Science and the dead: destructive sampling of archaeological human remains for scientific analysis

Second edition

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Contents

1. Introduction Page 4

2. Overview of destructive sampling Page 5

3. Radiocarbon dating Page 11

4. Stable isotopes and ancient diets Page 14

5. Stable isotopes and geographic origins of people in the past Page 17

6. DNA Page 19

7. Proteomics Page 22

8. Microscopy Page 24

9. Case studies Page 27

10. Procedures and terms of access for human remains: the example of Barton-upon-Humber Page 31

11. Further reading Page 38

12. Where to get advice Page 38

13. Acknowledgements Page

Executive summary

Scientific analyses involving destruction of samples of bones or teeth from human remains are widely used in archaeology. Institutions responsible for curating archaeological human remains in the long or medium term, principally museums, university departments and commercial archaeological contractors, are increasingly receiving requests from researchers to sample remains in their care. Clegy and others responsible for historic churchyards and other burial grounds are also receiving an increasing number of requests from those wishing to exhume ancient burials for research purposes. The purpose of this document is to provide a framework to help organisations in responding to such requests.

This document represdents an update to the first edition of Science and the Dead, published in 2013. This edition is intended not only to reflect advances that have taken place in scientific techniques but also in other areas. The remit remains as before, skeletal remains more than 100 years old from burial sites in England.

Legal and ethical considerations pertaining to destructive sampling for the purposes of scientific research are set out. There then follow sections devoted to some of the more commonly applied techniques: radiocarbon dating, stable isotopic analyses to study ancient diets, isotopic analyses to study geographical origins of people, ancient DNA studies, proteomics (the study of proteins), and microscopy. In each of these sections, the science behind the technique is summarised, the sorts of information that it can yield are outlined and the bone or tooth samples that are likely to be needed are described. Some case studies are then given for illustrative purposes.

The main recommendations are as follows:

- In general, the benefit of generating new knowledge by the application of techniques that require destructive sampling needs to be weighed against the imperative of preservation of skeletal collections intact and, in the case of Church burial grounds, the presumption of the Church against disturbance of remains.

- When faced with a request for destructive sampling the following need to be assessed:

    - The likelihood of obtaining useful knowledge and the value of that knowledge

    - Whether that knowledge could be obtained by non-destructive analyses

    - The experience and competence of those who intend to undertake the work.

    - The effects of the destructive analyses on the future research potential of the remains.

- Expert casework advice should be sought, if needed, from APABE or other sources

- For burials of known identity, permission should be sought from surviving family members, if known

- If sampling is approved, it should be minimally destructive commensurate with the purposes of the research, and removal of any material should be properly documented.

- Decisions concerning destructive sampling should be made in the public interest and in an accountable manner.

1. **Introduction**

Scientific research on ancient human remains uses a mixture of non-destructive and destructive methods. The former include visual observation and measurements of bones and teeth, augmented by medical imaging techniques such as radiography. Destructive analyses entail the removal of small samples of bones or teeth, either for biomolecular analyses, of which radiocarbon, DNA and stable isotope analyses are perhaps the most familiar, or for the purposes of microscopic examination.

Archaeological science is a rapidly changing field. Since the production of the first edition of this guideline in 2013, several major innovations in laboratory techniques for the study of bone or tooth samples from human remains have taken place. Palaeoproteomics, the identification and study of protein residues, is emerging as an important research field. The advent of next generation sequencing has revolutionised the way in which we study ancient DNA. Sampling requirements for many techniques have altered. For example, many stable isotope studies now use samples from teeth rather than bones. The study of dental calculus (calcified dental plaque) is a young but rapidly growing field, and a variety of biomolecular and microscopic techniques have been applied.

These sorts of technical advances have increased the information that can potentially be obtained from destructive sampling, so the number and diversity of requests from researchers has increased. At the same time, there are also more options for mitigating the impact of destructive sampling on skeletal collections. For example, there are more sophisticated ways of making images of skeletal parts prior to sampling. Advances in information technology for data storage and sharing mean that there are increased options for making the primary data from destructive analyses more widely available.

The above considerations indicated a need for an updated edition of this guideline, and the desirability of this was supported by informal consultations within the sector. Whilst we have updated every section of this document, we have retained the general layout of the first edition. After a brief outline of some of the legal, ethical and scientific considerations of destructive sampling, there are sections devoted to the different techniques. These give a brief outline of the science, examples of what can be learnt and the nature of the samples that are customarily required. In a document such as this it would be impossible to cover every scientific technique that involves destructive sampling of human remains, and this is not the aim. Instead, we concentrate on those that are currently among the more frequently used.

As for the first edition, the target audience is museum staff and others responsible for curating or otherwise caring for ancient human remains. These latter include organisations ranging from commercial archaeological contractors to the Church. An archaeological contractor conducting fieldwork on an archaeological site will be responsible for the excavated remains until their transfer to a museum or other institution (or reburial) upon completion of the post-excavation (assessment, analysis) phases of the project. They will frequently commission or permit destructive analyses on human remains as part of the assessment or analysis phases, or as part of collaborative or standalone research projects on the remains. Clergy and others responsible for churchyards and other historic burial grounds may face requests for exhumation of specific burials (for example, those thought to be of known historical

figures) for research purposes. Usually, such requests involve research that entails destructive analysis.

Commercial archaeological contractors may have suitably trained staff, either osteologists employed on a long-term basis, or else on short-term contracts associated with particular projects, available to advise on destructive sampling of human remains. This guideline may assist them in giving their advice. Some larger museums and other organisations may also have suitably trained staff to advise on casework in this way, and to formulate general policy, but for many this is not the case. This guidance aims to provide non-specialists with responsibility for human remains with relevant scientific and other information to aid them in their deliberations when they are faced with requests for destructive sampling from remains in their care. It also provides a framework to assist institutions to develop their own policies in this area. In addition, the reader is reminded that the Advisory Panel on the Archaeology of Human Burials in England (APABE) is available to provide specific casework advice on all areas regarding archaeological human remains. This encompasses destructive sampling, including that for the purposes of applying techniques not covered in this guideline (https://www.archaeologyuk.org/apabe/).

In keeping with APABE's remit, the scope of this document is restricted to remains over 100 years old (herein termed archaeological) from burial sites in England (different constraints apply to remains less than 100 years old, which are subject to the Human Tissue Act https://www.hta.gov.uk/policies/human-tissue-act-2004). The focus is on skeletal remains, as these are normally the only parts preserved in archaeological burials in England.

This document should be read in conjunction with the UK Governmental 'Guidance for the Care of Human Remains in Museums' http://www.culture.gov.uk/NR/rdonlyres/0017476B-3B86-46F3-BAB3-11E5A5F7F0A1/0/GuidanceHumanRemains11Oct.pdf , and for Christian burials, with 'Guidance for Best Practice for Treatment of Human Remains Excavated From Christian Burial Grounds in England' (2nd edition) (https://www.archaeologyuk.org/apabe/)

2. Overview of destructive sampling

2.1 Legal framework

In England, it is unlawful to disturb buried human remains without lawful authority. Secular burial law is generally aimed at regulating the way in which human remains or grave markers are cleared from burial grounds. Permission to excavate archaeological burials is administered via the Ministry of Justice. The secular legal system recognises the public benefit of scientific work on human remains. Destructive sampling of collections of human remains excavated from archaeological sites and curated in museums or other institutions is not normally subject to legal constraint. It is generally the curating institution which grants (or withholds) permission for destructive sampling of remains in its care. However, in cases where permission for exhumation is sought from the Ministry of Justice for the specific purpose of scientific research involving destructive sampling, the Ministry will evaluate carefully the proposals for destructive sampling when the application for the exhumation licence is considered.

In burial grounds under Church of England jurisdiction (mostly churchyards), ecclesiastical law applies rather than secular statutes. Human remains cannot be disturbed without ecclesiastical permission, usually issued in the form of a Faculty, or for cathedrals by the Cathedrals Fabric Commission. Ecclesiastical law is protective. It draws upon the principle that remains entrusted to the Church should normally lie undisturbed. This does not, however, mean that human remains should never be disturbed. Ecclesiastical law recognises that the living, including church congregations, have rights which may come into conflict with this principle. The Church also recognises that human remains, and the archaeological evidence for the rites that accompanied their burial, are important sources of scientific information and that this information is of legitimate academic and public interest. Analysis of human remains, including destructive analyses, is therefore potentially acceptable provided that the research aims are adequately justified. Under the Church system, as well as authorising exhumation of burials, the Consistory Court or Cathedrals Fabric Commission also regulates their treatment once exhumed, and therefore has the authority to grant or withhold permission for destructive sampling. Proposals to remove and / or destroy parts of skeletons are subject to rigorous scrutiny. This is particularly so when the personal identity of the individual is known and sensitivities are consequently heightened. There may be a requirement to destroy or return any material taken but not used for the sampling.

2.2 Ethical considerations

Several ethical considerations need to be borne in mind when considering applications for destructive sampling. Some of these fall under the general rubric of knowledge-based ethics. Analysis of human remains offers important insights into the human past, and provides benefits of other kinds, for example contributing to the development of forensic science. Most people would consider that the accrual of knowledge is a significant benefit for humanity. A museum or other institution holding archaeological remains for research purposes may be considered to have stewardship of that material. That is, they hold it in trust for the benefit of the wider community, and for the benefit of future generations. There is, therefore, a moral imperative toward the preservation of collections in ways which safeguard the information they contain. When it comes to destructive sampling there is a tension between the imperative to generate new knowledge and the imperative toward preserving collections intact. This dilemma lies at the heart of decisions concerning destructive sampling.

This description pre-supposes that remains are curated in long-term collections. It is with such collections, rather than with those that are slated for reburial, that this guidance is primarily concerned. It is APABE's position that collections of excavated human remains with long-term research potential be retained rather than reburied. Retention can be in a museum or similar institution or, for remains from Christian burial grounds, in church buildings (a Church Archive of Human Remains, or CAHR). Reburial of remains effectively means permanent loss of the information they contain, so that the ethical tension referred to in the previous paragraph will not apply. Nevertheless, competing demands of different researchers who may wish to sample the remains prior to reburial may still need to be managed.

In the case of requests to clergy for exhumations of skeletal remains from churchyards for research purposes, consideration will also need to be given to the Church's presumption against disturbance of remains. Particular care needs to be taken when there is a focus on remains of a specific known historic personage or other identified individuals. Ethical considerations in such cases are complex, but key questions include whether the project is in the wider public interest and whether it has the support of living descendants.

2.3 Scientific considerations

2.3.1 What is research?

Research is not aimless data-gathering but should always be directed at answering clear and specific questions. Research may be applied or methodological. Applied research involves using remains to find out about the past. In applied research, questions to be addressed may concern the ancient population represented by the burials in question, or they may be about the general time period or region that the population comes from, especially if the skeletal collection is one of several that are being used for a research project. In methodological research, remains are studied with the aim of testing or improving existing techniques of gaining information from skeletons (e.g. ways of assessing sex or age of the person, or to study their diet) or developing new ones.

Sometimes research focuses on a single or some few skeletons, for example to study some unusual disease, to build up an 'osteobiography' of a person's life from their bones, or to identify or establish links with remains of a particular historical personage. As was mentioned in Section 2.2, in the case of this last, it is important that the research questions are of wider interest – for example establishing family connections with some named figure using analysis of an exhumed burial may be of great personal interest for the individual researcher(s) involved but be of little wider significance.

However, nowadays, most scientific research is quantitative. It involves identifying statistical patterning in data, so usually large numbers of skeletons (>100) are needed. This means that researchers often look for large collections, or they may combine skeletons from a number of different sites.

Results of destructive analyses rarely make sense in isolation. Usually they require morphological data, recorded using non-destructive osteological techniques, to enable their interpretation. Therefore, research projects normally require access to collections to record some osteological aspects of the skeletons in addition to taking samples. Increasingly, projects are also combining different destructive techniques. This not only maximises the ability to address research questions but, because the same samples can sometimes be used for more than one analysis, it often helps to optimise information gain and minimise destruction.

Traditionally, the audience for scientific research in archaeology has been an academic one. However, researchers are increasingly aware of a need to disseminate results to a wider audience. This is particularly pertinent for work involving human remains, as this aspect of archaeology holds special interest for the public. For applied research, it is legitimate to ask researchers what their plans are for disseminating the results of their work to the wider public (particularly local

community engagement) and to take this into account when considering applications. In addition, providing information to use as a basis for community engagement projects may itself be a legitimate reason to conduct destructive analyses, although this work should still be purposive and question-led.

2.3.2 General considerations regarding destructive sampling

As a generality, two trends have been apparent over recent years.

- Samples required for biomolecular analyses have become smaller. This means that, in general, application of these techniques is less damaging to collections than was previously the case. However, the situation is a little more complex than this. For example, a small sample removed from a tooth may result in greater information loss to future researchers than a larger bone sample taken as a rib fragment or removed from a long-bone: teeth are smaller and are particularly rich in biological information.

- Microscopy of a cut section allows visualisation down to a level that is currently beyond that adequately captured by even the most sophisticated non-destructive methods. However, rapid advances in micro-CT and other imaging techniques have meant that studies of some microstructural features that once required sections to be physically removed from bones or teeth can now be accomplished non-destructively (although some advanced imaging techniques are currently limited in their availability to researchers).

There is every reason to believe that these two trends will continue in future. This does not mean that requests for sampling should be routinely refused, but it does emphasise the need to assess them rigorously.

The extent and nature of existing skeletal collections mean that extant archives of skeletal material, and our knowledge of earlier skeletal biology, vary temporally and geographically. Remains from earlier prehistoric times (Palaeolithic, Mesolithic) are rare in England, but curated collections become progressively more plentiful for more recent periods. Patterns of skeletal survival, intensity of archaeological investigation and other factors mean that regional disparities exist in availability of remains from different periods and from different types of burial grounds. Such factors need to be taken account of when evaluating requests for destructive sampling, with rare remains generally demanding a more cautious approach.

The nature of the research, the types of samples requested, and the measures to be taken to mitigate the impact of the destructive sampling need to be considered.

Research programme

- Any programme of destructive analysis should be carried out within a coherent research programme and should stand a realistic chance of advancing knowledge.

- The questions to be addressed by the work should be of general archaeological, historical or methodological significance, and clear hypotheses should be tested.

- Destructive analyses should only be considered if the research questions cannot be addressed adequately using non-destructive techniques.

- The researchers must be sufficiently competent and experienced to conduct the work proposed.

- Adequate funding for the work should be in place. If permission is sought in advance of funding being obtained then permission, if granted, should be time-limited rather than open-ended.

Sampling programme

- If the feasibility of a technique is questionable, then thought should be given to conducting a pilot study on a small number of samples, with permission to proceed further being contingent upon the results.

- Sampling should be kept to a minimum compatible with fulfilling the aims of the project. Oversampling, leading to stockpiling of material by researchers, is unacceptable.

- The number, location on the skeleton, the size of samples, and the methods by which the researchers intend to remove them, should be made explicit.

- The likely effect of sampling on future research potential of the remains is a key issue. To this end, the location in the skeleton from which a sample is to be taken should be carefully considered:-

    - Sampling from anatomical landmarks (points from which measurements are taken) or from areas important for sex or age determination should be avoided.

    - Unless the study specifically requires it, sampling from diseased bone should be avoided.

    - If a tooth is to be sampled, then its antimere (the corresponding tooth from the opposite side of the jaw) should preferably be present.

    - Samples should preferably be taken from bones or teeth that are already incomplete, damaged or fragmentary.

    - In the past, chemical consolidants may have been applied to archaeological bone to try and strengthen it. This may interfere with some scientific analyses, so sampling such areas is best avoided.

    - If appropriate, thought should be given to the visual impact of sampling – for example on the suitability of the specimen for future museum display.

- All sampling should be fully documented.

- Any un-used bone or tooth fragments, as well as slides and embedded microscopy samples should be returned to the collection.

- Any un-used bone or tooth powder, or liquid extracts from samples sometimes remain with the laboratory that conducted the analyses, but should be returned to the organisation holding the collection if requested. Use of these residues by researchers for purposes other than those that were part of the agreed project would require express permission.

Mitigation programme

- The bone, tooth or dental calculus deposit that is to be sampled, and the skeleton from which it came, should have been recorded by an osteologist to an appropriate level prior to sampling.

- Unless exhumation is for the specific purpose of research involving destructive analysis, sampling should not normally be permitted on-site during excavation.

- It is usual to take photographs before and after sample. In addition, it may be appropriate to produce a surface cast of the parts to be destroyed or to conduct a surface or micro-CT scan or both. The aim in these cases is to produce a physical or virtual model of the part that future researchers may use for morphological study. It is becoming increasingly common to ask researchers to mitigate the impact of their work in this way. In practice, this is more usually done for teeth, especially when crowns are sampled, but it is also appropriate in some instances for bones (for example, if they are diseased). Care needs to be taken that resolution of scans and quality of casts are sufficient to be useful.

- Publications arising from the scientific analyses should be lodged with the organisation which granted access to the remains.

- Data resulting from studies should be published fully if appropriate. A copy of the raw data and recording protocols should be archived with the organisation holding the remains. This will aid collections management, and help prioritisation of future research applications that they may receive. In instances where the raw data do not form part of the academic publication of the work, or have not otherwise been disseminated, the holding institution should normally be able to manage and control release of the data to other researchers as appropriate, perhaps following an embargo period agreed with the laboratory that generated it.

- DNA and proteomic analyses produce very large amounts of data. This is best disseminated using online data-sharing platforms. These are also becoming available as ways of disseminating other sorts of data. Holding organisations may opt to retain and manage access to these data.

- The implications of sharing DNA or other data from identified individuals require careful consideration.

General

- For work involving the exhumation of burials of identified individuals, in addition to legal authorisations, permission should be sought from surviving family members, if known.

- Casework advice should be sought from APABE or other sources as necessary to aid decision-making.

- Decisions concerning permissions for destructive sampling should be made in the public interest. Those making decisions should be willing to be held accountable for their judgements.

3. Radiocarbon dating

3.1 The science

Isotopes are atoms of a chemical element with different masses. Some are radioactive and steadily decay, transmuting into other elements. Others are stable - they are non-radioactive and do not change in abundance over time. Carbon has three naturally occurring isotopes: ^{12}C, ^{13}C, and ^{14}C. These three isotopes do not occur equally, with carbon in the atmosphere and biosphere consisting of 99% ^{12}C, 1% ^{13}C and about one part in a million million of ^{14}C. ^{14}C is different from the other two isotopes in that it is radioactive with a half-life of 5730±40 years. From this it derives its name radiocarbon.

Radiocarbon is formed in the upper atmosphere by the interaction of neutrons, produced by cosmic rays, with nitrogen atoms. Once radiocarbon has been produced it rapidly forms carbon dioxide and mixes through the atmosphere, dissolves in the ocean, and enters the terrestrial food chain through photosynthesis. Consequently, the ^{14}C content of a living terrestrial organism is in equilibrium with that of the contemporary atmosphere.

When a plant, human, or animal dies it no longer takes in ^{14}C and thus over time the proportion of radiocarbon falls at a rate that is determined by the law of radioactive decay. By measuring the proportion of ^{14}C that remains, it is possible to estimate the time since the organism died.

Unfortunately, as the production of radiocarbon in the atmosphere is not constant, a year in the radiocarbon age timescale does not have an equivalent interval in the calendar timescale and for this reason calibration is required. Progress in the extent and resolution of the data available for calibration means the current internationally agreed calibration curve extends to 55,000 years before present. This provides a common standard and means that all calibrated dates are comparable.

Radiocarbon is present in such low abundance it puts a statistical limit on the precision of a radiocarbon determination. A fundamental aim during measurement is

therefore to measure the isotope ratio as accurately and precisely as possible. The two main methods of measuring ^{14}C are decay counting methods (using liquid scintillation and gas proportional counters) and accelerator mass spectrometry (AMS) where the radiocarbon atoms are directly detected. Since the mid-1980s the introduction of accelerators for the direct detection of radiocarbon has allowed a whole range of much smaller samples to be measured.

Un-burnt human bone is one of the most complex materials commonly used in radiocarbon dating. Following the death of an individual degradation of a bone's molecular structure and the incorporation of exogenous molecules as a result of chemical and environmental processes can influence subsequent radiocarbon measurements. Research into effective pretreatment methods to minimise the problems of contamination continues with the aim of reducing the contaminants present in the sample from the environment and to minimise the addition of further contaminants. With human bone and dentine attempts to improve on the widely used simple extraction of protein ('collagen') have included molecular-size reduction using ultrafiltration, and the selection of individual amino acids.

Bones that have undergone burning at high temperatures (ie cremation) no longer contain organic carbon and so until relatively recently have not been suitable for radiocarbon dating. In the last twenty years the successful dating of the inorganic bone matrix (re-crystallised bio-apatite) content of cremated bone has therefore meant it is now possible to date this common burial practice for periods when it was the dominant funerary rite.

Humans have a markedly variable and mixed diet and as such frequently derive carbon from more than one reservoir. The measurement of carbon and nitrogen stable isotope ratios (see Section 4) can be used to determine the potential for diet-induced radiocarbon offsets if an individual has taken up carbon from a reservoir not in equilibrium with the terrestrial biosphere, for example – marine or carbonate-rich freshwater resources. For technical reasons, this issue affects radiocarbon measurements on unburnt bone, but not those on cremated bone. In practice, dietary effects have not been found to be significant for interpreting radiocarbon dates on human bone from England before the Viking period.

Calcined bone may exhibit an age-at-death offset derived from the incorporation of carbon from the pyre fuel during the cremation process (if for example the wood was from long lived species, eg oak). The scale of such offsets is currently uncertain, as is their prevalence in the past.

Age-at-death offsets may also exist in un-burnt human bone. The offset arises from the time it takes carbon from the diet to be incorporated into bone collagen. As individuals become older than this, the average difference between the time when bone collagen was laid down and the date of death goes up, particularly in men. Given life-expectancy in the past, bone turnover offsets are unlikely to be of practical relevance except for the most high-precision applications.

3.2 What can we learn from radiocarbon dating?

Chronology provides a fundamental structure for understanding the past, with timing unravelling the sequence of past events and the tempo of change. Increasingly

refined chronological frameworks from burial grounds, eg Barton-on-Humber are enhancing understanding and appreciation of the value of such assemblages, particularly when combined with other investigations such as stable isotope analysis.

The ability to chronologically divide the human population for cemeteries such as St Mary Spital, London (10,516 bodies) using radiocarbon dating and archaeological phasing means that it is possible to track developments in demographic change and variations in health of the population that lived and died in this part of Mediaeval; London. The radiocarbon dating programme for this site also identified a case of pre-Columbian syphilis and mass burial pits predating the Black Death of AD 1348.

Radiocarbon dating of a selection of the 50 or more bodies once present in Aveline's Hole, Burrington Combe, Somerset confirmed the site as one of the largest early Mesolithic burial sites in Europe. The results suggest use of the cave for burial over, at the most, a century or two, in the mid to late ninth millennium cal BC.

Exploiting information about the relative age of formation and eruption of human teeth radiocarbon dates on slices of dentine from the first and third molars of individual burials from Palace Green Library, Durham were 'wiggle-matched'. Wiggle-matching involves matching the shape of a series of radiocarbon dates separated by a known number of calendar years against the calibration curve. The chronological evidence suggests the burials are those of imprisoned Scottish soldiers from the Battle of Dunbar in AD 1650.

Dating of single bones such as the human maxilla from Kent's Cavern, Torquay, one of the most important Palaeolithic sites in the country, sheds light on the origins of the earliest anatomically modern humans in Europe.

When considering individual radiocarbon dates, it must be remembered that the bandwidth of calibrated radiocarbon dates is not only a function of the errors quoted on radiocarbon determinations and on the calibration data, but also on the shape of the calibration curve. Thus, for some time periods the bandwidth is relatively large, for example c 750–400 cal BC for a person who actually died in 500 BC, ie where the actual ages falls on a 'plateau'. It can also be relatively precise: cal AD 1400–1470 for a person who died in AD 1425, where it falls on a 'steep' section of the calibration curve.

In the last twenty years the use of a Bayesian approach has proved to be the most effective method available for producing estimates of chronology. In archaeological terms this means that we analyse new data we have collected about a problem (the 'standardised likelihoods' — radiocarbon dates) in the context of our existing experience and knowledge about that problem (our 'prior beliefs' —for example the stratigraphic relationship between graves). This allows us to arrive at a new understanding of chronology which incorporates both our existing understanding of the problem and our new data ('posterior belief').

3.3 Sampling for radiocarbon dating

The most common human remains submitted for dating are unburnt bones from which typically 1g of bone is needed for AMS dating and c 200g for liquid scintillation and gas proportional counters. Samples from the larger dense bones of the body

(femur, tibia, upper arm bone, or jaw) are preferred as these typically have better collagen preservation and less sedimentary contamination than more porous bone.

Sampling of complete unburnt bones and teeth for AMS dating is usually undertaken with a mechanical drilling kit and special care should be taken to avoid any areas that may have been consolidated or treated with chemical preservatives.

The preservation of un-burnt bone can be greatly influenced by the burial environment resulting in chemical and physical degradation. Over 90% of the collagen content can be lost in some environments, which restricts the potential for radiocarbon and stable isotope analysis. A rapid technique, determining the %N content of whole bone, that requires very little material (<5mg bone), has been shown to be very successful in predicting whether a bone is suitable for dating. This pre-screening method reduces the amount of destructive sampling, in addition to saving time and money spent on un-successful dating.

For teeth, the preferred samples are incisors, canines, and molars, and attempts should be made where possible to leave enamel in good condition for other researchers (eg strontium and oxygen isotopes) when sampling the dentine.

For cremated bone, a 2g sample that needs to be fully calcined (ie completely white or grey) not just burnt is required.

In exceptional circumstances other material suitable for dating can also be preserved, eg hair, skin, and soft body tissue.

For large human bone assemblages, the use of Bayesian simulation models to identify the minimum number of samples needed to provide meaningful answers has proved especially valuable.

4. Stable isotopes and ancient diets

4.1. The science

Most chemical elements exist as mixtures of two or more stable isotopes. For some elements, the stable isotope ratios differ in different classes of foods, and these differences are passed on to the tissues of the consumer. Hence measurement of stable isotope ratios in skeletal remains can be used to study ancient diets. The most widely used elements in this respect are carbon and nitrogen.

Carbon stable isotope ratios differ in plants using different photosynthetic pathways to manufacture carbohydrates from atmospheric carbon dioxide. Most temperate zone vegetation uses the so-called C3 pathway. Some plants that are native to warmer regions, such as maize, use the C4 pathway. In addition, both carbon and nitrogen stable isotope ratios differ in marine and terrestrial foods. In north-west Europe there are no indigenous C4 foods, so most stable isotope work has concentrated on studying marine contributions to diets. For nitrogen isotopes, there is a small trophic level effect, so ratios change as one ascends a food chain. In principle, this means that it is possible to say something about the relative importance of meat versus plant foods, but limitations in our knowledge of other sources of variability in nitrogen isotope ratios in bone mean that this is often difficult

in practice. Because fully breastfed infants are exclusively consuming a product of the mother's body, they are one trophic level higher. Nitrogen isotope ratios have been used to study the duration of breastfeeding in past societies.

Dietary stable isotope studies normally focus on collagen from bone or tooth dentine. In living people, collagen in bone is continually renewed. During infancy, this process is rapid, but by adulthood it slows down so that analysing adult bone collagen gives a measure of diet averaged over years or decades. Collagen in dentine is not renewed, so this gives indications of diet whilst the dentine was forming as the tooth developed during childhood. All the nitrogen in collagen, and most of the carbon, comes from dietary protein, so results tell us mainly about the protein part of the diet. Carbon stable isotopes can also be analysed in tooth enamel (the mineral contains a little carbon in the form of carbonate). Like dentine, enamel is not renewed once formed, so this too gives a child-diet signal, but unlike collagen results appear to reflect whole diet rather than being biased toward protein.

Although the great majority of dietary isotopic analyses involve carbon and nitrogen, some other elements are also sometimes used. Of these, sulphur is the most important. Sulphur stable isotope ratios differ in marine and terrestrial environments and, depending upon local geology, may be different in terrestrial versus freshwater foods. Sulphur stable isotope ratios in collagen may help identify consumption of foods from marine and coastal, and in some instances, freshwater ecosystems.

Researchers often find it useful to have local isotopic values from archaeological faunal remains (and plant remains if possible), to provide a baseline to help interpret the human data. These can either obtained from the literature or from conducting archaeofaunal and archaeobotanical isotopic analyses as part of the project.

Usually, bone and tooth samples from burials on English archaeological sites contain sufficient intact collagen for successful stable isotope determinations, so stable isotope work normally produces usable results. However, in cases where collagen survival is uncertain, measurement of nitrogen content, as described in Section 3.3, can be used as a pre-screening technique. Dietary information cannot be obtained from isotopic analysis of cremated bone.

4.2 What can we learn about diet from stable isotope analysis?

To study diet, carbon and nitrogen stable isotopes are usually used together. Most work attempts to address questions of broad archaeological or historical interest, so sampling involves multiple skeletons rather than single burials. Currently, most studies use anything from about 30 to more than 100 skeletons, often from several archaeological sites, depending upon the questions to be investigated.

Many studies have looked at the way in which diet changed with the advent of farming in the Neolithic period. In Britain, results show that prior to the Neolithic, coastal groups relied heavily on seafood, but these resources were largely abandoned with the introduction of farming. In most parts of Britain, this change in diet seems to have occurred abruptly, but in some other parts of Europe it was a much more gradual process with marine foods continuing to be exploited in significant quantities well into the Neolithic.

In Romano-British times, isotope data suggest that consumption of marine foods was greater than in immediately preceding or succeeding periods. Trading networks associated with the Roman Empire meant that these foods were available inland as well as on the coast, although in some locations at least, they were more available to the wealthy than to the poor.

In the Mediaeval period, at a British rural site, nitrogen isotope data suggested that breastfeeding was continued until children were about 18 months old. This prolonged period of breastfeeding seems to have had beneficial results: infant mortality in that community appeared low by premodern standards.

At a burial ground associated with the Great Irish Famine of the mid-19th century, sampling of teeth indicated a rise in carbon stable isotope ratios in some child burials just prior to death. Because of the close dating of burials associated with that particular event, this could be linked to the documented use of food made from maize flour, imported from North America, as an emergency famine-relief measure.

4.3 Sampling for carbon and nitrogen stable isotope work

Typically, a bone sample of less than a gram is taken for carbon and nitrogen stable isotope determinations. For adults this is normally taken from a long-bone shaft. Collagen in these locations is renewed more slowly than in other bones such as ribs, and so provides a good indication of long-term diet. When bone samples from infants and children are used to assess age of weaning, typically bones with more rapid turnover (those that are rich in spongy bone, e.g. ribs) are sampled so that the delay with which the weaning signal is manifest is minimised.

Archaeological bones are often fragmentary. If an appropriately sized bone fragment is present then this is normally taken for analysis. Otherwise a small saw is used to cut a piece of bone of suitable size. Care is taken not to cut completely through an intact or minimally damaged bone and to minimise visual impact beyond the area sampled. An alternative is to clean the bone surface and then use cutting burr to generate a sample of bone powder.

As mentioned above (section 3.1), carbon and nitrogen stable isotope determinations are routinely conducted on bone samples submitted for radiocarbon dating. This is because it is important to detect individuals who consumed significant amounts of marine foods as incorporation of marine carbon into skeletal collagen tends to make radiocarbon dates too old, and a correction is needed for this. When both radiocarbon dating and dietary studies are envisaged, with careful planning it may be possible to minimise destruction of material.

Sampling of teeth from adult skeletons can be used to study diet when that person was a child. Recent technical developments mean that many samples, of tissue which developed at different ages, may be obtained from a single tooth. For example, a first molar may yield about 10-15 dentine subsamples, each of which corresponds to about 9 months of development, and together they span the period from about birth to ten years of age. Unlike sampling bone of infants and children who died at different ages, it enables details such as duration of breastfeeding and other dietary transitions in children to be reconstructed for individuals who survived to become adults rather than those who died in childhood. Microsampling dentine is

increasingly used as a way of studying breastfeeding and diet in infancy and childhood. Conducting this sort of analysis normally involves cutting the tooth in half vertically, and sampling one half.

5. Stable isotopes and geographical origins of people in the past

5.1. The science

The two most important elements that have been used in isotopic studies to trace geographic origins are strontium and oxygen. Strontium isotope ratios vary in different types of rock. There are therefore systematic differences in plants and animals in areas with different geology, and these are passed on to the tissues of consumers. Oxygen isotopes vary in rainwater in different regions according to factors which include climate, altitude and distance from the coast. Oxygen isotope ratios vary in different living organisms, and hence in different foods, but this does not matter very much for human studies as the isotopic composition of drinking water is the prime determinant of the oxygen isotopic composition of human tissues (an exception is suckling infants - during breastfeeding their oxygen isotope ratios are altered).

Unlike most carbon and nitrogen stable isotope work, strontium and oxygen isotope analyses use the mineral part of skeletal tissues and not collagen. The mineral part of bone and dentine is vulnerable to changes in composition during burial, but dental enamel appears highly resistant (as is cremated bone or dentine, due to structural changes undergone on burning). Therefore, most strontium and oxygen work on unburnt human remains uses dental enamel. Because dental tissues are not continually renewed, the isotopic composition of dental enamel reflects that in the locale in which the person lived as a child when the enamel was forming. An approximate local baseline for oxygen or strontium values in the location in which the individual was buried (and by implication lived immediately prior to death) can be established from geological or rainfall maps. More precise values can be obtained for strontium from local plant or water values or from archaeofaunal remains; for oxygen isotopes, modern local surface or well waters can be sampled. If the isotopic composition of dental enamel differs from baseline values, then the person likely spent at least part of their childhood elsewhere.

Oxygen isotope ratios in waters in Britain overlap with those in other locations, for example in continental Europe, particularly north-western areas and parts of the Mediterranean basin. Strontium isotopes will be similar in regions of similar geology regardless of geographic separation. Oxygen isotopes are generally most useful for distinguishing among individuals on a fairly large spatial scale; strontium isotopes may, depending on geology, enable smaller spatial distinctions to be made. In practice, most workers use both strontium and oxygen isotopes in combination to narrow down the number of possible locations where a person may have spent their childhood. An exception is for cremated bone where only strontium isotopes are studied; alterations in oxygen isotope ratios during burning mean that they cannot be used for this purpose.

Some other elements in dental enamel are also sometimes studied. By far the most important is lead. In pre-industrial populations, lead isotope ratios tend to reflect local geology. Lead levels in the natural environment are generally low, so in societies with lead metallurgical technologies, most intake is from contamination from lead

artifacts or industrial pollution, so isotope ratios reflect the lead ores used. In either case, lead isotopes in dental enamel can be used to help identify migrants, and lead concentrations can be used to quantify lead burden, which can be high, especially in post-Mediaeval industrialised populations.

5.2. What can we learn about mobility from stable isotope studies?
A study of bone from some of the cremation burials from Stonehenge, dating from about the time of the monument's initial construction, showed that about 60% had strontium isotope ratios consistent with local geology. The remainder had signals indicating origins further afield. One of the places that would have been consistent with the data was west Wales, the likely place of origin of the famous bluestones used in an early phase of construction of the monument. These non-locals also had lower carbon isotope ratios in their bones. In cremations, the carbon isotope ratio does not reflect diet but derives mainly from the wood used for the pyre. The lower ratios mean that the wood used in these cases came from a more heavily forested environment than that around Stonehenge. At least some of these non-locals may have died and been cremated elsewhere and their burnt remains brought to Stonehenge for burial.

Strontium and oxygen studies at the Roman fort at Catterick, North Yorkshire, indicated that burials dating from the 2nd-3rd centuries AD showed greater isotopic diversity than burials from the 4th century. This seemed consistent with the idea that, in the Roman army, an early policy of more diverse recruitment was later supplanted by more extensive recruitment from the local population.

Strontium, oxygen and lead isotopic analyses show that Roman towns contained migrants from continental Europe and further afield. That is perhaps of little surprise considering the far-flung nature of the Roman Empire. However, isotope data show that the smaller scale polities of post-Roman times still had far-reaching connections. Dental enamel strontium and oxygen isotopes from a cemetery at the 7th-9th century AD Royal centre at Bamburgh, Northumberland, revealed that non-locals were in the majority. Many were from western Scotland and Ireland, regions with links to Northumberland since Early Christian times. Others came from Scandinavia and nearby regions, attesting to the importance of trading, cultural and other links across the North Sea. Southern migrants came from as far as the Mediterranean. In Early Mediaeval times, the seat of the Kings of Northumbria was a cosmopolitan, multinational trading and political hub.

At a mass grave in Dorset, dating to about AD1000, the occupants, who had been executed by decapitation, showed origins outside Britain. The isotopic evidence was consistent with Scandinavia and nearby areas of Europe associated with Viking expansion. It is likely that this was a site of mass execution of Viking raiders captured by the English.

5.3 Sampling dental enamel for isotopic analysis
A vertical section of enamel, normally about 1mm thick, is removed using a rotary cutting disc. This causes noticeable damage to the tooth crown but, with care, leaves most of it intact. Because different teeth form at different times during childhood, sampling more than one tooth per individual allows patterns of mobility over longer periods of childhood to be constructed. For example, the enamel crowns of the three

permanent molars mineralise at between approximately the first few months of life and three years, 3 – 8 years, and 9-13 years. Provided they have not been worn down by use, analysing enamel from all three allows coverage of most of infancy and childhood.

The enamel may be analysed as a bulk sample, but more detailed migration timelines can be built up by subsampling parts of the vertical section that formed at different times during the tooth's development. This can be accomplished by micromilling, a technique that allows very small samples to be removed. For strontium, an alternative is laser ablation in which a laser is used to remove multiple microsamples from the enamel section.

6. DNA.

6.1 The Science

DNA contains an organism's genetic information. It is encoded in the sequence of chemical bases which form part of the repeating subunits (nucleotides) which make up the molecule. In human cells, DNA is located in the chromosomes of the nucleus and also outside the nucleus in the mitochondria. The chromosomes consist of the X and Y sex chromosomes, plus the autosomes. Nuclear DNA comes from both parents; mitochondrial DNA comes solely from the mother. In the skeleton, DNA is present in bone, and in the dentine and cementum of the teeth. It is not present in dental enamel. Studies of ancient DNA (aDNA) in archaeological human remains have concentrated on human DNA and on DNA from pathogens from infections that were present at time of death.

DNA molecules decay rapidly in the soil, undergoing progressive fragmentation and other damage. However, since the 1980s, techniques have been available to analyse aDNA. Traditionally, this involved single-locus PCR, in which a specific part of a DNA molecule was targeted, amplified and the sequence of bases determined. This process has now been almost entirely superseded by next generation sequencing (NGS), also known as high throughput sequencing. Rather than targeting a specific sequence, NGS permits all the DNA in a sample to be sequenced (although most workers chemically enrich the sample in the DNA of interest because most DNA in ancient samples is just contamination from the soil). This produces vast amounts of sequence data so bioinformatic techniques (computer algorithms) are used to analyse it. This allows much more of the genome to be studied. It permits sequencing of DNA fragments too small to be accessed by single locus PCR, important given the degraded nature of aDNA. It also enables new techniques for the authentication of ancient sequences to be applied, another key advantage given the issues of contamination with modern DNA that bedevilled the early days of aDNA work.

Because DNA deteriorates rapidly in the soil, analyses of human remains sometimes fail to provide useful data. Nevertheless, the advent of NGS means that this happens less often than previously. Although single-locus PCR is still occasionally used (e.g. for some studies of pathogen DNA), the advantages of NGS make the balance between destruction of material and the likelihood of producing useful information much more favourable.

6.2 What can we learn from aDNA?

At a broad level, study of ancient human DNA can tell us about relationships between different human populations, and about migrations and population history. At a smaller scale it can be used to reconstruct patterns of kinship within burial grounds and communities. It can be used for probabalistic determination of sex of skeletons where this is not possible on osteological grounds. aDNA can also be used to make probabalistic statements about some phenotypic (bodily) features such as hair, skin or eye colour, as well as aspects such as lactase persistence (which determines whether a person can digest milk) or genetic predisposition to various diseases.

Because of the genome-wide nature of data from NGS, studies aimed at broad population-historical objectives normally also produce genotypic data relating to the sex of individuals and to phenotypic features. Typically, studies looking at population history / migrations involve sampling hundreds of burials from sites spread over wide temporal and geographic areas so that secular and spatial trends can be discerned. Often, the data from newly analysed burials are combined with archived data from previous aDNA studies, and DNA data from modern populations are also used to aid interpretation. Work aimed at some of the other objectives discussed above characteristically uses smaller numbers, often concentrating on one specific burial ground or even one particular burial that may be of special interest.

To study the question of whether the arrival of the Beaker cultural package at the Neolithic-Bronze Age transition in parts of Europe was primarily a process of cultural diffusion or whether it involved significant migration of people, a DNA study of 400 burials from over 130 prehistoric European sites was undertaken. This data was combined with previously published data to produce a dataset from nearly 700 burials stretching over Europe and western Asia. Of the analysed burials, 120 were from Britain, where results showed a genetic discontinuity between Neolithic burials, primarily from communal tombs, and Beaker period and later burials. This appears to support the idea of significant immigration at around the Neolithic-Bronze Age transition in Britain, but the nature and scale of the migration required to produce the observed DNA results is still debated.

A DNA study to investigate kinship was undertaken at a small 7th century AD burial ground in Germany. The interments were accompanied by rich grave goods. In all cases where sex could be determined, they were male. The burial ground was thought to belong to a household of a powerful family. The DNA study showed that some were indeed close kin, but this was not so in all cases, and one individual had ancestry far away in southern Europe. Kinship was a prime determinant of membership of this household group but it was clearly not the only factor, other considerations, presumably social or political allegiances, sometimes with individuals of very different ancestry, were also important.

Sex determination in adult skeletons is usually straightforward based on osteological features. However the relevant features may be missing or damaged and in children they are insufficiently developed to be reliable. In such instances, DNA studies may be used to determine sex if this would address a pressing research question. Among Romano-British populations, infanticide (killing of newborn children) seems to have been commonly practiced to limit family size. Some have suggested people in Roman times preferred male children, so more female infants may have been killed.

However, aDNA analysis of newborn infant bones from Roman sites in Britain does not appear to support this, boys and girls being present in similar numbers.

Study of phenotypic traits may help provide information for facial reconstruction (for example, for presenting archaeology to the public) and sometimes for helping to put a name to a skeleton. Studies of the skeleton from Leicester thought to be Richard III showed that there was a 96% probability of blue eye colour and a 77% probability of blond hair. These results were consistent with other findings that pointed to this skeleton being that of King Richard. Trends through time have also been investigated. For example, data from studies primarily concerned with population history have also shown that, in Europe, Mesolithic people probably showed a variety of skin pigmentations; DNA associated with lighter tones only increased in later prehistory.

Study of DNA from pathogens in human remains can help us to understand infectious disease in ancient times. It can help confirm diagnosis when the skeletal lesions are ambiguous. It also opens up the possibility of studying infections that leave no trace on the bones. The bubonic plague bacillus has been detected in mass graves linked to documented plague outbreaks, confirming what some had doubted, that this bacterium was indeed responsible for the Mediaeval and post-Mediaeval outbreaks in Europe. More surprisingly, it has also been detected in Neolithic and Bronze Age burials from Europe and Asia showing that there has been a long relationship between this pathogen and human populations.

Some pathogens, for example those that cause tuberculosis and leprosy, show phylogeography – different strains exist in different parts of the world. Studying pathogen DNA from diseased skeletons from different places and times helps us to understand how these diseases spread among human populations in the past. DNA from oral bacteria (oral microbiome) is relevant for understanding general health and susceptibility to dental disease.

6.3 Sampling for aDNA

For ancient human DNA, the best sampling site appears to be the otic capsule. This is a section of the petrous temporal bone at the base of the skull that contains the sensory apparatus of the inner ear. The technique that seems to maximise DNA recovery involves sandblasting to remove the surface of the petrous part of the temporal bone, and to then physically remove the cochlea and surrounding dense bone. This typically yields a sample of up to about 300mg, normally plenty for analysis. The method requires the temporal bone to be present as a separate element, which is the case in fragmentary skulls or in immature remains. The procedure destroys much of the petrous part of the temporal bone.

An alternative to sandblasting is the cranial base drilling technique. This accesses the otic capsule by drilling into it from the external surface of the cranial base. A small (3-4mm) cutting burr is used to generate 200-300mg of powder. This is much less destructive than the sandblasting technique and can be used on intact skulls. It shows somewhat less good DNA recovery, but that does not appear to be critical unless DNA survival is marginal. As for the sandblasting method, each otic capsule can effectively be only sampled once using this approach, and ear ossicles (see below) should first be removed if present in the ear canal. For rare collections a CT

scan can be undertaken prior to sampling to plan the trajectory of the drill through the petrous bone to minimise damage to anatomically informative areas.

An alternative source for ancient human DNA is the ear ossicles. There is less published research on DNA yields than for the petrous temporal bone, but using ossicles obviates the need to cut or drill and speeds up laboratory processing, and some laboratories are beginning to use them routinely. Ossicles are often preserved in sediment within the ear canals, and can be dislodged without damaging the skull. One ossicle (we have three in each ear) may suffice. Although they are very small, their destruction still entails loss of knowledge, as would be the case with any other bone. Tooth cementum may also be useful for aDNA studies but more work is needed to confirm this. When the above tissues are not available, tooth dentine is a better source of aDNA than the rest of the skeleton.

Petrous temporal bones and ear ossicles are not good sources of pathogen DNA. For this, skeletal lesions that were active at time of death (if there are any) appear to be good sampling sites. For diseases spread via the bloodstream, dentine from tooth roots may also be a good option. Dental calculus contains DNA from of the oral microbial community, which has an important role in human health.

Cremated bone is not generally suitable for DNA studies.

7. Proteomics

7.1 The Science

Proteins are made up of amino acids. Different proteins have different sequences of amino acids. This can be used as a basis for identifying protein fragments preserved in an ancient sample. Modern laboratory techniques potentially enable hundreds of different proteins to be identified in a single sample.

A protein may differ slightly in its amino acid sequence depending upon which species manufactured it. For example, bone collagen sequences differ between species. Proteomic analysis, normally by a method known as ‘zooarchaeology by mass spectrometry’, or ZooMS for short, uses this to identify whether a bone fragment is human or not in instances where this is not possible on morphological grounds.

Proteomics is a major focus in the study of dental calculus. Most of this work is directed toward the study of diet, but there are difficulties. Meat proteins are difficult to assign to species, and hence to distinguish from the human host. Distinguishing plant proteins is more straightforward, and they can provide useful information, but they are often found in low abundance, despite the fact that plant foods must have been ubiquitous in most diets. Reasons for this are unclear, but may include poor survival of these proteins. β-lactoglobulin, a protein that specifically occurs in milk, appears to survive rather better and is quite often found in calculus. This helps in the study of consumption of dairy products in the past.

A variety of other types of biomolecular analyses have also been carried out on dental calculus, and researchers often combine different types of analyses. DNA work has chiefly focused on the study of the oral microbiome, the bacteria that live in the human mouth. Metabolomics looks at the array of small molecules derived from

dietary and other sources. This potentially provides insights into the nature of the oral biofilm, and into dietary components and other ingested substances.

Proteomics offers another option for sex identification in human remains. In humans, the amino acid sequence of amelogenin proteins, which play a part in tooth enamel formation, show minor differences between males and females. Peptides (protein fragments) from these remain in trace amounts within the enamel after it has formed. These peptides are present in both the deciduous and permanent teeth. The crowns of the former begin mineralising when the child is still in the womb, so the technique can potentially identify sex from foetal life onward. Depending on results, for males the method may provide a definite sex, but for females, sex determination is always probabalistic, although in the few studies published to date, sex assignments carry a quite high degree of certainty.

7.2. What can we learn from proteomics?

A late Mesolithic shell midden in Scotland contained some disarticulated human as well as animal remains. There were also some bone fragments that were considered morphologically as possibly human. These were subject to analysis by ZooMS. Fourteen out of 20 proved to be human. This was important, as human remains from this period are scarce. Once identified, they were subject to radiocarbon dating and carbon and nitrogen stable isotope analysis. This enabled important additional dietary data to be collected for this key transitional period in prehistory. Identification by proteomics can release dormant research potential within unidentified bone fragments.

β-lactoglobulin is found in animal milk (humans do not produce it), specifically in the whey fraction. Protein fragments from β-lactoglobulin in ancient dental calculus may be identifiable to species. For example, calculus from British Neolithic remains showed evidence for consumption of bovine, sheep and goat milk, as did calculus from late Bronze Age burials from Mongolia. These groups not only lived in different environments but also practiced very different types of subsistence, the former being settled agriculturalists, the latter steppe herders. The results show that, despite these differences, both exploited multiple species for dairy products.

Sex assessment from peptides in tooth enamel is a new technique and is still being perfected. A potential advantage over sex assessment using DNA stems from the fact that proteins are more resistant to degradation in the soil. Both proteomic and DNA sex assessment were carried out on 55 archaeological burials from California and the results compared to conventional osteological sex assessment, a method of proven reliability in adults. The proteomic method invariably produced a sex assessment, and there was good agreement with the osteology. The DNA sometimes failed to do so or else produced results that were in conflict with the osteological and / or proteomic sex when DNA survival was poor. There was no sample age effect for the peptides: the amelogenin signal remained stable over the 2000 years spanned by the interments. However, the DNA levels decreased in the older burials. Overall, the study supports the value of proteomic sex identification when osteological indicators are not available.

7.3. Sampling for proteomics

For ZooMS, a bone fragment is drilled to yield about a 5-50mg sample. Because the fragment will not have been previously identified as human, it will probably not be part of a human skeletal collection but more likely kept as part of an archaeozoological assemblage. Nevertheless, the general ethical and scientific principles for justification of sampling are similar. There should be some *a priori* morphological or other reason for suspecting that fragments might be human, and an archaeologically meaningful reason for identifying them as such.

For proteomics in dental calculus, only a very small sample (about 15mg) is generally needed. To put that into context, the total amount of dental calculus on the teeth of a skeleton varies from zero to 500mg or more. It tends to build up incrementally during the lifetime of an individual. This means that in general most adults show it, but deposits are less extensive and less common in children. For example, in a large Mediaeval rural population, calculus deposits were noted in 89% of adults but in only 22% of children. Often, dental calculus tends to separate from the tooth during storage of remains, so sometimes loose pieces can be taken from within the bag containing the dentition instead of breaking off a fresh sample.

In an initial application of sex assessment from peptides in tooth enamel, the procedure was to first abrade the enamel surface with a dental burr and then to dip it in acid to solubilise a sample of the peptides. This method was said to lead to minimal damage to the tooth crown, but the surface erosion would prevent subsequent study of microfeatures, and the effects on biomolecular analyses are unknown. The method is still being developed, and later workers have argued that a sampling approach that involves physical removal of a piece of enamel offers a more valid and sensitive basis for sex assignment, and hence is the method of choice. A sample of about 20mg of enamel is needed. This corresponds to a fragment of enamel measuring about 1 x 7mm.

8. Microscopy

8.1 The science

Under the microscope, the tissue of bones and teeth is not amorphous, but has a regular structure. Microscopic study of skeletal remains generally necessitates cutting sections of bones or teeth. Light and scanning electron microscopy are the chief modalities used for the study of archaeological human remains.

There are some microstructural differences between human and non-human skeletal tissue. Using these it may, in many cases, be possible to determine whether small bone fragments are human or not by examining cut sections under the microscope. This may be especially useful where this distinction is not possible using proteomics, for example if the collagen content has been destroyed through burning.

Bone disease may cause alterations at the microstructural level, so microscopy may aid diagnosis. It is of more value in some diseases than others. It is of particular utility for conditions that upset bone metabolism, such as Paget's disease of bone or vitamin D deficiency. Paget's disease of bone is a disease of the elderly in which the bones become fragile, thickened and pitted. The gross appearance is not particularly distinctive, but the microstructural changes are firmly diagnostic. Vitamin D deficiency disease, primarily caused by lack of exposure of the skin to sunlight, was a significant health threat in the past, especially in the smoky cities of the Industrial

Revolution. Rickets, caused by vitamin D deficiency in children, is quite easy to recognise visually in child skeletons, but osteomalacia, the equivalent condition in adults is much harder to diagnose because the bone changes are often very subtle. However, the effects of vitamin D deficiency on bone mineralisation can be observed directly under the microscope, allowing for firm diagnosis.

Dental enamel is deposited in incremental layers. Irregularities in these layers often relate to episodes of disease or malnutrition during childhood, so the study of these features can tell us about childhood conditions. Some of these layers outcrop on the surface (as perikymata), so they can be studied without cutting a section, either through simple visual inspection of the enamel, or through making an impression of the surface using dental impression material and using that to make a cast for microscopic study, which permits more detailed analysis (Although conventionally viewed as non-destructive, application of dental impression material may cause damage to very fragile specimens, so care is needed.) However, some layers of enamel are hidden inside the enamel crown, so for the study of these, it is necessary to section the tooth.

Cementum coats the tooth roots and helps anchor them in their sockets. Unlike enamel, layers of cementum continue to form throughout life. Cutting a section from the tooth and counting these layers under the microscope holds promise as a way of estimating age at death in adult skeletons. Although the technique, known as cementochronology, is still being perfected, it is potentially quite important, as other ageing methods work poorly, especially once a person is past middle age. Study of cementum also has some other potential applications: for example recent work suggests that number of pregnancies that a woman experienced may be detectable.

Microscopy is an important focus of studies of dental calculus. Entrapped particles of food such as starch granules, can tell us about diet. Other particles found in calculus are non-dietary in origin, having been introduced into the mouth when objects are grasped between the teeth, or else having been inhaled. Fungal spores or pollen grains can give clues to the natural environment, dust particles or larger fragments from manufacturing processes may give clues to environmental pollution, activity patterns or even occupation.

Soil-dwelling micro-organisms attack the collagen of bones and teeth during burial. This process, known as bioerosion, causes progressive degradation of microstructural features. When severe this may limit the information that can be gained, for example, in diagnosis of disease from microscopic study of bone or dentine, although some information can normally be gained even from severely degraded sections. The study of microstructural deterioration of bone during burial can be a research aim in itself, helping us to understand the mechanisms by which skeletal tissues degrade and the timescales over which this occurs in different burial environments. Sometimes, post-depositional changes to cementum can prevent accurate line counts, making it hard to apply cementochronology. Whether this is a problem or not varies from site to site. A pilot study to investigate this may be worthwhile prior to large-scale sampling of teeth.

8.2. What can we learn from the microscopic study of bones and teeth?

Microscopic study of bone samples from four adult skeletons from a burial ground associated with a 19th-early 20th century psychiatric hospital confirmed what had been suspected from looking at the bones, that these individuals were suffering from vitamin D deficiency. Psychiatric treatment at that time would have involved some patients spending long hours confined indoors. In the days before foods were fortified with vitamin D, this would have led to vitamin D deficiency, something that even today continues to be a risk for institutionalised populations.

A study of microscopic sections of deciduous teeth among those that died in childhood in a prehistoric population found that those showing more disruptions in enamel formation in parts of the teeth that formed before birth tended to die younger. Prenatal problems, presumably associated with maternal illnesses and malnutrition, appear to have predisposed to death in early childhood.

In dental calculus, the most common dietary particles are starch granules. Sometimes they can be identified to species, in which case they can tell us something about the plants consumed. However, quantitative inferences about diet are difficult. An individual will ingest billions of starch granules over a lifetime but only a miniscule proportion of these (normally less than 100) will be recovered from a dental calculus sample. Other particles relate to non-food items. A wooden fragment recovered from a piece of calculus from a Neanderthal seems to derive from a piece of wood inserted into the mouth as a tooth-pick. Calculus from a Middle Neolithic burial from near the site of Stonehenge provided evidence for bast fibres, with nettles being the most likely origin. Nettles provide useful fibres, so these remains may be a result of fibre processing. Birch pollen, micro-charcoal and soot were also present, giving clues as to his living environment. Traces of lapis lazuli, a pigment used for blue colouration, were found in calculus from a female burial from a Mediaeval nunnery. This suggested she may have been involved in pigment preparation and / or manuscript production.

8.3 Sampling for microscopic studies

Samples for microscopy taken from bone generally need only be a few millimetres thick. The other dimensions of the section depend upon the purpose of the study. A section can be separated by making two closely spaced parallel saw-cuts, or else a plug of bone can be removed using a suitable drill. If a full-thickness of cortical bone is required, a half section is normally taken so as not to cut completely through the bone. Bone or tooth samples need to be prepared, a process that normally involves embedding in resin and grinding and polishing the surface to be examined.

To study disease, if distinct bone lesions are present (for example, a tumour), a section is generally cut from the lesion. Of course, this may compromise future studies of the lesion. For conditions where the metabolism of the skeleton as a whole is affected, such as rickets or osteomalacia, the sampling site is not constrained in this way. So, for example, most burials contain numerous rib fragments, which are of limited use for morphological studies, and might therefore be sampled.

A microscopic section of a tooth may be made to study growth markers and growth disruptions in enamel and dentine . The tooth is sectioned vertically. For cementochronology, on the other hand, a transverse (horizontal) section is used. The root is placed in embedding material. The area of interest is normally the middle

of the root of a single rooted tooth. Enough is normally removed to make several (sometimes up to about eight) thin sections, as evidence is normally combined from different sections to produce an age estimate. In practice, most of the root may be removed and one, or sometimes two teeth per individual are sampled. The sections are normally mounted on glass slides for viewing using a light microscope.

For calculus, a sample of less than 50mg is normally taken, and then decalcified to release the inclusions. However, a larger sample, is likely to yield a larger and possibly more diverse number of microscopic particles.

9. Case studies

9.1 Exhumation of remains thought to be of particular historical individuals

Cases involving the exhumation of single burials usually involve attempting to identify the remains of prominent individuals. The best-known project of this type is of course the excavation in 2012 in Leicester that recovered the skeleton subsequently identified as that of King Richard III. The resultant publicity led to in a spike in requests to clergy and others responsible for historic burial grounds for projects aimed at exhuming the bones of other historical figures. Over the years, projects have been proposed to exhume remains purported to be of Shakespeare, King Alfred the Great, King Harold II, the Mediaeval Duke of Clarence, and the early Saxon Christian St Eanswythe (which was successfully carried out following advice from APABE, see below), among others.

In addition to those outlined earlier in this document, some other caveats need to be considered in projects that involve exhumation of burials from historic burial grounds. Prominent personages are often interred in elaborate tombs and these are often in a fragile state. Dismantling them to access the human remains may involve significant risk of damage. Reburial of human remains, and organic or other artifacts that accompany them, after examination, tends to result in their renewed deterioration.

The quality of information regarding archaeological context, and the nature of the supporting historical evidence concerning the targeted burial are crucial to the success of projects. These need to provide firm support that the remains of the specific, sought-after person lie within a particular tomb, or at a precise, marked location. If this is not the case, then it is unlikely that scientific analyses can establish identity. As a generality, burials beneath churches and in churchyards are very densely packed and intercut, with later burials disturbing and displacing earlier ones. Depositum plates bearing the identity of the deceased are rarely part of coffin furniture, even for the rich, prior to the 18th century. Historical evidence that a person was buried beneath a certain church, or a certain part of a churchyard, is normally insufficient to enable remains for analysis to be correctly targeted. For example, a project was conceived to test the hypothesis that a particular burial in a stone coffin at Bosham Church was that of King Harold II, killed at Hastings in 1066. There was some historical evidence that suggested Bosham as a burial site but it was weak – other locations are more likely. Even leaving this aside, there seemed no reason to think that this particular individual rather than any other buried under the church might be King Harold II, making the whole project rather speculative. This, and other concerns over the application, led to Faculty permission for the work being refused.

Even projects involving the exhumation and study of a single skeleton are a major undertaking and will necessitate the assembly of a multidisciplinary professional project team. Project costs may run into tens of thousands of pounds, and applicants should demonstrate that they have the funding in place or have proper plans to secure it once permission is granted.

As with any scientific work, the project needs to have clear aims. For example, human remains held in elaborately decorated mortuary chests at Winchester Cathedral are identified epigraphically as particular Anglo-Norman Bishops and Royalty, but despoilation during the civil war now makes these identifications doubtful. A major scientific project was conceived to test whether the original remains are still inside. This was supported by the cathedral authorities as it had a clear hypothesis to test and, whatever the results, they would be important to the understanding and public appreciation of the history of the cathedral.

Although popular dissemination of work is often important (see below), there should be a commitment to publication of results in the scientific literature, and this is needed whatever the results. For example, the various analyses carried out on the skeleton identified as Richard III have been published in detail in scientific journals, enabling experts in the field to evaluate the validity of the identification and other interpretations.

Whilst, for ancient burials, personal identifications are unlikely to be unequivocal, they are most likely to be convincing when they are based on multiple lines of evidence. For example, contrary to popular belief, ancient DNA analyses are generally not conclusive on their own. In most cases, relevant scientific evidence is combined with the archaeological context and historical information relating to the place and manner of burial and (if available) to physical attributes of the person. In the case of the skeleton identified as Richard III, no one line of evidence was conclusive on its own in establishing identity. Nevertheless, combining the archaeological context in which the burial was found, with the historical background information about Richard and his life, death and burial, with osteological observations on the skeleton, and DNA and other biomolecular analyses, enabled a compelling case to be assembled.

In most projects, a staged approach to post-excavation analysis is taken. If basic osteological study (e.g. age and sex determination) is compatible with the putative identification, then a first round of destructive sampling may be merited. Radiocarbon dating is often the first resort. Only if the date produced is compatible with the known date of death of the sought individual are further analyses (e.g. DNA, isotopes) generally worthwhile.

The public fascination for ancient human remains is magnified when they appear to be of known historical figures. There is thus likely to be considerable wider interest in projects, but care needs to be taken to ensure that this is handled sensitively and ethically. Some projects have been funded from the outset by television companies. Whilst this is not necessarily problematic, care needs to be taken in such instances. It may be difficult to retain control over how the work is presented and, potentially, to avoid sensationalising or trivialising of results.

Properly handled, projects of this nature can provide important opportunities for community engagement. In Folkestone, the study of remains that had been concealed in a church wall at the Reformation, supported the idea that they were likely to be of 7th century St Eanswyth, one of the earliest female saints and an important figure in the adoption of Christianity in Britain. The study of the remains was used not only to support the identification but to explore aspects of her life. Engagement with the local community was integral to the project. The work was associated with the development of a much broader public appreciation of the history of Folkestone and what has made it significant and distinctive as a community.

9.2 Multidisciplinary study of a museum collection

Many museums hold assemblages of human remains as part of their permanent collection or on long term loan. The research conducted on such collections reflects the interests of the wider research community across a variety of disciplines (archaeological, clinical, forensic, and anthropological) and technological improvements over a period of many decades. In addition to the immediate impact of destructive sampling on an assemblage, curators need to consider the possible impact of sampling on future research directions and consider the likelihood that less destructive or more informative techniques will be developed.

The human osteological assemblage from Christ Church Spitalfields (CCS), London is held at the Natural History Museum in London under a Faculty and with permission from the friends of Christ Church Spitalfields. The collection is managed according to the same policies and procedures pertaining to other human remains held by the Natural History Museum, including those relating to destructive analysis. The CCS assemblage includes skeletons of adults and juveniles who were identified from the inscriptions on their coffin plates. The assemblage has been used to investigate human health, environment, diet and life history in 18th and 19th century London. The coffin plate series has been used to test and develop a range of analytical techniques in forensic and clinical research as well as osteoarchaeology and to undertake other research that relies on the documented biographical details.

This section reports on some of research projects involving destructive sampling that have been conducted on bone and dental tissues. Some proposals that would have involved more extensive sampling or sectioning of bones have been refused. Nitrogen stable isotope values in bone samples from infants and children from CCS were used to explore nursing behaviour in 18th- and 19th-century London. The study demonstrated that elevation in nitrogen stable isotope ratio associated with breastfeeding could be detected in the infant ribs by the age of 5–6 weeks, reflecting rapid bone turnover in new-born infants. This was possible because age at death was documented for this series of skeletons. Samples were taken from anatomically uninformative rib fragments, either by selecting small existing fragments or in a few cases by removing a small piece of bone the broken end of a broken rib. No whole ribs were included in the study. The bone fragments were cleaned and sampled in the lab, and any remaining bone was returned to the collection. A pilot study on a small sample was undertaken before proceeding with the full analysis. Complete analytical results were presented in a peer reviewed journals and copies of the publications were added to the collection archive.

Cortical bone has a dynamic microstructure that is renewed throughout adult life by the gradual replacement of bone (bone turnover). This remodelling alters the microstructural organisation of the bone and produces secondary osteons. Individuals from CCS were included in a study that investigated biomechanical and other influences on bone microstructure. Polished thin sections were prepared from bone blocks cut from the femoral midshafts and cross sections from midsternal ribs, and secondary osteons were measured on digital images captured with a camera mounted on a microscope. Within the sample studied, the patterning of osteon dimensions was not clearly linked to age, sex anatomical regions or inferred levels of physical activity. A subsequent study by a different team used the same bone blocks to investigate the presence of adult vitamin D deficiency osteomalacia. Analysis of the long bone sections using scanning electron microscopy confirmed the presence of microscopic features, consistent with osteomalacia, confirming the original diagnosis based on macroscopic features of the skeleton. The sampling of the femoral midsections generated valuable research results, but some other types of research involving the shape and bone microstructure of the femoral midsection that could not have been envisaged at the time of the original sampling have not been possible. This type of sampling is no longer recommended for long term museum collections.

Calculus from Spitalfields dentitions was included in a study of food proteins in calculus from teeth from archaeological sites spanning the iron age to post medieval periods. The research was able to identify consumption of dairy products, cereal grains, and legumes, and further research involving metagenomic analyses will be conducted on the same samples. The amount and distribution of calculus are fully recorded before sampling. Calculus is carefully removed using a non-metal implement that will not damage the tooth surface or jawbone. Photographs should be taken before and after sampling.

Growth markers occur in all dental tissues and capture a detailed record of the growth of those tissues. Growth markers within the structure of dental tissues are often studied using histological techniques. Teeth from five children from CCS who had a precisely documented age at death provided an opportunity to determine whether cross-striations in enamel reflect daily growth increments. The study demonstrated that the number of cross striations forming after birth corresponded to the number of days between birth and death, confirming that these markers reflect a daily rhythm of enamel matrix secretion. For this study histological sections of each tooth were prepared in a longitudinal plane from the cusp tip to the root apex. The tooth blocks from each side of the section were retained. Teeth were only sampled if the antimere was present. Digital photographs were taken using a camera mounted on the microscope, and these images or the tooth sections could be used for further research.

Some studies are preferentially conducted on high resolution replicas rather than directly on bones and teeth. Casts or replicas may also be produced prior to destructive testing to mitigate the loss of information caused by sampling. Replicas can be used for research on tooth and bone surfaces that is not possible using CT scans. High precision dental impression material is coated onto the tooth or bone surface using a syringe, and gently prised away from the surface once set. The condition of each tooth or bone must assessed in advance and teeth or bones that

are fragile, or those with cracked or otherwise damaged surface should not be included. For teeth that are still in the jaw, particular care must be taken to prevent the impression material spreading into the gap between the tooth and the jawbone. High resolution replicas of the multiple tooth crowns from young adult dentitions from CSS were used to investigate the number and age distribution of macroscopic and microscopic enamel defects in the permanent teeth. Enamel defects were matched across the dentition by reference to tooth formation schedules and by counting the number of perikymata (surface growth markers) between defects on simultaneously forming teeth. The condition of each tooth or bone was assessed in advance and teeth that were fragile, or those with cracked or otherwise damaged surface were excluded because casting could have caused further damage to the tooth.

There is a risk of mixing with all studies involving the removal and subsequent return of multiple bones or bone fragments of the same type from a collection, and this should always be discussed with staff at the holding institution. Samples or bones removed from the collections should be placed in individually numbered bags and a label with the correct number should accompany the bone at each stage of the research. A note should be left in the box until the bone is returned. When returning a sample or a sampled bone to the collection the antimere should be compared where possible or other matching criterial should be considered such as size and condition.

10. Procedures and terms of access for the human remains: the example of St Peter's Church Barton-upon-Humber

St Peter's Church Barton-upon-Humber is no longer used for worship and is under the care of English Heritage and Historic England. Part of the church is used for the storage of over 2800 burials excavated during archaeological investigations in the church and churchyard. The church is still consecrated. Placement of remains here satisfies a wish expressed by the Church that human remains should be returned to consecrated ground after excavation, and at the same time allows the remains to continue to be accessed by researchers. They are an internationally important collection and are much in demand for research. The procedures for managing access to this collection, including for the purposes of destructive sampling, are described below, as are the terms under which access may be granted.

10.1 Pro-formas for access to remains

Proformas help ensure that the correct information is gathered prior to considering an application, and that different applications are treated fairly and openly. Set out below are the proformas which applicants need to complete to request access to the human remains stored at St Peter's Church. Note that part B of the form refers to requests for destructive sampling.

APPLICATION FOR RESEARCH ACCESS TO THE HISTORIC ENGLAND HUMAN SKELETAL COLLECTION AT BARTON-ON-HUMBER

PART A: to be completed by all applicants

Applicant
Name Academic affiliation

Email:
Telephone:
Postal address:

Academic status of applicant:
Masters student/PhD student/University employee (please specify job title)/ Other (please specify)

Name of supervisor (students only)

Email
Telephone
Postal address

FOR STUDENT APPLICATIONS A SUPERVISOR'S LETTER OF RECOMMENDATION SHOULD BE SUBMITTED ALONG WITH THE COMPLETED FORMS

Aims & purpose of research. Please explain briefly the nature of your research and why the skeletal material you are requesting access to is needed for it. Include the overall rationale for your research and how the Barton collection contributes to this. Please also specify whether you require access to the entire collection or a subset of it (eg juvenile skeletons etc). Please summarise the above in no more than 500 words.

Data to be recorded and methods to be used

Dates when access will be required

Is loan of material requested? YES/NO

If yes please give details of material required; loan period requested; where material will be kept whilst on loan

Does the work involve destructive analysis YES/NO

If yes, please fill in PART B of this form

Is publication intended? YES/NO If yes please give details

I have read and accept the procedures and terms of access
Signature of applicant (scanned signature acceptable)

Countersignature by supervisor (students only; scanned signature acceptable)

PART B: to be completed by applicants conducting destructive analyses

Please detail what destructive techniques are to be used

What specific research questions will the analyses address?

What is the likelihood of useful information being obtained?

Please indicate:

(a) what skeletal elements are to be sampled and at what location on the bone
(b) how many specimens will be sampled
(c) how much tissue will be taken from each specimen
(d) how samples will be removed.

Please also specify the context numbers that you intend to sample from, if known at this stage

Please email completed forms to Simon Mays: simon.mays@historicengland.org.uk

10.2 Procedure for considering request for access to remains

A Barton Human Remains Research Committee (BHRRC) was set up to administer access to the remains at St Peter's Church. The BHRRC is comprised of representatives of Historic England, English Heritage and Parochial Council of St Mary's Church Barton-Upon-Humber, as well as external expertise in human remains. The aim was to assemble a committee with a mixture of expertise and experience in Church, curatorial, archaeological and scientific matters. The BHRRC considers requests for access to human remains using the flowchart set out below. Where the proposed work involves destructive analyses, it considers the proposals against the criteria set out in Section 2.3 of the current document.

10.3 Standard terms of access to the human remains from St Peter's Church, Barton-Upon-Humber

<u>Procedures for consideration of applications</u>

Access to the skeletal material kept at Barton is normally restricted to suitably qualified individuals conducting research in a relevant discipline, although requests for access for other reasons will be considered.

Postgraduate students may be granted access provided a supervisor's letter of recommendation is submitted. Undergraduate applications will be considered only in exceptional circumstances.

Applications will be considered by the Barton Human Remains Research Committee (BHRRC) and applicants will be informed of their decision.

The BHRRC reserves the right to seek external advice as necessary.

<u>General terms of access</u>

Applicants are reminded that it is a legal and ethical obligation that human remains be at all times treated with respect.

Human skeletal remains are fragile. Applicants should handle remains with care at all times.

Any material removed from boxes for study on- or off- site should be returned to its correct bag and box after study.

The BHRRC should be informed of any problems associated with the curation of the collection eg damage to or deterioration of specimens.

The BHRRC should be provided with a copy of the dissertation, thesis or published articles based on the study of material in the collection.

English Heritage and Historic England should receive acknowledgement in any published articles based on study of remains in the collection.

Whilst working on the remains at Barton, all reasonable requests from English Heritage or Historic England staff at Barton Church should be complied with.

The BHRRC reserves the right to confer additional terms of access in individual cases as it sees fit.

The BHRRC reserves the right to terminate access if the access conditions are violated.

Additional conditions covering loans of material

Researchers must not remove any remains from site without written permission from the BHRRC.

In cases where permission has been granted by the BHRRC to remove remains from site, a loan agreement form must be completed and countersigned by the English Heritage curator / registrar at the time remains are taken.

In cases where BHRRC grants permission for loan of material, a date by which material should be returned will be specified.

Loans will normally be for a period less than 6 months. Requests for loans for periods of more than 6 months will only be considered in exceptional circumstances.

Researchers should be able to provide safe and secure transportation for remains borrowed, and details should be agreed with the curator.

Researchers should ensure that any remains borrowed are kept in a secure store under conditions which ensure the physical integrity of the remains and which comply with standards set out in ‘Guidance for the Care of Human Remains in Museums’ (DCMS, 2005) and ‘Guidance for Best Practice for treatment of Human Remains Excavated from Christian Burial Grounds in England’ (APABE, 2017). These and any further conditions of loan will be incorporated into a loan agreement signed by both parties.

Additional conditions covering destructive analyses

No samples should be removed for destructive analysis without written permission from the BHRRC.

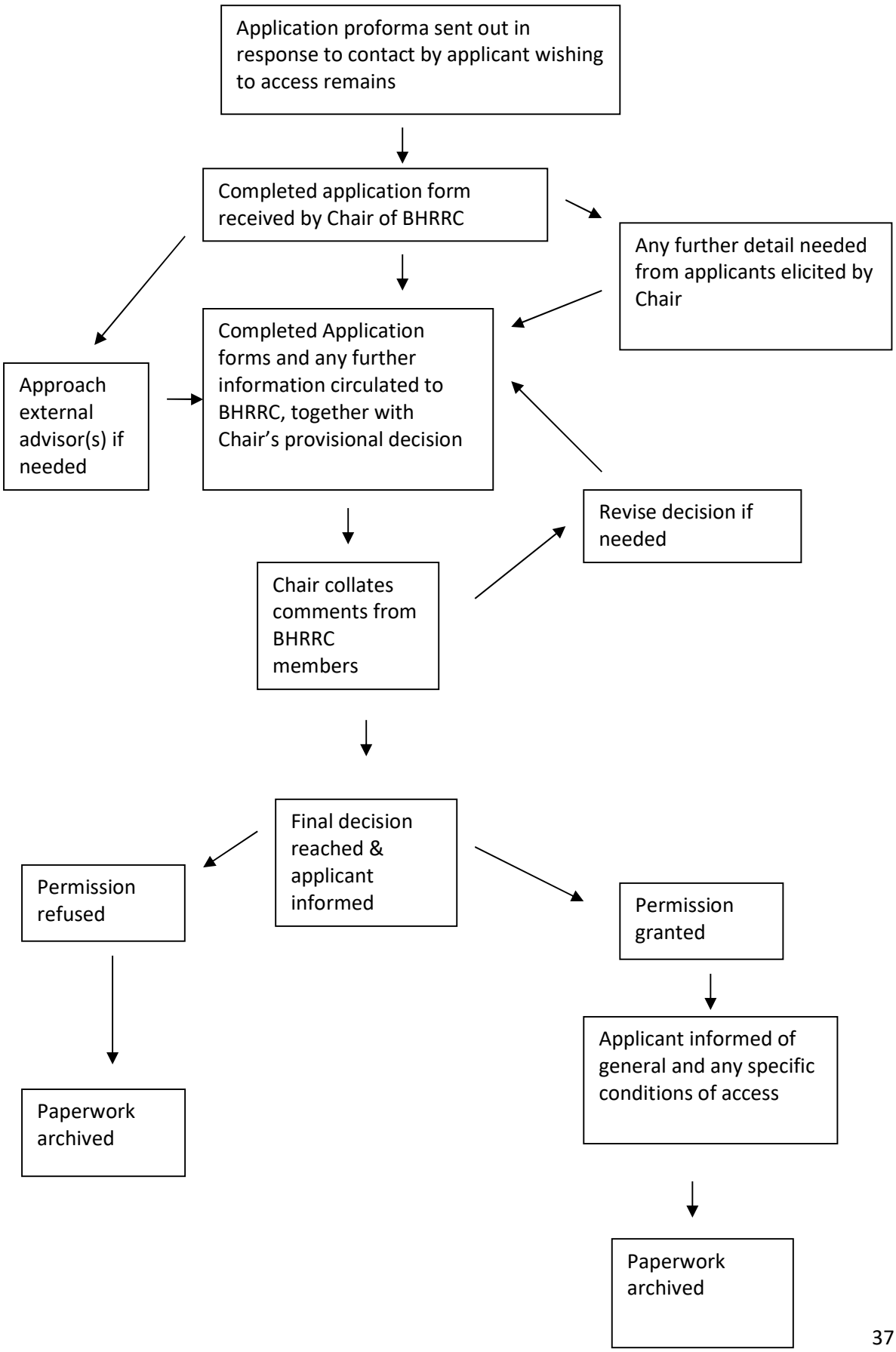
For permission for destructive analysis to be given, the BHRRC needs, minimally, to satisfy itself that the research questions could not be adequately addressed using non-destructive techniques; that the analyses have a realistic prospect of producing

useful knowledge; that the sampling strategy is designed to keep damage to the collection to a minimum.

When samples are removed for destructive analysis, a list of the samples taken should be presented to the BHRCC. In addition, a note should be placed in the box from whence each specimen was taken giving brief details of the sample removed; the analysis that will be performed on it; the date the sample was removed; the name and affiliation of the researcher who took the sample.

Any unused samples removed for destructive analysis should be returned by the researcher to their correct bag and box.

Flowchart summarising procedures for consideration of requests for access to the human remains stored in St Peter's Church, Barton-Upon-Humber



11. Further Reading

Mays S. 2021. *The Archaeology of Human Bones*, 3rd edition. Routledge, London. This book forms a general introduction to the scientific study of human skeletal remains from archaeological sites. It contains more detail on the techniques discussed here, and many of the examples of the archaeological application of the different techniques used in this guideline are drawn from studies discussed in this book.

Roberts, C. (2018). *Human Remains in Archaeology: A Handbook,* 2nd edition. Practical Handbooks for Archaeology, No. 19. Council for British Archaeology, York. A useful review of the excavation and study of human remains, with a British emphasis.

12. Where to get advice

The Advisory Panel on the Archaeology of Burials in England (APABE) gives free casework advice to professionals involved in archaeological projects in England dealing with human remains. Its members cover a wide range of expertise, and its remit encompasses advice on ethical and legal matters as well as scientific advice. APABE can be contacted via its website: https://www.archaeologyuk.org/apabe/