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Analysing Milk and Dairy Consumption in Ancient Societies: Bioarchaeological, Evolutionary and Human Social Perspectives

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Abstract: Background: In prehistoric societies, especially in the Neolithic period, the study of the palaeodiet assumes special importance as it is one of the points in human history characterised by important changes in diet. In this context, the study of food intolerances is even more significant. Methods: Some of the individuals studied were analysed from a genetic point of view, while a systematic literature review was performed from a genetic perspective, verifying the persistence or absence of lactase in adulthood, and information from necropolises regarding the presence of biomarkers linked to possible consumption of dairy products was analysed. Results: The results indicate a clear majority of individuals with hypolactasia in adulthood, although in a Pyrenean necropolis, studied here for the first time, the lactase persistence allele was already detected. Dairy consumption was also verified to be widespread in very early Neolithic periods. Conclusions: From a population perspective, this study enables a deeper understanding of past populations' daily lives, expanding our perspective on their dietary patterns. From an evolutionary standpoint, it illuminates a pivotal point in human history and evolution within the European context, where past and modern dairy consumption, particularly cheese, has profound implications for both present and past economies.

Keywords: Neolithic; lactose; ancient DNA; milk; dairy; cheese

1. Introduction

One of the most multidisciplinary areas in Neolithic investigations is the study of the palaeodiet. Given the important "revolution" that this period entails, the timing of the exploitation of domestic animals, as well as their "secondary" products, becomes important to comprehend the appearance of certain health problems, which may include gastrointestinal disorders such as lactose intolerance. As Copley et al. (2005) reported, the fact that all humans produce lactase during childhood while only some populations retain this capacity in adulthood seems to suggest that at some point in the past, milk intake could have been tolerated in adulthood due to persistent lactase production [1]. However, it is important to note that the milking of ruminant animals in the past may not have been restricted to certain populations. This is because milk can be transformed into butter, cheese or yoghurt, which contain little to no lactose. Therefore, it is theoretically possible that most individuals consumed dairy products in the past, probably in their fermented form. Lipid residue analyses can detect traces of dairy lipids in the walls of ceramic vessels used for food storage. This helps to determine the emergence and spread of dairying in ancient times, as well as the potential importance of dairy compared to other foods [1].

1.1. Lactose Digestion in Humans

Lactose is a disaccharide composed of two monosaccharides, glucose and galactose, linked together by a β-1→4 bond [2]. The process of hydrolysis of this bond is dependent upon the presence of a specific enzyme, known as lactase, which functions in the digestion of lactose into its constituent components. This process facilitates the uptake of glucose and galactose by the intestine [3]

The expression of intestinal lactase is subject to developmental regulation [2]. According to Liebert et al. (2017), lactase persistence (LP) has a genetic basis with a dominant pattern of inheritance [4]. This trait is hypothesised to be a result of multiple SNPs in a regulatory region, including a particular transcriptional enhancer called MCM6, which is located upstream of the LCT gene [5]. Furthermore, LP is categorised as an autosomal dominant trait, enabling the continuous production of the enzyme lactase throughout an individual's adult life. The condition of lactase non-persistence is the ancestral state of humans [6]. In humans, the onset of intestinal lactase activity occurs during the third trimester of gestation, reaching its maximum level at birth [2,7,8]. Healthy children typically demonstrate elevated levels of lactase activity. However, following weaning, the manifestation of two distinct phenotypes becomes evident—lactase deficiency and lactase persistence. In individuals who are lactase deficient, the expression of lactase shows a gradual decline during childhood, which ultimately results in their inability to digest dietary lactose. This condition is classified as adult-type hypolactasia [3]. It is important to emphasise that, according to Savaiano and Levitt, despite a genetic intolerance to lactose in low concentrations, it is not completely impossible for lactose to be digested due to the microbiome itself, specifically due to microbial β-galactosidase [9,10]. In contrast to lactase-deficient individuals, LP individuals continue to express elevated levels of lactase activity after childhood (i.e., normolactasia) and retain the capacity to digest lactose [3]. A specific genetic trait enables the continued activity of intestinal lactase into adulthood [11]. In individuals of European descent, hypolactasia has been observed to be associated with two distinct allelic variants located in the upstream region of the lactase gene (LCT)—C/T-13910 (rs4988235) and G/A-22018 (rs182549) [12,13]. Previous studies conducted by Lewinsky et al. (2005) indicated that the −13910T allele can directly affect LCT gene promoter activity [14], which may result in a possible mutation that allows continued production of the enzyme in adult life [15]. For instance, in contemporary Finnish populations, the −13910C (rs4988235) polymorphism has been shown to be associated with the lactase-nonpersistent phenotype in 100% of cases, while the −22018G (rs182549) polymorphism has been demonstrated to be linked to this phenotype in approximately 97% of cases. According to Ségurel and Bon (2017) [13], the −13910T (rs4988235) polymorphism associated with the −22018A (rs182549) polymorphism is responsible for lactase persistence in Eurasia and Central and North African populations.

Globally, approximately 30% of individuals worldwide are lactase persistent in adulthood. However, the prevalence of LP varies between different populations and societies. It is a generally accepted fact that cultures that have historically relied on pastoralism and dairy products demonstrate a higher prevalence of lactase persistence than populations with a lower consumption of dairy. For instance, in Southeast Asia, the proportion of adults who are lactase deficient is reported to be at least 90% in the population, whereas in Scandinavia, the prevalence is approximately 10% [16]. However, it is necessary to understand whether these populations were able to digest lactose because they had lifestyles based on pastoralism or whether their lifestyles were based on pastoralism because they tolerated the digestion of this disaccharide. The study of lactose digestion is a significant area of research in the field of evolutionary human biology. To date, there is no clear perception of human behaviour in past societies with regard to milk consumption, which is closely associated with agriculture.

1.2. Evidence of Dairy Consumption Among Neolithic Individuals in Europe

In an attempt to construct a more comprehensive depiction of prehistoric milk utilisation and to determine the extent to which it explains selection on lactose persistence, Evershed et al. (2022) assembled a significant number of pottery containers from 366 archaeological sites in Europe and Southwest Asia. The authors generated time series illustrating the frequency of milk utilisation across prehistoric continental Europe from 7000 BC to AD 1500 [17]. The findings of this study, in concordance with others in the field [1,2,18–29], indicate that the exploitation of milk could have first occurred with the advent of the first farmers in the Mediterranean basin (excluding the region that would subsequently become modern-day Greece) and persisted throughout the Neolithic period. The subsequent arrival of the Neolithic period in southern Britain was accompanied by an immediate and substantial utilisation of milk, a development that is likely indicative of the entrance of dairy populations from adjacent regions of Europe [30], which then gradually decreased. The prehistoric Balkans comprised one of the primary regions for the early intensification of milk utilisation, which is consistent with the rise of livestock exploitation in this area [31]. However, an analysis of over 190 animal fats from more than 870 potsherds has revealed the surprising absence of milk use in geographically adjacent Neolithic site phases located in Greece. It is acknowledged that other types of containers may have been utilised for milk processing in this cultural context or have not been preserved to the present day for study. The prevailing perspective suggests that, while dairying persisted throughout the Neolithic period, its intensity exhibited significant fluctuations both geographically and temporally [32].

1.3. The Case of the Pyrenean Mountains

Based on their study of 35 Neolithic sites located between 900 and 1700 m asl, Antolín et al. (2018) [21] described the importance of domestic animals during the Neolithic period in the Pyrenees. The Pyrenees Mountains form the dividing line between Europe and the Iberian Peninsula, a region which, due to its different climate and orography, could have seen different evolutionary behaviours regarding the consumption of milk and dairy products.

Domestic animals were found to be the primary component of all faunal assemblages recovered in the Pyrenees area. The most exploited resource in settlements, regardless of their functions, was ovicaprines, particularly sheep. In both lowland and highland areas, it seems that the main purpose of animal husbandry was meat production. On the other hand, several Neolithic sites have provided evidence of milk production, as shown by pottery residue analyses. Examples include Can Sadurní Cave [20,23], Els Trocs Cave [33] and La Draga [20,21]. One important conclusion of the Antolin et al. (2018) study is that the authors did not observe any activities compatible with a typical transhumance (seasonal) model or specialized pastoralist. Instead, they found indicators of permanent communities with a mixed farming strategy [21]. However, another human settlement also located in the Pyrenees, La Feixa del Moro, seems to indicate the complete absence of dairy products. It cannot be confirmed whether this lack of information is due to the lack of material evidence reaching the present day to be evaluated or whether this population did not use cattle as a source of dairy products [34–36].

One of the characteristics of mountains like the Pyrenees is that, while on the one hand they act as bridges between very unequal regions like the Iberian Peninsula and the rest of continental Europe, due to their own climate and high altitude, they also act as an "island", and the evidence found here is of paramount importance when compared with human evidence in the rest of Europe. Thus, despite the simplicity of the concept of being able to digest the lactose present in dairy products, it is something that may have marked a turning point in situations of food scarcity, especially in regions as harsh as the Pyrenees.

1.4. Consumption of Secondary Products in Neolithic Europe

Analysing organic residues during archaeological investigations provides relevant results on subsistence and food habits, including social and geographical differences between different ancient populations [24]. As stated by Roffe-Salque (2017), the 1970s witnessed the first studies of residues, with a particular focus on organic compounds, in relation to archaeological ceramics, particularly remains found inside vessels [37]. The identification of organic compounds facilitates the interpretation of the use of these vessels, thereby enabling the development of hypotheses regarding the activities of ancient populations and the lifestyles of past civilisations. For instance, analyses of lipids (fats, waxes or resins) preserved in the residues can serve as an analytical tool to ascertain the specific use of the pottery.

Different studies have shown the relevance of the consumption of dairy products in different parts of the ancient world [28,38–54]. For example, Evans et al. (2023) [55] described a wide variety of populations where milk secondary products were observed, such as the victims of the Great Irish Famine (XIX century) [56], individuals in Neolithic Britain [2], populations from the Eastern Eurasian steppe [57], individuals in the Neolithic East African Meroitic [58], or populations from early Bronze Age China [59]. The presence of significant quantities of milk residues in pottery vessels from seventh-millennium sites in northwestern Anatolia offers the earliest evidence of milk processing, although the precise nature of this practice remains ambiguous. It is worth noting that pottery sherds perforated with minute apertures have been discovered at early Neolithic sites in temperate Europe dating back to the sixth millennium BC. These have been interpreted on the basis of typology as 'cheese strainers' [60], although a direct correlation with milk processing has yet to be conclusively demonstrated. The presence of organic residues within pottery vessels has provided direct evidence for the use of milk in the Neolithic period, as evidenced by analysis of the δ13C and δ13C values of major fatty acids in milk. This use has been identified in the Near East, southeastern Europe, northern Africa, Denmark and the British Isles [1,24,49,61]. The presence of considerable milk lipids in these particular vessels, which bear a close resemblance to modern-day cheese strainers [62], provides compelling evidence that these vessels were utilised for the purpose of dividing fat-rich milk curds from the lactose-containing whey. This new proof serves to highlight the significance of pottery in the processing of dairy products, specifically in the production of reduced-lactose milk products among prehistoric farming societies [63,64].

In this sense, it is interesting to understand if the populations that brought agriculture to Europe and came to dominate livestock farming would have the ability to use milk as a primary food. The aim of this work is to analyse the genetic predisposition studied to date of individuals belonging to prehistoric European societies, specifically Neolithic individuals, and to evaluate it against possible material records found in these groups. Moreover, the objective was also to explore the potential correlation between the challenges faced by ancestral European populations in digesting lactose and the emergence of a significant economic activity that is widely attributed to the European continent, including in the present era—the production and consumption of secondary products, mainly cheese.

2. Materials and Methods

In order to comprehend the problem previously outlined and to understand our results from La Feixa del Moro (modern Spain) in the European Neolithic context, two distinct approaches were undertaken. On the one hand, a small group of three individuals from the Neolithic period in the Pyrenees region, important for their archaeological significance, was analysed from a genetic point of view. On the other hand, genetic information from different prehistoric necropolises on the European continent published to date was collected for a systematic literature review. The information was compiled through a systematic review of the scientific literature using the following inclusion criteria: (a) the populations studied were prehistoric; (b) the genetic methodology applied was explicit, specifically indicating which SNPs were analysed, as well as which biomarkers related to the consumption of milk and its derivatives were considered; and (c) the results obtained were explicit. The exclusion filters were mainly (a) articles without an explicit methodology; (b) articles published before the year 2000; (c) articles where the results were not explained; and (d) articles where the populations did not have a date or an associated period.

The search for the systematic literature review was carried out using scientific databases, specifically, The National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/), ScienceDirect (https://www.sciencedirect.com/), Scientific Electronic Library Online (https://www.scielo.org/es, accessed on 18 December 2024) and Bielefeld Academic Search Engine (https://www.base-search.net/), from September to December 2024. The keywords used to carry out the systematic review of the scientific literature were "Prehistoric; Neolithic; lactose; ancient DNA; milk; dairy; cheese". The dates indicated are those mentioned by the authors of the studies, and no changes were made.

Case 1: LP genetic analysis. Four archaeological sites on Gotland in the Baltic Sea (4800–4200 BP) (Malmström et al., 2010) [18].

Samples were sourced from four archaeological sites on Gotland in the Baltic Sea, dating to the middle Neolithic period (4800–4200 BP). The initial set comprised 36 samples in total, with 14 originating from Gotland and the remaining 22 from mainland Sweden. The Gotland samples were selected from the following subsequent sites: Ajvide (n = 9) in the parish of Eksta, Visby town (n = 1), Ire (n = 2) in the parish of Hangvar and Fridtorp (n = 2) in the parish of Vasterhejde. It is notable that all samples were at least 1000 years younger than the first signs of agriculture in Scandinavia. Consequently, these samples are indicative of a non-agricultural lifestyle that predates the introduction of agriculture in the region. The 13910 (rs4988235) SNP was amplified.

Case 2: La Feixa del Moro archaeological site (3975–3790 cal. BC) (Andorran Pyrenees) (information first published in this study).

La Feixa del Moro (3975–3790 cal. BC) necropolis, located on the Andorran Pyrenees (1335 m altitude and 455 m above the valley floor), consists of a small necropolis constituted by only three late Neolithic tombs in cists, with ancient human remains in two of them. Specifically, we sampled the skeletons of an adult male individual (individual 1) simultaneously buried with a female adult (individual 2) and a male newborn (individual 3) [35,36]. Prior to this study, an anthropological study of the group was carried out [65], which described the age and sex attributions according to the criteria described therein and in [34]. A study of the diet was also carried out on the basis of carbon and nitrogen isotopes.

(a) Paleogenetic Analysis

Bone and dental samples were chosen by the archaeological investigators at the *Patrimoni Cultural de Andorra* (Principality of Andorra) repository. For individual 1 (adult male), two fragments were selected from the right femur and the left tibia; for individual 2 (adult female), a fragment from the right tibia and a molar fragment were selected; and finally, for individual 3 (neonate), one part of the clavicle and one rib were selected. The samples were sent in individual packages to a genetics laboratory, specifically to the degraded DNA section. Here, each area is dedicated to a specific task, specifically, an area for receiving and recording the samples received, one for cleaning the samples, another for the extraction of genetic material considered a priori highly degraded (low-template DNA), an area for the amplification of genetic material and a post-amplification zone, where the amplified information is analysed, whether fragments or sequences.

(a1) Genetic Material Extraction

Genetic material extraction was performed by pulverizing one of the samples and without physical destruction of the other sample according to Gomes et al. (2020) [36]. The extracts were then preserved at −20 °C until genetic amplification.

(a2) Lactose Persistence/Absence, Genetic Analysis

In the present investigation, one polymorphism contained in the SNP*for*ID 34-Plex forensic ancestry set of markers [66] was analysed, specifically, the 22018 SNP (rs182549).

Case 3: LP genetic analysis. Tollense battlefield archaeological site (Bronze Age, 3200 BP) (modern Germany) (Burger et al., 2020) [67].

The authors conducted an investigation into 14 Bronze Age warriors from the Tollense battlefield in northern Germany (3200 BP). This site is the oldest large-scale conflict location north of the Alps. The authors complemented these data with the genotypes of 18 individuals from the Bronze Age site Mokrin in Serbia (4100 to 3700 BP) and 37 individuals from Eastern Europe and the Pontic–Caspian Steppe region, predating both Bronze Age sites (5980 to 3980 BP).

The 13910 (rs4988235) SNP was amplified.

Case 4. LP genetic analysis. San Juan Ante Portam Latinam archaeological site (5070 ± 150 YBP) (modern Spain) (Plantinga et al., 2012) [27].

Lactase profiles were obtained from 26 samples, 19 of which were obtained from the site of San Juan Ante Portam Latinam located in Araba (Basque Country, Spain) and dated to 5070 ± 150 years before the present (YBP), thus corresponding to the late Neolithic period. Seven additional samples were obtained from the site of Longar, a location situated in the southern region of the province of Navarre (Spain) dated to 4450 ± 70 YBP, corresponding to the transition between the Neolithic and the Chalcolithic periods. The authors of the study analysed the 13910C (rs4988235) polymorphism. There is no mention of analysing products associated with cheese or milk derivatives.

Case 5: LP genetic analysis. Cave I of the Treilles archaeological site (3000 BC) (modern France) (Lacan et al., 2011) [19].

In the present study, the authors analysed the genetic material of 26 out of 29 ancient individuals from Cave I of Treilles (3000 BC) located in the Grands Causses region of Saint-Jean-et-Saint-Paul, Aveyron, France. The 13910 (rs4988235) SNP (single-nucleotide polymorphism) associated with lactase persistence was typed for these samples.

Case 6. LP genetic analysis. Five archaeological sites from central Europe (8th millennium BC–6th century AD) (modern Lithuania, Germany, Hungary and Poland) (Burger et al., 2007) [64].

Bone and tooth samples were obtained in eastern Germany, Hungary, northeastern Poland and northeastern Lithuania.

Three individuals were sourced from the Derenburg Meerenstieg II cemetery in the northern Harz region of eastern Germany, and the burial sites belong to the Neolithic Linear Pottery culture, indicating the initial farmers of central Europe.

Three additional individuals were sourced from the Szarvas site located on the Great Hungarian Plain in the District of Békés, southeastern Hungary. The skeletons belong to the Körös Culture, which emerged in eastern Hungary during the early 8th millennium BC.

Two skeletons were unearthed at the Kretuonas burial site in eastern Lithuania and are attributed to the middle Neolithic Narva culture. These skeletons were radiocarbon-dated to the 6th millennium BC.

Tooth samples from one Mesolithic individual were obtained from Drestwo, a site in the Suwalki region of northeastern Poland, which were radiocarbon-dated to the late 5th millennium cal. BP.

One skeleton was obtained from the early medieval Merovingian burial ground Eltville in Hesse, southwestern Germany, and was dated to the 4th to 6th centuries AD.

The 13,910 (rs4988235) and 22018 (rs182549) SNPs were amplified.

Case 7. LP genetic analysis. El Portalón archaeological site (late Neolithic period and early Bronze Age) (modern Spain) (Sverrisdóttir et al., 2014) [68].

Ancient DNA data from the skeletal remains of eight late Neolithic Iberian individuals were collected. The remains were excavated from the site of El Portalón in the Sierra de Atapuerca (modern Burgos, Spain). The authors do not present a specific date for the individuals but indicate that they belong to the late Neolithic and early Bronze Age northeastern Iberian region.

The 13910 (rs4988235) SNP was amplified.

Case 8. Organic residue analysis. Atlantic European archaeological sites (ca. *5500–3300 cal. BC) (modern France, Portugal, Spain and Western Baltic margin) (Cubas et al., 2020) [38].*

Pottery sherds were selected from archaeological sites from Portugal, Spain and France dated ca. 5500 and 3500 cal. BC and the Western Baltic margin dated between ca. 3950 and 3300 cal. BC. AMS radiocarbon (14C) dates attribute these sites to the early Neolithic, i.e., contemporary with the earliest introduction of domesticated animals and plants. Lipid analysis included extraction, gas chromatography–flame ion detection, chromatography–mass spectrometry and chromatography–combustion isotope ratio mass spectrometry.

Case 9. Organic residue analysis. Three archaeological sites from Britain (c. 3800–3090 cal. BC) (modern United Kingdom) (Charlton et al., 2019) [2].

Ten dental calculus samples were analysed from three British Neolithic burilas, including three from the site of Hambledon Hill (3680–3310 cal. BC and 3650–3370 cal. BC), five from Hazleton North (c. 3800–3620 cal. BC) and two from Banbury Lane (c. 3360–3090 cal. BC).

The analysis employed a shotgun metaproteomic approach utilising liquid chromatography tandem mass spectrometry (LC/MS-MS) to ascertain the presence of the milk protein β-lactoglobulin (BLG).

Case 10. Organic residue analysis. Sławęcinek archaeological site (3650–3100 cal. BC) (modern Poland) (Evans et al., 2023) [55].

The site of Sławęcinek is located near Inowrocław in central Poland and is dated to the late Neolithic period (ca. 3650–3100 cal. BC).

A proteomic analysis was conducted on the residues adhered to the vessel walls using three collared containers and a ceramic filter. Subsequently, a lipid residue analysis was performed on the vessels using the ceramic itself. Proteins were also examined in residues adhered to four contemporary vessels, where milk had either been warmed and coagulated or coagulated and strained. The extracted lipids were characterised using gas chromatography–mass spectrometry (GC-MS) techniques following acidification with methanol.

3. Results

Case 1. LP genetic analysis. Four archaeological sites on Gotland in the Baltic Sea (4800–4200 BP) (Malmström et al., 2010) [18].

In the original examination of 14 individuals, 10 provided reliable results. The authors of the study found that the T allele frequency was low (5%) in the examined middle Neolithic hunter–gatherer population, and that this frequency differs considerably from that in the contemporary Swedish population (74%).

Case 2. La Feixa del Moro archaeological site (3975–3790 cal. BC) (Andorran Pyrenees) (information first published in this study).

The results for the 22018 (rs182549) analysis, not yet published to date, reveal that individual 1 (adult man) had the *GA* genotype, whereas it was not possible to obtain results from individual 2 (adult woman), and individual 3 (infant) had the *GG* genotype.

Case 3. LP genetic analysis. Tollense battlefield archaeological site (Bronze Age, 3200 BP) (modern Germany) (Burger et al., 2020) [67].

As Burger et al. (2020) reported, lactase persistence was absent in nine early Neolithic Central Europeans (7500 YBP).

Case 4. LP genetic analysis. San Juan Ante Portam Latinam archaeological site (5070 ± 150 YBP) (modern Spain) (Plantinga et al., 2012) [27].

The study revealed that 31% of individuals from the San Juan Ante Portam Latinam necropolis in Araba and 14% of individuals from the Longar necropolis exhibited the polymorphism linked to lactase persistence. These findings suggest that in both contexts, the majority of adults were unable to properly digest lactose.

Case 5. LP genetic analysis. Cave I of the Treilles archaeological site (3000 BC) (modern France) (Lacan et al., 2011) [19].

The researchers reported the absence of lactase persistence in 26 samples from a late Neolithic burial site found in southern France. All of the samples were homozygous C/C for this marker, which suggests that the ancient Treilles population was unlikely to have been able to digest fresh milk.

Case 6. LP genetic analysis. Five archaeological sites from central Europe (8th millennium BC–6th century AD) (modern Lithuania, Germany, Hungary and Poland) (Burger et al., 2007) [64].

The authors identified 9 of 10 individuals as homozygous C at position 13910; all were Mesolithic and Neolithic samples. The one Medieval individual was heterozygous for the 13910-C/T polymorphism. This was the only heterozygous individual without allelic dropout. For the 22018-A/G polymorphism, all samples were G, except for the Medieval individual, which was A.

Case 7. LP genetic analysis. El Portalón archaeological site (late Neolithic period and early Bronze Age) (modern Spain) (Sverrisdóttir et al., 2014) [68].

In the present study, the T allele was not identified in any of the eight samples analysed.

*Case 8. Organic residue analysis. Atlantic European archaeological sites (*ca. *5500–3300 cal. BC) (modern France, Portugal, Spain, Western Baltic margin) (Cubas et al., 2020) [38].*

A total of 234 samples were obtained, with concentrations of lipids that could be reliably measured (i.e., >5 $\mu g\ g^{-1}$) being present in 95% of these samples. Previous studies conducted in the British Isles, Ireland and Northern Europe have also reported excellent preservation of lipids. The authors of the present study have found that as farming was progressively introduced along a northerly latitudinal gradient, there was an increase in the frequency of dairy products in pottery.

Case 9. Organic residue analysis. Three archaeological sites from Britain (c. 3800–3090 cal. BC) (modern United Kingdom) (Charlton et al., 2019) [2].

Proteins were successfully recovered from all 10 of the dental calculus samples examined. The findings from the British sites demonstrate that Neolithic populations consumed milk and/or dairy products derived from cattle, goats and/or sheep (or potentially combinations of these). This assertion is corroborated by the recent discovery of evidence for bovine milk consumption among the middle Neolithic individual unearthed at Stonehenge.

Case 10. Organic residue analysis. Sławęcinek archaeological site (3650–3100 cal. BC) (modern Poland) (Evans et al., 2023) [55].

The assemblage was found to contain exclusively dairy proteins, suggesting a diet consisting primarily of dairy products. Conversely, no meat, plant or yeast proteins were detected.

The proteomic analysis yielded data indicating that dairy taxonomy is specific to the subfamily level, demonstrating both Caprinae (probably sheep and/or goat) and Bovinae (probably cow) milk. This finding indicates that a diversified dairy species economy was being practised at Sławecinek during the TRB.

Further indication for milk production at Sławęcinek was provided by the mandibular mortality profiles of 17 cattle, which indicate that four of them were juveniles (approximately six months of age), five individuals were subadults (approximately 24 months) and the remaining eight mandibles belonged to adult individuals (greater than 2.5 years).

The results obtained above are summarised in Table 1 below.

Table 1. This table presents a synopsis of the results obtained from seven cases submitted to genetic analysis of lactase persistence (LP) and three cases evaluated by organic residue analysis. It should be noted that the dates indicated in this table correspond to the dates specified by the authors in the respective articles.

Case	Investigation	Date	Region	Result	Study
1	LP genetic analysis	4800–4200 BP	Gotland in the Baltic Sea	5% of the individuals showed lactase persistence 95% presented hypolactasia	Malmström et al., 2010 [18]
2	LP genetic analysis	3975–3790 BC	Andorran Pyrenees	Three individuals presented hypolactasia (one individual was heterozygous)	Present study
3	LP genetic analysis	3200 BP	Modern Germany	100% of the individuals presented hypolactasia	Burger et al., 2020 [67]
4	LP genetic analysis	5070 ± 150 BP	Modern Spain	31% of individuals from Araba and 14% of individuals from the Longar were lactase persistent	Plantinga et al., 2012 [27]
5	LP genetic analysis	3000 BC	Modern France	100% of the individuals presented hypolactasia	Lacan et al., 2011 [19]
6	LP genetic analysis	8th millennium BC–6th century AD	Central Europe	100% of the Mesolithic and Neolithic samples presented hypolactasia One Medieval individual was heterozygous for both polymorphisms	Burger et al., 2007 [64]
7	LP genetic analysis	Late Neolithic period and early Bronze Age	Modern Spain	100% of the individuals presented hypolactasia	Sverrisdóttir et al., 2014 [68]
8	Organic residue analysis	5500–3300 BC	Modern France, Portugal, Spain and Western Baltic margin)	95% of these samples presented lipids associated with dairy activity	Cubas et al., 2020 [38]
9	Organic residue analysis	3800–3090 BC	(modern United Kingdom)	The results from the 10 analysed samples indicated that Neolithic populations consumed milk and/or dairy products derived from cattle, goats and/or sheep (or potentially combinations of these)	Charlton et al., 2019 [2]
10	Organic residue analysis	3650–3100 cal. BC	(modern Poland)	The assemblage was found to contain exclusively dairy proteins, suggesting a diet consisting primarily of dairy products (Caprinae and Bovinae)	Evans et al., 2023 [55]

4. Discussion

Despite the development of agriculture and its gradual application in Europe, the different results observed in individuals from Neolithic times indicate that, at a genetic level, individuals would not have the capacity for complete digestion and absorption of lactose. On the other hand, it can also be seen from the studies presented that, although they are not yet very extensive, they already demonstrate the very early use of milk as a primary product for the production of cheese and/or other dairy products.

As far as geographical distribution is concerned, there appear to be no significant differences between observations from the Iberian Peninsula, the British Isles and the centre of Europe. The majority of individuals from this period did not seem to exhibit the alleles related to lactose digestion. However, one of the curious cases presented here is that of the three individuals analysed from the Andorran Pyrenees, where the allele associated with the possible digestion of lactose was observed.

4.1. The Case of the Pyrenees

Considering the small group analysed in this study, the results are quite significant given the previous study by Gomes et al., 2020 [36], which demonstrates the high probability of being a biological family. Thus, the GG homozygosity observed in the baby for the −22,018 SNP reveals that he would be lactose intolerant in adulthood, while the probable father, individual 1, being GA, would show lactose digestion. Considering the homozygosity observed in the baby, it could be assumed that the mother (individual 2) would be GG or GA. These genotypes in this small group produced an elevated frequency of polymorphism *G* associated with intolerance. This is consistent with the previous existence of important frequencies of hypolactasia described in the other Neolithic cases. According to Subirà et al. (2018) [34], the palaeodiet reconstruction indicates that the two adult individuals from La Feixa del Moro consumed mainly terrestrial resources, consisting of the meat of herbivores and derived products, as well as C3 plants and possibly cereals, as occurred in other societies in southern France [34]. This is of particular importance when considering the presence of plant remains of such significance at the site, which have led to the site being designated as a rural settlement associated with agricultural practices [69]. To date, La Feixa del Moro has yielded no evidence of milk or dairy product consumption. This can be attributed to a paucity of evidence available to analyse or to the absence of involvement in the production of by-products derived from milk. It is not possible to exclude individuals from having consumed this diet due to their inability for or discomfort in digesting lactose. Other Neolithic settlements with livestock have been described at approximately the same altitude, such as the Perafita Valley at 1100 m altitude, dating from (3123 ± 200 cal. BC) [70]. Therefore, to determine if there is a correlation between livestock and the ability to digest lactose in the East of the Pyrenees, it would be crucial to analyse individuals from the Perafita Valley or other sites analysed by Antolin et al. (2018) [21] in their study about husbandry in Neolithic Pyrenees, but in all the cases, as for La Feixa del Moro, human remains are scarce. This question is complex as populations may have kept cattle for meat production rather than milk consumption, as suggested by Antolín et al. (2018) [21]. On the other hand, a pertinent issue concerns the harsher and typically colder climate of the Pyrenees, which may have posed a challenge to livestock farming. While milk's reduced shelf life compared to other dairy products is a recognized disadvantage, this issue may have been less pronounced in the Pyrenees, potentially benefiting individuals with a genetic predisposition for prolonged lactase activity.

4.2. The Iberian Peninsula and Milk Consumption in the Neolithic Period

The study on La Feixa del Moro is not the first to find the presence of lactase persistence in an Iberian Neolithic population. The study by Plantinga et al. (2012) (*Case 4*) investigated lactase persistence in two burial sites from the late Neolithic period in Basque Country (5000–4500 YBP) [27]. They found an average frequency of lactase persistence of 27%. According to the authors, this value is much lower than that in the modern Basque population.

This could be consistent with the hypothesis that Neolithic and post-Neolithic evolutionary pressures by cattle domestication and consumption of dairy products led to high lactase persistence in Southern European populations.

The presence of the *A* allele in this small group from La Feixa del Moro is also consistent with a possible growing frequency of lactose tolerance during the Neolithic and post-Neolithic periods. However, despite being one of the areas of great interest in human evolution, there are still not enough studies to be able to generalise about milk consumption in the Iberian Peninsula. Compared to the overall results, all the data seem to indicate the same trend—Neolithic populations did not seem to produce lactase in adulthood.

4.3. Lactase Persistence and Bioarchaeological Investigations

The frequency of the LP allele (rs4988235-A) has been a subject of interest. Although this allele only reached appreciable frequencies by approximately 2000 BC [19], it is important to note that this is nearly three millennia after its initial detection (the earliest LP individual has been dated to approximately 4700–4600 BC); the origins of the allele, the start of selection and the emergence of appreciable frequencies of this allele could have been possibly separated by thousands of years [30]. The ten cases analysed here demonstrate that the intensity of milk usage did not influence selection on LP. This is evidenced by the fact that different populations were already in contact with milk but were still unable to digest lactose in adulthood. In addition, the ten cases collated here enable us to reach an understanding of the subject in accordance with Evershed et al. (2022) [17]. We demonstrate that the scale of prehistoric milk utilisation for the production of cheese and other secondary products does not assist in explaining the trajectories of European LP allele frequencies. Consequently, it is also unable to account for selection intensities [30]. In fact, there are cases in which the production of cheese and other milk derivatives—from cows, goats and sheep—precedes some genetically analysed individuals, but Neolithic adults continued to present hypolactasia in adulthood. While it is widely accepted that sheep were domesticated in the Near East at 9000 BC and that cattle and goats were domesticated at 7000 BC, there is an absence of direct evidence supporting the milking of these animals [71]. Evidence of dairying can be traced back to the period 4000–2900 BC across various regions, including the Sahara, Egypt and Mesopotamia, as illustrated by pictorial and written records [71,72]. In the case of Britain, however, evidence pertaining to the prehistoric period is restricted to secondary evidence associated with the procurement and use of dairy products, such as alleged ceramic "cheese" strainers dated from 4500 BC.

Milk is naturally produced by different mammals. Therefore, different types of milk can be described by the concentrations of their components or nutrients. For example, the approximate protein concentration of cow and goat milk is 3.3%, and that of ewe milk is 5.6%. The approximate fat content of cow milk is 3.6%, that of goat milk is 4.3%, and that of ewe milk is 7.6%. Furthermore, the approximate lactose content of cow milk is 4.7%, that of goat milk is 4.6%, and that of ewe milk is 4.2% [73]. Different concentrations could serve as characteristics to identify the specific animal from which milk and its dairy products were derived.

The natural degradation of dairy products directly affects the concentrations of their constituents. When old samples are analysed, those structures or parts of them that resist the passage of time can be found. Degradation can be a problem when differentiating dairy products, cheese, butter or yoghurt by the percentages of their main components mentioned above.

However, it is important to keep in mind the great challenge of analysing ancient samples, especially because the compounds being sought—sugars, proteins and lipids—degrade over time. Some lipids, components of dairy products, have a structure that can withstand the passage of time, which is why they can be found in various archaeological samples. For example, vessels were used to make cheese from milk. Cheese-making allows milk to be preserved, mainly because water is eliminated from its composition. When cheese is consumed, the nutritional resource is important because it contains more significant concentrations of proteins, sugars and fats.

Looking at the materials found in prehistoric societies, it is obvious that the values in question could not be the same. This is due to the potential selection of animals for different nutritional outcomes. Furthermore, the passage of time itself could result in significant degradation of these biomarkers given the ages of the objects in question, which could be estimated to be over 6000 years old. For example, if a ceramic object is found with a lipid content of 2%, we would not be able to determine whether the source was a cow or another animal due to the expected biological degradation over the millennia.

4.4. Bioarchaeological and Evolutionary Perspectives

The calcium assimilation and vitamin D hypotheses [17,74] suggest a link between lower protein (LP) intake and higher concentrations of vitamin D and bone mineral density. This relationship is hypothesised to result from the positive correlation between LP intake and milk consumption [17,71,72,75]. Nonetheless, these estimations are in close proximity to the null, and the substantial sample size imposes significant limitations on the magnitude of potential effects [17,24]. The hypothesis that increased milk consumption leads to elevated circulating insulin-like growth factor I (IGF-1) [17,76] has been postulated. This has prompted subsequent research by other researchers [29] who have proposed a model of LP selection driven by the fitness advantages of IGF-1, which has been shown to increase body size and lower the age of sexual maturation. However, no significant differences in IGF-1 levels were observed between LP and LNP individuals.

In addition to dietary shifts, a multitude of other factors have likely exerted influence on reproductive rates and fatality levels subsequent to the establishment of agrarian societies across Europe [17]. These include population growth and urbanization density, heightened mobility [17,77], closer proximity to livestock, recurrent crop failure, periods of food scarcity and population collapse [32] and general sanitation and hygiene standards. The preponderance of these factors suggests a heightened risk of infectious disease, particularly zoonotic infections.

An interesting point to mention is that the persistence of the lactase enzyme is now considered to be a dominant characteristic. However, as mentioned previously, it appears that the ancestral characteristic of humans and mammals in general is the non-persistence of this enzyme in adulthood. On the other hand, the results of this study show that a small proportion of Neolithic individuals would have had the characteristic 'persistence of lactase' in adulthood, and they would have therefore maintained the ancestral characteristic at a higher frequency. For all these reasons, a hypothesis that has been widely discussed before [63] is that the possible mutation that leads to the dominant trait arose during the Neolithic period. Whether by genetic drift or probably by natural selection, the trait is maintained in the majority of current European populations, not so much because it is dominant but because it provides selective advantages over other genotypes, especially in times of famine. Neolithic populations in which a significant proportion of individuals were able to digest lactose in adulthood would have been better prepared for periods of food shortages and/or pandemics. Conversely, individuals lacking persistent lactase in adulthood, when consuming milk as a raw material, may experience a deterioration in their health, potentially leading to premature mortality due to various digestive complications. In accordance with other authors [17,25], we propose that the establishment of the persistent lactase allele is attributable to the positive selection of these individuals in contexts of both dietary and health deterioration.

4.5. Socio-Economic Perspective

Considering the consumption of milk and its derivatives, there is also a possible social and economic perspective. In terms of calories, regarding the nutritional information of non-skimmed cheeses produced from cow's milk, the caloric intake per 100 g can be approximately 264–300 kcal [78], and for different types of non-skimmed cow milk, 200 mL of plain milk (without the addition of any other product) can contain approximately 67–120 kcal [78]. Non-skimmed butter contains approximately 700 kcal per 100 g [78]. These observations can help to develop a perspective on the consumption of milk and its derivatives at a time when alternatives during food absences would be particularly limited. An individual with lactose intolerance could feed on cheese since during its production, the concentration of this disaccharide is reduced, benefiting from the fact that the calorific intake could be almost three times higher than that with milk intake. On the other hand, on an economic level, the keeping and production of animals, not only for meat consumption but also for the development and sale of other secondary products, could have boosted one of the primitive forms of trade. This may be the case with respect to observations in *Case 10*, where it was determined that most of the animal skeletons belonged to adult animals, which were possibly reared to produce primary and secondary dairy products.

In the case of cheese and butter sale, compared to the sale of milk, it has some benefits, including ease of transport and a longer shelf life. In the latter case, the stability of the product may also have been very important given the possible lack of preservation methods and the fact that secondary milk products, due to their production methods, are less vulnerable to bacterial and parasite action, allowing not only stability but also a lower incidence of disease in populations, both human and animal.

The results of the analyses of these seven cases of European Neolithic populations, where hypolactasia predominated in adulthood, are quite curious given that the European continent is currently responsible for half of the world's cheese production [79]. According to the page "*Cheesonomics—World Economy of the Cheese Industry*", the major European cheese producers are Germany, responsible for exporting 14.5% of European cheese, followed by the Netherlands, France, Italy and Denmark [79]. Checking the cases compiled here from the European Neolithic period, the majority of these countries demonstrated that they did not digest lactose in adulthood. On the other hand, according to McClure, cheese production in Europe may be approximately 7200 years old. Therefore, it is very likely that this human inability to process milk as a primary product was a factor in boosting one of the oldest but also one of the most productive and profitable industries in European history, which continues to expand to this day.

5. Conclusions

Overall, the majority of individuals analysed from different regions during the European Neolithic period demonstrated a reduced ability to digest lactose. It is possible that the phenotype associated with lactose digestion arose through a possible mutation and became established in the population, not due to pressure associated with consumption but rather to selective pressure to provide a nutritional advantage to the population in the event of famine and/or disease events.

A fact that seems to be associated with this general inability to digest lactose in European territories since the beginning of human contact with animal milk is the development, production and marketing of secondary dairy products, mainly cheese.

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