

Ancient Biomolecules Unravel our History: A Technical Update with Examples from the Middle East

Sara Tomei^{1*}, Ambra D'Aurelio¹, Ferhan Sakal², Francesca Castorina^{3,4}, Faisal Al Naimi² and Cristina Martínez Labarga⁵

¹Omics Core, Integrated Genomics Services, Sidra Medicine, Doha, Qatar

²Department of Archaeology, Qatar Museums, Doha, Qatar

³CNR, Istituto di Geologia Ambientale E Geoingegneria, c/o Dipartimento di Scienze Della Terra, University of Rome "La Sapienza", Rome, Italy

⁴Department of Earth Sciences, University of Rome "La Sapienza", Rome, Italy

⁵Dipartimento di Biologia, Università degli Studi di Roma "Tor Vergata", Rome, Italy

*Corresponding author: Dr. Sara Tomei, Omics Core, Integrated Genomics Services, Sidra Medicine, Doha, Qatar

Received:  July 20, 2023

Published:  July 26, 2023

Abstract

Context: The study of ancient biomolecules represents a useful tool to address questions related to human history.

Objective: This manuscript provides an overview of the major categories of ancient biomolecules, highlighting their potentialities when applied to research.

Methods: This study gathered knowledge from recently published papers on paleogenomics, paleoproteomics, ancient lipids and stable isotope analyses with the aim of providing a technical and historical background on ancient biomolecules, and examples of their application in the Arabian Peninsula and Middle East in general.

Results: The progress seen in the past decade with regard to the study of ancient biomolecules has led to a dramatic expansion of the studies that apply those analyses. Increasing attention has also been paid to the development and optimization of protocols aimed at reducing and/or preventing the risk of contamination. While extensively applied to Western areas, the study of ancient biomolecules in the Middle East and the Arabian Peninsula has been limited.

Conclusions: Research on ancient biomolecules represents the most valuable source of information to understand our evolutionary past at an inconceivable level of detail, especially when applied to areas so far underrepresented in this field, such as the Middle East and the Arabian Peninsula in particular.

Keywords (3-5): Paleobiology; ancient DNA; Gulf; stable isotopes; strontium

Introduction

The Nobel Prize in Medicine and Physiology last year (2022) has been awarded to Svante Pääbo, one of the pioneers in paleogenomics research, whose studies underlined the importance of our evolutionary history not only for our present but also for our future [1]. The scientific community has recently seen a rapid expansion of ancient biomolecular studies, which have allowed previously unsolved questions on our human history

to be addressed [2,3]. Cappellini et al. have provided a review of the most recent applications of the ancient biomolecules [2]. By applying ancient biomolecular analyses, bioarchaeologists were able to shed light on the pathways and evolutionary processes that contributed to the current biodiversity [2]. The field of ancient biomolecules covers a broad spectrum of techniques, including the examination of ancient nucleotide and amino acid sequences, as well

as the analysis of lipids and stable isotopes [2,3]. The development and application of ancient biomolecular analyses has the potential to help bioarchaeology studies to be carried out in geographical areas potentially challenging, due to suboptimal environmental conditions, including heat and humidity [4]. The advent of next-generation sequencing (NGS) technologies has revolutionized the field of ancient DNA (aDNA), allowing an increased number of ancient genomes to be analyzed and the possibility to study extremely ancient remains [2,5-8]. As reviewed in early studies, the last decades also recorded a growing interest in environmental DNA (eDNA) [2,8-10]. Environmental DNA (eDNA) pertains to the genetic material gathered from environmental samples, which may include soil, seawater, or even samples taken directly from organisms [11]. The field of eDNA has also benefited from the introduction of NGS technologies as well as from the subsequent application of paleometagenomics [2,12,13]. Along with the advancement of sequencing technologies, increasing efforts have been focused on the development of protocols aimed at maximizing the retrieval of aDNA as well as reducing and/or preventing the risk of contamination [5,14-17].

Long bones and teeth dentine have historically been recognized as the most employed substrates for aDNA studies; however, in the last years, researchers have pointed out other skeletal elements such as petrous bones and tooth cementum as samples richer in aDNA [5,14,15,18]. Moreover, advancements in the paleogenomics field have facilitated the retrieval of a vast amount of genetic data from various substrates that were previously unexplored, such as archaeological materials, dental calculus, archaeobotanical remains, and more [18]. In addition to the advances in aDNA studies, investigations on ancient proteins recently underwent a significant expansion [2,19]. Paleoproteomics is the discipline that aims at identifying and characterizing the proteins of ancient remains [20]. Unfortunately, the field of paleoproteomics has advanced at a slower pace as compared to the field of aDNA [20]. Although the survival of ancient proteins has been investigated since the 1950s [21], it is only since the 2000s that technical advancements in mass spectrometry enhanced the possibility of recovering and characterizing ancient proteins [20]. The paleoproteomics field has not been spared from technical challenges. In fact, efforts aimed at directly sequencing ancient proteins have failed because of the need to start from highly purified, unmodified, and concentrated proteins [22,23]. Despite the challenges, there is a growing number of paleoproteomics studies being conducted, which significantly enhances the field's potential to deepen our understanding of species evolution and human culture [24].

Another class of biomolecules is represented by ancient lipids. As recently reviewed by Cappellini and colleagues [2], ancient lipids were firstly analyzed in the 1960s, but their application in archaeology was registered only in the following decade [2,25,26]. These molecules have the potential to elucidate past human activities [27,28]. Ancient lipids can be recovered from a broad range of materials as demonstrated by wide research [27-31]. Moreover, unlike aDNA and proteins, lipid biomarkers can be retrieved in high concentrations as proved by the analyses of potteries and bog

butters [28,32,33]. Similar to ancient lipids, the examination of stable isotopes has seen significant growth in recent decades and has become one of the most prevalent methods in bioarchaeological research. This approach enables the investigation of past human diet and residential mobility [34-37]. In the present day, stable isotope analysis finds application in a wide range of areas, including plant and livestock management, examination of past population mobility, understanding social dynamics, exploring development trends, and studying the variation of subsistence strategies [37-55].

The Middle East lies at the crossroads of the Old World, where Africa, Europe and Asia meet. Due to this geographical location, the Middle East has played a central role in human evolution. At the same time, the often-inhospitable nature of the climate in this region has contributed to geographic structuring both within the region and between the Middle East and other parts of the World [56]. Nevertheless, it is thought that despite the Middle East being characterized by an arid desertic environment, there have been humid periods in the past that resulted in what has been called "Green Arabia" and which might have facilitated human dispersals [57]. The Middle East contains some of the earliest evidence of modern humans outside of Africa, with fossils dating back approximately 180,000 years ago from the Levant and around 85,000 years ago from North West Arabia. [58, 59]. However, our understanding of the prehistoric societies and population movements of Middle Easterners remains limited. [56]. In this article we describe the different classes of ancient biomolecules, highlighting their potentialities and shortcomings and providing examples of their application in the Middle East and the Arabian Peninsula.

Stable Isotope Analysis: Investigating Human Diet and Mobility

Stable isotope signatures represent a widely employed method for investigating dietary habits and migrations of past human populations [37,60]. This well-known method has been frequently utilized in reconstructing the dietary habits of human populations from Prehistory to the present day (e.g. [41-43,49,50,55,61-69]) also represents a useful tool to explore broader issues such as the domestication of plant and animal species, the patterns of the Neolithization process etc. [37,70-73]. Moreover, stable isotopes provide evidence of population mobility allowing inferences on the structure, dynamics, and economy of human groups [37,74-76]. Situated in a strategically central location, the Arabian Peninsula has facilitated the movement of its inhabitants and traders, leading to the exchange and spread of animal and plant species across Africa, Europe, and Asia. However, due to its arid and often inhospitable climate, the Arabian Peninsula (and the Middle East in general) has received limited attention in stable isotope analysis studies aimed at understanding the Arabian paleoenvironment [77]. We report some examples below.

Carbon and nitrogen stable isotopes: dietary pattern reconstruction

Diet represents the convergence of social and cultural values that display a strong influence on human habits [78, 79]. The

analysis of carbon and nitrogen stable isotopes has received a growing interest from the scientific community as it provides a powerful tool to reconstruct dietary patterns in past populations by providing dietary data at the individual level [80]. The isotopic composition of bone proteins reflects that of the protein component of food consumed in the last 10-15 years prior to death [81,82]. Because stable isotope analysis refers to single individuals, the obtained data can be useful for evaluating variation and social dynamics within the analyzed samples [83]. As widely discussed in the literature, the isotopic values are reported as the ratio of heavy/light (C^{13}/C^{12} ; N^{15}/N^{14}) isotope expressed in delta (δ) per mil (‰) relative to internationally defined standards [84], the Pee Dee Belemnite, PDB [85] limestone fossil, then substituted by the Vienna Pee Dee Belemnite, V-PDB [86] and atmospheric nitrogen, AIR [87]. Photosynthesis is the main natural process responsible for carbon isotopic fractionation during which the preferential exchange of the lighter carbon isotope (^{12}C) is responsible for the depletion of $\sim -7\text{‰}$ in $\delta^{13}C$ from CO_2 to plants [88, 89].

Terrestrial plants follow three main photosynthetic pathways. The crassulacean acid metabolism (CAM) will not be discussed here since these plants are not relevant to human diets. Plants known as C3 plants (such as trees, shrubs, tubers, wheat, rice, flowering plants, and grasses from temperate regions) follow the Calvin-Benson photosynthetic pathway, which results in the synthesis of a three-carbon compound; they discriminate against the heavier isotope of carbon, and thus their tissues are enriched in ^{12}C showing a mean $\delta^{13}C$ value of -26.5‰ [90, 91]. Maize and other plants from hot and arid environments (e.g., savannah grasses, amaranths, sedges, millet, sugarcane, sorghum, etc.) synthesizing a four-carbon compound are generally classified as C4 plants; They have higher carbon ratios, resulting in more positive $\delta^{13}C$ values (mean $\delta^{13}C$ value of -12.5‰) [92-95]. Temperature, variation in atmospheric CO_2 , nutrients, water availability and the amount of light can affect the isotopic ratios of C3 plants whereas C4 species generally exhibit less variability [96,97]. The fractionation process continues in consumers implying an enrichment factor of approx. $+5\text{‰}$ into their bone proteins [98] plus a further enrichment of ca. 1‰ for each trophic level [99].

A similar small increase in ^{13}C has also been attested in the marine environment by previous research [98,100-103]; in fact, marine organisms possess higher $\delta^{13}C$ values for the main source of carbon in the environment is represented by dissolved bicarbonates ($\delta^{13}C$ value of about 0‰) [104]. The consumption of marine food webs, on the other hand, can complicate the assessment of C4 plant consumption [98] leading to an overlap of the carbon isotopic signatures of marine and terrestrial environments [61,90]. Another aquatic resource which is worth considering is freshwater (lacustrine and riverine) fish which was consumed by past human populations as attested by different isotopic archaeological studies [105-112]. Though aquatic, freshwater fish has $\delta^{13}C$ values more similar to terrestrial animals, although extremely variable because carbon can come from organic and inorganic carbon sources [113].

Nitrogen is present in the Earth's atmosphere (approx. 78%) however, numerous biochemical processes (e.g., fixation, ammonification, nitrification, denitrification, assimilation) are necessary to convert it from gas (N_2) to the most usable forms and vice versa, clearly influencing its fractionation [114]. Plants from the terrestrial environment may alternatively use nitrogen as ammonium (NH_4^+) thanks to the symbiosis with Rhizobium bacteria (e.g. pulses) or in the form of ammonia (NH_3) or nitrate (NO_3^-) [115]. As a consequence of the use of different forms of nitrogen these plants also differ in terms of $\delta^{15}N$ values which result very low in the former group (ca. 1‰) and higher in the latter one (ca. 9‰) [115]. Although nitrogen ratios can vary due to the use of different nitrogen sources, various environmental factors (such as humidity, pH, salt concentration, etc.) can influence the isotopic values [115]. Through successively higher trophic levels, an enrichment of 3-5‰ in $\delta^{15}N$ values is observed [116].

The source of nitrogen also determines a variation in $\delta^{15}N$ ratios between marine and terrestrial ecosystems as the use of ^{15}N enriched sources (e.g. nitrates from water) is responsible for the increase in nitrogen values in marine organisms with respect to the terrestrial ones [89,109]. A different situation may be observed for freshwater environments as in this case they show a higher variability due to the use of both terrestrial and aquatic materials [115]. Moreover, the $\delta^{15}N$ value also varies according to biological and cultural factors as breastfeeding practices [117-122], nutritional stress or metabolic disease [43,49,50,123-125]. The stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) of plants and animals consumed by humans could be useful for investigating dietary patterns, environments and the food groups consumed [126-129] although with some limitations [130,131]. Clearly, these data should refer to the specific environment in which past human population lived therefore it is extremely important to reconstruct the reference baseline by analyzing coeval faunal and plant remains if available [132-141].

Despite the potentialities of isotopic analysis from bone proteins, it may be possible that exposure to arid environments prevents proteins' extraction [54]. In this case, radiocarbon dating and stable isotope analysis on hydroxyapatite represent a valid substitute in the reconstruction of dietary patterns. The former relies on the absence of equilibrium between the marine environment and atmosphere where ^{14}C is produced, leading to the development of the marine effect reservoir (MRE) due to the differential age of the carbon in these two environments being the marine ca. 400 years older than that in the atmosphere [54]. Marine resources intake increases the individual's ^{14}C age with respect to the biological one. The difference in the estimates of the time of burial and MRE are thus useful for investigating the amount of marine sources' intake [54]. Alternatively, the isotopic $\delta^{13}C$ ratio of hydroxyapatite is useful to discriminate between subsistence strategies mainly based on C3 (average $\delta^{13}C$ values -12‰) or C4 (average $\delta^{13}C$ values -1‰) plants [142-144]. Moreover, unlike bone proteins, hydroxyapatite reflects the total diet and not only the protein components [143,145-148].

At present, there is a scarcity of studies on diet reconstruction in the Arabian Peninsula. However, Roberts and colleagues addressed this gap by utilizing 21 mammal tooth enamel samples and applying stable carbon and oxygen isotope analysis to investigate the middle Pleistocene paleoenvironment of Ti's al Ghadah in Saudi Arabia [77]. The $\delta^{13}\text{C}$ signatures from the fossil mammals associated with hominin presence showed a prevalence of C4 vegetation in the diets of herbivores. Additionally, the consistent C4 consumption by all animals was indicative of the high consumption of C4 grasslands. Even the $\delta^{18}\text{O}$ signature suggested that in the past the region probably experienced higher humidity [77]. The data produced in this study represents a clear example of how stable isotope analysis from fauna helps gain insights into paleoaridity and paleovegetation during periods of hominin migration [77]. Recently, another study employed stable carbon and nitrogen isotope analysis to examine the dietary patterns of adult human and animal bones excavated from Qalat al-Bahrayn [149]. Interestingly, they found that the diets consumed were dominantly terrestrial although samples were retrieved in proximity to the coast [149]. The diets consumed were heterogeneous, spanning from terrestrial diets to more mixed diets, which included C4 resources [149]. The same heterogeneity was found in the sheep, suggesting diversity also in feeding practices that might have included the use of dried fish [149]. The study is particularly relevant since it highlights diversity in the economic and ecological systems that one would not expect from a small island [149].

Stable isotopes of strontium and oxygen for the study of residential mobility

The mobility and migrations of past human groups represent an important topic for both the archaeological and forensic fields. Phenomena such as changes in economic patterns, social complexity, landscape, post-marital residence, population origins, mortuary practices, and economic activities are all considered motivating factors for movement. For this reason, population movement influences events such as subsistence, health, demography, socio-political organization, and economy. Even in this case, the human remains serve as a valuable source of information for investigating an individual's history by analyzing stable isotopes [150]. As widely reported in the literature, isotopes in bone and tooth remains reflect the chemical composition of the consumed foods and drinks which in turn are dependent on the local environment [151,152]. As a result, isotope ratios like $^{87}\text{Sr}/^{86}\text{Sr}$ ($\delta^{87}\text{Sr}$) and $^{18}\text{O}/^{16}\text{O}$ ($\delta_{18}\text{O}$) are extensively utilized to explore the origins and mobility patterns of ancient populations [150].

The fundamental principles behind employing combined strontium and oxygen isotope analysis of human tissues are rooted in their connection with the geological composition of the soils where food was cultivated and the origin of the drinking water. Strontium is naturally present in various isotopic variants (e.g. ^{88}Sr , ^{87}Sr , ^{86}Sr , ^{84}Sr etc.) [153, 154]; nonetheless, the only isotope suitable for comparing modern and archaeological data is ^{87}Sr , as it possesses both stable and radiogenic characteristics, being the decay product of the long-lived natural beta-emitting isotope ^{87}Rb , which has a

half-life of 48.4 billion years [154-156]. The unique geology of each region is reflected by the strontium abundance in the weathered rocks from which it can pass into the local ecosystems through its release into groundwater to the food chain maintaining the same $^{87}\text{Sr}/^{86}\text{Sr}$ ratio without appreciable fractionation [157-159]. As the amount of strontium varies among different areas, its uptake into the skeletal tissue reflects the geologic features and the bio-availability of the chemical element, providing evidence of the area of residence and mobility [160-162] as well as the presence of non-local individuals because it can be incorporated into enamel hydroxyapatite due to its similarity to calcium [161,163]. The Sr isotopic ratio of dental enamel provides the signal of the place of birth because it represents the individual's Sr intake throughout the crown formation and results in a static tissue extremely resistant to diagenetic alteration in the post-burial environment. Also, the teeth formed post-weaning accurately reflect the food and water consumed by individuals, therefore, it can be considered a birthplace signal. [161,163]. Oxygen's signatures depend on multiple pathways. However, although the overall oxygen signature is determined by different factors, the isotopic ratios in tooth enamel and skeletal remains are a proxy of the drinking water consumed [164-166] and its variations are ascribable to geographic and climatic factors [167-171]. The fractionation processes of oxygen isotopes are known and therefore the isotopic analysis allows the reconstruction of the consumed water sources ($\delta_{18}\text{Ow}$) and thus past human mobility [166-169,172-174].

As enamel formation takes place during childhood without any further remodeling, the strontium and oxygen signatures it contains mirror those of the environment in which individuals lived during their infancy [45]. Specifically, the development of the crowns of the permanent first molars initiates in the womb and reaches completion between the ages of 2.5 to 3.0 years. The formation of the second molars, otherwise, starts at 2.5-3.0 years to be completed in 7.0-8.0 years old children [175, 176]. Consequently, individuals drinking water from local sources will possess isotopic values in line with those of the environment, conversely the use of non-local water sources will cause an inconsistency between environmental and individual isotopic signatures. Despite the potentials of these isotopic analyses, the interpretation of data could be problematic. For instance, with regard to strontium, local environments generally display different inputs that do not necessarily reflect the $\delta^{87}\text{Sr}$ ratio of the geological substrate [161]. Therefore, it is important to distinguish strontium values from rocks and the bio-available one [62]. The local baseline could be assessed by several methods such as analyzing environmental samples including soil, freshwater, and coeval remains and/or modern ones; in this case however attention should be paid to the potential effect of human activities (e.g. fertilizers) on isotopic ratios [161,177-179].

A possible solution for data interpretation relies on the use of ancient faunal isotopic values as the baseline from which human values can be inferred [62,161,177,180,181]. Animals' (e.g. herbivores) subsistence is based on plants distributed in quite wide areas, therefore they possess more homogenized isotopic ratios

that more likely reflect local isotopic values [61,62]. Conversely, a broad array of factors, including flowing water, fluctuations in seasonal and annual rainfall, particular cooking and beverage preparation methods, diet, and others, can lead to inconsistencies and variations in the $\delta^{18}\text{O}$ of skeletal remains within a single archaeological site, consequently affecting the oxygen isotopic signatures [182-187]. Moreover, the reservoir effects that cause an enrichment in ^{18}O can also play a role in introducing heterogeneity within a local environment [185]. Furthermore, the isotopic incorporation may vary due to the number of years during which tooth enamel develops, leading to isotopic variability among teeth of the same individual [188-190]. Similar to the carbon and nitrogen stable isotope data, studies on strontium stable isotope analysis in the Arabian Peninsula are limited. We provide some examples below. Al-Shorman and El-Khoury applied strontium isotope analysis from rural sites in Barsinia, in Northern Jordan [191]. The earlier archeological excavations of the regions revealed that its occupation started during the Iron Age and continued until the Ottoman period. The site is particularly relevant as it was characterized by economic wealth, suggesting an enhancement of population dynamics. Yet, the study showed that all the individuals analyzed were raised in the area and their food was taken from spatially restricted localities in the region [191].

Recently, Ryan and colleagues applied strontium analysis to investigate the origin and the production of cotton in the ancient site of Mleiha, in the United Arab Emirates [192]. The authors found different isotopic signatures of cotton seeds and textiles as compared to modern plants retrieved from the same area. Hence, they should be considered “non-local”. Along with archaeobotanical and archaeological evidence, the authors proved the existence of a trade network between Southeaster Arabia and India [192]. Evidence on human migration from strontium and oxygen stable isotope analysis in the Arabian Peninsula is also limited. Human skeletal remains from Neolithic sites within the United Arab Emirates have been subjected to investigation in a recent article [193]. The isotope ratios of strontium ($^{87}\text{Sr}/^{86}\text{Sr}$) and oxygen ($^{18}\text{O}/^{16}\text{O}$) of the remains assessed suggest the population was resident in the coastal area. Yet, the data suggested that mobility occurred between the coast and the inland regions [193]. A very recent study from Wang and colleagues has applied a combined approach encompassing isotopic and genetic analyses of samples recovered from Nevalı Çori to study the Neolithization in the Fertile Crescent [194]. The authors analyzed 44 molar enamel from 28 human individuals thought to have lived between ca. 8700 to 7500 BCE for $^{87}\text{Sr}/^{86}\text{Sr}$, $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ analyses. The isotopic data indicated a reduction in mobility and growing resilience on domesticates by ca. 8300 BCE and contributed to the understanding of the Neolithization process in the Near East [194]. Regarding the Arabian Peninsula, a comprehensive investigation is necessary to establish a robust repository of Sr and O isotope variations within this region. This will serve as a foundational resource for archaeological studies focusing on human and animal mobility.

Ancient DNA (aDNA) analysis

Both the production and the analysis of human genetic data have undergone a complete transformation in the era of NGS, where genomes are being read at an unprecedented pace [195] and have allowed the completion of the human genome [196]. This phenomenon not only promises to transform the field of genetics but has far-reaching effects in understanding human prehistory when applied to the aDNA field. The study of aDNA is a powerful tool for discovering the origins, migration patterns, kinships, admixtures, and cultural shifts in past populations but it also allows retracing the origin of the modern mosaic of DNA sequences [197]. As stated above, archaeological skeletal series represent an important source of information about our past. The discovery of long-term DNA survival in skeletal remains [198, 199] and sediment [200, 201] has allowed human genetics to infer evolutionary processes [202]. Nevertheless, aDNA studies face specific challenges, one of which is represented by the retrieval of enough DNA endogenous content [202]. This is strictly dependent on post-mortem degradation processes. DNA starts decaying immediately after death as a result of the exposure to unmitigated insults by endonucleases, bacteria and fungi as well as by oxidation and hydrolysis in absence of the enzymatic repair mechanisms that help maintain the genomic integrity in living cells [203, 204]. DNA fragmentation is partly caused by depurination, a continuous process causing the increase of DNA molecule fragmentation with the age of the sample [205], although the deamination of cytosine residues towards the end of the molecules (resulting in apparent C to T or G to A substitution on 3' and 5' strand respectively) also represents one of the main damages observed on ancient DNA [203]. As a consequence, the endogenous DNA extracted from archaeological samples possesses features directly linkable to the abovementioned factors (e.g. reduction of fragments' length; lesions preventing the replication; lesions causing nucleotide misincorporation during the replication), moreover its content in archaeological specimens is often extremely low (<1%) [206]. Long-term DNA preservation is influenced by the environment; in particular it seems better in “niche” microenvironments forming during fossilization. Pruvost and colleagues [207] analyzed bones that were subjected to different post-excavation preservation conditions and discovered that freshly excavated fossil bones and teeth are the most suitable samples for amplifying aDNA.

Due to the difficulties related to aDNA retrieval and analysis, recent research focused on the identification of new substrates, the optimization of extraction methods as well as the control of contamination [208-210]. aDNA studies have been generally performed on teeth or dense bone fragments (e.g. cortical region of long bones) as those samples have been considered more enriched in endogenous DNA than the trabecular bone consisting of fewer osteocytes per gram [211]. Nevertheless, the petrous pyramid and the cementum layer in tooth root have been recently proved to be the most suitable substrates for aDNA analysis, as they display the highest degree of preservation of viable DNA [211, 212]. Certainly,

an important factor to evaluate is the invasiveness of the sampling procedures, especially for those skeletal elements not easily accessible (e.g., petrous bone). In fact, the extent of damages caused by a disruptive sampling of bone specimens should be limited especially for museum collections. Sirak and colleagues proposed an innovative minimally invasive method for accessing the petrous bone by drilling the cranial base [14]. A minimal-invasive protocol dedicated to sampling the tooth cementum of the roots has also been recently proposed and relies on the direct digestion of the target portion of the teeth [16]. Additionally, it has been proved that aDNA yield from petrous bone was in the order of hundreds-fold higher than other skeletal elements tested (including rib, tooth or long bones) and overall, it performed better than tooth cementum [213, 214]. Recently, another interesting study pointed to ear ossicles as an alternative source of aDNA, able to produce a DNA recovery similar to the cochlea; however, they offer the advantage of sampling (from intact skulls without significant damage to the surrounding material) [215].

aDNA is usually more preserved in permafrost or extremely cold climates [216-222] whereas high temperatures weaken DNA preservation directly and indirectly by enhancing the proliferation of fungi, bacteria and other microorganisms that contribute to endogenous DNA degradation and contamination [216, 217, 221, 223]. Despite that, researchers recently provided aDNA data from arid environments [214]. Contamination is of paramount concern in aDNA analysis due to the nature of aDNA itself (highly degraded and fragmented molecules) that can be easily mixed with exogenous molecules if strict precautions are not taken [224]. The problems of contamination may be partly solved by using ad-hoc facilities for aDNA extraction, however it is of paramount importance to pay extreme attention to avoid any carry-over between pre- and post-PCR laboratories [225]. The publication of the first ancient genome in 2010 was followed in the last years by an unprecedented and unexpected amount of genomic data [226-228]. The advent of NGS has significantly propelled the field of human aDNA forward. This progress has moved beyond the analysis of only a few hundred base pairs of mtDNA, which was susceptible to contamination and offered limited biological insights. Instead, NGS has enabled large-scale population studies, leading to a transformative shift in our understanding of human history [214,229]. Although inconceivable, partial and complete genomic sequences have been obtained from modern humans [230-232], Neanderthal [233, 234] and even Denisovans [235, 236]. Some of the genomes possess an extremely high depth as compared to that achieved in contemporary humans [228].

NGS has also played a vital role in enhancing our comprehension of the degradation processes that impact aDNA as time passes. For instance, the phenomenon of cytosine deamination, leading to C>U changes at regular cytosines and C>T changes at 5-methylated cytosines, has facilitated the improvement of distinguishing between endogenous and contaminant sequences. Additionally, it was also reported that the frequency of cytosine deamination increase with the age of a sample [237]. However, despite its numerous advantages, NGS is not exempt from encountering

technical challenges when applied to the field of aDNA. During the initial application of NGS methods in aDNA studies, standard library construction protocols were used. However, it soon became evident that these protocols resulted in a significant loss of DNA. There are two main approaches to NGS library construction: direct sequencing (shotgun metagenomics) and enrichment for specific sequence regions using hybridization capture, followed by sequencing. Hybridization enrichment becomes necessary when the endogenous DNA content is low in a given sample.

Among these methods, whole exome capture of ancient samples has shown great promise for aDNA studies. Exomes, which encompass the protein-coding regions of the genome, tend to be more conserved than other parts of the genome. Consequently, exome capture has the potential to be particularly suitable for investigating extinct species, where no closely related genome sequence information is available [238].

In a very recent study Wang and colleagues analyzed six individuals retrieved from Nevalı Çori to study the inner-community structure during the late Neolithic in the Southern Levant. The study indicated consanguinity and raised questions on how the Late Neolithic societies were structured internally and whether they were characterized by endogamy in terms of cultural behavior and social practices [194]. Despite the Arabian Peninsula played a critical role in the early structuring of modern humans, it is still underrepresented by large-scale genomic studies [4,56,239]. Unfortunately, the poor environmental conditions that characterize the Arabian Peninsula have not facilitated the recovery of aDNA, hence the aDNA evidence of the Arabian Peninsula is inferred from the application of modeling statistical and bioinformatic tools on modern genomic data [239,240]. DNA studies applied to the Arabian Peninsula have revealed that Arabians were subjected to a population bottleneck parallel to the peninsula's aridification around 6 kya. In fact, despite Arabia being characterized by arid weather, there have been several humid periods resulting in what has been called "green Arabia" [57]. In a recent study, Almarri and colleagues conducted an analysis of a high-coverage dataset comprising samples from the Arabian Peninsula, the Levant, and Iraq. Their aim was to gain insights into the population structure, demographic history, and admixture patterns of both modern and ancient humans in these regions. By employing a model based on published ancient genomes, the researchers successfully identified distinctions between the Levant and Arabia. Specifically, the Levant exhibited a higher European/Anatolian ancestry, while Arabia displayed higher African and Natufian ancestries [56]. However, it should be noted that this study made inferences on past populations based on modern DNA data. Additional studies applying aDNA are warranted to better understand the origin and connections of Arabian populations that are currently underrepresented in genome-wide studies [56].

Dental Calculus, a Valuable Tool to Investigate Microbiome

aDNA can be retrieved in a wide variety of substrates [241] some of which, like dental calculus, represent a valuable source

of ancient biomolecules that have the potential to provide useful information on oral ecology, metabolites, and ancient microbiomes. Dental calculus – tartar or mineralized dental plaque – is a mineral matrix covering the tooth surface is composed of bacteria, food, and environmental debris forming during time [242]. The development of dental calculus depends on the trapping of all the mentioned materials by the inorganic salts of saliva [243, 244]. Dental calculus is commonly detected in all human populations both past and present, and it is generally well preserved in archaeological contexts [242, 245] even if, due to dietary variations, its retrieval has been proven more challenging in ancient and modern foraging or hunter-gatherer groups [246] as despite the poor oral hygiene and the genetic pre-disposition the accumulation of larger deposits is related to the consumption of soft carbohydrates [245,247,248].

Dental calculus was partly overlooked until the 1960s-1970s whereas, afterwards, it received particular attention as it offers the chance to reconstruct the oral microbiome, diet, medicinal therapies, and paleoenvironment [43,242,244,249-257]. The analysis of archaeological calculus deposits can be performed following different methodologies. The most common approach is certainly the morphological examination, by light microscopy, of the entrapped material in samples decalcified by hydrochloric acid (HCl). In the 1990s and 2000s, the analysis of starch granules trapped in dental calculus deposits provided fundamental contributions to the reconstruction of the starchy components – roots, tubers, seeds – of the hominin and human diet [249-251,255,258,259], moreover, pyrolysis profiles were used to infer about cooking practices [249]. Furthermore, the presence of non-dietary debris (e.g. textile fiber) could also help in reconstructing craft activities and trade [260].

More recent approaches include DNA analysis and gas-chromatography that allow the identification of specific compounds attributable to the ingestion or consumption of plants and or volatile substances' inhalation [43,244,249,261-265]. Nowadays, the application of shotgun metagenomics determined a change in the field, because, as the bacterial community changes over different stimuli (e.g. diet, hygiene practices etc.), the analysis allows to investigate past human microbiome and also to make inferences on health status and diet during human evolutionary history [256,266,267]. The application of high-throughput sequencing has allowed researchers to move from the identification of calcified bacterial cells [268], dietary micro debris, host mtDNA [269] and biomolecules from a small range of selected species [269, 270] to the investigation of entire microbial communities [256,266]. The initial characterization of the microbiota of the ancient oral cavities was made possible by the target characterization of the 16S rRNA gene. This gene contains hypervariable regions that are informative of specific microbial communities [271]. Nevertheless, 16S rRNA sequencing has proved to generate skewed taxonomic data that can introduce biases when reconstructing the ancient microbiome [271]. For this reason, shotgun metagenomic sequencing is recommended for the analysis of the ancient microbiome over 16S rRNA sequencing [272, 273]. The reconstruction of a partial genome of an uncultured TM7 bacterium by Liu et al. [274] also pointed at shotgun metagenomics sequencing as a useful tool for

the identification of uncultivable bacteria as well as for the recovery of extinct microbial genomes for which there are no reference sequences [242].

The recent advancement of molecular methods made dental calculus accessible to a wide range of analyses, including metagenomics and metaproteomics; the latter, although less explored, has recently been applied to dental calculus samples [253,256]. The metaproteomic approach allows the simultaneous characterization of the levels of individual proteins expressed by both microbial community and host, providing the direct assessment of protein functions but also allowing the elucidation of the interactions between potential disease-causing microorganisms and their host [253,275,276]. The work by Christensen et al. (2018) led to the identification of oral disease-susceptible individuals that were not apparent from the macroscopic examination of the skeletal remains demonstrating that the molecular investigation of dental calculus represents a valuable source of additional data useful to enhance our understanding of the health status of past human populations [253]. Despite the challenges of the emerging techniques, metaproteomics promises to yield a unique understanding of the role of the microbiome in the health status and diseases of past populations [242]. Dental calculus has the potential to become one of the most invaluable sources of information concerning ancient skeletal remains. Its analysis allows the investigation of various aspects, including diet, health status, diseases, microbial presence, and even the cultural affinity of past human populations. [267]. When integrated with data acquired from various methods, the examination of dental calculus holds the potential to offer a comprehensive reconstruction of our history.

To the best of our knowledge, the analysis of dental calculus has not been utilized in the context of the Arabian Peninsula.

Ancient proteins

Ancient proteins represent another class of biomolecules commonly referred to as “paleoproteomics” [24]. While the discipline has been introduced in the 1950s [277], it is only in the 2000s that techniques based on mass spectrometry shaped this field [24, 278]. Despite still at its infancy, the study of ancient proteins has many applications in evolutionary biology and archaeology [24]. Its application to biological tissues (e.g., ancient bones and teeth) allows phylogenetic studies when aDNA could not be used because degraded [279]. Proteins are indeed better preserved than DNA [24]. This enabled the proteomic analysis of samples substantially older than the experimented aDNA temporal limit, with the oldest sample so far analyzed being 1.77 million years old [279]. Beyond being a valuable tool in taxonomic and phylogenetic studies, ancient proteins can be leveraged for a number of other different applications. During the life of an organism, proteins are usually the expression of a specific tissue or physiological condition [24]. Hence, their study can give, for example, invaluable information concerning the origin of the material analyzed and the conditions surrounding the death of an individual [280]. In a study of 2013, Maixner and colleagues were able to identify many proteins in the brain tissue of the Tirolean mummy Ötzi, some typically expressed

by brain cells and others in situations of stress, opening up again the possibility of the Tirolean man being injured at the head before his death [280].

Furthermore, paleoproteomics has been used in archaeology to investigate past cultures. Despite some limitations, mainly due to the high impact of degradation processes on proteins removed from their original tissue and the lack of references required for accurate identification of the vegetal species [23,24], rests of proteins in ancient pottery and dental calculus have indeed the potential to reconstruct the main foodstuff used in the past. With respect to lipids, they are more concentrated in plant foods and can better distinguish between different food when mixed, thanks to their being taxonomic- and tissue-specific [23,24]. For instance, in a study by Hendy and colleagues from 2018, ancient proteins extracted from 8000-year-old ceramic vessels from West Mound in Anatolia revealed precisely the diet of the population. Interestingly, the authors were also able to discover that specific vessels were used only for certain types of food [23,24]. However, just a few studies were able to successfully study paleoproteins from ceramics thus far [23,24].

Both animal and vegetal proteins serve as raw materials for the production of clothes and they were used in building materials, paintings and glue. Hence, the paleoproteomics analysis of these materials can help the interpretation of some cultural aspects and habits of our past [23,24]. For instance, proteins such as fibroin and keratins help identify materials such as silk and wool [23,24] and the proteomics analysis of glues and paintings has allowed the detection of a wide range of proteins from different sources [281, 282]. Amino acid racemization (AAR) dating relies on the detection of amino acids. In living tissues, amino acids naturally occur in the L-configuration. However, following death, they undergo racemization and convert into the D-configuration [20]. The time since death may be estimated by analyzing the rate of D- and L-amino acids [20,283]. The AAR dating works well in highly mineralized substrates, such as tooth enamel [20,284]. The technique has also been applied to the study of early human activity in Europe [284].

Several methods are employed for the study of ancient proteins [20]. One of the methods for assessing ancient proteins is through immunoassays, which however can generate false positives and negatives, especially when analyzing degraded samples. These assays use the reaction of a specific antibody binding to an antigen. Their advantage is their effectiveness on challenging materials, such as ceramics [23, 285], as shown in a study of 2000 by Craig and colleagues where their application has allowed the detection of milk proteins on ceramic pots, unravelling the issue concerning dairy diffusion on the Scottish Atlantic coast during the Iron Age [285]. On the other hand, they require pre-knowledge of the target proteins and the conservation of specific epitopes [23,285]. Therefore, because of their limitations, they have been replaced by other techniques for the study of ancient proteins [20]. The introduction of mass spectrometry (MS) has revolutionized the paleoproteomics field. MS is more reliable as compared to immunoassays because

the mass and charges of the ionized molecules are detected precisely [20]. Matrix-assisted laser desorption/ionization MS (MALDI-MS) was applied initially in 2000 by Ostrom and colleagues [278]. In this study, peptides were identified from the osteocalcin of a 53,000-year-old bison [278]. Subsequently, the practicality of applying MALDI-time-of-flight (TOF) MS to collagenase-digested and trypsin-digested bone collagen surpassed that of using MALDI-TOF on purified osteocalcin [24]. This led to the development of the so-called ZooMS (Zooarchaeology by MS) [24], whose low cost and high sample throughput advanced its application in archaeology, ecology and cultural heritage [286]. ZooMS produces peptides mass fingerprints that are checked against reference fingerprints of known taxa; thus, it is used to detect the taxonomies of archaeological remains. Nevertheless, even though ZooMS has proved useful to discriminate between family and genera-level taxa, it is not as efficient in differentiating species due to the lack of sufficient protein variability [20].

Successively, the use of tandem mass spectrometry (MS/MS) led to an improvement in data accuracy and taxonomic resolution. This technique is indeed able to analyze a mix of proteins and, thanks to the parallel development of new software, to define *de novo* sequences, allowing the determination of proteins sequences of extinct species for which genomic data could not be obtained [24]. This is evidently valuable for phylogenetic studies. The MS/MS method has been applied to obtain proteome sequences from a Pleistocene specimen of *Stephanorhinus* of 1.77 million years [279]. Multiple methods are currently available for sample preparation for mass analysis. The main steps usually include the demineralization of the tissue, protein solubilization and buffer exchange, protein digestion and peptide purification. The protocols must be adjusted by considering multiple factors, from the status and kind of sample to the target amount and types of proteins [24]. Numerous programs for paleoproteomics analysis have been developed, improving the accuracy of the results and the applicability of the field [24].

Similar to the aDNA field, the paleoproteomics field needs to deal with contamination issues specific to the ancient biomolecules field. The contamination discrimination is based on the use of markers of degradation that can differentiate truly endogenous ancient proteins from potential modern contaminants [20]. Protein fragmentation can occur due to enzymatic damage (especially from the burial environment) or through hydrolysis via bond cleavage. Mass shifts are detected as an effect of protein degradation [20]. As an example, during degradation, glutamine and asparagine undergo deamidation and are transformed into glutamic acid and aspartic acid, respectively. Environmental factors like temperature and pH can influence these deamidation modifications. However, despite these influences, deamidation serves as a significant marker of protein degradation [20]. Ramsøe and colleagues have recently developed a method for the authentication of ancient proteins, called deamiDATE 1.0 [287]. The method was utilized on shotgun proteomic data of bone collagen derived from modern, archaeological, and extinct taxa. Through this approach, the researchers successfully distinguished authentic ancient proteins from contaminants in a case study involving dental calculus from

the Neolithic period [287]. Moreover, further developments in the method could enable the study of cooking practices in the past by assuming that the cooking process accelerates deamination. This strategy could be, in principle, applied to proteins extracted from animal bones but also pottery and dental calculus [287].

Paleoproteomics is a promising field of bioarcheology of recent and fast development. It has the potential to elucidate our history and evolution being applicable to a number of different and very old (at least if mineralized samples) substrates. paleoproteomics studies are still limited. However, we expect to see further technological improvements parallel to the increasing interest in the field. To the best of our knowledge, there have been no human paleoproteomics studies conducted in the Arabian Peninsula. This lack of research is likely attributed to the extreme environmental conditions in the region, which can lead to the deterioration of the organic components of archaeological remains.

Ancient Lipids

Lipids are an important class of organic substances that can be recovered in association with a wide range of archaeological artifacts and substrates that can be biomolecularly and isotopically characterized. The research in this field focused on a wide variety of substrates. Pottery artifacts are archaeological findings allowing inferring food production, storage, and processing, even if their original function is not always obvious [288,289]. These objects usually absorb organic residues entrapping and preserving organic biomolecules, including ancient lipids within the substances cooked and/or stored in them [290]. It has been demonstrated that the chemical analysis of these residues may help to understand the function of these archaeological artifacts as well as to allow the investigation of the culinary, cultural and social practices of past human populations parallelly to the transitions in human history [291-293]. The analysis of steroidal compounds in soil, coprolites and sediments has been proved helpful in investigating waste disposal, dairy, and agricultural activities as well as dietary patterns [294-304]. Furthermore, lipids from bog bodies, mummies and human skeletal remains [305-311], as well as from plants have also been analyzed [312,313].

The incorporation pattern of lipids taken from the diet as well as the distribution of lipid tissues are both well-known [314,315]. The analysis of isotopic signatures of bone lipids represents a valuable source of additional information for the investigation of past dietary patterns [307,310,311,316-319], and it allows not only to implement data from the analysis of bone proteins but also to shorten the investigated timeframe as they possess a faster turnover than proteins [320-322]. In addition, as previously described, the isotopic composition of bone proteins allows inferring information on diet's proteins intake, whereas that of lipids returns the individual's whole diet [146,323,324]. Ancient lipids are less susceptible to degradation as compared to aDNA and proteins, due to their chemical structures (e.g., limited presence of functional groups, high abundance of aliphatic chains, rings and branches). Moreover, the entrapment in organic and mineral matrices enhances their preservation even more, reducing the

microbial degradation and the diffusion of the biomolecules [325].

Generally, the research in this field focuses on steroidal compounds and more hydrophobic molecules that possess a higher resistance to microbial and chemical degradation. This is due to fatty acids that are only rarely retrieved from archaeological samples, with the exception of specimens collected from arid environments [316]. Lipid analysis follows well-established protocols [326-328]. The most employed method to extract lipids from different substrates is the solvent extraction using intermediate polarity systems. According to the substrate's nature different preparation treatments may be needed; for instance, bone remains need pulverization whereas others (e.g. wax, fat, and other substances as resinous and bituminous residues) may be directly dissolved in solvents [28]. GC and GC-MS are employed to evaluate which compounds they originate from [27, 329], whereas stable carbon isotope analysis through GC-combustion-stable isotope ratio MS (GC-c-IRMS) identifies animal lipids in archaeological potsherds [290,330-333]. Currently, the use of acidified methanol extraction is commonly paired with gas chromatography-mass spectrometry (GC-MS) and gas chromatography combustion mass spectrometry (GC-C-MS) for analysis [334-336]. The two-step extraction procedure allowed the retrieval of a wider range of lipids with an appreciable amount of endogenous fatty acids from samples retrieved in hot environments [334].

The traditional interpretation approach based on the comparison with modern fat references [29,327,330,331,333,337,338], showed some limitations related to a higher variation in the diet of omnivores compared to herbivores and to variations in the faunal diet due to environmental, temporal, and cultural changes, etc. [339-342]. Moreover, it should be noticed that the ancient lipids recovered from archaeological artifacts do not necessarily derive from lipid tissue; for instance, the boiling of animal bones for preparing soups may cause the transfer of bone marrow lipids [336]. The application of GC-MS analysis has been proved useful to understand Nabataean funeral practices of monumental tombs of ancient Hegra, in Saudi Arabia [343]. A mixture of fatty acids and triterpenic compounds was retrieved from four textile samples and suggested the presence of vegetable oil and probably elemi resin. Although the exact botanic origin of the resin could not be identified, the results of these experiments suggested that the resinous material belonged to the genus *Canarium*, providing insights into how Nabataeans prepared dead bodies for their funerary practices [343]. GC and GC-MS have also been applied in the field of petroleum geochemistry to explore bituminous mixtures found in archaeological excavations [344]. Bitumen from natural sources in Iraq, Syria, Iran, Bahrain, and Kuwait has been utilized as a reference for calibrating biomarkers in hydrocarbon fractions [344]. The Middle East is rich in oil-stained rocks, deposits of solid bitumen and oil and gas shows, particularly in Iran in the Zagros mountain [344]. Evidence from Syria (Tell Atij, 6800 BCE) and Israel (Netiv Hagdud, 8900-7800 BCE) has shown that in the Neolithic time bitumen was used as an adhesive to glue flint elements to several tools [344]. Bitumen mixtures retrieved from Tell el'Oueili in Iraq have revealed the routes of Mesopotamian traders over time [344]. In Syria bitumen has been found to be used

as a hafting material during the Middle Paleolithic [344]. Bitumen was also found in balms of Egyptian mummies, dated between 1000 BCE to 400 BCE [344].

The use of GC-MS has allowed the characterization of mummies samples from Yemen and the elucidation of Yemeni methods for body preservation [345]. A more recent study has analyzed 31 samples retrieved from the ancient Harbour of Qāni' (Yemen) which led to the identification of frankincense and bitumen which was thought to have been imported from Iran [346]. Another study has also applied GC-MS along with other techniques to reconstruct the Yemeni commercial routes during the Middle Ages [347]. The majority of samples analyzed were made of a diterpenoid substance similar to East African copal [347]. Commercial routes and exchange networks represent an important subject to study as they can provide informative clues on past societies. In a very recent study, Suryanarayan and colleagues analyzed ceramic lipid residues from Hili, al Ain in the United Arab Emirates to shed light on the organic products that were part of the exchange networks that occurred in Arabia during the Bronze Age [348]. Their results indicated that the vessel studies were associated with the handling of products of animal and plant origin [21,348]. Although studies that apply GC and GC-MS in the Middle East do exist, they have been mainly focused on non-human remains. The Middle East and the Arabian Peninsula in particular could significantly benefit from additional studies aiming at analyzing lipid substrates in human archaeological samples.

Concluding Remarks

The technical advancements occurred during the last few decades determined a dramatic and previously inconceivable expansion of the availability of data from progressively older samples at a constantly increasing level of detail. The application of ancient biomolecular analysis is paving the way toward a refined characterization of population dynamics and socio-cultural changes. This review provides an up-to-date overview of the current methods for the study of ancient biomolecules. We hope that our review will facilitate the application of ancient biomolecules to studies to be carried out in underrepresented regions like the Arabian Peninsula.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

This work was supported by a grant from the Qatar National Research Fund (NPRP10-0208-170411) allotted to ST. The contents are solely the responsibility of the authors and do not necessarily represent the official views of the Qatar National Research Fund.

References

- Nguyen M (2022) Nobel Prize in Physiology or Medicine 2022: Understanding the past for heading to the future. *Mindsponge*.
- Enrico Cappellini, Ana Prohaska, Fernando Racimo, Frido Welker, Mikkel Winther Pedersen, et al. (2018) Ancient Biomolecules and Evolutionary Inference. *Annu Rev Biochem* 87(1): 1029-1060.
- Hofreiter M, M Collins, JR Stewart (2012) Ancient biomolecules in Quaternary palaeoecology. *Quaternary Science Reviews* 33(1): 1-13.
- Baldoni M, Muna Al Hashmi, Alice Enrica Bianchi, Ferhan Sakal, Faisal Al-Naimi, et al. (2021) Bioarchaeology-related studies in the Arabian Gulf: potentialities and shortcomings. *Homo* 72(1): 17-32.
- Pinhasi R, Daniel M Fernandes, Kendra Sirak, Olivia Cheronet (2019) Isolating the human cochlea to generate bone powder for ancient DNA analysis. *Nat Protoc* 14(4): 1194-1205.
- Rasmussen M, Yingrui Li, Stinus Lindgreen, Jakob Skou Pedersen, Anders Albrechtsen, et al. (2010) Ancient human genome sequence of an extinct Palaeo-Eskimo. *Nature* 463(7282): 757-762.
- Skoglund P, Bernd H Northoff, Michael V Shunkov, Anatoli P Derevianko, Svante Pääbo, et al. (2014) Separating endogenous ancient DNA from modern day contamination in a Siberian Neandertal. *Proc Natl Acad Sci U S A* 111(6): 2229-2234.
- Orlando L, Robin Allaby, Pontus Skoglund, Clio Der Sarkissian, Philipp W Stockhammer, et al. (2021) Ancient DNA analysis. *Nature Reviews Methods Primers* 1(1): 14-25.
- Willerslev E, Enrico Cappellini, Wouter Boomsma, Rasmus Nielsen, Martin B Hebsgaard, et al. (2007) Ancient biomolecules from deep ice cores reveal a forested southern Greenland. *Science* 317(5834): 111-114.
- Willerslev E, Anders J Hansen, Jonas Binladen, Tina B Brand, M Thomas P Gilbert, et al. (2003) Diverse plant and animal genetic records from Holocene and Pleistocene sediments. *Science* 300(5620): 791-795.
- Wommack KE, Daniel J Nasko, Jessica Chopyk, Eric G Sakowski (2015) Counts and sequences, observations that continue to change our understanding of viruses in nature. *J Microbiol* 53(3): 181-192.
- Pedersen MW, Søren Overballe Petersen, Luca Ermini, Clio Der Sarkissian, James Haile, et al. (2015) Ancient and modern environmental DNA. *Philos Trans R Soc Lond B Biol Sci* 370(1660): 20130383-20130390.
- Wang Y, Mikkel Winther Pedersen, Inger Greve Alsos, Bianca De Sanctis, Fernando Racimo, et al. (2021) Late Quaternary dynamics of Arctic biota from ancient environmental genomics. *Nature* 600(7887): 86-92.
- Sirak KA, Daniel M Fernandes, Olivia Cheronet, Mario Novak, Beatriz Gamarra, et al. (2017) A minimally-invasive method for sampling human petrous bones from the cranial base for ancient DNA analysis. *Biotechniques* 62(6): 283-289.
- Hansen HB, Peter B Damgaard, Ashot Margaryan, Jesper Stenderup, Niels Lynnerup, et al. (2017) Comparing Ancient DNA Preservation in Petrous Bone and Tooth Cementum. *PLoS One* 12(1): e0170940-e0170945.
- Harney E, Olivia Cheronet, Daniel M Fernandes, Kendra Sirak, Matthew Mah, et al. (2021) A minimally destructive protocol for DNA extraction from ancient teeth. *Genome Res* 31(3): 472-483.
- Pinhasi R, Daniel Fernandes, Kendra Sirak, Mario Novak, Sarah Connell, et al. (2015) Optimal Ancient DNA Yields from the Inner Ear Part of the Human Petrous Bone. *PLoS One* 10(6): e0129102-e0129110.
- Green EJ, CF Speller (2017) Novel Substrates as Sources of Ancient DNA: Prospects and Hurdles. *Genes (Basel)* 8(7): 180-185.
- Mackie M, Jessica Hendy, Abigail D Lowe, Alessandra Sperduti, Malin Holst, et al. (2017) Preservation of the metaproteome: variability of protein preservation in ancient dental calculus. *Sci Technol Archaeol Res* 3(1): 74-86.
- Hendy J (2021) Ancient protein analysis in archaeology. *Sci Adv* 7(3): eabb9314-eabb9320.
- (1950) National Academy of Sciences: Abstracts of Papers Presented at the Annual Meeting April 26-28, 1954, Washington, D.C. *Science* 119(3096): 576-588.
- Robbins LL, G Muyzer, K Brew (1993) Macromolecules from living and fossil biominerals. *Organic Geochemistry: Principles and Applications*, Macko (Eds): Springer US, Boston, MA pp. 799-816.
- Hendy J, Frido Welker, Beatrice Demarchi, Camilla Speller, Christina Warinner, et al. (2018) A guide to ancient protein studies. *Nat Ecol Evol* 2(5): 791-799.

24. Warinner C, K Korzow Richter, MJ Collins (2022) Paleoproteomics. *Chem Rev* 122(16): 13401-13446.
25. Evershed R (2008) Organic residue analysis in archaeology: the archaeological biomarker revolution. *Archaeometry* 50(6): 895-924.
26. Bergmann W (1963) *Geochemistry of lipids*, in *Organic Geochemistry*. ed. E Breger Pergamon: Oxford.
27. Evershed R (2008) Organic residue analysis in archaeology: the archaeological biomarker revolution. *Archaeometry* 50(6): 895-924.
28. Enrico Cappellini, Ana Prohaska, Fernando Racimo, Frido Welker, Mikkel Winther Pedersen, et al. (2018) Ancient Biomolecules and Evolutionary Inference. *Annu Rev Biochem* 87(1): 1029-1060.
29. Dunne J, Richard P Evershed, Mélanie Salque, Lucy Cramp, Silvia Bruni, et al. (2012) First dairying in green Saharan Africa in the fifth millennium BC. *Nature* 486(7403): 390-394.
30. Craig O, Val J Steele, Anders Fischer, Sönke Hartz, Søren H Andersen, et al. (2011) Ancient lipids reveal continuity in culinary practices across the transition to agriculture in Northern Europe. *Proc Natl Acad Sci U S A* 108(44): 17910-17915.
31. Lucquin A, Kevin Gibbs, Junzo Uchiyama, Hayley Saul, Mayumi Ajimoto, et al. (2016) Ancient lipids document continuity in the use of early hunter-gatherer pottery through 9,000 years of Japanese prehistory. *Proc Natl Acad Sci U S A* 113(15): 3991-3996.
32. Berstan R, Stephanie N Dudd, Mark S Copley, E David Morgan, Anita Quye, et al. (2004) Characterisation of 'bog butter' using a combination of molecular and isotopic techniques. *Analyst* 129(3): 270-275.
33. Charters S, RP Evershed, LJ Goad, A Leyden, PW Blinkhorn, et al. (1993) Quantification and distribution of lipid in archaeological ceramics: implications for sampling potsherds for organic residue analysis and the classification of vessel use. *Archaeometry* 35(2): 211-223.
34. Canti M, D Huisman (2015) Scientific advances in geoarchaeology during the last twenty years. *Journal of Archaeological Science* 56(1): 96-108.
35. Rehren T, I Freestone (2015) Ancient glass: from kaleidoscope to crystal ball. *Journal of Archaeological Science* 56(1): 233-241.
36. Szpak P (2014) Complexities of nitrogen isotope biogeochemistry in plant-soil systems: implications for the study of ancient agricultural and animal management practices. *Frontiers in plant science* 5(1): 288-295.
37. Makarewicz C, J Sealy (2015) Dietary reconstruction, mobility, and the analysis of ancient skeletal tissues: Expanding the prospects of stable isotope research in archaeology. *J Archaeol Sci* 56(1): 146-158.
38. Ambrose S, J Buikstra, H Krueger (2003) Status and gender differences in diet at Mound 72, Cahokia, revealed by isotopic analysis of bone. *Journal of Anthropological Archaeology* 22(3): 217-226.
39. Balasse M, Stanley H Ambrose, Andrew B Smith, T Douglas Price (2002) The seasonal mobility model for prehistoric herders in the south-western Cape of South Africa assessed by isotopic analysis of sheep tooth enamel. *Journal of Archaeological Science* 29(9): 917-932.
40. Balasse M, Hervé Bocherens, André Mariotti, Stanley H Ambrose (2001) Detection of dietary changes by intra-tooth carbon and nitrogen isotopic analysis: an experimental study of dentine collagen of cattle (*Bos taurus*). *Journal of Archaeological Science* 28(3): 235-245.
41. Baldoni M, Alessandra Nardi, Gundula Muldner, Roberta Lelli, Micaela Gnes, et al. (2016) Archaeo-biological reconstruction of the Italian medieval population of Colonna (8th-10th centuries CE). *Journal of Archaeological Science: Reports* 10(1): 483-494.
42. Baldoni M, Gabriele Scorrano, Michelle Alexander, Francesca Romana Stasolla, Luigi Tonino Marsella, et al. (2019) The medieval population of Leopoli-Cencelle (Viterbo, Latium): Dietary reconstruction through stable isotope analysis from bone proteins. *Journal of Archaeological Science: Reports* 24(1): 92-101.
43. Baldoni M, Gabriele Scorrano, Angelo Gismondi, Alessia D Agostino, Michelle Alexander, et al. (2018) Who were the miners of Allumiere? A multidisciplinary approach to reconstruct the osteobiography of an Italian worker community. *PLoS One* 13(10): e0205362-e0205368.
44. Barrett J, M Richards (2004) Identity, gender, religion and economy: new isotope and radiocarbon evidence for marine resource intensification in early historic Orkney, Scotland, UK. *European Journal of Archaeology* 7(3): 249-271.
45. Gregoricka L (2013) Residential mobility and social identity in the periphery: strontium isotope analysis of archaeological tooth enamel from southeastern Arabia. *Journal of Archaeological Science* 40(1): 452-464.
46. Makarewicz C, N Tuross (2006) Foddering by Mongolian pastoralists is recorded in the stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotopes of caprine dentinal collagen. *Journal of Archaeological Science* 33(6): 862-870.
47. Makarewicz C, N Tuross (2012) Finding fodder and tracking transhumance: isotopic detection of goat domestication processes in the Near East. *Current Anthropology* 53(4): 495-505.
48. Müldner G, K Britton, A Ervynck (2014) Inferring animal husbandry strategies in coastal zones through stable isotope analysis: new evidence from the Flemish coastal plain (Belgium, 1st-15th century AD). *Journal of Archaeological Science* 41(1): 322-332.
49. Scorrano G, arica Baldoni, Mauro Brilli, Mario Federico Rolfo, Gino Fornaciari, et al. (2019) Effect of Neolithic transition on an Italian community: Mora Cavorso (Jenne, Rome). *Archaeological and Anthropological Sciences* 11(4): 1443-1459.
50. Scorrano G, Mauro Brilli, Cristina Martínez Labarga, Francesca Giustini, Elsa Pacciani, et al. (2014) Palaeodiet reconstruction in a woman with probable celiac disease: a stable isotope analysis of bone remains from the archaeological site of Cosa (Italy). *Am J Phys Anthropol* 154(3): 349-356.
51. Sealy J (2006) Diet, Mobility, and Settlement Pattern among Holocene Hunter-Gatherers in Southernmost Africa. *Current Anthropology* 47(4): 569-595.
52. Somerville A, M Fauvelle, A Froehle (2013) Applying new approaches to modeling diet and status: isotopic evidence for commoner resiliency and elite variability in the Classic Maya lowlands. *Journal of Archaeological Science* 40(3): 1539-1553.
53. Tung T, K Knudson (2008) Social identities and geographical origins of Wari trophy heads from Conchopata, Peru. *Current Anthropology* 49(5): 915-925.
54. Zazzo A, O Munoz, J Saliege (2014) Diet and mobility in a late Neolithic population of coastal Oman inferred from radiocarbon dating and stable isotope analysis. *Am J Phys Anthropol* 153(3): 353-364.
55. Baldoni M, Angelo Gismondi, Michelle Alexander, Alessia D Agostino, Domitilla Tibaldi, et al. (2019) A multidisciplinary approach to investigate the osteobiography of the Roman Imperial population from Muracciotta Torresina (Palestrina, Rome, Italy). *Journal of Archaeological Science: Reports* 27(1): 101960-101965.
56. Almarri MA, Marc Haber, Reem A Lootah, Pille Hallast, Saeed Al Turki, et al. (2021) The genomic history of the Middle East. *Cell* 184(18): 4612-4625.e14.
57. Petraglia MD, Groucutt Huw S, Guagnin Maria, Breeze Paul S, Boivin, Nicole (2020) Human responses to climate and ecosystem change in ancient Arabia. *Proc Natl Acad Sci U S A* 117(15): 8263-8270.
58. Hershkovitz I, Gerhard W Weber, Rolf Quam, Mathieu Duval, Rainer Grün, et al. (2018) The earliest modern humans outside Africa. *Science* 359(6374): 456-459.
59. Groucutt HS, Rainer Grün, Iyad A S Zalmout, Nick A Drake, Simon J Armitage, et al. (2018) Homo sapiens in Arabia by 85,000 years ago. *Nat Ecol Evol* 2(5): 800-809.

60. Lee Thorp JA (2008) On isotopes and old bones. *Archaeometry* 50(6): 925-950.
61. Sealy J, NJ van der Merwe, A Sillen, FJ Kruger, HW Krueger (1991) $^{87}\text{Sr}/^{86}\text{Sr}$ as a dietary indicator in modern and archaeological bone. *Journal of Archaeological Science* 18(3): 399-416.
62. Sillen A, Grant Hall, Stephen Richardson, Richard Armstrong (1998) $^{87}\text{Sr}/^{86}\text{Sr}$ ratios in modern and fossil food-webs of the Sterkfontein Valley: implications for early hominid habitat preference. *Geochimica et Cosmochimica Acta* 62(14): 2463-2473.
63. De Angelis F, Gabriele Scorrano, Cristina Martínez Labarga, Francesca Giustini, Mauro Brilli, et al. (2019) Eneolithic subsistence economy in Central Italy: first dietary reconstructions through stable isotopes. *Archaeological and Anthropological Sciences* 11(1): 4171-4186.
64. Villalba Mouco V, Pilar Utrilla, Rafael Laborda, José Ignacio Lorenzo, Cristina Martínez Labarga, et al. (2018) Reconstruction of human subsistence and husbandry strategies from the Iberian Early Neolithic: A stable isotope approach. *Am J Phys Anthropol* 167(2): 257-271.
65. Kusaka S (2019) Stable isotope analysis of human bone hydroxyapatite and collagen for the reconstruction of dietary patterns of hunter-gatherers from Jomon populations. *International Journal of Osteoarchaeology* 29(1): 36-47.
66. Hatch K, Morgan A Crawford, Amanda W Kunz, Steven R Thomsen, Dennis L Eggett, et al. (2006) An objective means of diagnosing anorexia nervosa and bulimia nervosa using $^{15}\text{N}/^{14}\text{N}$ and $^{13}\text{C}/^{12}\text{C}$ ratios in hair. *Rapid Commun Mass Spectrom* 20(22): 3367-3373.
67. Nardoto G, Steven Silva, Carol Kendall, James R Ehleringer, Lesley A Chesson, et al. (2006) Geographical patterns of human diet derived from stable-isotope analysis of fingernails. *Am J Phys Anthropol* 131(1): 137-146.
68. O'Brien D (2015) Stable Isotope Ratios as Biomarkers of Diet for Health Research. *Annu Rev Nutr* 35(1): 565-594.
69. Buchardt B, V Bunch, P Helin (2007) Fingernails and diet: stable isotope signatures of a marine hunting community from modern Uummannaq, North Greenland. *Chemical Geology* 244(1-2): 316-329.
70. Noe Nygaard N (1988) $\delta^{13}\text{C}$ -values of dog bones reveal the nature of change in man's food resources at the Mesolithic-Neolithic transition, Denmark. *Chemical Geology* 73(1): 87-96.
71. Schwarcz H, J Melbye, M Katzenberg, Martin Knyf (1985) Stable isotopes in human skeletons of southern Ontario: reconstructing paleodiet. *J Archaeol Sci* 12(3): 187-206.
72. Sealy J, N van der Merwe (1985) Isotope assessment of Holocene human diets in the southwestern Cape, South Africa. *Nature* 315(6015): 138-140.
73. Walker P, M DeNiro (1986) Stable nitrogen and carbon isotope ratios in bone collagen as indices of prehistoric dietary dependence on marine and terrestrial resources in southern California. *Am J Phys Anthropol* 71(1): 51-61.
74. Barrett J, Cluny Johnstone, Jennifer Harland, Wim Van Neer, Anton Ervynck, et al. (2008) Detecting the medieval cod trade: a new method and first results. *Journal of Archaeological Science* 35(4): 850-861.
75. Guiry E, Stéphane Noël, Eric Tourigny, Vaughan Grimes (2012) A stable isotope method for identifying transatlantic origin of pig (*Sus scrofa*) remains at French and English fishing stations in Newfoundland. *Journal of Archaeological Science* 39(7): 2012-2022.
76. Meiggs D (2007) Visualizing the seasonal round: a theoretical experiment with strontium isotope profiles in ovicaprine teeth. *Anthropozoologica* 42(2): 107-128.
77. Roberts P, Mathew Stewart, Abdulaziz N Alagaili, Paul Breeze, Ian Candy, et al. (2018) Fossil herbivore stable isotopes reveal middle Pleistocene hominin palaeoenvironment in 'Green Arabia'. *Nat Ecol Evol* 2(12): 1871-1878.
78. Lightfoot E, M Slaus, TO Connell (2012) Changing cultures, changing cuisines: Cultural transitions and dietary change in Iron Age, Roman, and Early Medieval Croatia. *Am J Phys Anthropol* 148(4): 543-556.
79. Pearson M (2003) *Food, Culture and Identity in the Neolithic and Early Bronze Age*. BAR, Oxford.
80. Goude G, Domingo C Salazar García, Robert C Power, Maité Rivollat, Lionel Gourichon, et al. (2020) New insights on Neolithic food and mobility patterns in Mediterranean coastal populations. *Am J Phys Anthropol* 173(2): 218-235.
81. Hedges R (2004) Isotopes and red herrings: comments on Milner et al. and Lidén et al. *Antiquity* 78(299): 34-37.
82. Hedges R, John G Clement, C David L Thomas, Tamsin C O Connell (2007) Collagen turnover in the adult femoral mid-shaft: modeled from anthropogenic radiocarbon tracer measurements. *Am J Phys Anthropol* 133(2): 808-816.
83. Ambrose SH, B M Butler, D B Hanson, R L Hunter Anderson, H W Krueger (1997) Stable isotopic analysis of human diet in the Marianas Archipelago, western Pacific. *Am J Phys Anthropol* 104(3): 343-361.
84. McKinney C, JM Mccrea, S Epstein, HA Allen, HC Urey (1950) Improvements in mass spectrometers for the measurement of small differences in isotope abundance ratios. *Review of Scientific Instruments* 21(8): 724-730.
85. Craig H (1957) Isotopic standards for carbon and oxygen and correction factors for mass-spectrometric analysis of carbon dioxide. *Geochimica et cosmochimica acta* 12(1-2): 133-149.
86. Coplen T (1996) New guidelines for reporting stable hydrogen, carbon, and oxygen isotope-ratio data. *Geochimica et Cosmochimica Acta* 60(17): 3359-3360.
87. Mariotti A (1983) Atmospheric nitrogen is a reliable standard for natural ^{15}N abundance measurements. *Nature* 303(5919): 685-687.
88. Park R, S Epstein (1961) Metabolic fractionation of C^{13} & C^{12} in plants. *Plant Physiology* 36(2): 133-135.
89. Tykot R (1999) Stable isotopes and diet: you are what you eat. *Proceedings-International School of Physics Enrico Fermi*. 2004. IOS Press Ohmsha.
90. Tykot RH (2018) Bone Chemistry and Ancient Diet. in Smith, C. (Ed.), *Encyclopedia of Global Archaeology*. Springer pp. 1-11.
91. Schoeninger MJ (2011) Diet reconstruction and ecology using stable isotope ratios. Larsen CS (Ed.), *A companion to biological anthropology* Chichester, Wiley-Blackwell pp. 445-464.
92. Bender M (1968) Mass spectrometric studies of carbon 13 variations in corn and other grasses. *Radiocarbon* 10(2): 468-472.
93. Van der Merwe N (1982) Carbon isotopes, photosynthesis, and archaeology: different pathways of photosynthesis cause characteristic changes in carbon isotope ratios that make possible the study of prehistoric human diets. *American Scientist* 70(6): 596-606.
94. Vogel J (1980) Fractionation of the carbon isotopes during photosynthesis. Berlin, Heidelberg pp. 5-29.
95. Vogel J, N Van Der Merwe (1977) Isotopic evidence for early maize cultivation in New York State. *American Antiquity* 42(2): 238-242.
96. O Leary M (1981) Carbon isotope fractionation in plants. *Phytochemistry* 20(4): 553-567.
97. Tieszen L (1991) Natural variations in the carbon isotope values of plants: implications for archaeology, ecology, and paleoecology. *Journal of Archaeological Science* 18(3): 227-248.
98. Schoeninger M, M DeNiro (1984) Nitrogen and carbon isotopic composition of bone collagen from marine and terrestrial animals. *Geochimica et Cosmochimica acta* 48(4): 625-639.

99. Malainey M (2011) A Consumer's Guide to Archaeological Science: Analytical Techniques. Springer, New York.
100. Fry B, R Scalan, P Parker (1983) $^{13}\text{C}/^{12}\text{C}$ ratios in marine food webs of the Torres Strait, Queensland. *Marine and Freshwater Research* 34(5): 707-715.
101. McCounaughy T, C McRoy (1979) Food-web structure and the fractionation of carbon isotopes in the Bering Sea. *Marine biology* 53(3): 257-262.
102. Rau G (1983) Animal $^{13}\text{C}/^{12}\text{C}$ correlates with trophic level in pelagic food webs. *Ecology* 64(5): 1314-1318.
103. Schoeninger M (1985) Trophic level effects on $^{15}\text{N}/^{14}\text{N}$ and $^{13}\text{C}/^{12}\text{C}$ ratios in bone collagen and strontium levels in bone mineral. *Journal of human evolution* 14(5): 515-525.
104. Walker P, M De Niro (1986) Stable nitrogen and carbon isotope ratios in bone collagen as indices of prehistoric dietary dependence on marine and terrestrial resources in southern California. *Am J Phys Anthropol* 71(1): 51-61.
105. Day S (1996) Dogs, deer and diet at Star Carr: a reconsideration of C-isotope evidence from Early Mesolithic dog remains from the Vale of Pickering, Yorkshire, England. *Journal of Archaeological Science* 23(5): 783-787.
106. Dufour E, H Bocherens, A Mariotti (1999) Palaeodietary implications of isotopic variability in Eurasian lacustrine fish. *Journal of Archaeological Science* 26(6): 617-627.
107. Eriksson G, Karin Margarita Frei, Rachel Howcroft, Sara Gummeson, Fredrik Molin, et al. (2018) Diet and mobility among Mesolithic hunter-gatherers in Motala (Sweden)-The isotope perspective. *Journal of Archaeological Science: Reports* 17(1): 904-918.
108. Guede I, Luis Angel Ortega, Maria Cruz Zuluaga, Ainhoa Alonso Olazabal, Xabier Murelaga, et al. (2017) Isotope analyses to explore diet and mobility in a medieval Muslim population at Tauste (NE Spain). *PLoS One* 12(5): e0176572-e0176578.
109. Katzenberg M (1989) Stable isotope analysis of archaeological faunal remains from southern Ontario. *Journal of Archaeological Science* 16(3): 319-329.
110. Katzenberg M, A Weber (1999) Stable isotope ecology and palaeodiet in the Lake Baikal region of Siberia. *Journal of Archaeological Science* 26(6): 651-659.
111. Richards M, Karavanic, I, Pettitt, P, P Miracle (2015) Isotope and faunal evidence for high levels of freshwater fish consumption by Late Glacial humans at the Late Upper Paleolithic site of Šandaljia II, Istria, Croatia. *Journal of Archaeological Science* 61(1): 204-212.
112. Schwarcz H (1985) Stable isotopes in human skeletons of Southern Ontario: reconstructing palaeodiet. *Journal of Archaeological Science* 12(3): 187-206.
113. Katzenberg M, S Saunders (2008) *Biological Anthropology of the Human Skeleton* (2nd edn). Wiley-Liss, New York.
114. Doane TA (2017) The Abiotic Nitrogen Cycle. *ACS Earth Space Chem* 1(7): 411-421.
115. De Niro M Stable isotope and archaeology. *American Scientist* 1987(75): 182-191.
116. Jaouen K, Michael P Richards, Adeline Le Cabec, Frido Welker, William Rendu, et al. (2019) Exceptionally high $\delta^{15}\text{N}$ values in collagen single amino acids confirm Neandertals as high-trophic level carnivores. *Proc Natl Acad Sci U S A* 116(11): 4928-4933.
117. Crowder K, Janet Montgomery, Darren R. Gröcke, Kori L Filipek (2019) Childhood "stress" and stable isotope life histories in Transylvania. *International Journal of Osteoarchaeology* 29(4): 644-653.
118. Fogel M, N Tuross, W Owsley (1989) Nitrogen isotope tracers of human lactation in modern and archaeological populations. *Carnegie Institution of Washington, Washington DC* 88(1): 111-117.
119. Gardner K, Eric J Bartelink, Antoinette Martinez, Alan Leventhal, Rosemary Cambra (2018) Breastfeeding and weaning practices of the ancestral Ohlone Indians of California: A case study using stable isotope analysis of bone collagen. *International Journal of Osteoarchaeology* 28(5): 523-534.
120. Kwok C, S Garvie Lok, M Katzenberg (2018) Exploring variation in infant feeding practices in Byzantine Greece using stable isotope analysis of dentin serial sections. *International Journal of Osteoarchaeology* 28(5): 563-578.
121. Schurr M (2018) Exploring ideas about isotopic variation in breastfeeding and weaning within and between populations: Case studies from the American midcontinent. *International Journal of Osteoarchaeology* 28(5): 479-491.
122. Tsutaya T, M Yoneda (2015) Reconstruction of breastfeeding and weaning practices using stable isotope and trace element analyses: A review. *Am J Phys Anthropol* 156 (Suppl 59): 2-21.
123. Katzenberg M, N Lovell (1999) Stable isotope variation in pathological bone. *International Journal of Osteoarchaeology* 9(5): 316-324.
124. Mekota A, Gisela Grupe, Sandra Ufer, Ullrich Cuntz (2006) Serial analysis of stable nitrogen and carbon isotopes in hair: monitoring starvation and recovery phases of patients suffering from anorexia nervosa. *Rapid Commun Mass Spectrom* 20(10): 1604-1610.
125. Scorrano, G., (2018) The Stable Isotope Method in Human Paleopathology and Nutritional Stress Analysis. *Archaeology & Anthropology: Open Access* 1(1): 1-3.
126. Ambrose S, M DeNiro (1986) The isotopic ecology of East African mammals. *Oecologia* 69(3): 395-406.
127. Salazar García D, J Emili Aura, Carme R Olària, Sahra Talamo, Juan V Morales, et al. (2014) Isotope evidence for the use of marine resources in the Eastern Iberian Mesolithic. *Journal of Archaeological Science* 42(1): 231-240.
128. Sandias M, G Müldner, (2015) Diet and herding strategies in a changing environment: stable isotope analysis of Bronze Age and Late Antique skeletal remains from Ya'amün, Jordan. *Journal of Archaeological Science* 63(1): 24-32.
129. Weber A, Dustin White, Vladimir I Bazaliiskii, Olga I Goriunova, Nikolai A Savelev, et al. (2011) Hunter-gatherer foraging ranges, migrations, and travel in the middle Holocene Baikal region of Siberia: insights from carbon and nitrogen stable isotope signatures. *Journal of Anthropological Archaeology* 30(4): 523-548.
130. Craig O, Luca Bondioli, Luciano Fattore, Tom Higham, Robert Hedges, et al. (2013) Evaluating marine diets through radiocarbon dating and stable isotope analysis of victims of the AD79 eruption of Vesuvius. *Am J Phys Anthropol* 152(3): 345-352.
131. O'Connell T, C J Kneale, N Tasevska, G G C Kuhnle (2012) The diet-body offset in human nitrogen isotopic values: a controlled dietary study. *Am J Phys Anthropol* 149(3): 426-434.
132. Bocherens H, Dorothée G Drucker, Mietje Germonpré, Martina Láznicková-Galetová, Yuichi I Naito, et al. (2015) Reconstruction of the Gravettian food-web at Předmostí I using multi-isotopic tracking (^{13}C , ^{15}N , ^{34}S) of bone collagen. *Quaternary International* 359-360(1): 211-228.
133. Bocherens H, Emilia Hofman Kamińska, Dorothée G Drucker, Ulrich Schmölcke, Rafał Kowalczyk (2015) European bison as a refugee species? Evidence from isotopic data on Early Holocene bison and other large herbivores in northern Europe. *PLoS One* 10(2): e0115090-e0115095.

134. Casey M, D Post (2011) The problem of isotopic baseline: reconstructing the diet and trophic position of fossil animals. *Earth Sci Rev* 106(1-2): 131-148.
135. Koch P (2007) Isotopic study of the biology of modern and fossil vertebrates. *Stable Isotopes in Ecology and Environmental Science* (2nd Edition) R Michener, Lajtha K (Editor) Blackwell Publishing: Boston pp. 99-154.
136. Koch P (1998) Isotopic reconstruction of past continental environments. *Annu. Rev. Earth Planet Sci* 26(1): 573-613.
137. Martínez del Río C, Nathan Wolf, Scott A Carleton, Leonard Z Gannes (2009) Isotopic ecology ten years after a call for more laboratory experiments. *Biol Rev* 84(1): 91-111.
138. Rubenstein D, K Hobson (2004) From birds to butterflies: animal movement patterns and stable isotopes. *Trends Ecol Evol* 19(5): 256-263.
139. Samec C, H Yacobaccio, H Panarello, (2017) Carbon and nitrogen isotope composition of natural pastures in the dry Puna of Argentina: a baseline for the study of prehistoric herd management strategies. *Archaeological and Anthropological Sciences* 9(1): 153-163.
140. Szpak P, Jean François Millaire, Christine D White, Fred J Longstaffe (2014) Small scale camelid husbandry on the north coast of Peru (Virú Valley): insight from stable isotope analysis. *Journal of Anthropological Archaeology* 36(2): 110-129.
141. Ugan A, J Coltrain, (2011) Variation in collagen stable nitrogen values in black-tailed jackrabbits (*Lepus californicus*) in relation to small-scale differences in climate, soil, and topography. *Journal of Archaeological Science* 38(7): 1417-1429.
142. Dupras T, M Tocheri (2007) Reconstructing infant weaning histories at Roman period Kellis, Egypt using stable isotope analysis of dentition. *American Journal of Physical Anthropology* 134(1): 63-74.
143. Krueger H, C Sullivan (1984) Models for carbon isotope fractionation between diet and bone. *Stable isotopes in nutrition*. J Turnlund, Johnson P (Editor) American Chemical Society, Washington DC pp. 205-220.
144. Passey B, Todd F Robinson b, Linda K Ayliffe, Thure E Cerling, Matt Sponheimer, et al. (2005) Carbon isotope fractionation between diet, breath CO₂, and bioapatite in different mammals. *Journal of Archaeological Science* 32(10): 1459-1470.
145. Ambrose S, L Norr (1993) Experimental evidence for the relationship of the carbon isotope ratios of whole diet and dietary protein to those of bone collagen and carbonate. *Prehistoric human bone: archaeology at the molecular level*, J Lambert, G Grupe (Editors) Springer: Berlin pp. 1-37.
146. Jim S, S Ambrose, R Evershed (2004) Stable carbon isotopic evidence for differences in the dietary origin of bone cholesterol, collagen and apatite: implications for their use in palaeodietary reconstruction. *Geochimica et cosmochimica acta* 68(1): 61-72.
147. Tieszen L, T Fagre (1993) Effect of diet quality and composition on the isotopic composition of respiratory CO₂, bone collagen, bioapatite, and soft tissues, in *Prehistoric human bone*. Archaeology at the molecular level, JB Lambert, Grupe G (Editor) Berlin Springer pp. 121-155.
148. Wright L, H Schwarcz (1999) Correspondence between stable carbon, oxygen and nitrogen isotopes in human tooth enamel and dentine: infant diets at Kaminaljuyu. *Journal of Archaeological Science* 26(1): 1159-1170.
149. Bonham Smith C, J Littleton (2022) Multi-species analysis of stable carbon and nitrogen isotope data from Qal'at al-Bahrain. *Proceedings of the Seminar for Arabian Studies* 51(1): 35-54.
150. Ortega LA, C Alonso Fernández, I Guede, MC Zuluaga, A Alonso Olazabal, et al. (2021) Strontium and oxygen isotopes to trace mobility routes during the Bell Beaker period in the north of Spain. *Sci Rep* 11(1): 19553-19560.
151. Pellegrini M, John Pouncett, Mandy Jay, Mike Parker Pearson, Michael P Richards (2016) Tooth enamel oxygen "isoscapes" show a high degree of human mobility in prehistoric Britain. *Sci Rep* 6(1): 34986-34990.
152. Copeland SR, Matt Sponheimer, Darryl J de Ruiter, Julia A Lee Thorp, Daryl Codron, et al. (2011) Strontium isotope evidence for landscape use by early hominins. *Nature* 474(7349): 76-78.
153. Ezzo J (1994) Putting the "chemistry" back into archaeological bone chemistry analysis: modeling potential paleodietary indicators. *Journal of anthropological archaeology* 13(1): 1-34.
154. Faure G (1986) *Principles of Isotope Geology*. John Wiley & Sons, New York.
155. Dickin A (2005) *Radiogenic Isotope Geology*. Cambridge University Press, New York.
156. Ezzo J, T Price (2002) Migration, regional reorganization, and spatial group composition at Grasshopper Pueblo, Arizona. *Journal of Archaeological Science* 29(5): 499-520.
157. Price T, Clark M Johnson, Joseph A Ezzo, Jonathan Ericson, James H Burton (1994) Residential mobility in the prehistoric southwest United States: a preliminary study using strontium isotope analysis. *Journal of Archaeological Science* 21(3): 315-330.
158. Beard B, C Johnson (2000) Strontium isotope composition of skeletal material can determine the birth place and geographic mobility of humans and animals. *J Forensic Sci* 45(5): 1049-1061.
159. Hurst R, T Davis (1981) Strontium isotopes as tracers of airborne fly ash from coal-fired power plants. *Environmental Geology* 3(6): 363-367.
160. Ericson J (1985) Strontium isotope characterization in the study of prehistoric human ecology. *Journal of Human Evolution* 14(5): 503-514.
161. Price T, J Burton, R Bentley (2002) The characterization of biologically available strontium isotope ratios for the study of prehistoric migration. *Archaeometry* 44(1): 117-135.
162. Bentley R, Price TD, Lüning J, Gronenborn D, Wahl J, et al. (2002) Prehistoric migration in Europe: strontium isotope analysis of early Neolithic skeletons. *Current Anthropology* 43(5): 799-804.
163. Bentley R (2006) Strontium isotopes from the earth to the archaeological skeleton: a review. *Journal of archaeological method and theory* 13(3): 135-187.
164. Dupras T, H Schwarcz (2001) Strangers in a strange land: stable isotope evidence for human migration in the Dakhleh Oasis, Egypt. *Journal of Archaeological Science* 28(11): 1199-1208.
165. Luz B, Y Kolodny (1985) Oxygen isotope variations in phosphate of biogenic apatites, IV. Mammal teeth and bones. *Earth and planetary science letters* 75(1): 29-36.
166. Luz B, Y Kolodny, M Horowitz (1984) Fractionation of oxygen isotopes between mammalian bone-phosphate and environmental drinking water. *Geochimica et Cosmochimica Acta* 48(8): 1689-1693.
167. Daux V, Christophe Lécuyer, Marie Anne Héran, Romain Amiot, Laurent Simon, et al. (2008) Oxygen isotope fractionation between human phosphate and water revisited. *Journal of human evolution* 55(6): 1138-1147.
168. Kohn M (1996) Predicting animal $\delta^{18}O$: accounting for diet and physiological adaptation. *Geochimica et Cosmochimica Acta* 60(23): 4811-4829.

169. Longinelli A (1984) Oxygen isotopes in mammal bone phosphate: a new tool for paleohydrological and paleoclimatological research? *Geochimica et Cosmochimica Acta* 48(2): 385-390.
170. White C, Michael W Spence, Hilary Le Q Stuart Williams, Henry P Schwarcz (1998) Oxygen isotopes and the identification of geographical origins: the Valley of Oaxaca versus the Valley of Mexico. *Journal of Archaeological Science* 25(7): 643-655.
171. Dansgaard W (1964) Stable isotopes in precipitation. *Tellus* 16(4): 436-467.
172. Bryant J, P Froelich (1995) A model of oxygen isotope fractionation in body water of large mammals. *Geochimica et Cosmochimica Acta* 59(21): 4523-4537.
173. Iacumin P, G Venturelli (2015) The $\delta^{18}\text{O}$ of phosphate of ancient human biogenic apatite can really be used for quantitative palaeoclimate reconstruction. *European Scientific Journal* 11(9): 221-235.
174. Levinson A, B Luz, Y Kolodny (1987) Variations in oxygen isotopic compositions of human teeth and urinary stones. *Applied Geochemistry* 2(4): 367-371.
175. Hillson S (1996) *Dental Anthropology*. 1st edition ed., Cambridge University Press, London.
176. Ubelaker D (1989) *Human Skeletal Remains: Excavation, Analysis, Interpretation*. 2nd edition ed., Taraxacum, Washington.
177. Bentley R, T Price, E Stephan (2004) Determining the 'local' $^{87}\text{Sr}/^{86}\text{Sr}$ range for archaeological skeletons: a case study from Neolithic Europe. *Journal of Archaeological Science* 31(4): 365-375.
178. Evans J, C Chenery, A Fitzpatrick (2006) Bronze Age childhood migration of individuals near Stonehenge, revealed by strontium and oxygen isotope tooth enamel analysis. *Archaeometry* 48(2): 309-321.
179. Tütken T, T Vennemann, H Pfretzschner (2011) Nd and Sr isotope compositions in modern and fossil bones—Proxies for vertebrate provenance and taphonomy. *Geochimica et Cosmochimica Acta* 75(20): 5951-5970.
180. Koch P, Alex N Halliday, Lynn M Walter, Ralph F Stearley, Ted J Huston, et al. (1992) Sr isotopic composition of hydroxyapatite from recent and fossil salmon: the record of lifetime migration and diagenesis. *Earth and Planetary Science Letters* 108(4): p. 277-287.
181. Turner B, George D Kamenov, John D Kingston, George J Armelagos (2009) Insights into immigration and social class at Machu Picchu, Peru based on oxygen, strontium, and lead isotopic analysis. *Journal of archaeological science* 36(2): 317-332.
182. Brettell R, J Montgomery, J Evans (2012) Brewing and stewing: the effect of culturally mediated behaviour on the oxygen isotope composition of ingested fluids and the implications for human provenance studies. *Journal of Analytical Atomic Spectrometry* 27(5): 778-785.
183. Evans J, C Chenery, J Montgomery (2012) A summary of strontium and oxygen isotope variation in archaeological human tooth enamel excavated from Britain. *Journal of Analytical Atomic Spectrometry* 27(5): 754-764.
184. Knudson K (2009) Oxygen isotope analysis in a land of environmental extremes: the complexities of isotopic work in the Andes. *International Journal of osteoarchaeology* 19(2): 171-191.
185. Price T, James H Burton, Robert J Sharer, Jane E Buikstra, Lori E Wright, et al. (2010) Kings and commoners at Copan: Isotopic evidence for origins and movement in the Classic Maya period. *Journal of Anthropological Archaeology* 29(1): 15-32.
186. Rozanski K, L Araguas Araguas, R Gonfiantini (1993) Isotopic patterns in modern global precipitation. *Climate change in continental isotopic records*, P Swart, et al. Editors., American Geophysical Union: Washington DC pp. 1-36.
187. White C, T Price, F Longstaffe, (2007) Residential histories of the human sacrifices at the Moon Pyramid, Teotihuacan: evidence from oxygen and strontium isotopes. *Ancient Mesoamerica* 18(1): 159-172.
188. Fricke H, J O Neil (1996) Inter-and intra-tooth variation in the oxygen isotope composition of mammalian tooth enamel phosphate: implications for palaeoclimatological and palaeobiological research. *Palaeogeography, Palaeoclimatology, Palaeoecology* 126(1-2): 91-99.
189. Weidemann F (1999) Methodological and archaeological implications of intra-tooth isotopic variation ($\delta^{13}\text{C}$, $\delta^{18}\text{O}$) in herbivores from Ain Ghazal (Jordan, Neolithic). *J Archaeol Sci* 26(1): 697-704.
190. Wright L, H Schwarcz (1998) Stable carbon and oxygen isotopes in human tooth enamel: identifying breastfeeding and weaning in prehistory. *Am J Phys Anthropol* 106(1): 1-18.
191. Al Shorman A, L El Khouri (2011) Strontium isotope analysis of human tooth enamel from Barsinia: a late antiquity site in Northern Jordan. *Archaeol Anthropol Sci* (3): 263-269.
192. Ryan SE, Vladimir Dabrowski, Arnaud Dapigny, Caroline Gauthier, Eric Douville, et al. (2021) Strontium isotope evidence for a trade network between southeastern Arabia and India during Antiquity. *Sci Rep* 11(1): 303.
193. Kutterer A, HP Uerpmann (2017) Neolithic nomadism in south-east Arabia—strontium and oxygen isotope ratios in human tooth enamel from al-Buhais 18 and Umm al-Quwain 2 in the Emirates of Sharjah and Umm al-Quwain (UAE). *Arabian Archaeology and Epigraphy* 28(1): 75-89.
194. Wang X, Eirini Skourtanioti, Marion Benz, Julia Gresky, Jana Ilgner, et al. (2023) Isotopic and DNA analyses reveal multiscale PPNB mobility and migration across Southeastern Anatolia and the Southern Levant. *Proc Natl Acad Sci U S A* 120(4): e2210611120-e2210611125.
195. Pfeifer SP (2017) From next-generation resequencing reads to a high-quality variant data set. *Heredity (Edinb)* 118(2): 111-124.
196. Nurk S, Sergey Koren, Arang Rhie, Mikko Rautiainen, Andrey V Bzikadze, et al. (2022) The complete sequence of a human genome. *Science* 376(6588): 44-53.
197. Pont C, Stefanie Wagner, Antoine Kremer, Ludovic Orlando, Christophe Plomion, et al. (2019) Paleogenomics: reconstruction of plant evolutionary trajectories from modern and ancient DNA. *Genome Biol* 20(1): 29-35.
198. Meyer M, Qiaomei Fu, Ayinuer Aximu-Petri, Isabelle Glocke, Birgit Nickel, et al. (2014) A mitochondrial genome sequence of a hominin from Sima de los Huesos. *Nature* 505(7483): 403-406.
199. Orlando L, Aurélien Ginolhac, Guojie Zhang, Duane Froese, Anders Albrechtsen, et al. (2013) Recalibrating Equus evolution using the genome sequence of an early Middle Pleistocene horse. *Nature* 499(7456): 74-78.
200. Slon V, Charlotte Hopfe, Clemens L Weiß, Fabrizio Mafessoni, Marco de la Rasilla, et al. (2017) Neandertal and Denisovan DNA from Pleistocene sediments. *Science* 356(6338): 605-608.
201. Willerslev E, Anders J Hansen, Jonas Binladen, Tina B Brand, M Thomas P Gilbert, et al. (2003) Diverse plant and animal genetic records from Holocene and Pleistocene sediments. *Science* 300(5620): 791-795.
202. Rohland N, Isabelle Glocke, Ayinuer Aximu Petri, Matthias Meyer, et al. (2018) Extraction of highly degraded DNA from ancient bones, teeth and sediments for high-throughput sequencing. *Nat Protoc* 13(11): 2447-2461.
203. Dabney J, M Meyer, S Paabo, (2013) Ancient DNA damage. *Cold Spring Harb Perspect Biol* 5(7): a012567-a012570.
204. Lindahl T (1993) Instability and decay of the primary structure of DNA. *Nature* 362(6422): 709-715.

205. Dabney J, Michael Knappb, Isabelle Glockea, Marie Theres Gansaugea, Antje Weihmann, et al. (2013) Complete mitochondrial genome sequence of a Middle Pleistocene cave bear reconstructed from ultrashort DNA fragments. *Proc Natl Acad Sci U S A* 110(39): 15758-15763.
206. Der Sarkissian C, Morten E Allentoft, María C Ávila Arcos, Ross Barnett, Paula F Campos, et al. (2015) Ancient genomics. *Philos Trans R Soc Lond B Biol Sci* 370(1660): 20130387-20130390.
207. Pruvost M, Reinhard Schwarz, Virginia Bessa Correia, Sophie Champlot, Séverine Braguier, et al. (2007) Freshly excavated fossil bones are best for amplification of ancient DNA. *Proc Natl Acad Sci U S A* 104(3): 739-744.
208. Slatkin M, F Racimo (2016) Ancient DNA and human history. *Proc Natl Acad Sci U S A* 113(23): 6380-6387.
209. Korlevic P, Tobias Gerber, Marie-Theres Gansauge, Mateja Hajdinjak, Sarah Nagel, et al. (2015) Reducing microbial and human contamination in DNA extractions from ancient bones and teeth. *Biotechniques* 59(2): 87-93.
210. Briggs AW, Udo Stenzel, Philip L F Johnson, Richard E Green, Janet Kelso, et al. (2007) Patterns of damage in genomic DNA sequences from a Neandertal. *Proc Natl Acad Sci U S A* 104(37): 14616-14621.
211. Sosa C, E Vispe, C Núñez, M Baeta, Y Casalod, et al. (2013) Association between ancient bone preservation and dna yield: a multidisciplinary approach. *Am J Phys Anthropol* 151(1): 102-119.
212. Scorrano G, Federica Valentini, Cristina Martínez Labarga, Mario Federico Rolfo, Antonella Fiammenghi, et al. (2015) Methodological strategies to assess the degree of bone preservation for ancient DNA studies. *Ann Hum Biol* 42(1): 10-19.
213. Gamba C, Eppie R Jones, Matthew D Teasdale, Russell L McLaughlin, Gloria Gonzalez Fortes, et al. (2014) Genome flux and stasis in a five millennium transect of European prehistory. *Nat Commun* 5(1): 5257-5262.
214. Hansen H, Peter B Damgaard, Ashot Margaryan, Jesper Stenderup, Niels Lynnerup, et al. (2017) Comparing Ancient DNA Preservation in Petrous Bone and Tooth Cementum. *PLoS One* 12(1): e0170940-e0170945.
215. Sirak K, Daniel Fernandes, Olivia Cheronet, Eadaoin Harney, Matthew Mah et al. (2020) Human auditory ossicles as an alternative optimal source of ancient DNA. *Genome Res* 30(3): 427-436.
216. Gilbert M, Lynn P Tomsho, Snjezana Rendulic, Michael Packard, Daniela I Drautz, et al. (2007) Whole-genome shotgun sequencing of mitochondria from ancient hair shafts. *Science* 317(5846): 1927-1930.
217. Handt O, M Höss, M Krings, S Pääbo (1994) Ancient DNA: methodological challenges. *Experientia* 50(6): 524-529.
218. Hoss M, S Paabo, N Vereshchagin, (1994) Mammoth DNA sequences. *Nature* 370(6488): 333-334.
219. Poinar H, Carsten Schwarz, Ji Qi, Beth Shapiro, Ross D E Macphee, et al. (2006) Metagenomics to paleogenomics: large-scale sequencing of mammoth DNA. *Science* 311(5759): 392-394.
220. Rollo F, W Asci, I Marota (1994) Neolithic plant DNA from the Iceman's site: rare, long and nicked. *Ancient DNA Newsletter* 2(1): 21-23.
221. Rollo F, I Marota (1999) How microbial ancient DNA, found in association with human remains, can be interpreted. *Philos Trans R Soc Lond B Biol Sci* 354(1379): 111-119.
222. Rollo F, S Sassaroli, M Ubaldi (1995) Molecular phylogeny of the fungi of the Iceman's grass clothing. *Curr Genet* 28(3): 289-297.
223. Caramelli D, M Lari, (2004) *Metodi di analisi e applicazioni*. Angelo Pontecorboli editore. Firenze.
224. Cooper A, H Poinar (2000) Ancient DNA: do it right or not at all. *Science* 289(5482): 1139-1145.
225. Champlot S, Camille Berthelot, Mélanie Pruvost, E Andrew Bennett, Thierry Grange, et al. (2010) An efficient multistrategy DNA decontamination procedure of PCR reagents for hypersensitive PCR applications. *PLoS One* 5(9).
226. Pickrell J, Reich D (2014) Toward a new history and geography of human genes informed by ancient DNA. *Trends Genet* 30(9): 377-389.
227. Ron Pinhasi, Daniel M Fernandes, Kendra Sirak, Olivia Cheronet (2019) Isolating the human cochlea to generate bone powder for ancient DNA analysis. *Nat Protoc* 14(4): 1194-1205.
228. Slatkin M, Racimo F (2016) Ancient DNA and human history. *Proc Natl Acad Sci U S A* 113(23): 6380-6387.
229. Llamas B, Willerslev E, Orlando L (2017) Human evolution: a tale from ancient genomes. *Philos Trans R Soc Lond B Biol Sci* 372(1713): 20150484.
230. Morten E Allentoft, Martin Sikora, Karl Göran Sjögren, Simon Rasmussen, Morten Rasmussen, et al. (2015) Population genomics of Bronze Age Eurasia. *Nature* 522(7555): 167-172.
231. Wolfgang Haak, Iosif Lazaridis, Nick Patterson, Nadin Rohland, Swapan Mallick, et al. (2015) Massive migration from the steppe was a source for Indo-European languages in Europe. *Nature* 522 (7555): 207-211.
232. Morten Rasmussen, Yingrui Li, Stinus Lindgreen, Jakob Skou Pedersen, Anders Albrechtsen, et al. (2010) Ancient human genome sequence of an extinct Palaeo-Eskimo. *Nature* 463(7282): 757-762.
233. Richard E Green, Johannes Krause, Adrian W Briggs, Tomislav Maricic, Udo Stenzel, et al. (2010) A draft sequence of the Neandertal genome. *Science* 328(5979): 710-722.
234. Prufer K, Fernando Racimo, Nick Patterson, Flora Jay, Sriram Sankararaman, et al. (2014) The complete genome sequence of a Neanderthal from the Altai Mountains. *Nature* 505(7481): 43-49.
235. Meyer M, Martin Kircher, Marie-Theres Gansauge, Heng Li, Fernando Racimo, et al. (2012) A high-coverage genome sequence from an archaic Denisovan individual. *Science* 338(6104): 222-226.
236. Reich D, Richard E Green, Martin Kircher, Johannes Krause, Nick Patterson, et al. (2010) Genetic history of an archaic hominin group from Denisova Cave in Siberia. *Nature* 468(7327): 1053-1060.
237. Sawyer S, Johannes Krause, Katerina Guschanski, Vincent Savolainen, Svante Pääbo (2012) Temporal patterns of nucleotide misincorporations and DNA fragmentation in ancient DNA. *PLoS One* 7(3): 34131-34131.
238. Hofreiter M, Johanna L A Paijmans, Helen Goodchild, Camilla F Speller, Axel Barlow, et al. (2015) The future of ancient DNA: Technical advances and conceptual shifts. *Bioessays* 37(3): 284-293.
239. Ferreira J C, Farida Alshamali, Francesco Montinaro, Bruno Cavadas, Antonio Torroni, et al. (2021) Projecting Ancient Ancestry in Modern-Day Arabians and Iranians: A Key Role of the Past Exposed Arabo-Persian Gulf on Human Migrations. *Genome Biol Evol* 13(9): 1-1.
240. Razali R M, Juan Rodriguez Flores, Mohammadmehdi Ghorbani, Haroon Naeem, Waleed Aamer, et al. (2021) Thousands of Qatari genomes inform human migration history and improve imputation of Arab haplotypes. *Nat Commun* 12(1): 5929-5929.
241. Green E, C Speller (2017) Novel Substrates as Sources of Ancient DNA: Prospects and Hurdles. *Genes (Basel)* 8(7): 180-180.
242. Warinner C, C Speller, M Collins (2015) A new era in palaeomicrobiology: prospects for ancient dental calculus as a long-term record of the human oral microbiome. *Philos Trans R Soc Lond B Biol Sci* 370(1660): 20130376-20130376.

243. Cristiani E, Anita Radini, Dušan Borić, Harry K. Robson, Isabella Caricola, et al. (2018) Dental calculus and isotopes provide direct evidence of fish and plant consumption in the Mesolithic Mediterranean. *Sci Rep* 8(1): p. 8147-8147.
244. Gismondi A, Alessia D'Agostino, Lorena Canuti, Gabriele Di Marco, Cristina Martínez-Labarga, et al. (2018) Dental calculus reveals diet habits and medicinal plant use in the Early Medieval Italian population of Colonna. *Journal of Archaeological Science: Reports* 20(1): 556-564.
245. Lieverse A (1999) Diet and the aetiology of dental calculus. *International Journal of osteoarchaeology* 9(4): 219-232.
246. Aufderheide A, R Conrado (1998) *The Cambridge Encyclopedia of Human Paleopathology*. Cambridge: Cambridge University Press 44(1): 121-123.
247. Al Zahrani M, E Borawski, N Bissada (2004) Poor overall diet quality as a possible contributor to calculus formation. *Oral Health Prev Dent* 2(4): 345-349.
248. Arensburg B (1996) Ancient dental calculus and diet. *Human Evolution* 11(2): 139-145.
249. Hardy K, Stephen Buckley, Matthew J Collins, Almudena Estalrich, Don Brothwell, et al. (2012) Neanderthal medics? Evidence for food, cooking, and medicinal plants entrapped in dental calculus. *Naturwissenschaften* 99(8): 617-626.
250. Henry A, A Brooks, D Piperno (2011) Microfossils in calculus demonstrate consumption of plants and cooked foods in Neanderthal diets (Shanidar III, Iraq; Spy I and II, Belgium). *Proc Natl Acad Sci U S A* 108(2): 486-491.
251. Henry A, D Piperno (2008) Using plant microfossils from dental calculus to recover human diet: a case study from Tell al-Raqā'i, Syria. *Journal of Archaeological Science* 35(7):1943-1950.
252. Henry A, Peter S Ungar, Benjamin H Passey, Matt Sponheimer, Lloyd Rossouw, et al. (2012) The diet of *Australopithecus sediba*. *Nature* 487(7405): 90-93.
253. Jersie Christensen R, Liam T Lanigan, David Lyon, Meaghan Mackie, Daniel Belstrøm et al. (2018) Quantitative metaproteomics of medieval dental calculus reveals individual oral health status. *Nat Commun* 9(1): 4744-4744.
254. Li M, Xiaoyan Yang, Quansheng Ge, Xiaoyan Ren, Zhiwei Wan (2013) Starch grains analysis of stone knives from Changning site, Qinghai Province, Northwest China. *Journal of Archaeological Science* 40(4): 1667-1672.
255. Piperno D, T Dillehay (2008) Starch grains on human teeth reveal early broad crop diet in northern Peru. *Proc Natl Acad Sci U S A* 105(50): 19622-19627.
256. Warinner C, João F Matias Rodrigues, Rounak Vyas, Christian Trachsel, Natallia Shved, et al. (2014) Pathogens and host immunity in the ancient human oral cavity. *Nat Genet* 46(4): 336-344.
257. Wesolowski V, Sheila Maria Ferraz Mendonça de Souza, Karl J Reinhard, Gregório Ceccantini (2010) Evaluating microfossil content of dental calculus from Brazilian sambaquis. *Journal of Archaeological Science* 37(6): 1326-1338.
258. Cummings L, A Magennis (1997) A phytolith and starch record of food and grit in Mayan human tooth tartar. *Estado Actual De Los Estudios De Fitólitos en Suelos Y Plantas*. Centro de Ciencias Medioambientales, Madrid 211-218.
259. Hardy K, Tony Blakeney, Les Copeland, Jennifer Kirkham, Richard Wrangham, et al. (2009) Starch granules, dental calculus and new perspectives on ancient diet. *Journal of Archaeological Science* 36(2): 248-255.
260. Blatt S, B G Redmond, V Cassman, P W Sciulli (2011) Dirty teeth and ancient trade: evidence of cotton fibres in human dental calculus from Late Woodland, Ohio. *International Journal of Osteoarchaeology* 21(6): 669-678.
261. Buckley S, Donatella Usai, Tina Jakob, Anita Radini, Karen Hardy (2014) Dental calculus reveals unique insights into food items, cooking and plant processing in prehistoric central Sudan. *PLoS One* 9(7): 100808-100808.
262. Hardy K (2019) Paleomedicine and the use of plant secondary compounds in the Paleolithic and Early Neolithic. *Evolutionary Anthropology: Issues, News, and Reviews* 28(2): 60-71.
263. Hardy K, S Buckley (2016) Stable isotopes and mass spectrometry, in *Wild harvest: Plants in the hominin and pre-agrarian human worlds*, K. Hardy and M. LK Editors 241-250.
264. Hardy K, S Buckley, L Copel (2018) Pleistocene dental calculus: Recovering information on Paleolithic food items, medicines, paleoenvironment and microbes. *Evolutionary Anthropology* 27(5): 234-246.
265. Hardy K, Radini A, Buckley S, Sarig R, Copeland L, et al. (2016) Dental calculus reveals potential respiratory irritants and ingestion of essential plant-based nutrients at Lower Palaeolithic Qesem Cave Israel. *Quaternary International* 398: 129-135.
266. Adler JC, Dobney K, Weyrich LS, Kaidonis J, Walker AW (2013) Sequencing ancient calcified dental plaque shows changes in oral microbiota with dietary shifts of the Neolithic and Industrial revolutions. *Nat Genet* 45(4): 450-455.
267. Weyrich L, Dobney K, Cooper A (2015) Ancient DNA analysis of dental calculus. *J Hum Evol* 2015. 79: 119-124.
268. Dobney K, Brothwell D (1988) A scanning electron microscope study of archaeological dental calculus, in *Scanning electron microscopy in archaeology*, Olsen S(Ed.), BAR: Oxford: 372-385.
269. De La Fuente C, Flores S, Moraga M (2012) Human bacterial DNA from dental calculus: a new source of genetic material. *American Journal of Physical Anthropology* 147:127-127.
270. Linossier A, Gajardo M, Olavarria J (1996) Paleomicrobiological study in dental calculus: *Streptococcus mutans*. *Scanning Microsc* 10(4): 1005-1013.
271. Ziesemer KA, E Mann A, Sankaranarayanan K, Schroeder H, Ozga AT, et al. (2015) Corrigendum: Intrinsic challenges in ancient microbiome reconstruction using 16S rRNA gene amplification. *Sci Rep* 5: 16498.
272. Ziesemer KA, Ramos-Madriral J, Mann AE, Brandt BW, Sankaranarayanan K, et al. (2019) The efficacy of whole human genome capture on ancient dental calculus and dentin. *Am J Phys Anthropol* 168(3): 496-509.
273. Sawafuji R, Saso A, Suda W, Hattori M, Ueda S, et al. (2020) Ancient DNA analysis of food remains in human dental calculus from the Edo period, Japan. *PLoS One* 15(3): e0226654.
274. Liu B, Faller LL, Klitgord N, Mazumdar V, Ghodsi M, et al. (2012) Deep sequencing of the oral microbiome reveals signatures of periodontal disease. *PLoS One* 7(6): 37919.
275. Graham R, Graham C, McMullan G (2007) Microbial proteomics: a mass spectrometry primer for biologists. *Microb Cell Fact* 6: 26.
276. Hettich R, Pan C, Chourey C, Giannone RJ (2013) Metaproteomics: harnessing the power of high performance mass spectrometry to identify the suite of proteins that control metabolic activities in microbial communities. *Anal Chem* 85(9): 4203-4214.
277. Abelson PH (1954) *Paleobiochemistry: Organic Constituents of Fossils*. Carnegie Institution of Washington, Yearbook. 53: 97-101.

278. Ostrom PH, Schall M, Gandhi H, Shen T, Peter V Hauschka, et al. (2000) New strategies for characterizing ancient proteins using matrix-assisted laser desorption ionization mass spectrometry. *Geochimica et Cosmochimica Acta* 64(6): 1043-1050.
279. Cappellini E, Welker F, Pandolfi L, Ramos-Madriral J, Samodova D, et al. (2019) Early Pleistocene enamel proteome from Dmanisi resolves *Stephanorhinus* phylogeny. *Nature* 574(7776): 103-107.
280. Maixner F, Overath T, Linke D, Janko M, Guerriero G, et al. (2013) Paleoproteomic study of the Iceman's brain tissue. *Cell Mol Life Sci* 70(19): 3709-3722.
281. Bleicher N, Kelstrup C, Olsen JV, Cappellini E (2015) Molecular evidence of use of hide glue in 4th millennium BC Europe. *Journal of Archaeological Science* 63: 65-71.
282. Tokarski C, Martin E, Rolando C, Cren-Olivé C (2006) Identification of proteins in renaissance paintings by proteomics. *Analytical chemistry* 78(5): 1494-1502.
283. Bravenec AD, Ward KD, Ward TJ (2018) Amino acid racemization and its relation to geochronology and archaeometry. *J Sep Sci* 41(6): 1489-1506.
284. Griffin RC, Chamberlain AT, Hotz G, Penkman KEH, Collins MJ (2009) Age estimation of archaeological remains using amino acid racemization in dental enamel: a comparison of morphological, biochemical, and known ages-at-death. *Am J Phys Anthropol* 140(2): 244-252.
285. Craig O, Mulville J, Pearson MP, Sokol R, Gelsthorpe K, et al. (2000) Detecting milk proteins in ancient pots. *Nature* 408(6810): 312.
286. Richter KK, Codlin MC, Seabrook M, Christina Warinner (2022) A primer for ZooMS applications in archaeology. *Proc Natl Acad Sci U S A* 119(20): e2109323119.
287. Ramsøe A, van Vivian H, Paola P, Roman F, Ian B, et al. (2020) DeamiDATE 1.0: Site-specific deamidation as a tool to assess authenticity of members of ancient proteomes. *Journal of Archaeological Science* 115.
288. Gibson A, Woods A (1990) Prehistoric pottery for the archaeologist. Leicester: University Press.
289. Rice P (1987) Pottery analysis: a sourcebook. Edinburgh: University Press.
290. Craig OE, Saul E, Lucquin A, Nishida Y, Tache K, et al. (2013) Earliest evidence for the use of pottery. *Nature* 496(7445): 351-354.
291. Colonese ACC, Collins M, Lucquin A, Eustace M, Hancock Y, et al. (2014) Long-term resilience of late holocene coastal subsistence system in Southeastern South America. *PLoS One* 9(4): e93854.
292. Copley MS, Berstan R, Dudd SN, Docherty G, Mukherjee AJ, et al. (2003) Direct chemical evidence for widespread dairying in prehistoric Britain. *Proc Natl Acad Sci U S A* 100(4): 1524-1529.
293. Evershed RP, Payne S, Sherratt AG, Copley MS, Coolidge J, et al. (2008) Earliest date for milk use in the Near East and southeastern Europe linked to cattle herding. *Nature* 455(7212): 528-531.
294. Bethell P (1994) The study of molecular markers of human activity: the use of coprostanol in the soil as an indicator of human faecal material. *Journal of Archaeological Science* 21(5): 619-632.
295. Bull IP, Betancourt, Evershed R (1999) Chemical evidence for a structured agricultural manuring regime on the island of Psira, Crete during the Minoan Period. *Aegaeum* 20: 69-74.
296. Bull I D, Simpson I A, Van Bergen P F, Evershed R P (1999) Muck n molecules: organic geochemical methods for detecting ancient manuring. *Antiquity* 73(279): 86-96.
297. Evershed R, Bethell P (1996) Application of multimolecular biomarker techniques to the identification of fecal material in archaeological soils and sediments. *ACS Symp Ser* 625:157-172.
298. Evershed RP, Bethell PH, Reynolds PJ, Walsh NJ (1997) 5 β -Stigmastanol and related 5 β -stanols as biomarkers of manuring: analysis of modern experimental material and assessment of the archaeological potential. *Journal of Archaeological Science* 24(6): 485-495.
299. Knights BA, Camilla A Dickson, Dickson JH, Breeze DJ (1983) Evidence concerning the Roman military diet at Bearsden, Scotland, in the 2nd century AD. *Journal of Archaeological Science* 10(2): 139-152.
300. Pepe C, Dizabo P (1990) Étude d'une fosse du 13^{ème} siècle par les marqueurs biogéochimiques: chantier archéologique du Louvre (Paris). *ArchéoSciences, revue d'Archéométrie* 14(1): 23-28.
301. Pepe sem linkP C, Dizabo sem linkP, Scribe sem linkJ, Dagaut sem linkJ, Fillaux sem-linkA, et al. (1989) Les marqueurs biogéochimiques: application à l'archéologie. *ArchéoSciences revue d'Archéométrie* 13(13): 1-12.
302. Simpson IA, Bol R, Dockrill SJ, Petzke KJ, RP Evershed (1997) Compound-specific $\delta^{15}\text{N}$ amino acid signals in palaeosols as indicators of early land use: a preliminary study. *Archaeological Prospection* 4(3): 147-152.
303. Simpson IA, Dockrill SJ, Bull ID, Evershed RP (1998) Early anthropogenic soil formation at tofts Ness, Sanday, Orkney. *Journal of Archaeological Science* 25(8): 729-746.
304. Ian A Simpson, Pim F van Bergen, Vincent Perret, Mohamed M Elhmmali, David J Roberts, et al. (1999) Lipid biomarkers of manuring practice in relict anthropogenic soils. *The Holocene* 9(2): 223-229.
305. Evershed R, Connolly R (1988) Lipid preservation in Lindow Man. *Naturwissenschaften* 75(3): 143-145.
306. Evershed R, Connolly R (1994) Post-mortem transformations of sterols in bog body tissues. *Journal of archaeological science* 21(5): 577-583.
307. Richard P Evershed, Gordon Turner Walker, Robert EM Hedges, Noreen Tuross, Ann Leyden e (1995) Preliminary results for the analysis of lipids in ancient bone. *Journal of Archaeological Science* 22(2): 277-290.
308. Güllacar F, Susini A, Kohn M (1990) Preservation and post-mortem transformations of lipids in samples from a 4000-year-old Nubian mummy. *Journal of Archaeological Science* 17(6): 691-705.
309. Jim S (1998) Animal feeding experiments in the development of cholesterol as a palaeodietary indicator, in *Proceedings of archaeological sciences '97*, A. Millard, Editor., BAR: Oxford.
310. Stott A, Evershed R (1996) $\delta^{13}\text{C}$ analysis of cholesterol preserved in archaeological bones and teeth. *Anal Chem* 68(24): 4402-4408.
311. Stott AW, Evershed RP, Jim S, Jones V, Rogers JM, et al. (1999) Cholesterol as a new source of palaeodietary information: experimental approaches and archaeological applications. *Journal of Archaeological Science* 26(6): 705-716.
312. Donoghue KO, Clapham A, Evershed RP, Brown TA (1996) Remarkable preservation of biomolecules in ancient radish seeds. *Proc Biol Sci* 263(1370): 541-547.
313. Van Bergen PF, Bland HA, Horton MC, Evershed RP (1997) Chemical and morphological changes in archaeological seeds and fruits during preservation by desiccation. *Geochimica et Cosmochimica Acta* 61(9): 1919-1930.
314. Hodson L, Skeaff C, Fielding B, (2008) Fatty acid composition of adipose tissue and blood in humans and its use as a biomarker of dietary intake. *Prog Lipid Res* 47(5): 348-380.
315. Budge SM, Wooller MJ, Springer AM, Iverson SJ, McRoy CP, et al. (2008) Tracing carbon flow in an arctic marine food web using fatty acid-stable isotope analysis. *Oecologia* 157(1): 117-129.
316. Copley M, Susan Jim, Vicky Jones, Pam Rose, Alan Clapham, et al. (2004) Short-and long-term foraging and foddering strategies of domesticated

- animals from Qasr Ibrim, Egypt. *Journal of Archaeological Science* 31(9): 1273-1286.
317. Corr L, Michael P. Richards, Colin Grier, Alexander Mackie, Owen Beattie, et al. (2009) Probing dietary change of the Kwäday Dän Ts' inchi individual, an ancient glacier body from British Columbia: II. Deconvoluting whole skin and bone collagen $\delta^{13}\text{C}$ values via carbon isotope analysis of individual amino acids. *Journal of Archaeological Science*, 36(1): 12-18.
 318. Spangenberg J, Montserrat Ferrer, Stefanie Jacomet, Niels Bleicher, Jörg Schibler, et al. (2014) Molecular and isotopic characterization of lipids staining bone and antler tools in the Late Neolithic settlement, Zurich Opera Parking, Switzerland. *Organic geochemistry* 69(1): 11-25.
 319. Stott A, R Evershed, N Tuross (1997) Compound-specific approach to the $\delta^{13}\text{C}$ analysis of cholesterol in fossil bones. *Organic Geochemistry* 26(1-2): 99-103.
 320. Arner P, Samuel Bernard, Mehran Salehpour, Göran Possnert, Jakob Liebl, et al. (2011) Dynamics of human adipose lipid turnover in health and metabolic disease. *Nature* 478(7367): 110-113.
 321. Strawford A, F Antelo, M Christiansen, M K Hellerstein (2004) Adipose tissue triglyceride turnover, de novo lipogenesis, and cell proliferation in humans measured with $^2\text{H}_2\text{O}$. *Am J Physiol Endocrinol Metab* 286(4): E577-588.
 322. Tieszen L, T W Boutton, K G Tesdahl, N A Slade (1983) Fractionation and turnover of stable carbon isotopes in animal tissues: Implications for $\delta^{13}\text{C}$ analysis of diet. *Oecologia* 57(1-2): 32-37.
 323. Howland M, LT Corr, SMM Young, V Jones, S Jim, et al. (2003) Expression of the dietary isotope signal in the compound-specific $\delta^{13}\text{C}$ values of pig bone lipids and amino acids. *International Journal of Osteoarchaeology* 13(1-2): 54-65.
 324. Stott A, E Davies, R P Evershed (1997) Monitoring the routing of dietary and biosynthesised lipids through compound-specific stable isotope ($\delta^{13}\text{C}$) measurements at natural abundance. *Naturwissenschaften* 84(2): 82-86.
 325. Correa Ascencio M, R Evershed (2014) High throughput screening of organic residues in archaeological potsherds using direct acidified methanol extraction. *Analytical Methods* 6(5): 1330-1340.
 326. Craig O, M Forster, Sh Andersen, E Koch, P Crombé, et al. (2007) Molecular and isotopic demonstration of the processing of aquatic products in northern European prehistoric pottery. *Archaeometry* 49(1): 135-152.
 327. Dudd S, R Evershed (1998) Direct demonstration of milk as an element of archaeological economies. *Science* 282(5393): 1478-1481.
 328. Hansel F, R Evershed (2009) Formation of dihydroxy acids from Z-monounsaturated alkenoic acids and their use as biomarkers for the processing of marine commodities in archaeological pottery vessels. *Tetrahedron Letters* 50(40): 5562-5564.
 329. Evershed R, Stephanie N Dudd, Stephanie Charters, Hazel Mottram, Andrew W Stott, et al. (1999) Lipids as carriers of anthropogenic signals from prehistory. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* 354(1379): 19-31.
 330. Copley M, R Berstan, S N Dudd, G Docherty, A J Mukherjee, et al. (2003) Direct chemical evidence for widespread dairying in prehistoric Britain. *Proceedings of the National Academy of Sciences* 100(4): 1524-1529.
 331. Evershed R, Stephanie N Dudd, Mark S Copley, Anna Mutherjee, et al. (2002) Identification of animal fats via compound specific $\delta^{13}\text{C}$ values of individual fatty acids: assessments of results for reference fats and lipid extracts of archaeological pottery vessels. *Documenta praehistorica* 29(1): 73-96.
 332. Mukherjee A, A Gibson, R Evershed (2008) Trends in pig product processing at British Neolithic Grooved Ware sites traced through organic residues in potsherds. *Journal of Archaeological Science* 35(7): 2059-2073.
 333. Salque M, Peter I Bogucki, Joanna Pyzel, Iwona Sobkowiak Tabaka, Ryszard Grygiel, et al. (2013) Earliest evidence for cheese making in the sixth millennium BC in northern Europe. *Nature* 493(7433): 522-525.
 334. Colonese A, Thomas Farrell, Alexandre Lucquin, Daniel Firth, Sophy Charlton, et al. (2015) Archaeological bone lipids as palaeodietary markers. *Rapid Commun Mass Spectrom* 29(7): 611-618.
 335. Papakosta V, Rienk H Smittenberg, Kevin Gibbs, Peter Jordan, Sven Isaksson (2015) Extraction and derivatization of absorbed lipid residues from very small and very old samples of ceramic potsherds for molecular analysis by gas chromatography-mass spectrometry (GC-MS) and single compound stable carbon isotope analysis by gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS). *Microchemical Journal* 123(1): 196-200.
 336. Colonese A, A Lucquin, EP Guedes, R Thomas, J Best, et al. (2017) The identification of poultry processing in archaeological ceramic vessels using in-situ isotope references for organic residue analysis. *Journal of Archaeological Science* 78(1): 179-192.
 337. Gregg M, EB Banning, K Gibbs, GF Slater (2009) Subsistence practices and pottery use in Neolithic Jordan: molecular and isotopic evidence. *Journal of archaeological science* 36(4): 937-946.
 338. Spangenberg J, S Jacomet, J Schibler (2006) Chemical analyses of organic residues in archaeological pottery from Arbon Bleiche 3, Switzerland-evidence for dairying in the late Neolithic. *Journal of Archaeological Science* 33(1): 1-13.
 339. Castaneda I, Stefan Multiza, Enno Schefuss, Raquel A Lopes dos Santos, Jaap S Sinninghe Damsté, et al. (2009) Wet phases in the Sahara/Sahel region and human migration patterns in North Africa. *Proc Natl Acad Sci U S A* 106(48): 20159-20163.
 340. Goodfriend G (1990) Rainfall in the Negev Desert during the middle Holocene, based on ^{13}C of organic matter in land snail shells. *Quaternary Research* 34(2): 186-197.
 341. Hamilton J, R Thomas (2012) Pannage, pulses and pigs: isotopic and zooarchaeological evidence for changing pig management practices in later Medieval England. *Medieval Archaeology* 56(1): 234-259.
 342. Madgwick R, J Mulville, R Stevens (2012) Diversity in foddering strategy and herd management in late Bronze Age Britain: an isotopic investigation of pigs and other fauna from two midden sites. *Environmental Archaeology* 17(2): 126-140.
 343. Mathe C, P Archier, L Nehme, C Vieillescazes (2009) The study of Nabataean organic residues from Madâ 'In Sâlih, ancient Hegra, by gas chromatography - mass spectrometry. *Archaeometry* 51(4): 626-636.
 344. Connan J (1999) Use and trade of bitumen in antiquity and prehistory: molecular archaeology reveals secrets of past civilizations. *Phil Trans R Soc Lond B* 354(1): 33-50.
 345. Buckley SA (2007) A preliminary study on the materials employed in ancient Yemeni mummification and burial practices (summary). *Proceedings of the Seminar for Arabian Studies* pp. 37-45.
 346. Connan J, C Joliot, C Mathe (2018) Frankincense and bitumen of the middle period (1st century - 5th century AD) from the ancient Harbour of Qâni' (Yemen). *Journal of Historical Archaeology & Anthropological Sciences* 3(5): 696-722.
 347. Regert M, T Deviese, AS Le Hô (2008) Reconstructing ancient Yemeni commercial routes during the Middle Ages using structural characterization of terpenoid resins. *Archaeometry* 50(4): 668-695.

348. Suryanarayan A, Sophie Méry, Arnaud Mazuy, Martine Regert (2022) Foodstuffs and organic products in ancient SE Arabia: preliminary results of ceramic lipid residue analysis of vessels from Hili 8 and

Hili North Tomb A, al Ain, United Arab Emirates. Proceedings of the Seminar for Arabian Studies 51: 379-401.



This work is licensed under Creative Commons Attribution 4.0 License

To Submit Your Article Click Here:

[Submit Article](#)

DOI: [10.32474/JAAS.2023.08.000290](https://doi.org/10.32474/JAAS.2023.08.000290)



Journal Of Anthropological And Archaeological Sciences

Assets of Publishing with us

- Global archiving of articles
- Immediate, unrestricted online access
- Rigorous Peer Review Process
- Authors Retain Copyrights
- Unique DOI for all articles