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Dietary patterns during the early prehispanic settlement in La Gomera (Canary Islands)

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ABSTRACT

The dietary pattern of 10 adults interred in the Acceso al Pescante de Vallehermoso cave (La Gomera, Canary Islands), dated from 1600 to 1800 years BP, has been investigated using carbon and nitrogen stable isotope signatures, bone barium and strontium levels, and dental and oral pathologies. In addition, trabecular bone mass – as a parameter useful to evaluate overall nutrition – was also determined.

The majority of the studied individuals died before 35 years. Diet was mixed, mainly based on C_3 plants and probably sea snails. The prevalence of carious lesions was low, but the intensity of dental attrition was high. They did not show osteopenia, but bone mass was lower among those who died at earlier ages. Based on anatomical characteristics it was inferred that the majority of individuals buried in this cave were women. This sex determination was confirmed by DNA molecular sexing using the amelogenin gene. There were no gender differences in dietary pattern or bone mass.

1. Introduction

The Canary Islands should have been colonized by people of North African origin 2500 years ago (Navarro-Mederos, 1983), a hypothesis which has been reinforced by recent genetic studies (Pinto et al., 1996; Rando et al., 1999; Maca-Meyer et al., 2004). Archaeological remains differ from one island to another, although a high degree of similitude exists between neighbour islands, such as La Gomera and Tenerife or Fuerteventura and Lanzarote (Fig. 1). Lacking metal ores, and practising only coastal fishing, the economic system of the prehispanic people of the Islands was mainly based on livestock (goats, sheep and some pigs), agriculture, and the consumption of some small animals and vegetal wild species (Navarro-Mederos, 1983).

Although this general assertion may be valid, in general, for all the prehispanic population of the Archipelago, marked differences exist between some islands. For instance, agriculture was strongly developed in Gran Canaria, which led to a considerable demographic concentration in this island, so that according to chroniclers who arrived with the Spanish conquerors, it was already inhabited by nearly 50,000 individuals with a population density of 30 inh/km^2. This very high population density in an island with a subdesertic climate, in which the main economic activity was agriculture, led to widespread malnutrition and precocious mortality (Velasco-Vázquez et al., 1999; González-Reimers and Arnay-de-la-Rosa, 1992). In this sense, the finding of a high prevalence of osteopenia in a wide population sample may be interpreted as a marker of malnutrition. In contrast, only 300 men should have survived in El Hierro, an island in which shellfishing surely was a major economic activity. Consequently, in contrast with the high prevalence of osteoporosis found in Gran Canaria, individuals from El Hierro showed a well preserved bone mass (González-Reimers et al., 2004), perhaps due to a better nutritional status, and a more favourable relationship between available food and population needs. Indeed, the subdesertic climate of the Islands leads to irregular and scarce rainfall, and vicinity to the Sahara desert and Sahel facilitated the arrival from locust plagues which almost certainly devastated the fields (repeatedly

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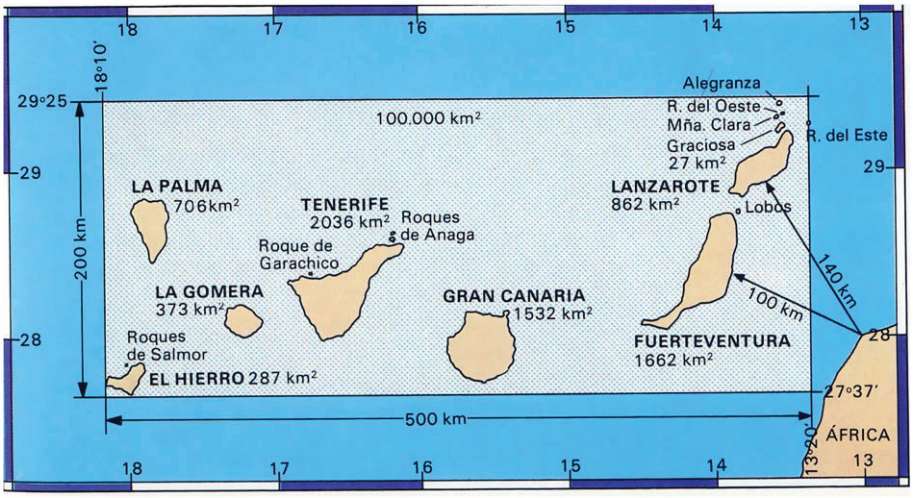

Fig. 1. The Canary Islands.

documented since short after Spanish conquest, Cola Benítez, 1996) and provoked widespread malnutrition, especially if food supply strongly relied on agriculture.

1.1. Economy and diet of the prehispanic population of La Gomera

La Gomera is one of the smallest islands (378 km^2) of the Canary Archipelago. Its origin is volcanic in nature, and the landscape is abrupt, with huge ravines which derive from a central plateau, covered by a dense forest towered by some ancient volcanoes. These volcanoes are inactive since three millions years, reaching the highest altitude in Garajonay peak (1487 m). Located very close to Tenerife, archaeological remains are similar between both islands. La Gomera was scarcely inhabited (a population of 2000–2500 inhabitants has been estimated on the basis of chroniclerśreports), and it was conquered by the Spaniards without war, although in later years bloody repressions killed many of the adult males of the island (Morales-Padrón, 1994). Little is known about economic pattern of the prehispanic society of La Gomera, although data from chroniclers and some archaeological remains allow an estimation of diet composition. Goatherding (*Capra hircus*) was the main economic activity, but livestock also included short-haired sheep (*Ovis aries*), and a few pigs *(Sus scrofa*). Seafood consumption seemed to be also important, mainly consisting of limpets (*Patella candei crenata, Patella ulysiponensis aspera*), other gastropoda (*Osilinus atratus, Thais haemastoma*) and some coastal fish. Indeed, there are shell accumulations in some coastal areas, especially in the northern coast, suggesting an important consumption of marine resources, at least in this area. Agriculture was rudimentary, and included cultivation of barley (*Hordeum* sp.) something reported by chroniclers and corroborated by archaeology. An intense gathering activity should have grown up, and some practices have survived until now, such as the exploitation of the Canarian palm (*Phoenix canariensis*) and the consumption of flour made from fern rhizomes (*Pteridium aquilinum*), or roots, bulbs, stalks, leaves and fruits, such as those of the "tagarnina" (*Sonchus gonzalezpadroni*) and reeds (*Scirpus holoscoenus*) (Navarro-Mederos, 1992).

Despite this estimation of the dietary pattern, there are no firm data derived from anthropological analysis, trace elements determination, or isotope analysis, which can confirm or refute it. Moreover, precise burial place of the relatively few anthropological remains preserved in museums and collections is unknown or confuse, and lack antiquity dates. A few analyses performed on these remains, nearly two decades ago (González-Reimers et al., 1991) reported high bone strontium levels, suggesting either marine or vegetal consumption.

1.2. The burial site

The site Acceso al Pescante de Vallehermoso is an intact collective burial cave, serependitiously discovered when a caterpillar destroyed part of a volcanic cave near the seashore (Fig. 2); the presence of human remains led to immediate stopping of works until archaeologists excavated the intact part of the cave, which represents the most important funerary site of Gomera subjected to systematic excavation and scientific analysis. The burial site showed initially two different spaces, one of which was completely destroyed by the caterpillar, so that only some bone fragments, belonging to at least eight individuals, could be recovered, but the most profound part of the second one was nearly intact. Altogether, in this second space there were remains of 13 individuals, 12 adults and 1 subadult, 10 of whom preserved the skeleton in anatomical position (Fig. 3), so they represented an opportunity to analyse in detail the archaeological remains with modern techniques. Considering the scarce information based on objective data available on dietary pattern of the primitive inhabitants of the island, we tried to approach to the dietary pattern of these individuals through combination of three methods, i.e, stable isotope analysis, bone trace elements, and analysis of dental caries, calculus, and dental wear.

Stable isotopes of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) ratios in bone collagen have been widely used to estimate dietary patterns of ancient human groups (DeNiro and Epstein, 1978, 1981). Collagen comprises about 20% of bone by weight and is remarkably resistant to post-mortem degradation (e.g., Ambrose, 1990). Stable isotopes in bone collagen provide direct information about the average protein diet of an individual over the last 5–10 years of his/her life (Dürrwächter et al., 2006), including: (1) the consumption of C_3 or C_4 plants, (2) the degree of carnivory, and (3) the utilization of aquatic resources. The estimated isotopic fractionation between diet and human bone collagen has been estimated generally about +5‰ for $\delta^{13}C$ and +3‰ for $\delta^{15}N$ (DeNiro and Epstein, 1978, 1981; Ambrose and DeNiro, 1986, 1989). However, in paleodietary studies of prehistoric humans is fundamental to check the quality of the extracted bone collagen prior the interpretation of the isotopic results. Carbon/nitrogen molar ratio, based on the content (in %) of

Fig. 2. Map of La Gomera with the burial cave (arrow).

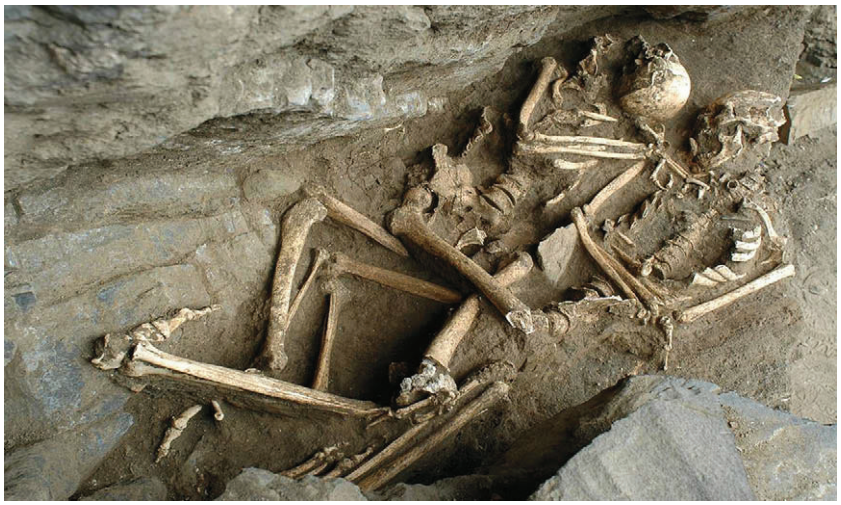

Fig. 3. Skeletons buried in the cave Pescante de Vallehermoso.

these elements on the sample, should be between 2.9 and 3.6 (DeNiro, 1985), the content of carbon should be above 13% and nitrogen above 5% (Ambrose, 1990), and yield collagen, expressed as the amount of freeze-dried gelatin relative to the dry weight of bone, should be above 1% (Ambrose, 1990). Unfortunately, anthropological remains do not always meet these requirements. Therefore, two additional methods may aid in diet reconstruction.

Trace elements in human bones have been generally used as direct paleodietary indicators (Fabig and Herrmann, 2002). Time and considerable research has down-regulated initial enthusiasm devoted to bone trace element analysis. The former idea that the Barium to Strontium ratio is a reliable proxy to differentiate between marine and terrestrial diet (Burton and Price, 1990) should be cautiously interpreted, because the possibility exists that, at least in some burial environments, diagenetic changes may obscure reliable interpretation (Fabig and Herrmann, 2002). More consistently, dental and oral pathology may provide information about dietary habits (Lukacs, 1992; Hillson, 2001). Carious lesions may indicate consumption of a vegetable-based diet, whereas a marine-based diet may be cariostatic. Although subjected to debate, calculus deposition may also indicate plant consumption, and an intense dental wear may develop with the consumption of sticky food, such as palm dates.

The aim of the present study was to infer dietary habits and overall nutrition of the early prehispanic population of La Gomera through the direct analysis of bones by multiple geochemical, anatomical and molecular techniques. Combination of these techniques may allow a more complete approach to infer dietary pattern. In addition, assessment of bone mass may permit the estimation of the overall nutritional status of the population. Moreover, anatomical sex determination was confirmed by DNA analysis using amelogenin gene.

2. Material and methods

2.1. Samples

Ten bone samples of adult individuals from La Gomera were collected in the collective burial of Acceso al Pescante de Vallehermoso (Fig. 3), which excavation was directed by some of us (JFNM, JCHM, AGM). Radiocarbon data (which was performed to seven samples of six individuals) ranged from 207 ± 40 to 457 ± 40 AC, suggesting that the cave was utilized as burial site for more than 200 years.

2.2. Age at death and anatomical sex estimation

Age at death was estimated by pubic symphysis inspection .Sex was estimated by pelvis inspection (Ubelaker, 1989).

2.3. Amelogenin molecular sexing

In humans, the amelogenin gene is present on both the X and the Y chromosome, but with size differences between them (Salido et al., 1992). These polymorphisms have been widely used for gender determination. In previous studies (Maca-Meyer et al., 2005; Arnay et al., 2007), an amelogenin essay was adapted to sex aboriginal remains to the Canary Islands. This protocol, with minor modification, has been followed in the present work.

2.3.1. DNA extraction

Archaeological samples for molecular analysis consisted, in all cases, of teeth without fractures. Whenever possible, teeth were directly taken from its mandible alveolus. A total of 20 teeth corresponding to 10 different individuals were analyzed.

Initial decontamination steps were carried out on all samples prior to extraction. Teeth were thoroughly washed with 15% HCl, rinsed with UV-treated ddH_2O and exposed to UV light for 10 min. In order to reconstruct teeth after extractions, they were transversely cut through the mid line, using a dentist electric saw, and the internal pulp and dentin drilled out using a dental drill. The powder was collected in 1.5 ml sterile tubes and DNA was extracted according to a modified GuSCN-silica-based protocol (Maca-Meyer et al., 2004).

2.3.2. Ancient DNA laboratory

To ensure the reliability of the results, strict measures were taken to avoid contamination, as recommended for ancient DNA work (Cooper and Poinar, 2000; Pääbo et al., 2004). Analyses were performed in three independently dedicated aDNA laboratories. First, the excavated material was decontaminated and processed to obtain powdered samples. Second, DNA extraction and pre-PCR procedures were carried out. PCR amplifications were performed in a third area. Finally, Post-PCR analyses were carried out in another physically isolated laboratory.

In each dedicated aDNA area, all personnel were required to wear lab coats, face shields, hats and multiple pairs of gloves. The equipment and work areas were constantly irradiated with UV lamps and frequently cleaned with bleach. All sample manipulations were performed in laminar flow cabinets, with dedicated pipettes and sterile filter tips (Tip One, Star Lab). Solutions were commercially acquired whenever possible; otherwise, they were autoclaved and UV-treated. All metallic material was sterilized in an oven at 200 °C for at least 4 h.

2.3.3. Mitochondrial DNA and amelogenin amplifications

As it is expected that there are approximately 3000 mtDNA molecules per cell (Iborra et al., 2004) compared to the two copies of the amelogenin genes, samples were first amplified for mtDNA fragment 4 following Maca-Meyer et al. (2004) protocol and sequenced using an ABIprism Analyzer as described previously (Fregel et al., in press). As all the samples gave positive results, all of them were tested for the amelogenin gene. In order to detect handling contamination, the mtDNA HVSI region was sequenced for all the researchers involved in the samples selected for molecular analysis manipulation (Table 1).

The X and Y amelogenin alleles were amplified using primer Amel-A (CCCTGGGCTCTGTAAAGAATAGTG) from Sullivan et al. (1993) and primer Amel-C (AATRYGGACCACTTGAGAAAC) described by Maca-Meyer et al. (2005). These primers amplify a small region in intron 1 of the amelogenin gene that encompasses a deletion polymorphism giving a product of 66 bp for the X allele and a product of 72 bp for the Y allele, so both products should be present in males but only one in females. The PCR was carried out in a 10 μl volume, containing 1 μl of 10× Tris–HCl buffer, 200 μM of each dNTP, 1 pmol of each primer, 5 mM $MgCl_2$, 15 ng of BSA, 1 unit of Taq polymerase (Ecogen) and 3 μl of DNA extract. If no amplification product was obtained, DNA was increased to 6 μl in subsequent PCRs. To overcome PCR inhibition, detectable by the lack of primer–dimers in the

Table 1
Haplotypes for researchers involved in the present work.

Researcher	HG	Haplotype	Charge
M.A.R.	K	093 189 224 311	Anthropologist
E. G.-R.	T2b	126 294 296 304	Anthropologist
A. G.	W	223 292 295	Anthropologist
J. C. H.	H	293	Archaeologist
J. F. N. M.	K	224 311	Archaeologist
R. F.	L3b3	223 278 311 362	Molecular researcher

reaction, DNA was lowered to 1 μl and/or the Taq and BSA amounts were doubled. Reactions were submitted to 40 amplification cycles with denaturation at 94 °C for 10 s, annealing at 45 °C for 10 s and extension at 72 °C for 10 s. PCR products were completely loaded in 10% acrylamide:bisacrylamide (19:1) gels, stained with ethidium bromide and visualized under UV.

2.3.4. Real-time PCR quantification

Real-time PCR quantification was carried out to assess the number of molecules used as template for PCR amplification. We used iQ™ SYBR® Green Supermix (BioRad) in a iCycler Thermal Cycler (BioRad). Primers and thermal cycling conditions were as previously specified. Tenfold serial dilutions of a purified and quantified standard were included in the experiment to determine the standard curve, in order to estimate the initial number of DNA molecules in each sample.

2.3.5. Contamination prevention and authentication

All the procedures followed to prevent contamination from modern sources and to authenticate results were as previously reported (Maca-Meyer et al., 2004; Arnay et al., 2007). One negative extract control and three negative PCR controls were included in each extraction and amplification to detect possible contamination of extraction and/or PCR reagents, in order to be sure that the products obtained were amplified from the ancient DNA extracts. To confirm the authenticity of the amplified products the following tests were carried out: (a) the correct electrophoretic migration of the X and Y amelogenin alleles was assessed running authenticated controls in parallel. (b) Faint bands, or bands with slightly different migration than controls, were re-amplified and RFLP checked using *Hinf*I digestion for the 66 bp X band and *Mbo*II digestion for the 72 bp Y chromosome band. (c) Initial successful male and female reactions were cloned, and 3 clones (females) or 7 clones (males) were sequenced to authenticate the amplified products as real X and Y amelogenin alleles. This cloning strategy was also applied in the cases of individual duplicates. (d) At least two additional amplifications were carried out for male extracts to confirm the presence of its sex specific band, and 4–5 additional amplifications were performed when only the female band was amplified the first time in order to diminish the possibility that the extract was from a male but only the X band, common to both sexes, was amplified. (e) Whenever possible, two independent tooth extractions from the same individual were carried out in different laboratories.

2.3.6. Cloning and sequencing

PCR products were ligated into pGEM-T vectors (Promega). Colonies were plated on selective Amp/IPTG/X-gal plates, and white colonies were selected.

PCR fragments were directly double-strand sequenced using the same primers as for amplification and clones were sequenced using M13 universal primers. Sequencing reactions were prepared in 10 μl volumes using the BigDye 3.1 Terminator Cycle Sequencing kit (Applied Biosystems) and the products were ethanol precipitated and run on an ABI PRISM 310 Genetic Analyzer (Applied Biosystems).

2.4. Stable isotopes

2.4.1. Bone collagen extraction

The collagen extraction used was established by Ambrose (1990) and Bocherens et al. (1991). Clean pieces of bone were ground in an agate mortar and pestle to a grain size less than 0.7 mm. About 200 mg of bone powder was weighed into a 2 ml Eppendorf centrifuge tube, and 1 M HCl was added to dissolve the mineral phase during 20 min. Samples were then centrifuged and the supernatant was poured off. The pellet was rinsed with distilled water and centrifuged three times. The remaining solid was plunged into 0.1 M NaOH for 20 h at room temperature to remove organic contaminants. Samples were again rinsed with distilled water three times by repeated centrifugations. The residue was then placed into 0.01 M HCl (pH = 2) in closed tubes, at 57 °C for 17 h, to solubilize the collagen. After centrifugation of the samples, the supernatant (containing solubilized collagen) was freeze-dried overnight. Yield collagen was expressed as the mass of freeze-dried collagen relative to the original dry weight of bone.

2.4.2. Plant sample preparation and sea shell organic matter extraction

Modern cereal samples (wheat and barley) were first cleaned with distilled water by sonication and then oven-dried at 40–50 °C overnight. Samples were lipid extracted using 2:1 chloroform:methanol mixture for 20 h prior isotope analysis. Dry plant tissues were then grounded and homogenized.

Sea shells and some body tissues of modern gastropod individuals were rinsed with distilled water while constant sonication. Clean shells were digested with 5 M HCl to eliminate completely the carbonate (between 1 and 5 days) until bubbling stopped. Samples were then centrifuged and the pellet (containing the shell organics) was first rinsed with DI water and then oven-dried at 40–50 °C overnight.

2.4.3. Stable isotope analysis

About 1 mg of freeze-dried bone collagen, ~1 mgr of shell organics and ~5 mgr of plant powder were weighted into tin capsules, crimped and analyzed on a Carlo Erba Elemental Analyzer (NC 2500), where combustion (oxidation and then reduction) of the sample occurred. The CO_2 and N_2 produced after combustion were analyzed using a Thermo Finnigan Delta Plus XL isotope ratio mass spectrometer. The δ values are defined as:

$$\delta^{13}C \text{ or } \delta^{15}N = [(R_{sample}/R_{standard}) - 1] \times 1000 \text{ (in } ‰ \text{ units)}$$

where $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$ ratios of the sample or standard. Stable isotope values are reported relative to the international standard Vienna-Pee Dee Belemnite (V-PDB) for carbon and Air for nitrogen. Multiple aliquots of in house standards were analyzed periodically as a check on the analytical precision throughout the analyses, which was ±0.1‰ for $\delta^{13}C$ and $\delta^{15}N$ values. Stable isotope analysis were performed by two of us (YY and CSR, at the Savannah River Ecology Laboratory, The University of Georgia, USA).

2.5. Assessment of carious lesions and dental calculus

The following parameters were recorded:

(a) Number of observed teeth.
(b) Number of teeth with carious lesions.
(c) Number of teeth with calculus deposition.
(d) Number of teeth with periodontal disease.
(e) Dental attrition.

We calculated:

- The proportion of individuals with at least one tooth with calculus deposition and with at least one carious lesion.
- The number of teeth with calculus deposition and the number of teeth with carious lesions.
- The proportion of teeth with each of the aforementioned alterations (in relation to the observed teeth) for each individual.

- The intensity of dental attrition following Brothwell (1972) for each teeth of the mandibles; we (MAR, AGM, EGR) calculated the mean value of the dental attrition score for each individual.

Dental carious lesions can easily be detected by the naked eye and a dental probe. Indeed, it has been shown that visual methods show little inter-observer variation, yielding reliable results (Rudney et al., 1983), as we have also tested elsewhere (Delgado-Darias et al., 2005). It is important to bear in mind that not every pit in the enamel is caused by caries since diagenetic changes can produce similar lesions in archaeological samples, so in some cases we needed the aid of a binocular microscope to distinguish among carious lesions from diagenetic changes. A brownish color and the general aspect of enamel destruction strongly suggest that the lesion observed is a carious lesion and not a postmortem alteration.

2.6. Trace elements determination

Bone samples of the inner cortical region of the tibiae were extracted for trace element analysis. They were dehydrated in a furnace at 100 °C during 4–7 days, and then dissolved in 65% HNO_3 (Merck p.a., Darmstadt, Germany) and 10% H_2O_2, in order to digest organic material. The digestion solutions were quantitatively transferred to volumetric flasks and diluted up to 10 ml with ultrapure water (Milli-Q OM-140 deionization system).

Prior to the analysis of each element we prepared a blank with ultrapure deionised water (Milli-Q system) and different solutions, at known concentrations, using certified standards of 1000 mg/kg for each of the elements analyzed (Aldrich, Milwaukee, Wisconsin, USA), which were further diluted. These solutions were used for the calibration of the apparatus.

Bone barium was measured using a Spectra A 220-Z atomic absorption spectrophotometer equipped with a Zeeman effect, a VARIAN GTA 110Z graphite furnace with pyrolized tubes provided with a Lvov platform, and a microcomputer-controlled AS-40 auto sampling system (Mulgrave, Victoria, Australia). Detection limits for barium were 0.04 μg/kg.

Strontium and calcium concentrations were determined with the aid of a Varian Spectra AA spectrophotometer (Mulgrave, Victoria, Australia) by flame atomic absorption spectrophotometry. Detection limit for Sr is 0.042 mg/kg. Samples destined for calcium analyses were further diluted (1:1000): Detection limit for calcium is 0.017 mg/kg.

Bone Ba, Sr and Ca were compared with those obtained from a modern sample, consisting of 13 individuals who died after traumatic injury at the intensive care unit of the Hospital Universitario de Canarias, and who were selected for kidney transplantation. According to their relatives, they ate a mixed diet.

2.6.1. Control for diagenesis

We performed two kinds of analysis in order to assess the importance of diagenesis:

(a) We took several random SEM microphotographs of bone samples from just beneath the place from which bone samples for trace element analysis were extracted, showing well-preserved trabeculae.
(b) We determined trace elements in the ground around the skeletal remains.

Trace elements analyses were performed at the department of Analytical Chemistry, of the University of La Laguna (Tenerife) by several of us (MAR, LGM, EGR).

2.7. Bone histomorphometry

Transiliac crest bone specimens were processed for undecalcified bone sample analysis. Briefly, samples were embedded in methylmetacrylate (Sigma Chemicals, St Louis, Missouri, USA), stored for 24 h at 4 °C and later polymerised at 32–34 °C for 3–4 days. Embedded samples were then cut in 9–12 μm thick slices with a Reichert-Jung microtome and stained with toluidin blue. Trabecular bone mass (TBM) was determined using an image analyzer equipped with the program "Image Measure 4.4a" (Microscience Inc.), at 40×. Results are presented as % of total area. Prehispanic data were compared with those of our modern control group (González-Reimers and Arnay-de-la-Rosa, 1992). This analysis was performed at the Hospital Universitario de Canarias (Tenerife), by one of us (EGR).

2.8. Statistics

Comparisons among several groups of samples were performed by means of Student's *t* test (pre-historic people vs. control group and men vs. women). A previous Kolmogorov–Smirnov test indicated normal distribution for most of the variables analyzed. Those variables without normal distribution were analyzed by Mann–Whitney's U-test. Statistical analyses were performed with the aid of SPSS (Statistical package for Social Sciences, Chicago, IL).

3. Results

The analysis was performed on 10 adult individuals with well-preserved skeletons (Fig. 3).

3.1. Carbon and nitrogen stable isotopes

Paleodietary studies of prehistoric humans are fundamental to check the quality of the extracted bone collagen prior the interpretation of the isotopic results. Carbon/nitrogen molar ratio, based on the content (in %) of these elements on the sample, should be between 2.9 and 3.6 (DeNiro, 1985), the content of carbon should be above 13% and nitrogen above 5% (Ambrose, 1990), and yield collagen, expressed as the amount of freeze-dried gelatin relative to the dry weight of bone, should be above 1% (Ambrose, 1990). Data relative to collagen yield and nitrogen and carbon proportion are shown in Table 2. Therefore, strictly speaking, only samples 3 and 4 would be appropriate for and adequate interpretation of the $\delta^{13}C$ and $\delta^{15}N$ values in the context of paleodietary analysis. However, $\delta^{13}C$ and $\delta^{15}N$ values of all the samples –including the non-appropriate ones – are similar (Table 2).

Carbon stable isotope values ranged from −21.2 to −19.3‰ (VPDB), averaging −19.7‰, suggesting an important contribution of C-3 plants in their diet, most likely by wheat and/or barley (Table 8). Nitrogen isotope fingerprint varied between 10.2 and 11.8‰ (AIR),

Table 2
Stable isotopes.

Sample	$\delta^{13}C$‰ (VPDB)	$\delta^{15}N$‰ (AIR)	% N	% C	Collagen yield	C/N
1	−19.5	10.3	3.4	10.0	2.2	3.4
2	−20.3	10.8	3.8	10.9	1.5	3.4
3	−19.8	11.8	6.3	17.6	1.0	3.3
4	−19.6	11.5	9.9	27.2	1.6	3.2
5	−19.3	10.5	0.9	3.4	1.4	4.4
6	−19.3	10.6	1.2	4.0	1.7	3.9
7	−19.3	10.2	1.5	4.9	1.2	3.8
8	−19.5	11.3	2.2	7.3	2.0	3.9
9	−21.2	10.2	1.3	4.8	1.3	4.3
10	−19.9	10.4	1.9	6.1	2.2	3.8

averaging 10.8‰ (Table 2), values compatible with consumption of marine snail species.

3.2. Bone mass

In Table 3 we show data relative to bone trace elements and bone mass. As shown all the individuals had TBM values within the normal range; indeed, no differences were observed between prehispanic individuals from Gomera (TBM = 22.54 ± 2.42 %) and modern controls (24.30 ± 5.17 % ($n = 13$); $t = 0.99$). No differences were observed between men and women regarding bone mass (Table 4), but individuals who died at younger ages (before 35) showed significantly ($p = 0.042$) less TBM than older individuals (Table 5).

3.3. Bone trace elements

Regarding trace elements, log Ba/Sr ranges from −0.51 to −0.82; statistically significant differences were observed between prehispanic individuals and modern controls regarding bone Sr, bone Ba, and log Ba/Sr ratios (Table 6). No differences were observed between men and women regarding trace elements (Table 4), or between age groups (Table 5).

In order to control for diagenesis, trace elements were also measured in soil samples adhered or in intimate contact with bones. Results are shown in Table 7, in which it becomes evident that soil content of these elements is by far lower than that observed in the bones.

3.4. Caries, calculi, and periodontal disease

Five individuals (62.5%) showed carious lesions, whereas two individuals (25%) had calculi. Mean number of carious lesions per affected individuals is 1.2. Proportion of teeth affected by carious lesions is low (7.39%), as well as proportion of teeth with calculus deposition (5.56%). No associations were observed between gender and caries or calculus. The intensity of dental attrition is relatively high (Table 9). All individuals show periodontal disease. No abscesses were recorded.

3.5. Mitochondrial DNA and amelogenin analysis

The mtDNA analysis gave positive results for the 10 teeth samples, with real-time PCR quantification giving around 3000 molecules used as initial template. In addition, two replicate samples, independently assayed in the Legal Medicine Laboratory of Las Palmas, produced identical haplotype in both laboratories. Although these preliminary results are only based on a 110 bp fragment of the HVSI region (m), the presence of the motif 163 172 219 in 5 of the 10 individuals confirms the presence of U6b1 haplotype in the aboriginal remains of La Gomera in high frequency, which is in agreement with the analysis performed in the extant population of the island. However, as a close parental relationship could exist in this burial site, these results have to be taken with caution.

Compared to mtDNA, molecular genetic sex estimation gave positive results in only 4 cases (3 females vs. 1 male). Moreover, using the amelogenin gene, only around 300–200 initial molecules were quantified by real-time PCR in the successfully amplified samples. These results could be attributed to differences in quantity and preservation between mitochondrial and genomic DNA. Nevertheless, for several samples 10-fold dilutions were necessary in order to overcome inhibition problems, so this quantification results could be underestimated. Molecular gender determination was always in agreement with pelvis inspection results.

4. Discussion

The individuals analyzed in this sample belong to a collective burial cave, partially destroyed by a caterpillar. La Gomera is one of the smallest islands of the Canary Archipelago (378 km^2). As the other islands, it was colonized by the prehispanic population probably 2500 years ago. In this sense, radiocarbon datings of this burial cave have yielded the most antique data for the population of this island – nearly 1800years BP. The cemetery was used during 200 years, as we can infer from the C14 data. Interestingly, the Canarian-specific mitochondrial haplogroup U6b1 was observed in 5 of the 10 cases. Although this result is in accordance with data obtained from other skeletal remains from the Archipelago (Maca-Meyer et al., 2004), and even from modern population of the Canaries (Rando et al., 1999), there is the possibility of parental relationship among the individuals that shared identical U6b1 haplotype. Nevertheless, no differences in dietary habits or nutritional status are observable among U6b1 carriers and the other individuals.

Bone collagen isotope analysis records mainly the protein fraction of the diet in ca. 5–10 years prior to death (Dürrwächter

Table 3
Results of histomorphometry and trace elements analysis.

Sample	TBM (%)	Strontium (mg/kg)	Barium (mg/kg)	Calcium (mg/kg)	Ba/Sr	Log Ba/Sr
1	24.00	304.60	54.74	257,504	0.15	−0.82
2	26.39	501.51	126.94	328,947	0.26	−0.59
3	22.14	375.38	88.94	263,000	0.25	−0.60
4	22.69	458.76	139.90	294,913	0.31	−0.51
5	20.15	412.89	94.64	303,594	0.23	−0.64
6	21.15	389.10	118.44	294,881	0.30	−0.52
7	19.36	418.33	101.82	274,889	0.24	−0.62
8	22.68	396.85	78.20	305,204	0.20	−0.70
9	26.27	415.92	73.58	290,259	0.18	−0.74
10	20.58	594.40	140.83	300,886	0.24	−0.62

Table 4
Trace elements in Gomera and controls.

	Groups	N	Mean	Std. deviation	
Sr	Gomera	10	426.78	78.14	$T = 11.33$, $p < 0.001$
	Control	11	121.8	41.62	
Ba	Gomera	10	96.80	40.31	$T = 9.95$, $p < 0.001$
	Control	12	9.60	12.43	
Log Ba/Sr	Gomera	10	−0.74	0.38	$T = 4.13$, $p = 0.01$
	Control	9	−1.26	0.46	
Ca	Gomera	10	291,407	21,325	$T = 3.62$, $p = 0.02$
	Control	8	254,707	21,404	

Table 5
Gender differences in trace elements and bone mass.

	Gender	N	Mean	Std. deviation	
Sr	Men	2	417.07	58.96	$T = 0.53$
	Women	7	447.00	74.74	
Ba	Men	2	114.42	36.03	$T = 0.44$
	Women	7	104.92	25.06	
Ba–Sr	Men	2	0.28	0.042	$T = 1.40$
	Women	7	0.24	0.039	
Log Ba/Sr	Men	2	−0.57	0.078	$T = 1.06$
	Women	7	−0.64	0.076	
VOT	Men	2	22.42	0.39	$T = 0.02$
	Women	7	22.37	2.89	

Table 6
Trabecular bone mass (TBM) and trace elements in younger and older individuals.

	Age at death	N	Mean	Std. deviation	
TBM	<35	6	21.32	1.72	$T = 2.42$, $p = 0.042$
	>35	4	24.37	2.27	
Sr	<35	6	429.69	95.57	$T = 0.14$, NS
	>35	4	422.42	55.27	
Ba	<35	6	100.06	50.41	$T = 0.30$, NS
	>35	4	91.92	24.22	
Log Ba/Sr	<35	6	−0.79	0.50	$T = 0.45$, NS
	>35	4	−0.67	0.07	

et al., 2006). Collagen is composed by aminoacids, including both essential and non-essential ones. Although non-essential aminoacids may be formed with carbon and nitrogen derived from non-protein sources, carbon and nitrogen of essential aminoacids can only derive from protein ingestion. Collagen shows a relatively fixed composition, with a carbon/nitrogen ratio of 2.9–3.6, a crucial aspect in paleodietary analysis (Valentin et al., 2006), because this range is considered to be characteristic of unaltered collagen (DeNiro, 1985). Also, too low proportions of nitrogen and carbon may obscure the interpretation of the results as paleodietary indicators. Therefore, strictly speaking, only two cases in this series from La Gomera are useful in dietary inference. Both results show a relatively high δ^{15}N, and δ^{13}C values (averaging 11.7‰ and −19.7‰, respectively) indicative of a consumption of C3 vegetables (Katzenberg, 2000; Ambrose et al., 2003). Indeed, δ^{13}C values of a local species of wheat –which is said that it was brought by the prehispanic population – fully support the hypothesis of an important C3 consumption by these people. Results from trace elements analysis also point to consumption of a mixed diet. Since marine environment is poor in barium, but not in Sr, it is said that log Ba/Sr values below −1.40 indicate a diet based on consumption of marine resources, and a value of −0.40 or higher, a diet based on terrestrial products (Burton and Price, 1990). Although diagenetic changes in Ba and Sr values cannot be excluded, the findings fully agree with the archaeological remains, since, although shell accumulations are present in some archaeological sites of the island (Fig. 4), they are lacking in many others (or are very scarce), in contrast with bone remains of goat, sheep, and pigs.

Consumption of goat products, such as meat and milk, and of wild species such as fruits of palm trees, which are abundant in La Gomera, probably completed the diet of these people. In this sense, an increase in δ^{15}N values has been observed with meat consumption (Thompson et al., 2008), and with consumption of marine species (Schoeninger et al., 1983).

It is important to remark the apparently good bone health of the population analyzed, with no cases of osteoporosis. As mentioned elsewhere (Velasco-Vázquez et al., 1999), in the population of other islands, such as Gran Canaria, the proportion of individuals with osteopenia is very high, reaching 30%, and also with a substantial proportion of individuals dead at relatively young ages. In this sense it is important to remind that osteopenia ensues as a disbalance between bone synthesis and bone resorption. This may be due either to decreased availability of aminoacids necessary to synthesize osteoid, as may happen in chronic starvation and or when a prolonged illness deviates metabolism to increased synthesis of acute phase reactants and increased muscle catabolism, or to increased bone resorption (Bourrin et al., 2000a, b). It is interesting, in this context, the finding of a lower TBM among those who died at earlier ages, perhaps suggesting either a prolonged illness or a more deficient nutritional status which prompted premature death.

The sample size ($n = 10$) is too small to extract conclusions valid for all the populations of La Gomera, but it is remarkable the low proportion of teeth affected by carious lesions: only 7%, something which supports the view of a mixed diet. Indeed, caries is a process in which bacteria may produce lactic acid from dietary carbohydrates. The proportion of carious lesions observed in this burial is among the lowest observed in the Canary Archipelago (Langsjoen, 1992; Velasco-Vázquez et al., 2001). By contrast there is an intense attrition, leading to pulpar exposure in many cases. Palm tree fruits are especially sticky, and might explain the results; otherwise, *Patella* and *Thais* bodies are also hard, and may lead to attrition, but consumption of roots of wild species may also lead to such a degree of attrition as intense as that observed in this study.

Table 7
Trace elements in soil samples.

Reference	Weight (g)	Calcium (mg/kg)	Barium (μg/kg)	Sr (μg/kg)
Indiv. 9 (soil)	2.56	89.84	32.03	173.44
Indiv. 7 (soil)	4.32	94.91	7.64	212.44
Indiv. 10 (soil)	4.78	75.31	5.60	205.96
Indiv. 8 (soil)	5.19	82.85	4.77	226.64
Indiv. 3 (soil)	5.04	56.55	6.00	173.21
Indiv. 1 (soil)	5.85	58.97	3.50	119.62

Table 8
Carbon and nitrogen isotopes of some species potentially consumed by the prehispanic inhabitants of the Canary Islands.

Sample	Species	Sample type	δ^{15}N‰ (AIR)	δ^{13}C‰ (VPDB)
Sea snails				
G-OA-1 (Modern)	*Osilinus attratus*	Body tissue	6.7	−18.6
G-OA-2 (Modern)	*Osilinus attratus*	Shell organic matter	7.0	−16.5
G-OA-3 (Modern)	*Osilinus attratus*	Body tissue	7.9	−18.4
G-PP-1 (Modern)	*Patella piperata*	Shell organic matter	3.3	−14.5
G-HC-1 (Modern)	*Haliotis coccinea*	Shell organic matter	3.2	−17.8
G-TH-1 (Modern)	*Thais haemastoma*	Shell organic matter	5.1	−15.0
G-PP-2 (Modern)	*Patella piperata*	Shell organic matter	4.4	−13.1
Plants (cereals)				
Barley (Modern)	*Hordeum* sp.	grain	3.4	−25.0
Wheat (Modern)	*Triticum* sp.	grain	2.0	−23.4
Terrestrial animals				
Goat (Prehistoric)	*Capra* sp.	Bone collagen	8.2	−18.5
Pig (Prehistoric)	*Sus* sp.	Bone collagen	5.9	−12.5
Fish				
Parrotfish (Prehistoric)	*Sparisoma cretensis*	Bone collagen	5.8	−12.7

Table 9
Oral pathology of the samples analyzed.

Sample	Observed teeth	Antemortem loss	Teeth with caries	Teeth with calculi	Mean attrition score
1	21	2	1	0	4.93
2	18	4	2	1	4.5
3	4	0	1	0	5.13
4	12	8	0	1	5.42
6	26	6	2	0	4.04
7	30	2	0	0	3.76
8	19	0	2	0	5.63
10	1	0	0	0	4

Fig. 4. Shell accumulation in Puntallana (Gomera).

5. Conclusions

The population buried in Acceso al Pescante de Vallehermoso belong to an antique prehispanic population who colonized the island of La Gomera. They died at relative young age (60% before 35 years), and they consumed a mixed diet with an important component of C3 vegetables. The proportion of caries is remarkably low, in contrast with the intensity of dental attrition, possibly in relation with consumption of sticky, hard food. They did not show osteopenia, but bone mass was lower among those who died at earlier ages. The majority of individuals interred in this cave were women; from our data no gender differences in dietary habits or nutritional status can be inferred from the analysis performed.

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