16. Multi-elemental analysis of human bone from Ballyhanna, Co. Donegal

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Concentrations of major, minor and trace elements within archaeological bone reflect elemental concentrations in diet and subsistence patterns of the sampled population. Through the determination of a variety of elements (substances, such as iron, that cannot be resolved by chemical means into simpler substances) it is also possible to obtain indications of the various diseases or environmental conditions that a population may have experienced. The various analytical techniques used to measure several elemental concentrations rapidly are generally referred to as multi-elemental analysis techniques. The main objective of this project is to generate quantitative multi-element data to aid in the reconstruction of the diet, environmental conditions and diseases that affected the population of a 'lost' medieval cemetery excavated at Ballyhanna, Co. Donegal, in advance of the construction of the N15 Bundoran–Ballyshannon Bypass (Ó Donnchadha 2007; MacDonagh, this volume). In order to generate relevant information it is also necessary to collect additional data in order to correct for interferences from the burial environment and particularly diagenetic factors (those elements originating in soil that are capable of contributing to post-mortem chemical alterations in a bone's inorganic component). This paper discusses some of the issues (including pitfalls) that must be considered when interpreting elemental results from archaeological bone samples with a view to the reconstruction of palaeodiets.

Diet

Multi-element chemical analysis can provide data for a diverse group of chemical elements associated with diet, disease, etc. The particular elements used in an analysis are chosen because of their apparent ability to distinguish between plant- and animal-based resources in the food chain (Gilbert 1985).

Originally, palaeodietary reconstruction was based on the primary assertion that elemental concentrations in bone would indicate the quantities in which they were initially consumed (Sandford 1993). A number of factors are responsible for elemental abundances in the living skeleton, however, ranging from food and water to cooking utensils, food preparation and occupation.

Once ingested, the presence of an element in the skeleton is influenced further by a host of metabolic and physiological processes ranging from absorption and excretion to skeletal growth and remodelling (ibid.). For example, pregnancy and lactation are believed to have an impact on the distribution of elements within the skeleton. All of these potential sources of elemental uptake and loss during life are collectively known as biogenic factors.

Trace elements have been determined as being particular to either plant or animal sources (Table 1). Palaeonutrition indicators can be found by determining the distribution/accumulation of certain trace elements in bone (Gilbert 1985).

Table 1—Mean elemental concentrations in parts per million for grains, vegetables, meats and nuts (Gilbert 1985).

Element	*Grains and cereals*	*Vegetables*[a]	*Meats*[b]	*Nuts*
Manganese	7.00	2.50	0.20	17.00
Copper	2.00	1.20	3.90	14.80
Zinc	17.70	6.00	30.60	34.00
Strontium	3.00	1.90	2.00	60.00
Vanadium	1.10	1.60	-	0.71
Cobalt	0.43	0.14	0.22	0.47
Molybdenum	1.79	0.51	4.82	-
Selenium	0.15	-	0.92	-
Magnesium	805.00	307.00	267.00	1970.00

Note: where no figure is shown this indicates an absence of sufficient data.
[a] The category of vegetables includes values for tubers, legumes and leafy material.
[b] The category of meats excludes fish and shellfish.

As various foodstuffs contain different elements in varying abundances, it is possible to try to associate certain quantities of trace elements with certain foods and to attempt to elucidate palaeodiet (Gilbert 1985). Elements such as zinc, copper, molybdenum and selenium are predominantly associated with animal protein, while elements such as strontium, magnesium, manganese, cobalt and nickel are essentially associated with vegetable sources (Martin et al. 1985). Hence, through the association of high quantities of trace elements with certain food sources and their presence in archaeological human bone, it may be possible to determine the prevailing character of the diet that was consumed.

At this stage it must be pointed out that the fact that an element is present in the diet, through whatever source, does not automatically mean that it will accumulate in human bone. Cobalt, which is a major constituent of vitamin B12, can be found predominantly in soft tissues of plants and animals, but it does not accumulate in sufficiently high quantities in bone to enable detection.

Another problem is the fact that there may be interaction between the various elements involved in diet, making it difficult to determine the absolute origins of the elements with regard to food. Therefore knowledge of the possible interactions of elements could enable educated estimates of elemental levels to be deduced. For example, zinc and copper are known antagonists (a substance that tends to nullify the action of another) so any entity that is capable of reducing the metabolic participation of zinc is qualified to increase resultant copper levels. This situation would be possible if the population were consuming grains, cereals or oats, all of which contain phytate (the main storage form of phosphorus in plant tissues), which is capable of complexing (binding) to important elements such as calcium, magnesium, iron and zinc, resulting in mineral deficiencies as these elements become unavailable to the body. Should a population introduce cereal into their diet while simultaneously maintaining their animal protein content (zinc component), copper levels would appear elevated owing to the inhibition of zinc by phytate complexation.

Only those elements which are thought to accumulate in bone tissue should be selected for multi-elemental analysis (Gilbert 1985). Lead bioaccumulates—it is difficult to excrete

and consequently the body retains it—and therefore its concentration will increase over time, provided that the metal constitutes some portion of the individual's intake (ingestion, respiration and/or direct contact). Other elements have a tendency to manifest themselves in bone up to some critical concentration. Once this exceeds the homeostatic level of the organism (the human body has an elaborate system for managing and regulating the amount of key trace metals), the element will be excreted in the urine, hair, perspiration and/or faeces. Although the exact bone capacity (the finite quantity of an element that is capable of being stored in bone) for certain elements has not been determined, it appears that, with the exception of those that fix firmly and are cumulative, a range of presence may be found in bone indicative of elemental consumption to the point of capacity.

Nuts and berries in a diet can considerably complicate matters as they contain extremely high quantities of trace elements (Gilbert 1985). Hence the presence of an unknown amount of nuts and berries in the diet could increase the amount of some elements present, creating ambiguity as regards dietary changes. If a population decided to terminate some food product while simultaneously increasing its consumption of nuts and berries, multi-elemental analysis would not be able to interpret this change in practice.

Diagenesis

Diagenesis describes the post-mortem alterations in the chemical constituents of bone, including both the loss of and increases to biogenic (necessary for the maintenance of life processes) concentrations (Sandford 1993). Naturally, bone has a very pale yellow or ivory colour. On decomposition of the protein component (mainly collagen) in a buried bone, the bone turns yellow. Although a form of diagenetic change, this does not by itself imply any change in the elemental composition of the inorganic fraction of bone. When a bone acquires the brown colour of its burial environment, however, chemical contamination by the soil is a distinct possibility. Furthermore, parameters that make contamination possible (e.g. the presence of moisture to transport soil minerals into the bone) can also result in other elements being removed from the bone by leaching. In conclusion, darker-coloured bones could differ markedly in composition from lighter-coloured bones.

Experimental methodology

The head and neck of the femur (thigh bone), comprising cortical bone that is expected to provide the greatest resistance to diagenetic alterations owing to its compact nature, were chosen as the standard sites from which to extract bone for investigation. Through consultation with Dr Andrew Macey, an orthopaedic surgeon at Sligo General Hospital, a standard method used in surgery for extracting bone samples was adapted for use in the present study. This method uses a manually operated 'coring' drill to collect the samples from the neck of the femur, terminating in the shaft of the femur (Illus. 1 & 2). Initially about a 1 cm depth of surface bone was removed from the coring area in order to avoid as far as possible any direct soil contamination of the analytical sample.

In order to determine whether the method of analysis was 'fit for purpose', a thorough method validation was carried out, including tests using a bone reference material. The

National Institute of Standards and Technology Standard Reference Material (SRM) 1486 (Animal Bone Meal) with certified elemental concentrations was chosen for this purpose.

Bone samples were dissolved with nitric acid and hydrogen peroxide using a microwave digestion system, which uses microwave energy to heat samples. A bone sample/SRM placed into a microwave digestion vessel with an acid is subjected to rapid heating and elevated pressures, causing the sample to digest or dissolve in a short time. The digested sample was then diluted to a specific volume with ultra-pure water, and further dilutions of this solution were made whenever necessary prior to analysis. Multi-element standard solutions were prepared from certified standards for each element. (A standard is a solution that contains known concentrations of an element; a multi-element standard is a solution that contains many elements at known concentrations; and certified standards are standards of a known purity that are used to prepare standards.)

Samples and standards were placed into the high-temperature plasma of an Inductively Coupled Plasma-Optical Emission Spectrometer (an instrument capable of determining the concentration of elements in a sample) and the emission data for each element were collected. Final results were calculated from linear best-fit calibrations generated from calibration standards. Standards with incrementally increasing concentrations would result in a corresponding proportionate increase in emission intensity. When this information is graphed, a linear relationship should be generated between concentration and emission (i.e. as concentration of the standard increases, so too should the emission intensity). A line of best fit ascertains the degree of correlation between measured instrumental variable (i.e. emission intensity) and standard concentration. This results in the generation of an equation for the line describing the relationship between emission intensity and concentration. The emission intensity generated for the sample is then substituted into the corresponding parameter in the equation and solved for the concentration of the sample.

Illus. 1—Analytical chemist Tasneem Bashir extracting bone samples at the Institute of Technology, Sligo (Deirdre McCarthy).

In this preliminary study only two representative disarticulated bone samples were analysed for a range of elements. The objective of the study was chiefly to validate the analytical methodology and to obtain an indication of the elemental concentrations in the Ballyhanna bone samples. Considering the limited dataset, it is not intended to comment on the significance of the results other than on the performance of the analytical methodology. A more detailed discussion of diet and other factors affecting the Ballyhanna population will be given when results for a greater number of samples are available.

The average results obtained for eight elements (calcium, magnesium, iron, strontium, zinc, lead, sodium and manganese) in the two Ballyhanna bone samples are presented in Table 2. This table also lists the certified content of the SRM and the result obtained for the analysis of the SRM. The final column includes, for comparison, the results obtained for these elements from modern-day human bone samples acquired by autopsy of a selected elderly Japanese population (Yoshinaga et al. 1995).

Table 2—Mean elemental concentrations for a Standard Reference Material (SRM), two samples from the Ballyhanna assemblage and modern-day samples obtained by autopsy (Yoshinaga et al. 1995).

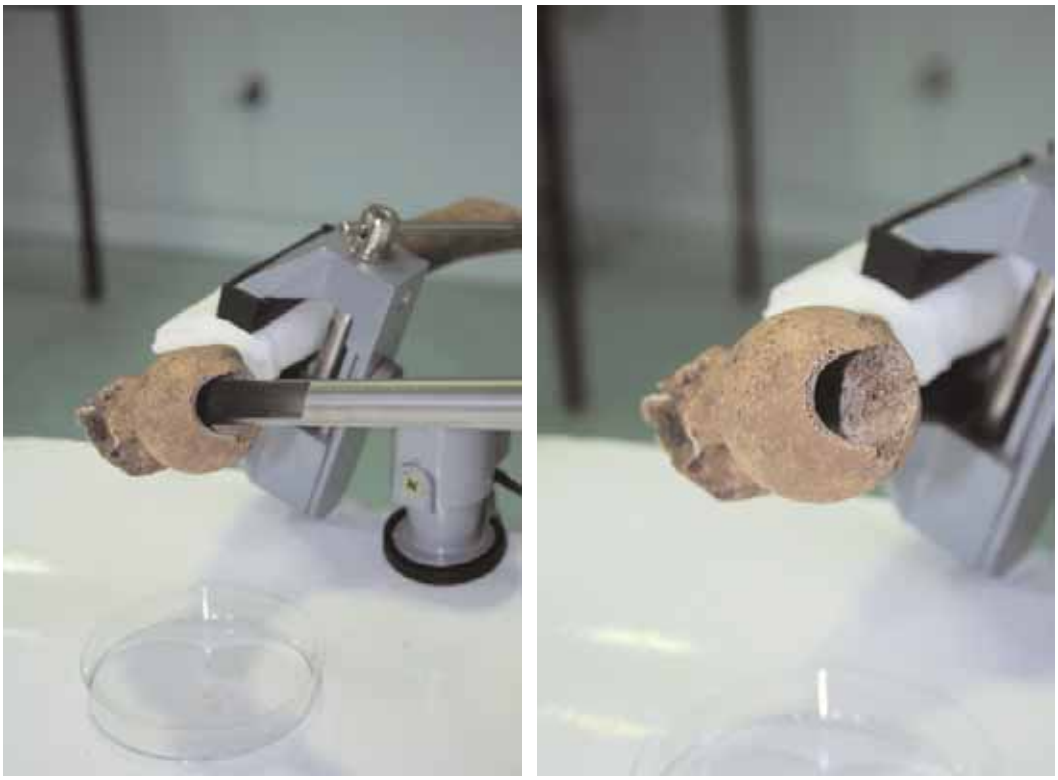
Element	*SRM spec µg/g*	*SRM µg/g±%RSD*	*Ballyhanna #7 µg/g±%RSD*	*Ballyhanna #14 µg/g±%RSD*	*Modern samples µg/g±SD*
Calcium*	26.58±0.24*	25.9±0.85	No result	26.52±1.10	24.6±6
Magnesium*	0.466±0.017*	0.456±0.519	0.109±1.0	0.102±0.54	0.286±0.20
Iron	99±8	104±2	2102±0.3	2630±0.5	71±64.2
Strontium	264±7	286±2	197±1	211±1	80.5±19.6
Zinc	147±16	163±1	144±1	155±4	149±19
Lead	1.335±0.014	1.036±12.48	2.972±10.52	2.521±12.45	6.85±3.41
•Sodium*	0.5*	0.4	0.6	0.6	0.518±0.46
•Manganese	1	1	433	400	<3.0

* Concentration weight percentage of the element.

•This symbol denotes non-certified concentrations of constituent elements, all other elements are certified.

Results

The results obtained for calcium in the disarticulated Ballyhanna bone samples are close to the levels normally expected for bone. The SRM result indicates that the analysis may be slightly underestimating the amount of calcium present. For magnesium, the SRM result indicates that the method is accurate and that both Ballyhanna sample results are consistent with each other. For iron, the SRM results are within the expected range; the results obtained for the Ballyhanna bone, however, are approximately 37 times the normal expected level. It is likely that these high levels of iron are a result of soil processes rather than biogenic factors. The SRM results obtained for strontium indicate that the results lie slightly outside the higher end of the range, which suggests that the analysis may be slightly



Illus. 2—Coring to collect a sample from the neck of a femur or thigh bone (Deirdre McCarthy).

overestimating the amount of strontium in the Ballyhanna bone samples. The sample results indicate that the strontium content is approximately three times greater than the contemporary bone levels. Again, such high levels could be attributed to soil processes. For zinc, the SRM results are within the expected range; the results for the Ballyhanna samples are consistent with each other and compare well with the reference levels determined by Yoshinaga et al. (1995). For lead, the SRM results are close to the expected range. The lead levels in the Ballyhanna samples are consistent but lower than those determined for modern-day bone. The sodium levels determined in the SRM are close to the expected values. The sodium levels in the Ballyhanna samples are consistent and compare closely with the modern-day samples. The manganese levels determined in the SRM are close to the expected values. As with the sample bone results for iron, it is likely that the high levels of manganese are a result of soil processes.

Conclusion

The results of this preliminary study indicate that the analytical methodology used in this study can provide the accuracy and precision required. Initial indications for the multi-element analysis of the Ballyhanna bone samples suggest that contamination from the burial environment will be a factor to consider in the final interpretation of the overall results. This is particularly obvious for iron and manganese. Results for the other six elements

presented here clearly indicate that the concentrations found are in the general range expected for bone. Further discussion on the significance of these results as indicators of past diet will follow when a more statistically significant number of sample results are available.

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