V., 2017. Non-traditional isotope perspectives in vertebrate palaeobiology. Palaeontology 60, 485–502. https://doi.org/10.1111/pala.12300.

Martin, J.E., Tacail, T., Cerling, T.E., Balter, V., 2018. Calcium isotopes in enamel of modern and Plio-Pleistocene East African mammals. Earth Planet Sci. Lett. 503, 227–235. https://doi.org/10.1016/j.epsl.2018.09.026.

Martin, J.E., Vincent, P., Tacail, T., Khaldoune, F., Jourani, E., Bardet, N., Balter, V., 2017. Calcium isotopic evidence for vulnerable marine ecosystem structure prior to the K/Pg extinction. Curr. Biol. 27, 1641–1644. https://doi.org/10.1016/j.cub.2017.04.043.

Meiklejohn, C., Chamberlain, A.T., Schulting, R.J., 2011. Radiocarbon Dating of Mesolithic Human Remains in Great Britain.

Melin, A.D., Crowley, B.E., Brown, S.T., Wheatley, P.V., Moritz, G.L., Yit Yu, F.T., Bernard, H., DePaolo, D.J., Jacobson, A.D., Dominy, N.J., 2014. Calcium and carbon stable isotope ratios as paleodietary indicators. Am. J. Phys. Anthropol. 154, 633–643. https://doi.org/10.1002/ajpa.22530.

Merrett, D.C., Meiklejohn, C., 2007. Is house 12 at Bouqras a charnel house? The Charnel House at Bouqras 5, 127–139.

Moore, A.M.T., Hillman, G.C., 1992. The pleistocene to holocene transition and human economy in southwest asia: the impact of the younger dryas. Am. Antiq. 57, 482–494. https://doi.org/10.2307/280936.

Moore, A.M.T., Hillman, G.C., Legge, A.J., 2000. Village on the Euphrates. From Foraging to Farming at Abu Hureyra. Oxford University Press.

Morgan, J.L.L., Skulan, J.L., Gordon, G.W., Romaniello, S.J., Smith, S.M., Anbar, A.D., 2012. Rapidly assessing changes in bone mineral balance using natural stable calcium isotopes. Proc. Natl. Acad. Sci. Unit. States Am. 109, 9989–9994. https://doi.org/10.1073/pnas.1119587109.

Murdock, G.P., 1967. Ethnographic atlas: a summary. Ethnology. https://doi.org/10.2307/3772751.

Nitsch, E.K., Humphrey, L.T., Hedges, R.E.M., 2010. The effect of parity status on δ15N: looking for the " pregnancy effect" in 18th and 19th century London. J. Archaeol. Sci. 37, 3191–3199. https://doi.org/10.1016/j.jas.2010.07.019.

Nitsch, E.K., Humphrey, L.T., Hedges, R.E.M., 2011. Using stable isotope analysis to examine the effect of economic change on breastfeeding practices in Spitalfields, London, UK. Am. J. Phys. Anthropol. 146, 619–628. https://doi.org/10.1002/ajpa.21623.

O'Brien, M.J., Laland, K.N., 2012. Genes, culture, and agriculture: an example of human niche construction. Curr. Anthropol. 53, 434–470. https://doi.org/10.1086/666585.

Oddy, D.J., 1970. Working-class diets in late nineteenth-century Britain. Econ. Hist. Rev. 23, 314. https://doi.org/10.2307/2593832.

Oross, K., Bánffy, E., 2009. Three successive waves of Neolithisation: LBK development in Transdanubia. Doc. Praehist. 36, 175–189. https://doi.org/10.4312/dp.36.11.

Oross, K., Marton, T., Whittle, A., Hedges, R.E.M., Cramp, L.J.E., 2008. Die Siedlung der Balaton-Lasinja-Kultur in Balatonszárszó-Kis-erdei-dűlő.

Paris, J.P., 1981. Géologie de la Nouvelle-Calédonie, un essai de synthèse. Mémoires du Bur. Rech. géologiques minières 113, 1–279.

Pearson, J.A., Hedges, R.E.M., Molleson, T.I., Özbek, M., 2010. Exploring the relationship between weaning and infant mortality: an isotope case study from Aşikli Höyük and Çayönü Tepesi. Am. J. Phys. Anthropol. 143, 448–457. https://doi.org/10.1002/ajpa.21335.

Pereira, P.C., 2014. Milk nutritional composition and its role in human health. Nutrition 30, 619–627. https://doi.org/10.1016/j.nut.2013.10.011.

R core team, 2013. R: A language and environment for statistical computing (https://www.r-project.org/). R Foundation for Statistical Computing, Vienna, Austria.

Ramirez Rozzi, F.V., Koudou, Y., Froment, A., Le Bouc, Y., Botton, J., 2015. Growth pattern from birth to adulthood in African pygmies of known age. Nat. Commun. 6, 1–12. https://doi.org/10.1038/ncomms8672.

Reyes-García, V., Gallois, S., Díaz-Reviriego, I., Fernández-Llamazares, Á., Napitupulu, L., 2018. Dietary patterns of children on three indigenous societies. J. Ethnobiol. 38, 244–260. https://doi.org/10.2993/0278-0771-38.2.244.

Reynard, L.M., Hedges, R.E.M., 2008. Stable hydrogen isotopes of bone collagen in palaeodietary and palaeoenvironmental reconstruction. J. Archaeol. Sci. 35, 1934–1942. https://doi.org/10.1016/j.jas.2007.12.004.

Reynard, L.M., Henderson, G.M., Hedges, R.E.M., 2010. Calcium isotope ratios in animal and human bone. Geochem. Cosmochim. Acta 74, 3735–3750. https://doi.org/10.1016/j.gca.2010.04.002.

Reynard, L.M., Henderson, G.M., Hedges, R.E.M., 2011. Calcium isotopes in archaeological bones and their relationship to dairy consumption. J. Archaeol. Sci. 38, 657–664. https://doi.org/10.1016/j.jas.2010.10.017.

Reynard, L.M., Pearson, J.A., Henderson, G.M., Hedges, R.E.M., 2013. Calcium isotopes in juvenile milk-consumers. Archaeometry 55, 946–957. https://doi.org/10.1111/j.1475-4754.2012.00715.x.

Rozenberg, S., Body, J.J., Bruyère, O., Bergmann, P., Brandi, M.L., Cooper, C., Devogelaer, J.P., Gielen, E., Goemaere, S., Kaufman, J.M., Rizzoli, R., Reginster, J.Y., 2016. Effects of dairy products consumption on health: benefits and beliefs—a commentary from the Belgian bone club and the European society for clinical and economic aspects of osteoporosis, osteoarthritis and musculoskeletal diseases. Calcif. Tissue Int. 98, 1–17. https://doi.org/10.1007/s00223-015-0062-x.

Saint Martin, M., 2007. Carte géologique harmonisée du département du Rhône. *BRGM/RP-55461-FR*, vols. 1–228.

Salque, M., Bogucki, P.I., Pyzel, J., Sobkowiak-Tabaka, I., Grygiel, R., Szmyt, M., Evershed, R.P., 2013. Earliest evidence for cheese making in the sixth millennium bc in northern Europe. Nature 493, 522–525. https://doi.org/10.1038/nature11698.

Schulting, R.J., 1996. Antlers, bone pins and flint blades: the Mesolithic cemeteries of Téviec and Hoëdic, Brittany. Antiquity 70, 335–350.

Schulting, R.J., Richards, M.P., 2000. The Use of Stable Isotopes in Studies of Subsistence and Seasonality in the British Mesolithic. In: Young, R. (Ed.), Mesolithic Lifeways: Current Research From Britain and Ireland. University of Leicester Press, Leicester, pp. 55–65.

Schulting, R.J., Richards, M.P., 2001. Dating women and becoming farmers: new palaeodietary and AMS dating evidence from the Breton Mesolithic cemeteries of Téviec and Hoëdic. J. Anthropol. Archaeol. 20, 314–344. https://doi.org/10.1006/jaar.2000.0370.

Schulting, R.J., Richards, M.P., 2002. Finding the coastal Mesolithic in southwest Britain: AMS dates and stable isotope results on human remains from Caldey Island, South Wales. Antiquity 76, 1011–1025. https://doi.org/10.1017/S0003598X00091821.

Schulting, R., Wysocki, M., 2002. The Mesolithic human skeletal collection from Aveline's Hole: a preliminary note. Proc. - Univ. Bristol Spelaeol. Soc. 22, 255–268.

Schulting, R.J., Gardiner, P.J., Hawkesworth, C.J., Murray, E., 2010. The mesolithic and neolithic human bone assemblage from totty pot. Proc. - Univ. Bristol Spelaeol. Soc. 25, 75–95.

Ségurel, L., Bon, C., 2017. On the evolution of lactase persistence in humans. Annu.

Rev. Genom. Hum. Genet. 18, 297–319. https://doi.org/10.1146/annurev-genom-091416-035340.

Sillen, A., 1986. Biogenic and diagenetic Sr/Ca in plio-pleistocene fossils of the omo shungura formation. Paleobiology 3, 311–323. https://doi.org/10.1017/S0094837300013816.

Skrzypek, E., Cruz Mermy, D., Chèvremont, P., Ménillet, F., 2008. Carte géologique harmonisée du département du Haut-Rhin, vols. 1–322. BRGM/RP-56029-FR.

Skulan, J., DePaolo, D.J., 1999. Calcium isotope fractionation between soft and mineralized tissues as a monitor of calcium use in vertebrates. Proc. Natl. Acad. Sci. Unit. States Am. 96, 13709–13713. https://doi.org/10.1073/pnas.96.24.13709.

Stiner, M.C., Buitenhuis, H., Duru, G., Kuhn, S.L., Mentzer, S.M., Munro, N.D., Pollath, N., Quade, J., Tsartsidou, G., Ozba aran, M., 2014. A forager-herder trade-off, from broad-spectrum hunting to sheep management at A kl Hoyuk, Turkey. Proc. Natl. Acad. Sci. Unit. States Am. 111, 8404–8409. https://doi.org/10.1073/pnas.1322723111.

Tacail, T., 2017. Physiologie isotopique du calcium chez les mammifères. Ecole Normale Supérieure de Lyon.

Tacail, T., Albalat, E., Télouk, P., Balter, V., 2014. A simplified protocol for measurement of Ca isotopes in biological samples. J. Anal. At. Spectrom. 29, 529–535. https://doi.org/10.1039/c3ja50337b.

Tacail, T., Martin, J.E., Arnaud-Godet, F., Thackeray, J.F., Cerling, T.E., Braga, J., Balter, V., 2019. Calcium isotopic patterns in enamel reflect different nursing behaviors among South African early hominins. Sci. Adv. 5, eaax3250. https://doi.org/10.1126/sciadv.aax3250.

Tacail, T., Télouk, P., Balter, V., 2016. Precise analysis of calcium stable isotope variations in biological apatites using laser ablation MC-ICPMS. J. Anal. At. Spectrom. 31, 152–162. https://doi.org/10.1039/C5JA00239G.

Tacail, T., Le Houedec, S., Skulan, J.L., 2020. New frontiers in calcium stable isotope geochemistry: perspectives in present and past vertebrate biology. Chem. Geol. 537, 119471. https://doi.org/10.1016/j.chemgeo.2020.119471.

Tacail, T., Thivichon-Prince, B., Martin, J.E., Charles, C., Viriot, L., Balter, V., 2017. Assessing human weaning practices with calcium isotopes in tooth enamel. Proc. Natl. Acad. Sci. 114, 6268–6273. https://doi.org/10.1073/pnas.1704412114.

Toutain, J.-C., 1971. La consommation alimentaire en France de 1789 à 1964, n°. *Cah. l'ISEA* **Tome V**, pp. 1909–2049.

Valentin, F., Clark, G., Parton, P., Reepmeyer, C., 2020. Mortuary practices of the first Polynesians: formative ethnogenesis in the Kingdom of Tonga. Antiquity 94, 999–1014. https://doi.org/10.15184/aqy.2020.89.

Van den Hende, R., Van den Hende, M., 1970. Carte Géologique à 1/500 000 de reconnaissance de la République Fédérale du Cameroun, Feuille Abong-Mbamg Est N: NA 33 NO E24. Dir. des Mines la Géologie.

Vigne, J.-D., 2011. The origins of animal domestication and husbandry: a major change in the history of humanity and the biosphere. Comptes Rendus Biol. 334, 171–181. https://doi.org/10.1016/j.crvi.2010.12.009.

Vigne, J.-D., Helmer, D., 2007. Was milk a "secondary product" in the Old World Neolithisation process? Its role in the domestication of cattle, sheep and goats. Anthropozoologica 42, 9–40.

Vissers, P.A., Streppel, M.T., Feskens, E.J., de Groot, L.C.P.G., 2011. The contribution of dairy products to micronutrient intake in The Netherlands. J. Am. Coll. Nutr. 30, 415S–421S. https://doi.org/10.1080/07315724.2011.10719985.

Warinner, C., Hendy, J., Speller, C., Cappellini, E., Fischer, R., Trachsel, C., Arneborg, J., Lynnerup, N., Craig, O.E., Swallow, D.M., Fotakis, A., Christensen, R.J., Olsen, J.V., Liebert, A., Montalva, N., Fiddyment, S., Charlton, S., Mackie, M., Canci, A., Bouwman, A., Rühli, F., Gilbert, M.T.P., Collins, M.J., 2014. Direct evidence of milk consumption from ancient human dental calculus. Sci. Rep. 4, 7104. https://doi.org/10.1038/srep07104.

Whittle, A., Anders, A., Bentley, A., Bickle, P., Cramp, L., Domboroczki, L., Fibiger, L., Hamilton, J., Hedges, R., Kalicz, N., Kovacs, N.E., Marton, T., Oross, K., Pap, I., Raczky, P., 2013. Hungary. In: Bickle, P., Whittle, A. (Eds.), The First Farmers of Central Europe: Diversity in LBK Lifeways, p. 608. Oxbow books.

Zamberlin, S., Antunac, N., Havranek, J., Samaržija, D., 2012. Mineral elements in milk and dairy products. Mljekarstvo 62, 111–125.