Calcium Isotopes in Sheep Dental Enamel: A New Approach to Studying Weaning and Dairying in the Archaeological Record

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ABSTRACT

Calcium isotope ratios ($^{44}Ca/^{42}Ca$) have shown promise as a milk dietary tracer. Previous studies have focused on bone but, due to homeostatic processes, $\delta^{44/42}Ca$ values are highly variable. This has greatly complicated the identification of mammal milk consumption through bone analysis, resulting in a search for an alternative. This thesis describes controlled studies to assess the effects of milk consumption on $\delta^{44/42}Ca$ values in bulk and sequential samples of dental enamel, using modern samples from Yorkshire, England, the Isle of Hoy, Scotland, and archaeological samples from Abu Hureyra, Syria. The samples from Yorkshire consisted of dental enamel, ewe milk, feed and bone. Dental enamel was sampled from the Hoy sheep.

Milk and plants are the greatest contributors of calcium in the mammal diet. Feed and ewe milk samples confirmed that milk has lower $\delta^{44/42}Ca$ values than plants in the diet, and with a common diet between ewes, uniform milk $\delta^{44/42}Ca$ values are produced. Also, there is a significant difference between bulk molar enamel $\delta^{44/42}Ca$ values between males and females, with males having higher values. Additionally, analysis of bulk and sequential samples of Hoy mature sheep molar enamel, although mass fractionation effects were small, produced $\delta^{44/42}Ca$ values with a clear isotopic offset between the enamel likely formed during nursing, weaning and the full conversion to a plant diet. The $\delta^{44/42}Ca$ values, once

associated with dental development chronologies, led to the identification of a clear pattern of nursing and weaning in the enamel of the first and second molars of sheep with known dietary and weaning histories. The modern results were used to identify patterns of ancient nursing and weaning. Although evidence was insufficient to establish early weaning of the Abu Hureyra sheep, this research successfully established the potential of this approach for investigating milk production and consumption in the archaeological record.

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CHAPTER 1

INTRODUCTION

The Neolithic transition in the Near East, beginning between 12,500 and 11,200 calibrated years before present (cal. BP), where humans changed from a means of subsistence based on hunting and gathering, to one based on domesticated plants and animals, is regarded as one of *the* pivotal events in human prehistory. Famously described by Vere Gordon Childe in 1934 as one of the two 'revolutionary' aspects of change in human progress (the other being the Industrial Revolution beginning in the 18th century) (Trigger 1989), and of equal influence, despite 80 years of new research the importance of this transition has not diminished. The concept of the Neolithic Revolution is still resonant, and the domestication of plants and animals has been studied extensively, from the morphological differences in the bones of wild and domesticated stock animals, to the genetic differences between wild and domesticated cereal crops. However, one very important, unique, aspect of this revolution has remained elusive: understanding the origin and contribution of dairy milk to Neolithic economies.

Gaining an understanding of dairying in the Neolithic is dependent on being able to evaluate 'the capacity of a milk oriented husbandry under prehistoric conditions' (Balasse and Tresset 2002: 853). With that in mind, why is gaining an understanding of a Neolithic community's capacity for milk production so important? What role does milk have in the development of specialised domesticate herd management (sheep, goat, cattle, horses, etc.) as well as in human nutrition? How can the capacity of milk production during the Neolithic be investigated? No mammal other than humans consumes milk after childhood, and certainly not the milk of another species. Dairy milk consumption represents an entirely different psychology of subsistence compared with that which led to the initial domestication of plants and animals. More than just managing a formerly wild species to

provide a more readily available, reliable and adaptable resource, dairy milk was something new. As initially adult humans were unlikely to have been able to digest milk in its raw state, it is even more intriguing that the earliest dairy milk was almost certainly used to create products that had been fermented, so a second innovation would have been necessary to even begin to exploit the resource.

So great are the benefits of milk to the human diet that it may have altered the genetics of some populations. A mutation on alleles responsible for lactase persistence allows the digestion of non-fermented, raw milk by humans. Research in ancient DNA has found that the mutation of the allele responsible for lactase persistence in European populations was almost absent during the early Neolithic (Burger et al. 2007, Itan et al. 2009), but that the spread of the allele mutation could be explained through a gene–culture co-evolutionary model, which essentially predicts that 'lactase persistence is only favoured in cultures practicing dairying, and dairying is more favoured in lactase persistent populations' (Itan et al. 2009: 1). It is now believed, based on computer simulation models, that the European lactase persistence allele underwent selection around 7500 BP in a population of dairy farmers in the central Balkans and Europe (Itan et al. 2009). The combination of the allele mutation and the availability of milk produced a strong selective advantage that resulted in the majority of northern Europeans being both lactase persistent and consumers of milk and dairy products.

Important though this is, lactase persistence is still a rare trait, and today only about 30% of the human population is able to digest non-fermented milk past weaning age (Tremaine et al. 1986). The reason these people, mainly northern Europeans and a few pastoralist groups in Africa, the Middle East and southern Asia, are able to digest raw non-human milk throughout life is down to some specific, highly selected, Mendelian dominant allele mutations (C/T−13910 in European, and G/C-14010, T/G-13915 and C/G-13907 in African, populations) (Tishkoff et al. 2006, Burger et al. 2007, Itan et al. 2009).

The advantage of dairy foods is that they are highly nutritious and contain more calcium than any other food in the mammal diet, with plants providing the second most significant source (Fransson and Lönnerdal 1983, Heaney et al. 1993). Lack of calcium in the diet has serious implications for mammal bone health, with deficiency resulting in osteoporosis and, when significant and persistent, leading to rickets in young mammals and osteomalacia in adults (Aloia et al. 2010). Dairying offers humans food(s) rich not only in calcium, but also in protein, fat and other minerals, and even lactase non-persistent humans may be able to consume dairy foods with reduced or eliminated lactose content, such as curd-based cheeses (the whey contains the majority of the lactose), and autodigested foods, such as aged cheeses and yogurts (most of the lactose is consumed by bacteria during production) (Fransson and Lönnerdal 1983, Kolars et al. 1984, McBean and Miller 1998).

Currently, it is believed that humans living in the Near East during the early Neolithic did not have the mutation for lactase persistence, although continued genetic research may change that position (Gerbault et al. 2011). Even if the Neolithic peoples of the Near East did not have the mutation, there is still evidence, from residues found in cooking ceramics (Evershed et al. 2008) (discussed in greater detail in Section 1.4), that they were consuming milk products, which strongly suggests that milk was being processed in preparation for human consumption, presumably in a way that reduced the lactose content. Dairy milk and foods would have provided Neolithic humans in the Near East with a seasonally renewable, ready source of nutrition, which, as the strong genetic selection for the lactase persistence variant indicates, could only be to their advantage (Tishkoff et al. 2006, Burger et al. 2007, Elwood et al. 2008).

Genetic modelling can tell us when the mutation occurred, and how important it was to the populations who had it, but as humans were probably consuming cooked or fermented dairy products long before the mutation arose, it cannot indicate when dairying began or how important milk was to the population. While the residues from cooking

ceramics provide important evidence of Neolithic milk production, processing and consumption (see Section 1.4.3), the Neolithic transition predates the use of pottery, and currently methods for detecting evidence of dairying in the archaeological record for this earlier period are limited. Needed are different approaches for investigating and evaluating milk use and production during the Neolithic that is not reliant on pottery, such as by learning about the lives of Neolithic animals potentially involved in dairy milk production.

Along with culling profiles (see Section 1.4.1), assessing milk production in the archaeological record through investigating possible dairy animals' lives involves knowing both the animals' (in the case of my research this refers to sheep) natural lactation length and the normal weaning age of the animals' offspring. Knowing this information provides a means of evaluating whether lamb weaning was early or natural. Early weaning is indicative of some outside influence, which normally means natural factors such as poor maternal nutrition/health and low milk quality (Hass 1990, Réale et al. 1999) and/or, in the case of domestic sheep, human management (see Chapter 3, Section 3.3). Ewes normally wean lambs between 150 and 160 days after birth (see Chapter 3, Table 3.1).

Lactation length is largely uniform for wild and domesticated sheep breeds and is closely linked to the age of lambs that are weaned naturally by their mothers (a lactation length of 150–160 days), with the main exception being dairy breeds (a lactation length of 180–240 days) (see Chapter 3, Table 3.1). Dairy sheep breeds have modified lactation lengths that extend milk production beyond what the lambs need to consume (lambs can be weaned without some form of replacement milk by 60 days) (Lyford 1988, McKusick et al. 2001). Extended lactation lengths are also a living testament to human involvement in selective breeding and management of sheep over time, as well as providing evidence that sheep milk is and has been a desired commodity by humans. Even though extended lactation length is important evidence of the human desire to consume sheep milk, the early domesticated sheep in the Neolithic probably had lactation lengths close to mouflon sheep

(~160 days) (Obregón et al. 1992), the closest living relative to the original wild sheep that, during the Neolithic, would have become the first sheep domesticates (Hiendleder et al. 2002, Pedrosa et al. 2005, Tapio et al. 2006, Zeder 2011). Additionally, it has only been in the last 150 years of better feeding and genetic selection that truly superior dairy sheep breeds have been produced (Haenlein 2007).

As lactation length and weaning age of Neolithic sheep is likely to be close to wild and non-dairy sheep breeds (150–160 days), detecting weaning timing in archaeological sheep could be a means of investigating possible human interactions with ewes and lambs, such as instigating early lamb weaning as part of managing and utilising dairy or mixed-subsistence (dairy and meat) herds. An understanding, additionally, of the causes behind weaning age/lactation length for sheep may be a means of investigating non-human related factors that influence when a ewe will choose to wean offspring, such as, as previously mentioned, poor maternal health and/or diet. Both the human and non-human factors influencing lactation length and lamb-weaning timing are of importance for archaeological investigation; based on my research originating as part of the Marie Curie Initial Training Network project Lactase Persistence in the early Cultural History of Europe (LeCHE), a project dedicated to researching dairying during the Neolithic, this thesis intends to discuss early weaning in regard to human involvement as part of managing sheep for milk production and human consumption.

This thesis will introduce a new approach for investigating lamb nursing lengths and weaning timing, by determining calcium isotope ratios in molar dental enamel, for the purpose of investigating, within the archaeological record, factors that may result in a lamb being weaned early. This approach has never been attempted before. Ethnographic evidence has demonstrated that early lamb weaning is a key component of sheep dairying (Vigne and Helmer 2007). However, before archaeological samples could be interpreted, a sound methodology was needed using modern sheep, with known dietary and environmental

variables, with a known natural length for lactation and information about weaning timing. As a result, this thesis is primarily a discussion of results from modern samples, but the successful outcome of the modern work has shown that the approach has strong potential for archaeological applications.

The reason sheep were chosen as the animal of focus is because they are known to be associated with the earliest livestock domestication, and are used for dairying in many parts of the world today. Sheep also mature more quickly than other modern animals used in dairying, such as cattle, so that generational information can be gathered within a shorter timeframe. The modern sheep used to test the methodology were chosen because they provided an experimental control, a supply of teeth for analysis and the crucial information needed about diet and weaning necessary for confident interpretation of the results.

Once the results derived from the modern samples were interpreted, it was possible to use the methodology on archaeological samples, which lack the corollary data about diet and weaning. To confirm its usefulness for archaeological material, a small pilot study was undertaken using the new methodology on teeth from the jaws of sheep excavated at the Epipalaeolithic–Neolithic site of Tell Abu Hureyra in Syria (~10,400 to 7000 BP). This site was selected because Tell Abu Hureyra has a history of human occupation during the Epipalaeolithic–Neolithic transition and through a significant part of the Pre-Pottery Neolithic (11,700–8250 cal. BP), the time period during which dairying possibly first occurred. It is also close to the Taurus–Zagros region (southern Turkey, Iraq and Iran), where sheep domestication is believed to have first occurred, based on DNA evidence (Hiendleder et al. 2002, Pedrosa et al. 2005, Tapio et al. 2006, Zeder 2011). The excavation yielded sheep jaws and teeth from contexts dated concurrently with the earliest evidence of dairying in the Near East.

Dental enamel was chosen rather than bone for this research because of the complexities of remodelling in bone and the large number of variables that affect bone

calcium isotope ratios. In contrast, dental enamel does not turn over once mineralisation has been completed, and is less affected by diagenesis than bone. To refine and correlate the results found with the new calcium isotope-based methodology, the oxygen and carbon isotope content of enamel apatite was measured to identify dietary change and seasonality.

To establish the context for the new methodology, this introductory chapter provides an overview of the background research and the objectives of this thesis. Additionally, the rationale for the sample and archaeological site selection will be discussed. This overview also provides an argument for the benefit of adding calcium isotope analysis of dental enamel to the few existing zooarchaeological and archaeological scientific methods of investigating dairying in the archaeological record.

1.1 Research background

Currently, milk and dairy food production for human consumption is a global industry involving vast amounts of money, millions of people and even more dairy-producing animals. Milk and dairy foods are highly nutritious and an excellent source of fat, protein, iron, copper, zinc and magnesium. Most significant in meeting dietary demand, milk is also an excellent source of calcium (Fransson and Lönnerdal 1983, Kolars et al. 1984). Plants are an important secondary source of calcium in mammal diets, but only a few of the *Brassica* genus contain close to as much as milk (Table 1.1) (Heaney et al. 1993). Milk and dairy foods are also a good way of 'consuming' a dairy animal without slaughtering it. This is as true today as it was in the past, but when studying ancient dairy milk production and consumption it has to be borne in mind that modern dairying has changed over time almost beyond recognition from its earliest origins.

Early domesticated sheep, goats and cattle in the Pre-Pottery Neolithic, specifically in the Taurus–Zagros (southern Turkey, Iraq and Iran) and Levant (Israel, southern Syria, south-east Turkey, Lebanon, Jordon, the Sinai Peninsula of Egypt and the State of

Table 1.1: Dairy and non-dairy food data from the United States Department of Agriculture, Nutrient Database for Standard Reference (2011)

Food	mg of Calcium/50 g	Food	mg of Calcium/50 g
Cheese, Parmesan	592	Milk, cow, all types	~60
Cheese, Gruyere	506	Yogurt, cow milk	~60
Cheese, Swiss	396	Mustard greens, cooked*	59
Cheese, Cheddar	360	Yogurt, Greek	55
Salmon, with bones	120	Kale, fresh, cooked*	36
Cheese, ricotta	104	Broccoli, fresh, cooked*	20
Milk, sheep	96	Butter, unsalted	12
Milk, Indian buffalo	84	Oats, cooked	5
Milk, goat	67	Lamb, leg, cooked	4
The foods listed are unfortified with calcium			
*Plant foods in the *Brassica* genus			

Palestine) regions of the Near East, would probably have been used for more than meat, bone and skins during this time (Legge and Rowley-Conwy 2000, Kuijt and Goring-Morris 2002, Byrd 2005). The early use of animals by humans for products that do not require the death of the animal has become commonly known as the 'secondary products revolution' and was first theorised by Andrew Sherratt (1981, 1983). Sherratt believed that there was a variety of reasons for the domestication and management of animals by humans for more than meat and skins, including as sources of milk, wool/hair and traction/transport (of goods and/or people). Sherratt's initial theory of the secondary products revolution has been further refined and expanded upon by later researchers and it has been found that the revolution occurred not in the Near East during the Chalcolithic and Early Bronze Age periods (~7500–4700 BP) (Gilead 1988), as Sherratt believed, but during the Pre-Pottery Neolithic (Vigne and Helmer 2007).

Animal domestication for both meat and skins and 'secondary products' was not the only significant change in human behaviour during the Pre-Pottery Neolithic, with

plants and other natural resources being managed in new ways and for new purposes. For the first time, human social and cultural interactions, as well as interactions with the natural world, would take place not in small, mobile hunter-gatherer bands but, as the Neolithic progressed, in larger, sedentary communities of agriculturalists (Moore 1998, Byrd 2005). It is no wonder much attention and research has gone into explaining the advent and progress of the Neolithic Revolution in the Near East and Europe. It provides new information not only about human diet and animal management practices, but insight into economy, work specialisation, religion, environment, culture and social structure. However, little is known about the origins of dairy milk production and use during the early Neolithic.

Where and when dairying originated has remained elusive, but the earliest evidence has been traced back to north-western Anatolia as early as 7000 years Before Christ (BC), based on ruminant milk fatty acids found during lipid analysis of potsherds from cooking vessels (Evershed et al. 2008). The high frequency of evidence from north-western Anatolia, however, points not to origination but to intensification (Evershed et al. 2008). This would seem to indicate that the origins of dairying preceded the use of cooking ceramics, placing it in the Pre-Pottery Neolithic, although after livestock domestication (sheep/goat ~11,000 and cattle ~10,000 cal. BP) (Zeder 2008). The lack of pottery for lipid analysis and the destructive nature of other types of analysis, such as nitrogen isotopes in bone and dentine to detect protein sources (meat, milk and plants) in the skeletons of humans and possible dairy animal domesticates, has made finding a new approach for detecting evidence of milk consumption important. Calcium isotopes in dental enamel may be a way to address this problem, as samples are discreet and small (~1.5 mg) and are not dependent on the presence of pottery from archaeological sites. Most importantly, calcium mineralised in skeletal tissue has only two main sources in the mammal diet, milk and plants (Chu et al. 2006, Reynard et al. 2011a). The exception to this is primarily carnivores, who probably consume calcium from bone, another excellent source (Heuser et al. 2011).

One of the key concepts for understanding isotope research is fractionation. Fractionation involves the different masses of isotopes of an element undergoing bond formation/cleavage, as part of (bio)chemical processes and reactions, and the mass-dependent bond energies of the different isotopes (Sharp 2007). Isotope mass differences have an effect on the energy requirements for participation in chemical reactions. Isotopes with higher bond energy requirements move more slowly through reactions, resulting in isotopes requiring less energy contributing in greater numbers and more quickly (Sulzman 2007). The end result may be a larger (enrichment) or smaller (depletion) number of greater mass isotopes in the reaction product than in the reactant (Reynard 2007). Isotope ratios are measured in order to detect fractionation between different materials, which provides information about the variables responsible for the fractionation, such as geochemical and biochemical processes.

Milk production in mammals has been shown to produce a calcium isotope fractionating effect between vertebrate diets and bone mineralisation (represented as $^{44/42}Ca_{diet\text{-}bone}$) (Skulan et al. 1997, Skulan and DePaolo 1999, Clementz et al. 2003). A very similar fractionation difference is seen between the diet of lactating mammals and the milk that is produced ($^{44/42}Ca_{diet\text{-}milk}$) (Chu et al. 2006). Fractionation due to (bio)chemical reactions in mammal tissues results in depletion of heavier calcium isotopes (^{44}Ca) in bone and milk/dairy foods relative to dietary input.

A limited amount of research using calcium isotope analysis of archaeological samples has been carried out, involving evidence of milk consumption by humans through the identification of plant–milk trophic differences. This was achieved by comparing human and faunal (cattle, sheep and goat) bone samples from individual archaeological sites, looking for differences that may be indicators of milk consumption, as the prevailing hypothesis was that the bones of humans consuming milk and/or dairy foods would show a depletion of ^{44}Ca compared with the bones from fauna at the same site.

However, over the course of my early calcium isotope research (Wright 2012, Hamilton et al. 2013) and the research undertaken by Reynard et al. (2010, 2011a), this approach was found to be problematic. Although calcium isotopes were able to demonstrate dietary information, the results were not necessarily indicative of milk or dairy food consumption. One of the sites demonstrating evidence of a dietary source of depleted calcium isotopes was the Epipalaeolithic site of Taforalt, Morocco, which had neither animal domestication nor agriculture, so milk could not have been the dietary source. Other dietary sources of calcium isotopes depleted in ^{44}Ca may be present, such as bone from small fish, which confounds the detection of evidence of milk and dairy food consumption. In addition, bone turnover and other physiological/metabolic processes can affect calcium isotope results and therefore require a better understanding (Reynard et al. 2010, Reynard et al. 2011a, Reynard et al. 2013). Essentially, a much better grasp of calcium isotope systematics is needed before bone can be productive in the study of diet. Dental enamel, which is also rich in calcium, was a logical tissue to analyse in its stead. Calcium isotopes in dental enamel have never been systematically analysed prior to this research, and it was therefore necessary to begin with very basic research questions and aims that could be expanded later.

1.2 Research objectives

The goal of this thesis is to detail the use of calcium isotopes in dental enamel as a dietary tracer of milk consumption and weaning timing in modern sheep, and to subsequently understand the potential of this technique for archaeological research. The usefulness of this approach has been stated by Balasse and Tresset (2002: 853): 'Evaluating the role of milk production in prehistoric subsistence economies requires a better estimation of the capacity of a milk-oriented husbandry under prehistoric conditions. Weaning pattern, which is linked to the length of lactation, is an important parameter in this estimation'. As such, this

research objective goes beyond merely trying to detect milk in the nursing and weaning diets of lambs, but to determine whether the weaning transition is natural or early.

Weaning is defined here as the transition from an exclusively milk diet to a mixture of milk and solid foods, and is complete when milk is no longer consumed. If a lamb is weaned early it is assumed that there has been either human intervention in the weaning process, and this intervention is indicative of the milk being used for human consumption, or non-human factors at play, such as ewe physiological stress and a shortened lactation length. Therefore, there is the potential to see the influence of human management and/or maternal physiological stress if early weaning in lambs is identified. If lamb-weaning timing can be detected reliably using calcium isotope ratio measurements in modern molar enamel samples from sheep with known dietary and weaning histories, the signature can potentially be used in interpreting archaeological samples.

More specifically, the identification of milk consumption and the weaning transition for sheep is achieved through analysis and quantification of the calcium isotope content in the dental enamel of first, second and third molars. Sheep molars are permanent teeth that mineralise over a period that includes birth and at least the first 2 years of life (Zazzo et al. 2010, Upex and Dobney 2012). The change in diet, from milk to plants, is identified through differences in the calcium isotope ratios ($^{44}Ca/^{42}Ca$) in the first and second molars. Enamel growth and mineralisation take place in two stages, saturation and maturation, that generally do not occur at the same time. The exception is the innermost layer of enamel at the enamel–dentine junction, which undergoes both stages of mineralisation in quick succession (Balasse 2003). Samples taken through the whole of the enamel layer reflect not a single period of mineralisation, but a succession of mineralisation events, which results in some amount of time-attenuation of the isotope signal (Balasse 2003, Zazzo et al. 2012); however, even with attenuation of the isotope signal, the chronology is preserved (Balasse et al. 2002). As a consequence of the majority of calcium

being mineralised during the second stage of enamel formation, such that possibly as much as 86% of the total calcium is obtained during maturation (Smith 1998), it is likely that the chronology largely reflects the calcium isotopes incorporated at that time.

A pattern of diet and dietary change can be seen when serial samples are taken along the length of sheep molars in segments perpendicular to the tooth growth axis. The samples can then be linked to a dental chronology, which places calcium isotope ratio results within the timeframe of a sheep's life. To do this, calcium isotope evidence of nursing and weaning was isolated through the analysis of molar samples from two modern herds with known management schedules, including dates for breeding, birth, nursing, weaning and slaughter. One important aspect of linking calcium isotope results to a dental chronology is in trying to understand the complicated timing and duration of sheep enamel mineralisation, which is discussed in detail in Chapter 3, and account for it during the interpretation of results. All of this information, along with the calcium isotope ratio values that identify evidence of fractionation between (1) a ewe's diet and her milk and (2) a lamb's dental enamel and the lamb's mother's milk, produces patterns of dental enamel that show the inclusion of milk in the nursing and weaning diets of modern sheep. These patterns, derived from modern sheep, have been used to interpret archaeological samples.

This approach is possible because mammal physiology does not synthesise calcium, so new calcium in a mammal's body is acquired through diet alone. As mentioned previously, the primary contributors to calcium in the mammal diet are milk and plant foods. Milk is depleted in ^{44}Ca relative to the diet of the milk producer, and is a source of depleted calcium isotopes for milk consumers, which is the reason why calcium isotopes have potential as a tool for tracing milk consumption. Sheep consume both milk and plants at different points in their lives, with the change from one to the other following a natural progression from birth to the age of approximately 5 months (Arnold et al. 1979, Obregón et al. 1992, Caumette et al. 2007, Chapman 2010).

Having established a nursing and weaning pattern for sheep from the two modern herds, it was then possible to apply the technique to the analysis of archaeological samples, specifically to sheep molar enamel samples from the Pre-Pottery Neolithic B (10,500–8250 cal. BP) settlement phase of Tell Abu Hureyra. Although only three sets of molars were analysed from the site, the purpose of the analysis was to identify whether it was possible to use the methodology on archaeological samples at all and, if so, to determine whether a meaningful signature could be read that could be correlated with weaning timing as set out by the modern samples.

Over the course of the research, a series of questions and topics was investigated. The use of calcium isotopes in dental enamel as a method for understanding nursing and weaning has never been undertaken before, so the questions were initially basic, and were used to organise the direction of the analysis systematically and from there generate new ideas.

Modern sheep samples

1. Are ewe milk samples depleted in ^{44}Ca relative to the diet?
2. Does the enamel have a calcium isotope content with ratios reflecting milk and plant consumption?
3. If so, is there a weaning transition?
4. If so, is the weaning transition abrupt or gradual?
5. Is it possible to link calcium isotope ratio results from serial, segmented sampling to a dental chronology?
6. If so, is it possible to identify an early weaning signature?
7. Can the calcium isotope content provide other information about the sheep's life outside of nursing and weaning, such as calcium incorporation *in utero* and movement/changes in plant diet?
8. Does sex play a role in the calcium isotope content?

Tell Abu Hureyra sheep samples

1. Does the enamel have a calcium isotope content with ratios indicating milk and plant consumption?
2. If so, is there a weaning transition?
3. If so, is the weaning transition abrupt or gradual?
4. Is it possible to link calcium isotope ratio results from serial, segmented sampling to a dental chronology?
5. If so, is it possible to identify early weaning in Tell Abu Hureyra sheep, based on the signature of modern sheep results?
6. Can the calcium isotope content provide other information about the sheep's life outside of nursing and weaning, such as calcium incorporation *in utero* and sheep movement/changes in plant diet?
7. If early weaning in sheep is indicated from the site of Tell Abu Hureyra, how do these results fit within the timeline of animal management for the purposes of identifying dairying in the archaeological record?

1.3 Sample selection

Mammals are the group of living organisms involved in dairying research. Mammals both produce (if they are female and have offspring) and partake of milk at some point in their lives. Milk and plants being the two main providers of calcium in the mammal diet, the selection of a herbivorous mammal was ideal for this research. The specific requirements for selecting the study species were:

1. rapid maturation after birth (1–2 years), because modern samples were necessary for building a foundation of information that could be used to interpret the archaeological results, and it was important to analyse samples from multiple years to identify yearly variation;

2. readily available and commonly slaughtered in the UK, so that close proximity allowed easy access to the animals for sampling;
3. a known historical use as a dairy animal;
4. known to be one of the earliest domesticates in the Near East during the early Neolithic.

Sheep met all the above criteria, and were therefore selected. The modern herds of sheep were from two locations in the UK. The first was a farm in Yorkshire. The farmer was a former zooarchaeologist who understood the research and was willing to provide the samples needed and documentary information about the life of the herd. The samples included feed samples (grass and supplementary feeds), molars and bone from male and female lambs (born in 2011 and 2012) paired with ewe milk samples (May 2010 and April 2011), and ewe bone samples also paired with milk samples. All the lambs were weaned 1 month early, at 4 months of age. The second source was from the Loft and White Hamars Grazing Project, South Walls, Hoy, Orkney, which began in the late 1980s. Both teeth and documentary information about the life of the herd were available, making it appropriate for analysis. These samples were molars from rams and wethers (castrated rams) raised only on pasture grasslands, and naturally weaned at about 5 months old. The two modern herds provided the contrast necessary to test whether differences could be seen in the isotopic signature, with one group being weaned early and the other allowed to wean naturally.

The samples from the site of Tell Abu Hureyra were selected because it is close to the region where sheep were first domesticated, and the molar samples were extracted from jaws excavated from the Pre-Pottery Neolithic B phase of the settlement, specifically from a trench dated to ~9300–7800 cal. BP (Moore 2000a). Anatolian cooking pots with evidence of milk fat residues dated from around 9000 BP have provided the earliest dates for evidence of dairying (Evershed et al. 2008), and the jaws and teeth from Tell Abu Hureyra are from a site context that brackets the Anatolian date. These jaws and teeth therefore had

a reasonable chance, based on the potsherd evidence, of being from sheep that could have been exploited for milk.

1.4 Investigating dairying in the archaeological record

As discussed earlier in the chapter, Sherratt's model of the secondary products revolution (1981, 1983) examined the human transition from only using animal domesticates as a source of meat, bone and hides (primary products), to also include products repeatedly produced by a living animal (secondary products). Sherratt's secondary products fell into three groups: milk, wool and traction (the use of animal strength to pull or carry). Sheep provide two of the three key secondary products: wool and milk. The first domesticates (~11,000 BP) would have been morphologically like their forbearers (Legge and Rowley-Conwy 2000), namely the wild mouflon (*Ovis orientalis*) native to the Taurus–Zagros regions (Pedrosa et al. 2005, Zeder 2008, Zeder 2011), with similar life cycles (time of rut, breeding, lambing and weaning) (Santiago-Moreno et al. 2000, Garel et al. 2005, Santiago-Moreno et al. 2005a, Pipia et al. 2008).

It is believed there were at least three distinct episodes of sheep domestication in the Taurus–Zagros regions, based on mitochondrial (mt) DNA results, which show three distinct maternal lineages from three different populations of subspecies of wild mouflon sheep (Hiendleder et al. 2002, Pedrosa et al. 2005, Tapio et al. 2006, Zeder 2011). The regions where these domestication events probably took place, and the sub-species associated with those regions, are: eastern Iran (*Ovis gmelini isphahanica* and *Ovis gmelini laristanica*); north-western Iran and eastern Turkey (*Ovis gmelini gmelini*); and central Anatolia (*Ovis gmelini anatolica)* (Hiendleder et al. 2002, Pedrosa et al. 2005). Although DNA research has established the probable locations for these domestication centres, sheep bone assemblages excavated at archaeological sites in the Near East provide the most direct evidence for a domestication timeline, and the assemblages allow zooarchaeological

assessment of sheep mortality profiles that may indicate domestication. As such, evidence from the Near East was chosen for use in this research, as physical samples were required.

Assessing milk's contribution to the economies of ancient communities and societies has traditionally been researched using a limited number of techniques. These methods include: (1) zooarchaeological modelling of mortality/slaughter/culling profiles (Zeder 2008, Polák and Frynta 2009, Zeder 2011); (2) use of nitrogen and carbon isotopes in cattle dentine to isolate the weaning transition and determine whether it occurred early or naturally (Balasse et al. 2001, Balasse and Tresset 2002), as well as nitrogen isotopes in archaeological cattle bone and dentine to establish weaning timing, and, once linked to the cattle's culling ages, identify a post-lactation slaughter profile (Gillis et al. 2013); and (3) the chemical analysis of residues contained in cooking potsherds (Evershed et al. 2008, Salque et al. 2013). These different methods are discussed in more detail in this section.

1.4.1 Mortality profiles and culling patterns

Traditionally, morphometric traits have been used to identify livestock domestication at Neolithic archaeological sites in the Near East. The most significant indicator was taken to be a reduction in sheep body size, in what was believed to be a direct result of domestication. It is now known that domestication does not affect sheep size in females, although it does affect males to a small degree, resulting in a reduction in sexual dimorphism (Zeder et al. 2005, Polák and Frynta 2009). Although morphology alone is not evidence for domestication, sexual dimorphism does allow the creation of age- and sex-specific mortality profiles (Zeder 2011). Over the last 30+ years, many different models have been designed to explain the age and sex distributions in sheep (as well as goats, cattle and pigs) in the archaeological record. These models are used to infer human hunting strategies, domestication timing and processes, herd maintenance and specialised herding strategies related to secondary products, such as dairy milk production. The work by Payne

(1973) presented three explanative models (A, B and C) for archaeological assemblages of sheep and goat skeletal remains.

Payne (1973) suggested that a meat production model (model A) would involve the majority of sheep slaughter happening when sheep were between the ages of 18 and 30 months (with ~30% of the herd slaughtered). Males would be slaughtered in increasing numbers between 1 (~30% slaughtered) and 3 years old (~90% slaughtered) until, at 4 years old, only a tiny percentage remained alive, with no males alive over 8 years old. Females would be slowly and steadily slaughtered (~5–10% slaughtered in each age group per year), as fertility declined, with no female over the age of 10 surviving in the herd. This creates a culling profile, after the first initial kill off of both male and female lambs, with prime meat males being killed as young adults and females as older adults.

Payne's (1973) model C related to the culling of a wool production herd, where the slaughter of both males and females would resemble that of the females in model A. The short initial kill off of both male and female lambs (~30% of total slaughtered) would transition into a slow steady slaughter of both males and females (~5–10% slaughtered in each age group per year) until there were no sheep older than age of 10 years.

The most relevant model to this thesis is Payne's (1973) model B, the dairy production model. Unlike the meat and wool models, there is a significant slaughter of lambs, mostly males (~90% males and ~10% females slaughtered), within the first few months of life. Male numbers then steadily decline until the sheep are 3 years old, and then only a tiny percentage are kept alive. Females would again be steadily slaughtered (~5–10% slaughtered in each age group per year) as fertility declined, with no female sheep over the age of 10 surviving. Essentially, the management of females remains the same in all three models, and the evidence of which type of herd production model is being expressed is visible in the age at death of the male sheep.

Payne's (1973) model B is an idealised model, with the herd focused not only on dairying but also on creating product surplus. His model does not take into account a more complicated, subsistence or mixed product approach to herd management. Vigne and Helmer (2007) have created a complementary alternative to model B, a 'production' model, that accounts for non-surplus, subsistence or 'exploitation' dairying (Figure 1.1).

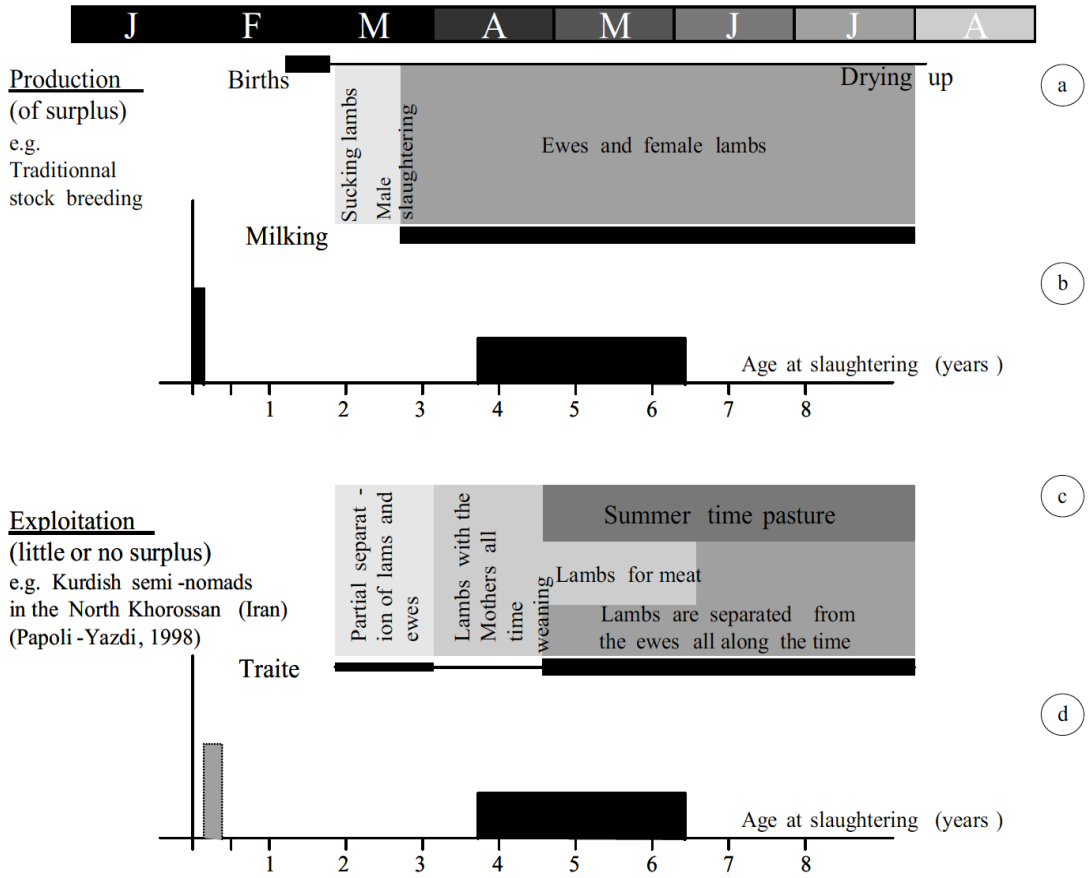

Figure 1.1: Payne's (1973) production (model B) and Vigne and Helmer's (2007) exploitation dairying models. The patterns of sheep herd management (circled a and c) and the imagined or possible archaeological slaughter/culling profile (circled b and d) are shown. The black milking/trait bar shows roughly the amount of milk taken for human consumption from March to August. Image reproduced from Vigne and Helmer (2007: 22).

Vigne and Helmer (2007) refer to, as an example of the milk exploitation model, the ethnographic work by Papoli-Yazdi, who studied Iranian Kurdish semi-nomadic herding methods. The Kurds are dairy sheepherders and have a highly refined herd management strategy. During the first month after birth, lambs and ewes are kept together

except for a few hours a day, with humans and lambs sharing the available milk, which initially has a low fat content. During the second month, ewes and lambs are kept together with no milk exploitation by humans, so the lambs get all the milk they need during a period of rapid growth. During the third month, the herd is moved to pastures rich enough to produce milk with a high fat content, suitable for making butter. At this time the lambs are either slaughtered, or weaned and kept alive to become part of the dairy herd or for future meat consumption. The age-at-death slaughter profile proposed by Vigne and Helmer (2007) is very similar to Payne's (1973) milk model, but clearly the herd management systems (production/surplus versus exploitation/subsistence) generating the profile are very different.

Payne (1973) fully acknowledged that his models may not cover all the possible reasons for why and at what age an animal was killed. Herds 'are not usually kept for a single product, particularly in subsistence economies; the balance drawn between the conflicting requirements of the approaches described above depends on the relative importance of the different products, which is determined in a subsistence economy by the needs of the family or group, or in a cash economy by market forces' (Payne 1973: 282). However, his models do form a good foundation to build or expand on, as Vigne and Helmer (2007) and others have done, and mortality profiles provide important clues for identifying animal management approaches in the archaeological record.

Modelling the mortality profiles of sheep is very useful for interpreting calcium isotope ratios from archaeological enamel samples pertaining to ancient dairying. The modelling of mortality profiles and calcium isotope analysis of dental enamel to investigate nursing and weaning are complementary approaches that both rely on the zooarchaeological assessment of age at death, sex and the taxonomic separation of sheep and goat, which is especially important when analysing samples from the Near East, where both sheep and goat were (and are) ubiquitous. Sheep and goat have similar skeletal morphologies, but very

different dietary behaviours, with sheep tending to be grazers and goats browsers (Balasse and Ambrose 2005, Vigne and Helmer 2007). Humans may also have exploited them for different reasons, for example wool from sheep and meat from goats. Sheep and goat samples analysed together may mask these differences. Mortality models could act as a check for calcium isotope results for supporting, countering and/or adding complexity to the findings.

1.4.2 Investigating nursing and weaning using nitrogen isotope analysis

Analysing mammal tissues for nitrogen isotope content, specifically the collagen content of skeletal tissues in archaeological samples, is the most common method for researching nursing and weaning in the archaeological record. The method is based on detecting the nitrogen isotope ratios (^{15}N) in collagen (bone and dentine), which is routed through proteins in animal diets. ^{15}N is enriched in animal bodies through preferential excretion of ^{14}N in urea, resulting in tissues more enriched in ^{15}N than the items in the animal's diet (Hedges and Reynard 2007). Not only is nitrogen enriched in an individual animal's tissues, it has been seen to have a step-wise increase with each trophic level (Schoeninger and DeNiro 1984, Hedges and Reynard 2007, O'Connell et al. 2012). Therefore there is the potential for the ^{15}N content of collagen from skeletal tissues from archaeological sites to provide trophic-level information about the protein diet of an individual in the past (Balasse and Tresset 2002, Hedges and Reynard 2007). Additionally, nitrogen isotope composition of bone collagen and tooth dentine is able to provide information about nursing length because breast feeding and nursing produce a trophic-level effect between mothers and infants (Fogel et al. 1997, Schurr 1997, Wright and Schwarcz 1999, Dupras et al. 2001, Richards et al. 2002, Fuller et al. 2003, Clayton et al. 2006, Jay et al. 2008, Nitsch et al. 2011, Howcroft et al. 2012).

Two studies, Balasse et al. (2001) and Balasse and Tresset (2002), have worked with nitrogen isotopes to collect information about weaning timing/length in modern and

archaeological cattle. First, modern cows were studied (Balasse et al. 2001) from an experimental farm with controlled diets. From birth to 7 months old, the calves were kept with their mothers and allowed to nurse freely. The mothers were fed with C_3-only plants and feeds, and at 7 months old the calves, along with their mothers, were moved to C_3 grass pastures. The calves were progressively weaned off milk and onto grass, with full separation/weaning occurred between 9 and 10 months old. Once weaning was complete, the calves were fed a mixed C_3/C_4 diet until they were slaughtered at 17–18 months old. Although weaning was gradual (occurring in the early months after birth through to the removal of the calves from the mothers), the introduction of the mixed C_3/C_4 diet was very abrupt.

The nature of dentine formation means that there is an overlap of older and newer dentine, reflecting an equilibrium process. In this study (Balasse et al. 2001), the process tempered the abrupt feeding change from the C_3-only to the mixed C_3/C_4 diet, and produced results demonstrating a gradual dietary change over a number of months. Although enamel does not develop in the same manner as dentine, it will also show a mix of older and younger mineralised calcium, which is something that has to be considered during interpretation. The conclusion of Balasse et al. (2001) was that, although it was difficult to determine the rate of dietary change through nitrogen isotope analysis of dentine, dietary change linked to nursing and weaning was discernible, and there was potential for archaeological application.

Balasse and Tresset (2002) applied this approach to cattle excavated from the Neolithic site (~ 4000 BC) of Bercy (Paris) in France. The dentine nitrogen isotope ratios of first and second molars, compared with modern cattle (Balasse et al. 2001), showed that Bercy Neolithic calves were possibly weaned early, but the results could alternatively reflect a shorter natural lactation length for Bercy Neolithic cows. If weaning was artificially induced, the shorter weaning length would be evidence of humans wanting a

greater proportion of the cows' milk. The ultimate conclusion was that more work was needed at Bercy and other Neolithic sites to confirm the pattern, although there was every indication that nitrogen analysis of tooth dentine in cattle has the potential to identify weaning timing in calves.

A recent study by Gillis et al. (2013) utilised $\delta^{15}N$ values of bone and dentine collagen, in conjunction with cattle-culling profiles, to uncover evidence of the mixed use of cattle herds at the fifth millennium cal. BC site of Borduşani-Popină, Romania, for both milk production and meat exploitation. Specifically, the $\delta^{15}N$ values showed that calves tended to survive until a post-lactation slaughter. A post-lactation slaughter is indicative of an animal management style where humans depend on the presence of offspring during milking in order to get the mother or dairy animal to let-down her milk. The culling profile of calves at Borduşani-Popină fit the profile of a post-lactation slaughter, with the calves tending to be old enough to be going through the weaning process or having only recently completed it at the time of death. Additionally, the culling profile of adult cattle at the site tended to be between the ages of 4–9 years, which suggests possible dairy cows that have passed their milk production or reproductive prime. Gillis et al. (2013) thought it likely that calves and herders were sharing the milk produced by the cows and that, once the usefulness of the calves for milking was over, the calves were slaughtered for meat. This speaks to a sophisticated system of herd management and animal utilisation.

Nitrogen isotopes have an established history of being used in weaning studies, and pairing calcium isotope analysis of enamel with nitrogen isotope analysis of dentine has the potential to create useful complementary approaches. As will be discussed in Chapter 2, Reynard et al. (2013) did pair calcium and nitrogen isotope data from the bone of human infants/juveniles and adults, to look for age- and diet-related relationships. From the Turkish archaeological site of Aşıklı Höyük, it was determined that the non-adults were significantly depleted in ^{44}Ca and enriched in ^{15}N relative to the adults, and the calcium

isotope results were probably interrelated with the nitrogen findings. At Aşıklı Höyük, the factor most likely affecting the isotopes in the bone of non-adults was childhood nursing, but these findings were tempered by the results from Spitalfields in the UK, which had non-adults enriched in ^{15}N relative to adults but no depletion of ^{44}Ca between the ages. The research by Reynard et al. (2013) involved bone, which is subject to the calcium regulatory process, making it difficult to predict and interpret calcium isotope content.

It has also been suggested that nitrogen isotope analysis, combined with strontium/calcium (Sr/Ca) ratio analysis, could be used to detect the addition of foods other than milk to the diet (Katzenberg et al. 1996, Mays 2003). Calcium isotopes could be used in place of Sr/Ca, as changes in enrichment and depletion of ^{44}Ca results reflect diet, including the transition from milk to plants during weaning. Analysis of enamel content for calcium isotopes, and dentine content for nitrogen isotopes together, might be a fruitful area for future work because both approaches are tracing different constituents of the same food, milk, and can provide clues to understanding nursing and weaning/lactation length.

1.4.3 Chemical analysis of Neolithic artefacts for dairy milk residues

While the zooarchaeological modelling of mortality profiles, and the use of nitrogen isotopes, has potential for direct integration with data derived from calcium isotope analysis, it is unlikely that milk residues on potsherds could be used to create a milk calcium isotope value for comparison with enamel results. This does not diminish the importance of lipid residue analysis of potsherds as an approach for investigating ancient dairying. In fact, this method has been able to provide the earliest evidence of Neolithic dairying in the Near East, along with cheese making in northern Europe (Evershed et al. 2008, Salque et al. 2013).

Until recently there were no artefacts from the Neolithic that could be linked directly to milk and dairying. This changed with the chemical analysis of milk fat residues from potsherds of Neolithic cooking ceramics (Copley et al. 2005, Craig et al. 2005,

Evershed et al. 2008, Salque et al. 2013). This research has pushed the earliest date for Neolithic dairying in the Near East to as early as 7000 BC (Evershed et al. 2008). The technique of using lipid residues to identify milk fat on potsherds from cooking vessels is possible because the fatty acids contained in mammal fats have stable carbon isotope values specific to milk, ruminant adipose and non-ruminant adipose, allowing the fats to be distinguished from each other (Copley et al. 2005, Evershed et al. 2008). 'The approach rests upon differences in the $\delta^{13}C$ value of the $C_{18:0}$ (in $C_{x:y}$, x is the number of carbon atoms in the fatty acid, and y is the number of double bonds) fatty acid of milk and carcass fats' (Evershed et al. 2008: 528). The carbon isotope differences are created because carcass fat and milk fat are generated by different dietary routes of carbon, resulting in distinct $^{13}C/^{12}C$ ratios (Evershed et al. 2008).

This method is the only one that shows direct evidence of milk and milk processing at archaeological sites. Although such data cannot be integrated directly with calcium isotope research, the data from potsherds containing milk fat residues will be useful for identifying future sites for investigation using calcium isotope analysis. It will also be beneficial in the future if the methodology is able to detect sheep as well as cattle milk fat residues in potsherds.

Taken together, these methods provide important clues about animal management and dairying in the archaeological record. Each, along with calcium isotopes in dental enamel, approaches questions regarding animal use by ancient people from different, divergent starting points. But however they are arrived at, gaining new insights into animal domestication and dairying has implications that extend beyond the individual topics towards a better understanding of one of the most important events in human prehistory: the transition from hunting and gathering to agropastoralism.

1.5 Potential benefits of calcium isotope analysis

Detecting nursing and weaning timing through the analysis of enamel $^{44/42}Ca$, in bulk and sequential samples from sheep molars, has the potential to add to our understanding of the lives of ancient sheep and the factors that affected nursing length/weaning timing. To be of the greatest value, zooarchaeological assessment is also important for determination of sex and age at death, as well as the taxonomic separation of sheep and goat. All of the current methods, including zooarchaeological mortality models, nitrogen isotope analysis of dentine for weaning information and chemical analysis of ceramic residues, have vital roles. They are complementary approaches, all contributing to the debate of ancient dairying, but using calcium isotopes has benefits over some of these other techniques. These benefits include: (1) only a small sample size is needed for analysis (~1 mg of powdered enamel) in comparison with carbon and nitrogen analyses; (2) the use of enamel, which is less compositionally altered by diagenesis in the burial environment compared with dentine (Kohn et al. 1999, Dauphin and Williams 2004, Zazzo et al. 2010); (3) damage to the teeth is limited, unlike the collagen extraction process for nitrogen and carbon analyses, to individual sample locations on the tooth; and (4) the methodology is not limited to archaeological periods and sites containing ceramic artefacts, which came into use approximately 2000 years after sheep domestication is currently thought to have occurred (Aurenche et al. 2006, Zeder 2008).

1.6 Chapter summary

Work to understand Childe's Neolithic Revolution continues today, and can be seen in the efforts of researchers to uncover new and better approaches to unlocking the transition from the Epipalaeolithic/Mesolithic hunter-gatherer approach to subsistence, to Neolithic farming. While much is now known and understood about the Neolithic, continued research has produced far more questions than answers, especially within Sherratt's (1981, 1983)

secondary products revolution and, in particular, the origins and development of the use of milk in the food economies of ancient peoples.

With or without lactase persistence, sheep and other dairy animals provided ancient people with a potential food source rich in nutrients that was seasonally renewable and without the limitation and finality of slaughter. There is also supporting evidence that a desire for milk and other secondary products produced the opportunity for imposing genetic selection, adding another level of complexity to animal management (Pereira et al. 2006, Taberlet et al. 2008, Kijas et al. 2009). Evidence of early dairying in the Near East has been found in residues in cooking potsherds, which supports the idea that ancient humans did take advantage of livestock milk production for their own benefit, but this technique is limited to periods when pottery was in use. As the majority of potsherd evidence looks more like intensification of a maturing tradition, rather than the invention of a new subsistence practice (Evershed et al. 2008), the implication is that the origins of dairying predates the ceramic residue evidence, and is therefore older than the advent of pottery itself (Aurenche et al. 2001).

How then can dairy milk production and consumption be investigated if ceramics are not available to be analysed? The research in this thesis details the development of a new method with potential application for studying possible Neolithic dairying (as well as non-human influences on lactation and weaning timing) when ceramics may or may not be available, by establishing the calcium isotope content of sheep dental enamel and from there determining periods of nursing and weaning. This is a new approach that required substantiation through analysis of modern sheep prior to application on archaeological samples. The modern sheep used to develop the methodology were selected because of the availability of background information regarding birth and death dates, lamb weaning timing and ewe lactation length as well as diet during nursing, weaning and as mature animals. For one of the two modern herds, milk and feed samples were analysed in

conjunction with the dental enamel. To refine the timing of the results, the oxygen and carbon isotope contents of the dental enamel were measured as part of identifying dietary (carbon) and seasonal (oxygen) change linked to sheep enamel formation. Once the modern nursing and weaning results and data were combined, it was possible to see a pattern of dietary change related to nursing and weaning. The successful outcome of the modern analysis meant the methodology could be tested on archaeological samples, and archaeological sheep teeth from the early Neolithic part of the settlement of Tell Abu Hureyra in Syria were analysed. The teeth were specifically selected from the period of the site (Moore 2000a) concurrent with the oldest dates for milk residues on cooking ceramics (~7000 BC) (Evershed et al. 2008) and the site was chosen because of its close proximity to the region currently believed to be the centre for sheep domestication, the Taurus–Zagros area (Hiendleder et al. 2002, Pedrosa et al. 2005, Tapio et al. 2006, Zeder 2011).

Dental enamel was chosen for analysis after bone proved to be too unpredictable for reliably identifying milk consumption, probably because of homeostatic processes and remodelling. Bone and dentine are also more susceptible to diagenetic alteration than dental enamel, and dental enamel does not remodel once mineralisation has been completed. This provides a chronology of enamel isotope results that, even with the complicated two-stage mineralisation process involved in enamel formation, can link a sheep's diet and life to enamel formation.

There are only a few existing methods for investigating dairying: zooarchaeological mortality profiles; identifying weaning timing using nitrogen isotope analysis of bone and dentine; and chemical residue analysis of potsherds. Each contributes to the Neolithic dairying discussion; however, the addition of a new method that can work in conjunction with other methods, but is not reliant on them, can only be useful. Calcium isotope analysis of dental enamel has the potential to be this new method.

CHAPTER 2

CALCIUM ISOTOPE RESEARCH

This chapter will begin by presenting the key concepts of calcium homeostasis, including a definition of homeostasis and which organs are involved, before moving on to introduce isotopes. Even though calcium is the focus of this research, the analysis includes oxygen and carbon isotopes to augment the interpretation of some of the calcium results. The principles of isotope fractionation will be outlined, as well as the isotopic offsets found in the biosphere. Finally, this chapter will discuss those aspects of isotopes most relevant to research questions concerning diet/food, mineralised tissues and milk, including the work done by Dr Linda Reynard. Reynard and associates have applied what is known to date about bone, milk and calcium isotopes to questions concerning dairying and human milk consumption in the archaeological record. This application was my original research focus, and some of my results are discussed in this chapter along with Reynard et al.'s (2010, 2011a, 2013), but it also became the impetus for a change in direction that led to the research now detailed in this thesis: the investigation of ancient sheep diet and lamb nursing and weaning timing, through incremental sampling of dental enamel and the determination of calcium isotope ratios. Ratios determined by the source of calcium in the diet enable the identification of milk (indicative of nursing animals), plant foods (adult animals) and a mix of the two (weaning animals) in the diet. The goal was to determine whether ewes in ancient herds were possibly managed for 'secondary product' (milk) purposes or not, through the identification of early or abnormal weaning patterns in their lambs. This was investigated by comparing enamel calcium isotope ratio results from modern sheep, with known diets, ewes with known lactation lengths and lambs with known weaning ages, to enamel calcium isotope ratio results from sheep identified from archaeological sites. This is a new and unique approach to investigating the lives of sheep in the archaeological record as well as possible human management of those sheep.

2.1 Calcium and calcium isotopes: an introduction

Although biological calcium isotope research is still largely experimental, it does have advantages over other areas of isotopic research, as it can be used to detect possible consumption of plants and/or milk, which are the two greatest providers of calcium in the mammal diet. This is because of the systematic calcium isotope differences seen when looking at the isotope ratios of the two dietary items. When comparing their isotope ratios with each other, plants, milk, bone and dental enamel consistently show enrichment or depletion of greater mass calcium isotopes (^{44}Ca). This means calcium isotopes can be used as a tool for addressing questions relevant to diet and dietary change.

Bone is the focus for the majority of this chapter, because bone acts as a calcium reservoir in the vertebrate body and is therefore at the centre of calcium homeostasis. It is also the tissue known to produce the greatest diet–vertebrate tissue isotopic offset, and is therefore the most important factor influencing bone calcium balance, as well as calcium isotope ratios. Diet- and organism-specific factors such as sex and age also have to be considered. Age is relevant, as calcium metabolic processes differ throughout the course of a vertebrate's life, especially early and late in life. Sex, especially for mammal females during pregnancy and lactation, requires a significant, often sacrificial, change in calcium balance. All of these factors affect bone calcium balance and isotope ratios. Additionally, the information gained about calcium isotopes in bone was the foundation for initiating this brand-new research into calcium isotopes in dental enamel. Factors that alter bone calcium isotopes may or may not also affect dental enamel; however, both are calcium-rich mineralised tissues and bone is the only one of the two that has had any significant history of analysis.

2.2 Mammal calcium homeostasis

Homeostasis occurs in higher order animals such as mammals and is the preservation of stable internal physiological conditions, such as mineral balance and blood pH, as a defence

against changeable external, environmental conditions. Calcium is an essential dietary element for vertebrates, and greatly abundant in mineralised tissues. Bone acts as a reservoir for calcium homeostasis and is extremely important, along with the intestinal absorption of dietary calcium and urinary excretion, in maintaining proper calcium levels in blood plasma. Calcium homeostasis affects all aspects of bone calcium balance and calcium isotope incorporation into vertebrate tissues (further details of calcium homeostasis can be found in Appendix A).

Calcium, once absorbed into the body through the intestines, has many functions as part of calcium homeostasis, which in turn is key to overall vertebrate homeostasis. Calcium is very important for heart, thyroid, nerve and muscle function, and is found in skin, hair, blood, muscle and especially mineralised tissues (Menon et al. 1985, McDonald et al. 1994, Kempson et al. 2003, Hirata et al. 2008, Aloia et al. 2010). The mammalian body is composed predominantly of water, carbon and some nitrogen (oxygen ~65%, hydrogen ~10%, carbon ~18% and nitrogen ~3%), but there is also a significant quantity of calcium (1.2–1.5%) (Miller et al. 1991, McDonald et al. 1995). Calcium is certainly the most abundant mineral, with 98–99% being contained in the skeletal tissue, while the majority of the remaining 1–2% is spread throughout the body and is tightly regulated in the blood plasma (8.0–12.0 mg/dL or 2.0–3.0 mmol/L) and extracellular fluid (Sharp 2005, National Research Council 2007).

The calcium homeostatic cycle begins with the calcium levels in the blood plasma. Calcium levels in blood plasma are kept within a very narrow range and any fluctuations are instantly detected and compensated for, through the release of parathyroid hormones (PTH) that in turn activate the major sites of calcium regulation, the bone, kidneys and intestines (Rizzoli and Bonjour 1999, Ball 2004, National Research Council 2007, Gardella et al. 2010) (Figure 2.1). It is particularly important that bone acts as a reservoir for calcium, because calcium from the diet is not supplied continuously and/or may not be of

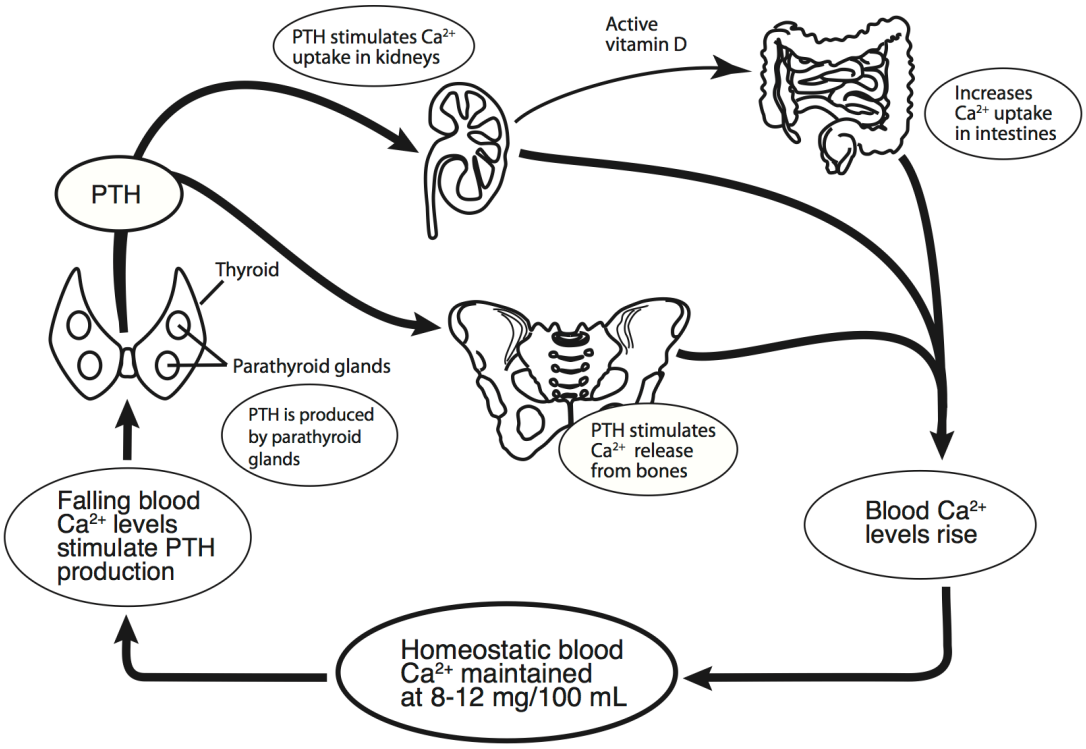

Figure 2.1: Calcium homeostasis.

sufficient quantity to meet homeostatic needs. The kidneys regulate calcium excretion and re-uptake, while also producing vitamin D-based hormones that stimulate an intestinal absorption of calcium.

Some of the calcium absorbed from the diet is utilised directly in maintaining the bone calcium balance, replacing any that has been lost to homeostatic regulation. This is largely what makes bone a dynamic tissue that changes over time. It should be noted, however, that the bone balance can be disrupted by factors such as old age and prolonged bed rest, resulting in more bone being lost than is replaced, such as with osteoporosis. Calcium homeostatic processes therefore also affect the movement and incorporation of the different isotopes of calcium.

2.3 Stable calcium isotopes: background and definition

Atoms of an element have, in equal amounts, a fixed number of protons and electrons, with the configuration of electrons influencing the overall chemical properties of an atom.

Neutrons are contained along with protons in the nucleus of the atom, but unlike protons and electrons the numbers of neutrons in an atom are not fixed. While protons do not have any effect on the atom's chemical properties, the varied neutron numbers in atoms may result in compounds with small chemical and physical differences. These atoms with differing numbers of neutrons are called isotopes, with most elements having two or more naturally occurring isotopes (Sharp 2007). Even though the focus of this chapter is calcium, the information in this section is applicable to isotopes in general.

Although neutrons have no charge, they do add mass to an atom, and therefore the different isotopes of an element have different masses (Sharp 2007). It is this that makes the current research possible. The mass differences have an effect on how much energy is required for the different isotopes to participate in chemical reactions, with isotopes requiring higher energy tending to move more slowly through the reaction, or only undergoing a chemical reaction after the isotopes requiring less energy have completed the reaction process (Sulzman 2007). The result of this chemical reaction may be a larger or smaller number (or amount) of isotopes with a greater mass, compared with the starting material. This behaviour is called fractionation, and can be seen in a wide range of biological, atmospheric, oceanic and geological chemical processes (Sharp 2007, Sulzman 2007, Clementz 2012). Because of the fractionation that occurs in the natural world, an element's isotopic ratios are not uniform, and it is these ratios that can be found using mass spectrometry analysis.

Calcium has six naturally occurring stable isotopes, ^{40}Ca, ^{42}Ca, ^{43}Ca, ^{44}Ca, ^{46}Ca and ^{48}Ca. The global natural abundance of calcium isotopes is ^{40}Ca = 96.941%, ^{42}Ca = 0.647%, ^{43}Ca = 0.135%, ^{44}Ca = 2.086%, ^{46}Ca = 0.004% and ^{48}Ca = 0.187% (de Laeter et al. 2003). The calcium isotopes monitored in this research were ^{42}Ca, ^{43}Ca and ^{44}Ca, as part of determining the ratios of ^{44}Ca/^{42}Ca and ^{43}Ca/^{42}Ca. The mass difference between ^{42}Ca, ^{43}Ca and ^{44}Ca is very small, resulting in fractionation effects that are also very small. These were compared with the isotopes of carbon and oxygen, which have larger mass differences between their isotopes. Depending on the instrumentation used in the analysis, the results

are given as $\delta^{44/40}Ca$ or $\delta^{44/42}Ca$. The results measured as $\delta^{44/40}Ca$ are double the magnitude of $\delta^{44/42}Ca$ (Reynard et al. 2013).

2.4 Isotopes: analysis and notation

After samples have been analysed for calcium isotopes using a multicollector–inductively coupled plasma–mass spectrometer (MC-ICP-MS), the results are reported as the ratios of $^{44}Ca/^{42}Ca$, $^{43}Ca/^{42}Ca$ and $^{44}Ca/^{43}Ca$. Once the isotope ratios for a sample have been determined, a mathematical formula known as the delta formula is applied. Delta (δ) values are a standard way of reporting isotopic data based on the formula:

$$\delta = ((R_{sample} - R_{standard})/R_{standard}) \times 1000.$$

In this formula, R is the ratio (or abundance) of heavy to light isotopes (Sharp 2007). The sample isotope ratio is compared with or normalised to the isotope ratio of a standard. This allows the δ-value to be reported in parts per thousand, which is typically denoted as per mil (‰) (Sharp 2007). Use of the delta formula magnifies small relative differences between the isotopic ratios of samples and standards (Sharp 2007), which is especially important for isotopes from heavier elements that have small isotopic mass differences. All data results discussed in this research are reported as δ-values, indicating that the results represent the measure of heavy versus light isotopes found in a sample, relative to a standard with a known isotope ratio, and the unit used is per mil (‰). The standards used in this research were NIST SRM 915a (915a), NIST SRM 915b (915b), Alfa Aesar (Alfa), Fisher07, High-Purity Standards calcium (HPS_{new} Calcium), International Association for the Physical Sciences of the Oceans (IAPSO) seawater and an Oxford in-house archaeological bone standard. These standards, with the exception of the bone standard, are geologically based.

The epsilon formula is used to analyse fractionation. Fractionation is shown as an offset between substrate and product, as a result of the physical properties inherent in isotopes with different atomic masses; this is denoted as epsilon (ε) (Passey et al. 2005).

This formula can be used, for example, to determine the isotopic offsets between the diet (δ_A) of a mammal and that mammal's dental enamel (δ_B) (the fractionation of δ_A relative to δ_B) (Passey et al. 2005). This isotopic offset or ε-value is determined by using the results of both the delta formula and the alpha formula (Passey et al. 2005):

$$\alpha_{A-B} = (1000 + \delta_A)/(1000 + \delta_B)$$

then

$$\varepsilon_{A-B} = (\alpha_{A-B} - 1) \times 1000.$$

If the epsilon formula is denoted as ε*, the implication is that the fractionation is not associated with chemical equilibrium (Cerling and Harris 1999). This is relevant because there are two main types of fractionation: equilibrium and kinetic. Equilibrium fractionation is where the substrate or product of a chemical reaction has the majority of the greater mass isotopes. This is because the bond strength is greater for isotopes with a greater mass, or, conversely, bonds of isotopes with a lower mass are more easily broken (Ben-David and Flaherty 2012). The key point of this type of fractionation is that it is not permanent: the reaction is reversible (Ben-David and Flaherty 2012). Kinetic fractionation, which is the type of fractionation presented in this thesis, is more pronounced than equilibrium fractionation, and involves either a non-reversible/unidirectional chemical reaction, or a change of phase for a single type of molecule (solid to liquid) (Sulzman 2007). Interactions between molecular velocity and bond strength affect kinetic fractionation (Sulzman 2007). The fractionation resulting from evaporation, dissociation reactions, diffusion and enzymatic processes are all examples of kinetic fractionation (Sulzman 2007, Ben-David and Flaherty 2012).

2.5 Calcium isotopes: distribution in the geosphere

The initial source of calcium in the diet of terrestrial mammals is found in soil and freshwater, and there is little variability in the calcium isotope ratios between these different sources. Water sources have a maximum $\delta^{44/40}$Ca range of ~1‰ between rain/snow, river,

ground and hydrothermic waters (Schmitt and Stille 2005). Additionally, analysis of precipitation has shown that atmospheric calcium, even in close proximity to sea and ocean sources, is derived from carbonate minerals, generally resulting in very similar calcium isotope values between freshwater and rock samples (Hippler et al. 2003, Schmitt and Stille 2005). Differences have been reported for the Ganges River depending on whether samples were collected in the dry or wet season (Schmitt et al. 2003), but these differences are small and still fall within the normal ranges calculated for precipitation and freshwater (Schmitt et al. 2003, Schmitt and Stille 2005). It is possible to distinguish freshwater from seawater. Seawater is the most enriched in heavier calcium isotopes of all geologic materials and has a largely uniform calcium isotope content due to a long residence time (~1 Ma) (Skulan et al. 1997, Zhu and MacDougall 1998, Blättler et al. 2011). Similar to freshwater, rock and soil have been found to have little variation in $\delta^{44/40}$Ca values, with a maximum range of difference of ~1‰ (Figure 2.2).

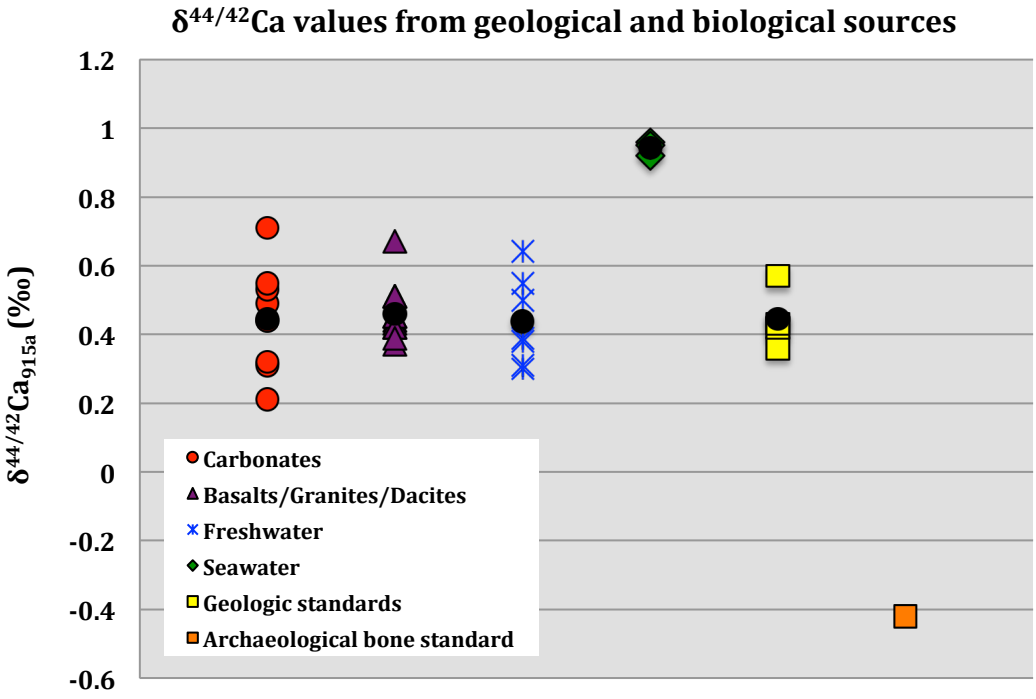

Figure 2.2: $\delta^{44/42}$Ca values for rock, soil, freshwater and seawater (data from Schmitt et al. 2003, Kasemann et al. 2005, Chu et al. 2006, Tipper et al. 2006, 2008, Reynard et al. 2010, Hindshaw et al. 2011). Some results were converted from $\delta^{44/40}$Ca to $\delta^{44/42}$Ca as well as being adjusted to reflect the standard 915a (see Chapter 4 for conversion between standards). Included in the yellow and orange datasets are the standards discussed in Section 2.4: Fisher07, HPS_{new} Calcium, Alfa (geologically based standards) and the in-house Oxford archaeological bone standard (Reynard et al. 2010).

It has been reported that 'calcium isotopic variations in nature are systematic and largely controlled by biological activity' (Skulan et al. 1997: 2505). Overall, both seawater and geological samples are enriched in greater mass calcium isotopes relative to biological samples, meaning ^{44}Ca is found in greater abundance in geological and especially seawater samples than in samples from biological organisms (Skulan et al. 1997, Schmitt et al. 2003, Kasemann et al. 2005, Tipper et al. 2006, 2008, Reynard et al. 2010, Hindshaw et al. 2011). This demonstrates that calcium isotopes with greater masses involved in biological processes are discriminated against, or depleted through, biological fractionation, especially during mineralisation processes (Skulan et al. 1997). The enrichment found in seawater is not the result of an external input of calcium isotopes of greater mass, such as from weathering or river runoff, but to the preference by marine animals to incorporate calcium isotopes of lesser mass during mineralisation processes (Skulan et al. 1997). Over millions of years, this has resulted in a significant enrichment of heavier calcium isotopes in seawater, making it the most enriched of all calcium sources on Earth (Skulan et al. 1997). The isotopic composition of seawater has been largely uniform over time, as is the case with modern seawater (Blättler et al. 2011).

2.6 Vertebrate tissues and calcium isotopes

Published research involving calcium isotopes and biological systems is extremely limited, and there are significant gaps in understanding. This section will primarily discuss important case studies involving research into calcium isotope behaviour related to calcium-regulating/homeostatic functions in vertebrate, especially mammal, physiology. This section also focuses on bone, because bone, compared with dietary input, has a much greater calcium isotope offset (fractionation) than between diet and soft tissues.

Plants and organisms have a preference for utilising calcium isotopes with less mass during biological processes, especially for mineralisation processes (Chu et al. 2006, Von Blanckenburg et al. 2009). Although there is evidence of fractionation occurring

between diet and muscle tissue, the difference is small, and generally muscle tissue reflects the calcium isotopic values of the diet (Skulan and DePaolo 1999). As a result, calcium-rich mineralised tissues, such as bone and shell, have become the most relevant tissues for studying calcium isotopes in organisms, although of course bone needs to be considered with regard to the overall homeostatic process as well as its interaction with the other tissues within an organism. What is known about vertebrate tissue mineralisation and calcium isotopes does not currently include calcium movement and biochemistry at the cellular level. Some unknown cellular and biochemical process is generating the fractionation during bone mineralisation: the nature of this process remains unclear at this time. It is, however, largely uniform throughout the skeleton, removing the need to select bone samples from a single skeletal element or from a particular location on a bone (Chu et al. 2006, Reynard et al. 2010). Ratios of calcium isotopes in bone are expected to reflect 10 or more years of human life or, for shorter-lived mammals, a lifetime average (Nanci and Ten Cate 2008, Reynard et al. 2010).

Although the cause(s) of calcium isotope fractionation during mineralisation is not understood, the effect can be seen. The comparison of calcium isotope ratios found between two groups of samples, such as the offsets between diet and organism, or organism and organism, can be used to address biological fractionation processes. This means trophic differences in a food chain can be identified, as they essentially represent the relationship between diet–bone mineralisation of different organisms and species. Isotopic offsets between vertebrates of the same species can also detect differences between sub-groups, such as age and sex.

Specific to bone and other vertebrate tissues, there is a 1.3‰ ($\delta^{44/40}Ca$) or 0.64‰ ($\delta^{44/42}Ca$) isotopic offset between bone and diet, with bone being depleted in greater mass calcium isotopes (Skulan and DePaolo 1999, Hirata et al. 2008, Reynard et al. 2010). Additionally, for $\delta^{44/42}Ca$ there is a slight difference of 0.17‰ between diet and soft tissues (Reynard et al. 2010). These calcium isotope ratio offsets have been seen between the bone

and muscle tissue of deer (Chu et al. 2006), mice (Hirata et al. 2008), fish, horse, seal and chicken (Skulan and DePaolo 1999) (Figure 2.3). Although it is known that mineralisation processes are responsible for generating the isotopic offset of calcium isotopes between diet and bone (Skulan et al. 1997, Skulan and DePaolo 1999), there is no consensus about what aspect of the mineralisation process results in the fractionation (Lemarchand et al. 2004).

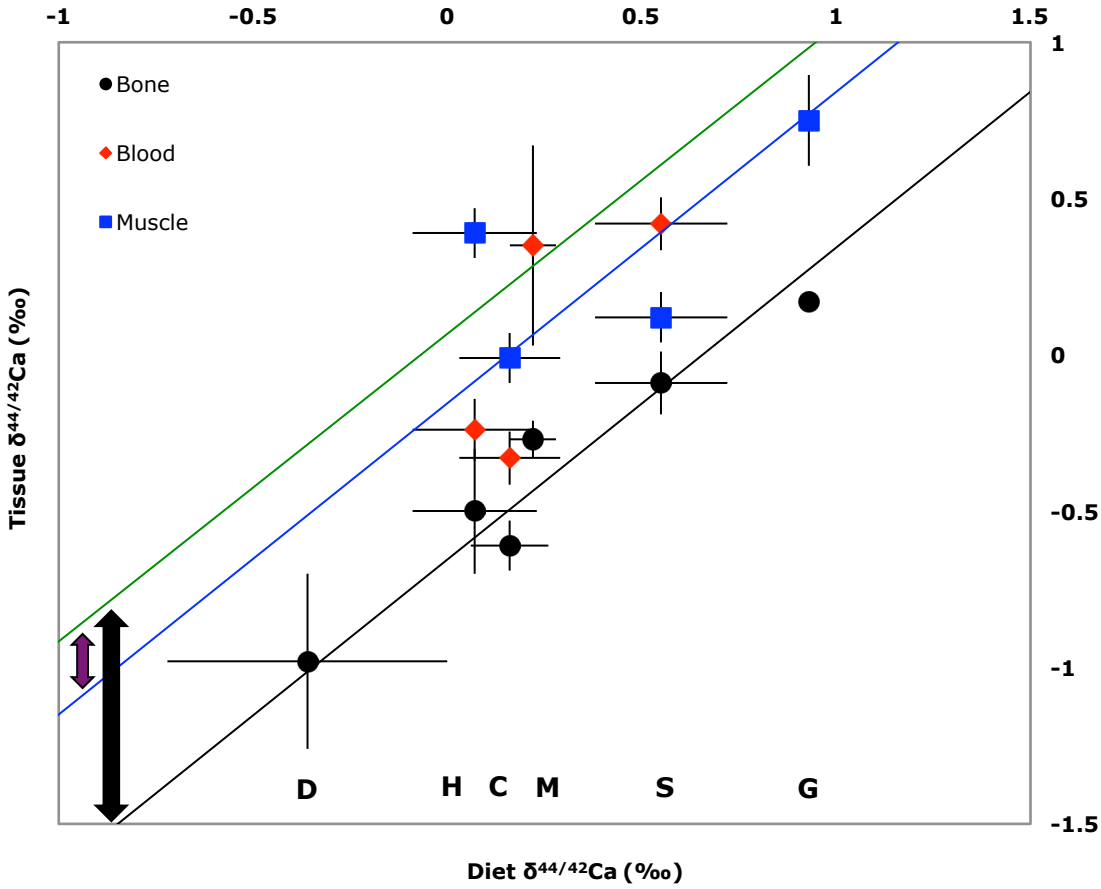

Figure 2.3: $\delta^{44/42}$Ca findings for animal tissues of bone, muscle and blood versus the animal's diet. The horse (H, *n* = 1), chicken (C, *n* = 1), seal (S, *n* = 1) and fish (G, *n* = 1) (Skulan and DePaolo 1999) were reported with 2 standard deviations of error (SD). The mice (M, *n* = 4) (Hirata et al. 2008) and deer (D, *n* = 3) (Chu et al. 2006) were reported with 2 SD based on the mean results. Additionally, the lines represent the averages of bone (black), soft tissue (muscle and blood) (blue) and diet (green). The black arrow reflects the $\delta^{44/42}$Ca diet–bone offset of 0.64‰ and the purple arrow reflects the $\delta^{44/42}$Ca diet–soft tissue offset of 0.17‰ reported by Reynard et al. (2010). The graph is based on Reynard et al. (2011b).

Typically it has been found that blood calcium isotope ratios fall in the range between muscle and bone, although this can vary significantly between animals of the same species (mice) as well as between the muscle tissue and bone of an individual animal (Skulan and DePaolo 1999, Hirata et al. 2008). The variability in blood arises from the

calcium retention time (the amount of time spent in the tissue), which can be years in bone, days in muscle, and hours in blood (Hirata et al. 2008). This variability is also indicative of the blood's role in correcting calcium imbalances and in maintaining the body's calcium homeostasis, which may result in blood containing calcium isotopes from the diet, the bones of the skeleton (depleted in ^{44}Ca relative to the diet) or both (Hoenderop et al. 2005, Hirata et al. 2008). Biological fractionation within an individual organism is important, but calcium isotopes were only first utilised in archaeological science when individual organisms from different trophic levels of a food chain were compared and distinguished based on their calcium isotope ratios. This was the initial direction for the research presented in this thesis, and will be discussed in detail in Section 2.7.

In acknowledging that biological processes discriminate against greater mass calcium isotopes during mineralisation, resulting in depleted $\delta^{44}Ca$ bone values relative to diet, the path was paved for using calcium isotopes to detect trophic differences between organisms (Skulan et al. 1997). The transition from plants, which tend to have $\delta^{44/40}Ca$ values close to the geological average, to herbivores, shows a plant–herbivore isotopic offset with a depletion of greater mass calcium isotopes of between 0.8‰ and 1.5‰ (Skulan et al. 1997, Skulan and DePaolo 1999). Similarly, evidence of trophic differences can be seen in the calcium isotope ratios ($\delta^{44/40}Ca$) of the bone of omnivorous freshwater turtles and the bone of their main predator, carnivorous freshwater alligators, with a 1.5‰ depletion of ^{44}Ca in the alligator bone (Skulan et al. 1997). Both of these examples demonstrate trophic differences in terrestrial and freshwater environments, but there is also evidence from marine environments based on the trophic differences, seen by Clementz et al. (2003). Their research compared the trophic levels of seals and fish, and found a uniform depletion of greater mass calcium isotopes in the seals relative to fish ($\delta^{44/40}Ca_{fish–seal}$ offset of about 1.7‰). The results of their study confirmed that dietary information was contained in the bones and teeth of marine mammals, and could be used to distinguish 'herbivorous and molluscivorous marine mammals from higher trophic level carnivores, and can therefore

provide information on relationships among consumers within marine foodwebs' (Clementz et al. 2003:35).

Recently, calcium isotope studies detecting trophic differences in food chains have involved fossil bone and teeth. The work by Heuser et al. (2011) used bone and dental tissue samples from herbivore and carnivore dinosaurs, as well as a modern bird and a modern reptile, to research trophic differences. However, their results were not able to demonstrate systematic trophic differences between herbivorous and carnivorous dinosaurs, as the offset between the two groups was small. They felt, in agreement with Clementz et al. (2003) and Clementz (2012), that this result is probably explained by the lack of an isotopically depleted ^{44}Ca food source, i.e. bone, in the carnivorous dinosaurs' diet.

It is common in modern carnivores for little or no bone to be consumed, even when large quantities of meat are eaten, such as with cheetahs (Schubert et al. 2010), which is problematic for calcium isotope research seeking evidence of food chains. Heuser et al. (2011) did see a general isotopic offset between the $\delta^{44/42}Ca$ values of mammals and reptiles/birds, with mammals being more depleted in ^{44}Ca than reptiles/birds. They speculated that the reason mammal bone tends to be more depleted is due to systematic differences between mammal and reptile reproductive strategies and thermophysiology. However, important variables were not included in the results, namely the $\delta^{44/42}Ca$ values for the mammal and reptile diets, which may or may not account for some of the differences.

Significant to this thesis is that Heuser et al. (2011) also specifically analysed both bone and dental tissues, which had previously only been undertaken a few times, and never methodically. Their work involved the systematic analysis of mammal tissues, including dental enamel, to understand reptile/bird/dinosaur calcium isotope metabolic processes better (Heuser et al. 2011). They concluded that studying food chains across different ecosystems or using all calcium isotope data together irrespective of source location and

environment, i.e. combining all data together, tends to obscure trophic differences that would be more readily apparent in local food chains.

The use of calcium isotopes to investigate trophic differences and food chains has therefore had mixed success. Similarly mixed results have been found when studying calcium isotope ratios in bone from sub-groups within a species. Nevertheless, this area of research has provided additional information relevant to understanding factors apart from diet that may influence calcium isotope fractionation during mineralisation. A recent study of modern male and female sheep from a single herd with identical diets (as part of identifying differences attributable to the animal's sex) found that females and males of the same species have different physiological requirements, which result in differing levels of enrichment or depletion of calcium isotopes in the bones. The female sheep of this particular herd were found to be 0.14 ± 0.08‰ [$\delta^{44/42}$Ca, 2 standard deviations of error (SD)] more enriched in ^{44}Ca relative to the males (Figure 2.4).

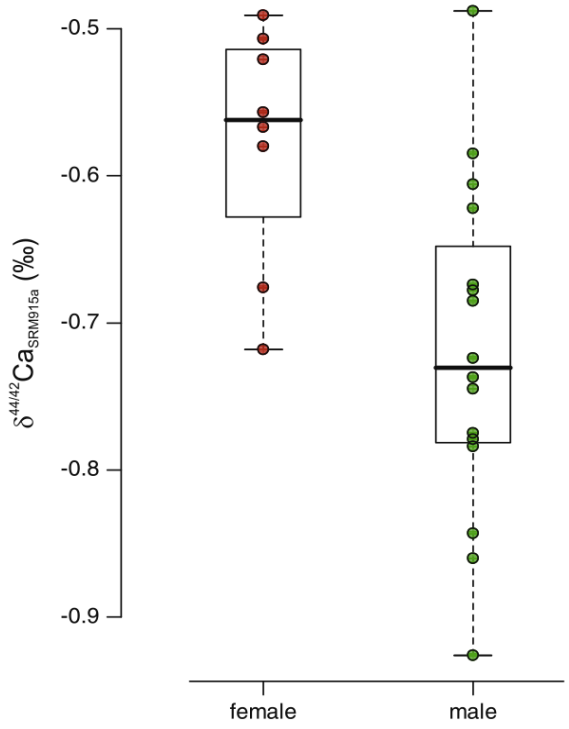

Figure 2.4: Graph from Reynard et al. (2010) detailing $\delta^{44/42}$Ca results for a modern herd of sheep separated by sex.

This significant difference seen between the sheep sexes (ANOVA, $p = 0.004$) (see Chapter 4 for information on the statistical techniques used) was probably due to physiological differences between males and females and/or the fact that the females had nursing lambs. Because milk is a significant source of calcium in the diets of infants and growing mammals (as well as the diets of adult humans when lactose is not an issue), it draws calcium from the physiological stores of the milk-producing mammal (Sanford 1992, Cross et al. 1995, Kovacs and Kronenberg 2006, Gardella et al. 2010).

Chu et al. (2006) found that through a combination of either the release of calcium isotopes with less mass from a mammal mother's bones, and/or an unknown calcium isotope fractionating process that occurs during milk production, a diet–milk isotopic offset is produced. Specifically, Chu et al. (2006) found that for modern sheep there is a $\delta^{44/42}$Ca diet–milk offset of 0.54‰ and 0.62‰ ($n = 2$). Milk is depleted in ^{44}Ca relative to diet, and as calcium from the mother's skeleton is known to be released during lactation to provide (along with dietary calcium) the calcium found in her milk (Kovacs and Kronenberg 2006), it is logical to assume that at least some of the depleted calcium isotopes found in milk are from the mother's skeleton. This is borne out by the results illustrated in Figure 2.4.

Other research investigating differences between and within the sexes, this time involving humans, has not been as promising. Restrictions on research involving modern human tissue samples creates a host of difficulties, and as a result archaeological samples have been used instead. This is not an issue in and of itself, especially when the research inquiry is archaeological, but it would be beneficial to have samples from modern people, with known life histories, for comparison. Reynard et al. (2010, 2011a) investigated human bones found at the Epipalaeolithic site of Taforalt in Morocco, the Epipalaeolithic–Neolithic site of Tell Abu Hureyra in Syria, the Neolithic sites of Magura in Romania and Balatonszárszó in Hungary, and the Iron Age site of Danebury in England. It was found that the $\delta^{44/42}$Ca difference seen between adult human males and females behaved similarly in both degree and direction as the sheep discussed above, with a $\delta^{44/42}$Ca difference in means

of 0.15‰, although a high variance ultimately contributed to there being no significant difference (t-test = –1.29, df = 20, p = 0.21). Thus it was possible to regard all adult humans equally, but it also meant that sex was not a significant enough factor in biological processes to be distinguishable through calcium isotope analysis.

Reynard et al. (2013) went further in researching sex and the factors that might be expected to influence female calcium isotope ratios, by analysing bones from the site of Christ Church Spitalfields, England. The excavated individuals were identified by grave markers and had personal information recorded, such as the number of births for women and age at death for all. The number of births (along with assumed nursing of an unknown length of time for each infant) proved to have no effect on maternal skeletal calcium isotope ratios.

Part of Reynard et al.'s (2013) research also analysed bone samples from women and men from the Anatolian Neolithic site of Aşıklı Höyük. It was found that males and females were not significantly different (t-test, p = 0.54), and it was therefore unlikely that pregnancy and lactation had had an effect on female $\delta^{44/42}$Ca values at this site. It was concluded by Reynard et al. (2013), with regard to both Spitalfields and Aşıklı Höyük, that pregnancy and lactation (unlike the previously discussed, shorter lived, modern sheep) did not alter the calcium isotope ratios in the women's skeletons to a large enough degree to be detectable over the course of their lives.

Although sex was shown in modern sheep to produce statistically significant differences that were not seen in humans found at archaeological sites, it was thought that age might be a factor that could distinguish groups of humans from each other through calcium isotope analysis. Age and calcium homeostasis/bone balance has been studied using calcium isotopes by monitoring the calcium isotope composition of urine. Only about 2% of the calcium released by the blood and entering the kidneys ends up being excreted in urine (Hoenderop et al. 2005). The rest is reabsorbed and remains part of the homeostatic process (Hoenderop et al. 2005).

A number of studies have used the changes in stable calcium isotope ratios in urine to monitor bone mineral balance. This is used in both clinical and research settings to detect changes to the calcium isotope composition in urine in human subjects whose bone mineral balance has been disrupted, especially with regard to instigating bone loss. Bone calcium equilibrium and homeostasis is usually dependent on age. Growing bone in infant and juvenile vertebrates requires significant amounts of dietary calcium, with little calcium being released from the bone. Elderly vertebrates release more calcium from their bones than is incorporated, resulting in a negative bone calcium balance. Pregnancy and especially lactation are also sources of bone calcium imbalance, with the mother's bones helping to supply calcium to the developing foetus during the last months of pregnancy and during the production of milk (Sanford 1992, Cross et al. 1995, Kovacs and Kronenberg 2006, Gardella et al. 2010) (Figure 2.5) (see Appendix A, Section A.3.2).

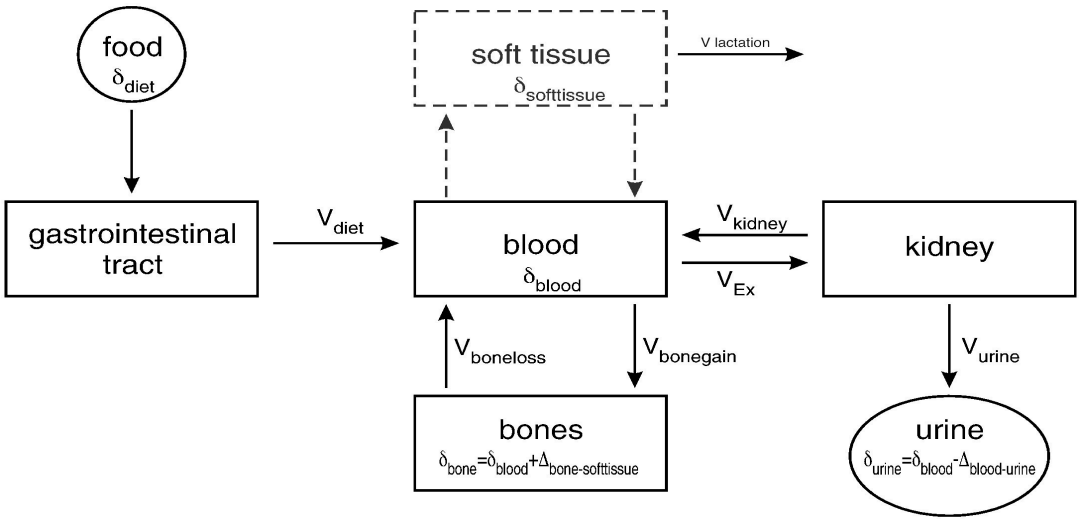

Figure 2.5: A simple illustration of calcium isotope movement in the mammal body (Skulan and DePaolo 1999, Chu et al. 2006, Heuser and Eisenhauer 2010, Reynard et al. 2010). Heuser and Eisenhauer (2010) used V to label the calcium fluxes between boxes; the δ-values correspond to the specific Ca isotopic composition of a certain box and the Δ-values correspond to the isotope difference between compartments due to fractionation processes.

The vast majority of calcium isotope urine studies have been undertaken on humans in an effort to understand and treat osteoporosis. These studies have a side benefit of helping our understanding of some of the complexity of the relationship between calcium

homeostasis, bone and calcium isotopes. One of the studies most relevant to this research is by Heuser and Eisenhauer (2010), who investigated age and dietary input. Although too small in comparison with the diet–bone isotopic offset to create a noticeable impact, it is worth noting that Heuser and Eisenhauer (2010) showed evidence for the first time of a possible fractionation effect in the glomerular filtrate and residue in the kidneys (see Appendix A, Section A.3.1). This finding demonstrates that, although mineralisation is by far the most significant source of fractionation in vertebrate tissues, it is not the only one.

Even more relevant are Heuser and Eisenhauer's (2010) findings for a 60-year-old woman with osteoporosis and a 4-year-old boy. Firstly, it was determined that the calcium content in their diets fell within the range of error for analysis (~ ± 0.10‰), based on modelling the European diet calcium average of 14% and Chu et al.'s (2006) food results. This meant the subjects could be considered equally, and that physiological processes (probably age-related, rather than diet-related) would be the source of calcium isotope offsets.

Secondly, neither subject was in bone equilibrium (bone loss = bone gain) and between them they represented the extremes of bone loss and gain: the young, healthy boy gaining calcium in his bone through growth, and the older woman losing bone calcium through osteoporosis. Applying this information towards understanding bone calcium metabolic processes as a result of urine analysis, and modelled in relation to bone calcium equilibriums (bone gain/bone loss versus $\delta^{44/42}Ca_{urine}$), the boy's urine results showed bone gain (an average calcium concentration of 87 mg/L and a $\delta^{44/42}Ca$ result of 0.74‰) and the woman's results showed bone loss (an average calcium concentration of 165 mg/L and a $\delta^{44/42}Ca$ result of 0.18‰). The growing boy was shown to have an enrichment of ^{44}Ca in his urine, reflecting the fact that isotopes with lesser mass in the diet were probably being used for extensive bone mineralisation. The research by Heuser and Eisenhauer (2010) uncovered not only a possibly unaccounted for (small) source of calcium isotope

fractionation in the kidneys but also that age influences the calcium isotope ratios in human bone.

Reynard et al. (2010, 2011a) looked at human age as part of their research at the sites of Taforalt, Tell Abu Hureyra, Magura, Balatonszárszó and Danebury. Age categories were assigned based on human osteological assessment, and grouped as infant, child, young adult, mid-adult, old adult and a non-specific category of adult. Similar to what was seen for sex at these sites, there was no significant difference associated with human age. Nor at these sites was there any evidence that pairing sex and age had an effect (ANOVA, p = 0.49).

As previously discussed by Heuser and Eisenhauer (2010) in their research involving calcium concentration and isotope ratios in urine (as a means of detecting calcium loss or gain in bone), a growing boy was shown to have an enrichment of greater mass calcium isotopes in his urine relative to both his diet and bone equilibrium constant, reflecting the fact that the isotopes with lower mass from the diet were probably being used for bone growth. The results for this boy were significantly different to those from the older woman with osteoporosis, but it is not clear how bone growth affects the bone $\delta^{44/42}Ca$ results. The main part of the study by Reynard et al. (2013) addressed this idea. Reynard et al. (2013) were interested in determining whether, assuming the quantity of dietary calcium is uniform across ages, dietary calcium sources from plants (enriched in ^{44}Ca) or milk (depleted in ^{44}Ca) could be detected in bones from infants and children from archaeological sites (Spitalfields and Aşıklı Höyük) compared with adults from the same sites.

Milk provides a supply of calcium that is depleted in ^{44}Ca, relative to the diet of the milk producer, by between 0.54‰ and 0.62‰ (this is discussed in further detail in Section 2.7) (Chu et al. 2006). Additionally, human milk has been found to have the lowest $\delta^{44/42}Ca_{915a}$ values of a variety of milk samples analysed, with results between –0.98‰ and –1.15‰ (n = 4) (Chu et al. 2006), although the mothers' diet was not included in the study so no diet–breast milk offset is known. As a result, humans that consume milk are expected to

have lower $\delta^{44/42}$Ca values compared with non-milk consumers (Reynard et al. 2010, 2011a), and nursing children might be expected to have lower $\delta^{44/42}$Ca values compared with non-nursing children (Reynard et al. 2013). This information, in regard to milk being a source depleted in ^{44}Ca, is the basis for using calcium isotope ratio results in dental enamel to study sheep weaning.

The findings for Aşıklı Höyük showed a significant difference between infants/juveniles and adults. The children were significantly depleted in ^{44}Ca relative to the adults (*t*-test, $p = 0.002$), and when $\delta^{44/42}$Ca was plotted against the nitrogen isotope δ^{15}N there was a clear correlation ($r^2 = 0.51$, $p = 0.001$). Even when one infant outlier was removed, the correlation remained ($r^2 = 0.39$, $p = 0.01$). However, Spitalfields showed no correlation with age ($r^2 = 0.03$, $p = 0.41$) or δ^{15}N ($r^2 = 0.006$, $p = 0.72$).

The conclusions reached by Reynard et al. (2013), with regard to both infant/juvenile milk consumption and bone growth effects on calcium isotopes, were that the infant/juvenile $\delta^{44/42}$Ca results from the site of Aşıklı Höyük were probably related to δ^{15}N, and that both sets of isotopes were responding to a common factor or input, probably milk. At Spitalfields, however, while the δ^{15}N infant/juvenile results did indicate milk consumption, the $\delta^{44/42}$Ca adult and children results were similar.

The different findings for Aşıklı Höyük and Spitalfields mean that other factors affecting calcium isotope ratios in bone need to be taken into account, such as bone growth. Growing bone requires a different level of dietary calcium input and, as indicated in the study by Heuser and Eisenhauer (2010), homeostatic processes are different for children, and changes in calcium isotope ratios may be indicative of those differences. Skulan and DePaolo (1999) considered that, as bone production/mineralisation exceeds bone resorption during growth, dietary calcium should be utilised to a greater degree than would be seen with bone in equilibrium. This means that the normal diet–bone calcium isotope offset of about 0.65‰ (Skulan and DePaolo 1999, Chu et al. 2006) would be reduced in growing bone. This is because calcium loss is low while the use of dietary calcium is high, resulting

in calcium isotope ratios enriched with ^{44}Ca in growing bone, compared with bone in equilibrium.

Reynard et al. (2013) found that bone growth was not likely to be a large enough factor to obscure other influences on calcium isotopes in bone. Additionally, when bone growth and questions of milk consumption were considered together, and in view of the dissimilar results from Spitalfields and Aşıklı Höyük, it was thought that, as bone growth decreased, so did consumption of breast milk (a dietary source depleted in ^{44}Ca). Plants (enriched in ^{44}Ca relative to milk), in the absence of dairy milk, would become the primary contributor of calcium to the diet of growing children. These two simultaneous and opposing influences on the $\delta^{44/42}Ca$ ratio could result in the impact of either factor being masked or muted. The ultimate conclusion regarding bone growth and infant/juvenile milk consumption at these two archaeological sites was that, although the $\delta^{15}N$ results demonstrated milk consumption, the $\delta^{44/42}Ca$ ratio results showed that factors other than milk may be affecting bone.

These results were the reason why the modern lambs and ewes studied in this research were also analysed for bone calcium isotope ratios. Although it was a small sample group, it was done to add to the debate regarding diet, bone status (growth vs. equilibrium), pregnancy/lactation and calcium isotopes. Additionally, understanding calcium isotope incorporation in growing bone is important for trying to understand calcium isotope incorporation in growing and maturing dental enamel. Unlike bone, dental enamel is not part of calcium homeostasis and is not 'in equilibrium' once it finishes growing. It was expected that, like immature bone, dental enamel during mineralisation would be influenced by growth, by having calcium isotope ratio values closer to those of the diet. The modern bone and enamel results are discussed in Chapters 5 and 6.

2.7 Calcium isotopes: human consumption of dairy milk in the archaeological record

The research by Reynard et al. (2013) did not discuss the effect of dietary calcium on adult bone or bone in homeostatic equilibrium, but some of their earlier research had, as part of investigating human consumption of dairy milk and foods in the archaeological record (Reynard et al. 2010, 2011a). The foundation for that and subsequent research (Reynard et al. 2013), as well as my research of both dental enamel and bone, was the work by Chu et al. (2006), along with the idea of milk providing a trophic difference between the producer and consumer. Chu et al. (2006) identified the first evidence of isotopic offsets between dietary input and milk production using two ewes from two farms. The result, as previously mentioned, was a $\delta^{44/42}Ca_{diet-milk}$ offset of 0.54‰ and 0.62‰, which is similar to the diet–bone offset of ~0.64‰ seen by others (Skulan and DePaolo 1999, Hirata et al. 2008, Reynard et al. 2010).

In the research of Reynard et al. (2010, 2011a), calcium isotope analysis was undertaken on human and faunal bones from archaeological sites to determine whether a calcium isotope signature indicative of dairy consumption could be seen. The idea was to investigate trophic differences between fauna (mainly dairy animals) and humans based on an attempt to detect isotopic offsets between dairy producers and consumers. The basic premise was that, compared with the plants that both humans and dairy animals would have consumed, milk and dairy foods provide not only the greatest source of calcium in the human diet, but also a food source depleted in ^{44}Ca. As a result, long-term adult human dairy milk consumers should be distinguishable from the non-dairy consuming humans and fauna within an archaeological assemblage.

Reynard et al.'s (2010, 2011a) research focused primarily on the periods leading up to and including the Neolithic, because the Neolithic people lived in a time of significant dietary change, as mobile hunting and gathering gave way to sedentary farming and animal domestication (discussed in Chapters 1 and 7). The Neolithic gave rise to new human approaches not just to subsistence but to all the aspects of human life, which became

possible by shaping nature to human needs instead of relying on what nature provided.

As well as Aşıklı Höyük, the Epipalaeolithic, Neolithic and Iron Age archaeological sites discussed in Section 2.6 were also used in the study of human milk/dairy food consumption by Reynard et al. (2010, 2011a). Trophic differences seen in bone samples indicative of milk and dairy consumption needed to be interpreted for each site individually, because of a $\delta^{44/42}$Ca difference of 0.4‰ between the average faunal results at the sites. Reynard et al. (2010) attributed these faunal bone differences to differences in the environment of each site, which, according to Chu et al. (2006), would have affected the specific, local diets.

Reynard et al. (2010, 2011a) found that, with the exception of the site of Balatonszárszó, humans at each site were more depleted in ^{44}Ca relative to the fauna. The most significant human–faunal offsets were seen for the sites of Tell Abu Hureyra (0.29‰), Taforalt (0.24‰) and Danebury (0.41‰) (Figure 2.6). The humans at all of the sites had a much greater range of results compared with the fauna. An example of this is the site of Taforalt, where the standard error of the mean (SEM) for humans was 1.5–3 times larger than that of the fauna. The different species of fauna at each site did not demonstrate a statistical difference between animal groups, which meant that possible dairy-producing fauna could be grouped and considered together. The uniformity of the faunal results, especially of possible dairy animals, seemed to indicate that the calcium isotopes in these animals' diets were from a narrow, uniform source, probably plant based or, in the case of the cheetah, muscle and intestinal contents from local plant-consuming animals. Reynard et al. (2010) also looked at the meat contribution to the human diet as a possible source of the $\delta^{44/42}Ca_{human–faunal}$ offset at the sites of Taforalt (0.24‰), Tell Abu Hureyra (0.29‰), Magura (0.12‰) and Danebury (0.41‰), but it was found that meat was unlikely to be the source of the offsets because the amount of meat needed to be consumed to provide the offset would have been unrealistic.

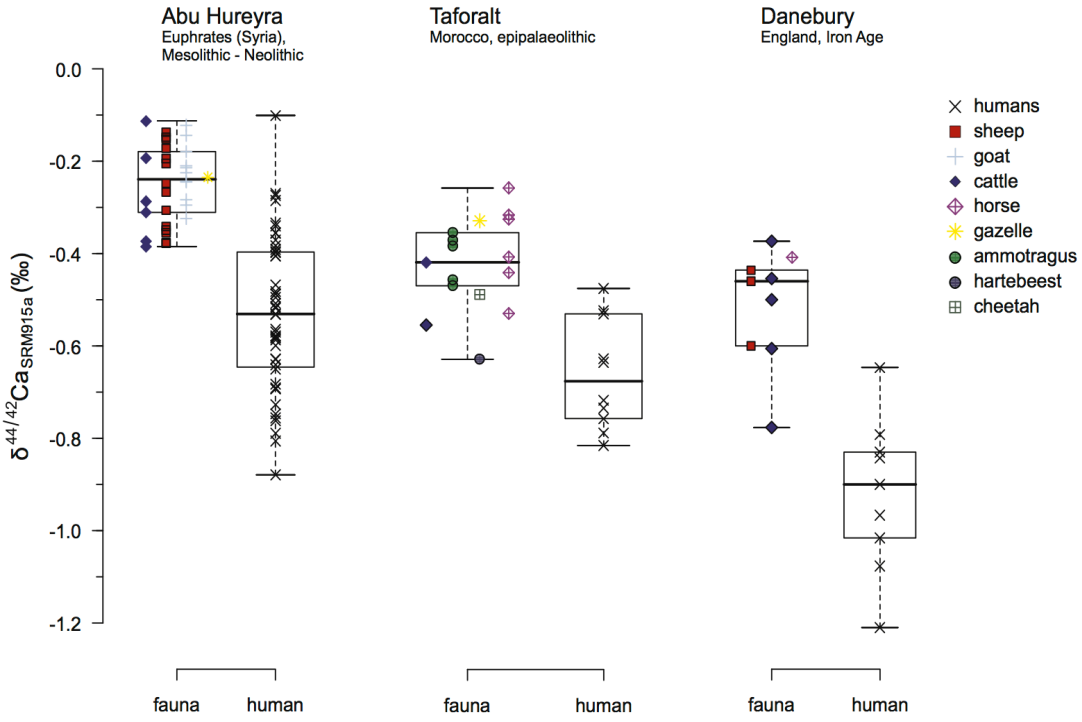

Figure 2.6: Graph from Reynard et al. (2011a) detailing $\delta^{44/42}$Ca results of humans and fauna from the sites of Tell Abu Hureyra, Taforalt and Danebury. The fauna show an enrichment of $\delta^{44/42}$Ca relative to the humans, although the humans show greater $\delta^{44/42}$Ca diversity than the fauna.

Unlike the offsets seen with the sites discussed previously, there was no mean difference between the human and faunal remains found at the Linearbandkeramik (LBK) Neolithic site of Balatonszárszó (Reynard et al. 2010). This same pattern was seen by me when analysing the bones from human males, cattle and sheep/goat from the LBK Neolithic site of Ensisheim, France (Table 2.1 and Figure 2.7): the mean results were not significantly different (ANOVA, $p = 0.98$) (Table 2.2). There was also no significant difference between the means of the human males and the fauna (combined cattle and sheep/goat data) (t-test, $p = 0.93$) (Table 2.3). However, a high variation was seen between the human males from Ensisheim, with 1 SD being 0.13‰. This high variance in the Ensisheim human results was consistent with the findings for all the humans from the archaeological sites studied by Reynard et al. (2010, 2011a). Because of one outlying data point, a similarly high variation from the mean was also seen for the Ensisheim sheep/goat (SD = 0.11‰).

Table 2.1: Calcium results for LBK Ensisheim bone samples (1 SD and 1 SEM)

Cattle	**$\delta^{44/42}$Ca (‰), 1 SD**	**$\delta^{43/42}$Ca (‰), 1 SD**	***n***	**Human males**	**$\delta^{44/42}$Ca (‰), 1 SD**	**$\delta^{43/42}$Ca (‰), 1 SD**	***n***
LBK 347	–0.81, 0.05	–0.38, 0.05	8	LBK 303	–0.57, 0.04	–0.25, 0.06	5
LBK 348	–0.71, 0.05	–0.36, 0.08	8	LBK 308	–0.99, 0.03	–0.48, 0.07	7
LBK 351	0.68, 0.06	–0.33, 0.08	3	LBK 310	–0.71, 0.06	–0.36, 0.08	9
LBK 353	–0.85, 0.09	–0.44, 0.08	3	LBK 311	–0.93, 0.02	–0.55, 0.05	6
LBK 359	–0.66, 0.09	–0.33, 0.07	3	LBK 313	–0.80, 0.05	–0.42, 0.05	7
	Mean (‰), 1 SE	**Mean (‰), 1 SE**		LBK 317	–0.56, 0.05	–0.27, 0.08	9
	–0.74, 0.04	–0.37, 0.02		LBK 318	–0.91, 0.06	–0.50, 0.03	3
				LBK 320	–0.84, 0.09	————	1
				LBK 330	–0.69, 0.04	–0.34, 0.07	4
				LBK 331	–0.71, 0.02	–0.46, 0.00	2
Sheep or goat	**$\delta^{44/42}$Ca (‰), 1 SD**	**$\delta^{43/42}$Ca (‰), 1 SD**	***n***	LBK 332	–0.64, 0.01	–0.23, 0.06	3
LBK 346	–0.83, 0.05	–0.41, 0.05	7	LBK 333	–0.76, 0.00	–0.40, 0.04	2
LBK 349	–0.59, 0.03	–0.33, 0.07	8	LBK 335	–0.75, 0.01	–0.34, 0.07	3
LBK 350	–0.81, 0.04	–0.39, 0.06	6	LBK 336	–0.43, 0.02	–0.22, 0.09	4
LBK 352	–0.79, 0.02	–0.41, 0.08	3	LBK 338	–0.74, 0.08	–0.33, 0.06	5
	Mean (‰), 1 SEM	**Mean (‰), 1 SEM**			**Mean (‰), 1 SEM**	**Mean (‰), 1 SEM**	
	–0.76, 0.06	–0.38, 0.02			–0.73, 0.04	–0.37, 0.03	

Table 2.2: ANOVA results for human males, cattle and sheep/goat from Ensisheim, France

	Sum of squares	**df**	**Mean square**	***F***	***p*-value**
Between groups	4.8469E-04	2	2.4234E-04	0.002	0.98
Within groups	0.2714	19	1.4285E-02		
Total	0.2719 21				

Table 2.3: Result of the *t*-test on the combination of humans and fauna from Ensisheim, France

SD	***t***	**df**	***p*-value**
0.117	–0.896E-01	20	0.93

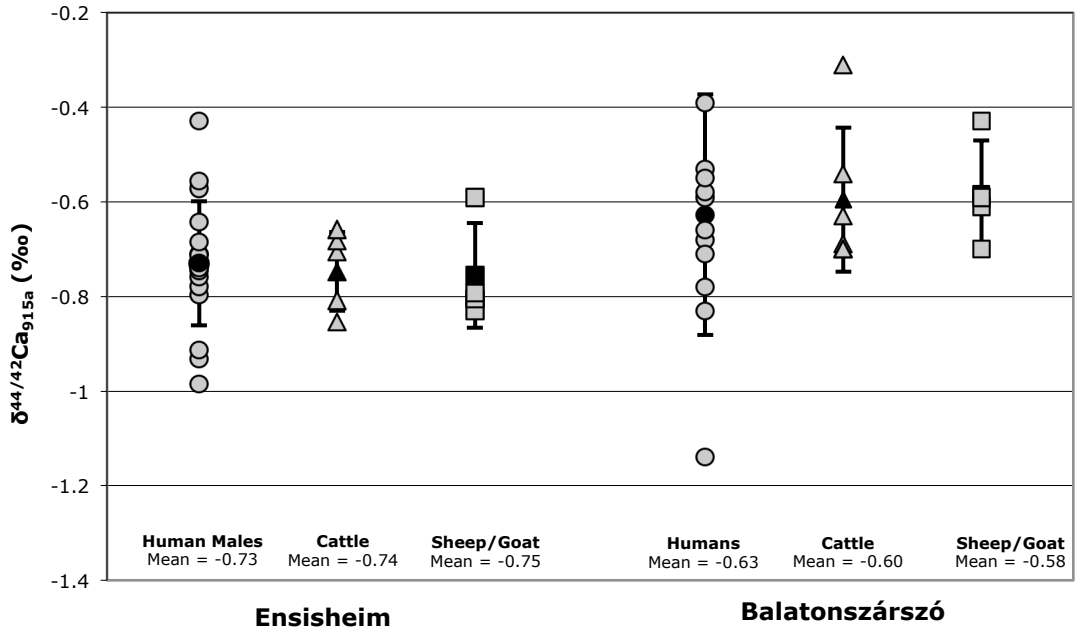

Figure 2.7: $\delta^{44/42}$Ca results for the Neolithic sites of Ensisheim, France, and Balatonszárszó, Hungary (1 SD). Graph reproduced from Hamilton et al. (2013).

These results should be borne in mind when considering the larger groups of archaeological human and faunal results of Reynard et al. (2010, 2011a). As milk and dairy foods, relative to plants, are depleted in ^{44}Ca, and assuming milk/dairy foods were consumed by the Ensisheim and Balatonszárszó Neolithic people, then an offset would be expected between dairy-consuming humans and the fauna at both sites, as seen at Taforalt, Tell Abu Hureyra, Magura and Danebury. The logical conclusion to draw from the Ensisheim and Balatonszárszó data is that humans living at these sites were not milk and dairy consumers.

This conclusion then needs to be taken into consideration when looking at archaeological sites that do show faunal–human bone calcium isotope offsets, as the time periods of these sites are significant when interpreting the results. Danebury, an Iron Age site, and Magura, a Neolithic site, are from time periods where it is reasonable to suppose that milk and dairy food would have contributed to the human diet, and therefore any human–faunal offsets could be due to dairy consumption.

There is iconographic, artefactual and biochemical evidence of dairying, such as ruminant milk fatty acids identified from early Neolithic cooking potsherds, dated as early as 7000 Before Christ (BC), in Anatolia (Evershed et al. 2008). Tell Abu Hureyra is an Epipalaeolithic–Neolithic site that spans the period in the Near East when herbivore domestication was first occurring, so it is a reasonable assumption that some of the world's first dairying would have taken place there starting in the early Neolithic. The $\delta^{44/42}Ca$ offset between humans and fauna at that site supports the theory that dairying was contributing to the human diet. Much of the material from Tell Abu Hureyra is from the Pre-Pottery Neolithic, so there is an absence of artefactual evidence of milk and dairy food processing to support the idea of dairying occurring at the site.

Unlike the results discussed thus far, which indicate that human–faunal offsets could be due to a dairy contribution to the human diet, the results for the Epipalaeolithic humans from Tell Abu Hureyra and all the humans from Taforalt suggest something else entirely. Epipalaeolithic humans from both Tell Abu Hureyra and Taforalt pre-date animal domestication, making it unlikely that these people would have had access to milk and dairy foods. Dairying and milk consumption by humans is widely believed to have been preceded by animal domestication. It is unlikely that Epipalaeolithic/Mesolithic people were catching and milking wild animals, especially on a regular basis. I can testify personally to the difficulty of catching, controlling and milking very reluctant non-dairying domestic sheep. It is hard to imagine succeeding with the wild progenitors. This also does not take into account the difficulty of getting a wild animal to let her milk down once captured, something humans struggled with even once animals had been domesticated. Thus the human–faunal bone offsets seen for the Epipalaeolithic humans and animals from Tell Abu Hureyra (0.29‰) and Taforalt (0.24‰) probably cannot be explained by a milk and dairy food contribution to the human diet. These results in fact cast doubt on the idea that any of the human–faunal offsets seen from the archaeological sites mentioned were due to dairy foods being present in the human diet.

A possible explanation for the discrepancies in results could lie with bone diagenesis. However, this is not a likely cause of the human–faunal offset, as the faunal and human remains at the same site would be expected to have been affected in a similar way. Calcium is the major element in the skeleton, making up about 27% of fresh bone and about 40% of fresh enamel (by weight), so biogenic calcium is expected to be preserved in greater quantities compared with other isotopes (Heuser et al. 2011). For calcium isotope ratios to be altered, significant diagenetic changes would be necessary (Reynard et al. 2010). If diagenesis is altering the biogenic calcium in bones from an archaeological site, it would be expected that all bones, human and faunal, would be altered to a similar degree. However, as each archaeological site was studied on an individual basis, the human–faunal offset is therefore still informative (Reynard et al. 2010).

A final problem is the significant variation in the results for humans within each site, which is not easily explained but probably caused by both diet and human metabolic processes. There is a greater diversity of calcium sources in the human diet compared with most fauna, as humans are omnivores and are able to acquire a wider range of foods. Individual human metabolic processes may also be a factor in the variation of human results (Reynard et al. 2010, 2011a), even though humans have similar metabolic processes as other mammals. In regard to ^{44}Ca, it might be that the enriched faunal (fast-maturing mammals) and depleted human (slow-maturing mammal) variation is due not to different metabolic processes between mammals, but the length of time that those processes are at work.

Thus the human and faunal results for Ensisheim and the other sites mentioned cast doubt on the usefulness of analysing calcium isotopes in bone as a means of identifying past human dairy consumption, at least until further fundamental investigations into calcium isotope systematics in mammals are complete. Reynard et al. (2013: 12), at the end of all their work investigating human nursing and dairy milk consumption, said ‘bone Ca isotopes are influenced by a complex series of factors, and that even in individuals

consuming high amounts of milk there is no single straightforward relationship between dietary Ca and bone Ca isotopes'. Because of the difficulties in interpreting the bone analysis findings from the archaeological sites, I only analysed bone samples from the site of Ensisheim before re-evaluating the research direction of this thesis. The research focus thus switched from bone to dental enamel. Diagenesis was not considered to have been a major issue for bone, and dental enamel is even less susceptible.

2.8 Calcium isotopes: dental enamel, milk consumption and mammal weaning

The discussion of the research into calcium isotopes and biological fractionation given in this chapter highlights the need for a greater understanding of the causes and factors mediating the composition of calcium isotopes in mammal tissues (Reynard et al. 2010, Reynard et al. 2011a, Clementz 2012). To that end, I first undertook to determine the make-up of calcium isotopes contained in mammal tissues, from both individual mammals, in this case sheep, and groups of mammals with the same life history, in this case a herd of sheep living on the same farm, with the same diet and herd management. Secondly, I compared the characteristics of the diet of these mammals (grass/feed for the ewes and milk for the lambs) with their body tissues. It was hoped that by expanding on the work begun by Skulan and DePaolo (1999), Chu et al. (2006) and Hirata et al. (2008), a greater understanding of calcium isotopes in mammal physiology could be achieved, and a new method created for detecting weaning and weaning timing.

One way of understanding calcium isotope movement and incorporation into mammal tissues is to study systematically the mineral tissue of dental enamel, which until now has received little attention. The earliest published discussion of calcium isotopes and teeth (roots) is in Russell et al. (1978), who considered whether biological processes resulted in fractionation or not. They concluded that determining the processes and mechanisms creating calcium isotope fractionation would be difficult, but that there was fractionation to a level of ~2.5‰ in nature. Bone took the place of dental tissue in later

studies, probably because bone is more readily available for destructive analysis than teeth. However, there have been some instances between Russell et al. (1978) and now where teeth have been analysed for calcium isotopes, but mainly as a supplement to bone analysis.

Skulan and DePaolo (1999) analysed manta teeth and, although not discussed directly, they reported that, for fish, reptiles, birds and mammals, calcium isotope results were related to diet and not phylogeny or environmental factors. Clementz et al. (2003), as previously discussed, used bone and dental enamel to look at trophic differences between marine mammals. They found that the calcium isotope content of both bone and teeth provided dietary information related to trophic levels within a marine food chain.

Reynard et al. (2010) undertook the most recent analysis of teeth from mammals. They first used teeth to look at intra-individual variability of skeletal elements. They concluded that calcium isotope ratios are largely uniform and that the part of the skeleton used for sampling is not important. The exception was dentine in two teeth (enamel was not analysed), which had a difference greater than 1 SD of measurement uncertainty (~0.8‰ difference or less), with a difference of 0.13‰. Reynard et al. (2011a) then analysed teeth (archaeological dentine and enamel) as part of a diagenetic/leaching study of skeletal tissue in order to detect the degree of alteration of calcium isotopes attributable to diagenesis. They concluded that diagenetic changes were minor and negligible, with enamel affected the least.

Another recent study, by Heuser et al. (2011), looked at calcium isotope ratios in teeth to a much greater degree than previous studies. However, the teeth they studied were from modern reptiles and ancient dinosaurs, rather than mammals. They reported, tentatively, that enamel and dentine samples from both modern reptiles and herbivorous and carnivorous Late Triassic to Late Cretaceous dinosaurs had a $\delta^{44/40}Ca_{915a}$ $\Delta_{dentine-enamel}$ offset of +0.3‰ to +0.4‰. They suggested that the offset was due to differences in the process of biomineralisation of enamel (ameloblasts) and dentine (odontoblasts). They concluded that no obvious trophic differences were seen, based on the $\delta^{44/40}Ca$ results, between herbivorous

and carnivorous dinosaur skeletal tissues. This is most logically explained by the carnivorous dinosaurs not having a dietary source depleted in ^{44}Ca, i.e. they consumed soft tissue and little or no bone.

The calcium isotope content of dental tissue has therefore been studied to only a small degree, with little specific analysis of mammal teeth. Certainly there has been no specific calcium isotope research involving dental tissue to address questions of nursing/lactation duration and weaning. Dental enamel, as the most mineralised (Hubbard 2000, Fowler et al. 2005) and least diagenetically altered (Zazzo et al. 2010, Heuser et al. 2011, Reynard et al. 2011a) skeletal tissue, is an obvious choice for further research into calcium isotopes, sheep nursing/lactation duration, weaning and dairying in the archaeological record.

Thus the purpose of using dental enamel rather than bone was to enable relevant questions to be asked about dental mineralisation, but also, in line with the initial direction of my research, milk consumption. In this case, the purpose was not to detect human milk consumption as seen through human–faunal trophic differences, but to identify mammal infant (lamb) milk consumption/mother's (ewe's) lactation period and weaning timing. The results could then be used to infer human interactions with ewes and lambs, such as early lamb weaning as part of managing and utilising dairy herds or possibly the duration of milk production by ewes. This was achieved by tracking the calcium isotope offsets between permanent teeth (first, second and third molars) from the same sheep, as well as between serial incremental/sequential samples from the same tooth. The dental samples were then linked to dental development/chronology and the mammal's diet over time.

Additionally, samples of dental enamel from the same locations as those taken for calcium isotope analysis were used for oxygen and carbon analysis. The sinusoidal variation of oxygen isotope ratios over time, due to seasonal changes of temperature and precipitation, were used to supplement dental development data in establishing the chronology and time scale of the calcium and carbon isotope results. The modern findings

were used to inform the archaeological results by providing clues about the weaning timing of lambs and lactation duration of ewes at archaeological sites, which in turn could be used to infer information regarding human herd management and/or non-human factors that affect sheep lactation/weaning. The results for the modern and archaeological samples are presented in detail in Chapters 5, 6 and 7.

2.9 Chapter summary

This chapter has provided a general background into calcium isotopes, including the sources of fractionation discovered within the geosphere. More specifically this chapter has discussed the link between calcium homeostasis and sources of calcium isotope fractionation via physiological processes, the most significant being bone mineralisation. The fractionation of calcium isotopes during mineralisation, seen as the depletion of ^{44}Ca in bone compared with the diet, has provided important information towards an understanding of the homeostatic processes that govern bone calcium isotope ratios. What has been learnt about calcium isotopes and bone mineralisation provides the basis for understanding the calcium isotopes found in dental enamel.

The discovery that milk and dairy foods are fractionated to a similar degree and in a similar direction as bone brought about a new focus of calcium isotope research to address questions of mammal milk consumption. However, the archaeological research thus far has proved to be too complicated to interpret, and has highlighted the need for more work on calcium isotope systematics and involvement in calcium homeostasis. Specifically, additional work is needed on modern mammal tissue samples to expand the range of results and aid in understanding calcium isotope mass balances. As discussed by Reynard et al. (2010, 2011a, 2013), calcium isotope metabolism in mammals and other vertebrates is still little understood, so modern results need to be used to validate and inform the interpretation of archaeological findings.

Lastly, this chapter has presented the reasoning behind the research for this thesis. The goals of the research were as follows. Firstly, to try and fulfil the need for more analysis of modern mammal tissue samples. Secondly, to utilise modern sheep dental enamel to address questions of weaning by detecting any associated dietary changes (milk to milk/plants to plants) and linking those changes to dental development. Thirdly, once the modern samples results were determined, to interpret the sheep dental enamel results from archaeological sites by utilising the modern sample results in order to identify positively or negatively evidence of early lamb weaning.

CHAPTER 3

LINKING MOLAR ENAMEL SAMPLES TO DIETARY AND LIFE EVENTS

This chapter discusses what is known about dental development and enamel formation rates, and my sampling strategy for modern sheep molars. Key to interpreting sheep molar enamel bulk and serial, segmented sample results for nursing and weaning lengths is bringing together the following data from modern sheep:

• a dental development chronology;

• enamel formation rates;

• weaning timing;

• oxygen and carbon isotope analysis for seasonal cycling, and for dietary changes linked to lamb nursing/weaning;

• information collected about the lives of the modern sheep being analysed.

The life information of the sheep included sex, birth date, lamb weaning age (and whether it was managed or natural), lactation length of ewes and death date. The ultimate goal of consolidating this varied data was to identify the time at which weaning began and ended; by identifying weaning timing in modern sheep, this approach could then be applied to investigate the weaning timing and the possible causes for that timing of ancient sheep.

3.1 Sheep molar development, enamel formation rates and sampling strategy

This section provides an overview of the current understanding of sheep molar dental development and enamel formation, developed over the last 15 years. This was used as a basis for formulating the sampling strategy.

3.1.1 Sheep molar development and dental chronologies

The timing of molar growth is species specific (Balasse et al. 2012b). However, health, nutrition and breed may, within species parameters, affect the development rate of sheep

teeth. Dental development in improved or crossbreeds may be faster and have more inter-individual variation than traditional or unimproved breeds (Upex 2009, Upex and Dobney 2012). This appears to be the explanation for the inter-individual variation seen in third molar (M3) development in the improved Suffolk crossbreed sheep analysed by Zazzo et al. (2010), which is not seen in the unimproved North Ronaldsay sheep (Balasse et al. 2005). Nutrition also makes a difference to sheep dental development, with malnutrition or specific dietary deficiencies resulting in developmental and eruption delays (Frankin 1950, Upex 2009, Popkin et al. 2012, Upex and Dobney 2012). However, dental eruption and development is not different between the sexes, nor affected, in the case of males, by castration (Davis 2000). This is important, because the molar enamel samples analysed in this research came from ewe, ram and wether (castrated) sheep. Wear is not thought to be a significant issue as 'once the teeth are scaled into the developmental chronology, the variation of a few millimetres of enamel becomes irrelevant, as it is broader time scales that are under investigation' (Upex and Dobney 2012: 262), but due consideration needs to be given to this factor when selecting teeth to sample. Upex and Dobney (2012)'s statement is based on the amount of enamel that is lost to wear as described in Payne (1973), which, if wear falls between stages three to six, only amounts to a few millimetres lost (Upex and Dobney 2012). The modern sheep analysed in this research fell between stages one and six.

Figure 3.1, from Upex and Dobney (2012), presents the mandibular molar dental development timings from their study of Shetland-breed sheep (these sheep are from the same herd of Hoy sheep analysed for calcium isotopes, discussed in Chapter 6) as well as other dental chronologies currently available. These other studies were for three non-Shetland breed sheep: the traditional Middle East native Awassi (Weinreb and Sharav 1964), southern Pre-Alps (Milhaud and Nezit 1991) and Suffolk cross (Zazzo et al. 2010). Although molar development rates may vary between breeds, the majority of the variation is seen in the lower portion (closer to the cemento-enamel junction) of the second molars (M2s) and in the overall development of the M3s, which contain enamel formed after weaning is complete and plants have fully replaced milk. Although this largely removes the

issue with regard to using calcium isotopes to study nursing and weaning, it may be an issue if there are future attempts to detect evidence of foddering or changes in the plant diet related to animal movement/herding.

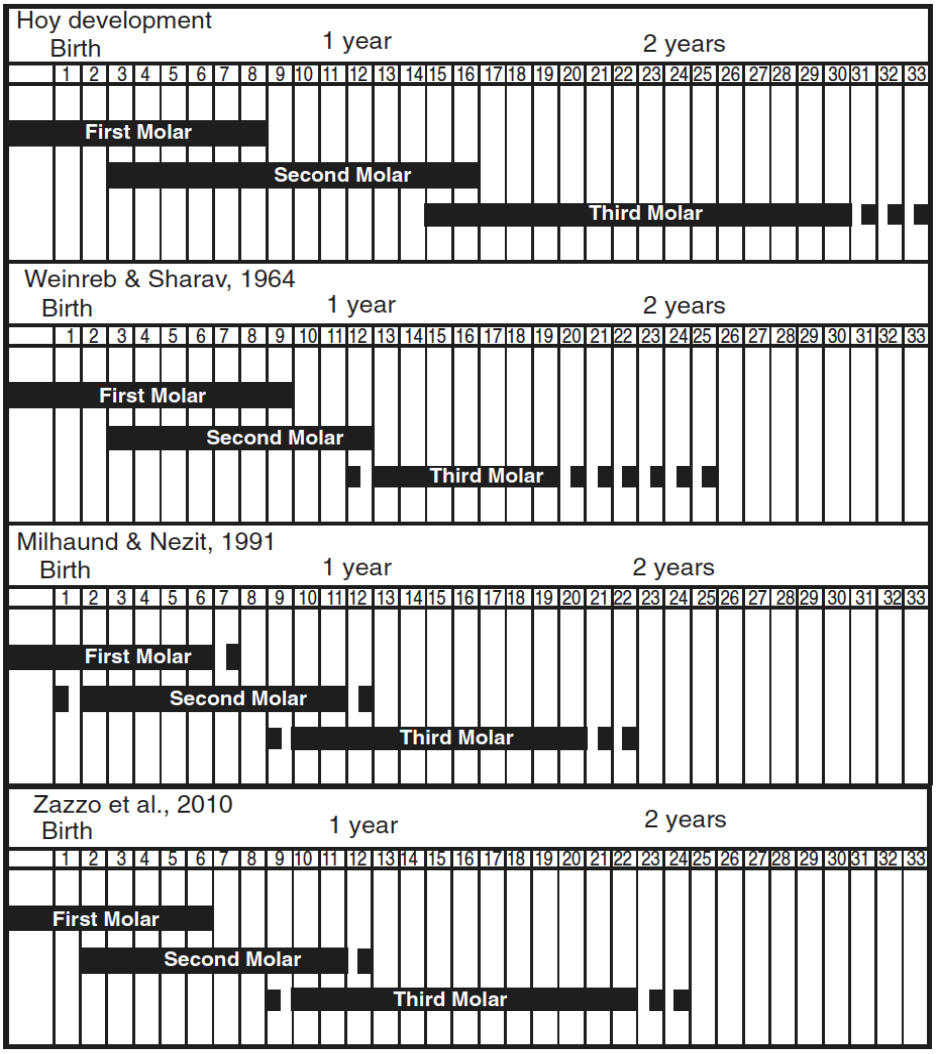

Figure 3.1: Sheep dental development and chronology for four breeds of sheep: Shetland (Upex and Dobney 2012), Awassi (Weinreb and Sharav 1964), southern Pre-Alps (Milhaud and Nezit 1991) and Suffolk cross (Zazzo et al. 2010). Image reproduced from Upex and Dobney (2012: 263).

However, the variation in molar dental development seen between breeds may not be due to true differences, but rather factors such as a lack of clarity regarding what parts of the molars were analysed for rates of dental development (Upex 2009, Upex and Dobney 2012). This is important, because the lingual face of the molars begins to develop and is completed before the buccal face, the side with the thickest enamel (Zazzo et al. 2012), and

similarly the enamel on the anterior cusp starts to form before the posterior (Upex 2009, Upex and Dobney 2012), which may result in dental development timing discrepancies between studies. Additionally, the sheep breeds analysed were a mix of improved breeds (southern Pre-Alps and Suffolk cross) and traditional breeds (Awassi and Shetland), with crossbreeds and improved breeds possibly reaching puberty and maturing more rapidly (Leymaster 2002).

Because of the close agreement in dental development between the first molars (M1) and the upper, earlier formed portion of the M2s in the unimproved breeds of Shetland (Upex and Dobney 2012) and Awassi (Weinreb and Sharav 1964) sheep, Upex and Dobney's (2012) dental development was selected for organising the calcium isotope ratio values generated from the analysis of the serial incremental enamel samples. Another reason for using this dental development chronology was that the herd of Shetland sheep used to produce it was the same herd as used for the Loft and White Hamars Grazing Project, on Hoy, Orkney, and which was used for half of the calcium isotope analysis of modern sheep. The remaining modern sheep enamel samples analysed came from another Shetland herd. The archaeological sheep samples were from early Neolithic Tell Abu Hureyra and were therefore far from being 'improved'.

Based on Upex (2009) and Upex and Dobney (2012), but bearing in mind Weinreb and Sharav's (1964) dental chronology for the traditional Awassi breed sheep from the Middle East, and the fact that there will be individual sheep variation, the following timing plan was used. The M1 begins to develop *in utero* approximately 3 months before birth. [This varies slightly from Witter and Míšek (1999), who reported M1 growth beginning 2 months before birth.] The M1 is fully formed 8 months after birth. [Payne (1973) has eruption of the M1 occurring at 3 months.] Upex and Dobney (2012) deemed molar development to be complete when root development has begun on the posterior buccal cusp. The M2 begins to develop around 2 months after birth and the enamel continues to grow and develop over the next 14 months. Development of the M2 overlaps with that of

the M1 for about 6 months. The M3 begins to develop as the M2 is being completed, with an overlap probably occurring for a few months.

3.1.2 Enamel formation

Dental enamel is formed in between the apical surface of the enamel epithelium (ameloblasts) and dentine through what is known as amelogenesis (Hubbard 2000). Amelogenesis involves two distinct stages or phases: saturation and maturation (Suga 1982, Hubbard 2000). The enamel formed during the saturation phase is produced by ameloblast cells and is in the form of an enamel fluid (matrix) that is rich in protein (~35%) and poor in mineral (compared with the final mineral content of the mature tooth) (Hubbard 2000, Balasse 2003, Fowler et al. 2005, Hillson 2005). Maturation involves a change in behaviour by the ameloblast, resulting in the protein being replaced with successive layers of mineralisation, through deproteination, hypermineralisation and loss of water (Hubbard 2000, Fowler et al. 2005). The enamel forms crystals of bioapatite from the two main components of enamel, calcium and phosphorus (80%), and hardens into the most highly mineralised (>95% by weight) and hardest tissue in the mammal body (Simmer and Fincham 1995, Fincham et al. 1999, Hubbard 2000, Fowler et al. 2005). More details about enamel formation, especially the participation of calcium in the process, are given in Appendix A, Section A.3.5.

Enamel is unique amongst all the forms of mineralised tissue in vertebrate bodies (Fincham et al. 1999). There is no resorption of old or creation of new enamel once the enamel has matured (Fincham et al. 1999). Enamel's static state therefore has a very different behaviour compared with bone, which is constantly remodelled (Hillson 2005). Additionally, dental enamel is less easily compromised by burial environments, and thus provides a means of gaining information about specific periods of time in a mammal's life for archaeological analysis (Dauphin and Williams 2004).

Enamel grows with the occlusal plane or apex of the crown portion developing first and progressing towards what will become the cemento-enamel junction or cervix of the

tooth, where the enamel and the cementum of the roots meet. Although a reasonable timing has been established for Shetland sheep molar development (Upex and Dobney 2012), the timing of mineralisation is not as clear (Balasse et al. 2012b). Using light and scanning electron microscopy, the rate of the saturation phase of sheep enamel formation has recently been found to be varied over the course of molar development (Kierdorf et al. 2012). 'A marked reduction of enamel extension rates in cervical compared with more cuspal crown portions of the sheep and goat molars was recorded, with formation of the cervical 25% of the crown taking about the same time as that of the upper 75% of the crown' (Kierdorf et al. 2012: 484). It is unclear, however, if the maturation process follows fully the pattern found for saturation, although results from oxygen isotope analysis have hinted at variation in tooth growth rates towards the cervical portion of mandibular M2s (Balasse et al. 2012b) and overall M3s have been found to have varied growth rates, which decreases over time (Zazzo et al. 2012).

Suga (1982) describes the maturation process for enamel in three stages. The first involves secondary mineralisation, i.e. mineralisation after the saturation phase is complete, beginning with increased mineralisation at the surface of the immature enamel and progressing obliquely inwards towards the deeper layers. While this additional mineralisation is occurring, the greatest degree of mineralisation is taking place in the narrow surface layer, with mineralisation decreasing with enamel depth. The second stage begins shortly after secondary mineralisation reaches the innermost layers and is a reversal, with the innermost layer beginning to increase mineralisation and moving slowly back towards the surface. This results in the outermost enamel layer being the most highly mineralised, apart from the highly mineralised innermost enamel layer. The third stage of enamel maturation and mineralisation involves rapid mineralisation from the middle layers of the enamel, progressing outwards towards the surface. This completes the maturation and mineralisation process and produces a subsurface layer that is overall the most highly mineralised of all the different layers. In sheep, this third stage takes approximately one-third of the time required for the whole maturation process to be completed.

Specific to sheep, once the secretion phase is complete, enamel mineralisation occurs rapidly in the innermost enamel layer that forms the dentine-enamel junction (Suga 1982, Balasse 2003). This innermost enamel is different from the rest of the enamel being formed in that there may be time delays between the conclusion of saturation and start of maturation for the different layers, resulting in maturation taking approximately 4 months to reach completion (Zazzo et al. 2010). Bearing in mind Suga (1982)'s timing for the maturation process, being two-thirds of the complete enamel development, this results in a period of 6 months from the start of the saturation phase to completion of the maturation phase (Zazzo et al. 2010, Balasse et al. 2012b).

The ramifications of enamel taking 6 months to form is that mature enamel will have gained protein during the saturation phase that is later replaced during the maturation phase with successive layers of late-stage mineral content, resulting in mature enamel layers of varied thicknesses and amounts of mineralisation. This is highly likely to cause isotope ratio signal blunting or attenuation (Balasse 2003). Essentially, mature enamel represents, for isotope ratio results, a time-averaged signal reflecting the mammal's body during the period it took the enamel to form (Passey and Cerling 2002). More specifically, and because it takes a number of months to finish forming, 'stable isotope analysis of tooth enamel returns a time-averaged signal attenuated in its amplitude relative to the input signal' (Zazzo et al. 2012: 1). Long-term dietary behaviours are less attenuated than short-term changes, although the mineralisation rate at the time of enamel formation also plays a part in the degree of signal muting of isotopic values (Passey and Cerling 2002, Zazzo et al. 2010, Zazzo et al. 2012).

Sampling (discussed in greater detail in Section 3.1.3) and analysis of enamel for isotopic analysis can only produce qualitative results if we obtain a greater understanding of the mineralisation processes and maturation (Zazzo et al. 2010). It has been found, in sheep M3s, that there is variability in tooth growth rates and that linear growth rate models do not reflect actual mineralisation rates in the upper and lower portions of the tooth (Zazzo et al. 2012). More specifically, although referring to average values, 'one millimetre of enamel is

deposited over 6–7 days in the upper 10 mm of the crown but takes nearly a month in the lower 10 mm' (Zazzo et al. 2012: 6). However, if, as Upex (2009) and Upex and Dobney (2012) suggest, traditional, unimproved breeds of sheep take longer to complete their molars than improved breeds, it would logically follow that the saturation and maturation stages also take longer for these breeds. Although much progress has been made in the past few years, the specifics regarding enamel maturation rates remain unclear. It does appear, however, that it is reasonable to assume that the enamel maturation phase may follow the pattern observed during the saturation phase.

The isotopes that have been discussed thus far, in regard to enamel formation rates, are specific to ^{18}O and ^{13}C. These isotopes are incorporated as part of carbonate inclusion in the hydroxyapatite enamel matrix through the substitution of hydroxyl ions by carbonate (OH-to-CO_3) (Elliott et al. 1985). Hydroxyapatite crystals precipitate early in the secretory stage and continue to grow and thicken until the completion of the maturation stage (Brookes et al. 1995). Calcium's inclusion differs from carbonates, with as much as 86% of the total enamel calcium in rat incisors accrued during the maturation stage alone (Smith 1998, Hubbard 2000). Although all enamel formation involves calcium, much of the calcium involved in saturation is bound in ligands and then replaced during maturation (see Appendix A, Section A.3.5) (Takano 1995, Hubbard 2000). Although this is currently unknown, it is predicted that the calcium contained in samples of mature enamel will reflect the high volume of calcium incorporated during maturation, with the calcium contribution to the isotope ratios from the saturation phase being overwhelmed by maturation calcium. If this is the case, then the isotopic $\delta^{44/42}Ca$ values should reflect dietary input over the later 4 months (approximately) of enamel formation instead of a full 6 months. Another unknown is whether the pattern of enamel formation rate change, with slowing of enamel formation in the crown near the cemento-enamel junction, as indicated by ^{18}O and ^{13}C, also occurs during calcium accretion. This uncertainty, as well as the uncertainty of whether the calcium accreted during saturation and/or maturation is the source of the isotopes measured in sheep molar enamel samples, will be discussed in the modern results chapters (Chapters

5 and 6). It is hoped that these modern calcium isotope results will indicate whether calcium inclusion during enamel formation follows the model seen with carbonates or not.

3.1.3 Sheep molar enamel sampling

Bulk and serial, sequential and incremental, dental enamel samples for calcium, oxygen and carbon apatite isotope analysis were collected from the same enamel sampling sites (Chapter 4 will detail the method of sample collection). As discussed in Section 3.1.2, enamel grows with the cuspal crown portion developing first and progressing until the cemento-enamel junction or cervix of the tooth is formed. Enamel, unlike bone, being free from remodelling or physiological alteration, provides a record over time of a sheep's dietary history, and dental development and eruption, unlike bone maturation, follow a 'constant sequence, broadly comparable between different individuals' (Hillson 2005). Teeth with high crowns, such as sheep molars, record dietary and seasonal change, which can be observed through serial, incremental sampling down the length of the tooth, with samples perpendicular to the growth axis (Zazzo et al. 2010). The challenge then is to link that dietary history to a specific time period in the sheep's life. A way of doing this is through linking isotope results to dental development and enamel formation rates in modern sheep reference sets. Modern reference sets, with background information about the animals' diets and life events, are necessary for reducing uncertainty about results from archaeological samples (Blaise and Balasse 2011).

Forming a timeline of life events, such as birth and weaning, based on the known management of a modern reference group, and then linking those events to dietary isotope results from enamel analysis, can be done but remains imprecise as a result of the uncertainties surrounding the variations in enamel formation rates. As discussed in Section 3.1.2, much has been learned regarding enamel formation processes and rates, such that, even with an inherited level of uncertainty, it is still worth attempting to link calcium isotope ratio results to sheep life dietary events. It is hoped that the use of oxygen isotopes as part of establishing seasonal patterns of change, and a timeframe of dietary events

observed through calcium isotopes, may be of use in reducing some of the uncertainty. Detecting possible changes in plant diet linked to ewe pregnancy and lamb nursing and weaning through carbon apatite results may, by sharing similar uncertainties with calcium isotopes regarding timing, provide further evidence of sheep diet and life events.

Figure 3.2 shows the enamel sampling approach that was used for this research, with samples collected every 3 mm through the full depth of the enamel, progressing down the tooth following the direction of tooth growth. Although there is uncertainty regarding mineralisation rates, the results from isotopic studies have shown that the chronology of diet and key dietary events is preserved (Balasse 2003). However, it must be kept in mind that a sample taken from the full thickness of the enamel reflects the diet during the whole period it took for that sampled enamel portion to form and be fully mineralised (Passey and Cerling 2002, Balasse 2003, Zazzo et al. 2012).

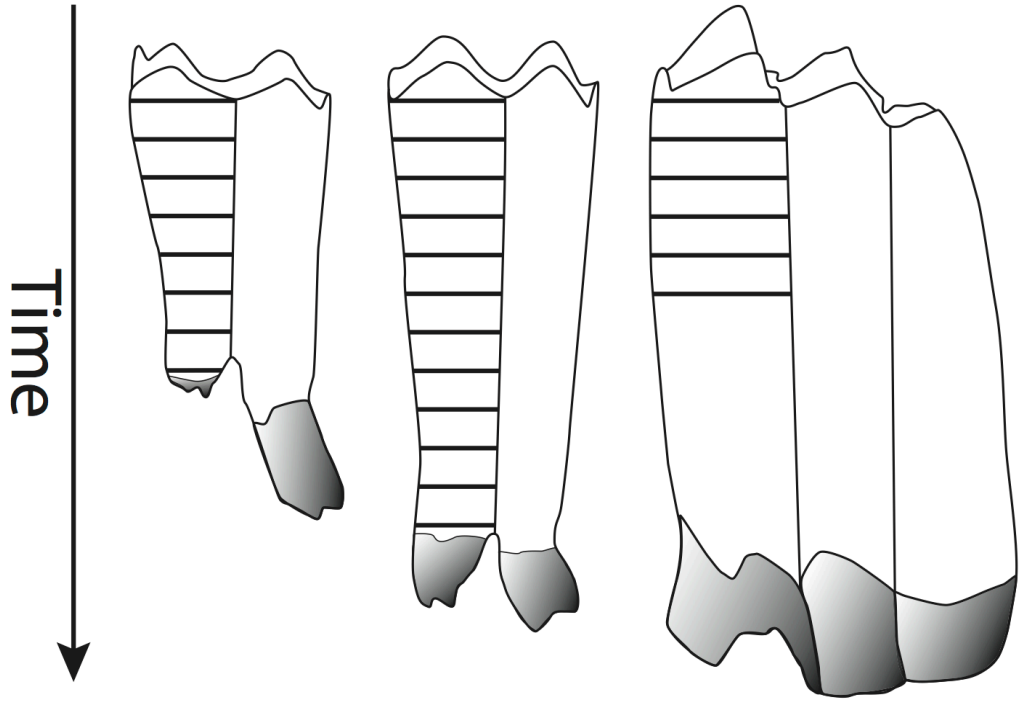

Figure 3.2: Enamel sampling following the growth direction. The image shows M1s, M2s and M3s (shown from left to right) with samples from near the apex of the crown containing the greatest amount of the 'oldest' calcium, and the enamel just above the cemento-enamel junction containing the greatest amount of the 'youngest' enamel. Image based on Vigne and Helmer (2007: 15).

An additional issue to consider is the non-steady state of enamel formation across the crown (Balasse et al. 2012b, Kierdorf et al. 2012, Zazzo et al. 2012). Because it is unknown if calcium enamel accretion follows the pattern seen with carbonates, Chapters 5, 6 and 7 present the isotope results both in a steady growth rate form and, based on the findings of Kierdorf et al. (2012), with decreased enamel formation in the bottom (cervical) 25% of the molars. Additionally, while a dental development and chronology chart are used to provide the structure and timing of known sheep life events, it should be remembered that it is imprecise and only intended as a means of organising the calcium isotope results with as few uncertainties and unknown variables as possible.

3.2 Oxygen and carbon isotope analysis of enamel: seasonality and dietary change

The investigation of milk consumption and weaning in this project relies primarily on the analysis of calcium isotopes. However, determination of the timing of the different events is imprecise, as discussed in Section 3.1. Oxygen isotopes were added to the project to help create a timeframe of when lamb milk consumption was occurring, when weaning began and ended and when plant foods formed the whole of the diet. Carbon was added to track changes in the plant diet that may have coincided with nursing and weaning.

Carbon and oxygen analyses have become common in archaeological research concerned with diet, tracking or detecting mobility, seasonality, and in determining environmental conditions/change over time (Tieszen et al. 1983, Delgado Huertas et al. 1995, Kohn et al. 1996, Cerling and Harris 1999, Wright and Schwarcz 1999, Balasse et al. 2001, Balasse et al. 2002, Richards et al. 2002, Balasse and Ambrose 2005, Balasse et al. 2005, Balasse and Tresset 2007, Henton et al. 2010, Zazzo et al. 2010, Blaise and Balasse 2011, Balasse et al. 2012b, Tornero et al. 2013). As a result, and because calcium isotope analysis is both the focus of this research and a new technique for analysing diet that requires a detailed discussion, the well-known history of carbon and oxygen isotope analysis in archaeological research will not be discussed in detail here, but only in regard to

its relevance to this research. Analysis of enamel for oxygen and carbon isotope ratios has the potential to help establish a better understanding of the timing of diet linked to life events, such as birth and weaning.

3.2.1 Oxygen isotopes: sheep enamel and seasonality

Oxygen has three naturally occurring isotopes, ^{16}O, ^{17}O and ^{18}O, with a natural abundance of 99.759% for ^{16}O, 0.037% for ^{17}O and 0.204% for ^{18}O (Ehleringer and Rundel 1989). Vienna Standard Mean Ocean Water (VSMOW) reflects the isotopic composition of freshwater and is used to measure oxygen isotope compositions. Vienna Pee Dee Belemnite (VPDB), a marine carbonate, is frequently used to measure oxygen isotope compositions of carbonates. Carbonates in dental enamel were measured for this research and are expressed relative to $\delta^{18}O_{VPDB}$.

Meteoric water is the source of oxygen isotopes incorporated into sheep and other animal tissues. Winter precipitation (rain and snow) has lower $\delta^{18}O$ values as a result of lower temperatures, and summer rain condenses and evaporates at warmer temperatures and thus tends to have higher $\delta^{18}O$ values (Gat 1996). The oxygen isotope content of precipitation is also dependent on environmental factors that influence temperature and precipitation, such as latitude, altitude, land mass size and proximity to large bodies of water (Gat 1996, Barbour et al. 2005, Henton et al. 2010). In middle and high latitudes, seasonal temperature fluctuations result in precipitation and soil water with $\delta^{18}O$ values that rise and fall in reflection of the cyclical temperature shifts (Gat 1996, Barbour et al. 2005).

The oxygen isotope composition of plants is derived from water taken up directly by the plant as well as evaporation during transpiration and the exchange of isotopes between plant water and organic molecules, such as sugars (Clark and Fritz 1997). However, there is little oxygen isotope fractionation due to plant uptake of soil water, and as a result plants largely reflect meteoric waters (Gat 1996, Barbour 2007). Drinking water may also reflect meteoric $\delta^{18}O$ values but high temperatures and evaporation may lead to fractionation that creates $\delta^{18}O$ values that are different from precipitation and plants (Gat

1996). Sheep depend on both meteoric and plant waters for hydration (Schröder et al. 1997).

Although different factors may influence water $\delta^{18}O$ values, if the source of water for sheep is from precipitation (drinking water and plants) and the location where the sheep live is at high or middle latitudes, then changes in temperature and rainfall will follow a seasonal pattern. This pattern will be recorded in sheep enamel through oxygen isotope incorporation into the carbonate and phosphate components and, although the carbonate fraction may be more attenuated than the phosphate (Pellegrini et al. 2011), it was this fraction that was analysed for this research because of the need for a smaller sample size and to allow for the simultaneous production of carbon apatite isotope results. $\delta^{18}O_{carbonate}$ values can be found by analysis of serial incremental or sequential samples taken along the tooth following the growth axis, making it possible to access the seasonal information recorded in the enamel (Balasse and Tresset 2007), typically appearing as a sinusoidal curve (Balasse et al. 2002, Balasse et al. 2009, Blaise and Balasse 2011, Stevens et al. 2011, Balasse et al. 2012a, Balasse et al. 2012b). Additionally, after the development of a model using a cosine function that eliminates the issue of variable crown height from different sheep, it has become possible to quantify timings down to individual months, which facilitates inter-sheep comparisons (Balasse et al. 2012b). The oxygen isotope analysis was used to produce a timeframe for interpretation by grounding the calcium and carbon isotope results within a season, which in the case of the modern samples could then be linked to specific recorded dates and management practices.

3.2.2 Carbon apatite isotopes: sheep enamel and dietary change

Carbon has two naturally occurring stable isotopes, ^{12}C and ^{13}C, with natural abundances of 98.89% and 1.11%, respectively (Ehleringer and Rundel 1989). The marine carbonate VPDB is used to measure carbon isotope compositions. Carbonates in dental enamel were measured for this research and are expressed relative to $\delta^{13}C_{VPDB}$.

Tooth enamel carbonate is created directly and in equilibrium with blood bicarbonate (Passey et al. 2005) and thus reflects all dietary carbon that has been metabolised. The isotopic composition reflects that of the diet. The diet of sheep, during the weaning transition and once weaning has been completed, is made up of plant foods. The majority of terrestrial plants are either C_3 or C_4 plants. The distinction between the two is down to the different photosynthetic pathways used by C_3 and C_4 plants, which results in differential carbon isotope fractionation (Jones et al. 1979, Kohn and Cerling 2002, Passey et al. 2005). The difference in isotope fractionation is because C_3 plants discriminate against ^{13}C more than C_4 plants, and therefore are more depleted in ^{13}C (Bender 1971, Farquhar et al. 1989). The result is that, in general, C_4 plants have $\delta^{13}C_{VPDB}$ values that fall between –10 and –20‰ and C_3 plants have $\delta^{13}C_{VPDB}$ values that fall between –22 and –34‰ (Gannes et al. 1998).

A number of environmental factors may influence $\delta^{13}C$ values, especially for C_3 plants, such as light, altitude, temperature, soil nutrient content and water availability (Körner et al. 1988, O'Leary 1988, Farquhar et al. 1989, van der Merwe and Medina 1991). Increases in light duration and intensity produce more positive $\delta^{13}C$ values, while low light has been found to produce the opposite effect (O'Leary 1988, Heaton 1999). In forest environments, declining amounts of light, along with the recycling of CO_2 depleted in ^{13}C, produces an increasingly more negative gradient of $\delta^{13}C$ values moving from the forest canopy down to the forest floor (canopy effect) (Tieszen 1991, van der Merwe and Medina 1991). The $\delta^{13}C$ values in C_3 plants are also affected by altitude, temperature and soil nutrient content. There is enrichment of ^{13}C with increasing altitude, and depletion of ^{13}C with increasing temperature and low soil nutrient content (Körner et al. 1988, O'Leary 1988, Tieszen 1991). There is also a negative correlation between water availability and C_3 plant $\delta^{13}C$ values, with increasing water stress or aridity resulting in higher $\delta^{13}C$ values (Johnson et al. 1989, Heaton 1999).

These environmental factors need to be kept in mind when interpreting animal samples for the contribution of isotopically distinct food groups (C_3 versus C_4 plants)

(Tieszen 1991, Ambrose and Norr 1993). Animal dietary carbon is supplied by proteins and other dietary constituents, although the carbon in collagen is mainly derived from dietary protein and amino acids, especially with protein-rich diets (Ambrose and Norr 1993, Jim et al. 2006). Carbon in enamel apatite is derived from biocarbonates in the blood, and reflects more of the diet as a whole because carbon is also being contributed by lipids and carbohydrates as well as protein (Schwarcz 2000, Jim et al. 2004).

Carbon isotope fractionation (ε*) results in enrichment of ^{13}C between large ruminants' enamel and their diet ($\delta^{13}C_{diet\text{-}enamel}$) by 14.1 ± 0.5‰ (Cerling and Harris 1999). Other research has found similar results for cattle: 14.6‰ (Passey et al. 2005). Non-ruminant animals have a similar enrichment, although generally not to the same degree (~12‰) (Krueger and Sullivan 1984, Lee-Thorp and van der Merwe 1987). It is believed that the methane produced in the rumen, the foregut fermentation chamber that breaks down vegetable matter (Langer 1994, Stevens and Hume 1995, McSweeney et al. 2005), by established gut microflora is the cause of the enriched $\delta^{13}C$ values in the enamel of ruminant animals compared with non-ruminant rodents, rabbits and pigs (Metges et al. 1990, Cerling and Harris 1999, Passey et al. 2005, Zazzo et al. 2010).

Carbon isotope analysis of enamel has the potential to provide information regarding nursing and weaning. In dairy cows, the milk has been found to mirror the cows' diet, with a small amount of ^{13}C enrichment (1.3‰) (Metges et al. 1990). However, in a study by Camin et al. (2008) analysing the constituent parts of milk, milk lipids were found to be depleted in ^{13}C relative to milk protein for cows from two farms by an average of 2‰. This is because lipids, plant or animal, are depleted in ^{13}C relative to carbohydrates and proteins (DeNiro and Epstein 1978, Post et al. 2007). Milk has a high lipid content and, unlike collagen, the lipid and carbohydrate contribution to the diet, as well as protein, is routed to the carbonate of enamel (Schwarcz 2000, Jim et al. 2004). Thus lipid-rich milk has the potential to produce $\delta^{13}C$ enamel values indicative of nursing and weaning.

3.3 Nursing and weaning

This chapter has outlined how serial incremental sampling of enamel for isotope analysis can be used to identify and link diet and dietary change to the stages of a sheep's life. The life events of greatest interest to this research are lamb nursing/weaning and ewe lactation, as part of the development of a new approach to investigating the lives of sheep in the archaeological record. An understanding of sheep lactation length (as well as the reasons why lactation length may differ from the norm), nursing and weaning patterns and social behaviours is necessary and is crucial to interpreting calcium isotope ratio values.

Typically lambs nurse from birth to about 5 months old, with non-dairy ewes supplying milk in decreasing amounts from around the first month onwards (Arnold et al. 1979, Festa-Bianchet 1988, Hass 1990, Obregón et al. 1992, Chapman 2010). Although milk availability for lambs is continuously in decline, observations of domestic breeds of sheep have shown that once ewes begin to reject suckling attempts by lambs consistently for a period of a week, then weaning is complete (Arnold et al. 1979). This results in an abrupt end to the weaning transition (Arnold et al. 1979). Unlike naturally weaned sheep, lambs born to heavily managed, specialised dairy ewes usually only have a month of access to milk and will either be weaned fully at that time or switched to a milk substitute (McKusick et al. 2001). As discussed in Chapter 1, models of traditional methods of dairy sheep management will have lambs killed very young (<2 months old), especially unnecessary ram lambs, with barren young ewes killed as well (Payne 1973). Table 3.1 provides information on lamb weaning timing and ewe lactation length for different sheep breeds.

Humans may begin the weaning transition for lambs as early as 3 weeks (Brown 1964) but there is a trade-off if all access to ewe milk is denied. Separation and nutritional stress on lambs has been shown to affect their immune system's resistance to parasites, and results in lower than normal lamb maturation weights (Watson and Gill 1991, Shaw et al. 1995). Up to 4 months of age, lambs remaining with their mothers have improved parasite resistance and maturation weight gain (Shaw et al. 1995). This is a benefit from having

access to milk, but suckling also creates and maintains the bond between ewe and lamb and is a comfort response for lambs (Arnold et al. 1979, Hass 1990). The Kurdish semi-nomadic herdsman discussed in Vigne and Helmer (2007) slaughtered or weaned their lambs at the start of the third month. From studies of immune system resistance to parasites, it would seem that weaning at that age is too early. Perhaps for the Kurds' herds parasites are not an issue, or the benefit of full access to fat-rich milk for making butter (*rughan*) exceeds the cost of having sheep with compromised immune systems, as selling the butter is the only non-subsistence dairy income for these people.

Table 3.1: Weaning ages and lactation lengths (managed and natural) of wild and domestic sheep

***Ovis* breeds**	**Weaning completion**	**Lactation length**	**References**
European Mouflon (*orientalis musimon*)	~160 days	~160 days	Obregón et al. (1992)
North American Bighorn (*canadensis*)	130–150 days	130–150 days	Festa-Bianchet (1988), Hass (1990), Festa-Bianchet et al. (1994)
Cyprus Fat-tailed (*aries*)	~158 days	~158 days	Louca (1972)
Dorset Horn (*aries*)	~160 days	~160 days	Arnold et al. (1979)
Mehraban (*aries*)	~144 days	~144 days	Zamiri et al. (2001)
Merino (*aries*)	~150 days	~150 days	Arnold et al. (1979)
North Ronaldsay (*aries*)	~150 days	~150 days	Caumette et al. (2007)
Shetland (*aries*)	~150 days	~150 days	Chapman (2010)
Assaf (dairy) (*aries*)	Managed, ~30 days	~180 days	Gutiérrez et al. (2007)
Awassi (dairy) (*aries*)	Managed, ~30 days	~200 days	Gootwine and Goot (1996)
Chios (dairy) (*aries*)	——	~209 days	Louca (1972)
East-Friesian (dairy) (*aries*)	Managed, 30 days	~186 days	Gootwine and Goot (1996), McKusick et al. (2001)
Sarda (dairy) (*aries*)	Managed, ~30 days	~240 days	Cappio-Borlino et al. (1997)

The start of the weaning transition may also vary naturally as a result of environmental conditions, maternal stress or poor milk quality (Hass 1990, Réale et al. 1999). The addition of plant foods to the lamb's diet generally begins 2 weeks after birth

but, under extremely poor milk and environmental conditions in European mouflon populations, grazing has been observed to begin during the third day of life and to be intensive by days 10–15 (Réale et al. 1999). However, the microflora in the rumen is generally not functional until a lamb is 8 weeks old (Lyford 1988). As a result, nutrient absorption would, in cases of intensive early plant consumption, be insufficient (Réale et al. 1999). Specific to calcium nutrition, in sheep and goats it has been found that the functioning rumen is a significant site of calcium absorption, with up to 50% of total calcium absorption in the gastrointestinal tract occurring in the rumen (Schröder et al. 1997). In the absence of a functioning rumen, milk remains the primary dietary contributor. As a result of all these different factors, it is likely that ancient people involved in sheep dairying weighed the costs and benefits of when to wean their lambs and, depending on food availability, quality and animal health (parasite load), selected an optimal weaning age that was earlier than when the weaning transition was likely to be naturally complete (~5 months).

It should be noted that an alternative reason for early lamb weaning, i.e. that domesticated ewes have a different lactation length compared with wild ewes, is unlikely. This is based on the mouflon (*Ovis orientalis*), the closest genetic link to the wild progenitor of modern sheep (Hiendleder et al. 2002, Pedrosa et al. 2005, Tapio et al. 2006), which, unless under significant dietary and/or physiological stress, will lactate for an equivalent length of time as non-dairy domestic sheep breeds, 150–160 days or ~5 months. When considering $\delta^{44/42}$Ca values and observing what may be indications of early weaning, it is useful to look for evidence of physiological stress, such as enamel hypoplasia, in order to try to clarify the source of possible early weaning, i.e. whether it was human managed or the result of nutritional deficiency.

3.4 Chapter summary

The process of developing a new means of investigating nursing and weaning through the use of an isotope not used previously for this purpose and on a tissue little analysed for this isotope is complicated. There are more questions than answers. However, there is a foundation of knowledge from other isotopic research that can be used as a basis from which to start. Thus oxygen and carbon isotope analyses were used in conjunction with the analysis of calcium isotopes in enamel. It was decided, after in-depth consideration, that a modern reference source of data was needed to provide a context for any archaeological results, and a modern dental chronology was needed to frame the results in connection with known timings of dietary and life events. Enamel mineralisation rates probably raise the most questions, but this is a common issue for researchers needing to link isotope ratio values, time, life history, diet and tooth growth data with each other. This chapter provides the necessary information as it is currently understood as a foundation for the later discussion of the isotope results provided in Chapters 5, 6 and 7.

CHAPTER 4

MATERIALS AND METHODS

This chapter presents the methodology for choosing appropriate samples, and the techniques used for extracting, processing and analysing the samples for calcium, oxygen and carbon isotopes. An overview of the instrumentation used to analyse the stable light isotopes of carbon and oxygen, and a discussion of the instrumentation used to analyse the heavier isotopes of calcium, are provided. The approaches for validating, reporting and analysing the results are also detailed. The laboratories involved in the sampling, pretreatment, chemistry and analysis were the Department of Earth Sciences, University of Oxford (referred to hereafter as Earth Sciences), the Research Laboratory for Archaeology and History of Art, University of Oxford (RLAHA), the Department of Archaeological Science, University of Bradford (Bradford), and the BioArCh laboratory, University of York (York).

4.1 Samples for calcium, oxygen and carbon apatite analysis

In the mammal diet, the two main sources of calcium are milk and plants. With the exception of humans, milk ceases to contribute to the mammal diet after weaning. This fundamental principle is pivotal to the research presented here, and thus milk and plant samples were collected and analysed, along with modern dental enamel and bone, to validate whether calcium isotope differences can be detected reliably and related to milk-based and plant-based diets. The technique developed was used to assess modern sheep results and archaeological sheep dental enamel samples from teeth excavated from the Neolithic phases of the site of Tell Abu Hureyra in Syria.

As part of assessing the plant food contribution to sheep enamel, modern dietary samples from Highfield Farm in Yorkshire, England, were collected for analysis. The dietary samples consisted of grass (the main and most consistent dietary contributor), hay, a

vitamin-supplemented mixed feed and dried sugar beet flakes. Mandibles from freshly slaughtered sheep from Highfield Farm were collected in the autumn/winter of 2010 and the autumn/winter of 2011. Bone samples were collected from the mandibles, and first and second molars were extracted for sampling. Only mandibles from animals under 1 year of age were collected because the abattoir's veterinarian could not release mandibles from sheep older than 12 months due to restrictions regarding the spread of scrapie (a fatal, degenerative neurological disease that is one of the group of transmissible spongiform encephalopathies and is similar to bovine spongiform encephalopathy) (Baron et al. 1999, Stack et al. 2002). With the exception of one lamb that was 5 months old, lambs were between 7 and 7.5 months old at the time of slaughter. Some bone samples from older Highfield Farm sheep were acquired as waste from butchering of the torso and limbs.

The enamel from the Highfield Farm sheep teeth, especially the second molars, contained significant amounts of immature enamel and were only approximately half structurally complete (Figure 4.1). To increase the dataset, Professor Terry O'Connor provided modern mature sheep jaws from the University of York's zooarchaeological reference collection. These mandibles were from a herd of managed modern rams and castrates raised on pastures that were part of a native plant restoration/grazing project run in the 1990s on the island of Hoy, Orkney. The teeth from these mandibles had mature enamel on all the first and second molars and the majority of the third molars (Figure 4.2). Using the Highfield Farm and Hoy modern sheep enamel results, methods were developed for identifying the contribution of milk and plants to the diet of sheep, including the mixed diet consumed during weaning.

Figure 4.1: Photograph of Highfield Farm lamb HF35's first (right) and second (left) molars. Although the crowns were essentially structurally complete, the first molars from the Highfield Farm lambs contained a small amount of immature enamel adjacent to the cemento-enamel junction. The second molars were approximately half formed and contained progressively less mature enamel down the length of the tooth away from the apex.

Figure 4.2: Photograph of Hoy sheep 800's first (left), second (middle) and third (right) molars. The first, second and some of the third molars of the Hoy sheep were structurally complete and had fully developed enamel, in contrast to the Highfield Farm lamb molars.

Once the methodology using modern teeth and their associated plant and milk samples had been defined (Section 4.2.1), an archaeological site with potential for researching early Neolithic sheep husbandry, through investigating weaning timing and lactation lengths, was selected. The site chosen was the Epipalaeolithic–Neolithic site of Tell Abu Hureyra, Syria. Tell Abu Hureyra, during the Pre-Pottery Neolithic (11,700–8250 cal. BP), was positioned for the human occupants to take advantage of living on the edge of the lush Euphrates River valley and the drier steppes, and was on the path of a yearly gazelle migration (see Chapter 7 for greater details) (Legge and Rowley-Conwy 1987, Moore 1998). Based on samples excavated and recovered through floatation, the plants near Neolithic Tell Abu Hureyra were commonly associated with woodland, steppe, woodland–steppe and dryland (cultivated) environments (de Moulins 2000). In addition to this naturally rich environment, the area around Tell Abu Hureyra was well suited to the development of, and eventual dependence on, cultivated domesticated cereal grasses (wheat, rye, barley and einkorn) and domesticated animals (sheep, goat, cattle and pigs) due to the warm and wet climate during the Holocene's climatic optimum (11,000–7000 BP), which followed the less hospitable period of the Younger Dryas (13,000 cal. BP–11,600 cal. BP) (Severinghaus et al. 1998, Severinghaus and Brook 1999, Hillman 2000, de Moulins 2000, van der Plicht et al. 2004).

As a result of the promising environmental conditions, Tell Abu Hureyra has a long history of Neolithic human occupation and, most importantly for the purposes of my research, through a large part of the Pre-Pottery Neolithic B (PPNB) (10,500–8250 cal. BP). This is relevant because excavation of the PPNB phase of the settlement site yielded sheep jaws and teeth from a dated context concomitant with the earliest direct evidence of dairying in the Near East (~7000 BC) (Evershed et al. 2008). Additionally, Tell Abu Hureyra is near the Taurus–Zagros region (in southern Turkey, Iraq and Iran), which has been identified through sheep mitochondrial (mt) DNA analysis as the most likely location of the origin of sheep domestication (Hiendleder et al. 2002, Pedrosa et al. 2005, Tapio et

al. 2006, Zeder 2011). It was also during this period that the site's faunal record demonstrates a change from a seasonal dependence on gazelle to, possibly early domesticated, sheep and goats (Moore 1998, Legge and Rowley-Conwy 2000). Therefore beginning to investigate the lives of sheep during this period at Tell Abu Hureyra through calcium isotope analysis is an excellent step in developing a new means of accessing individual sheep life histories (nursing and weaning), which in turn has the potential to address, once larger groups of sheep are analysed, the questions of human utilisation and management of sheep, not only at Tell Abu Hureyra during the Pre-Pottery Neolithic, but in different locations and during different periods in the archaeological record. Additionally, calcium isotope analysis of excavated sheep molars from archaeological sites has the potential to indicate if human management of the sheep was, or was not, part of a milk-based economy. This is possible if the variables associated with assessing the capacity of milk production in the archaeological record are known, namely weaning age, lactation length and herd size/slaughter profiles. Lastly, as discussed in Chapters 1 and 3, there is also the potential to investigate non-human factors that may affect Pre-Pottery Neolithic ewe lactation length and/or when a ewe may choose to wean an offspring early, such as maternal dietary stress.

The curator of the zooarchaeological portion of the Cambridge collection, Professor Tony Legge, provided the archaeological sheep mandibles and teeth from Tell Abu Hureyra. The mandibles from the site had been damaged during excavation and many of them had been reconstructed with glue. The extent of the glue used on the jaws and teeth was not readily apparent until the teeth were exposed, and as a result some of these samples required additional preparation before chemistry could begin (Section 4.2.1).

4.1.1 Dental enamel sampling

Dental enamel was selected because it is not subject to metabolic alterations over time, as occurs in bone, and is less susceptible to diagenetic alteration (Heuser et al. 2011). Additionally enamel provides 'snapshots' over time of the development of the tooth. This is

very important for research concerning nursing and weaning because certain teeth or even parts of teeth can be linked to both the diet and a particular period of a mammal's life. This is achieved by linking dental development to isotope results obtained from discrete portions of the enamel, which was done by taking serial, segmented samples from the first, second and third molars. Although bone was largely excluded from the project because of issues with high calcium isotope variability related to non-predictive metabolic processes and remodelling, it was also analysed in a few instances. The reason for this was because the bone samples were from sheep with known life histories and the results could be interpreted not only in reference to that information, but also in connection with the results of samples from their diet and the milk produced by the ewes. This was an attempt to establish whether animal age and ewe birth numbers/periods of lactation affected the calcium isotope ratio results, as suggested by the human results related to age in Heuser and Eisenhauer (2010) and the male and female sheep results in Reynard et al. (2010).

The surface of each tooth and bone sample was cleaned using a Swam-Blaster containing aluminium oxide powder. A diamond-tipped Dremel drill bit, using either a Dremel or dental drill, was then used to collect a powdered sample. Powdered samples were collected and weighed to between 1.5 and 2 mg. If carbonate analysis was being undertaken, then between 7 and 9 mg were taken, as the recommended sample size for carbonate analysis is at least 5 mg. In some instances not enough sample was taken initially to accommodate carbonate analysis, which was a later addition to the methodology, so secondary sampling was necessary. Any secondary sample was mixed with what remained after weighing the first sample for the calcium isotope analysis. A ground archaeological cattle femur was used as an in-house bone standard, and was also weighed to between 1.5 and 2 mg.

Bulk enamel samples were taken from just below the apex of the crown to just above the cemento-enamel junction, parallel to the tooth growth axis, in a single narrow strip through the full depth of the enamel. Serial segmented samples were taken

perpendicular to the growth axis of the tooth in grooves through the full depth of the enamel. The incremental sample sites were spaced 3 mm apart, progressing from just below the apex of the crown to just above the cemento-enamel junction (Figure 4.3). Bone samples, depending on the size of the bone, were taken over an area of about 2.5 × 2.5 cm and a depth of about 2 mm.

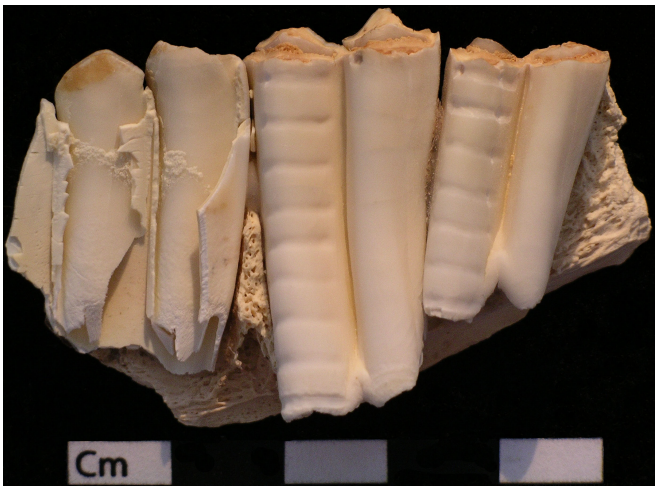

Figure 4.3: An example of incremental enamel sampling of, from right to left, first and second molars. The teeth shown are from the archaeological site of Tell Abu Hureyra and are identified as AH72, E33, B59.

4.1.2 Milk and dietary samples

The ewe, milk and feed samples were from Highfield Farm (Yorkshire) and were collected on 14 May 2010 and 19 April 2011. Ewes were corralled along with their lambs, and while I kept the ewe from escaping, the farmer (C. Chapman) milked the (very reluctant) ewe (Figure 4.4). Milk was collected in a measuring cup before being transferred to centrifuge tubes. The measuring cup was rinsed well with Milli-Q water (18.2 MΩ•cm) and methanol between each milk collection. If the milk was very thick, a small amount of Milli-Q water was added to make the transfer to the tubes easier. The milk samples were immediately placed in a cooler and kept cold until they could be placed in a freezer. Feed samples of grass, feed mix, sugar beet shavings and hay were collected in paper bags, to ensure the plant material could dry out, before being transferred to plastic bags. The first stage of preparation for these samples was to reduce them to more manageable sample sizes. Milk samples were measured out to 5–6 mL (depending on how watery the milk appeared) and

feed samples were weighed to ~1 g. Once all the samples were collected and their weights and volumes determined, they were prepared for instrumental analysis.

Figure 4.4: Collecting milk samples from reluctant Highfield Farm ewes.

4.2 Sample preparation and analysis: calcium from all samples

The process of preparing samples for calcium isotope analysis is very different from that undertaken for more traditional isotopes. Standards are critical to calcium isotope analysis, as they are directly involved not only in confirming that the instrument is functioning correctly, but that each individual sample analysed falls within accepted parameters as part of data assessment. However, before any samples, and some standards, can be analysed, it is necessary for the calcium to be isolated from all other elements in the sample/standard matrix.

As mentioned in Chapter 2, the difference in mass of the calcium isotopes ^{42}Ca, ^{43}Ca and ^{44}Ca is small and thus fractionation effects are also small, compared with the larger mass differences between the isotopes of carbon and oxygen. The small mass differences between calcium isotopes mean that samples have to be processed using column

chemistry/chromatography to separate calcium from organic materials and all other elements in the sample/standard matrix prior to instrumental analysis. Specifically it is to remove matrix elements, such as sodium (Na), magnesium (Mg), potassium (K) and especially strontium (Sr), from the sample, which could produce artificial isotope fractionation during chemistry or during instrumental analysis (Pietruszka and Reznik 2008).

A means of detecting issues in calcium isotope research is through the use of standards with known delta (δ) results. Two standards, High-Purity Standards calcium (HPS_{new} Calcium) and an in-house bone standard, are routinely run through column chemistry, usually in duplicate, alongside samples in order to detect issues with the individual batches of samples and with the methodology in general. Standards are also used to monitor instrument performance as well as in validating calcium isotope instrumental measurements/results.

The specific instrument monitored by calcium standards, as part of this sample analysis, was a multicollector–inductively coupled plasma–mass spectrometer (MC-ICP-MS), designated as Plasma 2, at Earth Sciences. This type of instrument uses a plasma source to ionise samples. If the samples have not been purified to contain only calcium, Plasma 2 will also ionise all other elements in the sample matrix, including organic material and other elements, which would reduce both instrumental accuracy and precision (Hirata et al. 2008, Pietruszka and Reznik 2008, Wombacher et al. 2009). Unlike instruments for analysing samples for carbon and oxygen, Plasma 2 does not generate δ-values; it produces sample and standard isotope measurements, which are entered into an Excel spread sheet containing macros, designed by me, that generate δ-values.

Figure 4.5 is a visual guide to the methods used to produce a purified calcium sample solution ready for instrumental analysis. Although most of the methods were the same for the samples and standards, there were some significant differences, especially

involving sample pretreatments. The details of the methods are given in Sections 4.2.1 and 4.2.2.

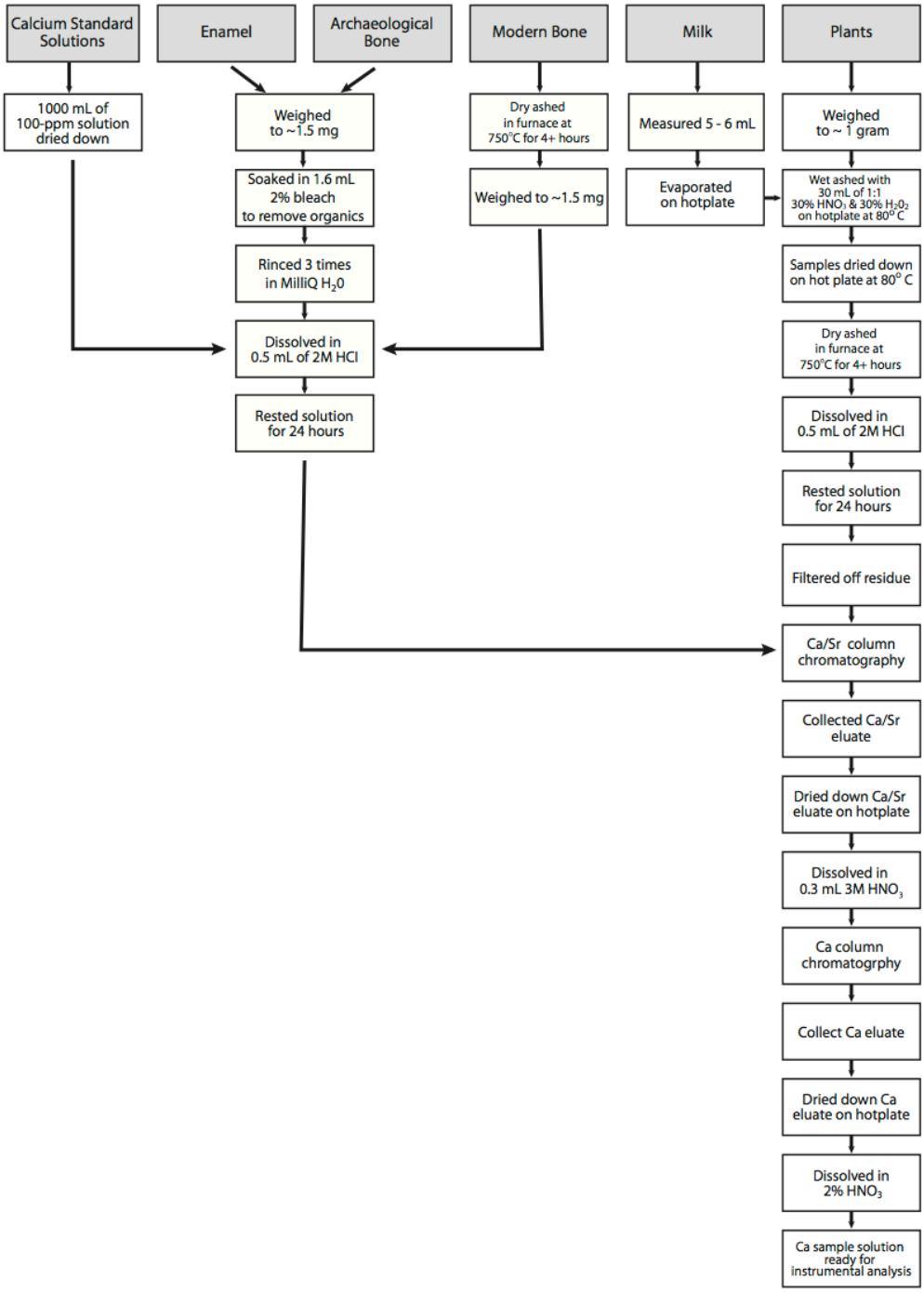

Figure 4.5: Flowchart of the preparations needed to produce sample solutions ready for instrumental analysis.

4.2.1 Sample and standard pretreatment for calcium isotope analysis

Prior to column chemistry, all of the samples required pretreatment, which was done at RLAHA. Powdered archaeological bone standards and samples, and modern and

archaeological dental enamel samples, were soaked in 2% bleach (NaOCl) solution overnight to dissolve extraneous organics. The samples were then centrifuged and rinsed three times in Milli-Q water (18.2 MΩ•cm) prior to being transported to the Class 1000 clean, metal-free laboratories at Earth Sciences, where they were dissolved in 0.5 mL of 2M quartz-distilled, concentrated hydrochloric acid (HCl).

Feed and milk samples required different processes. Milk samples were put in crucibles and dried down on a hotplate. Feed samples had been dried in paper bags before being reduced in size in a food processor and put in crucibles. The dried feed and milk samples were then wet-ashed with 30 mL of a 1:1 mixture of 30% ultra-pure hydrogen peroxide (H_2O_2) and 30% quartz-distilled concentrated nitric acid (HNO_3). The crucibles and contents were dried on a hotplate at 80°C to evaporate the acid and peroxide mixture. To ensure that all acid and H_2O_2 was removed, the samples were also dry-ashed for at least 4 hours in a furnace at 750°C. Some very fresh, modern bone samples also required dry-ashing in the same manner as the feed and milk samples, because the bone still contained significant amounts of organic material. Once cool, the dry-ashed samples were weighed to ~1.5 mg and transported to the Earth Sciences clean laboratories, where they were also dissolved in 0.5 mL of 2M HCl.

Although not included in the flow chart for enamel, an additional pretreatment to remove glue from some of the Tell Abu Hureyra enamel samples preceded the overnight soak in 2% NaOCl. Two protocols were used to remove the glue. The first involved 1.8 mL of acetone being added to each powdered enamel sample. The samples were agitated to help dissolve the glue, before the acetone was removed through centrifugation and four rinses in Milli-Q water. Acetone is commonly used to remove glue and other adhesives in archaeological samples that are to undergo radiocarbon dating or isotope analysis (Moore et al. 1989, Bruhn et al. 2006, Yuan et al. 2007). The samples were rinsed very thoroughly because acetone contains lead, which would create a contamination issue at the Earth Sciences laboratory, where lead isotope research was being carried out. All work with acetone was done at RLAHA.

Bone samples were collected from the jaws of sheep from the Neolithic site of Tell Abu Hureyra for Zooarchaeology by Mass Spectrometry (ZooMS) assessment. This was done to determine whether the extracted molars were definitively from sheep and not goat. It was found that one of the analysed sets of teeth was from a goat, which in fact provided useful information for future work (see Chapters 7 and 8).

A single rinse in acetone was found to be inadequate, because remnants of glue remained in the samples. This result was easily visible during instrumental analysis because the data was random, scattered and far outside of expected values. Thus the protocol was modified to include three washes of acetone before the four Milli-Q rinses (Figure 4.6). Instrumental results obtained after the second glue removal protocol were within the expected range of values. After the acetone treatments were complete, the enamel samples were soaked overnight in 2% NaOCl solution before centrifugation and three rinses in Milli-Q water (18.2 MΩ•cm). Samples were dissolved in 0.5 mL of 2M HCl at Earth Sciences.

Glue removal pretreatment **Date:**

Sample #	1.8 mL acetone	Heavy agitation	Centrifuge & pipette	1.8 mL acetone	Heavy agitation	Centrifuge & pipette	1.8 mL acetone	Heavy agitation	Centrifuge & pipette	Milli-Q rinse X4

Figure 4.6: Glue removal protocol.

Calcium standard solutions (solutions with known $\delta^{44/42}$Ca and $\delta^{43/42}$Ca values) intended for column chromatography alongside the samples also needed pretreatment. A

small quantity of the 1000-ppm bottled calcium reference standards of Alfa Aesar (Alfa) and HPS_{new} Calcium were diluted to 100 ppm. A 1000-mL aliquot of that solution was then dried down in a beaker and the calcium standard residue was dissolved in 0.5 mL of 2M HCl. All samples and standards dissolved in 0.5 mL of 2M HCl were then allowed to reach equilibrium by sitting at room temperature for at least 24 hours (or 6+ hours in a sealed beaker on a hotplate set to 120°C). This was the final step before the samples were ready to begin column chemistry/cation exchange chromatography.

4.2.2 Column chemistry/cation exchange chromatography

The purification of calcium from the rest of the sample matrix was undertaken through a two-part or series of column chemistry/cation-exchange chromatography. The column chemistry methodology used was based on Reynard et al. (2010, 2011a), which was based in turn on Chu et al. (2006). Some modifications were made because of issues identified early on in the project, related to incomplete calcium collection and resin cleanliness. The modified column chemistry methodology used is shown in Figure 4.7.

Column Chromatography to Collect Calcium: (AG50W-X12_BioRad resin) **Date:**

Ca/Sr Seperation Methodology

			Elute				*Collect Ca + Sr*		* Clean/store
Sample #	6 mL 6M HCl	6 mL 2M HCl	*Load Samples* 0.5 mL 2M HCl	0.5 mL 2M HCl	1 mL 2M HCl	10 mL 2M HCl	2 mL 6M HCl	10 mL 6M HCl	2 mL 6M HCl

Date:

Separation of Ca from Sr Methodology: (Sr-Spec resin)

						Collect Ca			
Sample #	2 mL 6M HCl	2 mL Mili Q H2O	2 mL 0.01M HNO3	2 mL 3M HNO3	2 mL 3M HNO3	*Load samples* 300 uL 3M HNO3	300 uL 3M HNO3	2000 uL 3M HNO3	Put resin in waste bottle

*** Cleaning: 1) 3 mL 6M HCl (drained through), 2) 10 mL MiliQ (drained through), 3) a bit of MiliQ is added to keep resin wet.**

Figure 4.7: Column chemistry methodology.

The first series of columns contained 1.6 mL of AG50W-X12 BioRad resin and was used to separate calcium and strontium from other elements. The resin was prepared for the loading of samples by being washed with 6 mL of 6M HCl, which elutes any residual calcium from previous analyses. The resin was reused up to 10 times and was cleaned with 6M HCl at the end of each run, as well as the precautionary cleaning carried out at the beginning. Once the 6M HCl had fully eluted, the columns were prepared further with the addition of 6 mL of 2M HCl, which matched the acid of the sample solutions. The samples, dissolved in 0.5 mL 2M HCl, were then loaded into the columns. A series of additions of 2M HCl, totalling 12 mL, was added to the columns to elute fully the non-calcium and strontium cations. At that concentration of HCl, the cations of calcium and strontium remain in, or 'stick to', the resin.

There was one additional step for the samples of plant material before they were loaded into the columns. Even with wet- and dry-ashing and treatment in 2M HCl, there was a very small amount of residue, probably plant siliceous phytoliths (Chu et al. 2006), that did not dissolve. This was removed from the sample through syringe filtration using a 0.2-μm sterile Nalgene filter. The filtered sample was then loaded into the columns and the method described above applied.

Once elution of waste cations was complete, the collection of calcium and strontium in the resin was undertaken. Savillex beakers were used to collect the calcium/strontium eluate when a total of 14 mL of 6M HCl was added to the columns (Figure 4.8). The in-house archaeological bone standard and HPS_{new} Calcium standard solution, and intermittently seawater, were run alongside each batch of samples (Figure 4.9).

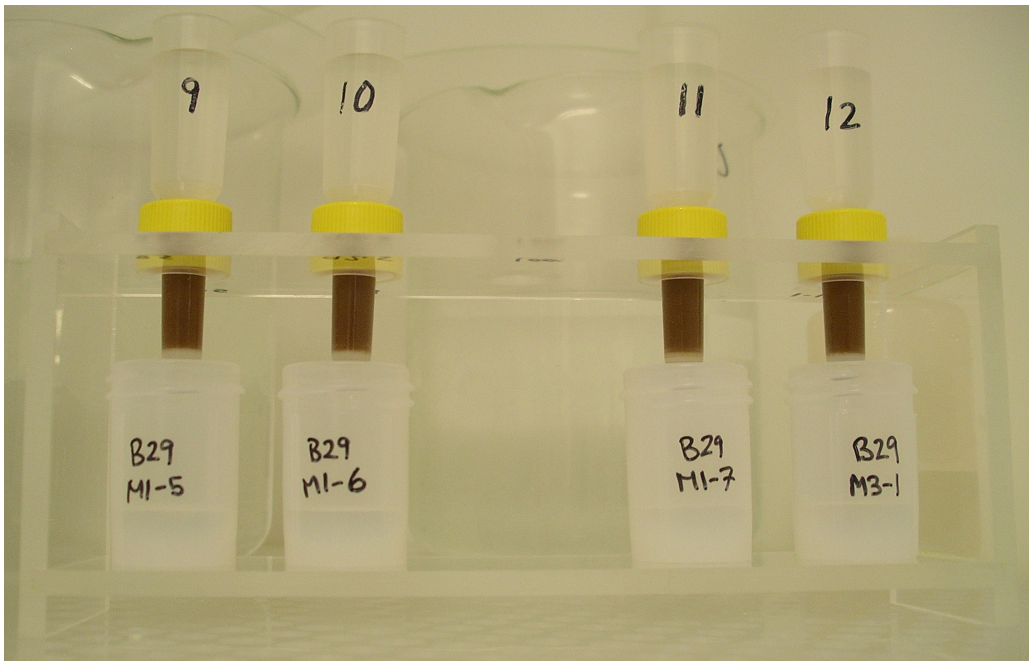

Figure 4.8: View of eluting columns with cation exchange resin and Savillex beakers, with calcium and strontium eluate.

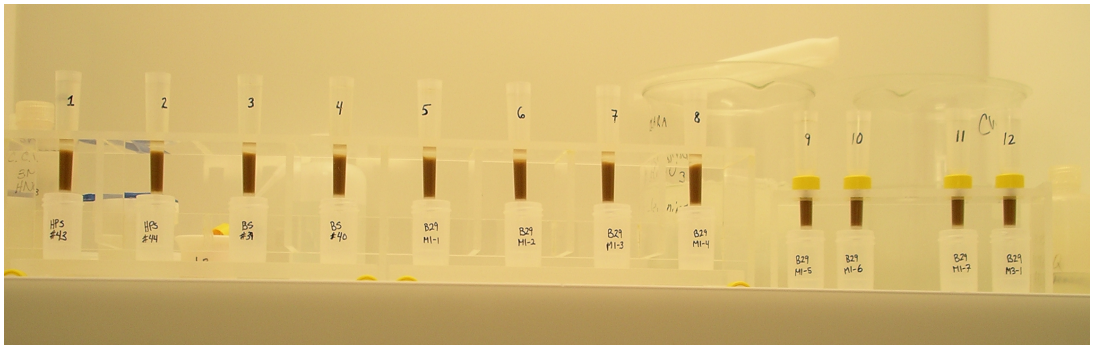

Figure 4.9: Columns with Savillex beakers. Two columns were run with HPS_{new} Calcium standard (columns 1 and 2) and two columns with the archaeological bone standard (columns 3 and 4). The remaining columns contained samples.

Once the collection was complete, each beaker was placed on a hotplate set to between 80°C and 120°C to evaporate and dry the sample. The temperature was dependent on how rapidly the samples needed to be dried. The samples could dry out and float away in the high negative pressure of the closed hotplate hoods if they were dried at a high temperature and not watched closely. If samples were left to dry overnight, lower temperatures were used to minimise this problem. Once samples were dry, they were dissolved in 0.3 mL 3M HNO_3 and, as with the sample preparation for the first columns, left

in sealed beakers to sit for 24-hours at room temperature or on a hotplate set to 120°C for at least 6 hours.

The resin used to separate and collect the calcium eluate from the calcium/strontium solution was 0.25 mL Sr-spec resin. As previously described, the first step in preparing the second columns for samples was to clean the resin with 2 mL of 6M HCl, followed by a 2-mL Milli-Q water rinse. Although the Sr-spec resin was never reused, the wash was still needed to prevent enrichment of heavier calcium isotopes in the samples/standards beyond what was in the samples/standards originally. It was observed during analysis, prior to the addition of the resin wash with HCl, that standards put through chemistry had a consistent enrichment in heavier calcium isotopes of or very near 0.1‰, compared with the standards analysed as stock solutions, which did not go through chemistry, and those reported in the literature. This tended to push the standards to the edge of and outside the accepted range of measurement precision for the Nu Plasma MC-ICP-MS, which is 0.1‰ in general (Halicz et al. 1999). Plasma 2 had a measurement precision of between 0.04–0.08‰ (1 SD) (see Appendix B). Plasma 2 was also used by Reynard et al. (2010, 2011a) and was in close agreement with their measurement precision of 0.06–0.08‰ (1 SD). The HCl wash fixed the problem and became part of the methodology.

The rest of the second column procedure was very similar to that of the first column, in that there was a series of steps to prepare the resin for the samples, with the addition of 2 mL of 0.01M HNO_3 and two additions of 2 mL of 3M HNO_3. Samples, dissolved in 0.3 mL of 3M HNO_3, were loaded into the columns and collection began immediately in Savillex beakers. The calcium eluate was collected with the addition of a total of 2.6 mL of 3M HNO_3 to the columns. Once all the calcium eluate was collected, the beakers were placed on a hotplate set to between 60°C and 90°C to evaporate and dry the samples. The samples from the second columns did not have the tendency to float away once dry, but the total eluate volume was much smaller upon completion of the second column compared with the first, and dried much more quickly, even at a lower temperature.

The Sr-spec resin was not reused and was placed in a waste resin container for later disposal. The columns were stored empty in a large Savillex beaker filled with 2% HNO_3. Once the samples were dry, they were diluted to a volume of 2 mL with 2% HNO_3. The sample solutions were left in sealed beakers to sit at least overnight at room temperature, or on a hotplate set to 120°C for at least 6 hours. After that the sample solutions were ready for instrumental analysis on Plasma 2.

As previously mentioned, the standards were run through column chemistry along with the samples. These standards, once analysed on Plasma 2, could be used to identify issues within the column chemistry methodology or possible sample contamination. Calcium standards are also key to assessing Plasma 2 sample results and determining whether or not they should be accepted or rejected. The selection, purpose and determined isotope ratio values of calcium standards are discussed in Appendix B.

4.2.3 Instrumentation, methodology for calcium isotope analysis and data validation

Calcium concentration checks and isotope measurements for ^{42}Ca, ^{43}Ca and ^{44}Ca were completed using Plasma 2, which is a Nu Plasma MC-ICP-MS instrument (Figure 4.10). It had a DSN-100 desolvation nebuliser system and a 12 Faraday cup collector array. The instrument was double-focusing, which meant it contained both an electrostatic analyser and a magnetic sector analyser (magnet). The electrostatic analyser focuses the ions heading toward the collector array by only allowing the calcium ions with a narrow range of kinetic (mass-to-charge ratios) energies to move towards the magnet or magnetic sector analyser. The magnetic sector analyser then separates the isotopes based on their mass through deflection. The heavier the isotopes, the less they are deflected away from the influence of the magnet. In the case of calcium isotopes, ^{44}Ca is deflected the least and ^{42}Ca the most, with ^{43}Ca falling between the two. Once separated the isotopes then enter the detection block and are collected/quantified by the Faraday cups assigned to collect ions for each mass of isotope. The operating parameters for Plasma 2 are listed in Table 4.1.

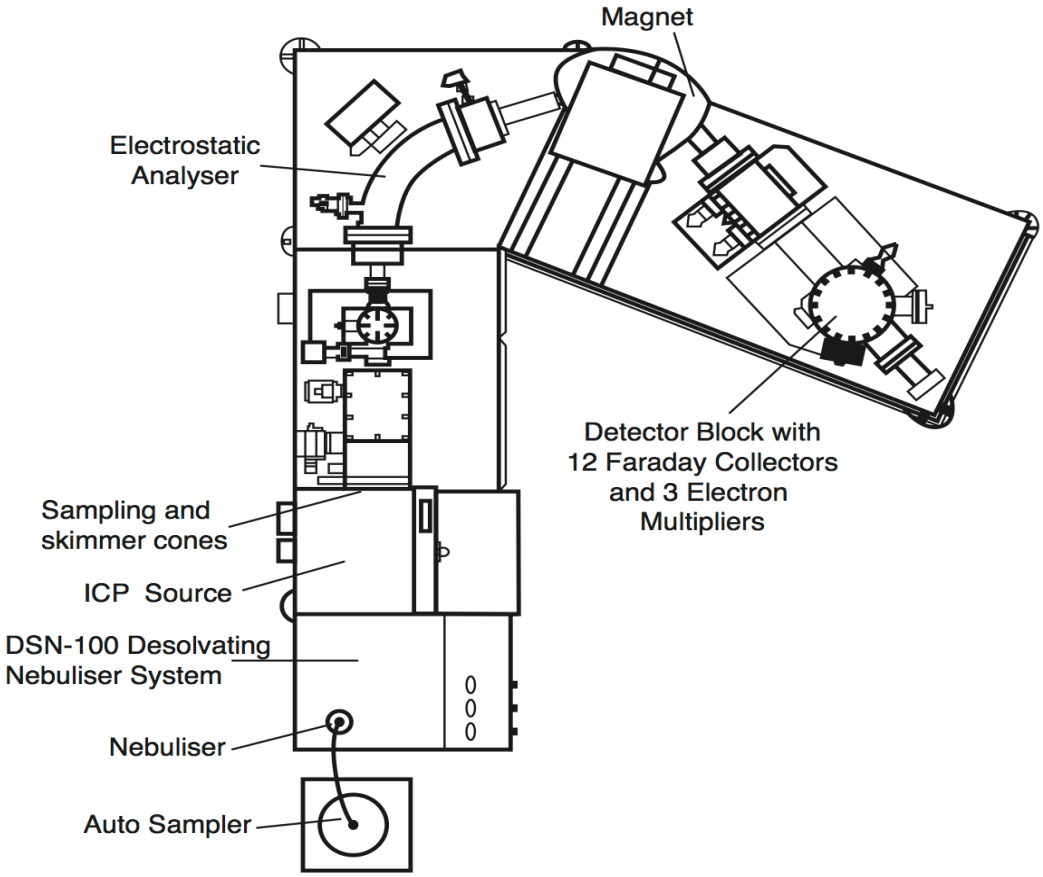

Figure 4.10: A schematic of Plasma 2, looking down on the instrument, with the locations of the auto sampler, DSN-100 nebuliser system, plasma chamber, cones, electrostatic analyser, magnetic sector analyser and Faraday collector array.

Table 4.1: Operating conditions and parameters of the Plasma 2 instrument

Argon (Ar) gas flow rates – cool gas	11.7 L/min
Ar gas flow rates – auxiliary gas	0.6–1.2 L/min
Desolvation nebuliser system	DSN 100
Nebuliser pressure	25.0 psi
Spray chamber temperature	105°C
Membrane temperature	115°C
Sweep gas flow (Ar)	2.50–3.50 L/min
Solution uptake rate	50 µL/min
Forward (rf) power	1300 W
Interface cones	Nickel, A-type geometry
Analyser vacuum	5×10^{-9} mbar
Acceleration voltage	4.0 kV
Focusing optics	Optimised for maximum intensity
Detector used	12 Faraday collectors
Typical Ca sensitivity (915a and Fisher07)	~0.7 and ~0.6 V/ppm, respectively
Sampling time	15 10-second integrations
Typical Ca introduced/analysis	4 µg

Concentration checks were a necessary first instrumental step in preparing for isotope analysis, especially as the method of isotope analysis utilised standard bracketing. This required all samples and standards being analysed for calcium isotopes to have the same calcium concentration (10 ppm ± 10%). Plasma 2 had a maximum sensitivity of 10 V, which, given the standards and samples being analysed, meant that there was a maximum acceptable concentration of about 15 ppm. Calcium isotope studies on an older, but nearly identical, instrument to Plasma 2, also at Earth Sciences, found that higher concentrations of standard solutions tended to reduce instrument sensitivity as the result of the build-up of residue on the sample and skimmer cones (Halicz et al. 1999). This is an issue regardless of the concentration of samples and standards, but the higher the concentration the more quickly cones build up deposits and sensitivity drops. The analysis undertaken by Chu et al. (2006) and Reynard et al. (2010, 2011a) used concentrations of 10 ppm, which I found to provide a large enough window of time (3–4 days) to complete most analyses while maintaining instrument sensitivity.

It was also crucial that the standards and samples upon completion of column chemistry were in 2% HNO_3. All concentration adjustments made to bring the samples and standards to 10 ppm were also done using only 2% HNO_3. This was critical because differences in the matrix between samples and standards can affect instrumental calcium isotope measurements and reproducibility (Fietzke et al. 2004). Details of instrumental analysis, issues with interferents and standard bracketing are given in Appendix C.

Once the analyses of the samples had been completed on Plasma 2 and the data was converted into δ-values, the means and standard deviations (SD) were determined. The SDs of the mean δ-values were important for completing a second culling of the data. Outliers were identified and removed if the SD was equal to or greater than 0.1. A final vetting of the results occurred when the mean δ-values were plotted in a three-isotope plot.

The example given here of a three-isotope plot is for human male samples from Ensisheim (France; see Chapter 2) as well as the standards of HPS_{new} Calcium CC, seawater

CC and the in-house bone CC (see Appendix B for an explanation of the standards). The data on a three-isotope plot should reflect the isotope mass differences between the isotope ratios of $\delta^{44/42}$Ca (plotted on the *y*-axis, with an isotope mass difference of 2) and $\delta^{43/42}$Ca (plotted on the *x*-axis, with an isotope mass difference of 1). As a result, the data should ideally plot on a trend line with a slope of two ($y = 2x$) (Figure 4.11). However, if Sr^{2+} affects instrumental measurements, which is more visible in $\delta^{43/42}$Ca results than in $\delta^{44/42}$Ca results due to the smaller mass difference in $\delta^{43/42}$Ca (Boulyga 2010), this may skew the data trend line away from $y = 2x$. In the three-isotope plot shown here, the line plotted as $y = 2x$ is defined as the mass fractionation line and is used in conjunction with the actual data trend line to identify $\delta^{44/42}$Ca and $\delta^{43/42}$Ca deviations away from a 2:1 ratio.

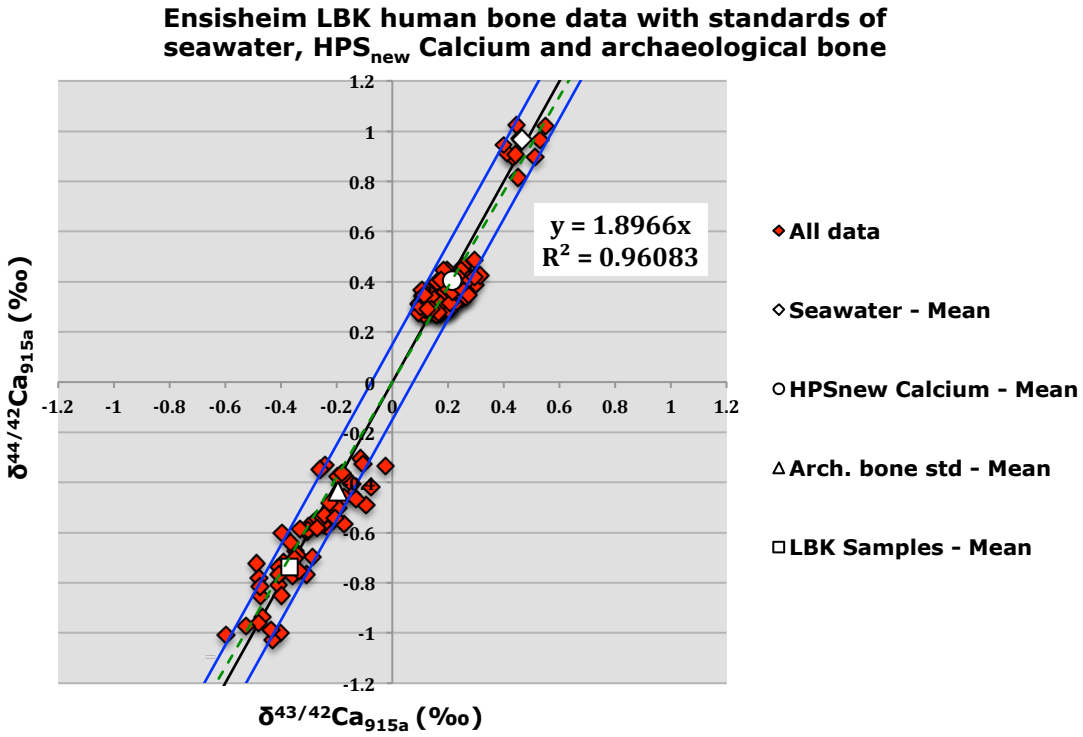

Figure 4.11: Three-isotope plot of $\delta^{44/42}$Ca‰ and $\delta^{43/42}$Ca‰ for human bone samples from Ensisheim, France (the white square is the mean), plus the standards of seawater CC, HPS_{new} Calcium CC and in-house bone CC (the white diamond, circle and triangle, respectively, are the means). The linear data trend line (dashed green line) for all of the plotted data has a slope of 1.9, close to the mass fractionation line with a slope of 2 (black solid line). R^2 is close to 1, demonstrating that 96% of the variation in *y* can be explained by the linear correlation between *x* and *y*. The blue lines are 0.15‰ to either side of the mass fractionation line. Data falling fully outside of the area between the blue lines is discarded.

If there is no or little deviation between the mass fractionation line and the data trend line, then it is likely that the Sr^{2+} contribution to the calcium results is non-existent or

negligible (Boulyga 2010). Following the recommendation by Reynard et al. (2010), results that were more than 0.15‰ from the mass fractionation line were discarded. This removed the $\delta^{44/42}Ca$ and $\delta^{43/42}Ca$ values that may have been affected by Sr^{2+} from the final results. All sample results along with the three-isotope plots can be found in Appendix D.

Although calcium was the focus of the analysis, it was not the only isotope that was measured in the samples. Measurement of the oxygen and carbon isotope contents of the dental enamel samples was also undertaken. Further mention of isotopes in this chapter therefore includes oxygen and carbon.

4.3 Sample preparation and analysis: oxygen and carbon apatite from dental enamel

A representative number of the dental enamel samples analysed for calcium isotopes underwent analysis for carbon and oxygen isotopes. The sample preparation involved a two-step process to clean and remove extraneous organics and absorbed, secondary carbonates in tooth enamel (Lee-Thorp et al. 1989). Once the samples had been prepared, they were analysed using isotope ratio mass spectrometers (IRMS). The instrument used was a Thermo Gasbench II at Bradford. The results were in δ-values and were used, once validated, to investigate seasonal patterns (oxygen) and milk versus plant foods in the diet (carbon).

4.3.1 Sample preparation for carbonate analysis

Although dental enamel is the safest tissue to analyse with regard to susceptibility to diagenetic alteration (Koch et al. 1997), it still requires cleaning and the removal of possible secondary carbonates acquired in the burial environment. The preparation of dental enamel samples for carbon apatite and oxygen analysis was undertaken at Bradford and York. It was based on the protocol developed at Bradford. Each powdered sample was weighed to between 5 and 7 mg and treated with 1.8 mL of ~1.7% NaOCl solution, agitated, and then left to sit for 30 minutes. The samples were then centrifuged and rinsed three times with

Milli-Q water (18.2 MΩ•cm). After the last rinse the samples were treated with 1.8 mL of 0.1M acetic acid (CH_3COOH), agitated, and left to sit for 5 minutes. The samples were then centrifuged for 4 minutes and the acetic acid pipetted off. The samples were rinsed three times with Milli-Q water before being placed in a freeze-drier overnight.

Some of the samples were found to contain contamination during analysis, which was probably residual acetic acid. Most of the samples that had been prepared for analysis had small amounts of enamel remaining for re-analysis. The contaminated samples were given three additional rinses with Milli-Q water before the samples were re-dried and weighed for a repeat analysis. The extra rinses solved the problem.

4.3.2 Instrumentation and methodology for carbon and oxygen isotope analysis

The analysis of dental enamel for oxygen and carbon isotopes requires an IRMS. This instrument measures the carbon and oxygen isotope content of dental enamel, which evolves as carbon dioxide (CO_2) gas during exposure to acid. An IRMS instrument consists of an ionisation source, a magnetic analyser to separate ions based on mass, and a detector that collects ions simultaneously using Faraday cups. The IRMS instrument used in the analysis of the samples for carbon and oxygen isotopes was a Thermo Delta V mass spectrometer connected to a Finnigan Gasbench II.

The analysis of samples involved first weighing each of the dental enamel samples to ~2 mg and then placing the samples in glass vials, septa-capped to seal the environment within the vial. During the analysis, atmospheric air was replaced with helium before H_3PO_4 was introduced. The samples analysed with a Thermo Delta V produced CO_2 when 100% H_3PO_4, kept at a temperature of 70°C, was introduced. CO_2 samples were analysed along with a CO_2 reference. The Thermo Delta V was in continuous flow and had an analytical precision of 0.2‰ or less for both $\delta^{13}C$ and $\delta^{18}O$ (Holzkämper et al. 2009, Pellegrini et al. 2011, Towers et al. 2011). This instrument has the advantage of using individual vials instead of a common acid bath, removing the issue of memory effect and cross-contamination.

4.3.3 Standards for carbonate analysis

Standards with known values, when analysed in reference to VPDB for carbon/oxygen and Vienna Standard Mean Ocean Water (VSMOW) for oxygen, were analysed before and after groups of samples. These standards were used for instrument calibration and monitoring. The standards used at Bradford were the international carbonate standards of IAEA-CO-1, IAEA-CO-8 and IAEA-NBS-19, as well as the in-house carbonate standard Merck $CaCO_3$ and the dental enamel standard BES. I added NOCZ, an Earth Sciences in-house carbonate standard, to the analysis at Bradford.

The carbonate-based standards were each weighed to ~0.15 mg and the enamel BES standard was weighed to ~2 mg. Once weighed, the standards were placed in glass vials for the Thermo Gasbench II. The δ-values for each of the standards, in relation to VPDB and/or VSMOW, are shown in Table 4.2.

Table 4.2: Standards used in carbon and oxygen analyses

Standard	**$\delta^{13}C_{VPDB}$ ‰**	**SD**	**$\delta^{18}O_{VSMOW}$ ‰**	**SD**
Bradford, international				
CO-1	2.49	0.03	28.41	0.07
CO-8	–5.76	0.03	7.55	0.19
NBS-19	1.95	0.09	28.65	0.26
Bradford, in-house				
BES	–11.1	–	25.5	–
Merck $CaCO_3$	–35.45	–	13.35	–
NOCZ	2.21	–	29.17	–
Earth Sciences, in-house				
NOCZ	2.09	0.1	–1.86	0.2

Oxygen results are reported relative to international standards with the delta notation. The samples and standards were carbonate-based and as a result the Bradford oxygen results were converted from the VSMOW scale to VPDB. The calculations for converting VSMOW to VPDB and vice versa are (Clark and Fritz 1997: 10, Sharp 2007: 29):

$$\delta^{18}O_{VSMOW} = \delta^{18}O_{VPDB} \times 1.03091 + 30.91$$

and

$$\delta^{18}O_{VPDB} = \delta^{18}O_{VSMOW} \times 0.97002 - 29.98.$$

4.3.4 Carbon and oxygen isotope data assessment

As previously mentioned in Section 4.3.1, there were contamination issues with some of the samples. This contamination, probably acetic acid, evolved late in the sample acid digestion and only affected the measurements taken towards the end of the run. A run was 10 measurements of CO_2, with each measurement lasting 10 minutes. Removing the results from the last measurements in a run usually brought the mean SD for the sample δ-value to an acceptable level (usually to 0.25 or less). Unfortunately the contamination was often carried over to the analysis of the next sample or standard by interfering with the correct measurements of the reference gases. Where the measurements of the reference gases were contaminated, the affected sample results were rejected outright.

Although the removal of measurement results from the end of a run, to compensate for contamination, produced a usable sample result, this was not an ideal situation. Removal of the measurements from late in the run meant that isotopes in the CO_2 that evolved late would not contribute to the overall sample result, possibly producing artificial isotope fractionation. As a result, the affected samples were prepared again and re-analysed. Appendix E shows all the carbon and oxygen isotope results.

The results from the calcium, oxygen and carbon isotope analyses were used predominantly to identify patterns of dietary and seasonal change over time. This was accomplished through the analysis of serial, segmented dental enamel samples. These samples were not conducive to statistical analysis. Bulk samples collected from the bone and dental enamel of modern sheep were appropriate for statistical analysis, as they involved groups containing such variables as age at death, sex and isotope results. Statistical methods were used to identify relationships between the variables and to determine the factors influencing them.

4.4 Statistical analysis

Statistical analysis was performed using statistical software Minitab 16. The tests used for results analysis were one-way ANOVA tests and two-sample *t*-tests. Graphs and tables were made using the Macintosh version of Excel from Microsoft Office 2011.

4.5 Chapter summary

This chapter has detailed the methodologies for sample selection, extraction, processing and analysis for calcium, oxygen and carbon apatite isotopes, and described the instruments used for the analyses. The methods of data validation, statistical analysis and reporting the results have also been described. The results that were obtained after implementing the full methodology are presented in Chapters 5, 6 and 7.

CHAPTER 5

HIGHFIELD FARM $\delta^{44/42}Ca$, $\delta^{18}O$ AND $\delta^{13}C$ SAMPLE RESULTS AND DISCUSSION

Previous attempts to investigate milk consumption using calcium isotopes in bone apatite gave ambiguous and unsatisfactory results. Therefore, calcium isotope analysis was performed on sheep molar enamel to develop a new approach for investigating the role of sheep weaning in dairying in the archaeological record. Calcium isotope analysis of dental enamel to investigate milk consumption, nursing/weaning and dairying has never been attempted before. A pilot study was therefore undertaken using enamel from lambs from Highfield Farm (Yorkshire) to determine whether enamel would be a suitable tissue for further investigation. There are two sources of dietary calcium: milk and plants, and the research by Chu et al. (2006) demonstrated that, for sheep, milk is depleted in ^{44}Ca relative to the plants in the diet. The pilot study using lamb molar enamel, coupled with feed and ewe milk samples, produced results that satisfactorily showed a weaning transition. Based on these results, the study was expanded with: (1) additional samples from Highfield Farm sheep, (2) sheep enamel samples from the Loft and White Hamars Grazing Project modern herd from Hoy (Orkney), and (3) enamel samples from sheep from the archaeological site of Tell Abu Hureyra (Syria).

Sheep were used for this study because once weaning is complete they consume only plant foods. This reduces dietary complexity for result interpretation and keeps the focus on the two sources of dietary calcium: milk and plants. Sheep are an ideal species for building a modern reference dataset due to their fast maturation rates, easy accessibility in the UK for sample collection and their history of use as a dairy animal. Sheep are relevant to researching questions regarding lactation, nursing and weaning, as well as, due to their domestication around 11,000 BP, animal management in the archaeological record (Zeder 2008).

To produce modern reference sets of calcium isotope data from sheep, samples were selected from animals living in managed situations, which allowed the recording of specific herd management details, individual animal vital statistics and dietary histories. The modern samples came from two locations in the UK: Highfield Farm in Yorkshire and the Loft and White Hamars Grazing Project, Hoy, Orkney (Chapter 6). Although not the focus of the research, bone samples were analysed for $\delta^{44/42}Ca$ values to add to the results reported in the literature. The majority of the analyses of Highfield Farm samples involved calcium isotopes, but a selection of key samples were also analysed for oxygen and carbon apatite isotope ratios in order to try to gain better resolution of the timing of nursing and weaning (oxygen) and to have supporting evidence of dietary change related to nursing and weaning (carbon). Appendix F contains the details of the results of the statistical tests. This chapter discusses the Highfield Farm results and the implications for using calcium isotope analysis of molar enamel as a method for investigating ancient sheep nursing, weaning and lactation in the archaeological record.

5.1 Highfield Farm samples

Highfield Farm is a small sheep farm in North Yorkshire, UK. The sheep, with the exception of one Wensleydale, are all of the Shetland breed. The small size of the farm and small number of animals made it possible to keep detailed records of these sheep's lives over a number of years. Although the mature ewes on this farm had all received names at birth, the herd is now becoming large enough that numbers are assigned to lambs (HF#) instead of names (Table 5.1).

Table 5.1: Highfield Farm sheep and samples

Sheep ID	Age at death	Sex	Kinship	Milk 2010	Milk 2011	Bone	Molars
HF29	7 months, 21 days	♂	Twin to HF30, lamb of Amber			X	X
HF30	7 months, 14 days	♀	Twin to HF29, lamb of Amber			X	X
HF33	7 months, 7 days	♀	Twin to HF44, lamb of Jet			X	X
HF35	7 months, 14 days	♀	Lamb of Coco				X
HF44	7 months, 14 days	♂	Twin to HF33, lamb of Jet			X	X
HF61	5 months, 6 days	♂	Lamb of Orla				X
Amber	Adult, still alive	♀	Mother of HF29 and HF30		X		
Coco	Adult, still alive	♀	Mother of HF35		X		
Fifi	4 years, 6 months, 8 days	♀	———	X	X	X	
Helena	7 years, 3 months, 18 days	♀	———	X		X	
Jet	Adult, still alive	♀	Mother of HF33 and HF44		X		
Stillborn	———	♂	———			X	

5.2 Highfield Farm milk and feed sample $\delta^{44/42}$Ca values: results and discussion

5.2.1 Highfield Farm milk and feed sample $\delta^{44/42}$Ca values: results

The milk from the Highfield Farm ewes was found to be depleted in ^{44}Ca relative to the diet. The range of isotope offset between the mean feed and the milk results was 0.35–0.43‰. However, grass played the greatest contribution to the diet over time and the range of isotope offsets between milk and grass was 0.43–0.51‰. Figure 5.1 and Table G.1 (see Appendix G for the tables prefixed G) contain the mean $\delta^{44/42}$Ca values for Highfield Farm ewe milk and feed samples.

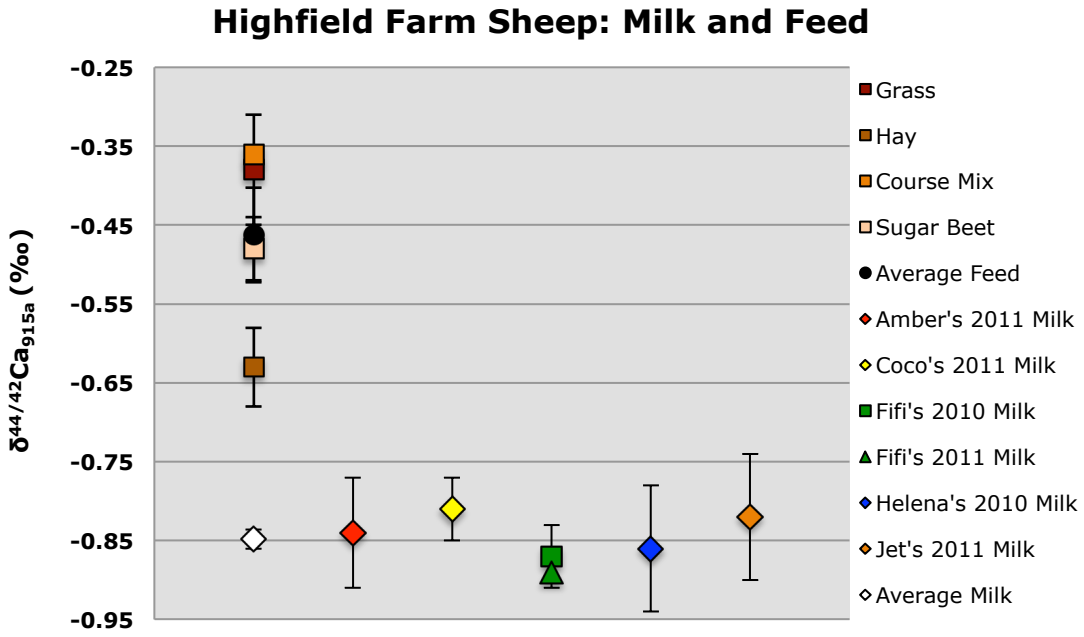

Figure 5.1: The milk collected in 2010 was from ewes Helena and Fifi. Milk collected in 2011 was from ewes Amber, Coco, Fifi and Jet. Feed samples were from 2011, but the foods in the diet were largely unchanged from one year to the next. The error bars are 1 standard deviation (SD) from the mean, except for Average Milk being 1 standard error of the mean (SEM).

5.2.2 Highfield Farm milk and feed sample $\delta^{44/42}$Ca values: discussion

Chu et al. (2006) showed differences between the grass or fodder, and milk produced, in two sheep, one from the UK and the other from France. The milk produced was depleted in ^{44}Ca by 0.54‰ compared with the grass consumed by the sheep (UK), and by 0.62‰ compared with the average feed consumed by the sheep (France). These results suggested that milk was a source of food depleted in ^{44}Ca. If true, then differences between milk and plants should be detectable by analysis of the calcium isotope ratios in calcium-rich mineralised tissues. However, due to only having two samples from two different farms, it is unknown if the ratios of calcium isotopes in milk are uniform over time in a single animal or whether different animals living in the same environment and consuming the same diet would produce similar $\delta^{44/42}$Ca milk values.

The milk analysed here was from five different ewes: Amber, Coco, Fifi, Helena and Jet. The milk from Helena was from 2010, the milk from Fifi was from both 2010 and 2011, and the milk from Amber, Coco and Jet was from 2011. This selection was made in order to determine whether $\delta^{44/42}$Ca results in collected milk were: (1) similar when taken

close to the time of lambing and from milk produced weeks later, (2) similar from a single ewe when taken from more than one year, (3) similar for a number of ewes living on the same farm with the same diet and (4) supportive of the results discussed in Chu et al. (2006). This was accomplished by analysis of the ewes' diet and milk. The main dietary contributor was grass, but during the last months of pregnancy and during the first few months of nursing the diet was supplemented with a grain-based, vitamin/mineral-enhanced course mix as well as dried sugar beets shreds. Hay was also offered as a supplement during periods when snow limited grass consumption.

The production of milk for lactation stimulates the release and transference of calcium from maternal bone to milk (Kovacs and Kronenberg 2006). Bones of the skeleton are depleted in ^{44}Ca relative to diet by an isotopic (ε^*) offset of ~0.65‰ (Skulan and DePaolo 1999, Chu et al. 2006, Hirata et al. 2008). Although the calcium in milk is largely supplied by ^{44}Ca-depleted bone, dietary intake and kidneys may also supply calcium for milk production. Normally in the kidneys, about 2% of calcium is removed to be excreted in urine, with the rest being reabsorbed and put back into homeostatic circulation (Bouillon et al. 2003, Hoenderop et al. 2005), but during lactation reabsorption of calcium increases (Kovacs and Kronenberg 2006, Gardella et al. 2010). Urine is enriched in ^{44}Ca relative to diet, with an individual's age affecting the amount (Heuser and Eisenhauer 2010). Increased reabsorption from the kidneys would probably result in blood calcium being slightly depleted in ^{44}Ca, which in turn would be available for milk production. Alternatively, mammary tissue uses similar cellular calcium regulatory processes in transporting calcium from the blood into the milk (VanHouten and Wysolmerski 2007) as used in urine production in the kidneys and in intestinal absorption (Hoenderop et al. 2005, Kovacs and Kronenberg 2006, Gardella et al. 2010). Although there are probably a number of factors that produce the final $\delta^{44/42}Ca$ milk value, the greatest supplier of calcium, and what probably has the most effect on the milk, is the ^{44}Ca-depleted maternal bone.

The mean ewe milk sample results for all milk samples were found to have no significant difference (ANOVA, $F_{5, 19} = 1.31$, $P = 0.301$, Table F.1; for tables prefixed with F, see Appendix F). Additionally, the ewe milk showed no significant difference between a time of production of 1–2 weeks after lambing (2011) or 4–6 weeks after lambing (2010) (independent samples *t* test: $t_9 = -1.24$, $P = 0.246$, Table F.2). The same was true for the mean values of the milk collected from Fifi in 2010 and 2011 (independent samples *t* test: $t_1 = 0.68$, $P = 0.618$, Table F.3). The physiological and biochemical processes that produce the calcium isotopic offset between diet and ewe milk appear to be stable and invariant when the diet remains uniform. Also, although not as large, the isotopic offsets between Highfield Farm ewe milk and grass (0.43–0.51‰, mean = 0.47‰) are in agreement with Chu et al. (2006)'s isotopic offsets between ewe milk and diet of 0.54 and 0.62‰.

These results are consistent with those of Chu et al. (2006), showing that milk is a dietary source depleted in ^{44}Ca relative to the plants in the diet. The next step was to determine whether the differences between the calcium isotope ratios in milk and plant foods could also be detected in mineralised tissues. Bone, as previously discussed in this thesis, has been shown to be an unreliable predictor of milk consumption. As dental enamel is not subject to turnover and remodelling, and is a tissue with linear growth that can be linked to time when sampled incrementally along the length of the tooth, it has the potential to be a better tissue for studying milk consumption.

5.3 Highfield Farm bulk enamel sample $\delta^{44/42}$Ca values: results and discussion

5.3.1 Highfield Farm bulk enamel sample $\delta^{44/42}$Ca values: results

The bulk enamel samples from the Highfield Farm lambs were found to be depleted in ^{44}Ca relative to the diet. The female lambs were more depleted than the males. The isotope offsets between the mean female and male first molar (M1) bulk $\delta^{44/42}$Ca values and the grass $\delta^{44/42}$Ca values were 0.14‰ and 0.28‰, respectively. The isotope offsets between the mean female and male second molar (M2) bulk $\delta^{44/42}$Ca values and the grass $\delta^{44/42}$Ca values

were 0.15‰ and 0.24‰, respectively. Figure 5.2 and Table G.2 contain the mean $\delta^{44/42}$Ca values for Highfield Farm bulk enamel samples.

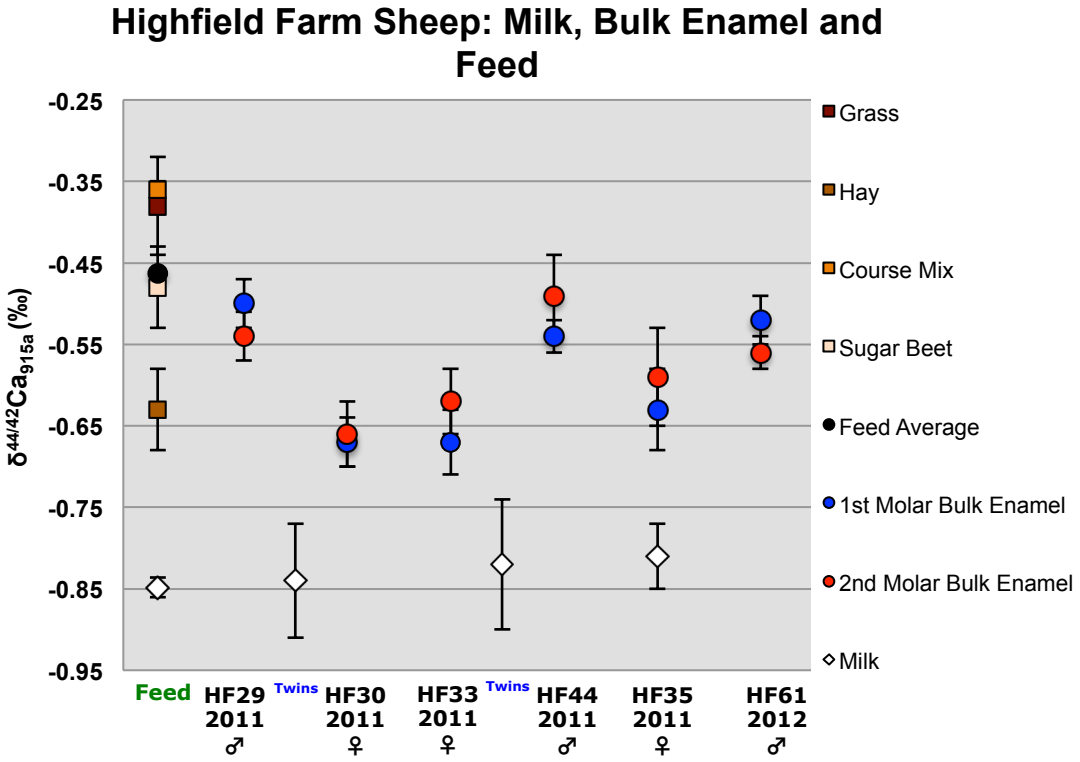

Figure 5.2: Mean M1 and M2 $\delta^{44/42}$Ca values for lambs HF29, HF30, HF33, HF35, HF44 (born 2011) and HF61 (born 2012). There were two sets of twins, each with a male (♂) and female (♀) lamb, which are indicated with the word Twins between the lambs. The mother of lambs HF29 and HF30 was Amber, and of lambs HF33 and HF44 was Jet. HF35's mother was Coco. The milk $\delta^{44/42}$Ca values from these ewes are plotted between the twin lambs and under HF35. HF61's mother's milk was not analysed. The error bars are 1 SD from the mean.

5.3.2 Highfield Farm bulk enamel sample $\delta^{44/42}$Ca values: discussion

For the Highfield Farm lambs HF29, HF30, HF33, HF35, HF44 and HF61, there was a significant difference between the mean M1 sample $\delta^{44/42}$Ca values (ANOVA, $F_{5,25} = 20.16$, $P = 0.000$, Table F.4). Specifically, the M1s for the male and female lambs were significantly different (independent samples t test: $t_{27} = 8.81$, $P = 0.000$, Table F.5), with females more depleted in ^{44}Ca than males. The mean lamb M2 sample results for all M2 samples were also found to have a significant difference (ANOVA, $F_{5,21} = 8.31$, $P = 0.000$, Table F.6), again the difference being attributable to sex (independent samples t test: $t_{24} = 4.84$, $P = 0.000$, Table F.7), with females more depleted in ^{44}Ca than males. Age differences between HF61 and the older male lambs did not produce any significant differences in both

the M1 (independent samples t test: $t_3 = 0.26$, $P = 0.810$, Table F.8) and M2 (independent samples t test: $t_8 = -2.04$, $P = 0.076$, Table F.9) $\delta^{44/42}$Ca values. The two sets of twins had a slight age difference between the males and females, with the males both being 7 days older than their female siblings at their time of death. It is unlikely, however, that 7 days would be enough of an age difference to account for the significant difference seen between the male and female lambs.

All of these lambs were weaned at 4 months of age, a month earlier than usual, and all had access to the same diet after weaning. Close to the time of weaning, male lambs display different behaviours compared with female lambs, such as increased play-fighting and interest in reproduction (Chapman 2010). These behavioural changes may also involve dietary differences between the sexes that reduce the amount of diet to enamel isotopic offset seen in the male lambs. However, a more tangible or testable reason for this difference between male and female lambs is that while both growing male and female lambs are mineralising bone and teeth, male Shetland lambs are also producing horns. Horn growth began very shortly after birth for the Shetlands at Highfield Farm and Figure 5.3 shows the amount of horn growth typically seen at about 4.5 months after birth.

Figure 5.3: Horn growth of one of the Highfield Farm ram lambs (right). The ewe lamb (left) next to him will not grow horns. These lambs are both 4.5 months old.

Based on the discussion of calcium isotopes, milk and enamel in Chapters 2 and 3, it is predicted that a lamb's M1s would be more depleted in ^{44}Ca than its M2s. This is because M1 are the teeth that should have enamel with the greatest bulk quantity of dietary calcium from milk, followed by M2, with the third molar (M3) not containing any calcium from milk. Thus it would be expected that M2s would have higher $\delta^{44/42}Ca$ values than M1s. For HF29 the M1 mean $\delta^{44/42}Ca$ value, in relation to the calcium standard NIST SRM 915a (915a) (see Chapter 2 and Appendix B) at a 95% confidence interval (CI), is (–0.53, –0.47), and for the M2 mean $\delta^{44/42}Ca$ value is (–0.57, –0.51). The overlap of the CI for HF29's M1 and M2 indicates that the true means are either very close or could possibly be the same. The same pattern was found for all of the lambs and was confirmed when no significant difference was found between the M1s and M2s of male lambs (independent samples *t* test: $t_{15} = -0.45$, $P = 0.656$, Table F.10) and M1s and M2s of female lambs (independent samples *t* test: $t_{29} = -1.85$, $P = 0.075$, Table F.11). This result is not in accordance with expectations.

In adult sheep weaned naturally at 5 months of age and with fully developed molars, the calcium for M1 enamel development would be from the periods of a lamb's life consisting of the last 2–3 months *in utero*, the 2 months of intense nursing, the 3 months of weaning and then about 3 months of a plant-only diet. The calcium for M2 enamel development would be from the periods of a lamb's life consisting of the entire weaning transition, lasting 3 months, and the conversion to a plant-only diet, for the remaining 11 months of M2 development.

The Highfield Farm lambs do not fit the profile of adult, naturally weaned sheep. They were slaughtered at about 7.5 months of age and were also weaned a month early. They had completely formed and mineralised M1s, but their M2s were only about half structurally formed (see Figure 4.1). The upper third of M2 enamel consists of hard, mature enamel, the middle section a mix of mature and immature enamel, and the lower portion consists exclusively of powdery immature enamel. Due to early weaning, the M1 enamel formation consisted of only 2 months of weaning instead of 3, which also resulted in an

additional month of a plant-only diet. The M2 also had a shortened weaning transition of only 2 months and an additional month of a plant-only diet. What is missing from having an incomplete M2 are the 12 months of a plant diet that would have provided the calcium in the lower half of the tooth, which would probably result in a higher $\delta^{44/42}$Ca value for the M2 compared with the M1.

As discussed in Chapter 3, the process of enamel formation (saturation and maturation phases) takes about 6 months, with the maturation process alone taking 4 months (Zazzo et al. 2010, Balasse et al. 2012b). This is significant because the lower two-thirds of M2s in the Highfield Farm lambs had immature enamel. Immature enamel would contain calcium accumulated over a shorter period of time than 6 months and would have less attenuation of an isotope signal. Although there would be less attenuation it would also be an incomplete record. Calcium in immature enamel may also be replaced during the maturation process. Chapter 6 will revisit the topic of M1 and M2 bulk sample analyses for adult sheep with fully developed M1 and M2 teeth. To summarise, the bulk enamel results from the Highfield Farm lambs indicated: (1) that M1 and M2 enamel from lambs of about 7.5 months of age was enriched in ^{44}Ca relative to their mother's milk and depleted in ^{44}Ca relative to grass, (2) that the enrichment in ^{44}Ca relative to milk was greater than the depletion in ^{44}Ca relative to grass, (3) that the M1s and M2s had similar $\delta^{44/42}$Ca values and (4) that both M1 and M2 were more enriched in ^{44}Ca in males relative to females.

5.4 Highfield Farm incremental enamel sample $\delta^{44/42}$Ca, δ^{18}O and δ^{13}C values: results and discussion

5.4.1 Highfield Farm incremental enamel sample $\delta^{44/42}$Ca, δ^{18}O and δ^{13}C values: results

For male lamb HF29 (Table G.3), the M1 incremental samples had a variation of 0.1‰ between the minimum and maximum $\delta^{44/42}$Ca values (Figure 5.4). The M2 had a greater offset between the minimum and maximum $\delta^{44/42}$Ca values, with 0.19‰ (Figure 5.5). The difference in $\delta^{44/42}$Ca values seen between the minimum and maximum values in both M1 and M2 was greater than the measurement precision, 0.04–0.08‰ (1 SD) (see Chapter 4).

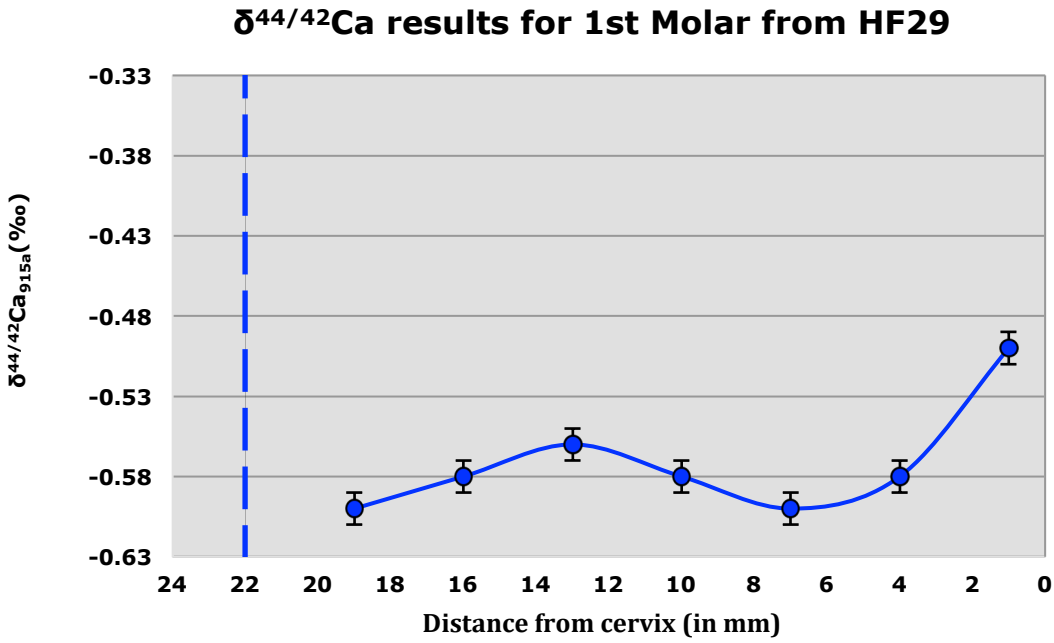

Figure 5.4: A graph of the $\delta^{44/42}$Ca results from incremental samples taken from lamb HF29's M1. Sampling was started 3 mm below the height of the apex of the crown (dashed line) and subsequently taken every 3 mm going down the length of the tooth. The last sample was taken 1 mm above the cervix (cemento-enamel junction). Temporal chronology progresses from left to right. Error bars are 1 SD for $\delta^{44/42}$Ca.

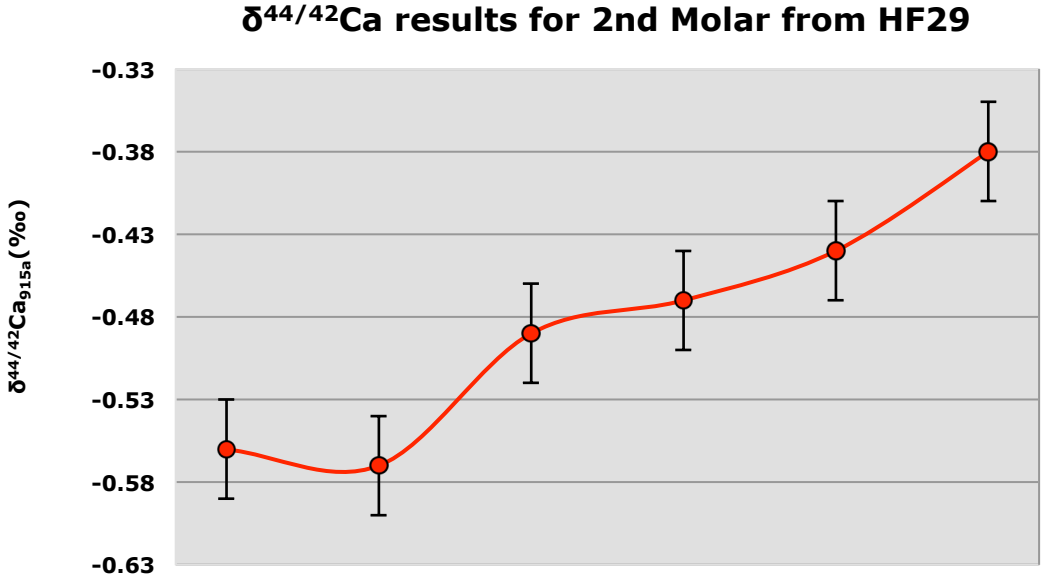

Figure 5.5: A graph of the $\delta^{44/42}$Ca results from incremental samples taken from lamb HF29's M2. Sampling was started 3 mm below the apex of the crown and subsequently taken every 3 mm going down the length of the tooth. This tooth was approximately half formed and crown height could not be established. Temporal chronology progresses from left to right. Error bars are 1 SD for $\delta^{44/42}$Ca.

Female lamb HF30's M1 and M2 incremental sample $\delta^{44/42}$Ca values (Table G.4) showed greater variation than seen with HF29. The M1 had a difference between the minimum and maximum $\delta^{44/42}$Ca values of 0.13‰ (Figure 5.6). The M2 had a greater

difference between the minimum and maximum $\delta^{44/42}$Ca values, of 0.25‰ (Figure 5.7). The $\delta^{44/42}$Ca values of 0.13‰ and 0.25‰ were greater than the 1 SD of measurement precision, 0.04–0.08‰ (see Chapter 4).

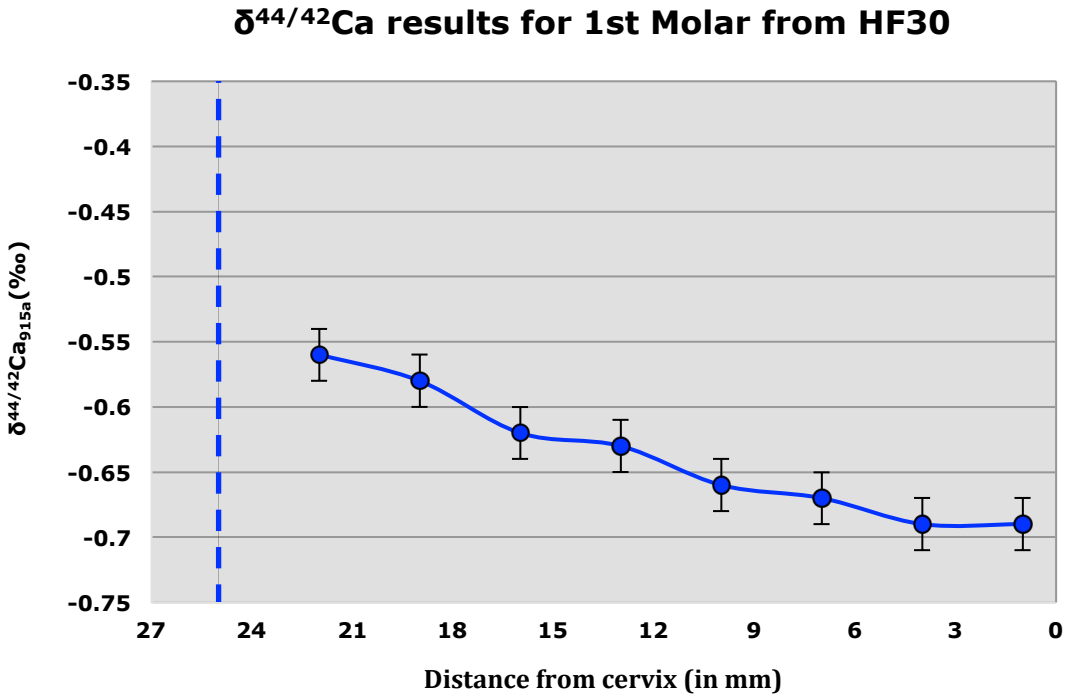

Figure 5.6: A graph of the $\delta^{44/42}$Ca results from incremental samples taken from lamb HF30's M1. Sampling was started 3 mm below the apex of the crown (dashed line) and subsequently taken every 3 mm going down the length of the tooth. The last sample was taken 1 mm above the cervix (cemento-enamel junction). Error bars are 1 SD for $\delta^{44/42}$Ca.

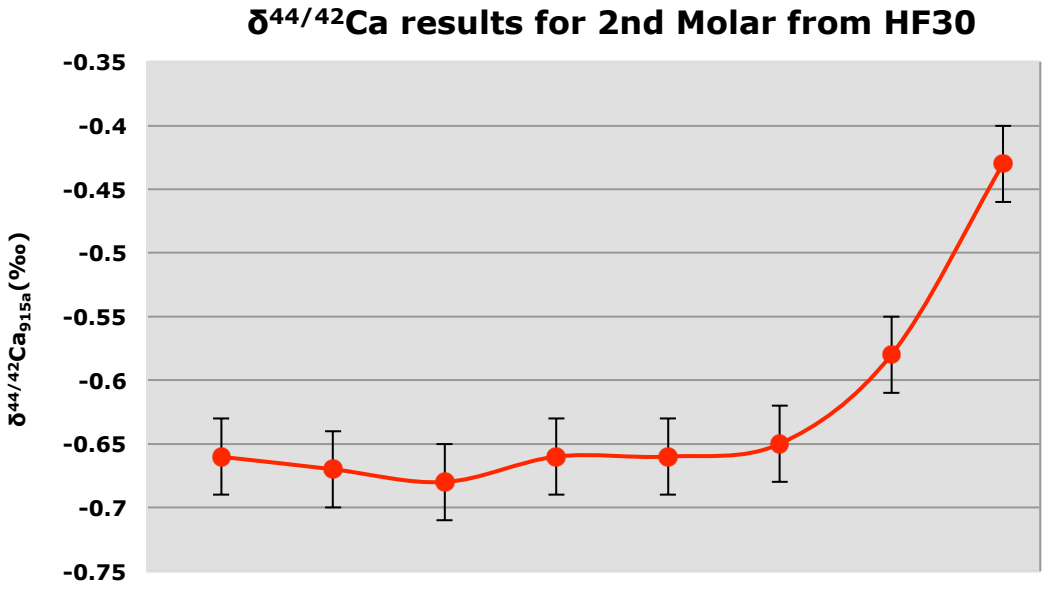

Figure 5.7: A graph of the $\delta^{44/42}$Ca results from incremental samples taken from lamb HF30's M2. Sampling was started 3 mm below the apex of the crown and subsequently taken every 3 mm going down the length of the tooth. This tooth was approximately half formed, making it impossible to establish crown height. Temporal chronology progresses from left to right. Error bars are 1 SD for $\delta^{44/42}$Ca.

The $\delta^{18}O$ and $\delta^{13}C$ values for incremental samples from HF29 are given in Table G.5 and for HF30 in Table G.6. Figure 5.8 shows the $\delta^{18}O$ values for HF29 and HF30 M1s and Figure 5.9 shows the $\delta^{18}O$ values for HF29 and HF30 M2s. Figure 5.10 shows the $\delta^{13}C$ values for HF29 and HF30 M1s and Figure 5.11 shows the $\delta^{13}C$ values for HF29 and HF30 M2s.

The M1s for HF29 had a difference between the minimum and maximum $\delta^{18}O$ values of 2.0‰, and a similar difference in $\delta^{18}O$ values of 1.5‰ was seen for HF30. The M2s for HF29 had a difference between the minimum and maximum $\delta^{18}O$ values of 3.1‰, and HF30 again had a similar difference of 2.9‰ for $\delta^{18}O$ values. Between the minimum and maximum values for the M1s and M2s for HF29 and HF30, there was a 4.7‰ isotope offset for HF29 and a 4.2‰ isotope offset for HF30. M1s and M2s from HF29 and HF30 both showed a similar steady depletion in ^{18}O, starting with samples taken from near the apex of the crown of both teeth and progressing towards the cervix.

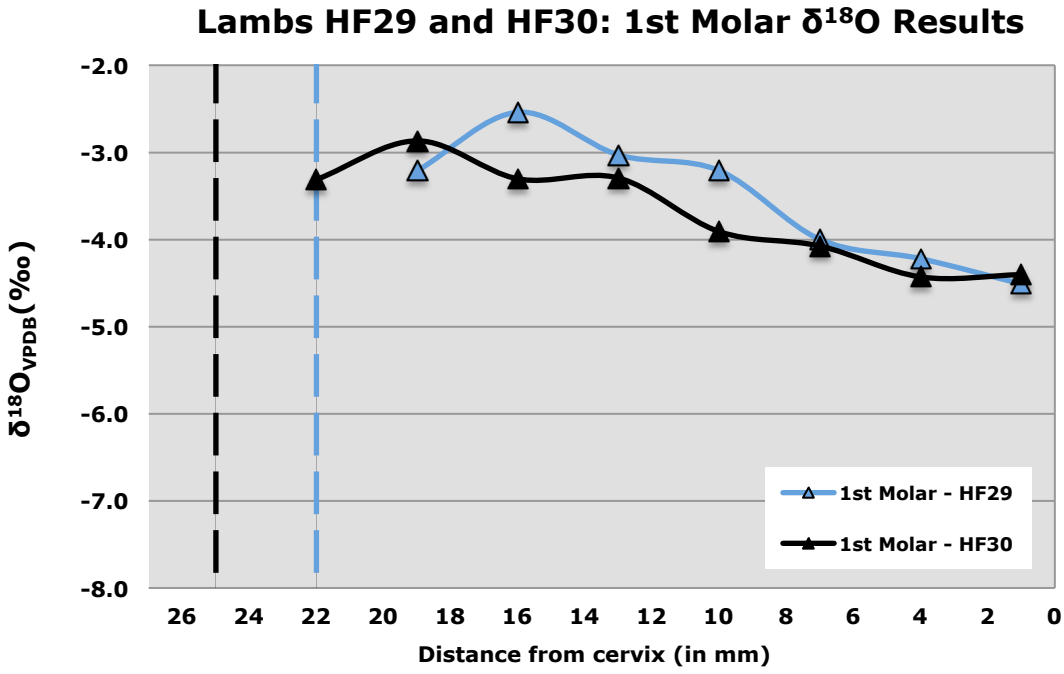

Figure 5.8: M1 incremental $\delta^{18}O$ results for HF29 and HF30. Samples were collected at 3-mm intervals, with the first sample taken 3 mm below the apex of the crown. The enamel adjacent to the cervix contains the greatest amount of youngest enamel. The crown heights are marked with dashed lines (HF29 – blue and HF30 – black). Temporal chronology progresses from left to right.

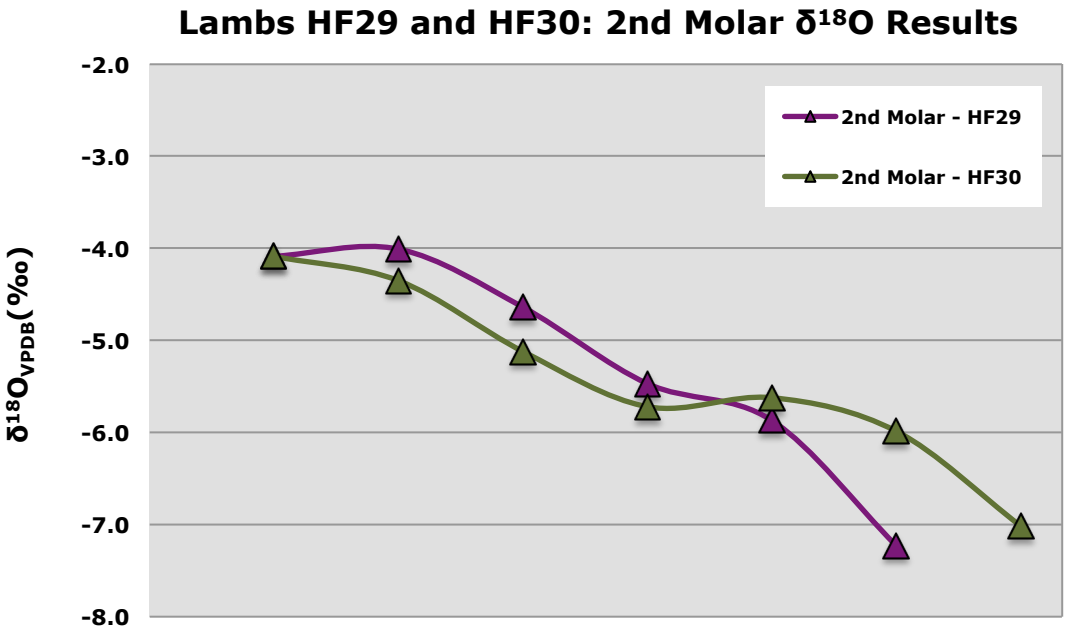

Figure 5.9: M2 incremental $\delta^{18}O$ results for HF29 and HF30. Samples were collected at 3-mm intervals, with the first sample taken 3 mm below the apex of the crown. The M2s were approximately half formed and it was not possible to establish crown height. Temporal chronology progresses from left to right.

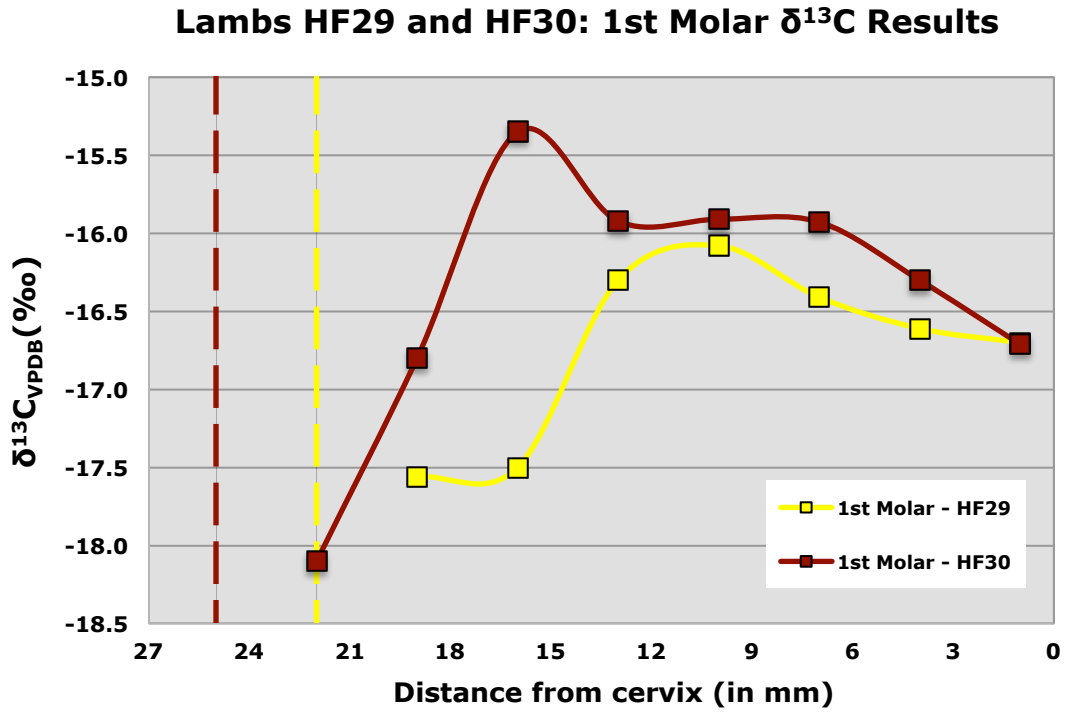

Figure 5.10: M1 incremental $\delta^{13}C$ results for HF29 and HF30. Samples were collected at 3-mm intervals, with the first sample taken 3 mm below the apex of the crown. The enamel adjacent to the cervix contains the greatest amount of youngest enamel. The crown heights are marked with dashed lines (HF29 – yellow and HF30 – rust). Temporal chronology progresses from left to right.

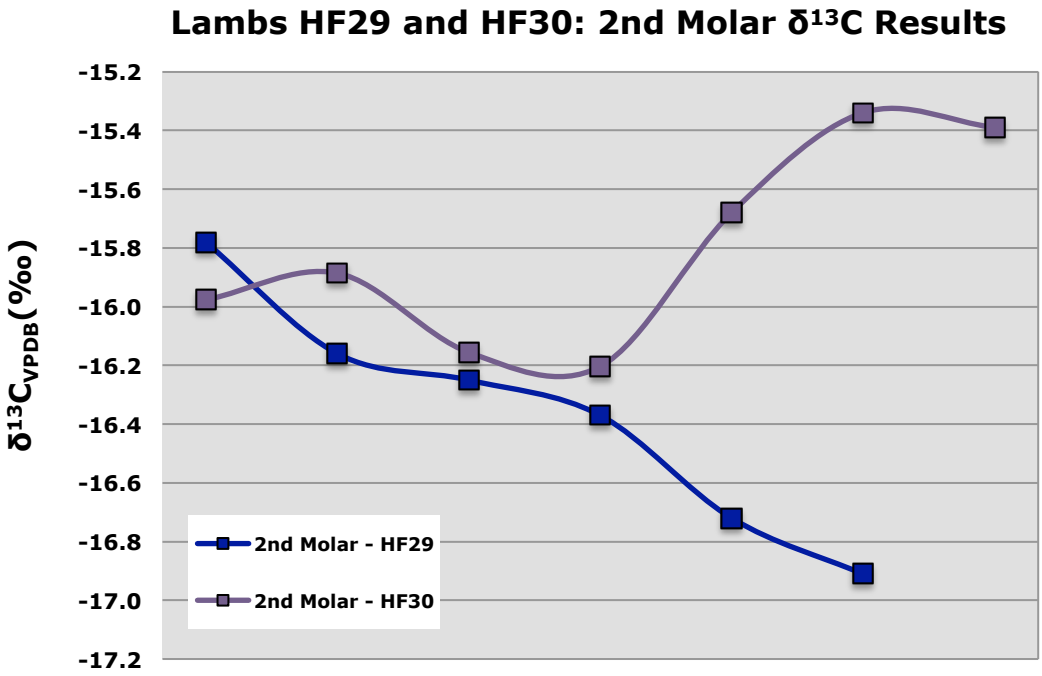

Figure 5.11: M2 incremental $\delta^{13}C$ results for HF29 and HF30. Samples were collected at 3-mm intervals, with the first sample taken 3 mm below the apex of the crown. The M2s were approximately half formed and it was not possible to establish crown height. Temporal chronology progresses from left to right.

The difference between the M1 minimum and maximum $\delta^{13}C$ values for HF29 and HF30 were 1.5‰ and 2.8‰, respectively. The difference between the M2 minimum and maximum $\delta^{13}C$ values for HF29 and HF30 were 1.1‰ and 0.9‰, respectively. Based on a 12–14.6‰ ^{13}C-enrichment between dietary input and enamel (Krueger and Sullivan 1984, Lee-Thorp and van der Merwe 1987, Cerling and Harris 1999, Passey et al. 2005), the Highfield Farm lamb molar enamel showed an overall C_3 diet.

5.4.2 Highfield Farm incremental enamel sample $\delta^{44/42}Ca$, $\delta^{18}O$ and $\delta^{13}C$ values: discussion

Bulk sample analyses produce results that reveal broad trends and differences between teeth, but overlook the more subtle changes in $\delta^{44/42}Ca$ values that could be used identify key dietary and life events. Serial incremental sampling, from the apex of the crown to the cemento-enamel junction, of sheep teeth for isotope analysis has the potential to provide isotope data with more precise timing. This is true, even with the attenuation of input signal, because of the length of time it takes for enamel to form and mature.

The rate of enamel formation going down the length of the tooth has been found to be variable during the initial saturation phase by Kierdorf et al. (2012), with similar evidence, based on ^{18}O and ^{13}C results, for the whole period of enamel formation found by Balasse et al. (2012a) and Zazzo et al. (2012). In all cases it was seen that the upper portion (apex/cuspal) of the sheep molars was growing more rapidly than the bottom portion (cervical) (see Chapter 3). Although variable mineralisation rates add uncertainty to the interpretation of isotope results from the analysis of serial incremental samples, the results do show a preservation of chronology of isotope incorporation (Balasse 2003), which may lead to information about the 'how, what and when' of key sheep life events (birth, nursing and weaning).

Dental enamel wear needs to be considered when interpreting incremental sample results. The Highfield Farm sheep were about 7.5 months old, which for the lambs of the same age in Upex and Dobney (2012)'s dental chronology were in Payne's (1973) stage three of wear. Therefore, it is unlikely that the Highfield Farm lambs had enough wear to consider this an issue during interpretation.

Sheep molar development begins *in utero* for the M1. In the last few months of pregnancy, the mother's gastrointestinal tract doubles its normal calcium absorption, resulting in the mother's diet providing the greatest amount of calcium for the rapidly developing foetal skeleton (Belkacemi et al. 2005, Kovacs 2006, Kovacs and Kronenberg 2006). Additionally, at the end of the pregnancy, as part of preparing for lactation, the mother's skeleton begins releasing calcium that may aid in foetal growth (Specker 2002). As the foetal teeth grow, calcium is accreted as part of the enamel extension along the tooth length, which results in a progression of $\delta^{44/42}Ca$ values that reflects calcium input over time. This leads to the prediction that, as the source of calcium changes, the $\delta^{44/42}Ca$ values resulting from the lamb's time *in utero* and during the first few months of nursing would shift to reflect the addition of plants to the milk diet. It is important to note that, although plant foods begin to be consumed within the first weeks of life, there is little or no

nutritional benefit until rumen microflora are established. Fifty per cent of total calcium absorption in the gastrointestinal tract occurs in the rumen (Schröder et al. 1997). The rumen is generally not functional until the lamb is 8 weeks old (Lyford 1988). Once weaning is complete, unless the plant source changes, the calcium ratio input should remain unchanged, stabilising the $\delta^{44/42}$Ca signal.

The rate of enamel formation has implications for interpreting the timing of changes in $\delta^{44/42}$Ca values. Rapid mineralisation is predicted to result in a decrease in isotopic offset between bone and dietary input (Skulan and DePaolo 1999). Calcium isotope fractionation related to bone is discussed in detail in Chapter 2, Appendix A and in Section 5.5 (Highfield Farm bone $\delta^{44/42}$Ca results). Due to the enamel in the cervix portion of the molar requiring more time for complete mineralisation than the enamel in the crown portion of the molar, assuming calcium accretion follows the same pattern seen with carbonates, the attenuation of input signal would be greater in the cervix portion of the molar.

Enamel, once mature, does not release calcium, but as part of the maturation process some calcium incorporated during saturation is lost or replaced (Hubbard 2000). Overall, the majority of calcium is incorporated during maturation (Smith 1998, Hubbard 2000) and it would be expected that the calcium $\delta^{44/42}$Ca values would reflect this phase of enamel formation. It then becomes a matter of deciding to interpret results based on a 6-month combined saturation and maturation enamel formation, with sample results reflecting an averaging of signal based on that length of time, or to interpret based on a 4-month maturation enamel formation alone, thereby reducing signal averaging by 2 months. If interpretation is based on the 4 months of maturation, it requires removal of the 2 months preceding maturation as a contributor to the $\delta^{44/42}$Ca values, which also means disregarding the diet and life events that took place during those months. The incremental results should clarify whether both saturation and maturation, or maturation alone, are contributing the calcium in the $\delta^{44/42}$Ca values.

Prior to analysing the $\delta^{44/42}$Ca values of the serial incremental enamel samples from the modern Highfield Farm and Hoy sheep, a model (Figure 5.12) was produced based on what was known about the isotope offsets between diet–soft tissue (0.17 ± 0.25‰) (Reynard et al. 2010; Section 2.6) and between average diet–milk (0.54‰) (Chu et al. 2006; Section 5.2.1), dental development (Upex and Dobney 2012) and sheep life events (pre-birth/*in utero*, birth, nursing, weaning and post-weaning). The model was used to see whether the modern sheep incremental enamel samples produced $\delta^{44/42}$Ca values that did or did not adhere to what was predicted, based on the known variables. Highfield Farm lambs HF29 and HF30, despite exhibiting differences between each other, both demonstrated aspects of the model. This was especially true in regard to sample values being depleted in ^{44}Ca in relation to nursing, with a continual enrichment in ^{44}Ca for the samples associated with weaning and weaning completion.

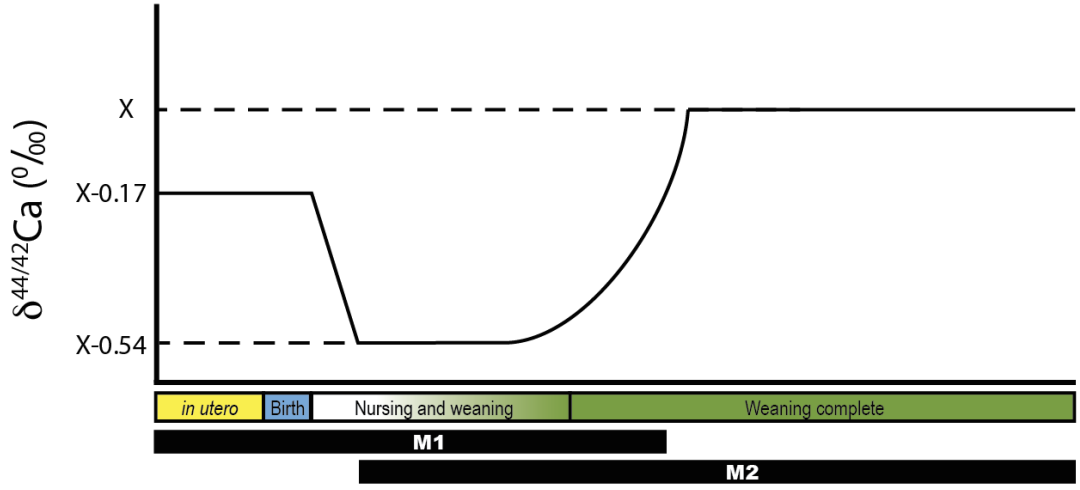

Figure 5.12: A model of the expected pattern of $\delta^{44/42}$Ca values from serial incremental sampling down the length of the first (M1) and second (M2) molars (apex of the crowns towards the cemento-enamel junctions, left to right on the figure). This pattern is based on the isotope offsets between diet–soft tissue and between average diet–milk, which are 0.17 ± 0.25‰ and 0.54‰, respectively. Both soft tissue and milk are more depleted in ^{44}Ca relative to diet (X-0.17 and X-0.54). The development and mineralisation of M1 and M2 occurs during important periods of a sheep's life: pre-birth/*in utero*, birth, nursing, weaning and post-weaning. Together these known variables have been combined to produce a model of the predicted results for the modern sheep enamel samples discussed in this chapter and in Chapter 6.

The bulk enamel sample $\delta^{44/42}$Ca values for M1s and M2s from HF29 (male) and HF30 (female) showed that there was a significant difference between the male and female lambs. Incremental sample analysis of the M1s and M2s from these two sheep provide

further evidence of how the male and female lambs differ from each other, also regarding $\delta^{44/42}$Ca values. Figure 5.13 shows the differences between HF29 (upper graph) and HF30 (lower graph). These lambs were twins that were raised together and milk was available to both lambs equally during nursing and weaning. They were also weaned at the same time and continued to have access to the same grass by being housed in different parts of the same pasture. Any differences therefore cannot be accounted for by diet alone.

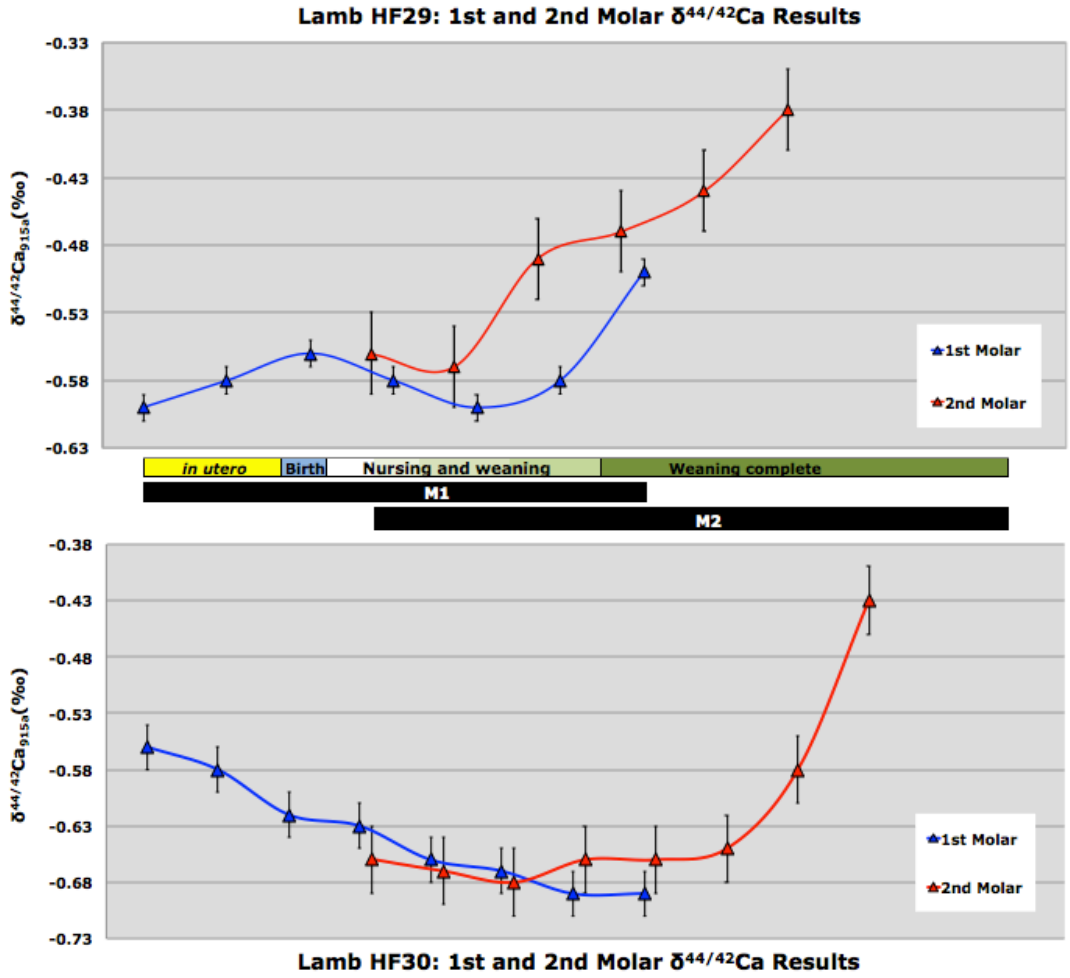

Figure 5.13: Male lamb HF29 (upper graph) and female lamb HF30 (lower graph), born on 3 April 2011. HF29 was slaughtered on 21 November 2011 and HF30 on 14 November 2011. The molars are associated with each other based on the dental development chronology developed by Upex (2009) and Upex and Dobney (2012). The timing of life events (*in utero*, birth, nursing and weaning) is based on a combination of what is known about the lives of these two lambs and on what is recorded in the enamel. The interval length between the samples for both the M1s and M2s is based on the number of samples divided by the length of the M1 bar on the graph. The error bars are 1 SEM.

The $\delta^{44/42}$Ca values from the M1 of HF29, although not a sinusoidal wave, has the appearance of one, with the crest in the crown half of the tooth and the trough in the

cervical half. The crest has a height of 0.04‰, which while less than measurement precision (0.04–0.08‰, 1 SD) is worth noting for comparison with other sheep. The trough also has a height of 0.04‰ on the side transitioning from the crest, but, as this is not a true sine wave, has a height of 0.1‰ on the other side. In general the M2 followed the pattern of the M1, but had samples with increasingly higher $\delta^{44/42}$Ca values near the cervix.

Unlike HF29, HF30 did not have incremental samples $\delta^{44/42}$Ca values that formed a sinusoidal wave-like pattern. Instead, the M1 had samples that showed a steady depletion of ^{44}Ca from the first sample taken adjacent to the apex of the crown to the last sample taken above the cervix. The apical half of the crown of HF30's M2 overlapped the M1 and then had a sharp change, with increasingly higher $\delta^{44/42}$Ca values in the cervical half of the crown.

It should be noted that without a completed M2 it was difficult to determine the ideal interval length between plotted samples for the M2s. The interval length between the samples for the M1s was determined by the total number of M1 samples divided by the length of the M1 bar on the graph. This interval length between each sample $\delta^{44/42}$Ca value was applied to both M1s and M2s. As a result, the M2 is shown in the graphs as having the same rate of development as the M1 while also being incomplete in regard to M2 dental development.

Possible reasons for HF29's results are that the enamel near the crown of the M1 was formed or partially formed while *in utero*. The steady enrichment of ^{44}Ca seen in the serial incremental samples taken within the 9 mm (crown height plus 6 mm) of enamel near the apex may be due to increases in both foetal skeletal calcium demand and maternal intestinal absorption of calcium to meet the needs of the foetus in the final months *in utero*. The higher *in utero* $\delta^{44/42}$Ca values reach a maximum near 'birth' on the timeline. However, HF30 does not have this same pattern of increasingly higher *in utero* $\delta^{44/42}$Ca values. Instead HF30's M1 shows steady depletion of ^{44}Ca in the serial incremental samples taken within the 9 mm (crown height plus 6 mm) of enamel near the apex. These lambs would have had

the same source of calcium while *in utero* and were too young for there to be a behavioural difference, which seems to indicate an *in utero* physiological difference between them. This difference may be a sex-specific difference or a normal difference between two individuals.

After the points on the graph associated with birth, there are additional differences between the lambs' M1s. While both demonstrate decreasing $\delta^{44/42}$Ca values, likely associated with nursing and weaning, HF29 has a fairly abrupt change after the sample with the lowest $\delta^{44/42}$Ca value, which is seen as increasing $\delta^{44/42}$Ca values until tooth completion. In contrast, HF30 shows a levelling off of signal input at the lowest $\delta^{44/42}$Ca value and remains there until tooth completion. Milk is both a food depleted in ^{44}Ca relative to plants and the predominant food after birth and through the weaning transition, which means that HF29 appears to have weaned earlier than HF30. The M2s, in having the upper one-third to one-half of the tooth following the pattern in the cervical half of the M1, also appears to show early weaning for HF29 compared with HF30. However, HF29 and HF30 were raised together in the same pasture, had the same access to their mother's milk, and were both weaned at 4 months of age.

A possible explanation for these differences between HF29 and HF30 is that the microflora in the male rumen is established sooner than in the female. There are known behavioural differences between the sheep sexes: perhaps male lambs begin eating plant foods earlier. Also, other social interactions and behaviours of male lambs may affect the amount and frequency of milk versus plants that is consumed. There is more calcium contained in milk than in plants (United States Department of Agriculture 2011), and so perhaps even the smallest amount of milk in the diet is enough to influence the results. As discussed in the section on bulk enamel results, there is also the possibility of metabolic differences between males and females. The most obvious difference between the sexes involving calcium is horn growth.

Because there are indications that the rate of enamel formation differs between the upper and lower portions of the molars, Figure 5.14 plots the incremental sample $\delta^{44/42}$Ca

values of the M1s from HF29 and HF30 but with the 25% of enamel adjacent to the cervix having a reduced rate of formation of 50%. Plotting the reduced rate of formation was done by doubling the length of the intervals between the samples that fall within the 25% of enamel adjacent to the cervix. Because the M2s were not complete and consisted of approximately the upper half of the tooth, the intervals between samples for the M2s were therefore equal to the intervals between samples in the apical half of the M1s.

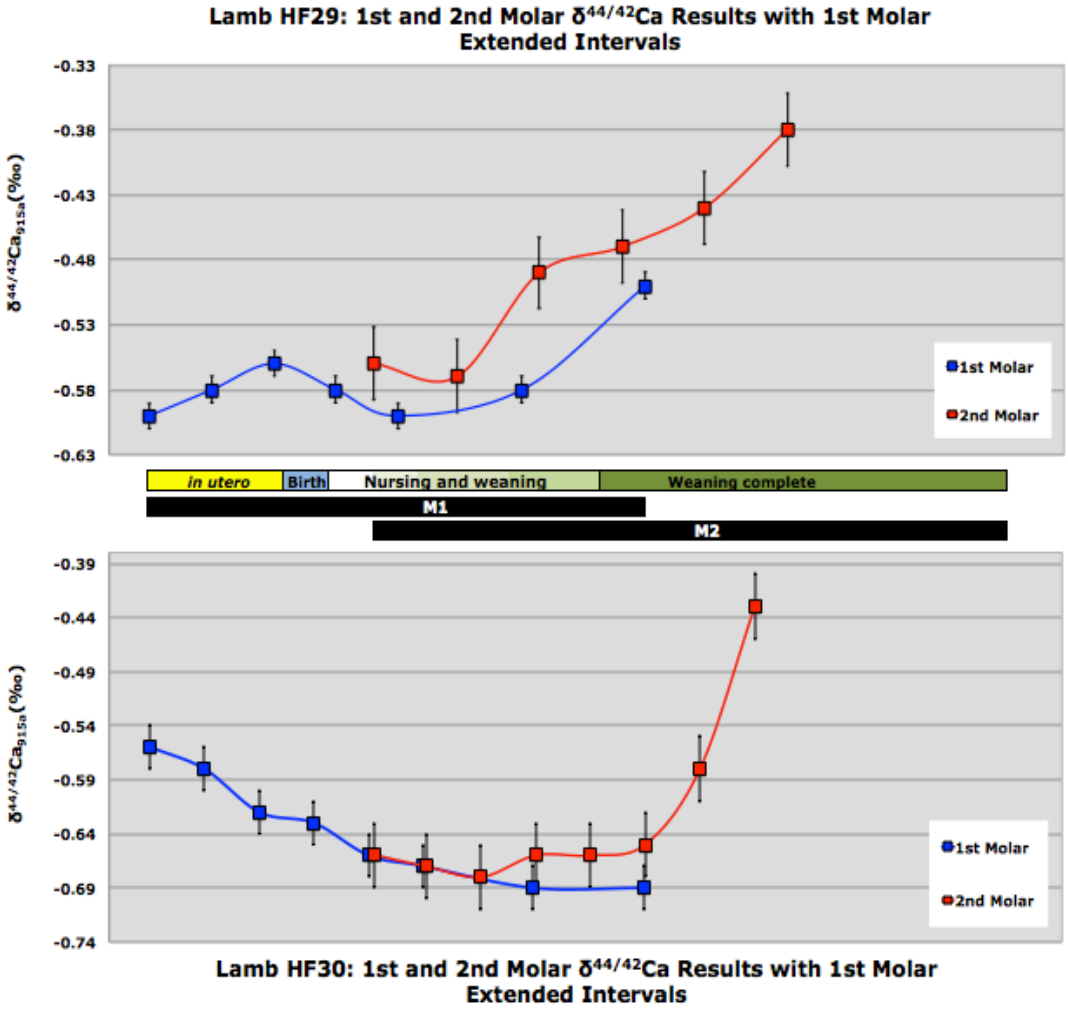

Figure 5.14: Male lamb HF29 (upper graph) and female lamb HF30 (lower graph) , born on 3 April 2011. HF29 was slaughtered on 21 November 2011 and HF30 on 14 November 2011. The molars are associated with each other based on the dental development chronology developed by Upex (2009) and Upex and Dobney (2012). The timing of life events (*in utero*, birth, nursing and weaning) is based on a combination of what is known about the lives of these two lambs and on what is recorded in the enamel. The interval length between the samples for both the M1s and M2s is based on the number of samples divided by the length of the M1 bar on the graph. The exception is for the interval lengths between the samples from the 25% of enamel near the cervix of the M1, which have doubled interval lengths. The error bars are 1 SEM.

The main difference between Figures 5.13 and 5.14 is that in Figure 5.14 HF29 has a period of very gradual enrichment in the 'trough portion' of the M1 that is more reflective of a period of milk consumption after birth followed by a more gradual increase of plant intake during weaning, instead of an immediate change. However, HF29 still appears to have been weaned earlier than HF30 in both figures. Also in Figure 5.14, the points of overlap between the M1s and M2s for HF29 more closely mirror each other, which is also true for HF30. It should be remembered that for any individual enamel sample, the $\delta^{44/42}$Ca values should reflect calcium input either during the whole of enamel formation (6 months) or from maturation alone (last 4 months of enamel formation). However, in both Figures 5.13 and 5.14, the $\delta^{44/42}$Ca values for HF29 and HF30 do not provide evidence of input of calcium from specific stages of enamel formation, and as a result both a 4- and 6-month enamel formation period is possible.

Overall, the graphs in Figure 5.14 seem to better reflect the timing of HF29 and HF30's life events and diet than Figure 5.13. This is based on the following factors: (1) the M1s and M2s at the points of overlap between the teeth more closely mirror each other, and (2) the $\delta^{44/42}$Ca values better reflect the enamel mineralisation process and the months *in utero*, the months of intense milk consumption after birth, the months of weaning and the remaining months once weaning was complete. However, these are only the first sets of teeth to be discussed and the Hoy sheep discussed in Chapter 6 provide additional information regarding enamel formation timing (maturation and saturation) and rates in different parts of the molars.

Also, it has to be concluded, based on both the bulk and incremental enamel sample calcium isotope results from lambs HF29 and HF30, that sex should be considered when producing modern reference datasets for future calcium isotope work. This may be a complicating factor for analysing teeth from archaeological sites if the jaws are not associated with parts of the sheep's skeleton that can be used for sex determination. DNA analysis of jawbones for the determination of sex may be a necessary step in calcium

analysis of molars from archaeological sites. As part of building a modern calcium isotope reference data collection, both male and female sheep need to be analysed to determine sex-specific patterns in the timing of life events, especially birth, nursing and weaning.

In addition to calcium isotope analysis, the carbonate in the incremental enamel samples from HF29 and HF30 were analysed in order to gain better resolution of the timing of nursing and weaning using $\delta^{18}O$ values. The $\delta^{18}O$ values were analysed in association with the average monthly high temperatures in the area around York. The temperature data was gathered by a weather station at 53°28´60´´N 2°15´0´´W. The $\delta^{13}C$ results were analysed to look for evidence of depletion in ^{13}C resulting from milk lipid consumption, as well as to identify in ewes the change from a mixed C_3/C_4 diet during pregnancy to a grass-only diet after giving birth.

It was not possible to see in the incremental $\delta^{18}O$ data the usual (or often) observed sinusoidal pattern in the M2, because it was only partially formed. A plot of sequential $\delta^{18}O$ values for HF29's M2s against average monthly high temperatures for the region around Highfield Farm shows that the data is apparently in keeping with the seasonal variation in temperature (the lower $\delta^{18}O$ values coincide with winter months, and the higher $\delta^{18}O$ values with summer) (Figure 5.15), although the annual sinusoidal cycle could not be observed to allow determination of the full cycle. Elsewhere a delay has been observed between the seasonal cycle of temperature and dental development by up to 5–6 months, resulting in enamel $\delta^{18}O$ values near the crown reflecting late summer/early autumn instead of spring (Balasse et al. 2012b). In this case, the (apparently) closer temporal match may be due to the incomplete development of the M2; the lower two-thirds of the tooth consisted of immature enamel. Immature enamel reflects only the early mineralisation stage, and lacks the full 5–6 month maturation phase; thus there should be less attenuation of incremental ^{18}O ratios. Neither HF29's nor HF30's M2s reflected a delay between the $\delta^{18}O$ values and seasonal temperature.

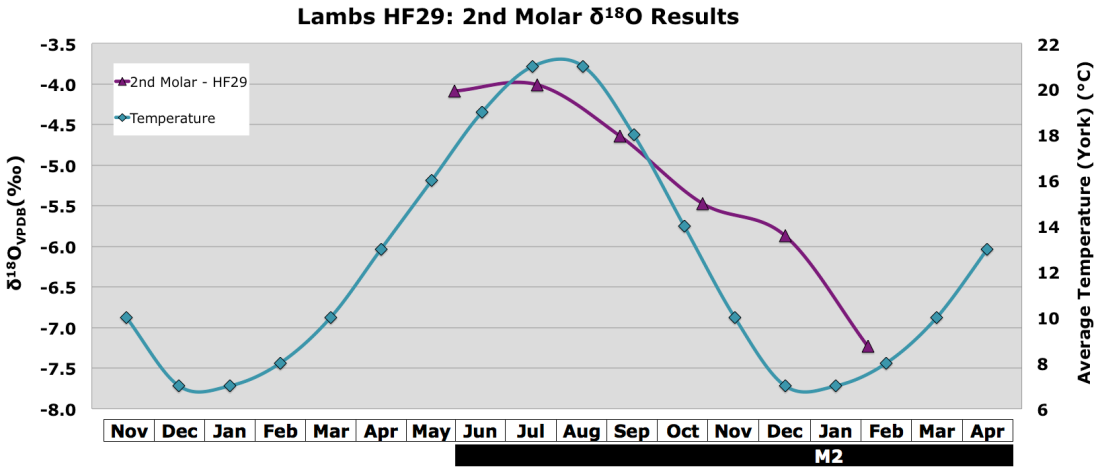

Figure 5.15: M2 incremental $\delta^{18}O$ results for HF29 in relation to average monthly temperatures and M2 dental development. Average monthly temperatures for the area around York, UK, were from a weather station located at 53°28´60´´N 2°15´0´´W.

As stated in Section 5.4.1, the enamel results for the Highfield Farm lambs show a C_3 diet, based on a 12–14.6‰ ^{13}C-enrichment between dietary input and enamel (Krueger and Sullivan 1984, Lee-Thorp and van der Merwe 1987, Cerling and Harris 1999, Passey et al. 2005) (Figure 5.16). Although the diet overall was from C_3 plants, there is evidence of variation within the C_3 diet that may be due to a lamb's life while *in utero* and during nursing. The crown and upper portion of the M1 for HF29 and HF30 were developing *in utero* for 2–3 months (Witter and Míšek 1999, Upex and Dobney 2012) and, as previously discussed, it takes approximately 6 months for enamel to form and fully mature (Zazzo et al. 2010, Balasse et al. 2012b). Therefore, the 9 mm (crown height plus the 6 mm of sampled enamel) of enamel from below the apex of the crown showed a steady increase in $\delta^{13}C$ values over time, which may reflect the *in utero* signal from acquiring C_3 plant carbonates from the mother's blood. Near the time of birth there is a slight lowering of $\delta^{13}C$ values by ~0.5‰. This may be due to the consumption of milk lipids during the period of intense nursing for the first 2 months after birth. Milk is rich in lipids, and lipids are depleted in ^{13}C compared with the carbohydrates and proteins in the diet (DeNiro and Epstein 1978, Post et al. 2007). Because increasing temperatures affect C_3 plants by lowering their $\delta^{13}C$ values, this may be another explanation for the results seen in enamel that would be forming in late spring, summer and early autumn (Körner et al. 1988, O'Leary 1988, Tieszen 1991). It may

also be a combination of milk lipids initially being the source of the lower $\delta^{13}C$ values during the intense months of milk consumption (April and May), with the trend of increasingly lower $\delta^{13}C$ values continuing once C_3 plants become a part of the diet about 2 months after birth, around June (based on HF29 and HF30's birth date).

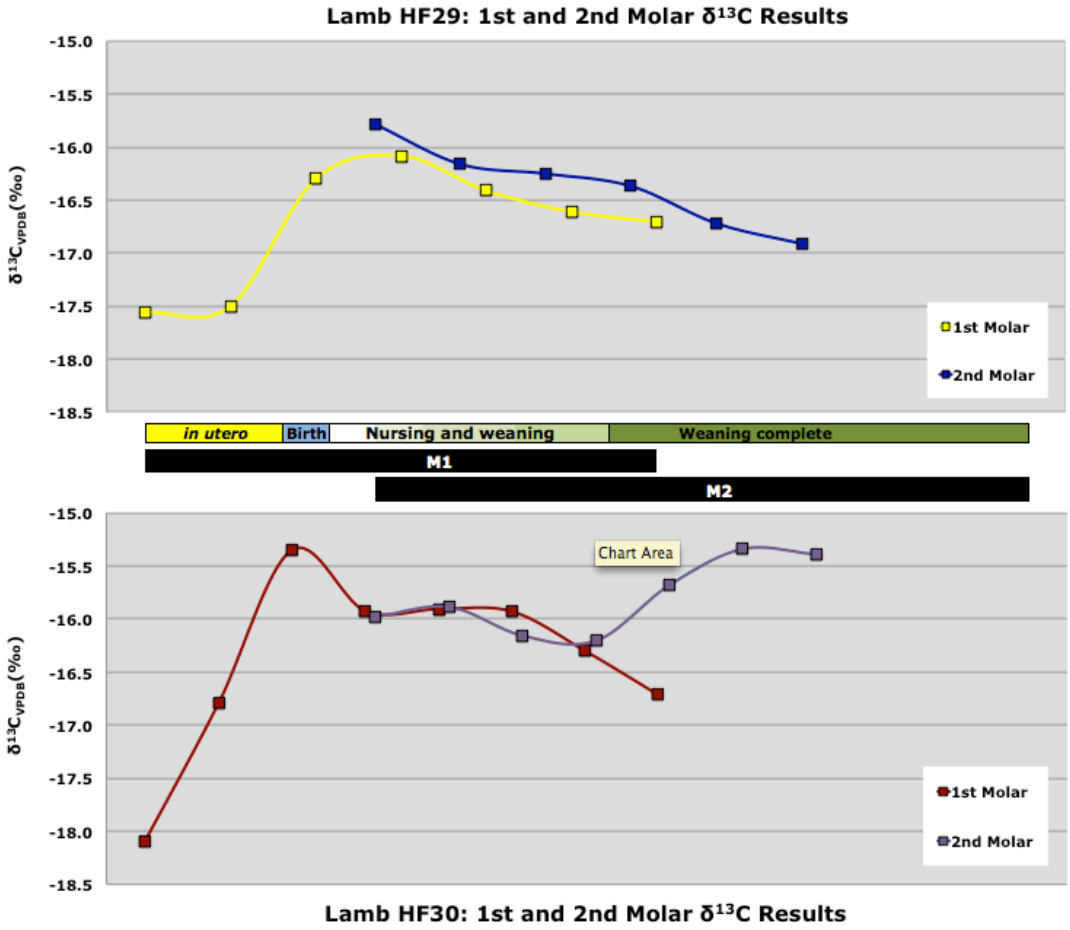

Figure 5.16: M1 and M2 incremental $\delta^{13}C$ results for HF29 (upper) and HF30 (lower). The molars are associated with each other based on the dental development chronology developed by Upex (2009) and Upex and Dobney (2012). The timing of life events (*in utero*, birth, nursing and weaning) is based on a combination of what is known about the lives of these two lambs and on what is recorded in the enamel. The interval length between the samples for both the M1s and M2s is based on the number of samples divided by the length of the M1 bar on the graph.

The M2s from HF29 closely follow the trend of progressively lower $\delta^{13}C$ values seen in the cervical half of the M1s. HF30, on the other hand, does not have an M2 with progressively lower $\delta^{13}C$ values after the fourth sample down from the apex of the crown. The samples taken from within the 9 mm of enamel above the cervix show a return to the

pre-milk and spring (March–April) $\delta^{13}C$ value. This may be due to C_3 plants having progressively higher $\delta^{13}C$ values in response to decreasing temperatures. However, while HF29 and HF30 have very similar M1 and M2 incremental sample $\delta^{18}O$ values, HF29 does not share the changed $\delta^{13}C$ values in the autumn, implying that decreasing temperatures are not driving the change. This also suggests that increasing temperatures may not be a contributing factor in the lower $\delta^{13}C$ values. Milk lipid consumption alone, however, does not explain the incremental sample $\delta^{13}C$ values for HF29 and HF30. Perhaps the same dietary, behavioural and/or metabolic differences suggested as an explanation for the disparities between HF29 and HF30's $\delta^{44/42}Ca$ values may also be the source of the differing $\delta^{13}C$ values.

5.5 Highfield Farm bone sample $\delta^{44/42}Ca$ values: results and discussion

5.5.1 Highfield Farm bone sample $\delta^{44/42}Ca$ values: results

The bone samples from the Highfield Farm sheep had $\delta^{44/42}Ca$ values that were depleted in ^{44}Ca relative to the diet. However, the bone $\delta^{44/42}Ca$ values also showed great variation between the sheep, with the range of bone $\delta^{44/42}Ca_{915a}$ values being between –0.68 and –0.42‰. The sheep had bone sample $\delta^{44/42}Ca$ values that were significantly different from each other (ANOVA, $F_{6,20} = 5.87$, $P = 0.001$, Table F.12). The isotope offset between the mean lamb (HF29, HF30, HF33 and HF44) bone $\delta^{44/42}Ca$ value and the grass $\delta^{44/42}Ca$ value was 0.17‰, with individual offsets of 0.21‰, 0.19‰, 0.16‰ and 0.10‰, respectively. The lambs had bone $\delta^{44/42}Ca$ values that were not significantly different from each other (ANOVA, $F_{3,11} = 1.44$, $P = 0.283$, Table F.13). The isotope offset between the mean adult ewe, Fifi and Helena, bone $\delta^{44/42}Ca$ values and the grass $\delta^{44/42}Ca$ value was 0.30‰ and 0.12‰, respectively. Helena and Fifi were found to be significantly different from each other (independent samples *t* test: $t_3 = –6.73$, $P = 0.007$, Table F.14). The stillborn lamb had an isotope offset between the mean bone $\delta^{44/42}Ca$ value and the grass $\delta^{44/42}Ca$ value of 0.28‰.

All of the bone samples were ashed in a furnace prior to calcium chromatography in order to remove organics. Ashed and unashed bone samples from HF30 were found to be significantly different (independent samples *t* test: $t_7 = 3.99$ $P = 0.005$, Table F.15), with the unashed bone being enriched in ^{44}Ca relative to the ashed, likely due to organics in the fresh bone. The isotope offset between HF30's mean unashed bone $\delta^{44/42}Ca$ value and the grass $\delta^{44/42}Ca$ value was 0.04‰. Figure 5.17 and Table G.7 show the bone $\delta^{44/42}Ca$ values discussed in this section.

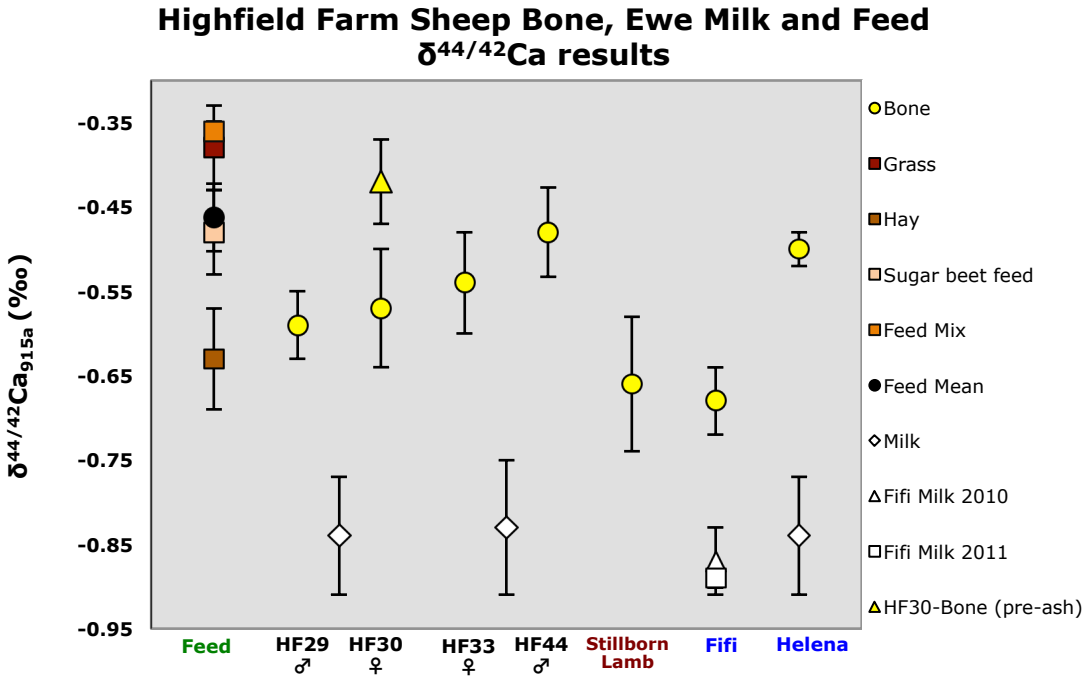

Figure 5.17: Lambs HF29 (♂), HF30 (♀), HF33 (♀) and HF44 (♂), mature ewes Fifi and Helena, and the stillborn lamb. Bone sample calcium isotope results, along with feed and milk sample results. The bone was all dry-ashed before chemistry and analysis, with the exception of HF30 where an ashed and unashed bone sample was analysed. The error bars are 1 SD.

5.5.2 Highfield Farm bone sample $\delta^{44/42}Ca$ values: discussion

As discussed in Chapter 2 and Appendix A, bone mineralisation, in a mammal that is in calcium homeostatic balance, produces a calcium isotopic offset between diet and bone, resulting in bone depleted in ^{44}Ca relative to diet. The reason for this is that during the mineralisation process lesser mass calcium isotopes are preferentially incorporated into bone apatite. A high demand for calcium during rapid mineralisation, such as during bone growth, increases the inclusion of greater mass calcium isotopes in mineralised tissue.

The male and female lambs from Highfield Farm were close in age, still young enough to have active skeletal growth, and the body size was not noticeably different between the lambs. The most obvious physical difference between the sexes was horn growth, with males growing horns and females not (see Figure 5.3). It is possible, therefore, that horn growth may be a factor in why the male lambs have dental enamel that is enriched in ^{44}Ca relative to female lambs, resulting in males having less diet–enamel isotope offset.

Sheep horns, along with cattle and goat horns, consist of a bony projection, core, covered in 'highly keratinized specialized epidermis' (Colville and Bassert 2009: 92). Horn core growth may mean that the pool (dietary input and tissue cycling) of calcium utilised for mineralisation, for male lambs, may not be utilised in the same manner as female lambs. Specifically, male lambs may require a greater amount of calcium from intestinal absorption compared with females. This is the case for male deer, which have increased intestinal calcium absorption and decreased bone resorption during antler growth, especially in young deer (Stephenson and Brown 1984). For deer, antler growth and mineralisation is the most significant osteogenic event in the animal's life, with antler mineralisation occurring at two to three times the rate of the bones of the skeleton (Cowan et al. 1968, Miller et al. 1995). Rapid mineralisation along with increased intestinal absorption of calcium and decreased bone resorption result in enrichment of ^{44}Ca in bone and a reduction in the diet–bone isotopic offset (Skulan and DePaolo 1999, Heuser and Eisenhauer 2010).

Horn, unlike antler, is not shed each year and continues to grow over the lifetime of the ram. Additionally, the amount of horn that grows each year is on a smaller scale than antler. Therefore, horn is not likely to be as significant an osteogenic event as yearly rapid antler growth. However, horn growth does have periods of rapid growth, namely during spring and summer (Lincoln et al. 1980, Santiago-Moreno et al. 2005b). This is due to changes in day length and light intensity, which directly affect horn growth, or at least the keratin sheath of the horn, with longer days producing an increased rate of growth (Lincoln et al. 1980). Additionally, sub-adult mouflon sheep have been found to have a higher rate of

horn growth than occurs in adults (Santiago-Moreno et al. 2005b). The Highfield Farm ram lambs were young, less than a year old at death, but lived long enough to have one spring and summer of 'rapid' horn growth before death in the autumn. Essentially they would have lived most of their lives during a period of rapid horn growth.

This does not mean that horn growth is the reason for the enamel calcium isotope differences between the sexes of Highfield Farm lambs. Horn core is an extension of the existing skeleton and it is not clear if 'rapid' horn core mineralisation is more rapid, or not, than the rest of the skeleton. If horn growth were responsible for enamel ^{44}Ca enrichment in males, relative to females, then it would be expected that the same would be true for male lamb bone. To test this, $\delta^{44/42}$Ca values from Highfield Farm lamb bone samples were compared. However, the bone results from the lambs were not significantly different from each other (Table F.13) and similarly there was no difference between males and females (independent samples *t* test: $t_{11} = 0.13$, $P = 0.898$, Table F.16). Overall, the bone results for the lambs were more enriched with ^{44}Ca relative to the bulk M1 and M2 enamel, the exception being male lamb HF29, which was more depleted (Figure 5.18).

These bone results would seem to indicate that horn core growth is not a factor in the enrichment of ^{44}Ca in male dental enamel compared with the female. Horn is, however, the only obvious difference between the male and female lambs (two sets of male and female twins and an unrelated male and female lamb all raised together in the same environment). To address the question of horn growth effect better, or lack of effect, on enamel and bone $\delta^{44/42}$Ca values, future sample collections should include horn. At this time there are too many questions surrounding horn to address properly its effect on male enamel $\delta^{44/42}$Ca values.

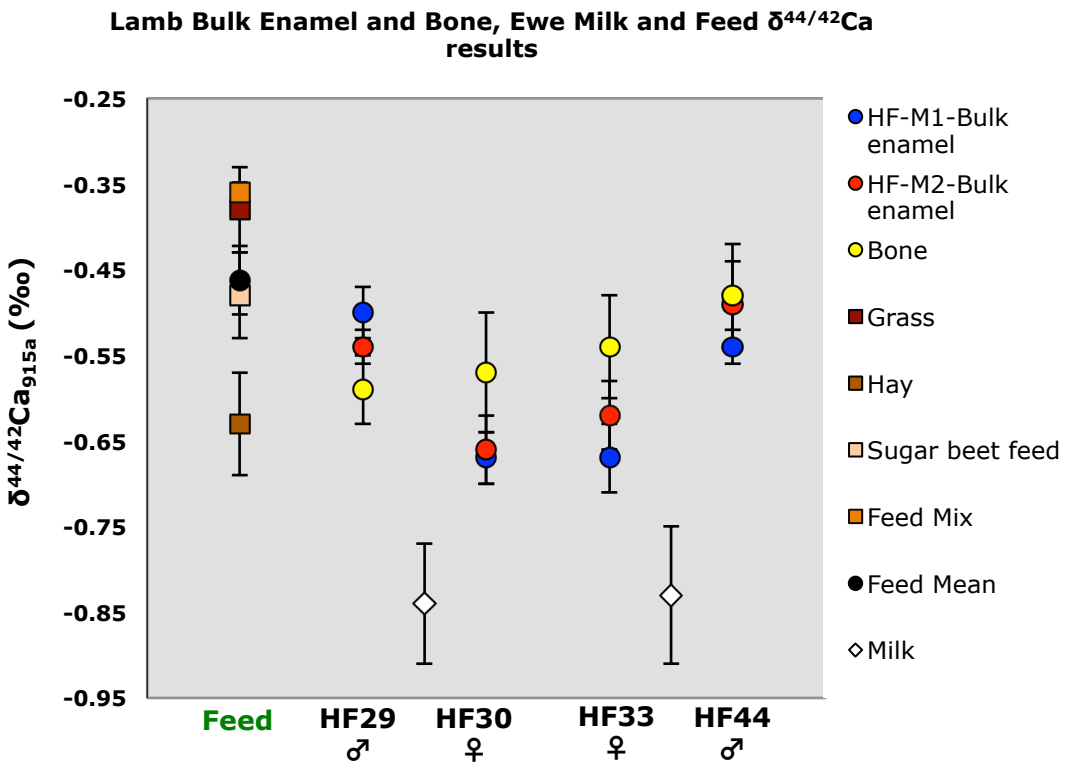

Figure 5.18: Lambs HF29, HF30, HF33 and HF44 were all either 7 months and 14 days old or 7 months and 21 days old at death. HF29 (♂) and HF30 (♀), and HF33 (♀) and HF44 (♂), were two sets of male and female twins. Included are the bone sample calcium isotope results, along with bulk enamel, feed and ewe milk results. The milk was from ewe Amber (mother of HF29 and HF30) and Jet (mother of HF33 and HF44). The error bars are 1 SD.

Preferentially, the horn samples collected, along with bone and dental samples, should be from primitive breed sheep. This is because horn growth in sheep breeds is variable. Some breeds have horn production as predominantly a male trait and rarely a female one (e.g. the Shetland breed), while other breeds have males and females that are both either naturally polled (do not produce horns) (e.g. the Est à Laine) or horn producers (e.g. the Manx Loaghtan). In the latter instances, the females that produce horns tend to produce fewer and/or smaller horns than the males, which in turn tend to produce multiple and/or large, robust horns. Horn growth in breeds where females and males both produce horns is considered to be a primitive breed morphological characteristic (Chessa et al. 2009). Well-known primitive breeds include the Mouflon, North Ronaldsay, Soay, Hebridean and Icelandic (Chessa et al. 2009). It is not surprising, therefore, that zooarchaeological analysis of Neolithic sheep in the Near East has found that both male and

female sheep produce horns and, like the primitive breeds that are descended from them, male horns tend to be larger and more robust than female horns (Zohary et al. 1998). If future work continues to focus on Neolithic sheep, then it would be appropriate for primitive breeds to be the focus of the research. Additionally, a larger sample size would be important because the Highfield Farm lamb results are from only six individuals, and small sample sizes produce uncertainty.

In addition to analysing bone from lambs HF29, HF30, HF33 and HF44, bone was analysed from the adult ewes Fifi and Helena as well as from a stillborn lamb. These samples generated bone $\delta^{44/42}$Ca values from animals with known life histories to add to the existing corpus of data: Skulan et al. (1997), Skulan and DePaolo (1999), Clementz et al. (2003), Chu et al. (2006), Hirata et al. (2008), Heuser and Eisenhauer (2010), Reynard et al. (2010), Reynard et al. (2011a) and Reynard et al. (2013). The commonly seen isotopic offset between diet and bone is ~0.65‰ (Skulan and DePaolo 1999, Chu et al. 2006). These results probably reflect animals with bone calcium balances in a steady state (equilibrium). Skulan and DePaolo (1999) proposed that the degree of fractionation between diet and bone is linked to calcium bone balance, meaning accretion is equal to loss. Bone calcium is also out of balance during young age, old age, lactation and, to a lesser extent, pregnancy (Specker 2002, Heuser and Eisenhauer 2010).

Bone that is not in equilibrium would produce a diet–bone fractionation that deviates from ~0.65‰. The bones of elderly individuals are not in a steady state due to calcium mineral loss exceeding accretion and probably resulting in bone depletion of ^{44}Ca. In the case of rapid mineralisation and bone accretion, more calcium is gained than is lost and the diet–bone isotopic offset is predicted to be reduced (Skulan and DePaolo 1999). Mammals with rapidly mineralising bone are either the young or adult females who are replenishing bone calcium stores after a period of lactation (Kovacs and Kronenberg 2006, Heuser and Eisenhauer 2010). These ideas have been supported by the research of Heuser and Eisenhauer (2010) discussed in Chapter 2.

The first few months after birth would have been the period of greatest growth and rapid mineralisation for the Highfield Farm lambs. Completion of lamb skeletal maturation, when the bones of the skeleton would be in calcium homeostatic equilibrium, is variable and can be affected by sex and, in males, castration (Davis 2000). The last bones in the sheep skeleton may fused as late as 4 years old (Moran and O'Connor 1994). However, the period of rapid skeletal growth and the attainment of adult body size occurs earlier and is likely closely aligned with sexual maturity. Female sheep sexual maturity is indicated by two factors: (1) reaching an adult or near adult body size (i.e. the ewe has the physiological reserves to sustain a pregnancy) and (2) the occurrence of photoperiod cues for the right season for breeding after an appropriate body size is reached (Foster et al. 1985). Female lambs, if not delayed by poor nutrition/body size, that are spring-born, reach sexual maturity between ~6.5 and 8.5 months (Foster et al. 1985, Quirke et al. 1985). Sexual maturity depends on different factors for male and female sheep. Male lambs do not require photoperiod cues and spermatogenesis begins around 10–15 weeks of age, irrespective of season (Courot et al. 1975). Breed, body growth and nutrition seem to dictate the age of ram puberty, which occurs between 6 and 10 months of age (Orgeur and Signoret 1984, Madani et al. 1989, Bunge et al. 1990, Mukasa-Mugerwa and Ezaz 1992, Salhab et al. 2001).

Rapid mineralisation of growing bone is predicted to result in a decrease in isotopic offset between lamb bone and dietary input. The Highfield Farm lambs were either 7 months and 2 weeks or 7 months and 3 weeks of age at the time of death and, due to a high-nutrition diet, were probably on the threshold of reaching their adult body sizes. This would seem to indicate that, while bone growth was not complete, the bone was no longer mineralising at the rapid rate it would have been in the preceding months since birth. However, although skeletal growth would have been slowing, the bone would not yet be in a steady state and should still reflect $\delta^{44/42}$Ca values linked to rapid mineralisation. This would therefore predict that the bone $\delta^{44/42}$Ca values would be close to those of the diet,

which consisted of milk for the first 4 months, the period of greatest calcium accretion, and then consisted of plants. Therefore, it would be anticipated that the bone $\delta^{44/42}$Ca values would be between the grass and milk $\delta^{44/42}$Ca values.

These lambs would also have had bone calcium accretion occurring *in utero* (~150 days to term) (Lok et al. 1996). Foetal bone calcium is largely sourced from the maternal diet. The last few months of pregnancy involve a near doubling in maternal intestinal calcium absorption to meet the needs of foetal skeletal growth and development (Belkacemi et al. 2005, Kovacs 2006, Kovacs and Kronenberg 2006). The mobilisation of calcium from the maternal skeleton contributes calcium to the foetus in the last months of pregnancy, but not to the same degree as the maternal diet (Specker 2002, Kovacs and Kronenberg 2006). Maternal bone calcium is released in preparation for the start of lactation (Specker 2002). Although small, there is fractionation (~0.17 ± 0.25‰) between an animal's diet and soft tissue, with soft tissue being depleted in ^{44}Ca relative to diet (Reynard et al. 2010). The placenta and the foetus' own calcium homeostatic processes have the potential to produce additional small fractionation with depletion in ^{44}Ca relative to the maternal dietary input (see Appendix A, Section A.3.3), but it would be expected that, even with small amounts of soft tissue fractionation, the calcium isotopes mineralised *in utero* would reflect the influence of the maternal diet, mainly grass and some supplemental feeds, more than the soft tissue fractionation. It was found that bone $\delta^{44/42}$Ca values for the Highfield Farm lambs were closer to grass than milk. This may have been due to the rapid mineralisation of calcium from the maternal diet occurring *in utero* as well as from plant consumption during the weaning transition.

Analysis of the bone from the stillborn lamb was a way of addressing the bone $\delta^{44/42}$Ca values acquired *in utero*. Based on what has just been discussed and the fact that the stillborn lamb had never consumed milk, it would be expected that the stillborn lamb would have $\delta^{44/42}$Ca values close to the maternal dietary values (–0.38‰). Instead, the stillborn lamb had a value (–0.66‰) closer to milk (mean = –0.85‰). A possible source of the

depletion of ^{44}Ca between the ewe's diet and the foetal bone may be soft tissue fractionation (0.17 ± 0.25‰) (Reynard et al. 2010) in the ewe's body. Another source of fractionation may be calcium transport from the ewe's blood to the foetus through the placenta. Placental epithelial transport of calcium is similar to that which occurs in the epithelial cells of the kidney and the intestinal lumen (Belkacemi et al. 2005, Hoenderop et al. 2005, Brown and Jüppner 2006, Kovacs and Kronenberg 2006). Research by Heuser and Eisenhauer (2010) found evidence of calcium fractionation occurring in the kidneys, probably due to epithelial cell transport. Between soft tissue fractionation and calcium's transport through the placenta, it is not unreasonable to see an isotope offset of 0.28‰ between the maternal dietary $\delta^{44/42}Ca_{grass}$ value and the stillborn lamb $\delta^{44/42}Ca_{bone}$ value.

There was no bone sample from the mother of the stillborn lamb, and as a result no comparison could be made between the two, but Reynard et al. (2010) did analyse a human mother and foetus from the Neolithic archaeological site of Bouqras, Syria. The foetus would have been actively growing at the time of death, and as a result the foetus would be expected to have a higher $\delta^{44/42}Ca_{bone}$ value than the mother (Reynard et al. 2010). The mother's skeleton would be unlikely to be greatly affected by the pregnancy unless it was near the time of birth and calcium was being liberated from the maternal skeleton in preparation for lactation. Reynard et al. (2010) found no significant difference between the mother ($\delta^{44/42}Ca$ = –0.60 ± 0.06‰, 2 SD) and the foetus ($\delta^{44/42}Ca$ = –0.66 ± 0.11‰, 2 SD). See Chapter 2 and Reynard et al. (2013) for a discussion of human $\delta^{44/42}Ca_{bone}$ values related to age.

While the bone samples from the human mother and foetus from Bouqras did not have an isotope offset, sheep do provide some evidence that frequent periods of lactation will affect maternal bone $\delta^{44/42}Ca$ values over time. Unlike bone mineralisation, it is thought that the release of calcium from the skeleton during demineralisation does not involve biological processes that result in fractionation of the calcium isotopes (Skulan and DePaolo 1999). The adult ewes Fifi and Helena, based on their ages, probably had bone in a

steady state. It would be expected, therefore, that, the ewes would have a diet–bone isotope offset close to 0.65‰. Lactation perturbs bone balance and results in a measurable decrease of calcium content in maternal bone, with, for humans, a typical loss of 280–400 mg (with a maximum of 1000 mg) of calcium per day (Kovacs and Kronenberg 2006). This would alter bone calcium isotope ratios due to the release of calcium depleted in ^{44}Ca from the skeleton. This results in a reduction in diet–bone fractionation in the lactating mammal.

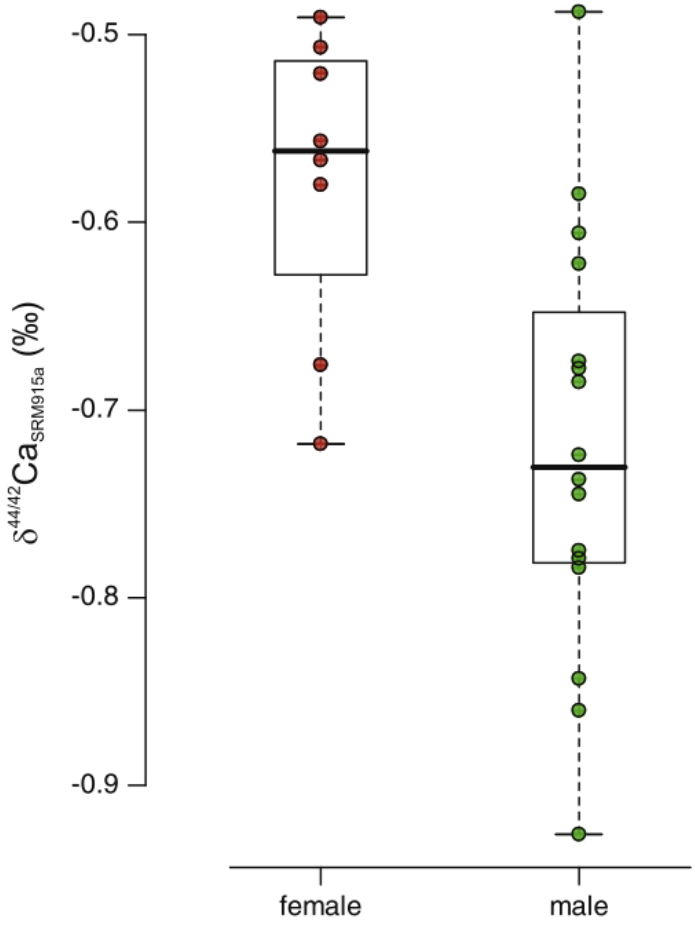

Figure 5.19: Graph from Reynard et al. (2010) detailing female and male bone $\delta^{44/42}Ca$ values from an experimental English Heritage herd of sheep.

After lambs are weaned and lactation ceases, maternal bone calcium is quickly restored (Kovacs and Kronenberg 2006), which results in a temporary period of rapid mineralisation. Rapid mineralisation, as discussed earlier, also results in a reduction of diet–bone fractionation. This is the probable explanation for the male–female bone offset seen between the adult male and female sheep in an English Heritage herd (Figure 5.19). Seasonal or near seasonal periods of lactation, with repetitive release of bone calcium depleted in ^{44}Ca, along with rapid mineralisation to replenish bone calcium after lactation, would over time produce enriched female $\delta^{44/42}Ca$ bone values relative to males.

When examining the Highfield Farm sheep in relation to age, the combined bone samples of the lambs (HF29, HF30, HF33, HF44 and the stillborn lamb) and the adult ewes (Helena and Fifi) were found to be not significantly different (independent samples *t* test: $t_{11} = -0.15$, $P = 0.883$, Table F.17). Adult ewes Helena and Fifi were, however, found to be significantly different from each other (Table F.14). The $\delta^{44/42}$Ca values for the stillborn lamb and Fifi were not significantly different (independent samples *t* test: $t_4 = -0.56$, $P = 0.605$, Table F.18) and these sheep were the most depleted in ^{44}Ca. Helena's results were not significantly different from lambs HF29, HF30, HF33 and HF44 (ANOVA, $F_{4,15} = 2.10$, $P = 0.132$, Table F.19).

A possible explanation for the offset between Fifi and Helena lies with how many periods of lactation each ewe had over the course of her life. Helena was approximately 3 years older than Fifi and had five births and a total of 10 lambs compared with Fifi's three births and five lambs. Nursing twin lambs requires an increase in milk supply and it has been documented that ewes produce more milk when multiple lambs are born (Gardner and Hogue 1964, Cardellino and Benson 2002), which would require an increase in maternal bone calcium. It is likely that the offset, with Helena more enriched in ^{44}Ca relative to Fifi, is due to the different number of periods of lactation and Helena's doubling of the number of lambs nursed. Additionally, the fact that Fifi had nursed five lambs during three periods of lactation may be the explanation for why she had a $diet_{grass}$–bone offset of 0.30‰ instead of 0.65‰. The even greater number of periods of lactation and number of lambs nursed would explain the small $diet_{grass}$–bone offset of 0.12‰ for Helena.

Overall, the diverse variability of bone in this small group of samples from Highfield Farm demonstrates the range of measurements that can be seen within a single herd. Similar variation of bone $\delta^{44/42}$Ca has been seen in the herbivore bone from the Neolithic archaeological sites of Ensisheim in France and Balatonszárszó in Hungary (see Chapter 2, Section 2.7). Although the humans from these archaeological sites had a higher variation from the mean [Ensisheim = 0.13‰ (1 SD) and Balatonszárszó = 0.15‰ (1 SD)]

than the herbivores, there was still a high variation in the herbivores. The variation from the mean, at 1 SD, seen for herbivores from different studies was: Highfield Farm (sheep, n = 7) = 0.10‰, Ensisheim (cattle and sheep/goat, n = 9) = 0.09‰ and Balatonszárszó (cattle and sheep/goat, n = 10) = 0.12‰ (Figure 5.20). Additionally, the modern sheep from the English Heritage experiment, at 1 SD, had similar high levels of variation from the mean for males (0.11‰, n = 16) and females (0.08‰, n = 8) as the archaeological herbivores. The range of $\delta^{44/42}$Ca bone values from Highfield Farm are in agreement with similar studies and demonstrates how the bone $\delta^{44/42}$Ca values reflect several influences over the course of a sheep's life.

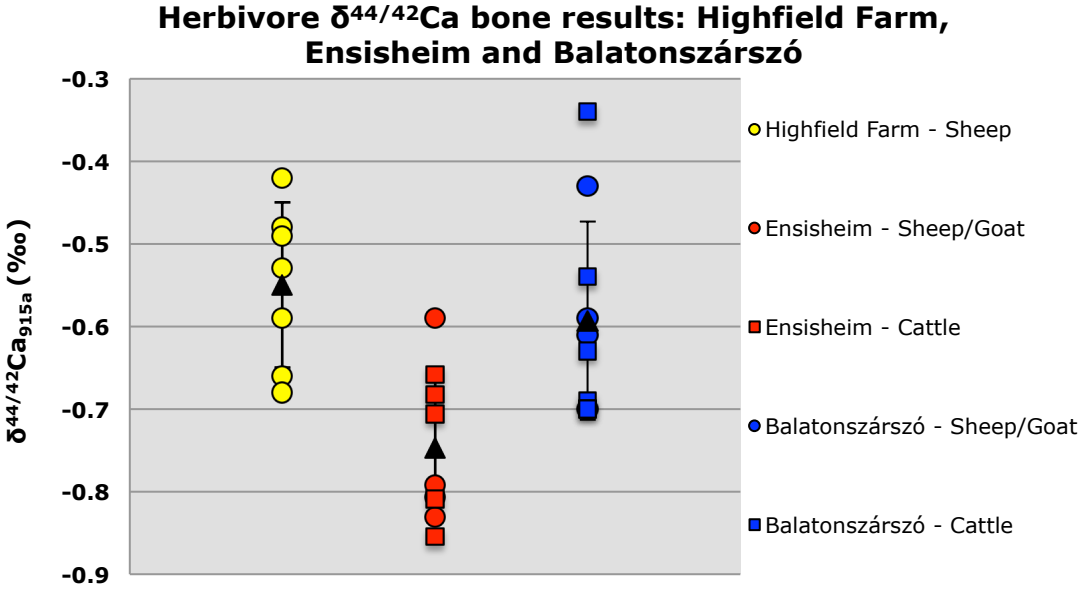

Figure 5.20: The ranges of bone $\delta^{44/42}$Ca values and variation from the mean (1 SD) of herbivores from modern Highfield Farm (sheep) compared with the Neolithic archaeological sites of Ensisheim, France (sheep/goat and cattle), and Balatonszárszó, Hungary (sheep/goat and cattle). Balatonszárszó data used with permission from Dr Linda Reynard.

5.6 Conclusion

The analysis of bone from animals with detailed life histories has demonstrated a variability reflective of the complexity inherent in mammal calcium homeostasis. Enamel, however, has potential for providing information regarding diet and dietary change linked to milk and plant consumption. Based on the Highfield Farm sample results it is not possible to identify

an exact timing for when dietary changes occur, and oxygen and carbon isotope analyses were not able to increase the resolution. Although the period of weaning completion was known, the results for HF29 and HF30 produced different patterns. It is not clear whether the differences are due to individual variation, immature enamel and/or sex. The bulk enamel results suggest that the difference is related to sex. Further analysis of male and female Highfield Farm lambs and/or male and female sheep from a different herd would help to identify the source of difference. Chapter 6, while only having samples from male sheep, does provide $\delta^{44/42}$Ca results that should help to clarify the contribution of individual variation and immature enamel to the results.

CHAPTER 6

LOFT AND WHITE HAMARS GRAZING PROJECT $\delta^{44/42}Ca$ SAMPLE RESULTS AND DISCUSSION

The Highfield Farm sheep data gave rise to several questions, namely does immature and mature enamel differ from each other and to what degree does complete versus incomplete second molars affect the ability to identify nursing and weaning timing? Therefore the Loft and White Hamars sheep were analysed, due to their being weaned naturally over a period of about 5 months instead of a shortened weaning period of 4 months. Unfortunately, there were no female sheep available to address questions regarding sex.

6.1 Loft and White Hamars Grazing Project samples

The second group of modern sheep enamel samples were from the Loft and White Hamars Grazing Project, Orkney, Scotland. This group of sheep will be referred to in the text as Hoy sheep rather than by the full name of the project. The majority of the Hoy sheep were born at the end of April, with a small number born at the start of May. The samples consisted of dental enamel from first, second and third mandibular molars. The sheep were a mix of rams and wethers (castrated rams), with the youngest being 1 year and 9 months old, and the oldest being 3 years and 3 months old (Table 6.1). The mean age for the group was 2 years and 5 months.

Table 6.1: Loft and White Hamars Grazing Project (Hoy) sheep

Sheep ID	Sex	Breed	Date of birth	Date of death
545	Ram	Shetland	22/04/1991	18/02/1993
549	Ram	Shetland	03/05/1991	18/02/1993
552	Wether	Shetland	25/04/1991	01/04/1993
668	Ram	Shetland	04/1992	09/1994
799	Wether	Shetland	04/1992	30/11/1994
800	Wether	Shetland	04/1992	09/1994
2914	Wether	Shetland	04/1990	07/1993

6.2 Hoy bulk enamel sample $\delta^{44/42}$Ca values: results and discussion

6.2.1 Hoy bulk enamel sample $\delta^{44/42}$Ca values: results

The bulk $\delta^{44/42}$Ca values for the first, second and third molar enamel results from sheep 545, 549, 552, 668, 799, 800 and 2914 are given in Table G.8 (for all tables prefixed G, see Appendix G). Figure 6.1 shows the bulk enamel molar results; Section 6.2.2 discusses the results.

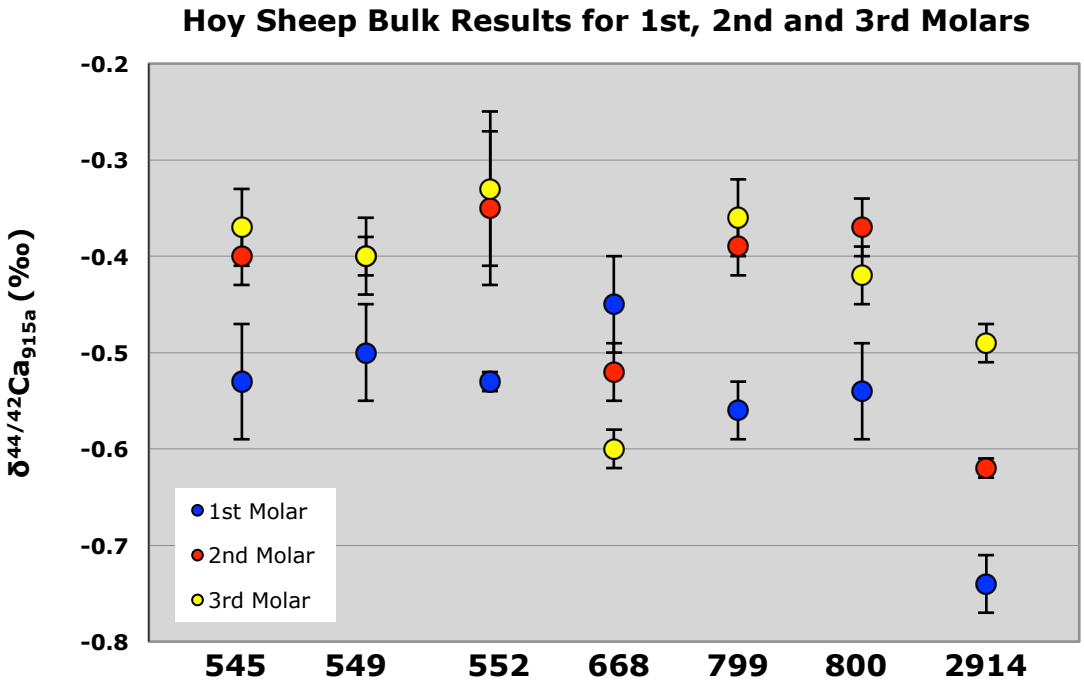

Figure 6.1: Mean first, second and third molar $\delta^{44/42}$Ca results for Hoy sheep 545, 549, 552, 668, 799, 800 and 2914. Error bars are 1 standard deviation (SD).

The results were used to establish what differences may be seen between: (1) molars within individual Hoy sheep; (2) molars between different Hoy sheep; (3) Hoy rams and wethers; (4) Highfield Farm and Hoy sheep; (5) sheep weaned at 4 months and sheep weaned at 5 months; and (6) mature and immature enamel. Each Hoy sheep had first molars that were significantly different (Tables F.20–F.26; for all tables prefixed F see Appendix F) in $\delta^{44/42}$Ca values from its second and third molars (Table 6.2). When the $\delta^{44/42}$Ca values from first molars from all of the Hoy sheep were compared, it was found that there was a significant difference between the first molars of individual sheep (ANOVA, $F_{6,20} = 15.72$, $P = 0.000$, Table F.27). However, the source of the difference was due to two of the seven

sheep having first molars that were outliers: sheep 668 and 2914. When these samples were removed from the statistical analysis, the remaining first molars were found to have no significant difference (ANOVA, $F_{4,15} = 0.87$, $P = 0.505$, Table F.28) in the $\delta^{44/42}$Ca values.

Table 6.2: ANOVA results for first, second and third molars for each Hoy sheep, with degrees of freedom (df), *F*-values, *P*-values and the statistics test identification from Appendix F

Sample	df factor	df error	*F*-value	*P*-value	Appendix F test ID
545	6	8	14.46	0.020	Table F.20
549	2	8	8.11	0.012	Table F.21
552	2	5	8.90	0.023	Table F.22
668	2	9	19.52	0.001	Table F.23
799	2	7	40.51	0.000	Table F.24
800	2	10	20.67	0.000	Table F.25
2914	2	7	48.20	0.000	Table F.26

Similarly, the second molars were significantly different in $\delta^{44/42}$Ca values between the Hoy sheep (ANOVA, $F_{6,18} = 20.97$, $P = 0.000$, Table F.29). However, once sheep 668 and 2914 data were removed there was no significant difference in $\delta^{44/42}$Ca values between the second molars (ANOVA, $F_{4,13} = 1.10$, $P = 0.398$, Table F.30). The same pattern of difference was repeated between the third molars for all the Hoy sheep (ANOVA, $F_{6,16} = 16.76$, $P = 0.000$, Table F.31) and again, once sheep 668 and 2914 data were removed, the difference in $\delta^{44/42}$Ca values was eliminated (ANOVA, $F_{4,10} = 2.01$, $P = 0.169$, Table F.32).

Sheep 668 had $\delta^{44/42}$Ca values that were not in keeping with the other first, second and third molar data, with the second and third molars being more depleted in ^{44}Ca than the first molars. The sheep all had access to the same diet and environment and it was not clear why there would be this difference in 668. The molars from 668 were re-sampled, put through column chemistry and analysed a second time. This did not change the results.

Although sheep 2914 was an outlier compared with sheep 545, 549, 552, 799 and 800, it did follow the pattern of having the first molar significantly more depleted in ^{44}Ca than the other two molars. However, sheep 2914 had shifted lower $\delta^{44/42}$Ca values for its first, second and third molars compared with the other sheep. The offset between the molar

results for sheep 2914 and the individual mean results for first, second and third molars for the other sheep was 0.21‰ for first molars, 0.24‰ for second molars and 0.11‰ for third molars.

6.2.2 Hoy bulk enamel sample $\delta^{44/42}$Ca values: discussion

These sheep were naturally weaned, which usually means that weaning is complete at 5 months of age. This is 1 month later than the Highfield Farm lambs. Also different was the fact that the Hoy sheep had fully developed second molars with completely mineralised enamel. These differences between the Highfield Farm and Hoy sheep seem to have resulted in the Hoy sheep having a significant difference in $\delta^{44/42}$Ca values between the first and second molars of sheep 545, 549, 552, 799, 800 and 2914. The average calcium isotope offset between the Hoy first and second molars was 0.15‰, whereas Highfield Farm mean bulk $\delta^{44/42}$Ca values had first and second molars with no offset.

Possible reasons for the Highfield Farm lambs not showing a similar pattern of bulk results is that the second molars were: (1) only about half formed and (2) much of the enamel was still immature. The second molar begins to form about 2 months after birth and over a 14-month period, based on Upex and Dobney's (2012) dental development chronology, with, for the Hoy sheep, 11 months of enamel formation occurring after the weaning transition is complete. The Highfield Farm lambs had second molars that stopped being formed between 7.5 and 8 months, which means that there were only 3.5–4 months of enamel formation after weaning was complete. As discussed and demonstrated in Chapter 5, milk produced by a sheep is more depleted in ^{44}Ca than the plants in the milk producer's diet. It is logical, therefore, based on the Highfield Farm first and second molar results, that the Hoy sheep second molars would show a significant enrichment in ^{44}Ca relative to the first molar due to having enamel that contained 7 additional months of calcium isotopes sourced from plants.

The Hoy sheep results establish that, with completely formed enamel, bulk analysis of molars produces $\delta^{44/42}$Ca values that, between individuals of the same sex and from the

same managed herd, show close agreement between sheep for each specific molar. This was also true for the Highfield Farm lambs, but the Hoy sheep also show the importance of having fully formed teeth and enamel. The Hoy sheep clearly demonstrate that, for naturally weaned sheep, the $\delta^{44/42}$Ca values in enamel reflect the difference in dietary input of milk between the first and second molars. Incremental samples were then analysed to see if there was similar agreement between individuals.

6.3 Hoy incremental enamel sample $\delta^{44/42}$Ca values: results and discussion

6.3.1 Hoy incremental enamel sample $\delta^{44/42}$Ca values: results

Table G.9 contains the $\delta^{44/42}$Ca values for sheep 545, and Table G.10 for sheep 799. Figures 6.2, 6.3 and 6.4 show the $\delta^{44/42}$Ca values from serial incremental samples from Hoy sheep 545 and 799. Figure 6.2 shows the first molar $\delta^{44/42}$Ca values for 545 and 799, while Figures 6.3 and 6.4 contain the second and third molars, respectively. Section 6.3.2 discusses the results.

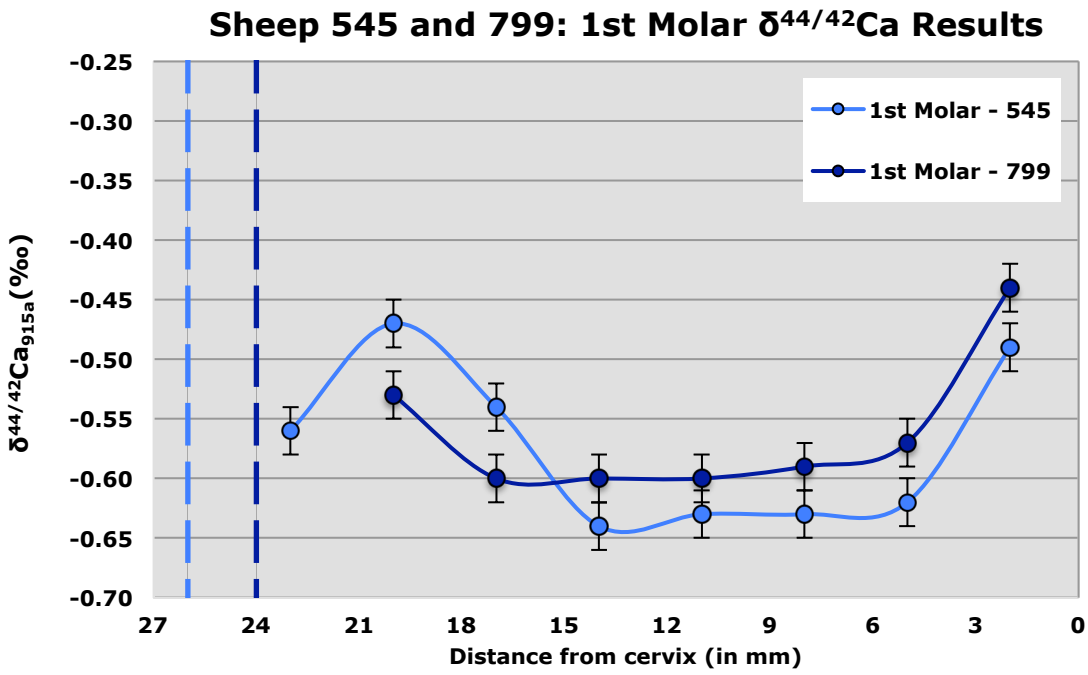

Figure 6.2: First molar incremental $\delta^{44/42}$Ca values for ram 545 and wether 799. Samples were collected at 3-mm intervals, with the first sample taken 3 mm and 4 mm, respectively, below the apex of the crown. Temporal chronology progresses from left to right. Error bars are 1 standard error of the mean (SEM) for both 545 and 799, 0.02‰.

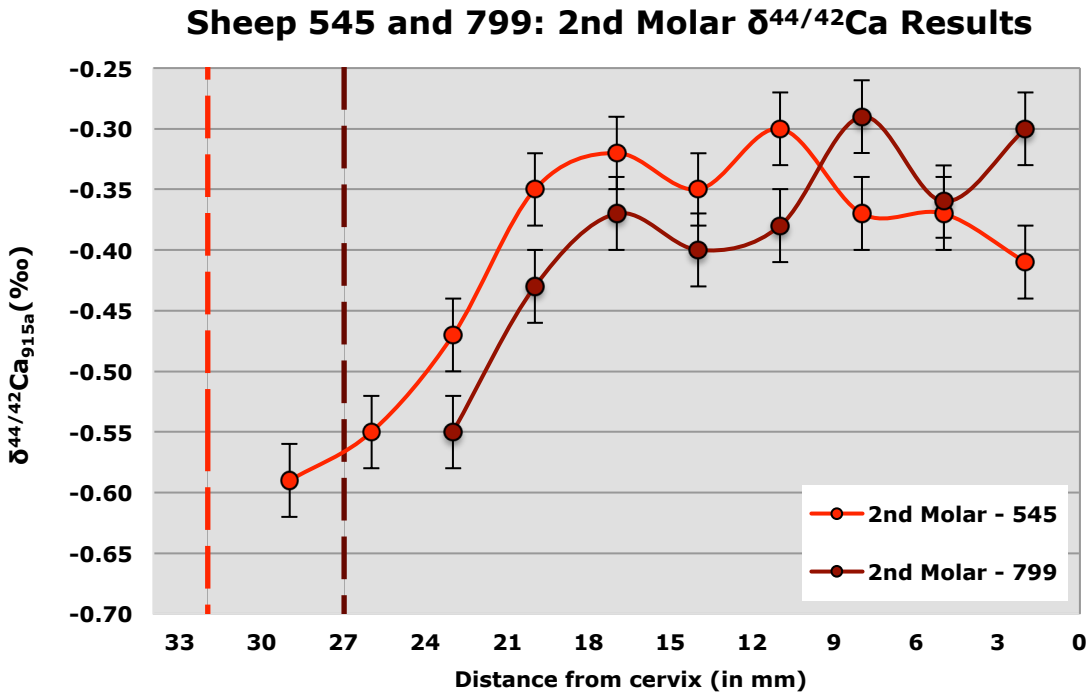

Figure 6.3: Second molar incremental $\delta^{44/42}$Ca results for ram 545 and wether 799. Samples were collected at 3-mm intervals with the first sample taken 3 mm and 4 mm, respectively, below the apex of the crown. Temporal chronology progresses from left to right. Error bars are 1 SEM for both 545 and 799, 0.03‰.

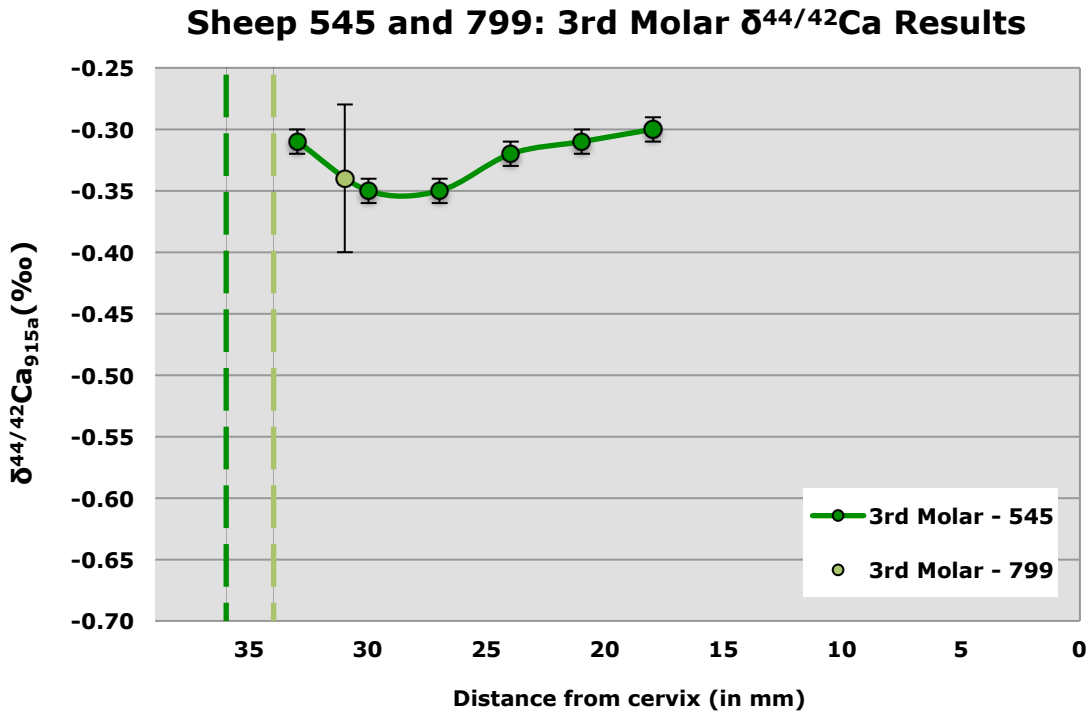

Figure 6.4: Third molar incremental $\delta^{44/42}$Ca results for ram 545 and a single result from below the apex of the crown for wether 799. Samples were collected at 3-mm intervals with the first sample taken 2 mm and 3 mm, respectively, below the apex of the crown. Temporal chronology progresses from left to right. Error bars are 1 SEM for 545, 0.02‰, and 1 SD for 799, 0.07‰.

The $\delta^{44/42}$Ca values from the analysis of serial incremental samples from the first, second and third molars from male sheep produced patterns of results that were similar to each other. Sheep 545 shared some similarities to Highfield Farm lamb HF29. The first molar of sheep 545 had a peak of enrichment in ^{44}Ca from samples taken within the 6 mm of enamel below the apex of the crown, which was similar to what was seen in the same area of enamel from HF29 (Chapter 5). This, along with the supporting evidence from HF29's second molar, suggests that HF29 did have a legitimate peak of enrichment in ^{44}Ca near the crown apex.

Hoy sheep 799 did not have a peak of enrichment in the first molar in the same way that sheep 545 did, but it did have enrichment in ^{44}Ca in the 3 mm of enamel near the apex of the crown. It appears that enrichment in ^{44}Ca occurs within the 9 mm of enamel near the apex of first molars of male sheep. This is important because this enrichment may be linked to the maternal diet and the lamb's life *in utero*.

The $\delta^{44/42}$Ca values between 5 and 14 mm of enamel above the cervix for sheep 545 and between 5 and 17 mm of enamel above the cervix for sheep 799 formed a plateau of low $\delta^{44/42}$Ca values. There was little variation between the $\delta^{44/42}$Ca values in this enamel (0.02–0.03‰). This plateau of results may be linked to milk consumption during nursing and weaning. Between the 2 and 5 mm of enamel above the cervix, both sheep then showed a steady increase in $\delta^{44/42}$Ca values, which may be due to the later stages of weaning and weaning completion.

The incremental samples in sheep 545 and 799 could be paired between the sheep based on their proximity in millimetres from the cervix of each molar. Overall the incremental sample $\delta^{44/42}$Ca values for the first molars from 545 and 799 were very similar. All but one pair of samples showed no significant difference (Table 6.3). Samples 545-M1-7 and 799-M1-6 (samples 5 mm from cervix) have a significant difference. Overall, the first molar incremental samples from adult male sheep that were weaned at 5 months of age and raised in the same environment and on the same diet produced enamel with comparable

$\delta^{44/42}$Ca values in the same sections of enamel (± 3 mm). This is important for understanding how much variation can be expected between male sheep enamel $\delta^{44/42}$Ca values.

Table 6.3: Statistical analysis of paired samples in the first molars of sheep 545 and 799

Sheep 545	Sheep 799	*t*-value	*P*-value	df	Distance from cervix	Appendix F test ID
545-M1-1	——	——	——	——	23 mm	———
545-M1-2	799-M1-1	2.17	0.118	3	20 mm	Table F.33
545-M1-3	799-M1-2	1.98	0.119	4	17 mm	Table F.34
545-M1-4	799-M1-3	–1.13	0.310	5	14 mm	Table F.35
545-M1-5	799-M1-4	–0.74	0.512	3	11 mm	Table F.36
545-M1-6	799-M1-5	–2.04	0.097	5	8 mm	Table F.37
545-M1-7	799-M1-6	–7.38	0.018	2	5 mm	Table F.38
545-M1-8	799-M1-7	–1.83	0.142	4	2 mm	Table F.39

The second molars of sheep 545 and 799 shared a similar pattern in $\delta^{44/42}$Ca values in the 10–12 mm of enamel below the apex of the crown, both showing a steady enrichment of ^{44}Ca. The samples within the next 19 mm of enamel for both sheep 545 and 799 were the most enriched in ^{44}Ca of all the second molar samples. The incremental samples in sheep 545 and 799 had individual samples that could be paired between the sheep based on their proximity in millimetres from the cervix of each molar. Overall, the incremental sample $\delta^{44/42}$Ca values for the second molars from sheep 545 and 799 showed no significant difference (Table 6.4). The exception was 545-M2-5 and 799-M2-3 (samples 17 mm from cervix), which did not have enough data to make a statistical assessment. Overall, the second molar incremental samples from adult male sheep that were weaned at 5 months of age and raised in the same environment and on the same diet produced enamel with comparable $\delta^{44/42}$Ca values in the same sections of the crown.

Table 6.4: Statistical analysis of paired samples in the second molars of sheep 545 and 799

Sheep 545	Sheep 799	*t*-value	*P*-value	df	Distance from cervix	Appendix F test ID
545-M2-1	——	——	——	——	29 mm	——
545-M2-2	——	——	——	——	26 mm	——
545-M2-3	799-M2-1	1.88	0.156	3	23 mm	Table F.40
545-M2-4	799-M2-2	2.72	0.113	2	20 mm	Table F.41
545-M2-5	799-M2-3	——	——	——	17 mm	——
545-M2-6	799-M2-4	2.66	0.076	3	14 mm	Table F.42
545-M2-7	799-M2-5	2.13	0.100	4	11 mm	Table F.43
545-M2-8	799-M2-6	0.47	0.672	3	8 mm	Table F.44
545-M2-9	799-M2-7	–0.27	0.806	3	5 mm	Table F.45
545-M2-10	799-M2-8	–3.26	0.083	2	2 mm	Table F.46

The third molar of sheep 545 had six samples taken from the 16 mm of enamel below the apex of the crown, the upper half of the tooth. Sheep 799 had only one sample taken 3 mm below the apex. The samples from both sheep 545 and 799 had results that were in agreement with the samples taken from within the 19 mm of enamel above the cervix of the second molar. This result demonstrated a steady signal input from a plant-only diet.

6.3.2 Hoy incremental enamel sample $\delta^{44/42}$Ca values: discussion

The Hoy sheep selected for incremental sample analysis were two sheep with very similar bulk $\delta^{44/42}$Ca values. They were of a similar age at the time of death: 2 years and 10 months for sheep 545, and 2 years and 7 months for sheep 799. Sheep 545 was born on 22 April 1991 and remained uncastrated. Sheep 799 was born in late April 1991 and was later castrated.

Dental enamel wear needs to be considered when interpreting incremental sample results. The amount of enamel lost to wear is typically related to sheep age (Payne 1973). At death, Hoy sheep 545 was 2 years and 10 months old and sheep 799 was 2 years and 7 months old, which, based on Upex and Dobney's (2012) dental chronology, were in the

fifth stage of Payne's (1973) wear, which probably means there was 6 mm of wear or less. Figure 6.5 shows the second molars from lamb HF29 and sheep 545. These two sheep were from different locations and the close alignment on the $\delta^{44/42}Ca_{915a}$ scale is coincidental. This figure shows that for HF29 the first and second samples taken from below the apex of the crown of the second molar had $\delta^{44/42}Ca$ values that mirrored $\delta^{44/42}Ca$ values from the middle of HF29's first molar. This was not the case for sheep 545, most probably because of wear.

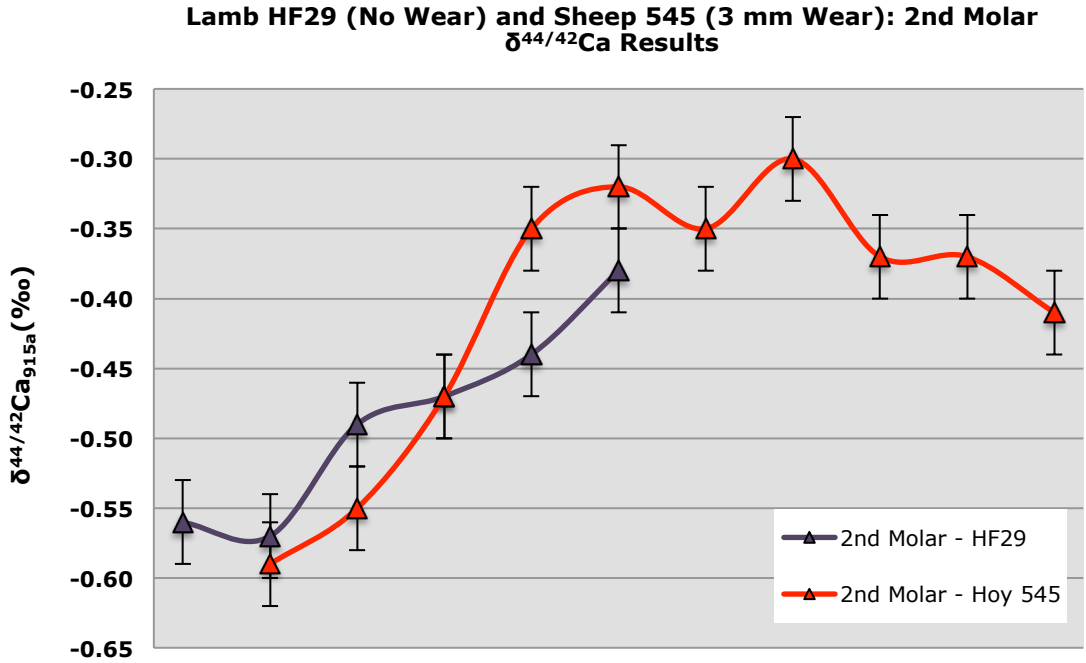

Figure 6.5: Second molar incremental $\delta^{44/42}Ca$ results for lamb HF29 and sheep Hoy 545. Lamb HF29 was 7 months and 21 days old at death and had little wear on the second molar and the tooth was only half formed. Hoy 545 was 2 years and 10 months old at death and may have had as much as 6 mm of wear. The graph demonstrates a possible missing overlap with the first molar due to wear in Hoy 545. Temporal chronology progresses from left to right. Error bars are 1 SEM.

Figure 6.5 also provides insight into mature versus immature enamel. The $\delta^{44/42}Ca$ values for HF29's second molar were generally in keeping with the pattern of enrichment in ^{44}Ca, as was seen in sheep 545. This was especially true for the second, third and fourth samples down from the crown apex, which were sampled from mature or nearly mature enamel. There was a slight change in the pattern with the fifth and sixth samples, showing depletion in ^{44}Ca in relation to the equivalent sheep 545 samples, probably because of the very immature enamel in the lower half of HF29's second molar. This implies that the

enamel maturation process will alter but not entirely overwrite the $\delta^{44/42}$Ca values in immature enamel.

Getting a sense of how wear may affect the molars of sheep 545 and 799 is important for interpreting the patterns of $\delta^{44/42}$Ca values not only in the individual molars, but also with the molars associated with each other within a dental development chronology. Due to sheep 545 and 799 having very similar $\delta^{44/42}$Ca values between their first, second and third molars, interpretation of one sheep would provide information about both. However, sheep 545 appears to have a more complete set of $\delta^{44/42}$Ca sample values, which suggests less wear than for sheep 799's molars. Therefore the first, second and third molars from sheep 545 were selected for further analysis of the data.

Figure 6.6 contains two graphs of the first (M1), second (M2) and third (M3) molars from sheep 545. The graphs show M1, M2 and M3 serial incremental sample $\delta^{44/42}$Ca values associated with sheep life events [*in utero* (maternal grass diet and maternal tissue cycling of calcium), birth, nursing (milk diet), weaning (milk and grass diet, with consumption of milk decreasing over time and of grass increasing) and post-weaning (grass-only diet)] and Upex and Dobney's (2012) dental chronology. The upper graph has equal interval lengths. M1 interval lengths were determined by dividing the length of the M1 bar on the graph by the number of sample intervals (seven intervals) and M2 interval lengths were determined by dividing the length of the M2 bar on the graph by the number of sample intervals (nine intervals). M3 was determined in the same manner as M1 and M2. The bottom graph is shown with a reduced rate of enamel mineralisation in the 25% of enamel near the cemento-enamel junction of M1 and M2, which is shown on the graph by doubling interval lengths between the $\delta^{44/42}$Ca sample values from within that region of the molars. The interval lengths in the lower graph were made by adding two extra intervals during division of the length of the tooth bars on the graph and then doubling the length of the intervals between the samples collected from within the 25% of the enamel near the cervix of M1 and M2.

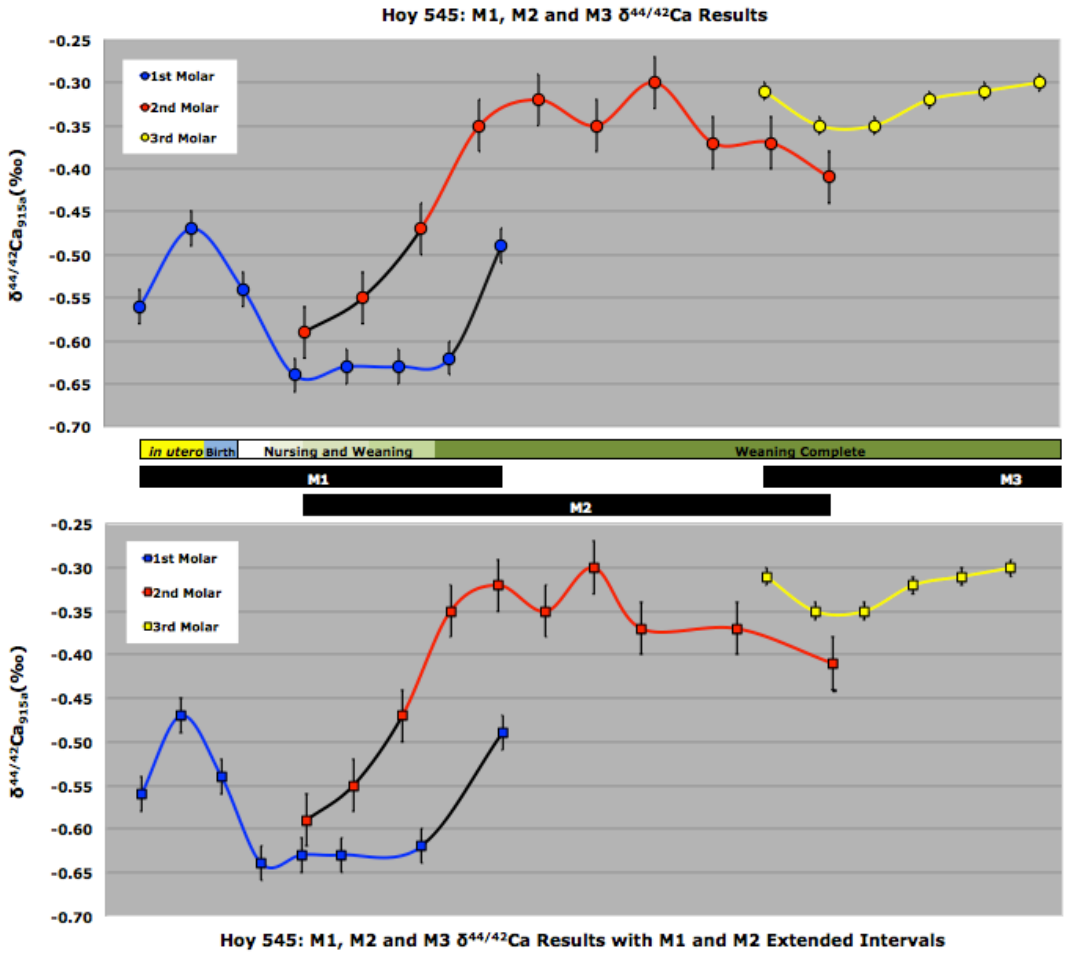

Figure 6.6: Ram sheep 545 was born on 22 April 1991 and was slaughtered on 18 February 1993. The graph accounts for the periods in the sheep's life: *in utero*, birth, nursing, weaning and lastly weaning completion. The molars are associated with each other based on the dental development chronology developed by Upex and Dobney (2012). The black intervals on the molars, near the cemento-enamel junction of M1 and near the apex of M2, are to highlight where M1 and M2 mirror each other. The top graph shows equal interval lengths between the sample $\delta^{44/42}$Ca values and the bottom graph shows the cervical 25% of the M1 and M2 having intervals with lengths twice as long as the rest. Error bars are 1 SEM.

The $\delta^{44/42}$Ca values for sheep 545 that show the most enrichment in ^{44}Ca are the ones linked to the post-weaning plant diet (moving from left to right, samples 4 to 10 on M2 and all M3 samples; Figure 6.6), which in turn provides a starting point for determining the maternal calcium input while *in utero*. This is because there is little fractionation between diet and soft tissue, but the fractionation that does occur results in the soft tissue being more depleted in ^{44}Ca (~0.17‰) than the diet (Reynard et al. 2010). Additionally, small amounts of fractionation may occur when calcium is transported through the epithelial cells of the placenta, resulting in the calcium available for foetal tissue

mineralisation being depleted in ^{44}Ca relative to the maternal dietary input (the same pasture grass was consumed by sheep 545 during weaning/post-weaning and by his mother during pregnancy). The isotope offset between the probable post-weaning plant diet $\delta^{44/42}Ca$ values (mean = –0.35‰) and the *in utero* $\delta^{44/42}Ca$ values (mean = –0.52‰) is 0.17‰ (the region on Figure 6.6 where $\delta^{44/42}Ca$ values on M1 are associated with *in utero*), which is what Reynard et al. (2010) predicted. It logically follows that the milk input, nursing and weaning would correspond with both the lowest $\delta^{44/42}Ca$ values on the graphs and would be located between the *in utero* and post-weaning plant diet regions. There is also a region on the graph after nursing and weaning where sample values show enrichment of ^{44}Ca as part of the transition to the post-weaning plant diet.

Birth on the graph is believed to be between the second and fourth samples down from the apex of the crown on M1. This is because during the last trimester (the last 6 weeks of pregnancy), when the lamb foetal skeleton is most rapidly developing, significant quantities of calcium are required for mineralisation. Maternal intestinal calcium absorption doubles during the third trimester as a result (see Appendix A, Section A.3.3), which might result in higher $\delta^{44/42}Ca$ values in the enamel that was formed during this period. This could be seen on the graph as an isotope offset between samples 1 and 2 on M1, with sample 2 being enriched in ^{44}Ca relative to sample 1. While enamel formed *in utero* may mainly show enrichment of ^{44}Ca, there may also be indications of *in utero* depletion of ^{44}Ca on the graph between samples 2 and 3, as a result of the mobilisation of maternal skeletal calcium towards the end of the pregnancy. Maternal skeletal calcium is mobilised during the late-stage foetal skeletal growth and in preparation for milk production (see Appendix A, Sections A.3.2 and A.3.3), and it has been found that calcium from bone resorption is depleted in ^{44}Ca (Chapter 2 and Appendix A). Inclusion of milk in the lambs' diet after birth would result in further depletion of ^{44}Ca in sample values. The placement of birth between samples 2 and 4 is in keeping with enamel mineralisation during a dietary change (maternal calcium input while *in utero* changing to milk calcium input from nursing), which involves

older and younger enamel being collected together in a sample. As Balasse et al. (2001) explains, 'because our sampling method cross-cuts growth layers, superposition creates a zone where the two signals are mixed'. They were discussing dentine, but, as discussed in Chapter 3, enamel mineralisation also progresses in a manner that creates a mixing of earlier and later formed enamel.

The only noticeable difference between the upper and lower graphs of Figure 6.6 involves the weaning transition seen on M1 between samples 7 and 8 and on M2 between samples 1 and 4. The weaning transition pattern made by the $\delta^{44/42}$Ca values of these M1 and M2 samples shares a closer resemblance in the lower graph. The lower graph also plots the M2 weaning transition nearer to the weaning transition on M1, but it is unclear at this time if the alignment in the upper or lower graph is more correct. The lower graph, far from being proof that calcium accretion follows the pattern of a reduced rate of enamel formation in the cervical 25% of molars, as indicated by ^{13}C and ^{18}O analysis, does lend some support, based on the better alignment of $\delta^{44/42}$Ca values, that calcium accretion during enamel formation does slow in the cervical portion of the molars.

In the end, it appears that the graphs in Figure 6.6 do show a similar pattern to the Figure 5.12 model. The model brought together the information from Reynard et al. (2010) regarding isotope offsets between diet–soft tissue (0.17 ± 0.25‰), which potentially could indicate an *in utero* signal, as well as the isotope offsets between diet–milk determined by Chu et al. (2006) and my work discussed in Section 5.2 (0.54‰). While in Figure 6.6 the M1 and M2 sample $\delta^{44/42}$Ca values do not directly overlap on the graph, the overall pattern is similar to the model, with a diet (post-weaning diet)–soft tissue (*in utero*) isotope offset of 0.17‰, which transitions to a more depleted ^{44}Ca signal that, while only being 0.29‰ instead of 0.54‰, reflects a diet (post-weaning diet)–milk (nursing and weaning) isotope offset. Lastly the graph and the model both show the milk signal transitioning to the post-weaning grass diet. While not exact, strong similarities between Figure 5.12 and Figure 6.6

are very encouraging and indicate that the incorporation of dietary calcium does accurately reflect diet and the sheep life events of nursing, weaning and weaning completion.

6.4 Conclusion

Between the Hoy (Orkney) and Highfield Farm (Yorkshire) sheep, the first modern reference set of $\delta^{44/42}$Ca values from bulk and incremental molar enamel samples for addressing questions related to sheep nursing and weaning has been created. The Hoy sheep could be used to address some of the questions generated by the Highfield Farm lamb enamel results and, while lacking adequate $\delta^{44/42}$Ca values from female sheep molar enamel, has produced an approach for organising and interpreting $\delta^{44/42}$Ca values from sheep with unknown life and dietary histories.

To that end, the research discussed in Chapter 7 presents the first application of what has been learned from the Highfield Farm and Hoy sheep enamel samples, to sheep (and a goat) from an archaeological site, the Neolithic site of Tell Abu Hureyra in Syria. The information gained from the analysis of the Highfield Farm and Hoy sheep has provided indicators of what to look for in archaeological data as well as how best to interpret them. Unlike the Highfield Farm and Hoy sheep, virtually nothing is known about the individual lives of the sheep from Tell Abu Hureyra, for example. This is obviously a limiting factor, but it was always the intention and part of the project design that the modern results would be used as a means of redressing this problem. The modern results have shown that both bulk and serial incremental enamel sample analysis are powerful tools for identifying the presence of milk in the diet of sheep. More specifically, it is possible to use isotope offsets between serial incremental sample $\delta^{44/42}$Ca values, in association with a dental development chronology, to identify periods of *in utero*, nursing, weaning and weaning completion. The technique of using $\delta^{44/42}$Ca values from modern sheep analyses was therefore applied to identify those periods in the lives of sheep from Tell Abu Hureyra. This was coupled with the use of ^{18}O analysis in order to establish the timing of sheep life

events better and to look for other evidence of dietary change through ^{13}C analysis. The approach of using calcium isotopes in dental enamel to investigate nursing and weaning in the archaeological record, if successful, will provide a new means of gaining access not only to information about the individual lives of ancient sheep, but also, with larger groups of animals analysed from a site, to information related to herd life and possibly even herd management by humans.

CHAPTER 7

PRE-POTTERY NEOLITHIC TELL ABU HUREYRA $\delta^{44/42}$Ca, δ^{18}O AND δ^{13}C SAMPLE RESULTS AND DISCUSSION

As discussed in previous chapters, dairying has been of pivotal importance to human development, but has always been elusive within the archaeological record. As such, the advent of dairying has always been of special interest to those studying where this is thought to have occurred, especially during the period of the Neolithic prior to the advent of pottery. The research carried out in this thesis is well placed to address this gap in archaeological knowledge and, to demonstrate its potential, sheep molars from the site of Tell Abu Hureyra, Syria, were analysed for $\delta^{44/42}$Ca, δ^{18}O and δ^{13}C values. This chapter sets out the archaeological background and context of the site of Tell Abu Hureyra, both historic and scientific. Tell Abu Hureyra has a history of human occupation beginning during the Epipalaeolithic–Neolithic transition, and continuing through a significant part of the Pre-Pottery Neolithic. It is also close to where sheep domestication is believed to have first occurred, and excavation has yielded sheep jaws and teeth from contexts dated concurrently with the earliest evidence of dairying.

To establish the background for the archaeological samples, this chapter begins with a brief account of how Tell Abu Hureyra and similar sites in the Near East were founded, grew and evolved during the Pre-Pottery Neolithic. Tell Abu Hureyra is a critical site for examining the development of herd animal domestication and the transition of people from hunting and gathering to agro-pastoralism. The domestication of sheep and goats was the essential first step in the development of dairying.

This chapter will also provide a background to the archaeological site of Tell Abu Hureyra and the source of the excavated material analysed. It will then discuss the incremental $\delta^{44/42}$Ca values for samples B29, B59 and B252, and what information can be discovered regarding the timing of nursing and weaning for these animals. Additionally,

$\delta^{18}O$ and $\delta^{13}C$ values for these samples will be discussed with regard to how these results aid in the interpretation of the $\delta^{44/42}Ca$ values.

7.1 Tell Abu Hureyra: site context within the Neolithic in the Near East

The human domestication of animals and plants during the Neolithic in the Near East, as a result of and/or evolving along with the creation of larger permanent settlements, led to new innovations and technological advancements. More complex human interactions, economies and ritual systems were also a product of the Neolithic and highlighted a fundamental shift in the way humans lived, reflecting a changing relationship between humans and their environment. Uncovering the origins of the first steps towards settled agriculturalism requires complicated and ongoing research. This is especially true when studying the very earliest periods of the Neolithic and during the pivotal Epipalaeolithic–Neolithic transition in the Near East. Humans are the global dominant species, for better or worse, and as a result nearly 7 billion people owe their way of life to these first farmers and the world they worked to modify.

The Epipalaeolithic and Neolithic in the Near East have been placed into different stages in the literature. Table 7.1 provides the dates of the Levantine periods, and Figure 7.1 presents key Epipalaeolithic and Neolithic sites shown within the Levantine dating chronology. The figure also includes climatic periods in the different regions during the Levantine periods and for the times the sites were occupied. The periods covered in the Levantine chronology are the Kebaran, the Geometric Kebaran, the Early Natufian, the Late Natufian, the Pre-Pottery Neolithic A (PPNA), the Pre-Pottery Neolithic B (PPNB) and the Pottery Neolithic (Kuijt and Goring-Morris 2002, Byrd 2005). PPNB is further broken down into early, middle, late and final/Pre-Pottery Neolithic C (Kuijt and Goring-Morris 2002). Dates for this region may also be discussed in the literature as broad stages rather than periods: Epipalaeolithic 1, Epipalaeolithic 2, Neolithic 1, Neolithic 2 and Neolithic 3 (Moore et al. 2000, Byrd 2005). The Neolithic part of Tell Abu Hureyra, also known as Tell

Abu Hureyra II, is the period from which the analysed samples originated and covers the whole of the Middle PPNB through to the Pottery Neolithic.

Table 7.1: Dates for Levantine periods and stages

	Uncalibrated and calibrated (cal.) dates before present (BP)
Levantine periods	
Kebaran	23,000–18,000
Geometric Kebaran	18,000–14,900/14,600
Early Natufian	14,900/14,600–12,500 (cal.)
Late Natufian	12,500 (cal.)–12,000 (cal.)/11,700
Pre-Pottery Neolithic A (PPNA)	12,000/11,700 (cal.)–10,650/10,500 (cal.)
Pre-Pottery Neolithic B (PPNB), early	10,500 (cal.)–10,100 (cal.)
Pre-Pottery Neolithic B (PPNB), middle	10,100 (cal.)–9250 (cal.)
Pre-Pottery Neolithic B (PPNB), late	9250 (cal.)–8700 (cal.)
Pre-Pottery Neolithic B (PPNB), final	8600 (cal.)–8250 (cal.)
Pottery Neolithic	8250 (cal.)–7800 (cal.)
Levantine stages	
Epipalaeolithic 1	20,000–12,500
Epipalaeolithic 2	12,500–10,000
Neolithic 1	10,000–9600
Neolithic 2	9000–8000
Neolithic 3	8000–7000

The earliest dates for Tell Abu Hureyra (Tell Abu Hureyra I) place it within the Epipalaeolithic–Neolithic transition. The Epipalaeolithic–Neolithic transition first began approximately 10,500–9200 cal. Before Christ (BC) in the Taurus–Zagros (southern Turkey, Iraq and Iran) and Levant (Israel, southern Syria, south-east Turkey, Lebanon, Jordon, the Sinai Peninsula of Egypt and the State of Palestine) regions of the Near East (the Fertile Crescent), where humans began to exploit their environment in new ways (Peters et al. 1999, Moore et al. 2000, Aurenche et al. 2001, Byrd 2005, Pedrosa et al. 2005, Asouti 2006, Starkovich and Stiner 2009). Before this time, during the Epipalaeolithic 1/Kebaran, Geometric Kebaran and Early Natufian periods, it is believed humans were largely mobile, lived in small familial groups and invested their efforts in collecting resources, especially in gathering plants and hunting animals for immediate or near

immediate consumption (Moore 1998, Moore et al. 2000, Byrd 2005). There is also evidence at this time (11,500 cal. BP) of the first cultivation of wild cereal plants, specifically rye and einkorn (Hillman 2000, Kuijt and Goring-Morris 2002, Zeder 2011).

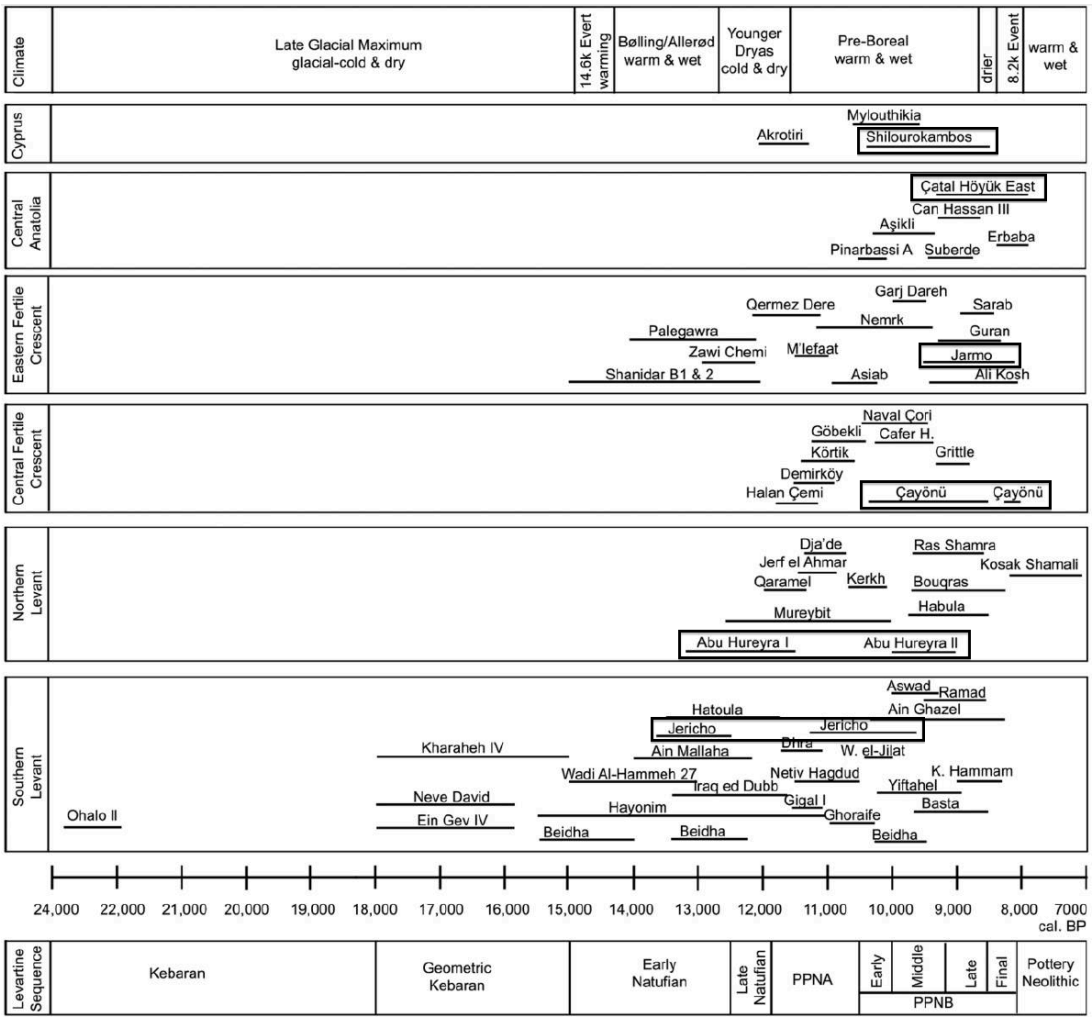

Figure 7.1: Timeline of Near East sites based on climatic conditions and Levantine chronology. Reproduced from Zeder (2011). The sites in boxes have been added to highlight the sites that appear in Figure 7.2.

The end of the Younger Dryas, a period marked by cold and drought (13,000 cal. BP–11,600 cal. BP), and the start of the Holocene's climatic optimum (11,000–7000 BP) during the PPNA, offered hunter-gatherers and early agriculturalists increased resources, and allowed for Neolithisation and the development of new subsistence strategies related to plant and animal domestication (Moore 1998, Severinghaus et al. 1998, Severinghaus and Brook 1999, Gupta 2004, van der Plicht et al. 2004, Pedrosa et al. 2005, Starkovich and

Stiner 2009, Zeder 2009). However, it is not clear whether humans began developing agriculture as a result of climate change or whether there were other pressures, such as population growth and food inadequacy (Starkovich and Stiner 2009).

One of these new human changes was sedentism, which probably began with seasonal hunter-gatherer camps becoming small permanent settlements during the Epipalaeolithic 2/Late Natufian period (Bar-Yosef 1998, Kuijt and Goring-Morris 2002, Byrd 2005). During the PPNA period, the first permanent food-producing communities began, although these first farmers still relied primarily on hunting and gathering (Bar-Yosef 1998, Hillman 2000, Moore et al. 2000, Byrd 2005, Pedrosa et al. 2005). A further change happened during the PPNB, with expanding domestication of wild plants and animals, a reduction of hunting and gathering, and the rise of the world's first villages, with people actively engaging in plant cultivation and herding livestock (Moore 1998, Moore et al. 2000, Kuijt and Goring-Morris 2002, Byrd 2005). This period corresponds with Tell Abu Hureyra II.

Genetic analysis of plant and animal remains from PPNA and early PPNB archaeological sites has provided new insight into the choices these first farmers were making in developing the first domesticates, and how these domesticates then spread throughout the Near East. It is believed the primary domestication of plants and animals occurred in the upper regions of the Euphrates and Tigris rivers, and involved crops of einkorn, pulses and emmer, and the domestication of sheep, goats, pigs and cattle was roughly contemporary (Figure 7.2) (Hillman 2000, Zeder 2008, Zeder 2011). The process of domestication is no longer believed to have been undertaken by one particular culture or group of people, but with 'a pluralistic process with initial domestication of various crops and livestock occurring, sometimes multiple times in the same species, across the entire region' (Zeder 2011: S230).

It is also during the PPNB that Neolithic people began to extend their control to exploiting their early livestock domesticates, for purposes other than just meat and skins

(Vigne and Helmer 2007). This expansion was dubbed the 'secondary products revolution' (Sherratt 1983). Sherratt's model (1981, 1983) examined the transition from using animal domesticates as a source of meat, bone and hides (primary products), to include products repeatedly produced by a living animal (secondary products), including milk, wool, manure and traction (the use of animal strength to pull or carry). Sheep provide two of the three key 'secondary products': wool and milk.

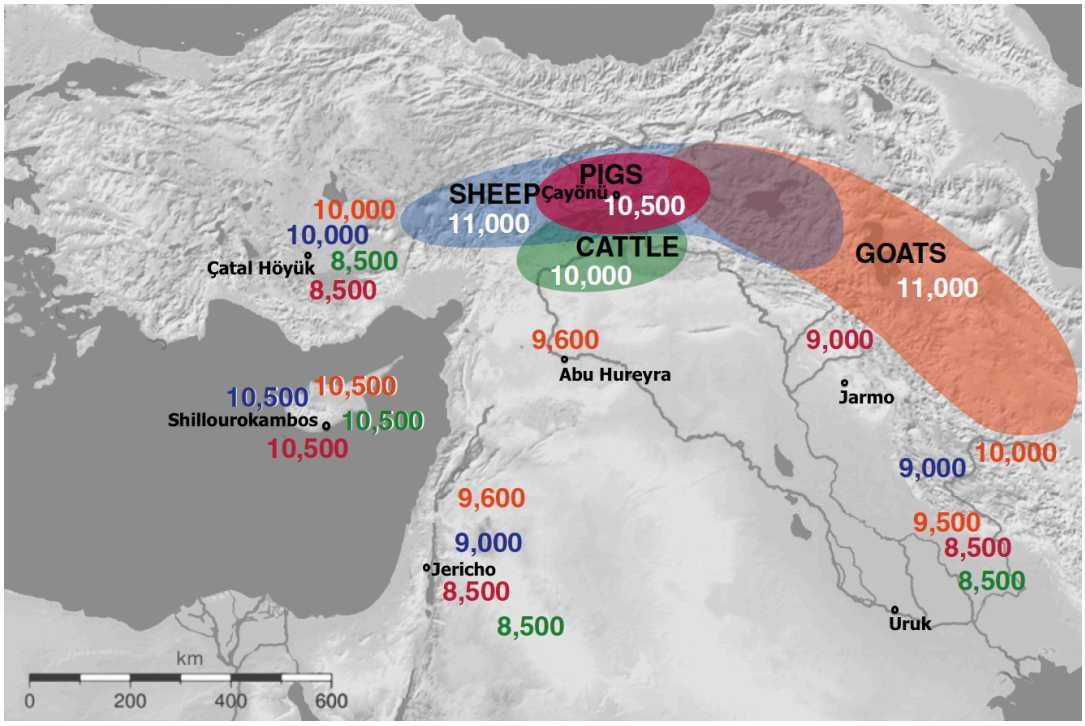

Figure 7.2: Regions and timing of livestock domestication in the Near East. The regions where it is believed livestock species where first domesticated (shaded) and the dates and directions for dispersal are shown. The dates are calibrated BP. The sites cover the late Epipalaeolithic and Pre-Pottery Neolithic and most are shown in the Levantine chronology in Figure 7.1. Image reproduced from Zeder (2008), with the addition of the locations of prominent Neolithic settlements.

As previously stated in Chapter 1, the first domesticates (~11,000 BP) were morphologically like their forbearers (Legge and Rowley-Conwy 2000) and kept similar life cycles (time of rut, breeding, lambing and nursing/weaning) (Santiago-Moreno et al. 2000, Garel et al. 2005, Santiago-Moreno et al. 2005a, Pipia et al. 2008). The closest modern link to these first domesticates is the 'wild' mouflon (*Ovis orientalis*), native to the Taurus–Zagros region (Pedrosa et al. 2005, Zeder 2008, Zeder 2011). It is believed there were at least three distinct episodes of sheep domestication in the Taurus–Zagros region,

based on mitochondrial (mt)DNA results, with maternal lineages producing three different population sub-species of mouflon (Hiendleder et al. 2002, Pedrosa et al. 2005, Tapio et al. 2006, Zeder 2011). The regions where these domestication events probably took place, and the sub-species associated with those regions, are: Iran (*Ovis gmelini isphahanica*, *Ovis gmelini laristanica* and *Ovis gmelini gmelini*), Turkey (*Ovis gmelini gmelini*) and central Anatolia (*Ovis gmelini anatolica)* (Hiendleder et al. 2002, Pedrosa et al. 2005). Tell Abu Hureyra lies approximately 200 km south of the epicentre of sheep domestication; therefore the PPNB people at the site would have been some of the first in the world to be involved in sheep domestication and management. It is therefore a useful site to look for evidence of early dairying.

7.2 Tell Abu Hureyra: the site

Prior to the formation of Lake al-Assad, the site of Tell Abu Hureyra sat on a low terrace in the Euphrates River valley and was well situated, being close to the river while also safe from flooding (Moore et al. 2000). Tell Abu Hureyra was also at the confluence of two environmental zones: the wet valley bottom to the north, and the dry steppes to the south (Moore et al. 2000). Tell Abu Hureyra was actually two successive settlements: Tell Abu Hureyra I (late Early Natufian through to early PPNA) and Tell Abu Hureyra II (middle PPNB through to the early Pottery Neolithic) (Moore 2000a, Moore 2000b, Kuijt and Goring-Morris 2002, Byrd 2005).

Tell Abu Hureyra was excavated in the autumn of 1972 and late summer–late autumn of 1973. It was a salvage dig in advance of flooding, because of the damming of the Euphrates River south-east of the site (Moore et al. 2000). Figure 7.3 shows the contour plan of Tell Abu Hureyra mound, with the boundaries and locations of excavation trenches marked.

The samples for this research were taken from three sets of jaws excavated from Trench E. Trench E was mainly from Abu Hureyra II (10,400 cal./9400 to at least 7800

cal./7000 BP; cal./uncalibrated dates for the Abu Hureyra II settlement), with dates from earlier than 8300 (8270 ± 100 BP, OXA-2167, and 8330 ± 100 BP, OXA-2168) to later than 7000 BP. There appears to have been occupation around Trench E beginning during the Abu Hureyra I settlement, around 9860 ± 220 BP (OXA-6996) and continuing until 8700 ± 240 BP (OXA-6995). The Abu Hureyra II settlement expanded around 8300 BP and the deposits covering the period from 8700 ± 240 BP (Abu Hureyra I) were then removed by the inhabitants. Overall, the area around Trench E was in continuous occupation from around 10,000 to 7000 BP.

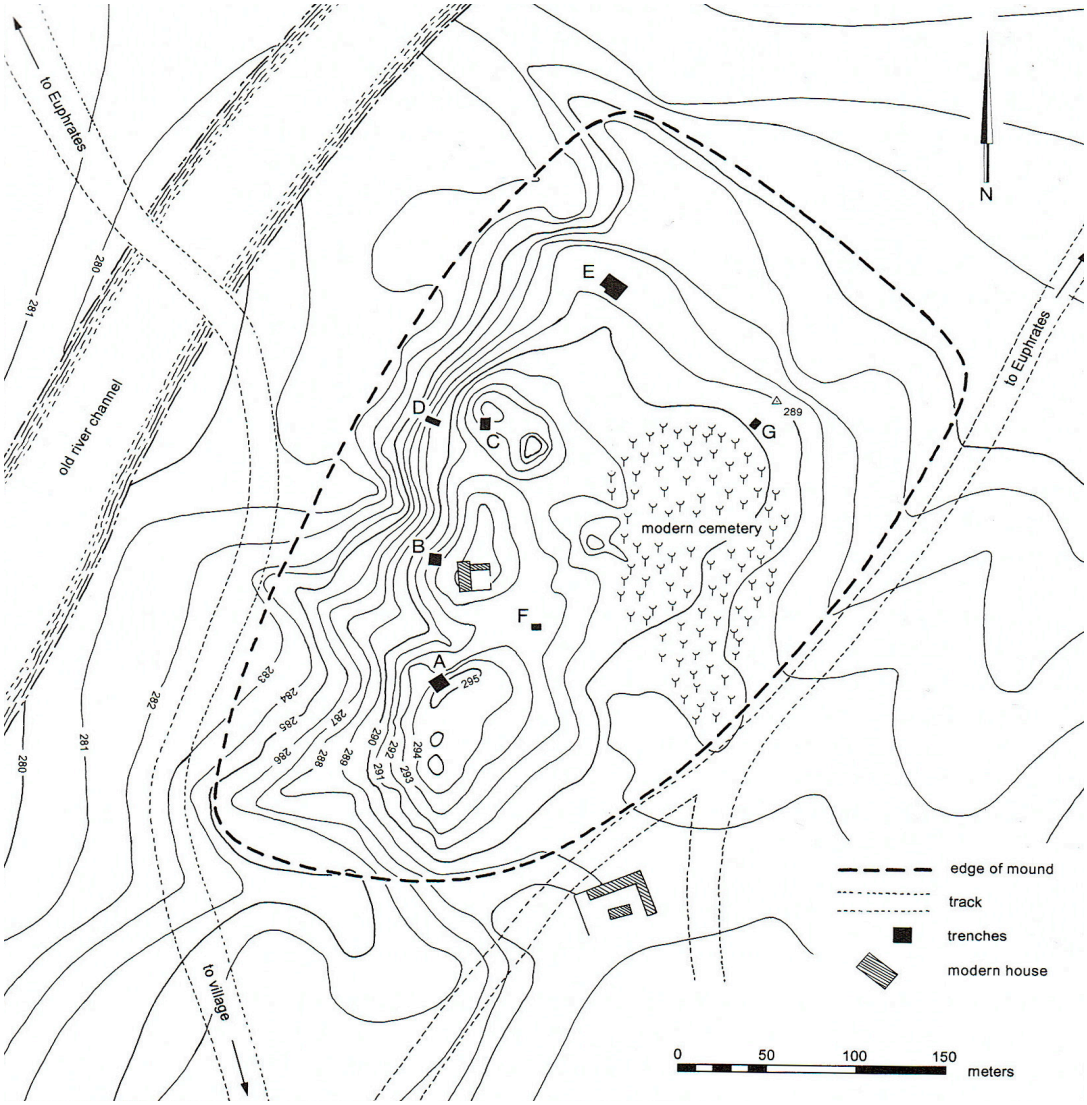

Figure 7.3: Image of Tell Abu Hureyra mound, including mound contours, boundaries and proximity to the Euphrates River channel. Included are modern additions to the mound and the excavation trenches. Reproduced from Moore et al. (2000: 34).

Trench E probably includes the very end of the middle PPNB (10,100–9250 cal. BP), the whole of the late and final PPNB (9250–8700 cal. BP and 8600–8250 cal. BP) and the Pottery Neolithic (8250 cal.–7800 cal. BP) (Kuijt and Goring-Morris 2002, Byrd 2005, Zeder 2011). Evidence of milk residues has been found in the remains of cooking vessels in the Near East during the late PPNB (~7000 BC) (Evershed et al. 2008) and, even though the ultimate goal is to use calcium isotopes in sheep enamel to investigate further back in the PPNB, this trench provided jaws that were of comparable age as the pottery vessel evidence.

The identifying codes for each of the three jaws from which molars were extracted for analysis were: (1) AH72, E, 33, B59, (2) AH73, E4, 20, B29 and (3) AH73, E, 160, B252, referred to hereafter as B59, B29 and B252, respectively. Sample B59 comes from a context dated (uncalibrated) to 8020 ± 100 BP (BM-1724R), B29 from a context dated to 7310 ± 120 BP (OXA-1232) and B252 from a context dated 5350 ± 400 BP (OX TL 196j) (Moore 2000a). Anthony Legge identified the jaws as probably sheep rather than goat. Jaws B29 and B59 had mandibular molars and B252 had maxillary molars. Legge also provided an age at death for the animals: sheep B59 and B252 were between the ages of 12 and 24 months and B29 was between the ages of 21 and 24 months. Zooarchaeology by Mass Spectrometry (ZooMS) analysis confirmed that B29 and B59 were sheep, but found that B252 was actually goat. Although not intentional, having both goat and sheep in the study proved to be interesting, as goats have an equally long history of being used as dairy animals.

7.3 Tell Abu Hureyra bulk enamel sample $\delta^{44/42}$Ca values: results and discussion

7.3.1 Abu Hureyra bulk enamel sample $\delta^{44/42}$Ca values: results

The bulk $\delta^{44/42}$Ca values from Tell Abu Hureyra sheep B59 and B29 as well as goat B252 are given in Table G.11 (for all tables prefixed G, see Appendix G). Figure 7.4 shows the

bulk enamel molar results. The first molar (M1) from B59 had the lowest $\delta^{44/42}$Ca value of the three molars, with the second molar (M2) having the second lowest and the third molar (M3) the highest $\delta^{44/42}$Ca value of the three. However, the M1, M2 and M3 bulk enamel samples from B59 were found to have no significant difference (ANOVA, $F_{2,5} = 5.39$, $P = 0.056$, Table F.47; for all tables prefixed F, see Appendix F).

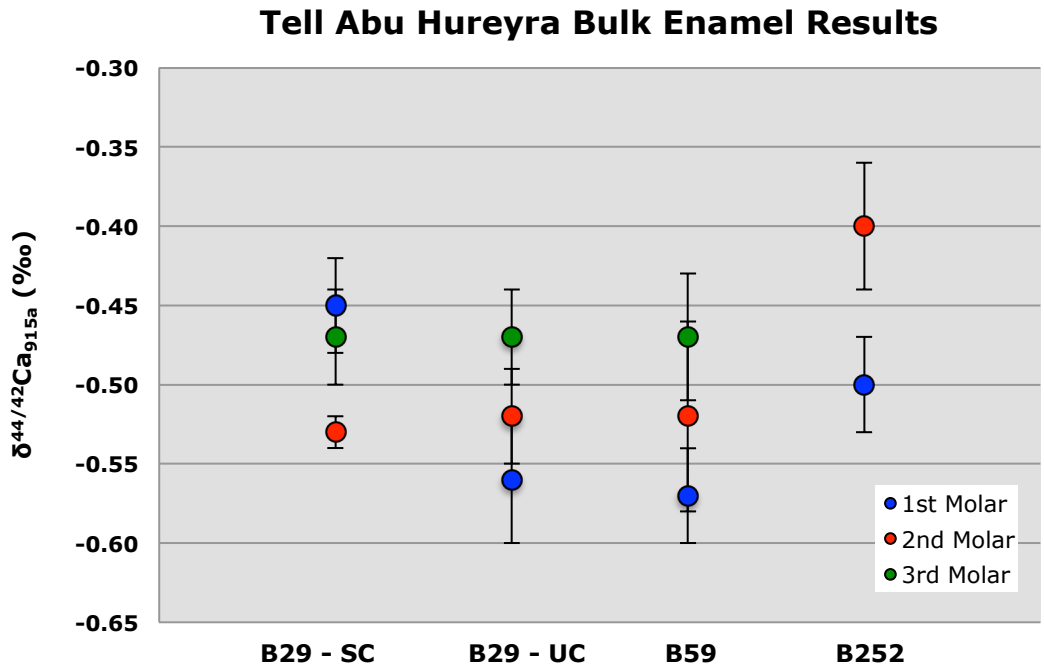

Figure 7.4: Mean M1, M2 and M3 $\delta^{44/42}$Ca values for sheep B59 and B29 as well as goat B252. Bulk samples from B29 were analysed twice. The first time was with a standard cleaning (SC) method and the second time was with an ultra cleaning (UC) method. The error bars are 1 standard deviation (SD) from the mean.

B29 had issues with glue contamination and as a result it was necessary for M1, M2 and M3 to be analysed twice. The methods for glue removal used prior to the first (standard clean, SC) and second (ultra clean, UC) analyses are discussed in Chapter 4. The first time that the molars from B29 (SC) were analysed, it was found that M2 had the lowest $\delta^{44/42}$Ca value of the three molars, with M3 having the second lowest and M1 the highest $\delta^{44/42}$Ca value. B29 (SC) had a significant difference between M1, M2 and M3 (ANOVA, $F_{2,6} = 6.00$, $P = 0.037$, Table F.48). The second time B29 (UC) was analysed, the results for M1, M2 and M3 changed to M1 to having the lowest $\delta^{44/42}$Ca value and M3 the highest, with M2 between. There was a significant difference between M1, M2 and M3 from B29 (UC)

(ANOVA, $F_{2, 11} = 6.83$, $P = 0.012$, Table F.49). M1 for B252 had the lower $\delta^{44/42}$Ca value of the two molars and it was found that there was a significant difference between them (independent samples *t* test: $t_3 = -3.45$, $P = 0.041$, Table F.50). Based on the results for B29 (UC), B59 and B252, as well as the bulk results from the Hoy sheep it is unlikely that the bulk results for B29 (SC) are correct.

7.3.2 Abu Hureyra bulk enamel sample $\delta^{44/42}$Ca values: discussion

The bulk enamel samples from the Tell Abu Hureyra animals cannot be compared to each other to the same degree as the sheep from Highfield Farm and Hoy because of their unknown life and dietary histories. However, all of these animals were excavated from the same archaeological site and it is likely that they were local animals, although this cannot be said with certainty. B59, B29 (UC) and B252 follow the pattern seen with Hoy sheep 545, 549, 552, 799, 800 and 2914, of having M1 with the lowest $\delta^{44/42}$Ca value of the three, M2 the second lowest and M3 the highest.

B59, unlike B29 (UC) and B252, showed no significant difference between molars. This may have implications regarding nursing length or the quantity of plant foods in B59's diet. The Hoy sheep lived on unimproved pasture and were weaned naturally by their mothers at around 5 months old, and had a significant difference between their M1s and other molars. It may be that B59 was weaned earlier, either by human management or because of poor nutrition (ewe lactation ceased earlier and/or the lamb consumed more plant foods earlier) (Hass 1990, Réale et al. 1999). The fact that B59 lived to be between the ages of 12 and 24 months indicates that it survived the approximate 8 weeks immediately after birth when the rumen microflora is being established in preparation for plant consumption (Lyford 1988) and when milk is providing nutrition. A lamb's immune system health is also dependent on the length of time a lamb nurses, with modern lambs that are weaned earlier than 4 months of age having a greater vulnerability to parasites as well as having less maturation weight gain (Shaw et al. 1995). This does not mean that B59 was not weaned, either by its mother or by humans, earlier than 4 months, just that its

health would have been more vulnerable than lambs that nursed longer. It is assumed that, by living to 12–24 months, B59 was a healthy animal slaughtered for food, but this cannot be said with any certainty. In the end it is likely that B59 nursed until at least 2 months of age and was weaned earlier than 5 months. Based on the modern Highfield Farm and Hoy sheep results, the analysis of incremental enamel samples from B59, B29 (UC) and B252 were used to try to provide additional clues as to when these animals were weaned.

7.4 Tell Abu Hureyra incremental enamel sample $\delta^{44/42}$Ca, δ^{18}O and δ^{18}C values: results and discussion

7.4.1 Tell Abu Hureyra incremental enamel sample $\delta^{44/42}$Ca, δ^{18}O and δ^{18}C values: results

For sheep B59 (Table G.12), the M1 incremental samples had a variation of 0.21‰ between the minimum and maximum $\delta^{44/42}$Ca values (Figure 7.5). The isotope offset for M2 was smaller than M1, being 0.13‰ (Figure 7.6). The difference in $\delta^{44/42}$Ca values between the minimum and maximum in M1 and in M2 was greater than the measurement precision, 0.04–0.08‰ (1 SD) (see Chapter 4).

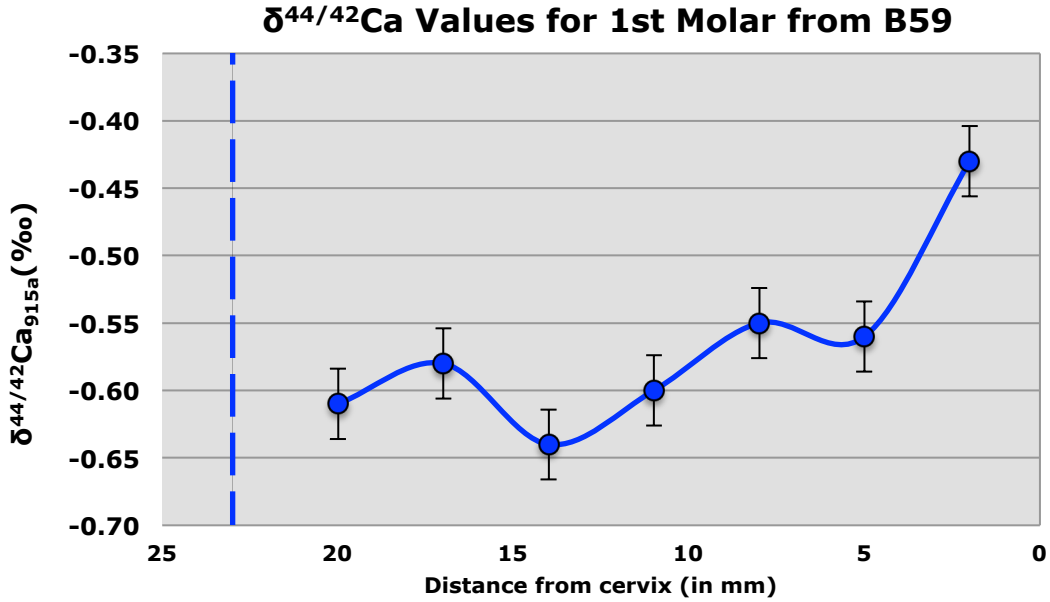

Figure 7.5: A graph of the $\delta^{44/42}$Ca values from incremental samples taken from sheep B59's M1. Sampling was started 3 mm below the apex of the crown (dashed line) and subsequently taken every 3 mm going down the length of the tooth. The last sample was taken 2 mm above the cervix (cemento-enamel junction). Temporal chronology progresses from left to right. Error bars are 1 standard error (SE) from the mean.

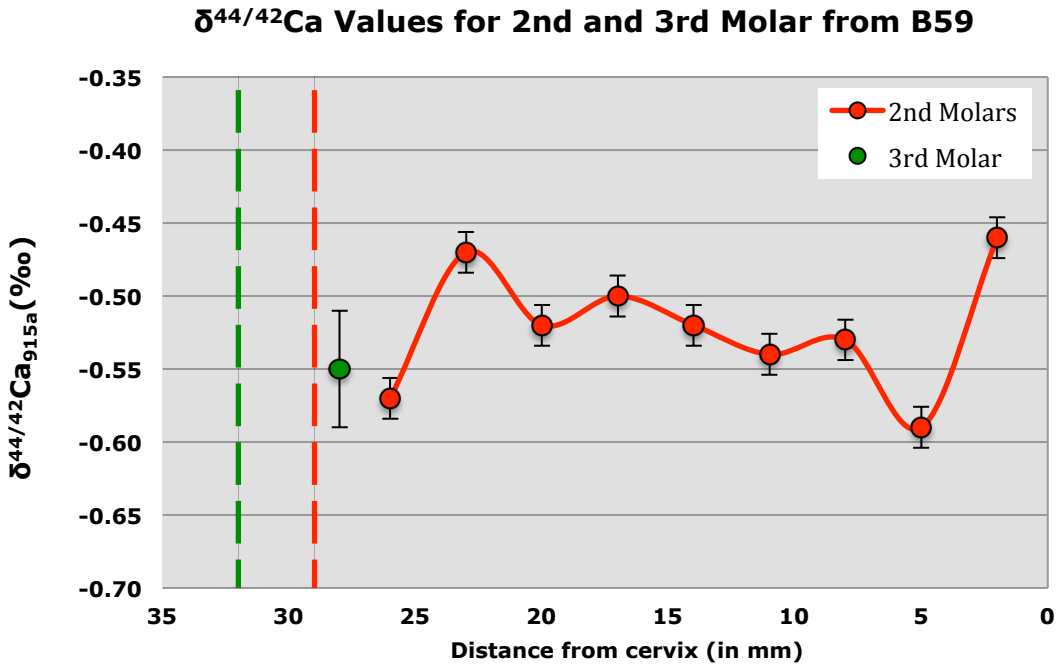

Figure 7.6: A graph of the $\delta^{44/42}$Ca values from incremental samples taken from sheep B59's M2 and M3. Sampling was started 3 mm and 4 mm, respectively, below the apex of the crown (dashed lines) and subsequently taken every 3 mm going down the length of M2. The last sample for M2 was taken 2 mm above the cervix (cemento-enamel junction). Temporal chronology progresses from left to right. Error bars are 1 SE from the mean.

For sheep B29 (Tables G.13 and G.14) there were significant issues with the results. The molars had large amounts of glue on the teeth and the enamel samples were cleaned two different ways and then analysed after each cleaning. Chapter 4 discusses the cleaning methods. However, neither method was able to remove the glue satisfactorily and, although the second method seemed to 'fix' the problem in the bulk $\delta^{44/42}$Ca values, when the incremental samples were analysed it was clear from the noisy and random nature of the signal that the samples were still probably being affected by remnants of the glue.

B252, like B59, did not have any issues with glue contamination, but it did provide a surprise. It was discovered from ZooMS that B252 was a goat and not a sheep (Table G.15). M1 for goat B252 had incremental samples with a variation of 0.11‰ between the minimum and maximum $\delta^{44/42}$Ca values (Figure 7.7). The isotope offset for M2 was slightly smaller than M1, 0.09‰ (Figure 7.8). The difference in $\delta^{44/42}$Ca values between the minimum and maximum in M1 and in M2 was greater than the measurement precision, 0.04–0.08‰ (1 SD) (see Chapter 4).

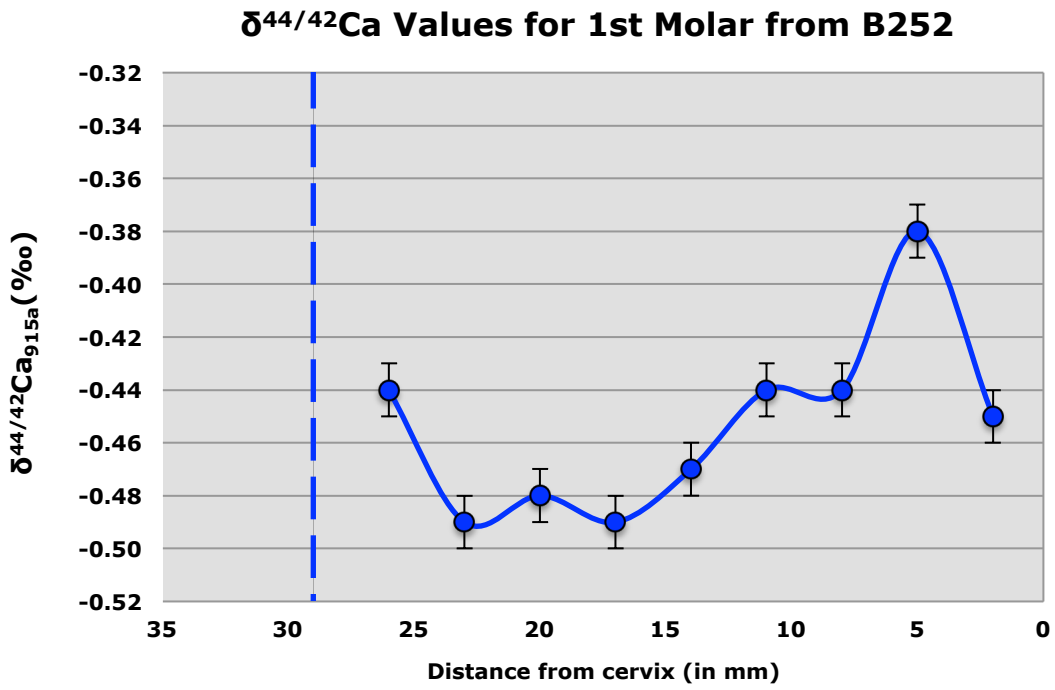

Figure 7.7: A graph of the $\delta^{44/42}$Ca values from incremental samples taken from goat B252's M1. Sampling was started 3 mm below the apex of the crown (dashed line) and subsequently taken every 3 mm going down the length of the tooth. The last sample was taken 2 mm above the cervix. Temporal chronology progresses from left to right. Error bars are 1 SE from the mean.

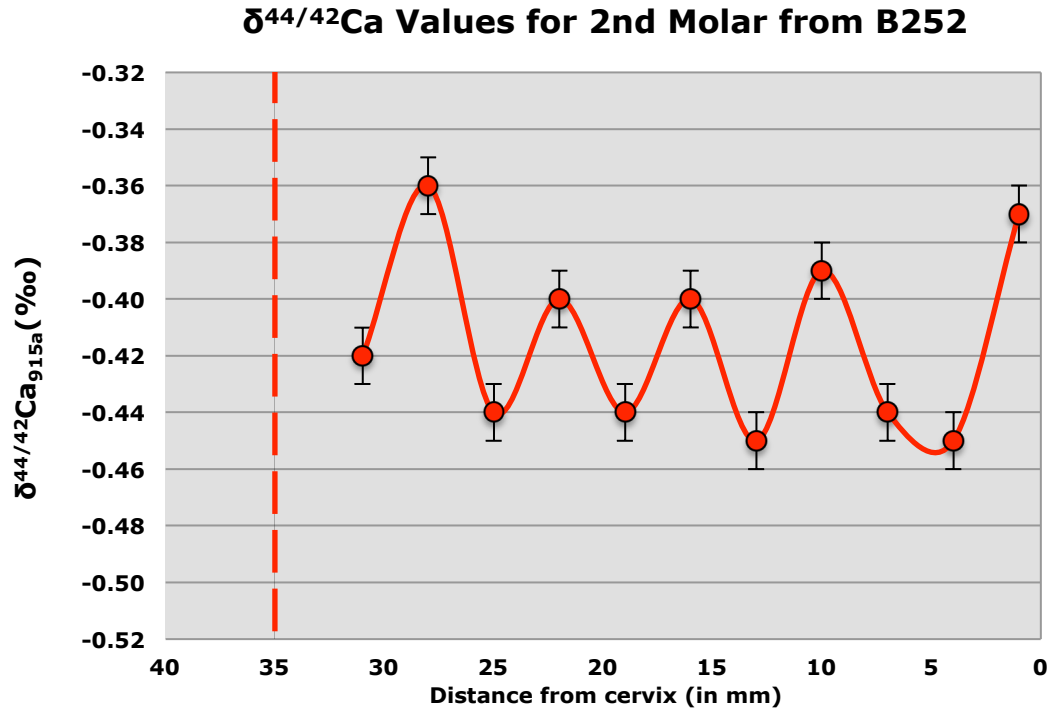

Figure 7.8: A graph of the $\delta^{44/42}$Ca values from incremental samples taken from goat B252's M2. Sampling was started 4 mm below the apex of the crown (dashed line) and subsequently taken every 3 mm going down the length of M2. The last sample for M2 was taken 1 mm above the cervix. Temporal chronology progresses from left to right. Error bars are 1 SE from the mean.

The $\delta^{18}O$ and $\delta^{13}C$ values for incremental samples from B59 are given in Table G.16 and for B252 in Table G.17. Tell Abu Hureyra molar enamel showed an overall C_3 diet based on a ^{13}C-enrichment of 12–14.6‰ between dietary input and enamel (Krueger and Sullivan 1984, Lee-Thorp and van der Merwe 1987, Cerling and Harris 1999, Passey et al. 2005). The difference between the M1 minimum and maximum $\delta^{13}C$ values for B59 and B252 was 2.1‰ and 2.3‰, respectively (Figure 7.9). The difference between the M2 minimum and maximum $\delta^{13}C$ values for B59 and B252 was 4.0‰ and 3.26‰, respectively (Figure 7.10). Figure 7.11 plots the $\delta^{18}O$ values for the M2s from B59 and B252. The M2s for B59 had a difference between the minimum and maximum $\delta^{18}O$ values of 5.5‰, and a difference in $\delta^{18}O$ values of 3.8‰ was seen for HF30.

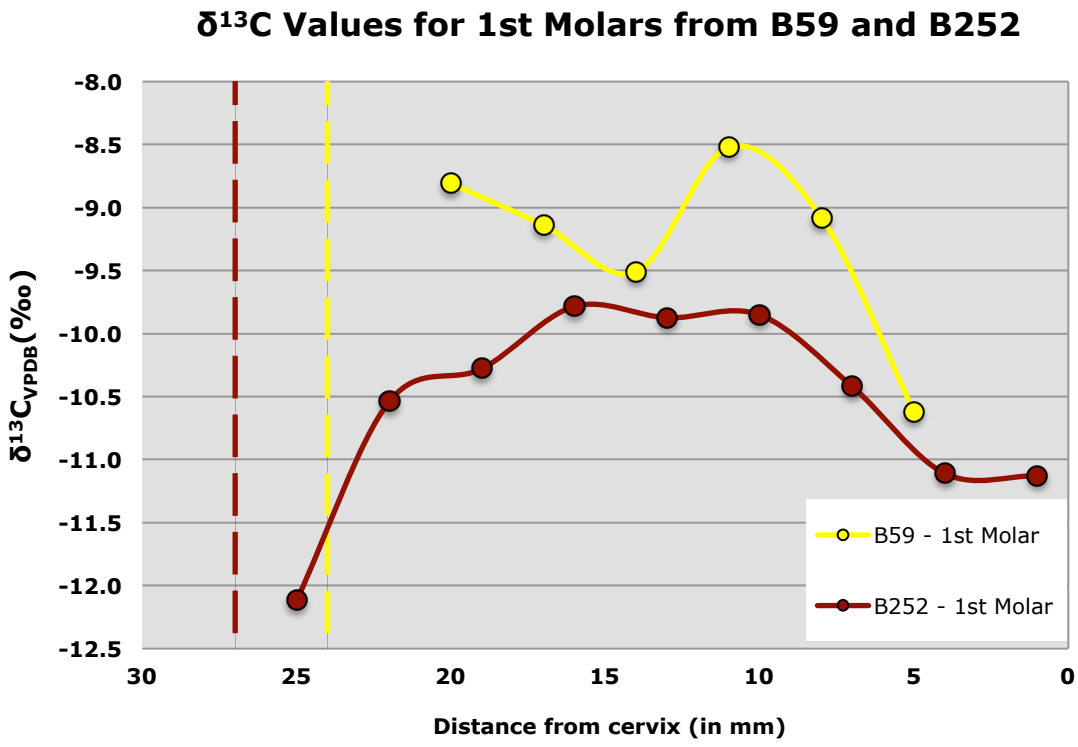

Figure 7.9: M1 incremental $\delta^{13}C$ values for B59 and B252. Samples were collected at 3-mm intervals, with the first sample taken 4 mm and 2 mm, respectively, below the apex of the crown. The crown heights are marked with dashed lines (B59 – yellow and B252 – rust). Temporal chronology progresses from left to right.

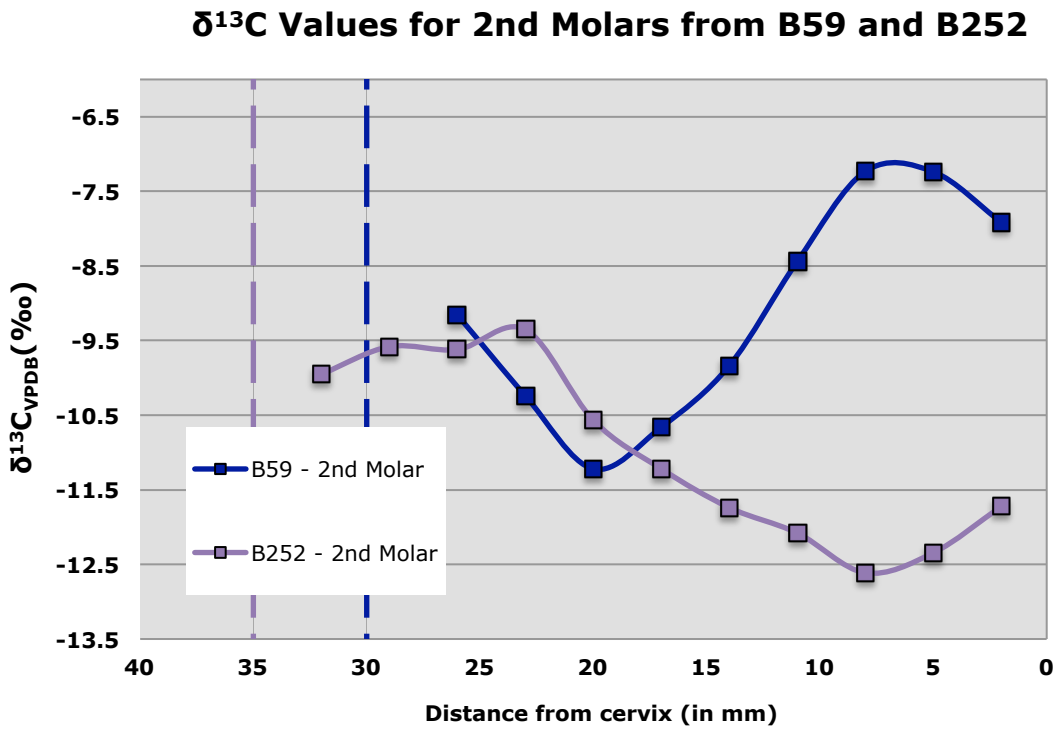

Figure 7.10: M2 (single sample taken 3 mm below crown) incremental $\delta^{13}C$ values for B59 and B252. Samples were collected at 3-mm intervals, with the first sample taken 3 mm below the apex of the crown. The crown heights are marked with dashed lines (B59 – navy blue and B252 – lavender). Temporal chronology progresses from left to right.

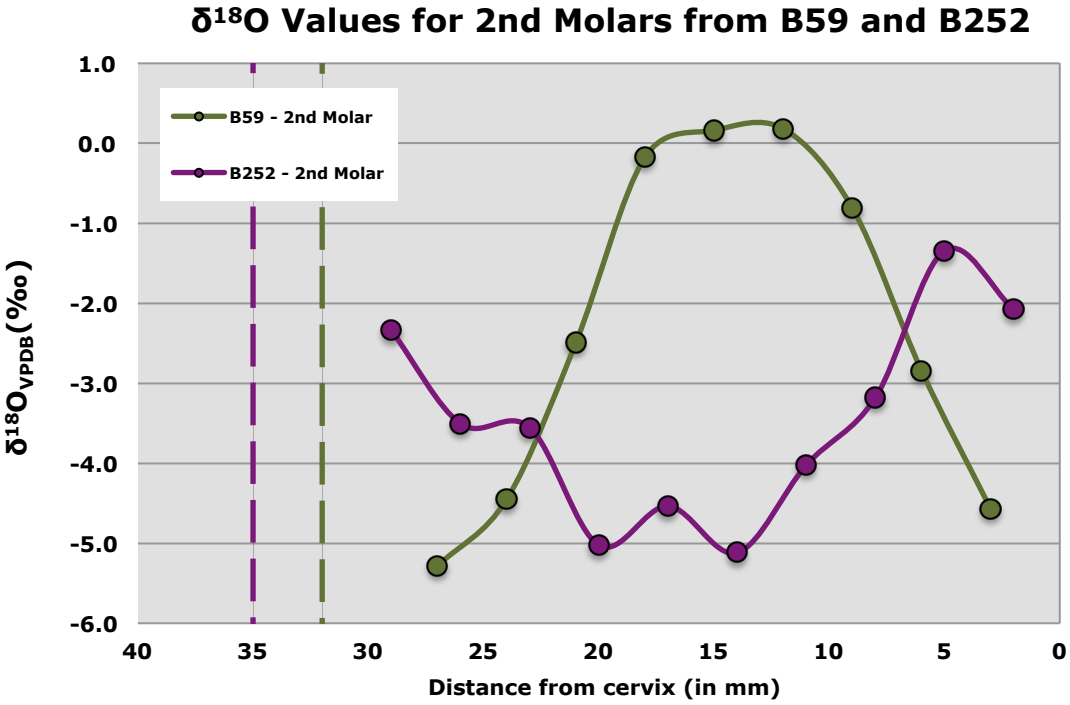

Figure 7.11: M2 incremental $\delta^{18}O$ results for B59 and B252. Samples were collected at 3-mm intervals, with the first sample taken 3 mm below the apex of the crown. The crown heights are marked with dashed lines (B59 – green and B252 – purple). Temporal chronology progresses from left to right.

7.4.2 Tell Abu Hureyra incremental enamel sample $\delta^{44/42}$Ca, δ^{18}O and δ^{18}C values: discussion

The sheep B59 came from the oldest context of all the samples analysed, dated to (uncalibrated) 8020 ± 100 BP (BM-1724R). It appears to have been deposited near the end of the late Preboreal climatic event (11,600 cal. BP–8200 cal. BP) and the whole of the brief 8.2-ka climatic event (8200 cal. BP–8040 cal. BP) (Severinghaus et al. 1998, Thomas et al. 2007, Maher et al. 2011). The Preboreal is characterised by warmer and wetter conditions after the cold, arid Younger Dryas (13,000 cal. BP–11,600 cal. BP) (Severinghaus et al. 1998, Severinghaus and Brook 1999, van der Plicht et al. 2004). The 8.2-ka climatic event was a return to colder and arid conditions. This is relevant because if life is difficult for ewes to such a degree that their nutrition suffers, then lactation lengths may be shorter than 5 months. Having an idea of climatic conditions is therefore important for interpreting $\delta^{44/42}$Ca values and accounting for all the reasons why a sheep may appear to have been weaned early.

As there was no life history information for the Tell Abu Hureyra sheep and goat, the interpretation of $\delta^{44/42}$Ca values was coupled with oxygen isotopes in order to try and establish the timing of sheep life events, specifically birth. The sources of δ^{18}O isotope offsets in sheep dental enamel are, as discussed in Chapter 3, Section 3.2.1, meteoric water and temperature. Lower δ^{18}O values are associated with winter precipitation, while summer, due to enrichment of ^{18}O from evaporation, is associated with higher δ^{18}O values (Gat 1996). Other influences on temperature and precipitation are proximity to large bodies of water, latitude, land mass size and altitude (Gat 1996, Barbour et al. 2005, Henton et al. 2010).

The temperature range for the region near Tell Abu Hureyra is 10–40°C (Moore et al. 2000), with clear seasonal temperature fluctuation (Figure 7.12). Sheep raised in regions with seasonal temperature fluctuation, in middle and high latitudes, have enamel δ^{18}O values that fluctuate in accordance with seasonal shifts in temperature (Gat 1996, Barbour et al. 2005). Sheep born in Europe during the spring, and that have lived long enough to

develop the enamel fully on their second molars, should have enamel serial incremental sample $\delta^{18}O$ values that form a sinusoidal wave.

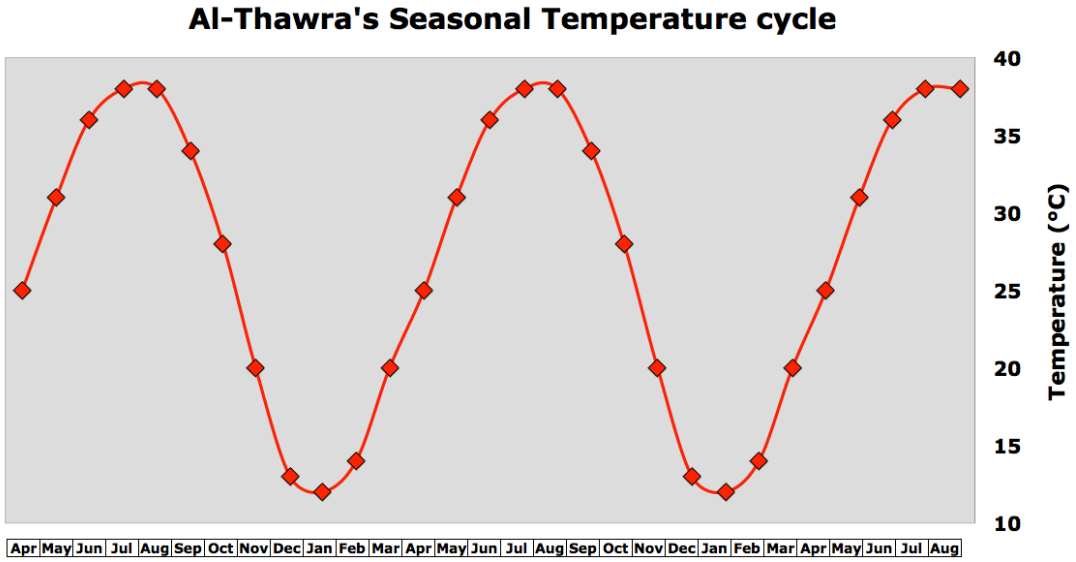

Figure 7.12: The mean monthly high temperatures from Al-Thawra, Syria. Al-Thawra is the closest modern community to the site of Tell Abu Hureyra.

The most accurate way to assess season of birth using oxygen isotopes is by applying a four-parameter, cosine formula-based, model. This model, which is detailed in Balasse et al. (2012b) and is further described and utilised in Tornero et al. (2013) and Balasse et al. (2013), is suitable for high crown molars that develop over approximately a single year. Sheep second molars have crowns that form over 12–14 months (Weinreb and Sharav 1964, Milhaud and Nezit 1991, Upex 2009, Zazzo et al. 2010, Upex and Dobney 2012), depending on breed, and therefore the enamel should include a full year of seasonally influenced $\delta^{18}O$ values.

The four-parameter model calculates the best fit for the $\delta^{18}O$ molar dataset, through the method of least squares, by determining the combined variation of period (X), amplitude (A), delay (Xo or x_o) and mean (M). Period (X) refers to the length of the tooth crown that has formed over approximately a single year. Amplitude (A) and mean (M) are determined by using the minimum and maximum $\delta^{18}O$ values within a second molar dataset, such that A = (max. – min.)/2 and M = (max. + min.)/2. Both A and M are reported in ‰. Delay (Xo or x_o) is also reported in mm and is dependent on when in the year the molar

began to grow and is determined by identifying the distance from the cemento-enamel junction to where the maximum $\delta^{18}O$ value is located on the crown.

Figures 7.13 and 7.14 show the application of the four-parameter model to the $\delta^{18}O$ datasets from B59 and B252, and include the results of the determination of the best fit for the combined variation of X, A, Xo and M. Additionally, Pearson's correlation coefficient (*R*) was calculated during modelling. *R* was found to be 0.995 for B59 and 0.938 for B252. If *R* is found to be ≥0.91 then there is a good fit between measured and modelled datasets.

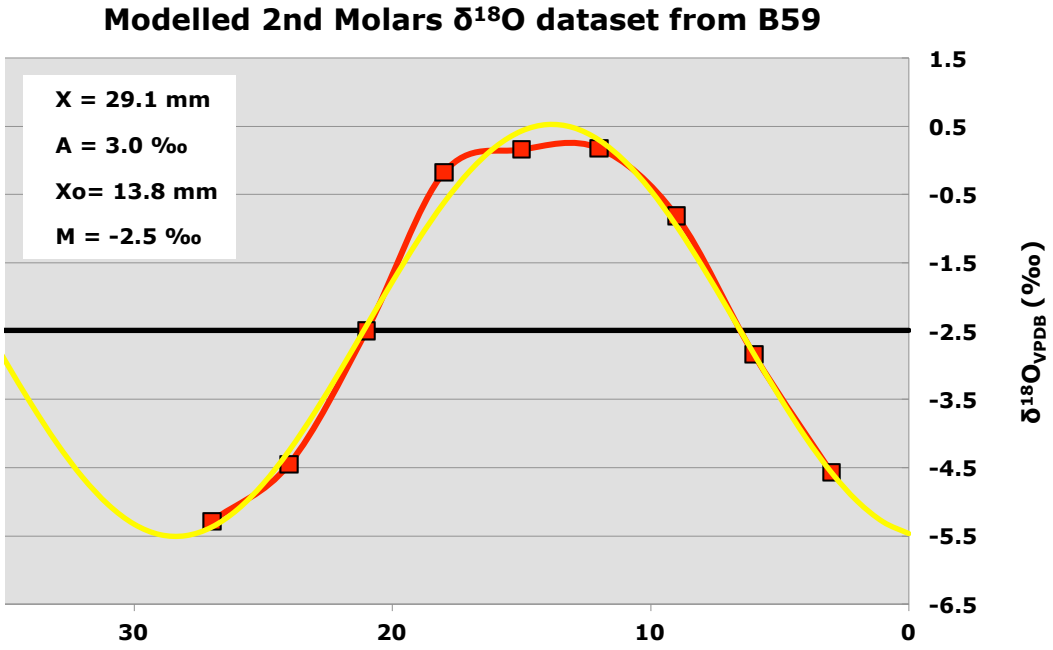

Figure 7.13: The result from the application of the four-parameter model to B59's second molar $\delta^{18}O$ dataset, which produced the calculated best fit (least squares) for the combined variation of X, A, Xo and M.

Once the results from calculating the best fit are known, the season of birth is determined by first normalising the location on the crown where the highest $\delta^{18}O$ value was measured (Xo) by the period (X), such that Xo/X. Then the normalised result from the sample with an unknown birth season is compared with the modern reference collections discussed in Blaise and Balasse (2011) and Balasse et al. (2012b) (Table 7.2).

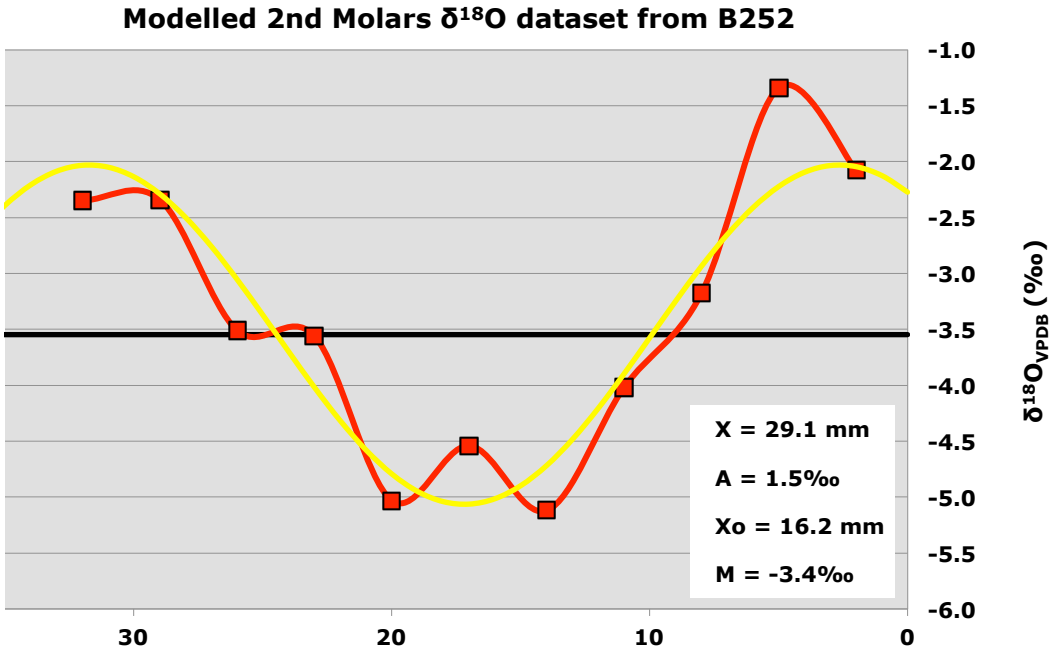

Figure 7.14: The result from the application of the four-parameter model to B252's second molar $\delta^{18}O$ dataset, which produced the calculated best fit (least squares) for the combined variation of X, A, Xo and M.

Figure 7.15 contains the results of the normalisation (Xo/X) of the sheep in the reference collections along with B59 and B252. The sheep in Figure 7.15 that were born in January–February are Ovis 0026 and Ovis 1216. Sheep Ovis 1511, Ovis 0522 and Ovis 0562 were born in September. All of the sheep from the ROU group were born in late April–early May. B59 and B252 have Xo/X values that fall between the sheep born in April–May and September. Both B59 and B252 appear to have been born mid–late summer, possibly June–July for B59 and July–August for B252, although those specific months cannot be substantiated.

Table 7.2: Results for the normalisation (Xo/X) of the modern reference sheep from Blaise and Balasse (2011) (Ovis) and Balasse et al. (2012b) (ROU)

	Jan–Feb	Jan–Feb	Sept	Sept	Sept	April–May	April–May
Sheep	Ovis 0026	Ovis 1216	Ovis 0522	Ovis 0562	Ovis 1511	ROU 01	ROU 04
Xo/X	0.12	0.14	0.75	0.76	0.64	0.28	0.19
	April–May	**April–May**	**April–May**	**April–May**	**April–May**	**April–May**	**April–May**
Sheep	ROU 06	ROU 07	ROU 08	ROU 09	ROU 11	ROU 16	ROU 17
Xo/X	0.31	0.33	0.31	0.23	0.28	0.28	0.25

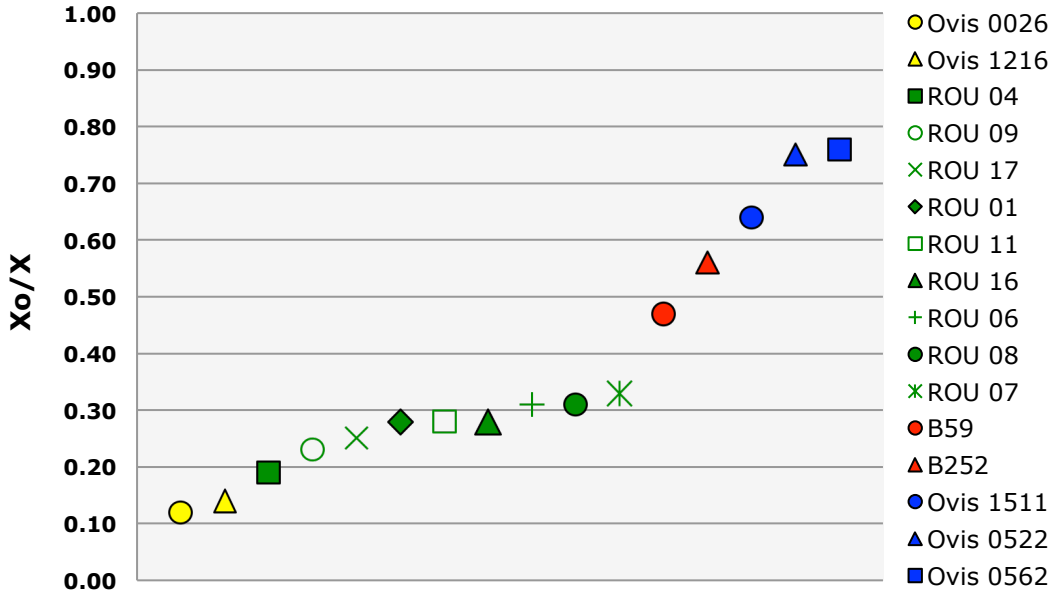

Figure 7.15: The results from the normalisation of sheep from modern reference populations as well as B59 (sheep) and B252 (goat). The Ovis sheep are from Blaise and Balasse (2011) and the ROU sheep are from Balasse et al. (2012b).

Mid- to late summer for the period of birth for B252 is unexpected, but not unreasonable. Wild goat (Alpine ibex and chamois) tend to give birth in the spring through to early summer (Blaise and Balasse 2011). Modern domestic goats, however, as a whole do not have a fixed season(s) of birth (Amoah et al. 1996, Bosso et al. 2007, Vargas et al. 2007). B252 was excavated from a context dated to 5350 ± 400 BP (Moore 2000a), which places it in the Levantian late Chalcolithic–early Bronze Age (Banning 2007). Goats were first domesticated ~11,000 BP and it is not unreasonable to consider that, after 5000–6000 years of possible human management, a goat could be born in mid- to late summer rather than spring to early summer.

B59 was excavated from a context dated to 8020 ± 100 BP (Moore 2000a). This places B59 within the transition between the final PPNB and the Pottery Neolithic as well as during the 8.2-ka climatic event (8200 cal. BP–8040 cal. BP), which was a colder period with arid conditions (Severinghaus et al. 1998, Thomas et al. 2007, Maher et al. 2011). Genetic and morphometric evidence has sheep domestication occurring during the late PPNA and early PPNB in the northern Levant (Peters et al. 1999, Hiendleder et al. 2002, Pedrosa et al. 2005, Tapio et al. 2006, Zeder 2011). B59 would therefore probably be a

domesticated sheep and, although unlikely this early in the Neolithic, possibly subject to human influence of birth timing.

Mid- to late summer for the period of birth for B59 is somewhat unexpected because, unlike goat, sheep tend to have a narrow window for lambing, which typically occurs in the spring (depending on latitude). April is a common month for modern sheep to lamb in northern latitudes, unless they are specifically managed to reproduce out of season. The mouflon, the closest genetic relative to the wild progenitor of modern sheep breeds, tends to lamb in April (Garel et al. 2005). Henton et al. (2010), using their own model for identifying birth season using $\delta^{18}O$ values, placed most of the births at Neolithic Çatalhöyük, central Anatolia, in late spring. If the lambing season for the upper Levant and central Anatolia is April–May then B59's birth being in mid- to late summer is outside the norm. If, however, the PPNB sheep of Tell Abu Hureyra lambed on the same schedule as the Bedouin flocks in the region around the site, December and January (Legge and Rowley-Conwy 2000), then that would make B59's birth very late indeed.

Although highly speculative, sheep B59 was dated to a context that coincides with a period marked by climatic change from warmer, wetter conditions towards colder, more arid conditions, which could potentially have influenced B59's lambing timing. It has been found that late-born lambs have low survival rates and may also be an indication of ewes going into rut in poor body condition, resulting in a late conception (Festa-Bianchet 1988). The uncertainty regarding B59's birth will be reduced or eliminated once Xo/X results are determined for a modern reference collection(s) of sheep from the northern Levant.

Establishing birth season using $\delta^{18}O$ datasets proved in the end to be of the most use for interpreting the calcium isotopes for the causes for possible early weaning. Figures 7.16 and 7.17 contain graphs of $\delta^{44/42}Ca$ values for Hoy sheep 545, sheep B59 and goat B252 as well as $\delta^{13}C$ values for both B59 and B252. All of the graphs in the figures have serial incremental sample isotope values associated with sheep life events and Upex and Dobney (2012)'s dental chronology. B59's and B252's $\delta^{44/42}Ca$ values (middle graphs) were compared with the $\delta^{44/42}Ca$ values of Hoy sheep 545 (upper graphs). $\delta^{13}C$ values from

B59 and B252 are shown in the lower graph of each figure and were used to assess changes within the C_3 diet that may be linked to nursing and weaning.

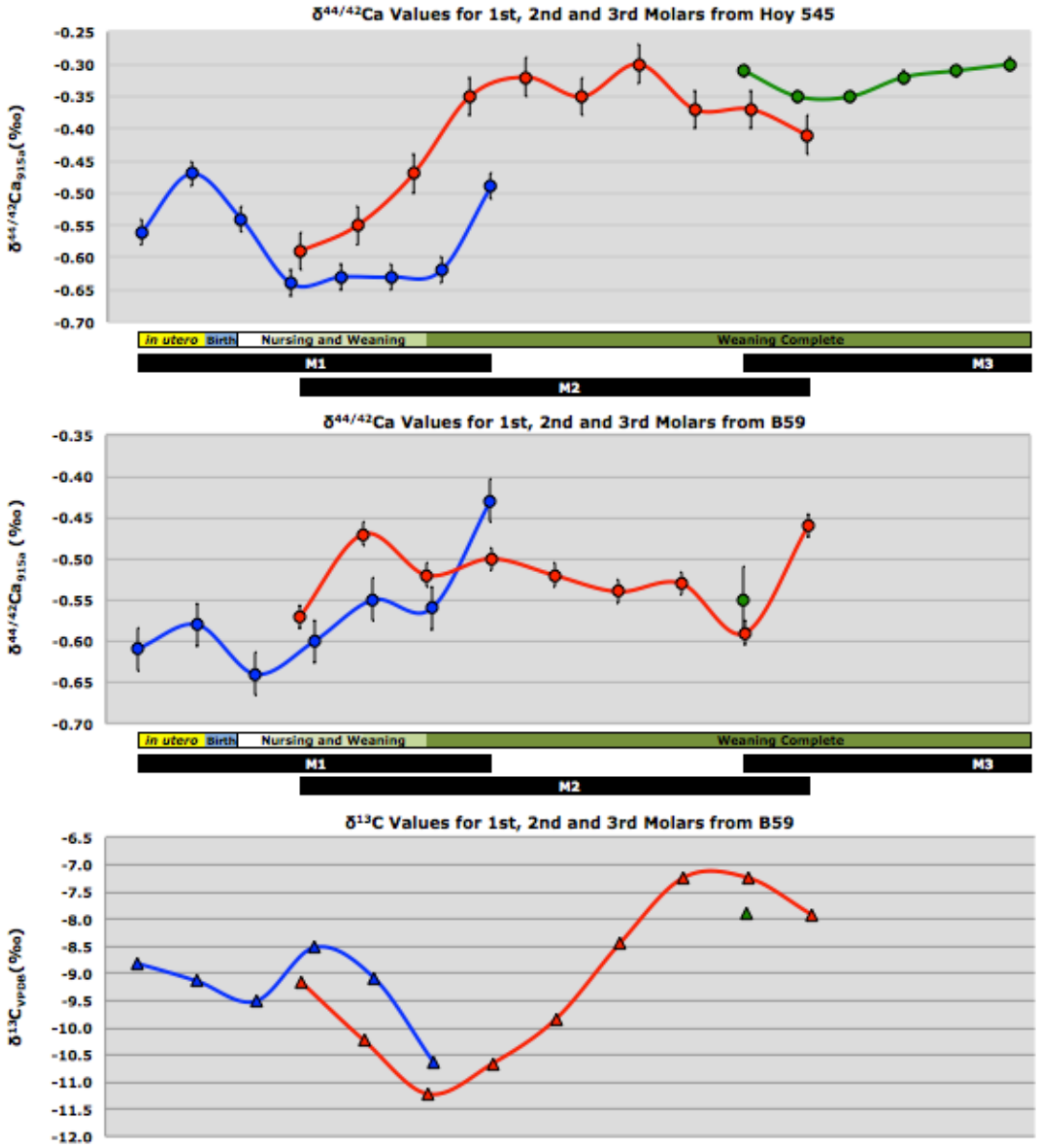

Figure 7.16: M1 (blue), M2 (red) and M3 (green) incremental enamel sample $\delta^{44/42}$Ca values for Hoy sheep 545 and Tell Abu Hureyra sheep B59 are shown in relation to each other, and B59's δ^{13}C values. All isotope values are plotted in relation to sheep life events and Upex and Dobney (2012)'s dental chronology. There was not enough enamel available from the last sample taken above the cemento-enamel junction of B59's M1 for δ^{13}C analysis. As a result the M1 δ^{13}C values are short by one sample on the graph.

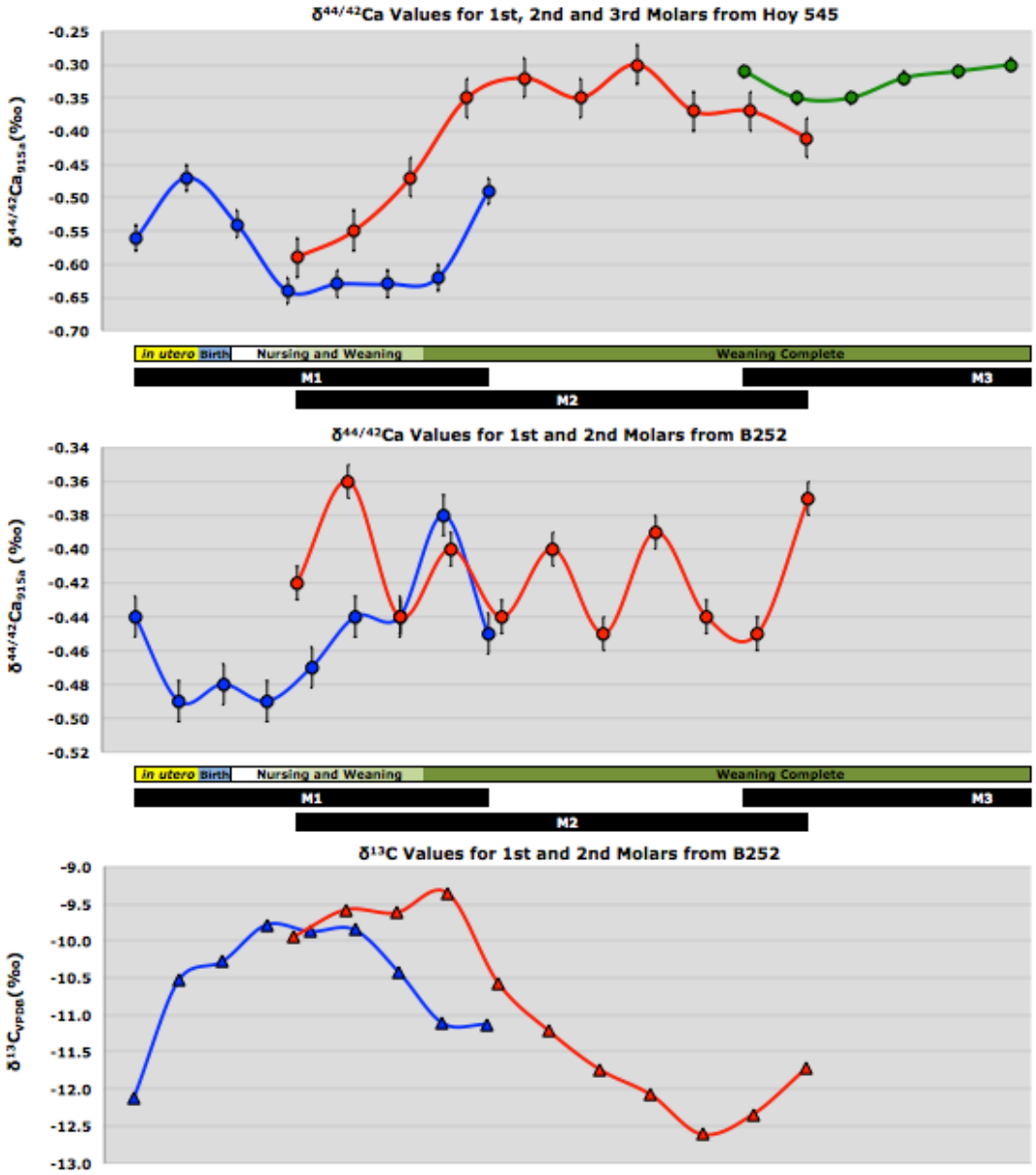

Figure 7.17: M1 (blue), M2 (red) and M3 (green) incremental enamel sample $\delta^{44/42}$Ca values for Hoy sheep 545 and Tell Abu Hureyra goat B252 are shown in relation to each other, and B252's δ^{13}C values. All isotope values are plotted in relation to sheep life events and Upex and Dobney (2012)'s dental chronology.

The carbon results for B59 and B252 were not particularly helpful for looking for evidence of dietary change associated with birth, nursing and weaning. The diets for both animals were C_3, although some samples were in the low C_3 range. As discussed earlier in the section, oxygen results for B59 indicated a mid- to late summer birth for this sheep, and as a result it appears that the highest and lowest δ^{13}C values correspond to changes in temperature and possibly water stress. B252, also born in the mid- to late summer, may

show some indications of more subtle influences on the C_3 diet, namely some similarities in the pattern of $\delta^{13}C$ values as Highfield Farm lamb HF29. However, decreases in temperature affect C_3 plants and result in higher $\delta^{13}C$ values (Körner et al. 1988, O'Leary 1988, Tieszen 1991). Additionally, increasing summer water stress or aridity affect C_3 plants by raising their $\delta^{13}C$ values (Johnson et al. 1989, Heaton 1999). The temperature range for the region near Tell Abu Hureyra is 10–40°C, with periods in the hottest part of the summer that are arid (Moore et al. 2000). It is likely that the environmental factors of aridity and decreasing temperatures after a mid- to late summer birth had the greatest effect on B252's enamel $\delta^{13}C$ values.

The interpretation of the patterns of incremental enamel sample $\delta^{44/42}Ca$ values for B59 and B252 have proven to be both interesting and exciting. B59 seems to clearly show in M1 a transition from calcium input that, I believe, is from the sheep's time *in utero* to the consumption of milk (nursing) after birth, which is seen as the lowest $\delta^{44/42}Ca$ value on the graph. B252 seems to have lost the *in utero* signal on the M1, probably through wear, but like B59 does show part of the transition between *in utero* and birth/nursing followed by lowest $\delta^{44/42}Ca$ values on the graph, likely due to milk consumption. The lowest $\delta^{44/42}Ca$ values on the M1 for both B59 and B252 are then followed by a transition of progressively higher $\delta^{44/42}Ca$ values that probably reflect the weaning transition. This same pattern was seen in all of the modern M1s from male sheep. That does not necessarily mean that B59 or B252 are males, it is just that these archaeological M1s seem to be reflecting the general pattern that was observed in the modern samples from sheep with known life histories. The most exciting part is that the main source of difference observed between the M1s of modern male sheep and B59 and B252 is in the area of the plotted incremental results that is linked to nursing and weaning. If in fact the enrichment in ^{44}Ca that is seen in the enamel near the apex of the crown is reflecting calcium input while the animals were *in utero*, and birth is located within the transition between the *in utero* signal and nursing, then it might be possible to begin to formulate a method for determining nursing length and weaning

timing. More research is obviously needed, but the identification of birth is important for future analysis.

When comparing B59 with the naturally weaned Hoy sheep 545, it was observed that the M1 seemed to show a very short period of nursing, with a seemingly rapid weaning transition. It would be tempting to say that this animal may have been used for dairying because it seems to have been weaned both early and abruptly. I think it is more likely that the sheep and its mother, due to a late lambing, had less time to prepare for winter and that the lamb only nursed for the first 2 months and until B59's rumen microflora was established. It has been observed in wild sheep and lightly managed modern breeds that ewes will cease lactation early in order to protect their own health in the lead-up to winter (Hass 1990, Réale et al. 1999). As a result I think it is more likely that this sheep was weaned early not as part of herd management by humans, but as a result of a late lambing, which may be linked in some way to nutritional stress due to climatic change. This is highly speculative, but good to keep in mind for future work.

M2 from B59 is interesting and puzzling. It seems that B59 might be able to communicate information about the sheep beyond weaning. Once weaning was complete, the Hoy sheep enamel $\delta^{44/42}$Ca values reflected the plant diet of the Hoy pastures and the results remained fairly static. B59's samples show more diversity of $\delta^{44/42}$Ca values in the M2s compared with the modern samples. It would be tempting to say that the $\delta^{44/42}$Ca values that are observed in the M2 are due to herding, but too many variables are unknown at this time. In order to investigate properly the potential of using $\delta^{44/42}$Ca values in sheep M2s and M3s to identify dietary changes linked to herding it would first be necessary to establish the soil and plant food $\delta^{44/42}$Ca values within a geographic region. Geologic samples (various rocks, soils and freshwaters) do not have a significant mean difference, but do have a range of 0.5–1.0‰ (Chapter 2, Section 2.5). This range is on par with the largest isotope offset recorded between $\delta^{44/42}Ca_{diet\text{-}milk}$, 0.62‰ (Chu et al. 2006). There is therefore potential for soil to create appreciable differences between plant $\delta^{44/42}$Ca values,

and in fact in an experiment it has been found that the 'amplitude of the Ca isotopic fractionation within plant organs is highly dependent on the composition of the nutritive solution' (Cobert et al. 2011: 5467). Additionally, it has been found in alpine plants and hardwood forests that there is fractionation between soil and plant roots (Page et al. 2008, Hindshaw et al. 2013) as well as species-specific differences between the amount of fractionation that occurs between roots and leaves (Hindshaw et al. 2013). Therefore, soil $\delta^{44/42}$Ca values and the fractionation differences between plant species have the potential to create plant-based isotope offsets that could be recorded in M2 and M3 enamel during mineralisation. However, due to do the attenuation of the calcium isotope signal, sheep would need to spend enough time (months) grazing in one location before moving to a new one with different soil $\delta^{44/42}$Ca values before a change would be recorded in the enamel. The same would be true for changes between plant types. This makes it unlikely that evidence of mobile herding would be recorded in sheep enamel.

B252's results demonstrate that goats also have the potential to provide information about nursing and weaning through calcium isotope analysis of dental enamel. It appears that B252 had a period of nursing that is more in keeping with that seen with Hoy 545. The weaning transition is similar, but there is a nice overlap of what may be a common dietary change seen in the last three samples of the M1 above the cervix, which is mirrored in the first three samples below the apex of the crown of the M2. Overlaps such as this will help determine the offsets seen between the enamel in one tooth and another. Unlike B59, however, B252's M2 has a pattern of change that is unlikely to be due to dietary input of calcium. The changes between the samples are too large and frequent to be easily acceptable, even taking the influence of soil and plant calcium isotope offsets into consideration. The results of B252's M2 are also unlikely to be due to analytical errors because there is a careful protocol for assessing the validity of results during each step of the analysis. It may be, although not visible on M2, that there was some glue contamination. The incremental sample results from B29 were not included in the thesis because of glue

contamination and the affect the glue had on B29's $\delta^{44/42}$Ca values. Even after using the ultra cleaning method for glue removal (Chapter 4), B29's results remained noisy and random in nature. Glue contamination seems to be the most logical explanation for B252's M2 results.

7.5 Conclusion

Based on the research detailed in this thesis, it is my recommendation that samples from sheep include milk, horn, bone and dental enamel as well as plant dietary samples. Bone and horn would be important because there are still so many unknowns regarding calcium isotope cycling in sheep, and other mammal bodies. The investigation of calcium isotopes in sheep mineralised tissue can only benefit from having samples that diversify, complement and augment the current collection. Milk and plant food samples provide the critical frame of reference needed for interpreting mineralised tissue samples. It would also be good in the future to consider collecting soil samples along with plant foods in order to examine the differences in $\delta^{44/42}$Ca values between plant types growing in the same soil and between plants and soils within a region.

The main focus, however, should be on sheep molar dental enamel. The molars that are critical for investigating nursing, weaning and weaning completion are the first and second molars (from the same animal). Neither molar is able to communicate the full story, but together all stages of a sheep's early life are included. The third molar, or at least the upper portion of the third molar, as long as the enamel is fully mineralised, is helpful for confirming the plant diet $\delta^{44/42}$Ca values associated with weaning completion on the second molar. It would therefore be useful to collect bulk and serial incremental samples from first, second and third molars. Bulk samples can provide information about nursing, weaning and weaning completion at a broad level. Serial incremental sampling of enamel provides better resolution of the details. The resolution could be enhanced by increasing the number of samples collected from the crown of the first and second molars by reducing the distance

between the incremental samples from 3 mm to 2 mm. However, reducing the sample distance further would be impractical at this time because calcium isotope analysis requires a significant investment in time for both column chemistry and analysing samples on an MC-ICP-MS, and analysis using a MC-ICP-MS is expensive. For these reasons it is important, if working with a modest, fixed budget and a set window of time for analysis, to balance how closely individual molars and individual sheep are investigated with the need to investigate as many sheep as possible.

When selecting sheep molars for analysis it is important to collect molars from individuals that have fully developed first and second molars but that do not have extensive crown wear. At between 12 and 18 months of age a sheep's second molar crown comes into its full height, just as it also comes into wear (Balasse et al. 2012b). Upex and Dobney (2012) place Shetland sheep second molar completion at around 14 months of age, which further narrows the range to 14–18 months. There may be difficulty in acquiring molars that are from that narrow age range, especially from archaeological assemblages. This makes it likely that molars will be from sheep older than 18 months who will have lost some crown height to wear. This may not be an issue if the wear is confined to within the *in utero* signal on the first molar and the important transition from *in utero* to birth/nursing is preserved. However, if some part of the *in utero* to birth/nursing transition is lost to wear, it would not be possible to quantify nursing length and weaning timing. Hoy sheep 545 was 26 months of age at death and there were still $\delta^{44/42}$Ca values indicative of an *in utero* signal in the first molar. Hoy sheep 799 was 31 months of age at death and the $\delta^{44/42}$Ca values indicative of an *in utero* signal had been lost, probably through wear, although at least part of the *in utero* to birth/nursing transition remained. As a result the ideal sheep age range for collecting samples for calcium isotope analysis is 14–26 months.

After all the analysis I have undertaken, I am convinced that calcium isotope analysis of dental enamel from herbivores has great potential to access key information regarding nursing and weaning, and I also feel it may help address other questions related to herd management and diet. It is an isotope that, at this time, benefits from being combined

with carbon and oxygen isotope analyses. Inclusion of nitrogen isotope analysis of molar dentine would be an especially welcome addition, based on nitrogen's ability to also provide information about nursing and weaning. Oxygen isotopes analysis and the establishment of season of birth is an important part of being able to best interpret calcium isotopes results because the birth season can affect both lactation and weaning length. Carbon appeared to be of more help with sheep from more temperate climates, but it is certainly worth collecting, along with oxygen isotopes, no matter what the geographic origin of the molars. As much as I am pleased with the final results of this research project, I am well aware that there is still a significant amount that is not known or not well understood. In my concluding chapter I will discuss what I feel has been achieved, what new questions have been generated and how my future plans are designed to tackle those questions.

CHAPTER 8

CONCLUSION

In the introduction of this thesis, I presented a list of questions that framed the initial goals of my research. The results that are presented in Chapters 5, 6 and 7 have provided many answers, and those that have not been answered will be addressed in the work that is intended for the future. The questions presented in Chapter 1 are repeated here, with a discussion of the answers that have or have not yet been provided.

8.1 Modern sheep samples

1. Are ewe milk samples depleted in ^{44}Ca relative to the diet?

Yes, as discussed in Chapter 5, milk samples are depleted in ^{44}Ca relative to diet, by an isotope offset of ~0.5‰. Not only that, but it has been seen that ewes living in the same environment and with access to the same diet and milk have isotope ratios that remain consistent between ewes as well as from one year to the next. This also confirms that the results seen by Chu et al. (2006) are correct.

2. Does the enamel have a calcium isotope content with ratios reflecting milk and plant consumption?

Yes, both the modern Highfield Farm and Loft and White Hamars Grazing Project sheep had enamel that reflected milk and plant consumption. It was observed that the parts of the molar enamel developing during nursing had the lowest $\delta^{44/42}$Ca values. Also, in the parts of the molars that were developed and mineralised after weaning was complete (the cervix portion of the second molar and the whole of the third molar) it was possible to see the isotopically distinct (from milk) plant $\delta^{44/42}$Ca values.

3. If so, is there a weaning transition?

The weaning transition was observed through progressively higher $\delta^{44/42}$Ca values in the cervical half of the first molar and the apical half of the second molar.

4. If so, is the weaning transition abrupt or gradual?

This was something that was difficult to establish because the only sheep that were weaned early and abruptly were also immature and lacked fully developed and mineralised second molars. The Hoy sheep were naturally weaned and were not suitable, as a result, for answering this question. Additionally, it may be that the 5–6 months of time needed for full enamel mineralisation means that abrupt changes in diet will not be noticeable or will seem to appear gradual. This will have to be taken into consideration for the selection of future sheep and herds.

5. Is it possible to link calcium isotope ratio results from serial, segmented sampling to a dental chronology?

Yes and no. Yes, in that recently a dental development chronology has been made using the same herd of Shetland breed Loft and White Hamars Grazing Project (Hoy) sheep as analysed here for calcium isotopes. The Highfield Farm lambs were also of the Shetland breed and the Hoy dental development chronology would be applicable. However, other breeds of modern sheep may not have the same dental development chronology, and sheep from archaeological sites may also be different. In the end, the dental development chronology was used as a tool to help organise all of the variables (diet and life events such as nursing and weaning) as well as providing a means of standardising how the incremental sample $\delta^{44/42}$Ca values from first and second molars were oriented in relation to each other.

6. If so, is it possible to identify an early weaning signature?

Yes, I believe so, but it is complicated by the fact that there may be a difference between male and female sheep. The Highfield Farm male lambs appeared to be weaned earlier than the female lambs. However, the male and female lambs were raised together until 4 months of age, when all the lambs were weaned. Further samples and research are needed to address possible sex-specific differences. It may be that the sex of the sheep needs to be determined prior to interpreting the calcium isotope results, in order to avoid false positives for early weaning.

7. Can the calcium isotope content provide other information about a sheep's life outside of nursing and weaning, such as calcium incorporation* in utero *and movement/changes in plant diet?

I believe being able to identify the *in utero* period is critical to being able to establish birth in the timeline for nursing and weaning. I do not believe much can be learned about the sheep's life while *in utero*; however, I think calcium isotope analysis of the cervical half of second molars and the whole of the third molars has the potential to provide new information about herding and foddering. The Tell Abu Hureyra sheep and goat that were analysed had very active looking second molars, with frequent small dietary changes that were isolated to the post-weaning period. This was very unlike the modern sheep that are kept year after year on the same pasture, as was the case with both the Highfield Farm and Hoy sheep.

8. Does sex play a role in the calcium isotope content?

As discussed for question 6, sex may very well play a role in the $\delta^{44/42}$Ca values that are seen in the analysis of dental enamel. Gaining a better understanding of how serious an issue sex may to understanding and interpreting results is one of the top priorities of my intended future work.

8.2 Tell Abu Hureyra sheep samples

1. Does the enamel have a calcium isotope content with ratios indicating milk and plant consumption?

Yes, the Tell Abu Hureyra sheep and goat both demonstrated distinct $\delta^{44/42}$Ca values in regions of the molars that, based on modern results, are associated with milk and plants. The $\delta^{44/42}$Ca values were also appropriate to dietary source by tooth location, i.e. the upper and middle portion of the first molar had the lowest $\delta^{44/42}$Ca values of any samples and the cervical half of the second molar and any third molars had the highest $\delta^{44/42}$Ca values. These values reflecting the fact that milk is a depleted food in relation to plants.

2. If so, is there a weaning transition?

Yes, there were clear weaning transitions seen in both Tell Abu Hureyra B59 and B252.

3. If so, is the weaning transition abrupt or gradual?

See the answer to the modern sheep samples, question 4.

4. Is it possible to link calcium isotope ratio results from serial, segmented sampling to a dental chronology?

In the absence of sheep life histories (modern samples), the assistance of oxygen isotope analysis in establishing the most likely point on the first molar to have enamel associated with birth is critical. Birth is the starting point from which to establish the timing of nursing and weaning.

5. If so, is it possible to identify early weaning in Tell Abu Hureyra sheep, based on the signature of modern sheep results?

I believe that by using the naturally weaned (Hoy) sheep pattern of incremental results, and if birth can be established, that it should be possible to determine whether the Tell Abu Hureyra sheep were weaned early. The first molar from sheep B59 certainly appears to show early weaning. While speculative at this point, it does appear that B59 was weaned earlier than the male lamb from Highfield Farm lamb, HF29.

6. Can the calcium isotope content provide other information about a sheep's life outside of nursing and weaning, such as calcium incorporation* in utero *and sheep movement/changes in plant diet?

See the answer to the modern sheep samples, question 7.

7. If early weaning in sheep is indicated from the site of Tell Abu Hureyra, how do these results fit within the timeline of animal management for the purposes of identifying dairying in the archaeological record?

Unfortunately, because of glue contamination, only two sets of teeth were successfully analysed. This is not a large enough sample size to provide an answer to this question. It will certainly be addressed in the work that is intended for the future.

8.3 Future work

8.3.1 Sheep

Ideally, because of issues of diagenesis, wear and incompletely formed enamel on molars, future analyses will focus on molars that have fully mineralised enamel from sheep between

the ages of 18 and 35 months [and in Payne's (1973) wear stage six or less]. This includes both modern and archaeological samples. These requirements have been decided largely based on issues encountered with establishing weaning timing in the incomplete molars from lambs from Highfield Farm.

8.3.2 Differences in weaning timing

Herds may be weaned naturally, or with a management strategy determined by human intervention. Management strategies vary, depending on the purpose of the herd (dairying, wool and/or meat production); this has been the case since the advent of the secondary products revolution and continues today. Therefore it is necessary to produce several modern datasets, reflecting differences in weaning timing. Two herds will be selected, one weaned naturally and one weaned between 2 and 4 months.

8.3.3 Differences in sex

As part of establishing weaning timings, differences in $\delta^{44/42}Ca$ values between males and females need to be addressed. Differences in calcium isotope ratio results were observed in the molar dental enamel of male and female lambs from Highfield Farm. These differences may have implications for the interpretation of nursing and weaning timing for samples from sheep derived from archaeological sites. As such, the following work must be carried out so that future results may be more confidentially understood. The differences between male and female lambs from Highfield Farm included statistically significant lower delta values within the bulk enamel results for the female lambs compared with the male lambs. In addition, the incremental enamel results showed a distinctly different pattern between male and female lambs. In both instances, the results indicated earlier weaning in male lambs compared with female lambs, despite the lambs being separated from the ewes at the same time as part of a managed weaning. To address this issue, there are several options.

1. Determine whether female sheep from the Hoy reference collections are available for comparison with the males already analysed as part of this research (if so, this could then comprise a full, naturally weaned dataset).

2. If female sheep from Hoy are not available, another modern herd with adult males and females would need to be identified [although if not part of an existing reference collection, licencing will be necessary to have samples released from an abattoir, because of laws in place to stop the spread of sheep spongiform encephalopathy (scrapie)].
3. Emma Svensson from Uppsala University is planning to investigate establishing sex of sheep based on ancient (a)DNA analysis from bone. This, along with the datasets of male and female sheep produced from herds with known weaning timing, should make it possible to include archaeological samples in future work. This being of course if sex proves to require special consideration.
4. It would be useful to analyse a modern herd derived from a location corresponding to the region where archaeological samples have been secured. Ideally, this herd would be managed using the most traditional methods possible, and would include samples from adult female sheep as well as male sheep.

8.3.4 Archaeological sheep samples

I am in discussion with archaeologists working at Çatalhöyük, a key site in the Near East for the Neolithic, and am exploring the possibility of gaining access to archaeological samples from the site, along with making contact with local residents carrying out traditional dairying practices, in order to gain a better understanding of the timing and duration of ewe lactation and lamb nursing and weaning. As such, it represents an ideal site for potential future work.

8.3.5 Goat

A pilot study using goat teeth should be carried out. This is because goat domestication parallels that of sheep in the archaeological record and goat are also a known dairy animal. First, it will need to be established whether there is a modern proxy for wild goat available that can provide data about natural goat lactation/nursing length (this is not available for cattle). If this can be found, modern samples for a male and female goat raised together

would be required initially, and work could then proceed as has been outlined for sheep in this thesis. Now that the ZooMS protocol can determine whether a sample is sheep or goat quickly and easily, it is important to begin work to establish the calcium patterns for goat, so that they can also be considered when found within an archaeological site, to give a more complete picture of their dairying practice.

8.3.6 Human

An opportunity has arisen to gain access to deciduous human teeth as part of research into nursing and weaning currently in preparation at Bradford University. I have been in discussion with Julia Beaumont and, if the project moves forward, she has offered to let me sample the teeth for calcium isotope analysis to complement the other isotope work planned by the team. This would be an excellent opportunity to expand this work to human samples. Work with calcium isotopes offers the potential to bring insight into the human weaning process, and the transition to dairy milk.

8.4 Summary

Overall, this research has answered the questions and met the goals as laid out in Chapter 1. Calcium isotopes have been successfully analysed in dental enamel for the first time for the purpose of identifying dietary information related to milk and plant consumption. The use of calcium isotopes to address questions in the archaeological record related to milk (nursing and dairy) and plant consumption has shown great potential. Further work is certainly necessary, but this thesis presents the critical first step.

REFERENCES

Abbott, NJ 2004. Evidence for bulk flow of brain interstitial fluid: significance for physiology and pathology. *Neurochemistry International*, 45, 545–552.

Abrahams, PW 2005. Geophagy and the involuntary ingestion of soil. *In:* Selinus, O, Alloway, B, Centeno, JA, Finkelman, RB, Fuge, R, Lindh, U & Smedley, P (eds) *Essentials of Medical Geology: Impacts of the Natural Environment on Public Health*. Burlington, MA: Elsevier Academic Press, 435–458.

Adams, RS 1975. Variability in mineral and trace element content of dairy cattle feeds. *Journal of Dairy Science*, 58, 1538–1548.

Adriano, DC 1986. *Trace Elements in the Terrestrial Environment.* New York, NY: Springer-Verlag.

Albarède, F & Beard, B 2004. Analytical methods for non-traditional isotopes. *Reviews in Mineralogy & Geochemistry*, 55, 113–152.

Alloway, BJ 2005. Bioavailability of elements in soil. *In:* Selinus, O, Alloway, B, Centeno, JA, Finkelman, RB, Fuge, R, Lindh, U & Smedley, P (eds) *Essentials of Medical Geology: Impacts of the Natural Environment on Public Health*. Burlington, MA: Elsevier Academic Press, 347–372.

Aloia, JF, Chen, D-GD, Yeh, JK & Chen, H 2010. Serum vitamin D metabolites and intestinal calcium absorption efficiency in women. *American Journal of Clinical Nutrition*, DOI 10.3945/ajcn.2010.29553.

Altimimi, HF & Schnetkamp, PPM 2007. Examining Ca^{2+} extrusion of Na^{+}/Ca^{2+}-K^{+} exchangers. *In:* Herchuelz, A, Blaustein, MP, Lytton, J & Philipson, KD (eds) *Sodium-Calcium Exchange and the Plasma Membrane Ca^{2+}-ATPase in Cell Function, 5th International Conference*. New York, NY: Annals of the New York Academy of Sciences, 29–33.

Ambrose, SH & Norr, L 1993. Experimental evidence for the relationship of the carbon isotope ratios of whole diet and dietary protein to those of bone collagen and carbonate. *In:* Lambert, JB & Grupe, G (eds) *Prehistoric Human Bone: Archaeology at the Molecular Level*. Heidelberg: Springer Berlin, 1–37.

Amoah, EA, Gelaye, S, Guthrie, P & Rexroad, CE 1996. Breeding season and aspects of reproduction of female goats. *Journal of Animal Science*, 74, 723–728.

Aoba, T & Moreno, E 1987. The enamel fluid in the early secretory stage of porcine amelogenesis: chemical composition and saturation with respect to enamel mineral. *Calcified Tissue International*, 41, 86–94.

Arnold, GW, Wallace, SR & Maller, RA 1979. Some factors envolved in natural weaning processes in sheep. *Applied Animal Ethology*, 5, 43–50.

Asouti, E 2006. Beyond the Pre-pottery Neolithic B interaction sphere. *Journal of World Prehistory*, 20, 87–126.

Aurenche, O, Galet, P, Ragnon-Caroline, E & Évin, J 2001. Proto-Neolithic and Neolithic cultures in the Middle East—the birth of agriculture, livestock raising, and ceramics: a calibrated ^{14}C chronology 12,500–5500 cal BC. *Radiocarbon*, 43, 1191–1202.

Avery, JK, Visser, RL & Knapp, DE 1961. The pattern of the mineralization of enamel. *Journal of Dental Research*, 40, 1004–1019.

Baker, PF, Blaustein, MP, Hodgkin, AL & Steinhardt, RA 1969. The influence of calcium on sodium efflux in squid axons. *Journal of Physiology*, 200, 431–458.

Balasse, M 2003. Potential biases in sampling design and interpretation of intra-tooth isotope analysis. *International Journal of Osteoarchaeology*, 13, 3–10.

Balasse, M & Ambrose, SH 2005. Distinguishing sheep and goats using dental morphology and stable carbon isotopes in C_4 grassland environments. *Journal of Archaeological Science*, 32, 691–702.

Balasse, M, Ambrose, SH, Smith, AB & Price, TD 2002. The seasonal mobility model for prehistoric herders in the south-western Cape of South Africa assessed by isotopic analysis of sheep tooth enamel. *Journal of Archaeological Science*, 29, 917–932.

Balasse, M, Bălăşescu, A, Janzen, A, Ughetto-Monfrin, JL, Mirea, P & Andreescu, R 2013. Early herding at Mağura-Boldul lui Moş Ivaňuş (early sixth millennium BC, Romania): environments and seasonality from stable isotope analysis. *European Journal of Archaeology,* 16, 221–246.

Balasse, M, Bocherens, H, Mariotti, A & Ambrose, SH 2001. Detection of dietary changes by intra-tooth carbon and nitrogen isotopic analysis: an experimental study of dentine collagen of cattle (*Bos taurus*). *Journal of Archaeological Science*, 28, 235–245.

Balasse, M, Boury, L, Ughetto-Monfrin, J & Tresset, A 2012a. Stable isotope insights ($\delta^{18}O$, $\delta^{13}C$) into cattle and sheep husbandry at Bercy (Paris, France, 4th millennium BC): birth seasonality and winter leaf foddering. *Environmental Archaeology*, 17, 29–44.

Balasse, M, Mainland, I & Richards, MP 2009. Stable isotope evidence for seasonal consumption of marine seaweed by modern and archaeological sheep in the Orkney archipelago (Scotland). *Environmental Archaeology*, 14, 1–14.

Balasse, M, Obein, G, Ughetto-Monfrin, J & Mainland, I 2012b. Investigating seasonality and season of birth in past herds: a reference set of sheep enamel stable oxygen isotope ratios. *Archaeometry*, 54, 349–368.

Balasse, M & Tresset, A 2002. Early weaning of Neolithic domestic cattle (Bercy, France) revealed by intra-tooth variation in nitrogen isotope ratios. *Journal of Archaeological Science*, 29, 853–859.

Balasse, M & Tresset, A 2007. Environmental constraints on the reproductive activity of domestic sheep and cattle: what latitude for the herder. *Anthropozoologica*, 42, 71–88.

Balasse, M, Tresset, A, Dobney, K & Ambrose, SH 2005. The use of isotope ratios to test for seaweed eating in sheep. *Journal of Zoology*, 266, 283–291.

Ball, GFM 2004. *Vitamins: Their Role in the Human Body*. Oxford: Blackwell Publishing Ltd.

Banning, EB 2007. Time and tradition in the transition from late Neolithic to Chalcolithic: summary and conclusions. *Paléorient*, 137–142.

Bar-Yosef, O 1998. The Natufian culture in the Levant, threshold to the origins of agriculture. *Evolutionary Anthropology: Issues, News, and Reviews*, 6, 159–177.

Barber, SA 1995. *Soil Nutrient Bioavailability: A Mechanistic Approach.* New York, NY: John Wiley & Sons Inc.

Barbour, M 2007. Stable oxygen isotope composition of plant tissue: a review. *Functional Plant Biology*, 34, 83–94.

Barbour, M, Cernusak, L & Farquhar, G 2005. Factors affecting the oxygen isotope ratio of plant organic material. *In:* Flanagan, L & Ehleringer, J (eds) *Stable Isotopes in Biosphere–Atmosphere Interactions: Processes and Biological Controls*. San Diego, CA: Elsevier Academic Press, 9–28.

Barnes, TG, Varner, LW, Blankenship, LH, Fillinger, TJ & Heineman, SC 1990. Macro and trace mineral content of selected South Texas deer forages. *Journal of Range Management*, 43, 220–223.

Baron, TG, Madec, JY & Calavas, D 1999. Similar signature of the prion protein in natural sheep scrapie and bovine spongiform encephalopathy-linked diseases. *Journal of Clinical Microbiology*, 37, 3701–3704.

Bawden, JW, Wolkoff, AS & Flowers, CE 1965. Maternal-fetal blood calcium relationships in sheep. *Obstetrics & Gynecology*, 25, 548–552.

Belkacemi, L, Bédard, I, Simoneau, L & Lafond, J 2005. Calcium channels, transporters and exchangers in placenta: a review. *Cell Calcium*, 37, 1–8.

Belkacemi, L, Nelson, DM, Desai, M & Ross, MG 2010. Maternal undernutrition influences placental-fetal development. *Biology of Reproduction*, 83, 325–331.

Ben-David, M & Flaherty, EA 2012. Stable isotopes in mammalian research: a beginner's guide. *Journal of Mammalogy*, 93, 312–328.

Bender, MM 1971. Variations in the $^{13}C/^{12}C$ ratios of plants in relation to the pathway of photosynthetic carbon dioxide fixation. *Phytochemistry*, 10, 1239–1244.

Beniash, E, Metzler, RA, Lam, RS & Gilbert, PUPA 2009. Transient amorphous calcium phosphate in forming enamel. *Journal of Structural Biology*, 166, 133–143.

Berger, LL 1995. Why do we need a new NRC data base? *Animal Feed Science and Technology*, 53, 99–107.

Berner, EK & Berner, RA 1987. *The Global Water Cycle: Geochemistry and Environment.* Upper Saddle River, NJ: Prentice-Hall, Inc.

Berner, EK & Berner, RA 1996. *Global Environment: Water, Air, and Geochemical Cycles.* Upper Saddle River, NJ: Prentice-Hall, Inc.

Black, RE, Williams, SM, Jones, IE & Goulding, A 2002. Children who avoid drinking cow milk have low dietary calcium intakes and poor bone health. *American Journal of Clinical Nutrition*, 76, 675–680.

Blaise, E & Balasse, M 2011. Seasonality and season of birth of modern and late Neolithic sheep from south-eastern France using tooth enamel $\delta^{18}O$ analysis. *Journal of Archaeological Science*, 38, 3085–3093.

Blättler, CL, Jenkyns, HC, Reynard, LM & Henderson, GM 2011. Significant increases in global weathering during Oceanic Anoxic Events 1a and 2 indicated by calcium isotopes. *Earth and Planetary Science Letters*, 309, 77–88.

Blaustein, MP, Juhaszova, M, Golovina, VA, Church, PJ & Stanley, EF 2002. Na/Ca exchanger and PMCA localization in neurons and astrocytes: functional implications. *Annals of the New York Academy of Sciences*, 976, 356–366.

Bosso, NA, Cisse, MF, Van der Waaij, EH, Fall, A & Van Arendonk, JAM 2007. Genetic and phenotypic parameters of body weight in West African Dwarf goat and Djallonke sheep. *Small Ruminant Research*, 67, 271–278.

Bouillon, R, Cromphaut, SV & Carmeliet, G 2003. Intestinal calcium absorption: molecular vitamin D mediated mechanisms. *Journal of Cellular Biochemistry*, 88, 332–339.

Boulyga, SF 2010. Calcium isotope analysis by mass spectrometry. *Mass Spectrometry Reviews*, 29, 685–716.

Braithwaite, GD, Glascock, RF & Riazuddin, S 1970. Calcium metabolism in pregnant ewes. *British Journal of Nutrition*, 24, 661–670.

Bredin, IP, Skinner, JD & Mitchell, G 2008. Can osteophagia provide giraffes with phosphorus and calcium? *Onderstepoort Journal of Veterinary Research*, 75, 1–9.

Bronner, F 1987. Calcium absorption. *In:* Johnson, LR, Christensen, J, Jacobson, ED, Jackson, MJ & Walsh, JH (eds) *Physiology of the Gastrointestinal Tract*. New York, NY: Raven, 1419–1435.

Bronner, F 2003. Mechanisms of intestinal calcium absorption. *Journal of Cellular Biochemistry*, 88, 387–393.

Brookes, SJ, Robinson, C, Kirkham, J & Bonass, WA 1995. Biochemistry and molecular biology of amelogenin proteins of developing dental enamel. *Archives of Oral Biology*, 40, 1–14.

Brown, EM & Jüppner, H 2006. Parathyroid hormone: synthesis, secretion, and action. *In:* Favus, MJ (ed.) *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*. Washington, DC: The American Society for Bone and Mineral Research.

Brown, TH 1964. The early weaning of lambs. *Journal of Agriculture Science*, 63, 191–204.

Bruhn, F, Duhr, A, Grootes, PM, Mintrop, A & Nadeau, M 2006. Chemical removal of conservation substances by 'Soxhlet'-type extraction. *Radiocarbon*, 43, 229–237.

Bunge, R, Thomas, DL & Stookey, JM 1990. Factors affecting productivity of Rambouillet ewes mated to ram lambs. *Journal of Animal Science*, 68, 2253–2262.

Burger, J, Kirchner, M, Bramanti, B, Haak, W & Thomas, MG 2007. Absence of the lactase-persistence-associated allele in early Neolithic Europeans. *Proceedings of the National Academy of Sciences, USA*, 104, 3736–3741.

Byrd, BF 2005. Reassessing the emergence of village life in the Near East. *Journal of Archaeological Research*, 13, 231–290.

Camin, F, Perini, M, Colombari, G, Bontempo, L & Versini, G 2008. Influence of dietary composition on the carbon, nitrogen, oxygen and hydrogen stable isotope ratios of milk. *Rapid Communications in Mass Spectrometry*, 22, 1690–1696.

Cardellino, RA & Benson, ME 2002. Lactation curves of commercial ewes rearing lambs. *Journal of Animal Science*, 80, 23–27.

Cappio-Borlino, A, Macciotta, NPP & Pulina, G 1997. The shape of sarda ewe lactation curve analysed by a compartimental model. *Livestock Production Science*, 51, 89-96.

Catterall, WA 1988. Structure and function of voltage-sensitive ion channels. *Science, New Series*, 242, 50–61.

Caumette, G, Ouypornkochagorn, S, Scrimgeour, CM, Raab, A & Feldmann, J 2007. Monitoring the arsenic and iodine exposure of seaweed-eating North Ronaldsay sheep from the gestational and suckling periods to adulthood by using horns as a dietary archive. *Environmental Science and Technology*, 41, 2673–2679.

Cerling, TE & Harris, JM 1999. Carbon isotope fractionation between diet and bioapatite in ungulate mammals and implications for ecological and paleoecological studies. *Oecologia*, 120, 347–363.

Chapman, C. 2010. *RE: Shetland sheep weaning age?* Type to Wright, C.

Chessa, B, Pereira, F, Arnaud, F, Amorim, A, Goyache, F, Mainland, I, Kao, RR, Pemberton, JM, Beraldi, D, Stear, MJ, Alberti, A, Pittau, M, Iannuzzi, L, Banabazi, MH, Kazwala, RR, Zhang, Y, Arranz, JJ, Ali, BA, Wang, Z, Uzun, M, Dione, MM, Olsaker, I, Holm, L, Saarma, U, Ahmad, S, Marzanov, N, Eythorsdottir, E, Holland, MJ, Ajmone-Marsan, P, Bruford, MW, Kantanen, J, Spencer, TE, Palmarini, M 2009. Revealing the history of sheep domestication using retrovirus integrations. *Science*, 324, 532–536.

Chichilo, P & Whittaker, CW 1961. Trace elements in agricultural limestones of the United States. *Agronomy Journal*, 53, 139–144.

Childe, VG 1934. Neolithic settlement in the west of Scotland. *The Scottish Geographical Magazine*, 50, 18–25.

Christakos, S, Dhawan, P, Benn, B, Porta, A, Hediger, M, Oh, GT, Jeung, E-B, Zhong, Y, Ajibade, D, Dhawan, K & Joshi, S 2007. Vitamin D: molecular mechanism of action. *Annals of the New York Academy of Sciences: Skeletal Biology and Medicine, Part A*, 1116, 340–348.

Chu, N-C, Henderson, GM, Belshaw, NS & Hedges, REM 2006. Establishing the potential of Ca isotopes as proxy for consumption of dairy products. *Applied Geochemistry*, 21, 1656–1667.

Clark, ID & Fritz, P 1997. *Environmental Isotopes in Hydrogeology*. Boca Raton, FL: CRC Press/Lewis Publishers.

Clayton, F, Sealy, J & Pfeiffer, S 2006. Weaning age among foragers at Matjes River Rock Shelter, South Africa, from stable nitrogen and carbon isotope analyses. *American Journal of Physical Anthropology*, 129, 311–317.

Clementz, MT 2012. New insight from old bones: stable isotope analysis of fossil mammals. *Journal of Mammalogy*, 93, 368–380.

Clementz, MT, Holden, P & Koch, PL 2003. Are calcium isotopes a reliable monitor of trophic level in marine settings? *International Journal of Osteoarchaeology*, 13, 29–36.

Cobert, F, Schmitt, AD, Bourgeade, P, Labolle, F, Badot, PM, Chabaux, F & Stille, P 2011. Experimental identification of Ca isotopic fractionations in higher plants. *Geochimica et Cosmochimica Acta*, 75, 5467–5482.

Colville, TP & Bassert, JM 2009. *Clinical Anatomy and Physiology Laboratory Manual for Veterinary Technicians*. St Louis, MO: Mosby Elsevier.

Copley, MS, Berstan, R, Mukherjee, AJ, Dudd, SN, Straker, V, Payne, S & Evershed, RP 2005. Dairying in antiquity. III. Evidence from absorbed lipid residues dating to the British Neolithic. *Journal of Archaeological Science*, 32, 523–546.

Courot, M, De Reviers, MM & Pelletier, J 1975. Variations in pituitary and blood LH during puberty in the male lamb. Relation to time of birth. *Annales De Biologie Animale, Biochimie, Biophysique*, 15, 509–516.

Cowan, RL, Hartsook, EW & Whelan, JB 1968. Calcium-strontium metabolism in white tailed deer as related to age and antler growth. *Experimental Biology and Medicine*, 129, 733–737.

Craig, OE, Chapman, J, Heron, C, Willis, LH, Bartosiewicz, L, Taylor, G, Whittle, A & Collins, M 2005. Did the first farmers of central and eastern Europe produce dairy foods? *Antiquity*, 79, 882–894.

Crawley, MJ 1983. *Herbivory: the Dynamics of Animal–Plant Interactions.* Oxford: Blackwell Scientific Publications.

Cross, NA, Hillman, LS, Allen, SH, Krause, GF & Vieira, NE 1995. Calcium homeostasis and bone metabolism during pregnancy, lactation, and postweaning: a longitudinal study. *American Journal of Clinical Nutrition*, 61, 514–523.

Crowell-Davis, SL & Houpt, KA 1985. Coprophagy by foals: effect of age and possible functions. *Equine Veterinary Journal*, 17, 17–19.

D'Haese, PC, Lamberts, LV & De Broe, ME 2004. Silicon. *In:* Merian, E, Anke, M, Ihnat, M & Stoeppler, M (eds) *Elements and their Compounds in the Environment*. Weinheim: Wiley-Vch, 1273–1284.

Dauphin, Y & Williams, CT 2004. Diagenetic trends of dental tissues. *Comptes Rendus Palevol*, 3, 583–590.

Davenport, HW 1982. *Physiology of the Digestive Tract*, 5th edn. Chicago, IL: Year Book Medical Publishers Inc.

Davis, SJM 2000. The effect of castration and age on the development of the Shetland sheep skeleton and a metric comparison between bones of males, females and castrates. *Journal of Archaeological Science*, 27, 373–390.

de Laeter, JR, Böhlke, JK, de Bièvre, P, Hidaka, H, Peiser, HS, Rosman, KJR & Taylor, PDP 2003. Atomic weights of the elements. *Pure and Applied Chemistry*, 75, 683–800.

de Moulins, D 2000. Abu Hureyra 2: plant remains from the Neolithic. *In:* Moore, A, Hillman, G & Legge, A (eds) *Village on the Euphrates: From Foraging to Farming at Abu Hureyra*. Oxford: Oxford University Press, 399–422.

Delgado Huertas, A, Iacumin, P, Stenni, B, Sánchez Chillón, B & Longinelli, A 1995. Oxygen isotope variations of phosphate in mammalian bone and tooth enamel. *Geochimica et Cosmochimica Acta*, 59, 4299–4305.

DeNiro, MJ & Epstein, S 1978. Influence of diet on the distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta*, 42, 495–506.

Douthitt, CB 2008. The evolution and applications of multicollector ICPMS (MC-ICPMS). *Analytical and Bioanalytical Chemistry*, 390, 437–440.

Dupras, TL, Schwarcz, HP & Fairgrieve, SI 2001. Infant feeding and weaning practices in Roman Egypt. *American Journal of Physical Anthropology*, 115, 204–212.

Ehara, T, Matsuoka, S & Noma, A 1989. Measurement of reversal potential of Na^+ - Ca^{2+} exchange current in single guinea-pig ventricular cells. *Journal of Physiology*, 410, 227–249.

Ehleringer, JR & Rundel, PW 1989. Stable isotopes: history, units, and instrumentation. *In:* Nagy, KA, Ehleringer, JR & Rundel, PW (eds) *Stable Isotopes in Ecological Research*. Berlin: Springer, 1–16.

Elliott, JC, Holcomb, DW & Young, RA 1985. Infrared determination of the degree of substitution of hydroxyl by carbonate ions in human dental enamel. *Calcified Tissue International*, 37, 372–375.

Elwood, PC, Givens, DI, Beswick, AD, Fehily, AM, Pickering, JE & Gallacher, J 2008. The survival advantage of milk and dairy consumption: an overview of evidence from cohort studies of vascular diseases, diabetes and cancer. *Journal of the American College of Nutrition*, 27, 723S–734S.

Evershed, RP, Payne, S, Sherratt, AG, Copley, MS, Coolidge, J, Urem-Kotsu, D, Kotsakis, K, Özdoan, M, Özdoan, AE, Nieuwenhuyse, O, Akkermans, PMMG, Bailey, D, Andeescu, R-R, Campbell, S, Farid, S, Hodder, I, Yalman, N, Özbaaran, M, Bçakc, E, Garfinkel, Y, Levy, T & Burton, MM 2008. Earliest date for milk use in the Near East and southeastern Europe linked to cattle herding. *Nature*, 455, 528–531.

Ewer, TK 1951. Rickets in sheep. 1. The experimental production of rickets in young sheep. *British Journal of Nutrition*, 5, 287–300.

Farquhar, GD, Ehleringer, JR & Hubick, KT 1989. Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Biology*, 40, 503–537.

Festa-Bianchet, M 1988. Nursing behaviour of bighorn sheep: correlates of ewe age, parasitism, lamb age, birthdate and sex. *Animal Behaviour*, 36, 1445–1454.

Festa-Bianchet, M, Jorgenson, JT & WUhart, WD 1994. Early weaning in bighorn sheep, *Ovis canadensis* affects growth of males but not of females. *Behavioral Ecology*, 5, 21–27.

Fietzke, J, Eisenhauer, A, Gussone, N, Bock, B, Liebetrau, V, Nägler, TF, Spero, HJ, Bijma, J & Dullo, C 2004. Direct measurement of $^{44}Ca/^{40}Ca$ ratios by MC–ICP–MS using the cool plasma technique. *Chemical Geology*, 206, 11–20.

Fincham, AG, Moradian-Oldak, J & Simmer, JP 1999. The structural biology of the developing dental enamel matrix. *Journal of Structural Biology*, 126, 270–299.

Fish, KD, Sauther, ML, Loudon, JE & Cuozzo, FP 2007. Coprophagy by wild ring-tailed lemurs (*Lemur catta*) in human-disturbed locations adjacent to the Beza Mahafaly Special Reserve, Madagascar. *American Journal of Primatology*, 69, 713–718.

Fleming, GA 1963. Distribution of major and trace elements in some common pasture species. *Journal of the Science of Food and Agriculture*, 14, 203–208.

Fogel, ML, Tuross, N, Johnson, BJ & Miller, GH 1997. Biogeochemical record of ancient humans. *Organic Geochemistry*, 27, 275–287.

Fogh-Andersen, N, Altura, BM, Altura, BT & Siggaard-Andersen, O 1995. Composition of interstitial fluid. *Clinical Chemistry*, 41, 1522–1525.

Foster, DL, Yellon, SM & Olster, DH 1985. Internal and external determinants of the timing of puberty in the female. *Journal of Reproduction and Fertility*, 75, 327–344.

Fowler, CE, Li, M, Mann, S & Margolis, HC 2005. Influence of surfactant assembly on the formation of calcium phosphate materials—a model for dental enamel formation. *Journal of Materials Chemistry*, 15, 3317–3325.

Frankin, M 1950. The influence of diet on dental development in the sheep. *Commonwealth Scientific and Industrial Research Organisation Bulletin*, 252, 34.

Franklin, IK, Winz, RA & Hubbard, MJ 2001. Endoplasmic reticulum Ca^{2+}-ATPase pump is up-regulated in calcium-transporting dental enamel cells: a non-housekeeping role for SERCA2b. *Biochemical Journal*, 358, 217–224.

Fransson, G-B & Lönnerdal, B 1983. Distribution of trace elements and minerals in human and cow's milk pediatric research. *Pediatric Research*, 17, 912–915.

Fuller, BT, Richards, MP & Mays, SA 2003. Stable carbon and nitrogen isotope variations in tooth dentine serial sections from Wharram Percy. *Journal of Archaeological Science*, 30, 1673–1684.

Gannes, LZ, Del Rio, CM & Koch, P 1998. Natural abundance variations in stable isotopes and their potential uses in animal physiological ecology. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 119, 725–737.

Gardella, TJ, Jüppner, H, Brown, EM, Kronenberg, HM & Potts, JT 2010. Parathyroid hormone and parathyroid hormone-related peptide in the regulation of calcium homeostasis and bone development. *In:* Jameson, JL, De Groot, LJ, de Kretser, D, Grossman, A, Marshall, JC, Melmed, S, Potts, JT & Weir, GC (eds) *Endocrinology: Adult and Pediatric*, 6th edn. Philadelphia, PA: Saunders Elsevier, 1040–1073.

Gardner, RW & Hogue, DE 1964. Effects of energy intake and number of lambs suckled on milk yield, milk composition and energetic efficiency of lactating ewes. *Journal of Animal Science*, 23, 935–942.

Garel, M, Cugnasse, JM, Gaillard, JM, Loison, A, Gibert, P, Douvre, P & Dubray, D 2005. Reproductive output of female mouflon (*Ovis gmelini musimon* × *Ovis* sp.): a comparative analysis. *Journal of Zoology*, 266, 65–71.

Garner, SC, Anderson, JJB & Ambrose, WW 1996. Skeletal tissues and mineralization. *In:* Anderson, JJB & Garner, SC (eds) *Calcium and Phosphorus in Health and Disease*. Boca Raton, FL: CRC Press Inc., 97–117.

Gat, JR 1996. Oxygen and hydrogen isotopes in the hydrologic cycle. *Annual Review of Earth and Planetary Sciences*, 24, 225–262.

Gerbault, P, Liebert, A, Itan, Y, Powell, A, Currat, M, Burger, J, Swallow, DM & Thomas, MG 2011. Evolution of lactase persistence: an example of human niche construction. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 366, 863–877.

Gilead, I 1988. The Chalcolithic period in the Levant. *Journal of World Prehistory*, *2*, 397–443.

Gillis, R, Bréhard, S, Bălăşescu, A, Ughetto-Monfrin, J, Popovici, D, Vigne, JD & Balasse, M 2013. Sophisticated cattle dairy husbandry at Borduşani-Popină (Romania, fifth millennium BC): the evidence from complementary analysis of mortality profiles and stable isotopes. *World Archaeology*, 45, 447–472.

Goldstein, GW 1979. Pathogenesis of brain edema and hemorrhage: role of the brain capillary. *Pediatrics*, 64, 357–360.

Gootwine, E & Goot, H 1996. Lamb and milk production of Awassi and East-Friesian sheep and their crosses under Mediterranean environment. *Small Ruminant Research*, 20, 255–260.

Graczyk, TK & Cranfield, MR 2003. Coprophagy and intestinal parasites: implications to human-habituated Mountain Gorillas (*Gorilla gorilla beringei*) of the Virunga Mountains and Bwindi Impenetrable Forest. *Primate Conservation*, 19, 58–64.

Gupta, AK 2004. Origin of agriculture and domestication of plants and animals linked to early Holocene climate amelioration. *Current Science*, 87, 54–59.

Gutiérrez, JP, Legaz, E & Goyache, F 2007. Genetic parameters affecting 180-days standardised milk yield, test-day milk yield and lactation length in Spanish Assaf (Assaf. E) dairy sheep. *Small Ruminant Research*, 70, 233–238.

Haenlein, GFW 2007. About the evolution of goat and sheep milk production. *Small Ruminant Research*, 68, 3–6.

Halicz, L, Galy, A, Belshaw, NS & O'Nions, RK 1999. High-precision measurement of calcium isotopes in carbonates and related materials by multiple collector inductively coupled plasma mass spectrometry (MC-ICP-MS). *Journal of Analytical Atomic Spectrometry*, 14, 1835–1838.

Hamilton, J, Bentley, RA, Bickle, P, Fibiger, L, Hedges, R, Reynard, L, Wright, C, Cullen, P, Dale, C, Nowell, G & Whittle, A 2013. Seeking out diversity: methodology. *In:* Bickle, P & Whittle, A (eds) *The First Farmers in Central Europe: Diversity in LBK Lifeways*. Oxford: Oxbow Books, 29–48.

Harris, LD & Bowman, GB 1980. Vertebrate predator subsystem. *In:* Breymeyer, AI & Van Dyne, GM (eds) *Grasslands, Systems Analysis and Man*. Cambridge: Cambridge University Press, 591–607.

Hass, CC 1990. Alternative maternal-care patterns in two herds of bighorn sheep. *Journal of Mammalogy*, 71, 24–35.

Heaney, RP 2009. Dairy and bone health. *Journal of the American College of Nutrition*, 28, 82S–90S.

Heaney, RP, Weaver, CM, Hinders, SM, Martin, B & Packard, PT 1993. Absorbability of calcium from brassica vegetables: broccoli, bok choy, and kale. *Journal of Food Science*, 58, 1378–1380.

Heaton, TH 1999. Spatial, species, and temporal variations in the $^{13}C/^{12}C$ ratios of C_3 plants: implications for palaeodiet studies. *Journal of Archaeological Science*, 26, 637–649.

Hedges, REM & Reynard, LM 2007. Nitrogen isotopes and the trophic level of humans in archaeology. *Journal of Archaeological Science*, 34, 1240–1251.

Henton, E, Meier-Augenstein, W & Kemp, HF 2010. The use of oxygen isotopes in sheep molars to investigate past herding practices at the Neolithic settlement of Çatalhöyük, central Anatolia. *Archaeometry*, 52, 429–449.

Heuser, A & Eisenhauer, A 2008. The calcium isotope composition ($\delta^{44}Ca/^{40}Ca$) of NIST SRM 915b and NIST SRM 1486. *Geostandards and Geoanalytical Research*, 32, 311–315.

Heuser, A & Eisenhauer, A 2010. A pilot study on the use of natural calcium isotope ($^{44}Ca/^{40}Ca$) fractionation in urine as a proxy for the human body calcium balance. *Bone*, 46, 889–896.

Heuser, A, Tütken, T, Gussone, N & Galer, SJG 2011. Calcium isotopes in fossil bones and teeth–diagenetic versus biogenic origin. *Geochimica et Cosmochimica Acta*, 75, 3419–3433.

Hiendleder, S, Kaupe, B, Wassmuth, R & Janke, A 2002. Molecular analysis of wild and domestic sheep questions current nomenclature and provides evidence for domestication from two different subspecies. *Proceedings of the Royal Society of London, Series B: Biological Sciences*, 269, 893–904.

Hilge, M, Aelen, J, Perrakis, A & Vuister, GW 2007. Structural basis for Ca^{2+} regulation in the Na^{+}/Ca^{2+} exchanger. *In:* Herchuelz, A, Blaustein, MP, Lytton, J & Philipson, KD (eds) *Sodium-Calcium Exchange and the Plasma Membrane Ca^{2+}-ATPase in Cell Function, 5th International Conference*. New York, NY: Annals of the New York Academy of Sciences, 7–15.

Hillman, GC 2000. Abu Hureyra 1: the Epipalaeolithic. *In:* Moore, A, Hillman, G & Legge, A (eds) *Village on the Euphrates: From Foraging to Farming at Abu Hureyra*. Oxford: Oxford University Press, 327–399.

Hillson, S 2005. *Teeth.* Cambridge: Cambridge University Press.

Hindshaw, RS, Reynolds, BC, Wiederhold, JG, Kiczka, M, Kretzschmar, R & Bourdon, B 2013. Calcium isotope fractionation in alpine plants. *Biogeochemistry*, 112, 373–388.

Hindshaw, RS, Reynolds, BC, Wiederhold, JG, Kretzschmar, R & Bourdon, B 2011. Calcium isotopes in a proglacial weathering environment: Damma glacier, Switzerland. *Geochimica et Cosmochimica Acta*, 75, 106–118.

Hippler, D, Schmitt, A-D, Gussone, N, Heuser, A, Stille, P, Eisenhauer, A & Nägler, TF 2003. Calcium isotopic composition of various reference materials and seawater. *Geostandards Newsletter*, Volume 27, 13–19.

Hirakawa, H 2001. Coprophagy in leporids and other mammalian herbivores. *Mammal Review*, 31, 61–80.

Hirata, T, Tanoshima, M, Suga, A, Tanaka, Y-K, Nagata, Y, Shinohara, A & Chiba, M 2008. Isotopic analysis of calcium in blood plasma and bone from mouse samples by multiple collector–ICP–mass spectrometry. *Analytical Sciences*, 24, 1501–1507.

Hoenderop, JGJ, Nilius, B & Bindels, RJM 2005. Calcium absorption across epithelia. *Physiological Reviews*, 85, 373–422.

Holmes, WN & Donaldson, EM 1969. The body compartments and the distribution of electrolytes. *In:* Hoar, WS & Randall, DJ (eds) *Fish Physiology, Volume I: Excretion, Ionic Regulation, and Metabolism*. London: Academic Press Inc., 1–89.

Holzkämper, S, Holmgren, K, Lee-Thorp, J, Talma, S, Mangini, A & Partridge, T 2009. Late Pleistocene stalagmite growth in Wolkberg Cave, South Africa. *Earth and Planetary Science Letters*, 282, 212–221.

Howcroft, R, Eriksson, G & Lidén, K 2012. Conformity in diversity? Isotopic investigations of infant feeding practices in two iron age populations from Southern Öland, Sweden. *American Journal of Physical Anthropology*, 149, 217–230.

Hubbard, MJ 2000. Calcium transport across the dental enamel epithelium. *Critical Reviews in Oral Biology & Medicine*, 11, 437–466.

Itan, Y, Powell, A, Beaumont, MA, Burger, J & Thomas, MG 2009. The origins of lactase persistence in Europe. *PLoS Computational Biology*, 5, 1–13.

Jay, M, Fuller, BT, Richards, MP, Knüsel, CJ & King, SS 2008. Iron age breastfeeding practices in Britain: isotopic evidence from Wetwang Slack, East Yorkshire. *American Journal of Physical Anthropology*, 136, 327–337.

Jefferies, RL. Herbivores, nutrients and trophic cascades in terrestrial environments. *In:* Olff, H, Brown, VK & Drent, RH, eds. *Herbivores: Between Plants and Predators, 38th Symposium of the British Ecological Society in Cooperation with the Netherlands Ecological Society, 1999 Wageningen Agricultural University, The Netherlands.* Oxford: Blackwell Science Ltd, 301–330.

Jim, S, Ambrose, SH & Evershed, RP 2004. Stable carbon isotopic evidence for differences in the dietary origin of bone cholesterol, collagen and apatite: implications for their use in palaeodietary reconstruction. *Geochimica et Cosmochimica Acta*, 68, 61–72.

Jim, S, Jones, V, Ambrose, SH & Evershed, RP 2006. Quantifying dietary macronutrient sources of carbon for bone collagen biosynthesis using natural abundance stable carbon isotope analysis. *British Journal of Nutrition*, 95, 1055–1062.

Johnson, DA, Asay, KH, Tieszen, LL, Ehleringer, J & Jefferson, P 1989. Carbon isotope discrimination: a possible selection criterion for improving range grass production in water-limited environments. *Crop Science*, 30, 338–343.

Johnson, JA & Kumar, R 1994. Renal and intestinal calcium transport: roles of vitamin D and vitamin D-dependent calcium binding proteins. *Seminars in Nephrology*, 14, 119–128.

Johnson, LR 2007a. Digestion and absorption. *In:* Johnson, LR (ed.) *Gastrointestinal Physiology*, 7th edn. Philadelphia, PA: Mosby Elsevier, 107–126.

Johnson, LR 2007b. Fluid and electrolyte absorption. *In:* Johnson, LR (ed.) *Gastrointestinal Physiology*, 7th edn. Philadelphia, PA: Mosby Elsevier, 127–136.

Jones, B 2005. Animals and Medical Geology. *In:* Selinus, O, Alloway, B, Centeno, JA, Finkelman, RB, Fuge, R, Lindh, U & Smedley, P (eds) *Essentials of Medical Geology: Impacts of the Natural Environment on Public Health*. Burlington, MA: Elsevier Academic Press.

Jones, RJ, Ludlow, MM, Troughton, JH & Blunt, CG 1979. Estimation of the proportion of C_3 and C_4 plant species in the diet of animals from the ratio of natural ^{12}C and ^{13}C isotopes in the faeces. *The Journal of Agricultural Science*, 92, 91–100.

Kähäri, J & Nissinen, H 1978. The mineral element contents of timothy (*Phleum pratense*) in Finland. I. The elements calcium, magnesium, phosphorus, potassium, chromium, cobalt, iron, copper, manganese, sodium and zinc. *Acta Agriculturae Scandinavica*, 20, 26–39.

Karbach, U 1992. Paracellular calcium transport across the small intestine. *Journal of Nutrition*, 122, 672–677.

Kasemann, SA, Hawkesworth, CJ, Prave, AR, Fallick, AE & Pearson, PN 2005. Boron and calcium isotope composition in Neoproterozoic carbonate rocks from Namibia: evidence for extreme environmental change. *Earth and Planetary Science Letters*, 231, 73–86.

Katzenberg, MA, Herring, D & Saunders, SR 1996. Weaning and infant mortality: evaluating the skeletal evidence. *American Journal of Physical Anthropology*, 101, 177–199.

Kempson, IM, Skinner, WM & Kirkbride, PK 2003. Calcium distribution in human hair by ToF-SIMS. *Biochimica et Biophysica Acta*, 1624, 1–5.

Kierdorf, H, Witzel, C, Upex, B, Dobney, K & Kierdorf, U 2012. Enamel hypoplasia in molars of sheep and goats, and its relationship to the pattern of tooth crown growth. *Journal of Anatomy*, 220, 484–495.

Kijas, JW, Townley, D, Dalrymple, BP, Heaton, MP, Maddox, JF, McGrath, A, Wilson, P, Ingersoll, RG, McCulloch, R, McWilliam, S, Tang, D, McEwan, J, Cockett, N, Oddy, VH, Nicholas, FW & Raadsma, H. 2009. A genome wide survey of SNP variation reveals the genetic structure of sheep breeds. *PLoS ONE*, 4.

Kirkham, J, Zhang, J, Brookes, SJ, Shore, RC, Wood, SR, Smith, DA, Wallwork, ML, Ryu, OH & Robinson, C 2000. Evidence for charge domains on developing enamel crystal surfaces. *Journal of Dental Research*, 79, 1943–1947.

Kitahara, Y, Suda, N, Kuroda, T, Beck, F, Hammond, VE & Takano, Y 2002. Disturbed tooth development in parathyroid hormone-related protein (PTHrP)–gene knockout mice. *Bone*, 30, 48–56.

Koch, PL, Tuross, N & Fogel, ML 1997. The effects of sample treatment and diagenesis on the isotopic integrity of carbonate in biogenic hydroxylapatite. *Journal of Archaeological Science*, 24, 417–429.

Kohn, MJ & Cerling, TE 2002. Stable isotope compositions of biological apatite. *Reviews in Mineralogy and Geochemistry*, 48, 455–488.

Kohn, MJ, Schoeninger, MJ & Barker, WW 1999. Altered states: effects of diagenesis on fossil tooth chemistry. *Geochimica et Cosmochimica Acta*, 63, 2737–2747.

Kohn, MJ, Schoeninger, MJ & Valley, JW 1996. Herbivore tooth oxygen isotope compositions: effects of diet and physiology. *Geochimica et Cosmochimica Acta*, 60, 3889–3896.

Kolars, JC, Levitt, MD, Aouji, M & Savaiano, DA 1984. Yogurt—an autodigesting source of lactose. *New England Journal of Medicine*, 310, 1–3.

Körner, C, Farquhar, GD & Roksandic, Z 1988. A global survey of carbon isotope discrimination in plants from high altitude. *Oecologia*, 74, 623–632.

Kovacs, CS 2006. Skeletal physiology: fetus and neonate. *In:* Favus, MJ (ed.) *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*. Washington, DC: The American Society for Bone and Mineral Research, 50–55.

Kovacs, CS & Kronenberg, HM 2006. Skeletal physiology: pregnancy and lactation. *In:* Favus, MJ (ed.) *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*, 6th edn. Washington, DC: The American Society for Bone and Mineral Research, 63–68.

Krueger, HW & Sullivan, CH 1984. Models for carbon isotope fractionation between diet and bone. *Stable Isotopes in Nutrition*, 258, 205–220.

Kuijt, I & Goring-Morris, N 2002. Foraging, farming, and social complexity in the Pre-Pottery Neolithic of the southern Levant: a review and synthesis. *Journal of World Prehistory*, 16, 361–440.

Langer, P 1994. Food and digestion of Cenozoic mammals in Europe. *In:* Chivers, DJ & Langer, P (eds) *The Digestive System in Mammals: Food, Form and Function*. Cambridge: Cambridge University Press, 9–24.

Lee-Thorp, JA, Sealy, JC & van der Merwe, NJ 1989. Stable carbon isotope ratio differences between bone collagen and bone apatite, and their relationship to diet. *Journal of Archaeological Science*, 16, 585–599.

Lee-Thorp, JA & van der Merwe, NJ 1987. Carbon isotope analysis of fossil bone apatite. *South African Journal of Science*, 83, 712–715.

Legge, A & Rowley-Conwy, P 1987. Gazelle killing in Stone Age Syria. *Scientific American*, 255, 88–95.

Legge, A & Rowley-Conwy, P 2000. The exploitation of animals. *In:* Moore, A, Hillman, G & Legge, A (eds) *Village on the Euphrates: From Foraging to Farming at Abu Hureyra*. Oxford: Oxford University Press, 423–471.

Lemarchand, D, Wasserburg, GJ & Papanastassiou, DA 2004. Rate-controlled calcium isotope fractionation in synthetic calcite. *Geochimica et Cosmochimica Acta*, 68, 4665–4678.

Leymaster, KA 2002. Fundamental aspects of crossbreeding of sheep: use of breed diversity to improve efficiency of meat production. *Sheep and Goat Research Journal*, 17, 50–59.

Lincoln, GA, Klandorf, H & Anderson, N 1980. Photoperiodic control of thyroid function and wool and horn growth in rams and the effect of cranial sympathectomy. *Endocrinology*, 107, 1543–1548.

Lindh, U 2005. Biological functions of the elements. *In:* Selinus, O, Alloway, B, Centeno, JA, Finkelman, RB, Fuge, R, Lindh, U & Smedley, P (eds) *Essentials of Medical Geology: Impacts of the Natural Environment on Public Health*. Burlington, VT: Elsevier Academic Press, 122–124.

Lok, F, Owens, JA, Mundy, L, Robinson, JS & Owens, PC 1996. Insulin-like growth factor I promotes growth selectively in fetal sheep in late gestation. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*, 270, R1148–R1155.

Louca, A 1972. The effect of suckling regime on growth rate and lactation performance of the Cyprus fat-tailed and Chios sheep. *Animal Production*, 15, 53–59.

Lyford, SJ 1988. Growth and development of the ruminant digestive system. *In:* Church, D (ed.) *The Ruminant Animal: Digestive Physiology and Nutrition*. Englewood Cliffs, NJ: Prentice Hall Inc., 44–63.

Madani, MOK, Rahal, MS, Zawia, MT & Eluwhaishi, BA 1989. Puberty and early sexual development in Libyan fat-tailed ram lambs. *British Veterinary Journal*, 145, 276–288.

Maher, LA, Banning, EB & Chazan, M 2011. Oasis or mirage? Assessing the role of abrupt climate change in the prehistory of the southern Levant. *Cambridge Archaeological Journal*, 21, 1–29.

Mason, B & Moore, CB 1982. *Principles of Geochemistry*. New York, NY: John Wiley & Sons, Inc.

Mays, S 2003. Bone strontium: calcium ratios and duration of breastfeeding in a Mediaeval skeletal population. *Journal of Archaeological Science*, 30, 731–741.

McBean, LD & Miller, GD 1998. Allaying fears and fallacies about lactose intolerance. *Journal of the American Dietetic Association*, 98, 671–676.

McDonald, P, Edwards, RA, Greenhalgh, JFD & Morgan, CA 1995. *Animal Nutrition.* Harlow: Longman.

McDonald, TF, Pelzer, S, Trautwein, W & Pelzer, DJ 1994. Regulation and modulation of calcium channels in cardiac, skeletal, and smooth muscle cells. *Physiological Reviews*, 74, 366–461.

McDowell, LR 1992. *Minerals in Animal and Human Nutrition.* London: Academic Press, Inc.

McKusick, BC, Thomas, DL & Berger, YM 2001. Effect of weaning system on commercial milk production and lamb growth of East Friesian dairy sheep. *Journal of Dairy Science*, 84, 1660–1668.

McSweeney, CS, Denman, SE & Mackie, RI 2005. Rumen bacteria. *In:* Makkar, HPS & McSweeney, CS (eds) *Methods in Gut Microbial Ecology for Ruminants*. The Netherlands: Springer, 23-37.

Menon, GK, Grayson, S & Elias, PM 1985. Ionic calcium reservoirs in mammalian epidermis: ultrastructural location by ion-capture cytochemistry. *The Journal of Investigative Dermatology*, 84, 508–512.

Metges, C, Kempe, K & Schmidt, H-L 1990. Dependence of the carbon-isotope contents of breath carbon dioxide, milk, serum and rumen fermentation products on the $\delta^{13}C$ value of food in dairy cows. *British Journal of Nutrition*, 63, 187–196.

Milhaud, G & Nezit, J 1991. Développement des molaires chez le mouton. *Etude Morphologique, Radiographique et Microdurométrique. Recueil de Médecine Vétérinaire*, 167, 121–127.

Miller, ER, Lei, X & Ullrey, DE 1991. Trace elements in animal nutrition. *In:* Mortvedt, JJ (ed.) *Micronutrients in Agriculture*, 2nd edn. Madison, WI: Soil Science Society of America, 593–662.

Miller, SC, Bowman, BM, & Jee, WSS 1995. Available animal models of osteopenia—small and large. *Bone*, 17, S117–S123.

Mittlefehldt, DW 1999. Calcium. *In:* Marshall, CP & Fairbridge, RW (eds) *Encyclopedia of Earth Sciences*. Dordrecht: Kluwer Academic Publishers, 51.

Moore, AMT 1998. The interaction between people and environment at the early village of Abu Hureyra on the Euphrates. *Travaux de la Maison de l'Orient Méditerranéen*, 28, 131–138.

Moore, AMT 2000a. The excavation of Abu Hureyra 2. *In:* Moore, A, Hillman, G & Legge, A (eds) *Village on the Euphrates: From Foraging to Farming at Abu Hureyra*. Oxford: Oxford University Press, 189–259.

Moore, AMT 2000b. The excavation of Abu Hureyra 1. *In:* Moore, A, Hillman, G & Legge, A (eds) *Village on the Euphrates: From Foraging to Farming at Abu Hureyra*. Oxford: Oxford University Press, 105–131.

Moore, AMT, Hillman, GC & Legge, AJ 2000. *Village on the Euphrates: From Foraging to Farming at Abu Hureyra.* Oxford: Oxford University Press.

Moore, KM, Murray, ML & Schoeninger, MJ 1989. Dietary reconstruction from bones treated with preservatives. *Journal of Archaeological Science*, 16, 437–446.

Moran, NC & O'Connor, TP 1994. Age attribution in domestic sheep by skeletal and dental maturation: a pilot study of available sources. *International Journal of Osteoarchaeology*, 4, 267–285.

Moreno, EC & Aoba, T 1987. Calcium binding in enamel fluid and driving force for enamel mineralization in the secretory stage of amelogenesis. *Advances in Dental Research*, 1, 245–251.

Mukasa-Mugerwa, E & Ezaz, Z 1992. Relationship of testicular growth and size to age, body weight and onset of puberty in Menz ram lambs. *Theriogenology*, 38, 979–988.

Nanci, A & Ten Cate, AR 2008. *Ten Cate's Oral Histology: Development, Structure, and Function.* St Louis, MO: Mosby Elsevier.

National Research Council 1977. *Nutrient Requirements of Rabbits*, 2nd edn. Washington, DC: The National Academies.

National Research Council 1985. *Nutrient Requirements of Sheep*, 6th edn. Washington, DC: The National Academies.

National Research Council 1989a. *Nutrient Requirements of Dairy Cattle, Updated*, 6th edn. Washington, DC: The National Academies.

National Research Council 1989b. *Nutrient Requirements of Horses*, 5th edn. Washington, DC: The National Academies.

National Research Council 2000. *Nutrient Requirements of Beef Cattle, Update*, 7th edn. Washington, DC: The National Academies.

National Research Council 2007. *Nutrient Requirements of Small Ruminants*. Washington, DC: The National Academies.

Nelson, DA, Norris, SA & Gilsanz, V 2006. Childhood and adolescence. *In:* Favus, MJ (ed.) *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*. Washington, DC: The American Society for Bone and Mineral Research, 55–63.

Nitsch, EK, Humphrey, LT & Hedges, REM 2011. Using stable isotope analysis to examine the effect of economic change on breastfeeding practices in Spitalfields, London, UK. *American Journal of Physical Anthropology*, 146, 619–628.

O'Connell, TC, Kneale, CJ, Tasevska, N & Kuhnle, GGC 2012. The diet-body offset in human nitrogen isotopic values: a controlled dietary study. *American Journal of Physical Anthropology*, 149, 426–434.

O'Leary, MH 1988. Carbon isotopes in photosynthesis. *Bioscience*, 38, 328–336.

Obregón, F, Arias de Reyna, L & Recuerda, P 1992. Nursing and suckling behaviour in the mouflon. *Ethology, Ecology and Evolution*, 4, 285–291.

Orgeur, P & Signoret, JP 1984. Sexual play and its functional significance in the domestic sheep (*Ovis aries L.*). *Physiology & Behavior*, 33, 111–118.

Ott, SM 2008. Histomorphometric measurements of bone turnover, mineralization, and volume. *Clinical Journal of the American Society of Nephrology*, 3, S151–S156.

Page, BD, Bullen, TD & Mitchell, MJ 2008. Influences of calcium availability and tree species on Ca isotope fractionation in soil and vegetation. *Biogeochemistry*, 88, 1–13.

Park, YW, Juárez, M, Ramos, M & Haenlein, GFW 2007. Physico-chemical characteristics of goat and sheep milk. *Small Ruminant Research*, 68, 88–113.

Passey, BH & Cerling, TE 2002. Tooth enamel mineralization in ungulates: implications for recovering a primary isotopic time-series. *Geochimica et Cosmochimica Acta*, 66, 3225–3234.

Passey, BH, Robinson, TF, Ayliffe, LK, Cerling, TE, Sponheimer, M, Dearing, MD, Roeder, BL & Ehleringer, JR 2005. Carbon isotope fractionation between diet, breath CO_2, and bioapatite in different mammals. *Journal of Archaeological Science*, 32, 1459–1470.

Payne, S 1973. Kill-off patterns in sheep and goats: the mandibles from Asvan Kale. *Anatolian Studies*, 23, 281–303.

Pedrosa, S, Uzun, M, Arranz, J-J, Gutiérrez-Gil, B, Primitivo, FS & Bayón, Y 2005. Evidence of three maternal lineages in near eastern sheep supporting multiple domestication events. *Proceedings of the Royal Society B: Biological Sciences*, 272, 2211–2217.

Pellegrini, M, Lee-Thorp, JA & Donahue, RE 2011. Exploring the variation of the δ^{18}Op and δ^{18}Oc relationship in enamel increments. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 310, 71–83.

Peng, JB, Brown, EM & Hediger, MA 2003. Epithelial Ca^{2+} entry channels: transcellular Ca^{2+} transport and beyond. *Journal of Physiology*, 551, 729–740.

Pereira, F, Davis, SJ, Pereira, L, McEvoy, B, Bradley, DG & Amorim, A 2006. Genetic signatures of a Mediterranean influence in Iberian Peninsula sheep husbandry. *Molecular Biology and Evolution*, 23, 1420–1426.

Peterlik, M & Stoeppler, M 2004. Calcium. *In:* Merian, E, Anke, M, Ihnat, M & Stoeppler, M (eds) *Elements and their Compounds in the Environment*. Weinheim: Wiley-Vch, 599–618.

Peters, J, Helmer, D, von den Driesch, A & Saña Segui, M 1999. Early animal husbandry in the Northern Levant. *Paléorient*, 25, 27–48.

Pietruszka, AJ & Reznik, AD 2008. Identification of a matrix effect in the MC-ICP-MS due to sample purification using ion exchange resin: an isotopic case study of molybdenum. *International Journal of Mass Spectrometry*, 270, 23–30.

Pipia, A, Ciuti, S, Grignolio, S, Luchetti, S, Madau, R & Apollonio, M 2008. Influence of sex, season, temperature and reproductive status on daily activity patterns in Sardinian mouflon (*Ovis orientalis musimon*). *Behaviour*, 145, 1723–1745.

Polák, J & Frynta, D 2009. Sexual size dimorphism in domestic goats, sheep, and their wild relatives. *Biological Journal of the Linnean Society*, 98, 872–883.

Popkin, PR, Baker, P, Worley, F, Payne, S & Hammon, A 2012. The Sheep Project. I. Determining skeletal growth, timing of epiphyseal fusion and morphometric variation in unimproved Shetland sheep of known age, sex, castration status and nutrition. *Journal of Archaeological Science*, 39, 1775–1792.

Post, DM, Layman, CA, Arrington, DA, Takimoto, G, Quattrochi, J & Montana, CG 2007. Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. *Oecologia*, 152, 179–189.

Quirke, JF, Stabenfeldt, GH & Bradford, GE 1985. Onset of puberty and duration of the breeding season in Suffolk, Rambouillet, Finnish Landrace, Dorset and Finn-Dorset ewe lambs. *Journal of Animal Science*, 60, 1463–1471.

Réale, D, Bousses, P & Chapuis, JL 1999. Nursing behaviour and mother–lamb relationships in mouflon under fluctuating population densities. *Behavioural Processes*, 47, 81–94.

Reynard, LM. 2007. *The Application of stable hydrogen isotope analysis to the study of ancient diet.* DPhil, University of Oxford.

Reynard, LM, Henderson, GM & Hedges, REM 2010. Calcium isotope ratios in animal and human bone. *Geochimica et Cosmochimica Acta*, 74, 3735–3750.

Reynard, LM, Henderson, GM & Hedges, REM 2011a. Calcium isotopes in archaeological bones and their relationship to dairy consumption. *Journal of Archaeological Science*, 38, 657–664.

Reynard, LM, Henderson, GM & Hedges, REM 2011b. Erratum to Reynard, L.M., Henderson, G.M. and Hedges, R.E.M. (2010) Calcium isotopes in human and animal bone, Geochimica et Cosmochimica Acta 74, 3735–3750. *Geochimica et Cosmochimica Acta*, 75, 2234–2235.

Reynard, LM, Pearson, JA, Henderson, GM & Hedges, REM 2013. Calcium isotopes in juvenile milk-consumers. *Archaeometry*, 55, 946–957.

Richards, MP, Mays, S & Fuller, BT 2002. Stable carbon and nitrogen isotope values of bone and teeth reflect weaning age at the Medieval Wharram Percy site, Yorkshire, UK. *American Journal of Physical Anthropology*, 119, 205–210.

Rizzoli, R & Bonjour, J-P 1999. Physiology of calcium and phosphate homeostasis. *In:* Seibel, MJ, Robins, SP & Bilezikian, JP (eds) *Dynamics of Bone and Cartilage Metabolism*. San Diego, CA: Academic Press, 345–357.

Robbins, CT 1993. *Wildlife Feeding and Nutrition.* London: Academic Press, Inc.

Robey, PG & Boskey, AL 2006. Extracellular matrix and biomineralization of bone. *In:* Favus, MJ (ed.) *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*, 6th edn. Washington, DC: The American Society for Bone and Mineral Research, 12–19.

Robinson, C, Connell, S, Kirkham, J, Shore, R & Smith, A 2004. Dental enamel—a biological ceramic: regular substructures in enamel hydroxyapatite crystals revealed by atomic force microscopy. *Journal of Materials Chemistry*, 14, 2242–2248.

Robinson, C, Kirkham, J & Hallsworth, AS 1988. Volume distribution and concentration of protein, mineral and water in developing bovine enamel. *Archives of Oral Biology*, 33, 159–162.

Russell, WA, Papanastassiou, DA & Tombrello, TA 1978. Ca isotope fractionation on the Earth and other solar system materials. *Geochimica et Cosmochimica Acta*, 42, 1075–1090.

Salhab, SA, Zarkawi, M, Wardeh, MF, Al-Masri, MR & Kassem, R 2001. Development of testicular dimensions and size, and their relationship to age, body weight and parental size in growing Awassi ram lambs. *Small Ruminant Research*, 40, 187–191.

Salque, M, Bogucki, PI, Pyzel, J, Sobkowiak-Tabaka, I, Grygiel, R, Szmyt, M & Evershed, RP 2013. Earliest evidence for cheese making in the sixth millennium BC in northern Europe. *Nature*, 493, 522–525.

Sanford, P. 1992. The small intestine. *In:* Hobsley, M, Saunders, KB & FitzSimons, JT (eds.) *Digestive System Physiology*. 2nd ed. London: Edward Arnold, 113–202.

Sansom, BF & Gleed, PT 1981. The ingestion of sow's faeces by suckling piglets. *British Journal of Nutrition*, 46, 451–456.

Santiago-Moreno, J, Gomez-Brunet, A, Gonzalez-Bulnes, A, Toledano-Diaz, A, Malpaux, B & Lopez-Sebastian, A 2005a. Differences in reproductive pattern between wild and domestic rams are not associated with inter-specific annual variations in plasma prolactin and melatonin concentrations. *Domestic Animal Endocrinology*, 28, 416–429.

Santiago-Moreno, J, Gomez-Brunet, A, Toledano-Díaz, A, González-Bulnes, A, Picazo, RA & López-Sebastián, A 2005b. Influence of age on the relationship between annual changes in horn growth rate and prolactin secretion in the European mouflon (*Ovis gmelini musimon*). *Animal Reproduction Science*, 85, 251–261.

Santiago-Moreno, J, López-Sebastián, A, González-Bulnes, A, Gómez-Brunet, A & Chemineau, P 2000. Seasonal changes in ovulatory activity, plasma prolactin, and melatonin concentrations, in Mouflon (*Ovis gmelini musimon*) and Manchega (*Ovis aries*) ewes. *Reproduction Nutrition Development*, 40, 421–430.

Schmitt, A-D, Chabaux, F & Stille, P 2003. The calcium riverine and hydrothermal isotopic fluxes and the oceanic calcium mass balance. *Earth and Planetary Science Letters*, 213, 503–518.

Schmitt, A-D & Stille, P 2005. The source of calcium in wet atmospheric deposits: Ca-Sr isotope evidence. *Geochimica et Cosmochimica Acta*, 69, 3463–3468.

Schoeninger, MJ & DeNiro, MJ 1984. Nitrogen and carbon isotopic composition of bone collagen from marine and terrestrial animals. *Geochimica et Cosmochimica Acta*, 48, 625–639.

Schröder, B, Rittmann, I, Pfeffer, E & Breves, G 1997. In vitro studies on calcium absorption from the gastrointestinal tract in small ruminants. *Journal of Comparative Physiology B*, 167, 43–51.

Schröder, B, Vössing, S & Breves, G 1999. In vitro studies on active calcium absorption from ovine rumen. *Journal of Comparative Physiology B*, 169, 487–494.

Schubert, BW, Ungar, PS & DeSantis, LRG 2010. Carnassial microwear and dietary behaviour in large carnivorans. *Journal of Zoology*, 280, 257–263.

Schümann, K & Elsenhans, B 2004. The iron group. *In:* Merian, E, Anke, M, Ihnat, M & Stoeppler, M (eds) *Elements and their Compounds in the Environment*. Weinheim: Wiley-Vch, 811–824.

Schurr, MR 1997. Stable nitrogen isotopes as evidence for the age of weaning at the Angel site: a comparison of isotopic and demographic measures of weaning age. *Journal of Archaeological Science*, 24, 919–927.

Schwarcz, HP 2000. Some biochemical aspects of carbon isotopic paleodiet studies. *In:* Ambrose, SH & Katzenberg, MA (eds) *Biogeochemical Approaches to Paleodietary Analysis*. New York, NY: Kluwer Academic/Plenum, 189–209.

Severinghaus, JP & Brook, EJ 1999. Abrupt climate change at the end of the last glacial period inferred from trapped air in polar ice. *Science*, 286, 930–934.

Severinghaus, JP, Sowers, T, Brook, EJ, Alley, RB & Bender, ML 1998. Timing of abrupt climate change at the end of the Younger Dryas interval from thermally fractionated gases in polar ice. *Nature*, 391, 141–146.

Shacklette, HT & Boerngen, JG 1984. *Element Concentrations in Soils and other Surficial Materials of the Conterminous United States. Professional Paper, 1270.* Washington DC: US Geological Survey.

Sharp, P 2005. Minerals and trace elements. *In:* Geissler, C & Powers, H (eds) *Human Nutrition*, 11th edn. London: Elsevier Ltd, 231–249.

Sharp, Z 2007. *Principles of Stable Isotope Geochemistry.* Upper Saddle River, NJ: Pearson Education Inc.

Shaw, KL, Nolan, JV, Lynch, JJ, Coverdale, OR & Gill, HS 1995. Effects of weaning, supplementation and gender on acquired immunity to *Haemonchus contortus* in lambs. *International Journal for Parasitology*, 25, 381–387.

Sherratt, AG 1981. Plough and pastoralism: aspects of the secondary products revolution. *In:* Hodder, I, Isaac, G & Hammond, N (eds) *Pattern of the Past: Studies in Honour of David Clarke*. Cambridge: Cambridge University Press, 261–305.

Sherratt, AG 1983. The secondary exploitation of animals in the old world. *World Archaeology*, 15, 90–104.

Shibukawa, Y, Kang, KJ, Kinjo, TG, Szerencsei, RT, Altimimi, HF, Pratikhya, P, Winkfein, RJ & Schnetkamp, PPM 2007. Structure–function relationships of the NCKX2 Na^{+}/Ca^{2+}-K^{+} exchanger. *In:* Herchuelz, A, Blaustein, MP, Lytton, J & Philipson, KD (eds) *Sodium-Calcium Exchange and the Plasma Membrane Ca^{2+}-ATPase in Cell Function, 5th International Conference*. New York, NY: Annals of the New York Academy of Sciences, 16–28.

Simmer, JP & Fincham, AG 1995. Molecular mechanisms of dental enamel formation. *Critical Reviews in Oral Biology & Medicine*, 6, 84–108.

Simpson, LA, Hearn, R, Merson, S & Catterick, T 2005. A comparison of double-focusing sector field ICP-MS, ICP-OES and octopole collision cell ICP-MS for the high-accuracy determination of calcium in human serum. *Talanta*, 65, 900–906.

Skulan, J & DePaolo, DJ 1999. Calcium isotope fractionation between soft and mineralized tissues as a monitor of calcium use in vertebrates. *Proceedings of the National Academy of Sciences, USA*, 96, 13709–13713.

Skulan, J, DePaolo, DJ & Owens, TL 1997. Biological control of calcium isotopic abundances in the global calcium cycle. *Geochimica et Cosmochimica Acta*, 61, 2505–2510.

Smith, CE 1998. Cellular and chemical events during enamel maturation. *Critical Reviews in Oral Biology & Medicine*, 9, 128–161.

Specker, BL 2002. Bone mineral changes during pregnancy and lactation. *Endocrine*, 17, 49–53.

Stack, M, Chaplin, M & Clark, J 2002. Differentiation of prion protein glycoforms from naturally occurring sheep scrapie, sheep-passaged scrapie strains (CH1641 and SSBP1), bovine spongiform encephalopathy (BSE) cases and Romney and Cheviot breed sheep experimentally inoculated with BSE using two monoclonal antibodies. *Acta Neuropathologica*, 104, 279–286.

Starkovich, BM & Stiner, MC 2009. Hallan Çemi Tepesi: high-ranked game exploitation alongside intensive seed processing at the Epipaleolithic-Neolithic transition in southeastern Turkey. *Anthropozoologica*, 44, 41–61.

Stephenson, DC & Brown, RD 1984. Calcium kinetics in male white-tailed deer. *The Journal of Nutrition*, 114, 1014–1024.

Stevens, CE & Hume, ID 1995. *Comparative Physiology of the Vertebrate Digestive System.* Cambridge: Cambridge University Press.

Stevens, RE, Balasse, M & O'Connell, TC 2011. Intra-tooth oxygen isotope variation in a known population of red deer: implications for past climate and seasonality reconstructions. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 301, 64–74.

Stokes, DL & Green, NM 2003. Structure and function of the calcium pump. *Annual Review of Biophysics and Biomolecular Structure*, 32, 445–468.

Strehler, EE, Caride, AJ, Filoteo, AG, Xiong, Y, Penniston, J, T. & Enyedi, A 2007. Plasma membrane Ca^{2+} ATPase as dynamic regulators of cellular calcium handling. *In:* Herchuelz, A, Blaustein, MP, Lytton, J & Philipson, KD (eds) *Sodium-Calcium Exchange and the Plasma Membrane Ca^{2+}-ATPase in Cell Function, 5th International Conference*. New York, NY: Annals of the New York Academy of Sciences, 226–236.

Suga, S 1982. Progressive mineralization pattern of developing enamel during the maturation stage. *Journal of Dental Research*, 61, 1532–1542.

Suleiman, A, Okine, E & Goonewardene, LA 1997. Relevance of National Research Council feed composition tables in Alberta. *Canadian Journal of Animal Science*, 77, 197–203.

Sulzman, EW 2007. Stable isotope chemistry and measurement: a primer. *In:* Michener, R & Lajtha, K (eds) *Stable Isotopes in Ecology and Environmental Science*, 2nd edn. Oxford: Blackwell Publishing, 1–21.

Taberlet, P, Valentini, A, Rezaei, HR, Naderi, S, Pompanon, F, Negrini, R & Ajmone-Marsan, P 2008. Are cattle, sheep, and goats endangered species? *Molecular Ecology*, 17, 275–284.

Takano, Y 1995. Enamel mineralization and the role of ameloblasts in calcium transport. *Connective Tissue Research*, 33, 127–137.

Takano, Y, Crenshaw, MA & Reith, EJ 1982. Correlation of ^{45}Ca incorporation with maturation ameloblast morphology in the rat incisor. *Calcified Tissue International*, 34, 211–213.

Talmage, RV & Elliott, JR 1958. Removal of calcium from bone as influenced by the parathyroids. *Endocrinology*, 62, 717–722.

Tang, VW & Goodenough, DA 2003. Paracellular ion channel at the tight junction. *Biophysical Journal*, 84, 1660–1673.

Tapio, M, Marzanov, N, Ozerov, M, Ćinkulov, M, Gonzarenko, G, Kiselyova, T, Murawski, M, Viinalass, H & Kantanen, J 2006. Sheep mitochondrial DNA variation in European, Caucasian, and Central Asian areas. *Molecular Biology and Evolution*, 23, 1776–1783.

Thirlwall, MF & Anczkiewicz, R 2004. Multidynamic isotope ratio analysis using MC–ICP–MS and the causes of secular drift in Hf, Nd and Pb isotope ratios. *International Journal of Mass Spectrometry*, 235, 59–81.

Thomas, ER, Wolff, EW, Mulvaney, R, Steffensen, JP, Johnsen, SJ, Arrowsmith, C, White, JWC, Vaughn, B & Popp, T 2007. The 8.2 ka event from Greenland ice cores. *Quaternary Science Reviews*, 26, 70–81.

Tieszen, LL 1991. Natural variations in the carbon isotope values of plants: implications for archaeology, ecology, and paleoecology. *Journal of Archaeological Science*, 18, 227–248.

Tieszen, LL, Boutton, TW, Tesdahl, KG & Slade, NA 1983. Fractionation and turnover of stable carbon isotopes in animal tissues: implications for $\delta^{13}C$ analysis of diet. *Oecologia*, 57, 32–37.

Tipper, ET, Galy, A & Bickle, MJ 2006. Riverine evidence for a fractionated reservoir of Ca and Mg on the continents: implications for the oceanic Ca cycle. *Earth and Planetary Science Letters*, 247, 267–279.

Tipper, ET, Galy, A & Bickle, MJ 2008. Calcium and magnesium isotope systematics in rivers draining the Himalaya-Tibetan-Plateau region: lithological or fractionation control? *Geochimica et Cosmochimica Acta*, 72, 1057–1075.

Tishkoff, SA, Reed, FA, Ranciaro, A, Voight, BF, Babbitt, CC, Silverman, JS, Powell, K, Mortensen, HM, Hirbo, JB, Osman, M, Ibrahim, M, Omar, SA, Lema, G, Nyambo, TB, Ghori, J, Bumpstead, S, Pritchard, JK, Wray, GA & Deloukas, P 2006. Convergent adaptation of human lactase persistence in Africa and Europe. *Nature Genetics*, 39, 31–40.

Tornero, C, Bălăşescu, A, Ughetto-Monfrin, J, Voinea, V & Balasse, M 2013. Seasonality and season of birth in early Eneolithic sheep from Cheia (Romania): methodological advances and implications for animal economy. *Journal of Archaeological Science*, 40, 4039–4055.

Towers, J, Jay, M, Mainland, I, Nehlich, O & Montgomery, J 2011. A calf for all seasons? The potential of stable isotope analysis to investigate prehistoric husbandry practices. *Journal of Archaeological Science*, 38, 1858–1868.

Tremaine, WJ, MD, Newcomer, AD, MD, Riggs, BL, MD & McGill, DB, MD 1986. Calcium absorption from milk in lactase-deficient and lactase-sufficient adults. *Digestive Diseases and Sciences*, 31, 376–378.

Trigger, BG 1989. *A History of Archaeological Thought.* Cambridge: Cambridge University Press.

Tsukita, S & Furuse, M 2000. Epithelial transport and barrier function: pathomechanisms in gastrointestinal disorders. *Annals of the New York Academy of Sciences*, 915, 129–135.

Ullrey, DE, Youatt, WG, Johnson, HE, Fay, LD, Schoepke, BL, Magee, WT & Keahey, KK 1973. Calcium requirements of weaned white-tailed deer fawns. *Journal of Wildlife Management*, 37, 187–194.

United States Department of Agriculture 2011. *National Nutrient Database for Standard Reference.* Available at: http://ndb.nal.usda.gov.

Upex, B 2009. *Enamel hypoplasia in archaeological and modern caprine populations: the development and application of a new methodological approach.* PhD, University of Durham. Available at: http://etheses.dur.ac.uk/182/.

Upex, B & Dobney, K 2012. Dental enamel hypoplasia as indicators of seasonal environmental and physiological impacts in modern sheep populations: a model for interpreting the zooarchaeological record. *Journal of Zoology*, 287, 259–268.

van der Merwe, NJ & Medina, E 1991. The canopy effect, carbon isotope ratios and foodwebs in Amazonia. *Journal of Archaeological Science*, 18, 249–259.

van der Plicht, J, van Geel, B, Bohncke, SJP, Bos, JAA, Blaauw, M, Speranza, AOM, Muscheler, R & Björck, S 2004. The preboreal climate reversal and a subsequent solar-forced climate shift. *Journal of Quaternary Science*, 19, 263–269.

VanHouten, J, Dann, P, McGeoch, G, Brown, EM, Krapcho, K, Neville, M & Wysolmerski, JJ 2004. The calcium-sensing receptor regulates mammary gland parathyroid hormone-related protein production and calcium transport. *Journal of Clinical Investigation*, 113, 598–608.

VanHouten, JN & Wysolmerski, JJ 2007. Transcellular calcium transport in mammary epithelial cells. *Journal of Mammary Gland Biology and Neoplasia*, 12, 223–235.

Vargas, S, Larbi, A & Sanchez, M 2007. Analysis of size and conformation of native Creole goat breeds and crossbreds used in smallholder agrosilvopastoral systems in Puebla, Mexico. *Tropical Animal Health and Production*, 39, 279–286.

Vennekens, R, Owsianik, G & Nilius, B 2008. Vanilloid transient receptor potential cation channels: an overview. *Current Pharmaceutical Design*, 14, 18–31.

Vigne, J-D & Helmer, D 2007. Was milk a "secondary product" in the Old World Neolithisation process? Its role in the domestication of cattle, sheep and goats. *Anthropozoologica*, 42, 9–40.

Von Blanckenburg, F, Von Wirén, N, Guelke, M, Weiss, DJ & Bullen, TD 2009. Fractionation of metal stable isotopes by higher plants. *Elements*, 5, 375–380.

Wadhwa, DR & Care, AD 2000. The absorption of calcium ions from the ovine reticulo-rumen. *Journal of Comparative Physiology B*, 170, 581–588.

Walsh, JN 1997. Inductively coupled plasma-atomic emission spectrometry (ICP-AES). *In:* Gill, R (ed.) *Modern Analytical Geochemistry: An Introduction to Quantitative Chemical Analysis for Earth, Environmental and Materials Scientists*. Harlow: Addison Wesley Longman, Ltd, 41–66.

Watson, DL & Gill, HS 1991. Effect of weaning on antibody responses and nematode parasitism in Merino lambs. *Research in Veterinary Science*, 51, 128–132.

Wedepohl, KH 1995. The composition of the continental crust. *Geochimica et Cosmochimica Acta*, 59, 1217–1232.

Weinreb, MM & Sharav, DMD 1964. Tooth development in sheep. *American Journal of Veterinary Research*, 25, 891–908.

Whitehead, DC 2000. *Nutrient Elements in Grassland: Soil-Plant-Animal Relationships.* Wallingford: CABI Publishing.

Whitehead, DC & Jones, EC 1969. Nutrient elements in the herbage of white clover, red clover, lucerne and sainfoin. *Journal of Science of the Food and Agriculture*, 20, 584–591.

Wieser, ME, Buhl, D, Bouman, C & Schwieters, J 2004. High precision calcium isotope ratio measurements using a magnetic sector multiple collector inductively coupled plasma mass spectrometer. *Journal of Analytical Atomic Spectrometry*, 19, 844–851.

Witter, K & Míšek, I 1999. Time programme of the early tooth development in the domestic sheep (*Ovis aries*, Ruminantia). *Acta Veterinaria Brno*, 68, 3–8.

Wombacher, F, Eisenhauer, A, Heuser, A & Weyer, S 2009. Separation of Mg, Ca and Fe from geological reference materials for stable isotope ratio analyses by MC-ICP-MS and double-spike TIMS. *Journal of Analytical Atomic Spectrometry*, 24, 627–636.

Wright, C 2012. A Weighty Matter. *In:* Feulner, F, Gerbault, P, Gillis, R, Hollund, H, Howcroft, R, Leonardi, M, Liebert, A, Raghavan, M, Salque, M, Sverrisdóttir, O, Teasdale, M, van Doorn, N & Wright, C (eds) *May Contain Traces of Milk: Investigating the Role of Dairy Farming and Milk Consumption in the European Neolithic*. Heslington: University of York, 159–174.

Wright, LE & Schwarcz, HP 1999. Correspondence between stable carbon, oxygen and nitrogen isotopes in human tooth enamel and dentine: infant diets at Kaminaljuyú. *Journal of Archaeological Science*, 26, 1159–1170.

Yamakoshi, Y, Tanabe, T, Oida, S, Hu, C-C, Simmer, JP & Fukae, M 2001. Calcium binding of enamel proteins and their derivatives with emphasis on the calcium-binding domain of porcine sheathlin. *Archives of Oral Biology*, 46, 1005–1014.

Yokel, RA 2004. Aluminum. *In:* Merian, E, Anke, M, Ihnat, M & Stoeppler, M (eds) *Elements and Their Compounds in the Environment, Occurrence, Analysis and Biological Relevance*. Weinheim: Wiley-Vch, 635–658.

Yuan, S, Wu, X, Liu, K, Guo, Z, Cheng, X, Pan, Y & Wang, J 2007. Removal of contaminants from oracle bones during sample pretreatment. *Radiocarbon*, 49, 211–216.

Zamiri, MJ, Qotbi, A & Izadifard, J 2001. Effect of daily oxytocin injection on milk yield and lactation length in sheep. *Small Ruminant Research*, 40, 179–185.

Zazzo, A, Balasse, M, Passey, BH, Moloney, AP, Monahan, FJ & Schmidt, O 2010. The isotope record of short-and long-term dietary changes in sheep tooth enamel: implications for quantitative reconstruction of paleodiets. *Geochimica et Cosmochimica Acta*, 74, 3571–3586.

Zazzo, A, Bendrey, R, Vella, D, Moloney, AP, Monahan, FJ & Schmidt, O 2012. A refined sampling strategy for intra-tooth stable isotope analysis of mammalian enamel. *Geochimica et Cosmochimica Acta*, 84, 1–13.

Zeder, MA 2008. Domestication and early agriculture in the Mediterranean Basin: origins, diffusion, and impact. *Proceedings of the National Academy of Sciences, USA*, 105, 11597–11604.

Zeder, MA 2009. The Neolithic macro-(r)evolution: macroevolutionary theory and the study of culture change. *Journal of Archaeological Research*, 17, 1–63.

Zeder, MA 2011. The origins of agriculture in the Near East. *Current Anthropology*, 52, S221–S235.

Zeder, MA, Vigne, JD & Helmer, D 2005. A view from the Zagros: new perspectives on livestock domestication in the Fertile Crescent. *In:* Vigne, JD, Peters, J & Helmer., D (eds) *The First Steps of Animal Domestication*. Oxford: Oxbow Books, 125–146.

Zohary, D, Tchernov, E & Horwitz, L 1998. The role of unconscious selection in the domestication of sheep and goats. *Journal of Zoology*, 245, 129–135.

Zhu, P & MacDougall, JD 1998. Calcium isotopes in the marine environment and the oceanic calcium cycle. *Geochimica et Cosmochimica Acta*, 62, 1691–1698.

APPENDIX A

MAMMAL CALCIUM HOMEOSTASIS

The purpose of this appendix is to provide the details of calcium homeostasis that were only touched on in Chapter 2. Understanding calcium homeostasis is an important first step in beginning to understand calcium isotope systematics. The kidneys and blood do not play a direct part in this research, but they do affect gastrointestinal (GI) tract calcium absorption from the diet, calcium absorption/release from bone and calcium levels in milk. The kidneys are a (small) source of calcium isotope fractionation (see Chapter 2), which means consideration of their involvement in calcium homeostatic is worthwhile. Obviously, calcium in the diet, dental enamel, bone and milk are integral parts of this research, and a background in general calcium homeostasis is essential for trying to understand sources of calcium isotope differences created by physiological processes. Although dental enamel does not participate in calcium homeostasis directly, it is subject to the influence of homeostasis during mineralisation and maturation.

A.1 Calcium and herbivores

Much of what is discussed in this section involves understanding the interactions of living organisms within an ecosystem and how these interactions influence dietary calcium supplies. Mammalian herbivores and their plant 'prey' hold important positions in an ecosystem because they both liberate and absorb nutrients that would normally be inaccessible to other living organisms (Crawley 1983, Jefferies 1999). Plants use sunlight to generate their own food in the form of sugars as well as take in nutrients from the soil (Alloway 2005).

With regard to calcium uptake from soils by plants, the most critical factors are that the supply of calcium is adequate to meet plant physiological needs, replenishable and provides for a whole growing season (Whitehead 2000). Generally this is not an issue

because of the large amount of calcium contained in the Earth's crust, which helps replenish soil supplies. In fact calcium (3.9%) is the fifth most plentiful element after oxygen (47.2%), silicon (28.8%), aluminium (8.0%) and iron (4.7%) by weight, and is found in more than 700 magmatic, sedimentary and metamorphic rock minerals, all containing between 1.0% and 3.63% calcium (Barber 1995, Wedepohl 1995, D'Haese et al. 2004, Peterlik and Stoeppler 2004, Schümann and Elsenhans 2004, Yokel 2004). Calcium enters plants and subsequently food systems of other living organisms through calcium salts and calcium carbonate (Whitehead 2000, Alloway 2005) (Table A.1). Table A.2 provides information on the amount of calcium available in feed crops and wild plants, as well as dietary calcium requirements for some herbivores, including domestic sheep.

Table A.1: Calcium mineral, soil and water sources in the environment

Calcium sources	mg/kg or ppm of dry matter	mg/L or ppm of solution	Reference
Earth's crust	38,500	–	Wedepohl (1995)
Average of conterminous soils (USA)	9200	–	Shacklette and Boerngen (1984)
Limestones (agricultural soils, USA)	179,800–397,600	–	Chichilo and Whittaker (1961) , Adriano (1986)
Carbonates	302,300		Mason and Moore (1982)
Basalts	~81,000	–	Mittlefehldt (1999)
Sandstones	39,100	–	Mason and Moore (1982)
Igneous rocks	36,300		Mason and Moore (1982)
Shales	22,100	–	Mason and Moore (1982)
Granites	2000–18,000	–	Mittlefehldt (1999)
River water (averages)	–	13.0 and 15.0	Berner and Berner (1987) , Walsh (1997)
Seawater	–	400.78	Berner and Berner (1996)
Rainfall (sea salt, soil dust and pollution)	–	0.1–3.0	Berner and Berner (1996)

Table A.2: Calcium availability in animal feeds and wild plants as well as some herbivore calcium requirements for maintenance, growth, pregnancy and lactation

Calcium in plant food	mg/kg or ppm	Mean	Reference
Grass/legume herbage (USA)	300–27,300	8600	Adams (1975)
Timothy (Finland)	1000–6700	2600	Kähäri and Nissinen (1978)
Perennial ryegrass	4000–8700	–	Fleming (1963), Whitehead (2000)
White clover	10,000–20,000	–	Whitehead (2000)
Cocksfoot	5700	–	Fleming (1963)
Red clover	18,400	–	Whitehead and Jones (1969)
Lucerne hay	13,100–18,200	16,400	Whitehead and Jones (1969), Berger (1995), Suleiman et al. (1997)
Sainfoin	9400	–	Whitehead and Jones (1969)
Barley (USA)	700	–	Berger (1995)
Barley (Alberta, Canada)	100–1700	600	Suleiman et al. (1997)
Maize silage (USA)	2500	–	Berger (1995)
Maize silage (Alberta, Canada)	900– 6200	2600	Suleiman et al. (1997)
Forbs, wild deer food (Texas, USA)	6100–35,500	–	Barnes et al. (1990)
Browse, wild deer food (Texas, USA)	1300–60,300	–	Barnes et al. (1990)
Grass, wild deer food (Texas, USA)	1700–5700	–	Barnes et al. (1990)
Cacti, wild deer food (Texas, USA)	18,500–52,100	–	Barnes et al. (1990)
Calcium requirements for some herbivores	**ppm of calcium in the diet**		**Reference**
Beef cattle, growing and finishing bulls	1700–5600		National Research Council (2000)
Beef cattle, pregnancy	1800–3700	–	National Research Council (2000)
Dairy cattle, lactating (20 kg milk/day)	6000	–	National Research Council (1989a), Whitehead (2000)
Ewe, non-lactating	2000–3200	–	National Research Council (1985), Whitehead (2000), National Research Council (2007)
Ewe, lactating	3200–3900	–	National Research Council (1985), Whitehead (2000)
Horses	2400–6800	–	National Research Council (1989b)
Rabbits, growing	4000	–	National Research Council (1977)
White-tail deer	4000		Ullrey et al. (1973)

Ingestion of plants by mammal herbivores makes it possible to pass energy and nutrients to other living organisms. This is done through the consumption of herbivore meat, milk or excreta (waste matter that has been processed microbially in the gut, i.e. urine and faeces) (Harris and Bowman 1980, Crawley 1983, Fransson and Lönnerdal 1983, Jefferies 1999, Park et al. 2007). In the case of dairy animals, there is a transfer and even consolidation of energy and nutrients absorbed from plant foods that are passed on to human consumers in addition to or instead of a dairy animal's offspring (Fransson and Lönnerdal 1983, Black et al. 2002, Park et al. 2007).

A.1.1 Dietary exceptions: geophagy, coprophagy and osteophagy

It should be noted that nutritional deficiencies, including calcium deficiency, may induce the consumption of non-food materials by mammals. In particular, both domestic and wild mammals may eat soil (geophagy or, for humans, pica), faeces (coprophagy) and/or bone (osteophagy) (Sansom and Gleed 1981, Crowell-Davis and Houpt 1985, Hirakawa 2001, Graczyk and Cranfield 2003, Abrahams 2005, Jones 2005, Fish et al. 2007, Bredin et al. 2008). Geophagy is the most common, but herbivores will also eat faeces and bone.

A.1.2 Calcium homeostasis: from ingestion to excretion

Blood, bone and other tissues work together to store, recycle and circulate calcium throughout the body (Lindh 2005). The excretion of sweat, urine and faeces is the final step of the process and is one way by which calcium can be removed from a mammal's system if necessary (Lindh 2005). Mammals' physiological reactions to a calcium imbalance, be it deficiency or excess, are similar for all species, and when chronic may present as rickets/osteomalacia (Figure A.1) or calcinosis (Ewer 1951, Jones 2005).

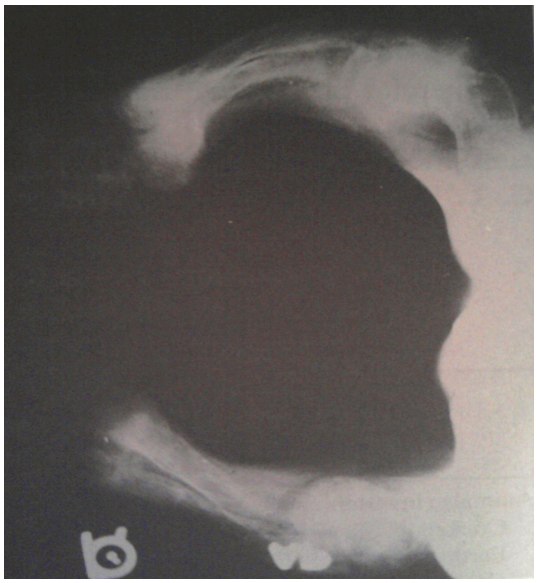

Figure A.1: X-ray of the front limbs of a lion. Calcium deficiency has led to rachitic deformation, poor mineralisation and bone fractures (Robbins 1993).

Epithelial calcium transport regulates Ca^{2+} across epithelia, which is how calcium passes from the environment and into/out of mammals (Hoenderop et al. 2005). Epithelial tissues responsible for calcium transport are found in the GI tract, kidneys, breasts and bone, and, along with blood, are a major agent of transport within the body (VanHouten et al. 2004, Hoenderop et al. 2005, Brown and Jüppner 2006, VanHouten and Wysolmerski 2007). The mechanics of epithelial calcium transport occur as either paracellular or transcellular transport (Bronner 1987).

Although gut morphology is specialised for different mammals (monogastric, ruminant, etc.), the mechanisms of calcium and other nutrient absorption from food is similar for all species (Davenport 1982, Schröder et al. 1999, Wadhwa and Care 2000). Absorption first involves the transition of calcium bound in food to an absorbable form via the combination of mechanical (mastication and stomach mixing) and chemical (hydrochloric acid and enzymatic) breakdown of food into smaller and more easily absorbed components (Sanford 1992, Stevens and Hume 1995). Hydrochloric acid, by forming soluble salts, as well as bile acids and lactose help with calcium absorption after the chyme (partially digested food) leaves the stomach and moves to the small intestine (Davenport 1982, Sanford 1992).

A.2 Mechanics of epithelial calcium transport and absorption in mammals

Calcium in the small intestine will be absorbed by either a paracellular calcium concentration-dependent diffusion process (the calcium travels through spaces between cells) or a vitamin D-dependent transcellular mechanism (the calcium travels through cells) (Bronner 1987, Sanford 1992, Bronner 2003, Hoenderop et al. 2005) (Figure A.2). The type of absorption that a mammal's body will use is dependent on the calcium content of the chyme that passes from the stomach into the small intestine (Bronner 2003). If the chyme produced by a meal is high in calcium, then the calcium will be absorbed by passive transport, the paracellular process, and not the vitamin D-dependent transcellular mechanism (Bronner 2003). The reverse situation results in active transport, the transcellular mechanism, when chyme calcium is low, although a small amount of paracellular diffusion usually also occurs (Bronner 2003). This is the mechanism that has

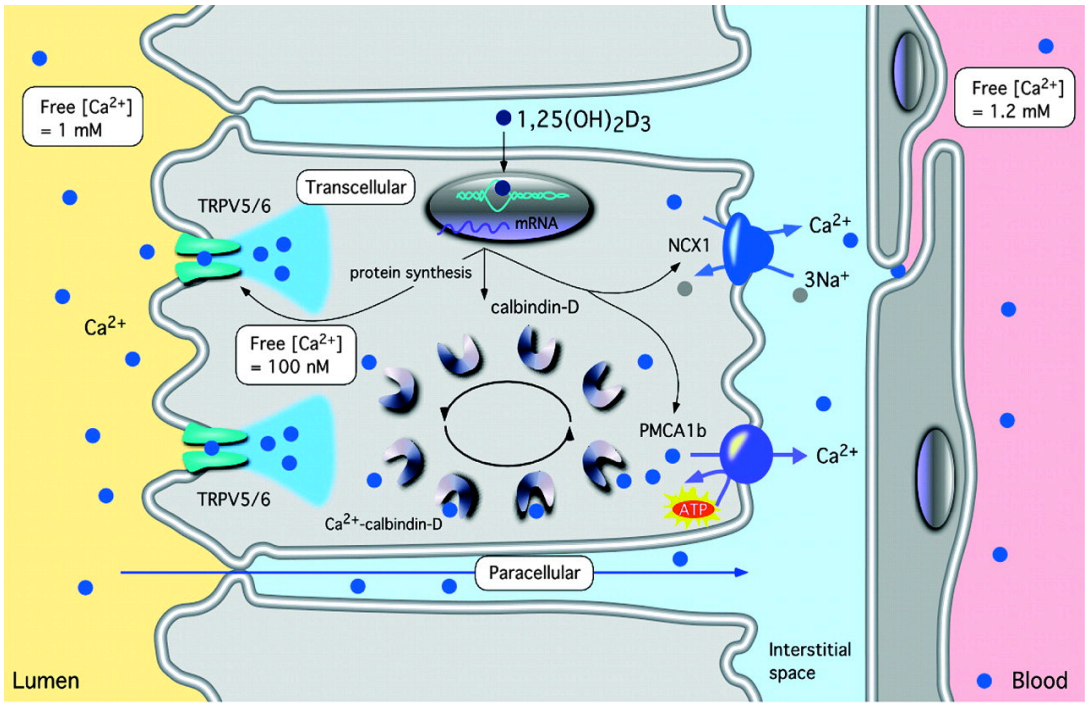

Figure A.2: Summary of the movement of calcium through both paracellular and transcellular pathways. All aspects of calcium absorption that are discussed in this appendix are detailed in this figure, including possible ion channel proteins TRPV 5/6, calbindin-D synthesis from 1,25-dihydroxycholecalciferol, calbindin-D ferrying calcium through the cytosol, extrusion of calcium through Na^+:Ca^{2+} exchange, the Ca^{2+}-ATPase calcium extrusion mechanism and lastly movement of calcium ions into the blood stream (from Hoenderop et al. 2005).

been seen in rumen absorption in goats. The low calcium content of the goat diet triggered the vitamin D-dependent transcellular absorption (Schröder et al. 1997). This mechanism is in fact common for mammals in general and not just goats (Bronner 2003). Most calcium is absorbed during paracellular calcium concentration-dependent diffusion (which occurs along the whole length of the small intestine, i.e. the duodenum, jejunum and ileum) and not the transcellular mechanism (which occurs mainly in the duodenum) (Bronner 1987, Karbach 1992, Bronner 2003).

Currently it is not known whether the process of moving calcium through intestinal epithelial cells, the topic to be discussed next, generates any calcium isotope fraction. There is evidence of a small amount of fractionation occurring in the kidneys (Section A.3.1), possibly due to both paracellular calcium concentration-dependent diffusion processes and vitamin D-dependent transcellular mechanisms. If this is the case, although there is no evidence at present to support the idea, it would have implications for understanding calcium isotope fractionation related to calcium homeostasis, as these types of cells (or very similar), which use these two types of calcium transport, are found in many organs and tissues of the mammalian body.

A.2.1 Paracellular calcium concentration-dependent diffusion process

The small intestine wall is lined with epithelia (continuous layers of cells) that have spaces between the individual cells allowing passive diffusion of ions and molecules (Sanford 1992, Hoenderop et al. 2005). These spaces are very narrow but can adjust to allow more or less diffusion to occur (Sanford 1992). The sugars of glucose (synthesised from glycerol and pyruvate) and galactose (produced by hydrolysis of the milk sugar lactose) interact with the intestinal epithelia, swelling or widening the intercellular space, and promoting increased absorption of calcium (Davenport 1982, Sanford 1992). Milk and most dairy foods are prime sources of both calcium and lactose (Sanford 1992).

The ion routes or paracellular pathways into the intercellular spaces of the epithelia are known as tight junctions, the area where two cells membranes join, forming a near

impermeable barrier to fluid (Hoenderop et al. 2005, Johnson 2007a, Johnson 2007b). Specifically, the tight junction barrier keeps solutes from diffusing through the epithelial cells into the lumen (inner cavity of the intestines), as well as maintaining cell polarity by preventing cell membrane lipids and proteins, both major structural components of the tight junctions, from diffusing into neighbouring cells (Tsukita and Furuse 2000, Tang and Goodenough 2003, Hoenderop et al. 2005). Most important for understanding how calcium moves through the epithelia, is that within these tight junctions are ion channels that facilitate paracellular ion transport (Tang and Goodenough 2003, Hoenderop et al. 2005). Tight junctions have 'biophysical properties with conventional ion channels, including size and charge selectivity, dependency of permeability on ion concentration, competition between permanent molecules, anomalous mole-fraction effects, and sensitivity to pH' (Tang and Goodenough 2003: 1660).

As simple as paracellular diffusion seems, there is an issue that researchers are becoming aware of. The permeability of calcium may be affected indirectly by hormones that alter the electrochemical gradient across the tight junction, and nominally put paracellular diffusion under physiological control (Hoenderop et al. 2005). Future research may find that paracellular diffusion is not entirely as passive as originally believed (Sanford 1992, Tang and Goodenough 2003, Hoenderop et al. 2005, Johnson 2007b).

Paracellular diffusion is the process responsible for calcium absorption when the small intestine is saturated with calcium (>5 mM) (Davenport 1982). However, if the concentration of calcium is between 1 mM and 5 mM then physiological regulation activates vitamin D-dependent transcellular absorption, replacing paracellular diffusion as the primary means of calcium absorption (Davenport 1982). Calcium deficiency (on a meal-by-meal basis) is the predictor of which of the two types of absorption occurs.

A.2.2 Vitamin D-dependent transcellular process

The transcellular process involves calcium movement across at least two plasma membranes and takes place in four stages (Bouillon et al. 2003, Hoenderop et al. 2005): (1)

transfer of luminal calcium through the cell membrane into the enterocyte/intestinal epithelial cell; (2) movement of calcium from the point of entry in the cell through the cytoplasm to the basolateral membrane; (3) calcium extrusion across the basolateral membrane into the interstitial fluid; and (4) movement from the interstitial fluid into the blood plasma (Sanford 1992, Fogh-Andersen et al. 1995, Hoenderop et al. 2005). Figure A.2 provides a visual explanation of this complex process.

A.2.2.1 Transfer of calcium into the enterocyte

The transfer of calcium from the lumen into the intestinal epithelial cells, as seen with paracellular diffusion, does not require energy expenditure (Sanford 1992). However, unlike with paracellular diffusion, there is a strong inwardly directed electrochemical gradient facilitating calcium's movement into the cell (Sanford 1992, Hoenderop et al. 2005). Specifically, the amount of intracellular Ca^{2+} is less than 1 μM, or less than $1/1000^{th}$ of what is found in the blood and extracellular fluid (Sanford 1992). The gradient across the cell membrane between the negative cytosolic side of the cell and the luminal side is approximately 35 mV (Sanford 1992). In addition to the cell membrane gradient, a protein carrier with an affinity for calcium ions is needed (Davenport 1982, Sanford 1992). This protein may be calbindin-D_{9k} and/or TRPV6, which will be discussed in more detail below (Johnson 2007b). Phospholipids and unsaturated fatty acids in the cell membrane are also important for regulating the cell permeability of calcium (Sanford 1992, Ball 2004).

The role that calcium ion channels (proteins that form pores across cell membranes and allow the passage of ions that are drawn into the cell by an electrochemical gradient) play in intestinal epithelial cells is not fully understood at present (Catterall 1988, Peng et al. 2003). Calcium-specific regulating ion channels, known as transient receptor potential vanilloid (TRPV) cation channels, function in kidney epithelia as part of calcium flow in homeostasis and excretion, but have only recently been found to function in small intestine epithelia (Peng et al. 2003, Christakos et al. 2007). There are six members of the TRPV family of ion channels, and 'TRPV5 and TRPV6 are unique compared to all other members

of the TRP family, as they constitute highly Ca^{2+} selective cation channels' (Vennekens et al. 2008: 25). Of the two vitamin D-dependent proteins, TRPV6 is by far the more dominant in the gastrointestinal tract (Hoenderop et al. 2005). TRPV5/6 are probably the ion channelling proteins researchers in the past predicted would be found, as part of the first stage of transcellular calcium transport into the cell, which have only been identified in the past few years (Peng et al. 2003).

A.2.2.2 Movement of calcium through the enterocyte cell

Once in the cell, calcium moves through the cytoplasm to the distal or basal layer of the cell's membrane (basolateral membrane) (Sanford 1992). This transcellular process is facilitated by the vitamin D_3-derived protein calbindin-D_{9k} (Bronner 2003, Christakos et al. 2007). It is calbindin-D_{9k}'s job to ferry calcium through the cell and to the basolateral membrane (Sanford 1992, Bronner 2003, Christakos et al. 2007). Vitamin D_3 is generated in mammals by ultraviolet radiation interacting with 7-dehydrocholesterol in the skin (McDowell 1992, Johnson 2007b). From the skin, vitamin D_3 is transported to the liver, where it is converted to 25-hydroxycholecalciferol (calcifediol) (Sanford 1992, Johnson 2007b). Parathyroid hormone (PTH) working in the kidneys converts calcifediol into 1,25-$(OH)_2$-cholecalciferol [calcitriol or $1,25(OH)_2D_3$] (Sanford 1992, Christakos et al. 2007, Johnson 2007b). Calcitriol is taken up by the nucleus of enterocyte cells, where it is finally converted into the calcium transport protein calbindin-D_{9k} (Bronner 2003, Christakos et al. 2007, Johnson 2007b). Calbindin-D_{9k}, as previously mentioned, may be involved in the cell membrane gates (TRPV6) transporting calcium into the enterocyte, but does not seem to play a role in calcium extrusion out of the cell (Ball 2004, Johnson 2007b).

Additionally, it is likely that the availability of calbindin-D_{9k} in the cell is a rate-limiting factor in the absorption of calcium (Hoenderop et al. 2005, Christakos et al. 2007). This is because the integrity of the enterocyte's own low calcium levels (for example free $[Ca^{2+}]$ in cytosol = 0.0001 mM, compared with free $[Ca^{2+}]$ in the lumen = 1 mM and blood stream = 1.2 mM) needs to be preserved in order for cell homeostasis and functionality to be

maintained (Hoenderop et al. 2005). 'Epithelial cells involved in transcellular Ca^{2+} transport are continuously challenged by substantial Ca^{2+} traffic through the cytosol, while simultaneously maintaining low levels of cytosolic Ca^{2+}' (Hoenderop et al. 2005: 378). Preventing dietary calcium from swamping the cytosol requires the calcium to be 'grabbed' as soon as it enters the cell, possibly even during transport into the cell, where calbindin-D_{9k} may act as a buffer between cell calcium and entrant calcium as well as shepherding calcium to the basolateral layer for removal from the cell (Bouillon et al. 2003, Hoenderop et al. 2005).

A.2.2.3 Transport across the basolateral membrane

Once the calcium is at the basolateral membrane, it is pumped out of the cell through another strong electrochemical gradient by two mechanisms. Unlike the entry into the cell, however, these pumps are working 'uphill' and require energy expenditure (Ball 2004, Hoenderop et al. 2005, Johnson 2007b).

(1) The first mechanism is a sodium/calcium (Na^+:Ca^{2+}) exchanger (NCX), which is dependent on the sodium/potassium (Na^+:K^+) pumps. The Na^+:K^+ pumps supply the necessary sodium ions needed for the calcium exchange that enables calcium movement from the cell to the interstitial fluid and the blood stream (Sanford 1992, Hoenderop et al. 2005, Hilge et al. 2007, Shibukawa et al. 2007). 'Na^+/Ca^{2+}- K^+ exchangers (NCKX) are plasma membrane transporters that are thought to mainly mediate Ca^{2+} extrusion (along with K^+) at the expense of the Na^+ electrochemical gradient' (Altimimi and Schnetkamp 2007: 29). The electrogenic ion exchange across the basolateral membrane involves one calcium ion for every three sodium ions (Baker et al. 1969, Ehara et al. 1989, Hoenderop et al. 2005). Although not yet fully understood, it appears that PTH regulate the ion exchange mechanism and there is a supportive interaction between vitamin D-facilitated absorption of calcium (Davenport 1982, Hoenderop et al. 2005).

(2) The calcium-specific cell extrusion mechanism, working in concordance with the Na^+:Ca^{2+} exchanger, is the plasma membrane Ca^{2+}-ATPase mechanism ($PMCA_{1b}$)

(Sanford 1992, Hoenderop et al. 2005, Johnson 2007b). ATPases are enzymes that catalyse the conversion of adenosine triphosphate (ATP) to adenosine diphosphate (ADP), which in turn releases stored energy that in this instance is used for calcium extrusion across the basolateral membrane and into the interstitial fluid (Ball 2004). 'Active transport of cations is achieved by a large family of ATP-dependent ion pumps, known as P-type ATPases' (Stokes and Green 2003: 445). The $PMCA_{1b}$ has a high affinity for calcium ions and has the job of 'fixing' or maintaining calcium levels in the epithelial cell as well as providing 80% of total Ca^{2+} cell extrusion (Blaustein et al. 2002, Ball 2004, Strehler et al. 2007). 'Plasma membrane Ca^{2+} ATPase ($PMCA_{1b}$) are essential components of the cellular toolkit to regulate and fine-tune cytosolic Ca^{2+} concentrations' (Strehler et al. 2007: 226). $PMCA_{1b}$ appears to be regulated by vitamin D-originating 1,25-dihydroxycholecalciferol, and when experimentally injected has been shown to increase $PMCA_{1b}$ pumping (Johnson and Kumar 1994).

A.2.2.4 Movement of calcium from interstitial fluids to the circulatory system

Calcium ions, once across the basolateral membrane, are contained in the interstitial fluid, which is the fluid that surrounds the cells throughout the body (Abbott 2004). Interstitial fluid is also very similar to plasma and is able to interact freely with blood plasma through the basement membrane surrounding capillaries and veins (Holmes and Donaldson 1969, Fogh-Andersen et al. 1995). 'Sugars, amino acids, ions, and drugs readily diffuse between endothelial cells into the interstitial fluid' and vice versa (Goldstein 1979: 357). This diffusional interaction facilitates the movement of calcium ions into the blood stream and begins the distribution of calcium into the various tissues throughout the body (Holmes and Donaldson 1969, Goldstein 1979, Fogh-Andersen et al. 1995).

A.2.3 Calcium absorption interferents: phytate and oxalates

Successful absorption of calcium has been the focus thus far, but it is also important to discuss the factors that inhibit or prevent calcium from being absorbed. The main inhibitors of calcium absorption are oxalates (oxalic acid), which form a highly insoluble complex

with calcium, and phytates (phytic acid), which, although less chemically binding than oxalates, occur in significant amounts in the intestines (Sharp 2005). Both oxalates and phytates are introduced into the GI tract through vegetable matter such as cereals/grains, spinach and rhubarb (Sanford 1992). Cereals tend to be the source of phytates in the diet, but they also contain an enzyme, phytase, that unbinds calcium from phytates (Sanford 1992). However, the risk of calcium deficiency increases when there is a 0.2 dietary phytate to calcium molar ratio (Sharp 2005).

A.3 Calcium homeostasis: blood, kidneys, lactation, pregnancy, bone and teeth

Calcium, once absorbed into the body, has many functions as part of mammalian homeostasis, which involves the maintenance of metabolic equilibrium and the prevention of disruptive changes in the body. Calcium is very important for heart, thyroid, nerve and muscle function, and is found in skin, hair, blood, muscle and especially bone (Menon et al. 1985, McDonald et al. 1994, Kempson et al. 2003, Hirata et al. 2008, Aloia et al. 2010). The mammalian body is predominantly made of water, carbon and some nitrogen (oxygen ~65%, hydrogen ~10%, carbon ~18% and nitrogen ~3%) but there is also a not insignificant quantity of calcium (1.2–1.5%) compared with other trace elements (Miller et al. 1991, McDonald et al. 1995).

Calcium is certainly the most abundant mineral in mammals, with 98–99% being contained in the skeletal tissue and the majority of the remaining 1–2% being tightly regulated in the blood plasma and extracellular fluid (Sharp 2005, National Research Council 2007). As previously discussed in Section A.2, the amount of calcium contained in the intracellular stores (cytosol) is very small, an example value being ~100 nmol/L in humans (Sharp 2005). The result is that, other than mineralised tissues, there are only trace amounts of calcium to be found in mammalian tissues (Robbins 1993). As discussed at the beginning of this appendix, calcium homeostasis is controlled through three major sites in the mammalian body: the GI tract/lumen, the kidneys and the bones of the skeleton, with

PTH and blood plasma mediating the complicated balance (Rizzoli and Bonjour 1999, Ball 2004).

Calcium absorption is the first stage in physiological homeostasis. There are clearly many factors influencing both the process and amount of calcium being absorbed in the GI tract and, as will be discussed in Section A.3.1, the amount excreted, but the result is a net absorption of calcium that is available to meet the needs of the body (Bouillon et al. 2003). The remainder of the appendix will discuss calcium movement in the blood, the kidneys and the skeleton, as excretion is the balance to absorption and bone acts as a balancing reservoir. Also discussed are other factors, such as pregnancy, lactation and foetal development, that may be a source of calcium homeostatic disruption.

A.3.1 Kidneys and blood

The kidneys are critical to calcium homeostasis by regulating calcium ion excretion (Hoenderop et al. 2005). A large amount of calcium is filtered from the blood through the kidneys each day, as much as 8–10 g for humans (Bouillon et al. 2003, Hoenderop et al. 2005). The calcium ions are filtered through the proximal section, the glomeruli, of a kidney structure called the nephron (Hoenderop et al. 2005). The distal portion of the nephron is responsible for calcium reabsorption (Bouillon et al. 2003). Ultimately less than 2% of calcium ions in the kidneys end up being excreted in urine, with the vast majority of calcium being reabsorbed in the remaining portions of the nephron through, as described in Section A.2, paracellular or transcellular transport (Bouillon et al. 2003, Hoenderop et al. 2005).

Calcium homeostasis in the kidneys is closely linked with PTH (Brown and Jüppner 2006, Gardella et al. 2010). PTH in the kidneys has two major functions: the first is to convert calcifediol (produced in the liver) into calcitriol (Sanford 1992, Brown and Jüppner 2006, Christakos et al. 2007, Johnson 2007b) and the second is to stimulate calcium reabsorption (in the nephron) (Hoenderop et al. 2005, Brown and Jüppner 2006). Both of these PTH functions play a part in regulating blood calcium within the narrow

limits of 8.0–12.0 mg/dL or 2.0–3.0 mmol/L (Sharp 2005). More specifically, low blood serum calcium results in the parathyroid gland calcium-sensing receptor releasing PTH or the parathyroid hormone-related protein (PTHrP), which in turn triggers increased calcium absorption by the intestines, reabsorption in the kidneys and release from the bone (Hoenderop et al. 2005, Kovacs and Kronenberg 2006, Gardella et al. 2010).

Calcitriol, unlike PTH's minute-by-minute control of calcium blood balance, is important for long-term or day-to-day/week-to-week calcium homeostasis, although the production and regulation of both hormones is interrelated, with one influencing the other (Gardella et al. 2010). Increased levels of calcitriol in the blood induces GI tract calcium absorption and calcium release from bone through reabsorption (Brown and Jüppner 2006).

Calcium isotope research by Heuser and Eisenhauer (2010) has found that homeostatic processes other than bone mineralisation can be a source of calcium isotope fractionation. They found that fractionation occurs during filtration of the blood in the kidneys and during the production of urine, which I speculate would make the nephron the logical part of the kidney responsible for the fractionation and paracellular or transcellular transport mechanism. Heuser and Eisenhauer (2010)'s evidence of fractionation in the kidneys is that $\delta^{44/40}Ca_{blood}$ and $\delta^{44/40}Ca_{diet}$ values are similar and both depleted in ^{44}Ca, in contrast to what is seen in the urine. The urine of both a boy and an older woman was enriched in ^{44}Ca [the $\delta^{44/40}Ca$ value for the boy was $1.47 \pm 0.29‰$ and for the woman $0.35 \pm 0.1‰$, 1 standard deviation (SD) for both], relative to both blood ($\delta^{44/40}Ca$ value of ~–1.3‰) [based on soft tissue information from Skulan and DePaolo (1999)] and diet ($\delta^{44/40}Ca$ of about $-1.02 \pm 0.1‰$). The lighter mass isotopes of calcium appeared to be recycled by the kidneys and returned to homeostatic circulation.

A.3.2 Calcium homeostasis: lactation

In addition to excretion, milk production by lactating females contributes to calcium loss from the body (Braithwaite et al. 1970, Cross et al. 1995). Unlike excretion, which is part of the process of maintaining calcium homeostasis, lactation is intended to transfer calcium

and other nutrients to a mammal's offspring. In fact pregnancy and lactation involve the mother sacrificing her own calcium for her young's benefit (Bawden et al. 1965, Braithwaite et al. 1970, Cross et al. 1995). The mother's sacrifice is rewarded by her being able to provide her offspring with milk that contains adequate amounts of calcium for proper growth and development, especially towards mineralised tissue production (Fransson and Lönnerdal 1983, Park et al. 2007, Heaney 2009). As is the case with all aspects of calcium regulation, PTH plays a vital role in calcium movement in the female mammal body during pregnancy and lactation.

During the transition from pregnancy (pregnancy will be discussed in Section A.3.3) to lactation, a calcium-sensing receptor (CaR) starts to be expressed on mammary epithelial cells and closely monitors calcium levels (VanHouten et al. 2004). PTHrP, a protein from the PTH family, which in blood is mediated through falling oestrogen levels, seems to be largely responsible for activating the release of calcium ions from bone as well as increasing reabsorption of calcium from urine in the kidneys, thereby making calcium available for milk production (Kovacs and Kronenberg 2006, Gardella et al. 2010). PTHrP is also believed to regulate breast development and blood flow, as well as the calcium content of milk (Kovacs and Kronenberg 2006).

Lactation has been found to produce a measurable, although temporary, reduction of calcium mineral content in maternal bone, with a typical loss of calcium per day of 280–400 mg (with a maximum of 1000 mg) (Kovacs and Kronenberg 2006). After weaning is complete, bone calcium is quickly restored (Kovacs and Kronenberg 2006). However, the male and female sheep shown in Figure 2.4 demonstrate that repeated, yearly pregnancies and periods of lactation (the greater culprit) can alter bone calcium isotope ratio values (Chapter 2, Section 2.6). Although ewes are more enriched in ^{44}Ca relative to the rams/wethers (castrated male sheep), that does not mean they are necessarily calcium deficient, but it clearly shows that calcium balance is not uniform between the sexes once reproduction begins. The ewe isotope ratios may reflect the release of calcium depleted in ^{44}Ca for milk production or it may be due to the efforts of the ewe's body to restore the

bone calcium balance, resulting in less fractionation occurring between the diet and the ewe's bones. It is probably a combination of both factors and is dependent on individual ewe physiology.

Returning to the discussion of PTH and milk production, PTHrP is synthesised in the mammary tissue via regulation by CaR and is passed on to offspring in significant amounts in the milk, indicating that it probably also imparts some kind of immunological benefit (VanHouten et al. 2004, Gardella et al. 2010). The CaR also regulates the transfer of calcium across mammary epithelial cells through a Ca^{2+}-ATPase extrusion mechanism and possibly TRPV6 ion channels (see Section A.2) (VanHouten et al. 2004, Hoenderop et al. 2005, VanHouten and Wysolmerski 2007). Animal studies have shown that, when there are issues with the gene that encodes Ca^{2+}-ATPase, calcium concentrations in milk are reduced, which provides evidence for Ca^{2+}-ATPase's importance in transferring calcium from the blood and breast epithelia into milk (VanHouten and Wysolmerski 2007).

As with bone, significant fractionation differences are seen when maternal diet and milk production are compared. This was first seen by Chu et al. (2006) (Chapter 2) in ewes and has been confirmed by my own analysis (Chapter 5). On the surface there appears to be a very similar use of epithelial cells in the breast tissue to transport calcium, but unlike the kidney's reabsorption of calcium there is a much greater amount of fractionation seen between the maternal diet and the milk that is produced. As bone supplies more calcium for milk production than the maternal diet, perhaps the level of calcium isotope fractionation seen between diet and milk arises from a combination of calcium isotopes depleted in ^{44}Ca being released by the bone, and a similar, although smaller, depletion of blood calcium isotopes due to calcium reabsorption from the kidneys, as well as a possible additional small fractionation as the calcium isotopes are transported across the breast epithelia.

A.3.3 Calcium homeostasis: pregnancy

While the calcium demands of lactation are met mainly by the release of calcium from the mother's bones and reabsorption of calcium from her kidneys, pregnancy is dominated by

increased maternal GI tract absorption of calcium (Kovacs and Kronenberg 2006). Calcium movement across the placenta, for foetal growth, requires a similar demand on a pregnant mammal, but lactation and pregnancy seem to have developed differently and have different means of adapting to the increased physiological demands (Kovacs and Kronenberg 2006). Over the course of a pregnancy, a human foetus will require as much as 33 g of calcium for skeletal growth (Kovacs 2006, Kovacs and Kronenberg 2006). During the last trimester, when the foetal skeleton is developing rapidly, the majority of the necessary calcium will be accreted (~80%) (Belkacemi et al. 2005, Kovacs 2006). Maternal GI tract absorption of calcium doubles at this time to meet the increased demand (Kovacs and Kronenberg 2006). Although the mother's skeleton mobilises calcium most significantly for milk production, in the last months of pregnancy it also provides calcium for foetal growth (Specker 2002).

The placenta is responsible for absorbing and supplying calcium to the foetus (Hoenderop et al. 2005). As a result of calcium transport across the placenta, aided by PTHrP, foetal blood calcium levels rise and exceed the levels of the mother (Kovacs and Kronenberg 2006). It is theorised that, similar to the process seen with the epithelial cells of the lumen (Section A.2), calcium is transported through the cells in the placenta, aided by calbindin-D_{9K} diffusion, from the mother to the foetus (Belkacemi et al. 2005, Hoenderop et al. 2005). PTHrP, but not PTH, regulates the transport of calcium through the placenta, and it is thought that the placenta as well as the parathryroid glands supplies the required PTHrP (Kovacs 2006). Although the process of how the placental cells transport calcium is generally understood, the specifics have yet to be fully determined (Belkacemi et al. 2005). Maternal health and nutrition affect placenta development and the body's ability to transport nutrients such as calcium, which has implications for foetal health and birth weight (Belkacemi et al. 2010).

With regard to calcium isotopes, it is unknown whether the placenta is a source of calcium isotope fractionation, as it has never been researched, but as the kidneys have provided evidence of fractionation it is not unreasonable to consider the placenta as a

possible source of small amounts of fractionation between maternal diet/blood and foetal blood. There is certainly evidence of calcium fractionation during milk production (Chu et al. 2006). However, the implications of placental fractionation of calcium isotopes are probably small and should not impede the comparison of mineralised tissues formed *in utero*, which for this thesis is essentially dental enamel, with maternal diet.

A.3.4 Bone

The skeleton in mammals has a number of significant roles in supporting life. Firstly, as a rigid, mechanical structure, it provides a place of attachment for skeletal muscles and protects the brain, heart, spinal cord and lungs. Secondly, the red bone marrow is a source of red blood cells (Garner et al. 1996, Gardella et al. 2010). Thirdly, the bones of a skeleton act as a metabolic buffer and mineral repository, with the capacity to both release and absorb and store minerals (predominantly calcium and phosphorus) (Garner et al. 1996, Sharp 2005). The latter function has been alluded to in the previous sections of this appendix.

The constituents of bone remain fairly constant over time for adult mammals, and consist of minerals (50–70%), an organic matrix/collagen (20–40%), water (5–10%) and lipids (<3%) (Robey and Boskey 2006). A turnover of bone occurs continuously throughout life, a full replacement taking on average 10 years (~5% per year for cortical bone and ~15% per year for trabecular bone) (Robey and Boskey 2006, Nanci and Ten Cate 2008). However, these rates are influenced by a mammal's diet, health, age and reproductive status, which are in turn linked to endocrine regulation such as is seen in the relationship between PTH, calcium homeostasis and bone cells (Gardella et al. 2010).

Ott (2008) has discussed the factors affecting bone turnover. The difference in the time it takes for cortical (compact) and trabecular (spongy) bone to turnover is related to how the two types of bone are resorbed and rebuilt. Bone is broken down and rebuilt by the movement of osteocytes around the bone surface, dissolving old bone and building new bone. The osteocytes do this by triggering specialised bone cells to work together in what is

known as a bone metabolic unit (BMU) to both resorb (osteoclasts) and rebuild (osteoblasts) bone. In cortical bone, BMUs drill tunnels into the bone and then fill them with new bone. In trabecular bone, BMUs can either carve out channels on the bone surface that are then refilled, or can flow/spread over the surface of the bone, triggering break down and rebuilding as they go. Ideally as much new bone is produced as is resorbed (equilibrium), although age, health, diet, pregnancy and lactation can affect this process and result in a negative bone balance.

The homeostatic role of bone is known to be critical, but the Ca^{2+} transport processes are not well understood (Hoenderop et al. 2005). Calcium is in continuous homeostatic circulation into and out of bone as a result of ion exchange at the points in the skeleton where extracellular fluids, such as blood plasma, interact with bone surfaces (Garner et al. 1996, Hoenderop et al. 2005). Bone resorption and formation are generally believed to occur too slowly to respond to rapid changes in blood plasma calcium concentrations (Hoenderop et al. 2005). However, there appear to be two pools of bone calcium available for release. Clinical administration of PTH results in the quick release of calcium from the surface of the bone in what is thought to be a pool of calcium available for rapid turnover (Talmage and Elliott 1958, Brown and Jüppner 2006). A second release of calcium, several hours after the initial PTH exposure, is from a pool of much more slowly released calcium (Talmage and Elliott 1958, Brown and Jüppner 2006). Unfortunately, it is unknown if the calcium isotope ratios are different between the quick and slow release calcium pools.

From what is known about calcium homeostasis involving bone cells, osteoclasts seem to be stimulated by cytokines or signalling from osteoblasts, and detect changes in calcium concentration when ambient calcium levels increase during bone resorption, which triggers calcium levels to increase greatly in the cytosol (intercellular fluids) (Hoenderop et al. 2005, Gardella et al. 2010). The movement of calcium into the cytosol involves both a release within the cell and an external input (Hoenderop et al. 2005). Osteoblasts give a

slight indication of calcium ion movement through the cells to the sites of active mineralisation, and a few studies have found evidence of mRNA from TRPV5 (resorption of Ca^{2+} in the kidneys) and TRPV6 (resorption of Ca^{2+} in the intestinal lumen) in these cells (as described in Section A.2) (Hoenderop et al. 2005, Brown and Jüppner 2006). It is known that osteoblasts are stimulated by and respond strongly to PTH and PTHrP through receptors sensitive to PTH and related hormones (Gardella et al. 2010). It is believed, but not fully understood, that PTH and PTHrP mediate long-term homeostatic calcium processes by interacting with osteoblasts, and indirectly with osteoclasts, by increasing their number/activity and also by implementing the release of calcium from bone (Hoenderop et al. 2005).

Foetal, neonate and young mammal bones are not in equilibrium and instead are rapidly growing. Similar to late-term foetal skeletal growth, the human neonatal skeleton requires approximately 150 mg/kg of calcium/day (Kovacs 2006). Unlike the foetus, which has calcium supplied by the mother's diet through the placenta, a neonate has to begin self-regulating calcium homeostasis quickly, through intestinal absorption and kidney reabsorption as well as the release and absorption of calcium from the skeleton (Kovacs 2006). The neonatal transition to calcium self-regulation usually occurs within hours or days of birth (Kovacs 2006). This transition is also occurring at a time of rapid skeletal development (Kovacs 2006), although a diet of calcium-rich milk, along with the sugars in milk (lactose and galactose) that aid in calcium absorption (Davenport 1982, Sanford 1992), should meet the demand.

Young mammal bone growth involves both PTH and PTHrP, but each has a specific task in the process. PTH maintains blood calcium levels (which PTHrP does as well, but to a lesser degree) and directs the calcium to the growing bone, while also possibly stimulating osteoblasts (Kovacs 2006). PTHrP works within the bone, more specifically in the growth plate, to stimulate bone growth (Kovacs 2006). The bones of young and adolescent mammals are focused on longitudinal growth and in developing

increased bone mass (Nelson et al. 2006). The change in skeletal mass from birth (~70–95 g) to adulthood (2400 g for young women and 3300 g for young men) is tremendous, and is the result of bone modelling and remodelling (Nelson et al. 2006). Overall childhood and adolescent bone growth is marked by more calcium being absorbed than is released, with 90% of peak bone mass in humans being acquired by 18 years of age (Nelson et al. 2006). Calcium isotope fractionation related to bone is discussed in detail in Chapter 2.

A.3.5 Dental enamel

Mature or fully formed dental enamel is the hardest and most highly mineralised (>95% by weight) tissue in the mammal body (Fowler et al. 2005). The two main components of enamel are calcium and phosphorus, in the form of hydroxyapatite (Simmer and Fincham 1995, Fowler et al. 2005). Mature enamel is formed from crystals of hydroxyapatite, whose early mineralised growth is stimulated and guided by enamel proteins in a lightly mineralised structure that forms the initial enamel matrix (Fincham et al. 1999, Robinson et al. 2004).

Dental enamel, once formed and mature, is removed from the processes of calcium homeostasis. This is because once the mineralisation processes are complete the dental tissue is unaltered by natural, healthy physiological processes (dental decay/caries destroy enamel). However, as the tooth is forming and grows, the same hormones involved in bone formation and calcium homeostasis are involved in dental development. The cells involved in enamel mineralisation are both similar and different in behaviour to the cells involved in bone mineralisation.

Tooth germ formation depends on, like so many other calcium-regulating systems, PTHrP. PTHrP is expressed in the epithelial cell component of the enamel organ (Kitahara et al. 2002). The enamel organ is made up of calcium-transport epithelia and is responsible for forming tooth enamel (Hubbard 2000, Kitahara et al. 2002). The enamel epithelium initially comprises layers of cuboidal cells that change in order to produce enamel (Nanci and Ten Cate 2008). These changes are known as: (1) differentiation, (2) secretion, (3)

maturation and (4) involution (Hubbard 2000, Franklin et al. 2001). Initially the cuboidal cells will differentiate (1) and become ameloblasts, resulting in these new cells secreting (2) a soft enamel matrix that is water- and protein-rich (50–60% and 20–30% by volume, respectively) and mineral poor (15–20% by volume) (Robinson et al. 1988, Hubbard 2000). The enamel matrix is produced by the ameloblasts making nanometer-sized particles of mineral that form a three-dimensional network that creates the rough framework or template of what will become the final enamel portion of the tooth (Smith 1998, Hubbard 2000, Beniash et al. 2009). The majority of the mineral portion of the enamel matrix during the secretory phase is in the form of ribbon-like hydroxyapatite crystals (Smith 1998). Maturation (3) involves dehydration, deproteination and hypermineralisation by the ameloblasts, resulting in crystal growth and a hard enamel matrix that, as previously mentioned, is >95% by weight hydroxylapatite mineral (Smith 1998, Hubbard 2000, Fowler et al. 2005). Of the mature enamel, 40% is calcium (Smith 1998). The ameloblasts involved in maturation change their morphology from being 'ruffled' to 'smooth' at their apex. Hubbard (2000: 443) describes this morphological change as a successive progression in 'migratory bands that move through the maturation zone much like a "Mexican wave" through a sports crowd'. At the end of the wave the smooth ameloblast will return to being ruffled (Hubbard 2000). The last change that the enamel epithelial cells/ameloblasts go through is involution (4), which involves apoptotic cell death (Smith 1998, Hubbard 2000).

The transfer of calcium into the enamel through the epithelial cells is not fully understood, but it is believed that both paracellular calcium concentration-dependent diffusion processes and vitamin D-dependent transcellular mechanisms are involved (Hubbard 2000). The method of calcium transport, paracellular or transcellular, is dependent on the enamel phase, secretion or maturation (Hubbard 2000). During the secretion phase, ameloblasts hinder calcium transport into the enamel matrix (Aoba and Moreno 1987, Hubbard 2000). When secreted enamel fluid was analysed chemically, it was found that the enamel epithelium was part of a barrier to calcium mineralisation of the

enamel matrix. Specifically, transcellular calcium transport was blocked by ameloblasts, although some paracellular transport probably occurred (Aoba and Moreno 1987, Hubbard 2000). The small amount of calcium transported into the enamel matrix was probably bound in peptides, proteins and other ligands, which were produced by the degradation of the enamel building proteins of amelogenin (Aoba and Moreno 1987, Moreno and Aoba 1987). As a result the enamel fluid was found to contain very low concentrations of calcium relative to blood serum levels (Aoba and Moreno 1987, Moreno and Aoba 1987, Hubbard 2000).

Only 12–15% of calcium in the enamel fluid remains in a free ion form available for mineralisation, with the remaining 85–88% being bound in ligands within the enamel matrix (Moreno and Aoba 1987). The calcium bound in protein ligands may actually be a reservoir for future enamel mineralisation. It is also thought that the proteins in the enamel matrix and enamel fluid trigger and regulate hydroxyapatite crystal formation, growth and possibly even crystal shape (Kirkham et al. 2000, Yamakoshi et al. 2001).

The period of saturation is when the enamel matrix is formed: ribbon-like enamel crystals are seeded and crystal elongation begins (Takano 1995, Smith 1998, Beniash et al. 2009). Elongation of the hydroxyapatite crystals involves ameloblast movement away from the dentine surface, with crystals forming in the ameloblasts' wake, resulting in organised crystal rods with inter-rod spaces that contain lipids, randomly orientated enamel crystals and proteins (Avery et al. 1961, Smith 1998). Elongation of the crystals continues until the full mature enamel thickness, from the dentino-enamel junction to the outermost enamel surface, is reached (Smith 1998). It is during the next phase, maturation, that the crystals expand and fill the spaces between the elongated crystal rods.

Although calcium is clearly incorporated into the enamel matrix during the saturation phase, it is during enamel maturation that significant quantities of calcium are incorporated, in what is believed to be the same manner as described in Section A.2. It is thought that calcium transport is dependent on ameloblast morphology, with ruffled cells

having a tight junction between cells that requires vitamin D-dependent transcellular mechanisms to move the calcium through the ameloblast (Hubbard 2000). It is when the ameloblast morphology becomes smooth that the space between cells allows calcium to move into the enamel matrix through paracellular calcium concentration-dependent diffusion (Hubbard 2000). As seen with the use of ^{45}Ca tracers, it seems that calcium is transported through ruffled ameloblasts (deproteination means a significant reduction in calcium ligands being formed) by transcellular mechanisms and is incorporated into an insoluble enamel mineral matrix (Hubbard 2000). However, calcium that passes between the smooth ameloblasts, through paracellular diffusion, appears to be vulnerable to aqueous removal and generally is not a supplier of mineralising calcium (Hubbard 2000). Once the ameloblasts cells have returned to being ruffled, they resume transcellular mechanisms for calcium transport and mineralise calcium (Takano et al. 1982, Hubbard 2000). It is also when the cells are ruffled that the enamel crystals increase in volume and expand into the spaces once held by water and proteins (Takano 1995, Smith 1998, Hubbard 2000).

Not only is the majority of calcium accretion in enamel happening during maturation, this is also when the most rapid rate of calcium transport is occurring, with, for example, 86% of total calcium in rat incisors being obtained during maturation (Smith 1998, Hubbard 2000). It has been found that not only are the processes for transporting calcium in enamel epithelia similar to intestinal epithelia (when ameloblasts are in the ruffled form), but that the rate of calcium flux in ameloblasts is very similar to that seen across the intestinal epithelia (Hubbard 2000). However, the different stages of enamel development and the implications for calcium ameloblast transport (or lack of transport) mean that enamel is more complicated than other tissues and organs involved with calcium homeostasis.

In order to understand fully the calcium isotope ratio values from samples of dental enamel, it is necessary to have a sense of how, when, why and how much/how quickly calcium enters the enamel matrix and is mineralised. Additionally, the specifics of the

epithelial cells and processes involved in calcium transport from the blood serum and into the enamel matrix help to anticipate at least one possible small source of fractionation between diet and enamel. A last consideration for calcium isotopes and enamel formation is that the initial secretion phase involves far less calcium mineralisation than the later maturation phase. As discussed, this is a result of calcium being actively regulated in the enamel matrix by ameloblasts and ligands. The fact that calcium's involvement in mineralisation seems to be most significant in the later maturation stage of enamel formation means that the calcium isotope ratios in enamel samples should reflect the animal's diet during that phase of enamel formation.

A.4 Chapter summary

Calcium homeostasis is an active, continuous process in the mammal body and relies initially on the amount of calcium ingested in the diet, which for herbivores comes from soil and plants, and then on internal, co-ordinated efforts by organs and tissues to monitor and regulate calcium. Cells, especially epithelial cells, facilitate the vast majority of the physical movement of calcium between organs and the blood, while PTH, triggered by blood calcium levels, provides direction. Although this appendix is concerned with calcium homeostasis, it is a subject directly relevant for understanding and interpreting the calcium isotope ratio values in this thesis. Specifically, the homeostatic topics related to possible epithelial cell calcium isotope fractionation, diet–milk fractionation and diet–bone fractionation, as well as the sources of foetal, neonate and young mammal skeletal calcium (the mother's diet, lactated milk and/or solid foods), are all integral to understanding the sheep bone and dental enamel results.

APPENDIX B

STANDARDS FOR CALCIUM ISOTOPE ANALYSIS AND STANDARD CALCIUM ISOTOPE RATIO RESULTS

B.1 Standards for calcium isotope analysis

The application of calcium isotopes in geochemistry and in archaeological science is in its infancy. As a result geochemists have been active in searching for, and developing, a suitable calcium carbonate standard to be a primary reference standard. The one selected by the majority of researchers has been NIST SRM 915a (915a) (Halicz et al. 1999, Hippler et al. 2003). Repeated analyses of this standard in relation to seawater samples were performed in a number of laboratories (Hippler et al. 2003) and it subsequently became the benchmark against which other standards were measured. However, this standard became unavailable after 2006, requiring the selection of new reference standards (Heuser and Eisenhauer 2008). This has been an issue for all researchers investigating calcium isotopes, especially as the reserves of 915a have become limited.

Reynard et al. (2010) compared the standard Fisher07 against 915a, thus allowing conversion of the results to 915a. Conversion of sample δ-values from one reference standard to another is acceptable as long as the conversion factors for the standards are known (Boulyga 2010). The conversion of results from Fisher07 to 915a comprises two very simple equations:

$$\delta^{44/42}Ca_{915a} = \delta^{44/42}Ca_{Fisher07} + 0.57‰$$

and

$$\delta^{43/42}Ca_{915a} = \delta^{43/42}Ca_{Fisher07} + 0.29‰.$$

The analyses conducted as part of this research were performed using the same stock solutions and multicollector–inductively coupled plasma–mass spectrometer (MC-ICP-MS) instrument at the Department of Earth Sciences, University of Oxford as used by

Reynard et al. (2010, 2011a, 2013). Both Reynard et al. (2010, 2011a, 2013) and I analysed the standards as part of validating the instrumental measurements and sample data. This included comparing the calcium carbonate standard 915a with the calcium carbonate standard NIST SRM 915b (915b). The intention was to replace 915a with 915b, but it was found to contain strontium and I only analysed it briefly. Alfa Aesar (Alfa) and High-Purity Standards calcium (HPS_{new} Calcium), as well as the non-calcium carbonate standards of International Association for the Physical Sciences of the Oceans (IAPSO) seawater and the in-house powdered archaeological cattle femur standard, were also analysed in relation to 915a, with what little remained of the stock reserves, and Fisher07. When the offsets were determined between the standards HPS_{new} Calcium, seawater and the in-house bone, the findings were consistent with the direct measurement offset between Fisher07 and 915a that was used to make the conversion equations shown above (Figure B.1). Fisher07 was therefore used as the replacement primary reference standard for 915a.

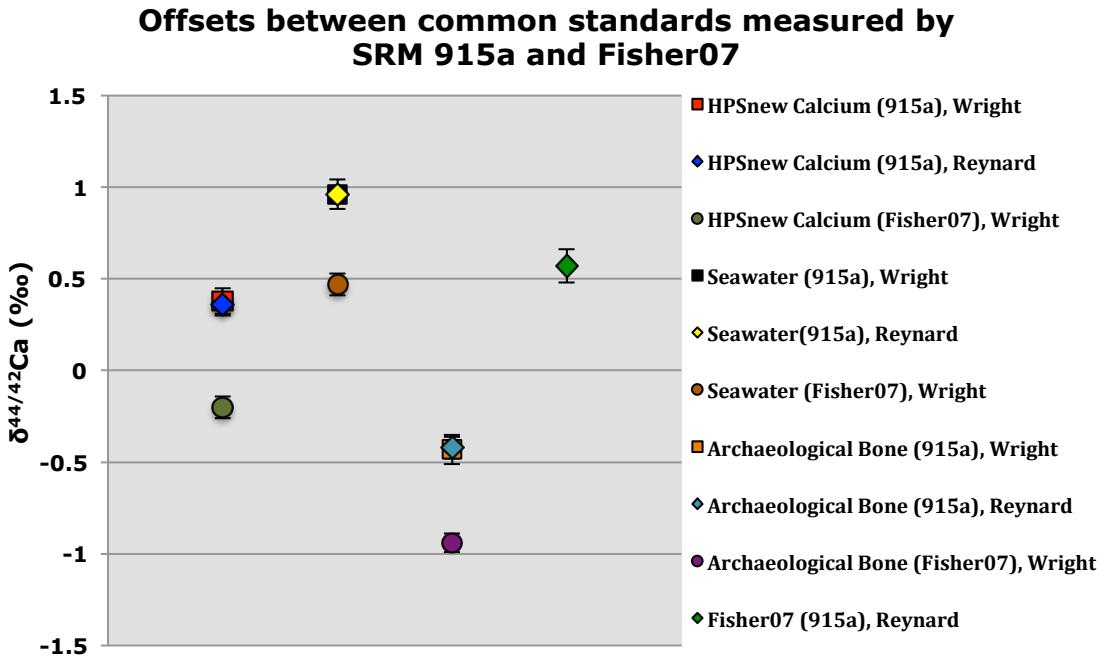

Figure B.1: The offsets between common standard measurements and primary reference standards 915a, as determined by Reynard et al. (2010) and me, and Fisher07. The offset for HPS_{new} Calcium was 0.58‰, for seawater 0.49‰ and the in-house archaeological bone 0.52‰. Both the individual offsets and the average of the offsets, 0.53‰, were within the error of Reynard et al. (2010)'s direct measurement of Fisher07 versus 915a, 0.57 ± 0.09‰.

Table B.1 presents my and Reynard et al. (2010)'s measurements of the different standards analysed. The standards I analysed are labelled according to whether they went through column chemistry/cation exchange chromatography prior to analysis (CC), were dilutions of stock solution that did not go through column chemistry prior to analysis (SS) or were dilutions of stock solution that were analysed specifically as part of standard bracketing (SB).

Towards the very end of the project an issue developed with the standard HPS_{new} Calcium. The problem seemed to occur in the column chemistry. Standards of seawater and archaeological bone run through column chemistry at the same time as HPS_{new} Calcium were within error and correct, but the HPS_{new} Calcium was found to be enriched in ^{44}Ca by an average of 0.20‰. This will also be discussed in Appendix C. There had been no issue when HPS_{new} Calcium had been run through column chemistry previously, and instrumental analysis of freshly made dilutions of HPS_{new} Calcium stock solution were within error, removing contamination as an issue. The source of the error was therefore probably due to a column matrix effect from the resin in the columns. There was no opportunity to determine whether the column matrix effect was the cause, because the problem did not arise until the analysis of the last few groups of samples at the end of the project.

Issues with HPS_{new} Calcium aside, the analysis of standards was key to assessing the instrument performance and the validity of the isotope measurements. MC-ICP-MSs are highly specialised analytical instruments that simultaneously measure more than one isotope. They are also designed and intended for the analysis of heavier isotopes, such as calcium, rather than carbon, oxygen and nitrogen.

Table B.1: Common results for standards between my research and Reynard et al. (2010) [1 standard deviation (SD)]

	Reynard, $\delta^{44/42}Ca_{915a}$ (‰)	**Reynard, $\delta^{43/42}Ca_{915a}$ (‰)**	***n***
Alfa	0.42 ± 0.05	0.21 ± 0.09	183
Arch. bone	–0.42 ± 0.06	–	74
Fisher07	0.57 ± 0.09	0.27 ± 0.08	19
HPS_{new} Calcium	0.36 ± 0.06	0.19 ± 0.08	154
915b	0.43 ± 0.09	0.21 ± 0.08	38
Seawater	0.96 ± 0.08	0.49 ± 0.08	13
	Wright, $\delta^{44/42}Ca_{915a}$ (‰)	**Wright, $\delta^{43/42}Ca_{915a}$ (‰)**	*n*
Alfa SB	0.39 ± 0.05	0.20 ± 0.04	45
Arch. bone CC	–0.43 ± 0.08	–0.21 ± 0.07	48
Fisher07 SS	–	–	–
HPS_{new} Calcium SB	0.35 ± 0.05	0.19 ± 0.07	378
HPS_{new} Calcium CC	0.38 ± 0.07	0.19 ± 0.08	187
915b SS	–	–	–
Seawater CC	0.96 ± 0.08	0.49 +/- 0.8	23
	Wright, $\delta^{44/42}Ca_{Fisher07}$ (‰)	**Wright, $\delta^{43/42}Ca_{Fisher07}$ (‰)**	*n*
Alfa	–	–	–
Arch. bone CC	-0.94 ± 0.05	–0.43 ± 0.07	127
HPS_{new} Calcium SB	–0.22 ± 0.04	–0.09 ± 0.05	1122
HPS_{new} Calcium CC	–0.21 ± 0.06	–0.10 ± 0.06	86
915b SS	–0.20 ± 0.04	–0.10 ± 0.04	8
Seawater CC	0.47 ± 0.06	0.26 ± 0.06	7

B.2 Standard calcium isotope ratio results

The methods of instrumental analysis, determination of delta (δ) values and validation of results are discussed in Chapters 2 and 4 and Appendix C. In this appendix, the standard results are presented in two groups: one group was measured with 915a, and the other with Fisher07. Calcium standard solutions (915b, Alfa and HPS_{new} Calcium), seawater and an in-house archaeological bone standard (archaeological bone) appear in both groups. If the standards went through column chemistry before analysis, they are labelled CC; if they did not go through column chemistry before analysis and were analysed alongside samples, they are labelled SS; and if they did not go through column chemistry before analysis and were specifically used in standard bracketing, they are labelled SB. Reported are the number of measurements (*n*) as well as the mean (AVERAGE), maximum (MAX) and

minimum (MIN) δ-value results in per mil (‰), including standard deviation (SD), for $^{44/42}$Ca, $^{43/42}$Ca and $^{44/43}$Ca. Included in the group measured with 915a are, when applicable, Reynard et al. (2010)'s mean results. The results were graphed and are shown in Figures B.2 and B.3.

Long-term tracking of standard results was critical to gauge instrumental reproducibility and measurement precision. As a consequence there was no sorting or discarding of standard results. Standards were measured with 915a over the course of the first year of research and Fisher07 over the next two. The results for 915a were in good agreement with those of Reynard et al. (2010). Thus it was shown that there was instrument reproducibility of results, even over long periods of time, which can be an issue for MC-ICP-MS instruments such as the Plasma 2.

B.2.1 Standards measured against 915a

Table B.2 Alfa (SB), *n* = 45

Mean $\delta^{44/42}$Ca‰	**Mean $\delta^{43/42}$Ca‰**	**Mean $\delta^{44/43}$Ca‰**
0.385941975	0.204788692	0.200546757
SD	SD	SD
0.048920601	0.044222146	0.167923417
MAX	MAX	MAX
0.516930907	0.295124948	1.248285153
MIN	MIN	MIN
0.30062284	0.118941421	0.061718109
Reynard et al. (2010)	Reynard et al. (2010)	
0.42 ± 0.05	0.21± 0.09	1 SD, *n* = 183

Table B.3 Archaeological bone (CC), *n* = 48

Mean $\delta^{44/42}$Ca‰	**Mean $\delta^{43/42}$Ca‰**	**Mean $\delta^{44/43}$Ca‰**
–0.433062297	–0.20691294	–0.229133915
SD	SD	SD
0.07831446	0.074718072	0.073167568
MAX	MAX	MAX
–0.27544298	–0.025274516	–0.068184495
MIN	MIN	MIN
–0.54587623	–0.363710395	–0.390474892
Reynard et al. (2010)	Reynard et al. (2010)	1 SD, *n* = 74
–0.42 ± 0.06	———	

Table B.4 HPS_{new} Calcium (CC), *n* = 187

Mean $\delta^{44/42}$Ca‰	**Mean $\delta^{43/42}$Ca‰**	**Mean $\delta^{44/43}$Ca‰**
0.381564598	0.194280803	0.186311424
SD	SD	SD
0.072839142	0.079994356	0.078972498
MAX	MAX	MAX
0.530258121	0.411132267	0.414611657
MIN	MIN	MIN
0.207980509	0.02292064	0.046392374
Reynard et al. (2010)	Reynard et al. (2010)	1 SD, *n* = 154
0.36 ± 0.06	0.19 ± 0.08	

Table B.5 HPS_{new} Calcium (SB), *n* = 378

Mean $\delta^{44/42}$Ca‰	**Mean $\delta^{43/42}$Ca‰**	**Mean $\delta^{44/43}$Ca‰**
0.345925078	0.186056453	0.159013413
SD	SD	SD
0.051930921	0.066754047	0.064489731
MAX	MAX	MAX
0.529869436	0.334673556	0.447535008
MIN	MIN	MIN
0.236442986	0.034407221	0.018572401

Table B.6 Seawater (CC), *n* = 23

Mean $\delta^{44/42}$Ca‰	Mean $\delta^{43/42}$Ca‰	Mean $\delta^{44/43}$Ca‰
0.957590231	0.486703812	0.479111252
SD	SD	SD
0.081033958	0.082862247	0.085049311
MAX	MAX	MAX
1.114344172	0.627106146	0.693352483
MIN	MIN	MIN
0.852965931	0.348678234	0.364508038
Reynard et al. (2010)	Reynard et al. (2010)	1 SD, *n* = 13
0.96 ± 0.08	0.49 ± 0.08	

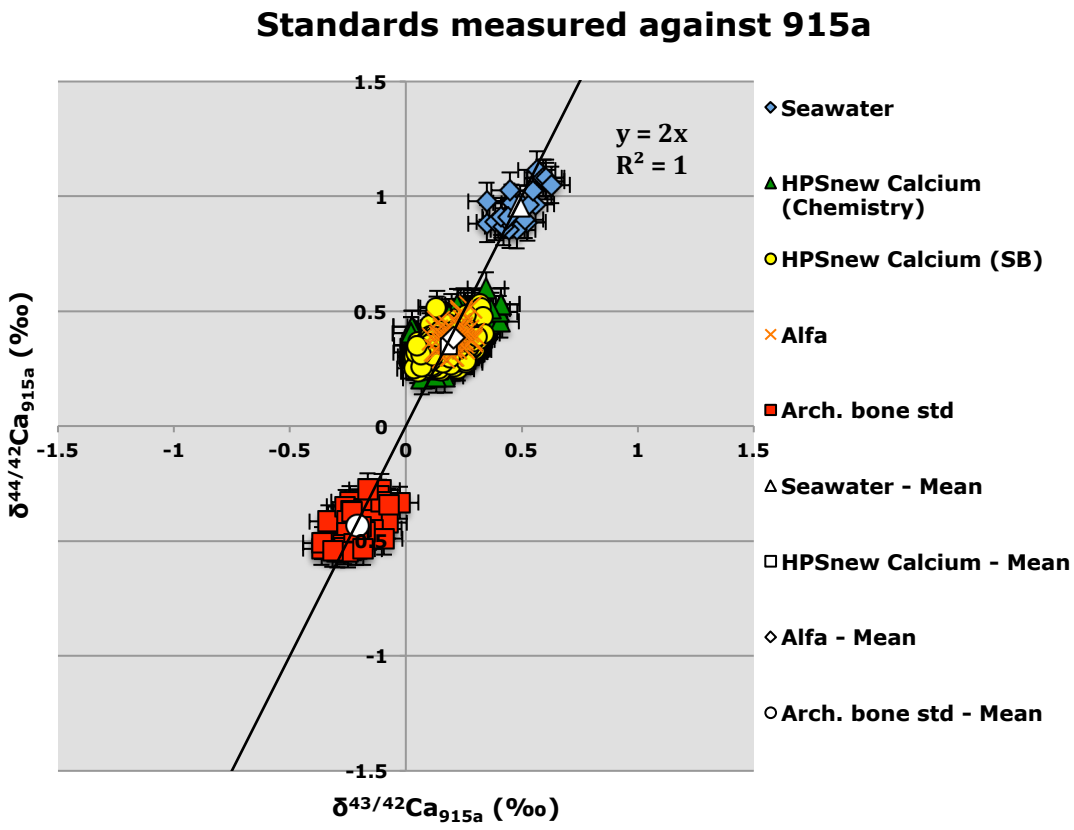

Figure B.2: Results for the seawater, HPS_{new} Calcium (Chemistry/CC and SB), Alfa and archaeological bone standards measured against the standard 915a. Mean results are in white and the black line is the mass fractionation line with a slope of 2:1. See Chapter 4 for a discussion of three-isotope plots and mass fractionation lines.

B.2.2 Standards measured against Fisher07

Table B.7 915b (SS), *n* = 8

Mean $\delta^{44/42}$Ca‰	**Mean $\delta^{43/42}$Ca‰**	**Mean $\delta^{44/43}$Ca‰**
–0.196484726	–0.100745811	–0.097114227
SD	SD	SD
0.035710681	0.038857625	0.040957105
MAX	MAX	MAX
–0.152930748	–0.05054427	–0.040216177
MIN	MIN	MIN
–0.243521405	–0.179301277	–0.161171344

Table B.8 Archaeological bone (CC), *n* = 127

Mean $\delta^{44/42}$Ca‰	**Mean $\delta^{43/42}$Ca‰**	**Mean $\delta^{44/43}$Ca‰**
–0.935457272	–0.433111998	–0.500816001
SD	SD	SD
0.050493825	0.069502321	0.066361661
MAX	MAX	MAX
–0.855734563	–0.223058756	–0.359652006
MIN	MIN	MIN
–1.085763937	–0.618159173	–0.6668962

Table B.9 HPS_{new} Calcium (SB), *n* = 1122

Mean $\delta^{44/42}$Ca‰	Mean $\delta^{43/42}$Ca‰	Mean $\delta^{44/43}$Ca‰
–0.223706756	–0.089826553	–0.129400402
SD	SD	SD
0.042734654	0.054207272	0.04710592
MAX	MAX	MAX
–0.10980126	0.062176882	0.04028447
MIN	MIN	MIN
–0.319042871	–0.218684898	–0.228927813

Table B.10 Seawater (CC), *n* = 7

Mean $\delta^{44/42}$Ca‰	Mean $\delta^{43/42}$Ca‰	Mean $\delta^{44/43}$Ca‰
0.472275366	0.258236149	0.215552599
SD	SD	SD
0.058341364	0.063112084	0.085839373
MAX	MAX	MAX
0.576890674	0.33571399	0.316273186
MIN	MIN	MIN
0.382363003	0.174854939	0.130193803

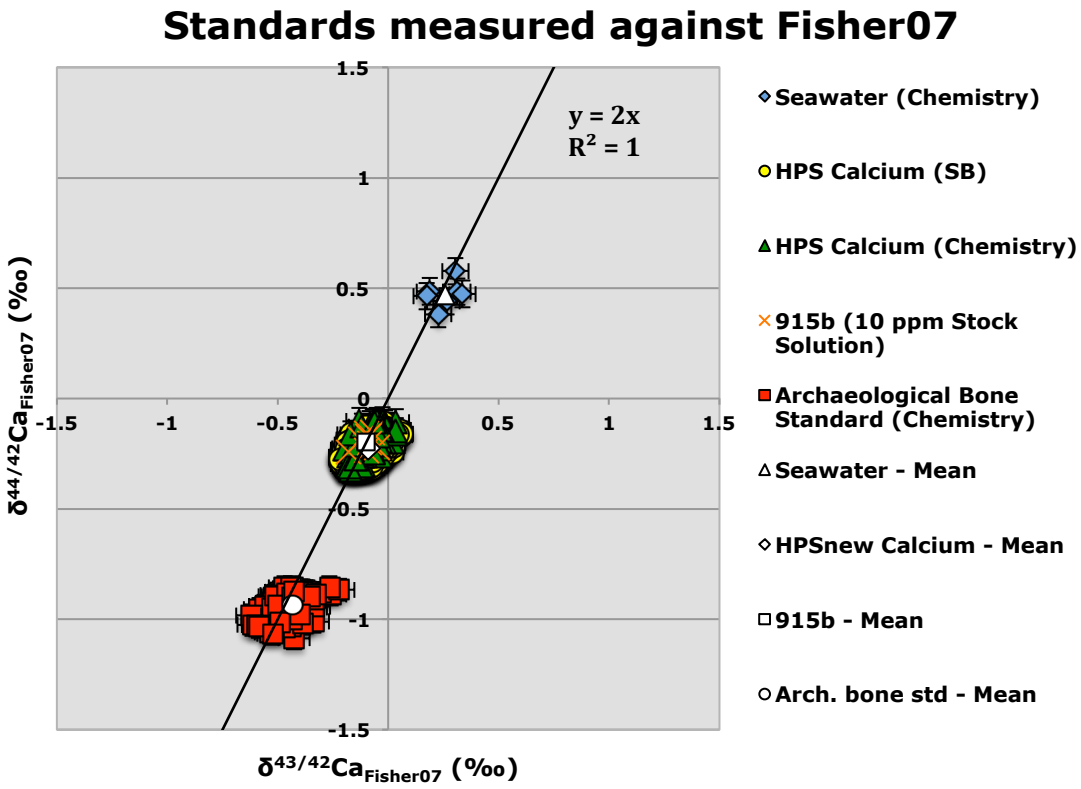

Figure B.3: Results for the seawater, HPS_{new} Calcium (Chemistry/CC and SB), Alfa and archaeological bone standards measured against the standard Fisher07. Mean results are in white and the black line is the mass fractionation line with a slope of 2:1. See Chapter 4, Section 4.2.3 for a discussion of three-isotope plots and mass fractionation lines.

APPENDIX C

INSTRUMENTATION AND STANDARD BRACKETING FOR CALCIUM ANALYSIS

C.1 MC-ICP-MS, TIMS and instrumental interferents

The multicollector–inductively coupled plasma–mass spectrometer (MC-ICP-MS) is not the only instrument available for measuring calcium isotopes. Thermal ionisation mass spectrometry (TIMS) is a second method for measuring the abundance of isotopes that are subject to fractionation processes (Thirlwall and Anczkiewicz 2004, Pietruszka and Reznik 2008, Boulyga 2010). However, MC-ICP-MS is currently the method more commonly used for non-light stable isotope measurements (Thirlwall and Anczkiewicz 2004). This is because plasma techniques provide a thorough ionisation of elements in samples, even ones that are traditionally difficult to ionise using TIMS (Pietruszka and Reznik 2008).

The one advantage TIMS has over MC-ICP-MS is specifically linked to the analysis of calcium isotopes. The carrier gas most often used in plasma instruments is argon (Ar), which can be a source of the calcium molecular interferents of $^{40}Ar^{+}$ (which is a bigger issue for desolvating systems that produce a dry aerosol) and $^{40}ArH_2^{+}$ (a bigger issue for standard nebulisers that produce a 'wetter' aerosol) as it shares the same mass as ^{40}Ca (Halicz et al. 1999). TIMS does not use an argon carrier gas and is able to detect the most abundant isotope of calcium, ^{40}Ca. This provides a greater mass difference between calcium isotopes (10% for $^{44/40}Ca$ compared with 4.8% for $^{44/42}Ca$ and 2.4% for $^{43/42}Ca$) when monitoring isotope ratios for fractionation. Therefore plasma instruments are designed to have the ability to detect, differentiate and quantify isotopes of an element with very small mass differences. In the early years of calcium isotope analysis TIMS was used more often, and as a result TIMS data discussed in relation to MC-ICP-MS data requires a mass conversion from a four- to a two-neutron mass difference.

In addition to $^{40}Ar^{+}$ and $^{40}ArH_2^{+}$ for ^{40}Ca, other calcium isotopes have interferents, although most of them are eliminated by calcium purification through column chemistry (Hirata et al. 2008, Boulyga 2010). The remaining interferents are related to the fact that samples are introduced into the instrument through a nebuliser as an aqueous aerosol (Boulyga 2010). Aqueous samples provide hydrogen, which can produce the mass interferents of $^{43}Ca^{1}H^{+}$ for both ^{44}Ca and ^{43}Ca, and $^{40}Ca^{1}H_2^{+}$ for ^{42}Ca (Simpson et al. 2005). To eliminate this problem, Plasma 2 (the MC-ICP-MS used in research) uses a DSN-100, which reduces aerosol droplet size in a heated spray chamber (105°C) and then finishes vapourising the aerosol via transport through a heated membrane wall (115°C) before the sample is introduced to the plasma chamber.

The separation of calcium from strontium by cation exchange chromatography/column chemistry largely eliminates the issue, but it is still important to monitor for doubly charged strontium (Sr^{2+}). As a result the mass (mass-to-charge ratio) 43.5 was monitored during instrumental analysis on Plasma 2 (Wieser et al. 2004). It was also possible to check for Sr^{2+} by plotting calcium isotope ratios, $\delta^{44/42}Ca$ and $\delta^{43/42}Ca$, on a three-isotope plot (Boulyga 2010). Three-isotope plots are discussed in detail in Section 4.2.3. The only time Sr^{2+} appeared to be a significant issue was during the analyses of the stock solution of NIST SRM 915b (915b), which did contain strontium. At the time of discovery it was decided, by me and other researchers at the Department of Earth Sciences, University of Oxford, that there was too much strontium in the standard causing background interference for use in sample analysis.

Although the standard 915b could not be used as a primary reference standard, the standards of NIST SRM 915a (915a) and Fisher07 were. These standards were used during analysis on Plasma 2 as part of standard bracketing. Standard bracketing is used for MC-ICP-MS because of the operating variation inherent in this type of instrumentation. The next section provides a summary of the reasons behind using standard bracketing and how it works during analysis and in validating measurement results.

C.2 Standard bracketing

The method or sequence of analysis on Plasma 2 involved standard bracketing. Standard bracketing is used to correct for instrumental mass bias as well as being critical to the validation of δ-values. The different causes of instrumental mass bias are, although very complex, generally understood, and result in inconstant transmission of the ion beam through the instrument and to the collectors (signal drift) (Thirlwall and Anczkiewicz 2004, Douthitt 2008). One source of mass bias in the MC-ICP-MS, already discussed, is the matrix of the samples and standards being analysed that may produce isotope-specific interferences; more specifically, isobaric and molecular interferences (Albarède and Beard 2004) such as Sr^{2+}, $^{43}Ca^{1}H^{+}$, $^{40}Ca^{1}H_{2}^{+}$, $^{40}Ar^{+}$ and $^{40}ArH_{2}^{+}$, which share the same mass as the calcium isotopes being analysed (Halicz et al. 1999, Simpson et al. 2005).

However, it is believed that the main source of instrumental mass bias is in the plasma source and in the interface between where the instrument is open to atmospheric influences, such as air, argon gas and plasma, and the vacuum of the rest of the instrument (Albarède and Beard 2004), neither of which can easily be 'fixed' and requires a means of accommodating the variability of ion transmission inherent in MC-ICP-MSs. Standard bracketing has been found to be the best solution because it involves short, discrete analysis sequences for each sample in conjunction with reference standards (Boulyga 2010). This compensates for instrument mass bias, which occurs over longer time periods of analysis (Albarède and Beard 2004, Wieser et al. 2004). 'It is possible to measure isotope abundance ratio differences relative to a standard reference material because the mass bias does not change significantly during a measurement sequence' (Wieser et al. 2004: 847).

Closely interrelated is the fact that standard bracketing deals with instrument mass bias as well as validating results. Isotope analysis undertaken by standard bracketing involves the analysis of a series of four HNO_3 blanks (2%, 10%, 2% and 2%) in order to wash away calcium residues in the instrument that are left over from the previous sample or standard analysed. This removes the possibility of memory effect altering the results of subsequently analysed samples and standards (Wieser et al. 2004: 847). The actual

bracketing of a sample with standards involves, for this research, the analysis of either standard 915a or Fisher07, followed by the standard High-Purity Standards calcium (HPS_{new} Calcium), the sample and then, lastly, the closing of the bracket with 915a or Fisher07 again (Figure C.1).

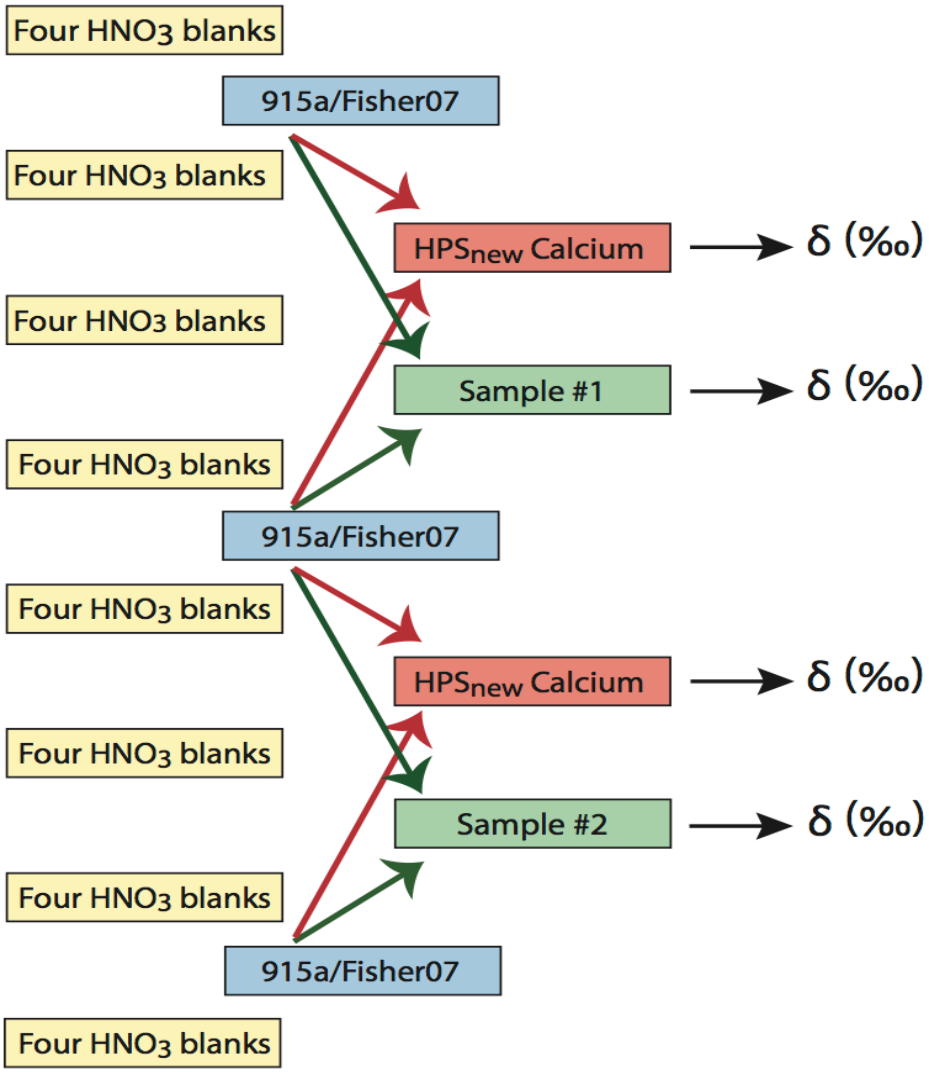

Figure C.1: A schematic of the standard bracketing method used (based on Wieser *et al.* 2004: 848). HNO_3 blanks were used to remove the chance of memory effect and standard 915a or Fisher07 was used to bracket both the HPS_{new} Calcium standard and each sample. The instrumental data for 915a/Fisher07 was averaged (red and green arrows) before being used in the delta equation along with the data from HPS_{new} Calcium and the samples. The δ-value for HPS_{new} Calcium is well-known compared with both 915a and Fisher07. If the δ-value of HPS_{new} Calcium is off by more than 0.1‰, then there is probably an instrument issue (mass bias) and the δ-value for the associated sample is automatically rejected.

The standards used in bracketing the sample were diluted from 1000 ppm stock solution to 10 ppm using 2% HNO_3 and were designated SB. The value of HPS_{new} Calcium SB is known when analysed against 915a or Fisher07 and provides a means of identifying when the instrument is not measuring correctly or is subject to strong instrumental mass biases. This is achieved by averaging the bracketed standard (915a or Fisher07) results for ^{42}Ca, ^{43}Ca and ^{44}Ca. The average result, along with HPS_{new} Calcium SB, is then entered into the formula for determining δ-values:

$$\delta=[(HPS_{new}\text{ Calcium SB})–(915a\ \textbf{or}\ Fisher07)]/(915a\ \textbf{or}\ Fisher07) \times 1000.$$

If the δ-value for HPS_{new} Calcium SB within a bracket is ±0.1‰ of $\delta^{44/42}Ca$ = 0.35‰ (0.25–0.45‰) and $\delta^{43/42}Ca$ = 0.19‰ (0.09–0.29‰) for 915a, and $\delta^{44/42}Ca$ = –0.22‰ (–0.12 to –0.32‰) and $\delta^{43/42}Ca$ = –0.09‰ (0.01 to –0.19‰) for Fisher07, then the associated sample within the bracket is provisionally accepted. Alternatively, if the δ-value for HPS_{new} Calcium SB is outside the bounds of ±0.1‰ then there is an issue with chemistry or the instrument is not measuring correctly and the sample data is automatically rejected. This is the first way in which sample results are validated.

As briefly discussed in Appendix B, HPS_{new} Calcium, seawater and the in-house archaeological bone were routinely run through column chemistry alongside samples. These standards, designated CC, held the same position in standard bracketing as the samples, with results rejected or provisionally accepted in the same manner. This was how the issues with HPS_{new} Calcium CC were spotted (mentioned in Appendix B) at the end of the project. The HPS_{new} Calcium CC results should have had, like HPS_{new} Calcium SB, a $\delta^{44/42}Ca$ = –0.22‰ (–0.12 to –0.32‰). Instead, the mean result for HPS_{new} Calcium CC was $\delta^{44/42}Ca$ = –0.02‰, a 0.20‰ enrichment of ^{44}Ca. The results for the standards of seawater CC and in-house bone standard CC that were run through column chemistry in the same sample batches as HPS_{new} Calcium CC were within their acceptable range of values for $\delta^{44/42}Ca$ and $\delta^{43/42}Ca$, thus it seemed likely that the issue was localised to HPS_{new} Calcium CC.

APPENDIX D

CALCIUM ISOTOPE RESULTS FOR SAMPLES

The methods used in instrumental analysis, the determination of delta values and result validation are discussed in Chapters 2 and 4. The samples were measured with the calcium standards of NIST SRM 915a or Fisher07. The standard used in the sample analysis is stated with each sample group. The three-isotope plots are all in respect to the standard NIST SRM 915a. Samples and standards analysed against Fisher07 were converted to NIST SRM 915a equivalent values. This was so that samples and standards that had gone through chemistry could be plotted in relation to the standards in the literature, which were all determined in relation to NIST SRM 915a. The conversion factor is based on the direct measurements between NIST SRM 915a and Fisher07 that were undertaken by Reynard et al. (2010) and involved:

$$\delta^{44/42}Ca_{915a} = \delta^{44/42}Ca_{Fisher07} + 0.57‰$$

and

$$\delta 43/42Ca_{915a} = \delta\ 43/42Ca_{Fisher07} + 0.29‰.$$

Mean sample and standard results are shown in a three-isotope plot at the bottom of each group of samples. The standards in the plots are either from the literature, Reynard et al. (2010) or have been put through column chemistry alongside the samples by me. The three-isotope plot should have standards and results that are on or close to a mass fractionation line with a slope of two, with $\delta^{44/42}$Ca‰ results on the vertical axis and $\delta^{43/42}$Ca‰ on the horizontal axis. Three-isotope plots are also a way to see whether the standards that went through column chemistry agree with the standards in the literature. This is a means of detecting possible sources of error due to column chemistry and a final assessment of data. In some cases the standards did not agree with the literature values and it is for this reason that at least two standards were run through column chemistry and two columns in each run of chemistry were dedicated to each of the standards. Additionally, each plot contains three

lines. The black line is the mass fractionation line and the two blue lines lie to either side of the mass fractionation line by 0.15‰. The data has already been sorted based on whether it fell between the blue lines or outside them. If a data point was outside but touching one of the blue lines then it was included.

D.1 Linienbandkeramik samples – Ensisheim, France (NIST SRM 915a)

D.1.1 Human males

LBK 303/Ens–003

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.595731491	–0.305365945	–0.278667104
–0.553239302	–0.278217842	–0.313161787
–0.502178716	–0.192921614	–0.306612612
–0.574935106	–0.300894184	–0.288097842
AVERAGE	AVERAGE	AVERAGE
–0.556521154	–0.269349896	–0.296634836
SD	SD	SD
0.040167961	0.052319915	0.01600433

LBK 308/Ens–008

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.001152108	–0.402155554	–0.604218374
–1.010112507	–0.597135587	–0.421229992
–1.027014323	–0.429777366	–0.632166099
–0.936761482	–0.466391121	–0.464747363
–0.960278609	–0.479999633	–0.442909575
–0.974081458	–0.526186389	–0.464828014
–0.989052118	–0.436440483	–0.529705317
AVERAGE	AVERAGE	AVERAGE
–0.985493229	–0.476869447	–0.508543533
SD	SD	SD
0.030899744	0.066395318	0.082304979

LBK 310/Ens–011

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.72415623	–0.486897407	–0.253980053
–0.779119595	–0.477530798	–0.287965816
–0.766318452	–0.307951114	–0.436966769
–0.674788808	–0.346923068	–0.334599441
–0.691368141	–0.339878287	–0.315928365
–0.566541514	–0.248449491	–0.32257064
–0.775198217	–0.35859431	–0.443233559
–0.702048186	–0.351530998	–0.374967074
–0.717258025	–0.390511938	–0.331446874
–0.696998922	–0.287060023	–0.414994302
AVERAGE	AVERAGE	AVERAGE
–0.709379609	–0.359532743	–0.351665289
SD	SD	SD
0.062226195	0.07598806	0.063803787

LBK 311/Ens–012

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.889815062	–0.496021447	–0.391912908
–0.94021063	–0.601680556	–0.317079388
–0.958131101	–0.548053718	–0.406767872
–0.950484747	–0.591621988	–0.372534289
–0.933089638	–0.489622531	–0.474087846
–0.922574895	–0.572191493	–0.350054367
AVERAGE	AVERAGE	AVERAGE
–0.932384346	–0.549865289	–0.385406112
SD	SD	SD
0.024333669	0.047871775	0.053753296

LBK 313/Ens–015

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.734749672	–0.406706755	–0.300558046
–0.8535822	–0.473112945	–0.349989314
–0.806622556	–0.408781922	–0.411939429
–0.754708367	–0.335809738	–0.409388646
–0.85213104	–0.397667325	–0.474225973
–0.814534021	–0.475598576	–0.343982324
–0.767068702	–0.406568493	–0.377933632
AVERAGE	AVERAGE	AVERAGE
–0.79762808	–0.414892251	–0.381145338
SD	SD	SD
0.046900283	0.047957051	0.056666244

LBK 317/Ens–019

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.58804517	–0.303293492	–0.269565999
–0.638710402	–0.365168862	–0.315877489
–0.601393014	–0.394901183	–0.21673437
–0.540242354	–0.25058681	–0.285216486
–0.529846601	–0.243608718	–0.257199255
–0.46519899	–0.130902377	–0.275658716
–0.480650604	–0.225374397	–0.23567353
–0.542909052	–0.206880382	–0.347140431
–0.585235824	–0.330856505	–0.241703077
–0.583458801	–0.271031343	–0.275708245
AVERAGE	AVERAGE	AVERAGE
–0.555569081	–0.272260407	–0.27204776
SD	SD	SD
0.054325483	0.078751655	0.038427277

LBK 318/Ens–020

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.929194264	–0.478893184	–0.47599325
–0.961410672	–0.526554366	–0.410687763
–0.85022649	–0.482067104	–0.362328939
AVERAGE	AVERAGE	AVERAGE
–0.913610475	–0.495838218	–0.416336651
SD	SD	SD
0.05720683	0.02664826	0.05704232

LBK 320/Ens–022

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.798851014	–0.396382608	–0.401817451
–0.783211563	–0.437212007	–0.327326177
–0.717960821	–0.291533812	–0.42733984
AVERAGE	AVERAGE	AVERAGE
–0.766674466	–0.375042809	–0.38549449
SD	SD	SD
0.042905857	0.075147018	0.051966456

LBK 330/Ens–032

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.666754435	–0.327569185	–0.342987452
–0.735348187	–0.446443903	–0.271787808
–0.692037589	–0.298370438	–0.356009597
–0.645973738	–0.282165101	–0.349666579
AVERAGE	AVERAGE	AVERAGE
–0.685028487	–0.338637157	–0.330112859
SD	SD	SD
0.038472562	0.07428615	0.039245194

LBK 331/Ens–033

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.770698196	–0.471644115	–0.287208121
–0.844044936	–0.442950094	–0.417913959
AVERAGE	AVERAGE	AVERAGE
–0.807371566	–0.457297104	–0.35256104
SD	SD	SD
0.051863977	0.020289737	0.092422985

LBK 332//Ens–034

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.643675286	–0.279993758	–0.365286626
–0.633134213	–0.250022364	–0.358955316
AVERAGE	AVERAGE	AVERAGE
–0.63840475	–0.265008061	–0.362120971
SD	SD	SD
0.007453664	0.021192975	0.004476912

LBK 333/Ens–035

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.755836502	–0.428344004	–0.346035728
–0.76019046	–0.369499111	–0.399337533
AVERAGE	AVERAGE	AVERAGE
–0.758013481	–0.398921557	–0.372686631
SD	SD	SD
0.003078713	0.041609623	0.037690068

LBK 335/Ens–037

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.758617957	–0.480967432	–0.262635064
–0.743080331	–0.383230565	–0.365293411
AVERAGE	AVERAGE	AVERAGE
–0.750849144	–0.432098998	–0.313964238
SD	SD	SD
0.010986761	0.069110401	0.072590413

LBK 336/Ens–038

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.452860095	–0.297715833	–0.120501658
–0.427039822	–0.148814634	–0.262493939
–0.424785299	–0.282249274	–0.142396345
–0.408804049	–0.135331344	–0.247533943
AVERAGE	AVERAGE	AVERAGE
–0.428372316	–0.216027771	–0.193231471
SD	SD	SD
0.018231937	0.085805489	0.072156967

LBK 338/Ens–040

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.786819917	–0.38014977	–0.398575017
–0.728220893	–0.311346758	–0.43854231
–0.778559494	–0.341914861	–0.436485211
–0.796303788	–0.378469013	–0.430080911
AVERAGE	AVERAGE	AVERAGE
–0.772476023	–0.3529701	–0.425920862
SD	SD	SD
0.030381106	0.032881859	0.018583215

D.1.2 Sheep/goat

LBK 346/EnR–001

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.830085278	–0.406173842	–0.420049099
–0.757680121	–0.410628354	–0.345238354
–0.817946782	–0.478949259	–0.329139394
–0.884578842	–0.457419739	–0.446179587
–0.892621988	–0.330911247	–0.53588742
–0.816784204	–0.365303098	–0.476898303
–0.806253226	–0.431737283	–0.359133127
AVERAGE	AVERAGE	AVERAGE
–0.829421492	–0.411588975	–0.416075041
SD	SD	SD
0.046569016	0.051194638	0.076151797

LBK 349/EnR–004

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.533630177	–0.380560508	–0.146160185
–0.574456594	–0.384931876	–0.192668647
–0.618050508	–0.3501207	–0.257763175
–0.566146174	–0.290118027	–0.257681549
–0.613815841	–0.298788756	–0.312919227
–0.586486887	–0.197615743	–0.39957255
–0.601808841	–0.310118833	–0.278701622
–0.622718122	–0.388076633	–0.241465136
AVERAGE	AVERAGE	AVERAGE
–0.589639143	–0.325041385	–0.260866511
SD	SD	SD
0.030620743	0.065071913	0.076037227

LBK 350/EnR–005

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.776880273	–0.378471437	–0.395132712
–0.745308917	–0.304015772	–0.403736739
–0.838781616	–0.448169744	–0.368677872
–0.802810406	–0.353860294	–0.458405863
–0.836501036	–0.385914134	–0.452096365
–0.834540597	–0.470746006	–0.359093104
AVERAGE	AVERAGE	AVERAGE
–0.805803808	–0.390196231	–0.406190442
SD	SD	SD
0.038368794	0.061241119	0.041442611

LBK 352/EnR–007

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.801649756	–0.488002694	–0.293642183
–0.769100636	–0.330563335	–0.458275275
–0.803904191	–0.401546528	–0.396157274
AVERAGE	AVERAGE	AVERAGE
–0.791551527	–0.406704186	–0.382691577
SD	SD	SD
0.01947569	0.0788463	0.083138484

D.1.3 Cattle

LBK 347/EnR–002

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.768365736	–0.3646468	–0.404495501
–0.790620458	–0.373724837	–0.385634493
–0.78272678	–0.400844076	–0.382014864
–0.776440812	–0.414458142	–0.344661626
–0.768885573	–0.290067268	–0.468706835
–0.854608333	–0.360887185	–0.498811836
–0.894016354	–0.418227359	–0.461536079
–0.836041939	–0.447791012	–0.386981329
AVERAGE	AVERAGE	AVERAGE
–0.808963248	–0.383830835	–0.41660532
SD	SD	SD
0.046885357	0.04824	0.053247492

LBK 348//EnR–003

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.694398613	–0.313719701	–0.376289413
–0.810788356	–0.454066663	–0.385327715
–0.613376761	–0.262501036	–0.347724577
–0.714491896	–0.492579089	–0.201766231
–0.693614615	–0.305709879	–0.371777157
–0.71889498	–0.386034734	–0.356219121
–0.692892235	–0.346878439	–0.3530308
–0.71088565	–0.305412226	–0.377680915
AVERAGE	AVERAGE	AVERAGE
–0.706167888	–0.358362721	–0.346226991
SD	SD	SD
0.05382711	0.080026286	0.059871368

LBK 351/EnR–006

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.639511029	–0.414457942	–0.265689588
–0.754895089	–0.323687745	–0.439688379
–0.650560789	–0.245524907	–0.414745117
AVERAGE	AVERAGE	AVERAGE
–0.681655636	–0.327890198	–0.373374361
SD	SD	SD
0.063667397	0.084544888	0.094087987

LBK 353/EnR–008

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.798819334	–0.357401417	–0.438927286
–0.804669929	–0.429272234	–0.380872166
–0.957136203	–0.520774784	–0.346674095
AVERAGE	AVERAGE	AVERAGE
–0.853541822	–0.435816145	–0.388824516
SD	SD	SD
0.089763045	0.081883035	0.046637889

LBK 359/EnR–014

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.671293018	–0.352812881	–0.315268256
–0.563069436	–0.245030125	–0.355237592
–0.740581748	–0.385623585	–0.356075736
AVERAGE	AVERAGE	AVERAGE
–0.658314734	–0.327822197	–0.342193861
SD	SD	SD
0.089464977	0.073552913	0.023322024

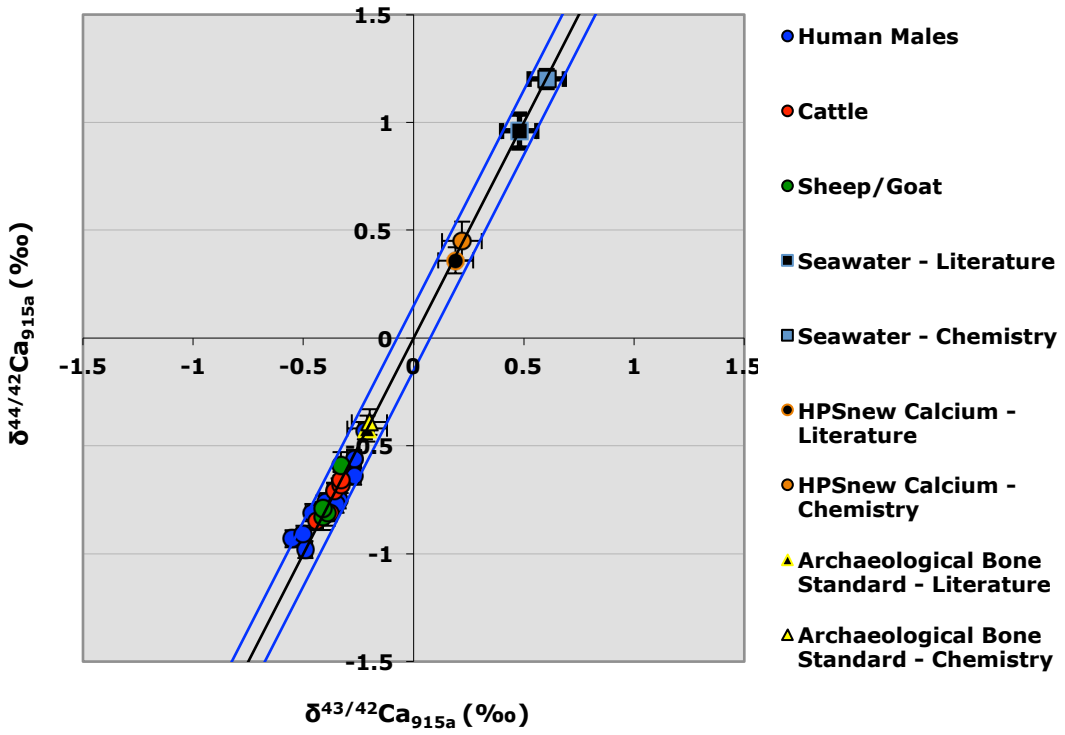

Figure D.1: Three-isotope plot of mean standards and Ensisheim bone samples.

D.2 Modern sheep samples – Highfield Farm, Yorkshire, UK (Standard Fisher07)

D.2.1 Highfield Farm, Yorkshire, UK – feed samples

Grass

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.90824194	–0.370124302	–0.569694718
–1.013316	–0.4881104	–0.5183229
–0.9413492	–0.3613557	–0.5833871
–1.006384205	–0.514904651	–0.514904651
–0.897200195	–0.3779954	–0.525065756
AVERAGE	AVERAGE	AVERAGE
–0.953298308	–0.422498091	–0.540849628
SD	SD	SD
0.054175852	0.072982605	0.0335105

Hay

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.280012302	–0.639148048	–0.639148048
–1.226941471	–0.588451637	–0.678045866
–1.246687597	–0.574646592	–0.677970299
–1.135845082	–0.464009409	–0.705497933
–1.181268577	–0.574213672	–0.597221835
–1.138581421	–0.654584041	–0.513674256
AVERAGE	AVERAGE	AVERAGE
–1.201556075	–0.5825089	–0.637110941
SD	SD	SD
0.059214823	0.067247143	0.070742448

Course mix

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.916692051	–0.425272692	–0.501563516
–0.936334918	–0.450508431	–0.492179302
AVERAGE	AVERAGE	AVERAGE
–0.926513484	–0.437890561	–0.496871409
SD	SD	SD
0.013889604	0.017844362	0.006635641

Sugar beet

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.042741	–0.468793088	–0.557147898
–1.002219098	–0.496168477	–0.519847016
–1.110039022	–0.537834595	–0.578920484
AVERAGE	AVERAGE	AVERAGE
–1.051666374	–0.500932053	–0.551971799
SD	SD	SD
0.054461277	0.03476638	0.02987495

D.2.2 Highfield Farm, Yorkshire, UK – Sheep #29

HF29 – 1st molar – 1st sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.148046681	–0.545094495	–0.59837354
–1.189861354	–0.595910535	–0.576686964
–1.157881662	–0.607525026	–0.539762504
AVERAGE	AVERAGE	AVERAGE
–1.165263232	–0.582843352	–0.57160767
SD	SD	SD
0.021862809	0.033203257	0.029633812

HF29 – 1st molar – 2nd sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.126601485	–0.540502	–0.54873682
–1.122734736	–0.499271799	–0.635550526
–1.149197204	–0.543148049	–0.604780558
AVERAGE	AVERAGE	AVERAGE
–1.132844475	–0.527640616	–0.596355968
SD	SD	SD
0.014293241	0.024603713	0.044015738

HF29 – 1st molar – 3rd sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.036710401	–0.538577327	–0.487028598
–1.092075529	–0.464902186	–0.620266591
–1.20967972	–0.556739073	–0.654172286
–1.173657033	–0.522140361	–0.666478606
AVERAGE	AVERAGE	AVERAGE
–1.128030671	–0.520589737	–0.60698652
SD	SD	SD
0.078274083	0.039723359	0.082324699

HF29 – 1st molar – 4th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.192534392	–0.568230182	–0.607672799
–1.12376215	–0.609533098	–0.539401079
–1.183523077	–0.512932571	–0.678831913
–1.107678956	–0.53155871	–0.561384791
AVERAGE	AVERAGE	AVERAGE
–1.151874644	–0.55556364	–0.596822645
SD	SD	SD
0.042420159	0.04268797	0.0616342

HF29 – 1st molar – 5th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.086266085	–0.529241758	–0.552095482
–1.198934661	–0.655846352	–0.527197172
–1.185366501	–0.513024614	–0.691342103
–1.207986115	–0.628023658	–0.582768647
–1.183533203	–0.657843286	–0.526945514
–1.186537365	–0.531355739	–0.641691337
AVERAGE	AVERAGE	AVERAGE
–1.174770655	–0.585889234	–0.587006709
SD	SD	SD
0.044379577	0.068320985	0.066779586

HF29 – 1st molar – 6th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.118370375	–0.535929101	–0.586066501
–1.159221083	–0.602562039	–0.53628611
–1.156557367	–0.437079024	–0.716019305
–1.093208123	–0.644300244	–0.508561824
AVERAGE	AVERAGE	AVERAGE
–1.131839237	–0.554967602	–0.586733435
SD	SD	SD
0.031804217	0.090380183	0.091961974

HF29 – 1st molar – 7th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.047565765	–0.561409967	–0.499328543
–1.06556734	–0.554457632	–0.520826875
–1.104099146	–0.653215938	–0.462039859
AVERAGE	AVERAGE	AVERAGE
–1.072410751	–0.589694513	–0.494065092
SD	SD	SD
0.028881309	0.055120889	0.029744852

HF29 – 2nd molar – 1st sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.169003724	–0.586429335	–0.579620302
–1.122158199	–0.497200943	–0.660528609
–1.111899321	–0.483167369	–0.61416297
AVERAGE	AVERAGE	AVERAGE
–1.134353748	–0.522265882	–0.61810396
SD	SD	SD
0.030443008	0.056008453	0.04059787

HF29 – 2nd molar – 2nd sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.12957653	–0.503507308	–0.613363898
–1.216055356	–0.566267057	–0.651057814
–1.234569663	–0.572897134	–0.654452292
–1.20266212	–0.54975514	–0.663635856
–1.200198555	–0.585293914	–0.608389568
–1.061271607	–0.440275783	–0.62442334
–1.077612423	–0.453934832	–0.605546809
–1.096251449	–0.543413187	–0.533826606
–1.175679015	–0.665256354	–0.511978751
AVERAGE	AVERAGE	AVERAGE
–1.154875191	–0.542288968	–0.607408326
SD	SD	SD
0.064877529	0.06915235	0.052595661

HF29 – 2nd molar – 3rd sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.119092865	–0.593376265	–0.539270683
–0.97736044	–0.513776348	–0.475139048
–1.078341896	–0.499460261	–0.577102079
AVERAGE	AVERAGE	AVERAGE
–1.058265067	–0.535537625	–0.530503937
SD	SD	SD
0.072968002	0.050598605	0.051543737

HF29 – 2nd molar – 4th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.03691887	–0.51035656	–0.52972829
–1.084905256	–0.531695741	–0.564372585
–1.04734155	–0.575289255	–0.492954471
–1.004007473	–0.487593954	–0.523964395
AVERAGE	AVERAGE	AVERAGE
–1.043293287	–0.526233878	–0.527754935
SD	SD	SD
0.03332643	0.037333609	0.029269933

HF29 – 2nd molar – 5th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.966776863	–0.515523233	–0.446758211
–1.065927927	–0.533500129	–0.526743055
AVERAGE	AVERAGE	AVERAGE
–1.016352395	–0.524511681	–0.486750633
SD	SD	SD
0.07011039	0.012711585	0.056557826

HF29 – 2nd molar – 6th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.902355962	–0.519793555	–0.39357024
–0.927684764	–0.482876287	–0.45239615
–0.938809293	–0.478229081	–0.45852516
–1.013101832	–0.414229234	–0.610748555
AVERAGE	AVERAGE	AVERAGE
–0.945487963	–0.473782039	–0.478810026
SD	SD	SD
0.047586985	0.043840916	0.092705205

HF29 – 2nd molar – 7th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.678458929	–0.262160571	–0.427662977
–0.740793504	–0.356350511	–0.343911983
–0.780678005	–0.414322674	–0.31622416
–0.672005616	–0.276151552	–0.396798333
–0.677873023	–0.294803173	–0.397394582
AVERAGE	AVERAGE	AVERAGE
–0.709961815	–0.320757696	–0.376398407
SD	SD	SD
0.048513318	0.06347878	0.045170124

HF29 – 1st molar – bulk sample

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.087256612	–0.450431817	–0.631645436
–1.123903626	–0.596743616	–0.513814173
–1.051659206	–0.555517297	–0.473618227
–1.078349641	–0.438670026	–0.675156711
–1.044768694	–0.417831774	–0.597495472
–1.046554617	–0.427110985	–0.622335337
AVERAGE	AVERAGE	AVERAGE
–1.072082066	–0.481050919	–0.585677559
SD	SD	SD
0.030886203	0.075590856	0.076582844

HF29 – 2nd molar – bulk sample

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.112882108	–0.459609516	–0.631668906
–1.136796394	–0.569227732	–0.492179302
–1.078306689	–0.42028869	–0.668954325
–1.087273218	–0.486624707	–0.600604319
–1.137882686	–0.562229642	–0.609731563
AVERAGE	AVERAGE	AVERAGE
–1.110628219	–0.499596057	–0.600627683
SD	SD	SD
0.027489983	0.064862112	0.06609845

HF29 – bone

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.111744665	–0.516393178	–0.605919366
–1.153574007	–0.624086036	–0.530714727
–1.167244105	–0.607404817	–0.57989351
–1.191364533	–0.52227491	–0.679046606
AVERAGE	AVERAGE	AVERAGE
–1.155981828	–0.567539735	–0.598893552
SD	SD	SD
0.033374136	0.056129563	0.061868753

D.2.3 Highfield Farm, Yorkshire, UK – Sheep #30

HF30 – 1st molar – 1st sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.169597324	–0.520308133	–0.629607689
–1.131648435	–0.572138903	–0.548260898
AVERAGE	AVERAGE	AVERAGE
–1.150622879	–0.546223518	–0.588934294
SD	SD	SD
0.026833917	0.036649889	0.057520868

HF30 – 1st molar – 2nd sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.193862746	–0.570941824	–0.598596245
–1.176329509	–0.614565902	–0.567496411
–1.168880412	–0.579424898	–0.579663423
–1.058029275	–0.519370688	–0.52669736
AVERAGE	AVERAGE	AVERAGE
–1.149275485	–0.571075828	–0.56811336
SD	SD	SD
0.061725651	0.039304508	0.03043176

HF30 – 1st molar – 3rd sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.199129774	–0.543235566	–0.669775749
–1.240146537	–0.646106233	–0.576558257
–1.165527736	–0.501147121	–0.672494956
–1.171898918	–0.523966889	–0.638213498
AVERAGE	AVERAGE	AVERAGE
–1.194175741	–0.553613952	–0.639260615
SD	SD	SD
0.033935338	0.064016273	0.044603352

HF30 – 1st molar – 4th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.151586503	–0.557259572	–0.592547551
–1.266204057	–0.633003174	–0.648072063
–1.220158849	–0.540360317	–0.688094722
–1.182578763	–0.581589141	–0.595013047
–1.190418555	–0.634287369	–0.594828708
AVERAGE	AVERAGE	AVERAGE
–1.202189345	–0.589299915	–0.623711218
SD	SD	SD
0.043314433	0.043055109	0.042917493

HF30 – 1st molar – 5th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.221562943	–0.612524985	–0.601845245
–1.286148848	–0.660582556	–0.607785861
–1.195376358	–0.5172283	–0.684865707
–1.159319562	–0.551577051	–0.734175725
AVERAGE	AVERAGE	AVERAGE
–1.215601928	–0.585478223	–0.657168135
SD	SD	SD
0.05350757	0.063716899	0.063761419

HF30 – 1st molar – 6th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.256152291	–0.545508531	–0.731801099
–1.21958517	–0.62987637	–0.585688742
AVERAGE	AVERAGE	AVERAGE
–1.237868731	–0.58769245	–0.658744921
SD	SD	SD
0.025856859	0.05965707	0.103317038

HF30 – 1st molar – 7th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.282738333	–0.639221529	–0.70662617
–1.273550694	–0.559419309	–0.700818655
–1.218106621	–0.572404582	–0.601185016
–1.282738333	–0.639221529	–0.70662617
AVERAGE	AVERAGE	AVERAGE
–1.264283495	–0.602566737	–0.678814003
SD	SD	SD
0.031087762	0.042656003	0.051825018

HF30 – 1st molar – 8th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.279806201	–0.552593043	–0.728928537
–1.242108329	–0.573197822	–0.651215292
–1.266452125	–0.590757066	–0.594987233
–1.234685696	–0.629711344	–0.625836514
AVERAGE	AVERAGE	AVERAGE
–1.255763088	–0.586564819	–0.650241894
SD	SD	SD
0.021000283	0.032720818	0.05727488

HF30 – 2nd molar – 1st sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.170522184	–0.594856672	–0.603761901
–1.264770509	–0.671709086	–0.626038232
–1.233045623	–0.572741638	–0.700301811
AVERAGE	AVERAGE	AVERAGE
–1.222779438	–0.613102465	–0.643367315
SD	SD	SD
0.047955527	0.051945362	0.050549099

HF30 – 2nd molar – 2nd sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.253337848	–0.624747345	–0.634873443
–1.240130157	–0.62007101	–0.656399586
AVERAGE	AVERAGE	AVERAGE
–1.246734002	–0.622409177	–0.645636515
SD	SD	SD
0.009339248	0.003306668	0.015221282

HF30 – 2nd molar – 3rd sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.290542896	–0.721318405	–0.579135632
–1.291162928	–0.679753452	–0.631582858
–1.257531645	–0.547531736	–0.728214758
AVERAGE	AVERAGE	AVERAGE
–1.279745823	–0.649534531	–0.646311083
SD	SD	SD
0.01924054	0.090748768	0.075622992

HF30 – 2nd molar – 4th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.204934113	–0.599491466	–0.625545804
–1.192231669	–0.575175429	–0.613664257
–1.297094508	–0.595710425	–0.725068789
–1.200295807	–0.610870743	–0.579024452
–1.242861368	–0.652168921	–0.616142945
AVERAGE	AVERAGE	AVERAGE
–1.227483493	–0.606683397	–0.63188925
SD	SD	SD
0.04351008	0.0285103	0.054994202

HF30 – 2nd molar – 5th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.235868927	–0.604010353	–0.702840777
–1.28122907	–0.709622155	–0.609909009
AVERAGE	AVERAGE	AVERAGE
–1.258548998	–0.656816254	–0.656374893
SD	SD	SD
0.032074465	0.074678821	0.065712683

HF30 – 2nd molar – 6th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.190854806	–0.570595029	–0.669422871
–1.225661358	–0.588765616	–0.628967842
–1.23162868	–0.562772054	–0.674884991
AVERAGE	AVERAGE	AVERAGE
–1.216048281	–0.574044233	–0.657758568
SD	SD	SD
0.022021254	0.013335632	0.025082626

HF30 – 2nd molar – 7th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.123348834	–0.519867687	–0.601144033
–1.187193136	–0.648550191	–0.554627733
–1.146430136	–0.528259591	–0.600701024
AVERAGE	AVERAGE	AVERAGE
–1.152324035	–0.565559156	–0.58549093
SD	SD	SD
0.032327655	0.071994721	0.02672923

HF30 – 2nd molar – 8th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.995070203	–0.425305933	–0.566586271
–1.002132378	–0.47584566	–0.563498895
–1.019034308	–0.44362411	–0.575837131
–0.964663165	–0.4412515	–0.498370236
–1.087740103	–0.657024186	–0.575623282
AVERAGE	AVERAGE	AVERAGE
–1.013728031	–0.488610278	–0.555983163
SD	SD	SD
0.045814512	0.095916929	0.032665256

HF30 – 1st molar – bulk sample

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.250912568	–0.705466562	–0.548084337
–1.257890241	–0.514275744	–0.739812106
–1.21632029	–0.603644359	–0.58194245
–1.279557785	–0.633655365	–0.66570681
–1.199528148	–0.52336071	–0.715119357
AVERAGE	AVERAGE	AVERAGE
–1.240841806	–0.596080548	–0.650133012
SD	SD	SD
0.032400307	0.079709753	0.083024082

HF30 – 2nd molar – bulk sample

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.269447307	–0.590621301	–0.702866892
–1.269468986	–0.664146434	–0.609992104
–1.209165789	–0.610520227	–0.575744444
–1.197713818	–0.530511289	–0.63796446
–1.200916961	–0.661087213	–0.523172842
AVERAGE	AVERAGE	AVERAGE
–1.229342572	–0.611377293	–0.609948148
SD	SD	SD
0.036857886	0.055286786	0.067302778

HF30 – bone (unashed)

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.007490722	–0.425036243	–0.557444674
–1.005539713	–0.440990023	–0.563502384
–0.908831021	–0.417992991	–0.507802824
–1.018155684	–0.473911843	–0.573346515
AVERAGE	AVERAGE	AVERAGE
–0.985004285	–0.439482775	–0.550524099
SD	SD	SD
0.051083999	0.024887384	0.029224991

HF30 – bone (ashed)

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.167368614	–0.681861112	–0.484405858
–1.19208933	–0.414521136	–0.782054942
–1.256640201	–0.660600801	–0.592492408
–1.037599401	–0.49042068	–0.524530328
–1.164799086	–0.432599601	–0.72271144
–1.078705553	–0.602717289	–0.458985889
AVERAGE	AVERAGE	AVERAGE
–1.149533698	–0.547120103	–0.594196811
SD	SD	SD
0.079220975	0.116650171	0.131908345

D.2.4 Highfield Farm, Yorkshire, UK – Sheep #33

HF33 – 1st molar – bulk sample

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.226731941	–0.611275037	–0.609992104
–1.192474477	–0.606035563	–0.631520292
–1.29728593	–0.447587503	–0.848097661
–1.21626153	–0.459067267	–0.711906796
–1.24603502	–0.65220187	–0.576040434
–1.232617884	–0.557913999	–0.650178954
AVERAGE	AVERAGE	AVERAGE
–1.235234464	–0.555680207	–0.671289374
SD	SD	SD
0.035311654	0.084812689	0.097742407

HF33 – 2nd molar – bulk sample

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.236744842	–0.576821872	–0.659542779
–1.162595687	–0.608307413	–0.588180664
–1.166566019	–0.564639778	–0.550991475
–1.147863935	–0.54657107	–0.591508905
–1.222870664	–0.70443057	–0.519937112
AVERAGE	AVERAGE	AVERAGE
–1.187328229	–0.600154141	–0.582032187
SD	SD	SD
0.039703479	0.062483717	0.052332657

HF33 – bone

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.09714456	–0.470217268	–0.618283161
–1.176298866	–0.573670593	–0.565241967
–1.11023043	–0.525222183	–0.512417586
–1.03766762	–0.490474886	–0.549326067
AVERAGE	AVERAGE	AVERAGE
–1.105335369	–0.514896232	–0.561317195
SD	SD	SD
0.056879384	0.045290407	0.043952566

D.2.5 Highfield Farm, Yorkshire, UK – Sheep #35

HF35 – 1st molar – bulk sample

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.253587523	–0.622763738	–0.619191212
–1.166595856	–0.626599493	–0.585032455
–1.19130546	–0.58543036	–0.625414105
–1.100075568	–0.466496154	–0.68429315
–1.182442995	–0.475155344	–0.631528112
–1.243080564	–0.741766507	–0.476901257
–1.229876047	–0.596582044	–0.618934442
AVERAGE	AVERAGE	AVERAGE
–1.195280573	–0.587827663	–0.605899248
SD	SD	SD
0.053148843	0.094797789	0.064038751

HF35 – 2nd molar – bulk sample

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.151367053	–0.494070011	–0.693618708
–1.135286671	–0.654085279	–0.498379493
–1.074144379	–0.445264588	–0.622138858
–1.144342046	–0.592281063	–0.47680971
–1.22480065	–0.543968491	–0.69954313
–1.224191849	–0.555315888	–0.665311288
AVERAGE	AVERAGE	AVERAGE
–1.159022108	–0.547497553	–0.609300198
SD	SD	SD
0.057646536	0.073138455	0.098399801

D.2.6 Highfield Farm, Yorkshire, UK – Sheep #44

HF44 – 1st molar – bulk sample

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.103382517	–0.469373387	–0.629681902
–1.108596898	–0.590811389	–0.514460684
–1.096386487	–0.547284961	–0.558008035
–1.141157313	–0.512609504	–0.598069444
AVERAGE	AVERAGE	AVERAGE
–1.112380804	–0.53001981	–0.575055016
SD	SD	SD
0.019825848	0.05155841	0.049920047

HF44 – 2nd molar – bulk sample

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.113274225	–0.499239637	–0.601766837
–1.005506754	–0.517447169	–0.48372993
–1.04634421	–0.462178401	–0.632365979
AVERAGE	AVERAGE	AVERAGE
–1.05504173	–0.492955069	–0.572620915
SD	SD	SD
0.054407647	0.028165245	0.078487466

HF44 – bone

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.006156995	–0.478942642	–0.530688374
–1.092371818	–0.499506248	–0.564509125
AVERAGE	AVERAGE	AVERAGE
–1.049264406	–0.489224445	–0.54759875
SD	SD	SD
0.060963086	0.014540666	0.023914882

D.2.7 Highfield Farm, Yorkshire, UK – Sheep #61

HF61 – 1st molar – bulk sample

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.130985871	–0.452226972	–0.697089538
–1.078733259	–0.442909483	–0.656415582
–1.071765351	–0.461840362	–0.617104017
AVERAGE	AVERAGE	AVERAGE
–1.09382816	–0.452325606	–0.656869712
SD	SD	SD
0.032367569	0.009465824	0.039994694

HF61 – 2nd molar – bulk sample

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.138082053	–0.508760581	–0.629599878
–1.109158624	–0.430424325	–0.682195927
–1.137214894	–0.409619146	–0.678953977
AVERAGE	AVERAGE	AVERAGE
–1.128151857	–0.449601351	–0.663583261
SD	SD	SD
0.016454336	0.052278818	0.029475079

D.2.8 Highfield Farm, Yorkshire, UK – Ewe, Amber

Milk – Amber

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.39516873	–0.648350385	–0.721386796
–1.353638384	–0.712594534	–0.643986774
–1.375699765	–0.664100649	–0.70887079
–1.504952974	–0.705489258	–0.761397753
AVERAGE	AVERAGE	AVERAGE
–1.407364963	–0.682633706	–0.708910528
SD	SD	SD
0.067234411	0.031298816	0.048736234

D.2.9 Highfield Farm, Yorkshire, UK – Ewe, Coco

Milk – Coco

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.400536303	–0.61375783	–0.789446799
–1.378533292	–0.576792711	–0.773850059
–1.448087898	–0.565301517	–0.810939638
–1.374714075	–0.588083949	–0.801408499
–1.336866039	–0.558696467	–0.78023407
–1.348013921	–0.556321839	–0.789485905
AVERAGE	AVERAGE	AVERAGE
–1.381125255	–0.576492385	–0.790894162
SD	SD	SD
0.039909509	0.021780492	0.013569005

D.2.10 Highfield Farm, Yorkshire, UK – Ewe, Fifi

Milk, 2010 – Fifi

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.409218435	–0.72106358	–0.692445513
–1.471010795	–0.550423642	–0.90006766
AVERAGE	AVERAGE	AVERAGE
–1.440114615	–0.635743611	–0.796256586
SD	SD	SD
0.043693797	0.120660658	0.146811029

Milk, 2011 – Fifi

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.457289384	–0.659359914	–0.81731083
–1.471010795	–0.550423642	–0.90006766
–1.469590614	–0.628411087	–0.862395225
–1.448408819	–0.681060983	–0.744241432
AVERAGE	AVERAGE	AVERAGE
–1.461574903	–0.629813906	–0.831003787
SD	SD	SD
0.010723791	0.057166457	0.067008567

Bone – Fifi

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.26016004	–0.631537217	–0.618194809
–1.210546348	–0.601256419	–0.658381004
–1.290444282	–0.6148178	–0.670253766
AVERAGE	AVERAGE	AVERAGE
–1.25371689	–0.615870478	–0.648943193
SD	SD	SD
0.040336777	0.015167821	0.027282558

D.2.11 Highfield Farm, Yorkshire, UK – Ewe, Helena

Milk – Helena

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.510270838	–0.724083001	–0.801919653
–1.414737178	–0.627515521	–0.767797103
–1.390092355	–0.609065188	–0.780016715
–1.342388769	–0.60880213	–0.705637674
–1.512149115	–0.838746889	–0.684038987
AVERAGE	AVERAGE	AVERAGE
–1.433927651	–0.681642546	–0.747882026
SD	SD	SD
0.075193174	0.099982374	0.050521858

Bone – Helena

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.070976536	–0.548467977	–0.525015921
–1.034371759	–0.516010136	–0.481314143
–1.085128316	–0.435027793	–0.669933627
–1.06435799	–0.563660249	–0.480618665
AVERAGE	AVERAGE	AVERAGE
–1.06370865	–0.515791539	–0.539220589
SD	SD	SD
0.021390801	0.05739336	0.089582388

D.2.12 Highfield Farm, Yorkshire, UK – Ewe, Jet

Milk – Jet

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.488815381	–0.696460464	–0.780248565
–1.399106506	–0.670817156	–0.680840529
–1.320286024	–0.618173378	–0.708879568
–1.340738232	–0.602254547	–0.729795188
AVERAGE	AVERAGE	AVERAGE
–1.387236536	–0.646926386	–0.729795188
SD	SD	SD
0.075506338	0.044146357	0.043804799

D.2.13 Highfield Farm, Yorkshire, UK – Stillborn lamb

Bone – stillborn lamb

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.199748024	–0.594587868	–0.608793967
–1.173093437	–0.495043806	–0.698135513
–1.314961001	–0.580001013	–0.72268454
AVERAGE	AVERAGE	AVERAGE
–1.229267488	–0.556544229	–0.676538007
SD	SD	SD
0.075399937	0.053757982	0.059938342

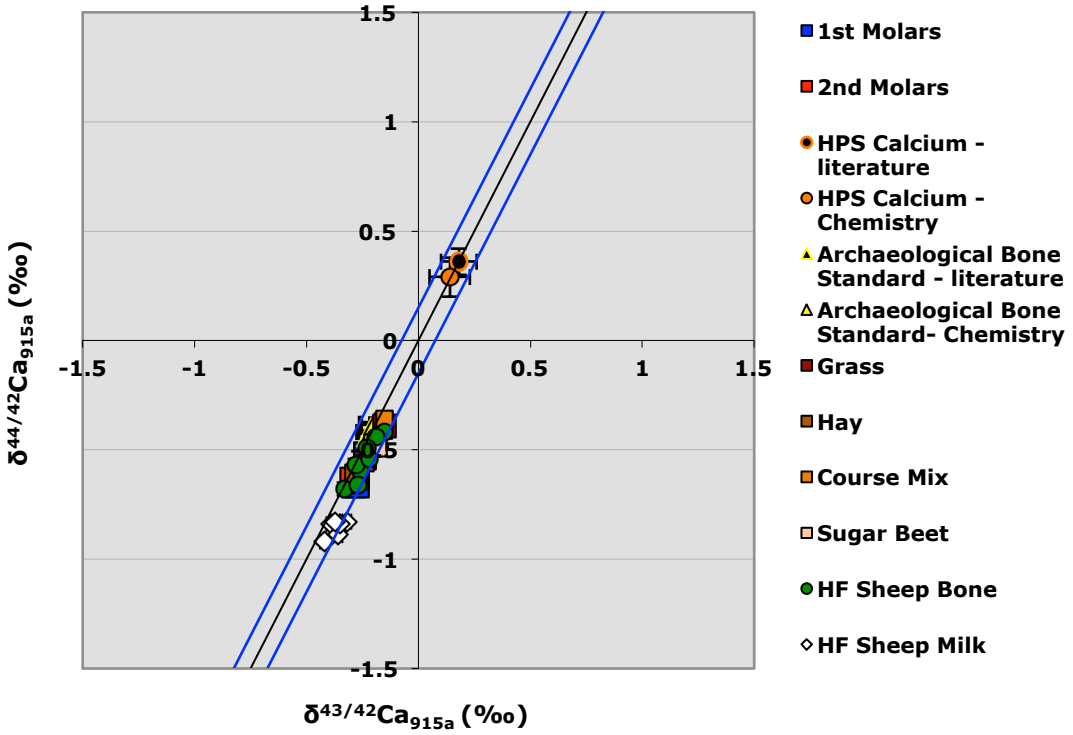

Figure D.2: Three-isotope plot of mean standards and Highfield Farm sheep feed, milk, bone and bulk enamel results.

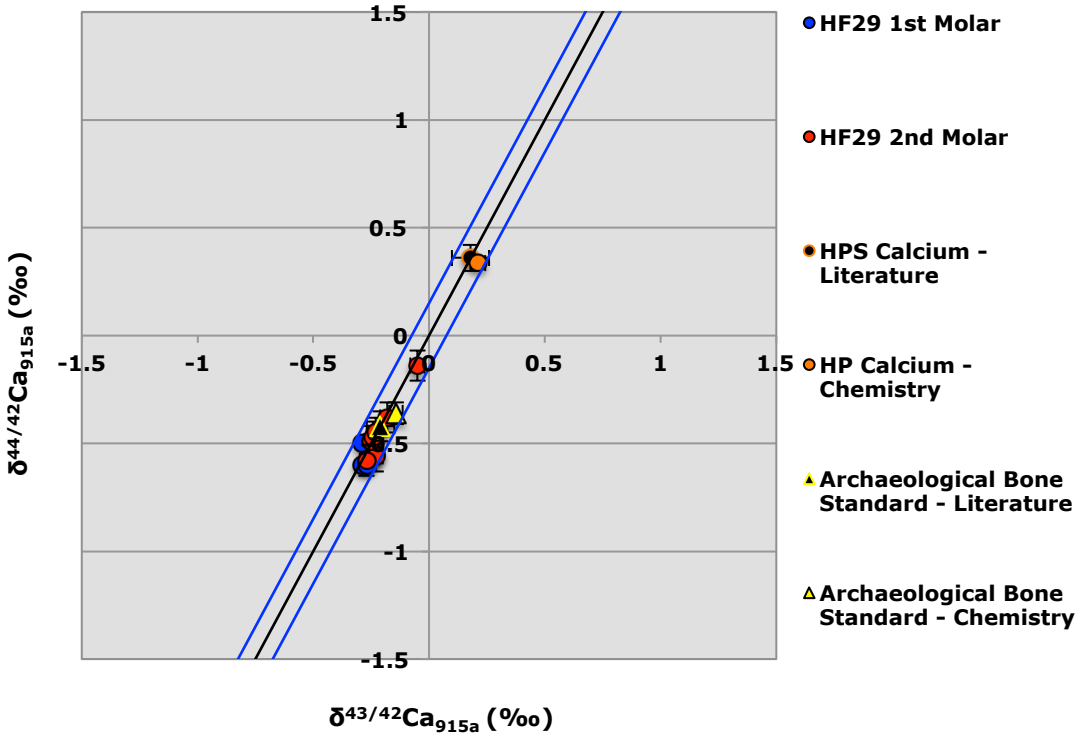

Figure D.3: Three-isotope plot of mean standards and Highfield Farm sheep HF29 incremental enamel sample results.

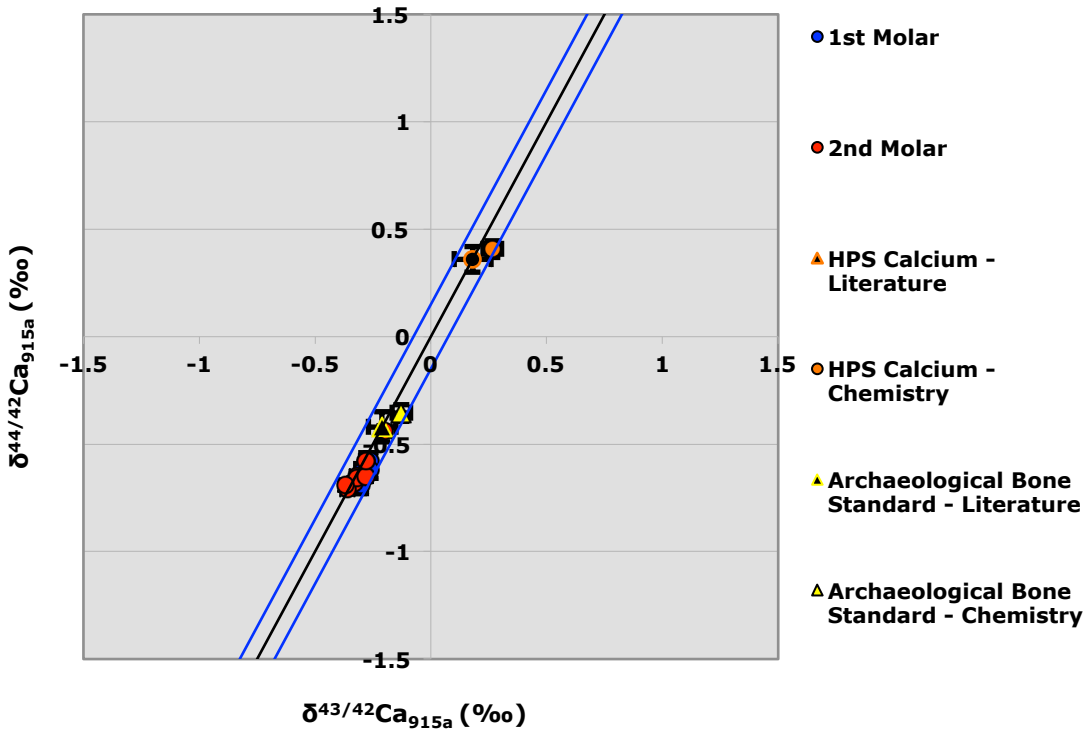

Figure D.4: Three-isotope plot of mean standards and Highfield Farm sheep HF30 incremental enamel sample results.

D.3 Modern sheep samples – The Loft and White Hamars Grazing Project, Hoy, Orkney, UK (Standard Fisher07)

D.3.1 LWHGP, Hoy, UK – Sheep #545

545 – 1st molar – 1st sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.183609556	–0.607064397	–0.587137365
–1.180765501	–0.510647694	–0.68196331
–1.055175272	–0.5904856	–0.467931217
–1.111237748	–0.581412718	–0.514553176
AVERAGE	AVERAGE	AVERAGE
–1.132697019	–0.572402602	–0.562896267
SD	SD	SD
0.061570521	0.042517783	0.093309533

545 – 1st molar – 2nd sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.003108503	–0.533893043	–0.460657101
–1.022414695	–0.439348109	–0.592018597
–1.085578334	–0.572204642	–0.480472661
AVERAGE	AVERAGE	AVERAGE
–1.037033844	–0.515148598	–0.511049453
SD	SD	SD
0.043134764	0.068382968	0.070817836

545 – 1st molar – 3rd sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.087652314	–0.476537406	–0.608887282
–1.158296208	–0.505835502	–0.653734497
–1.143345317	–0.624888003	–0.508211962
–1.053878844	–0.480218005	–0.598121341
AVERAGE	AVERAGE	AVERAGE
–1.110793171	–0.521869729	–0.59223877
SD	SD	SD
0.048617283	0.069904093	0.060975423

545 – 1st molar – 4th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.201907654	–0.501405315	–0.68807766
–1.175252181	–0.510380026	–0.653710193
–1.221230625	–0.523988564	–0.693653074
–1.16232254	–0.52845379	–0.631578947
–1.270488946	–0.726049555	–0.575865656
AVERAGE	AVERAGE	AVERAGE
–1.206240389	–0.55805545	–0.648577106
SD	SD	SD
0.042604	0.09452647	0.047943659

545 – 1st molar – 5th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.261751923	–0.689543	–0.57799359
–1.135417669	–0.634493058	–0.469736021
–1.210802064	–0.491997002	–0.73111644
AVERAGE	AVERAGE	AVERAGE
–1.202657219	–0.605344354	–0.592948683
SD	SD	SD
0.063559734	0.101947735	0.131330392

545 – 1st molar – 6th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.200789576	–0.645977075	–0.519262153
–1.163821298	–0.636805527	–0.534648631
–1.226431086	–0.469004331	–0.749685566
–1.216861863	–0.574503973	–0.653390353
AVERAGE	AVERAGE	AVERAGE
–1.201975956	–0.581572727	–0.614246676
SD	SD	SD
0.02754892	0.08148659	0.10837255

545 – 1st molar – 7th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.180831453	–0.591021964	–0.540812826
–1.202107962	–0.567812176	–0.625770596
–1.186918282	–0.585981933	–0.610018548
AVERAGE	AVERAGE	AVERAGE
–1.189952565	–0.581605358	–0.592200657
SD	SD	SD
0.010957993	0.012208168	0.04519473

545 – 1st molar – 8th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.081711362	–0.467327371	–0.583474029
–1.025005568	–0.497011031	–0.493245025
–1.083970642	–0.60247336	–0.505493756
–1.08327228	–0.632162109	–0.44644518
–1.036673758	–0.457247371	–0.576365304
AVERAGE	AVERAGE	AVERAGE
–1.062126722	–0.531244248	–0.521004659
SD	SD	SD
0.028869028	0.080608342	0.058175434

545 – 2nd molar – 1st sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.161654868	–0.560478888	–0.600429586
–1.187935351	–0.571736909	–0.603380789
–1.138819547	–0.477462836	–0.643345396
AVERAGE	AVERAGE	AVERAGE
–1.162803255	–0.536559544	–0.61571859
SD	SD	SD
0.024578032	0.051487877	0.023970976

545 – 2nd molar – 2nd sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.105411972	–0.493625345	–0.594107199
AVERAGE	AVERAGE	AVERAGE
–1.105411972	–0.493625345	–0.594107199
SD	SD	SD
———	———	———

545 – 2nd molar – 3rd sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.06056298	–0.512211536	–0.560151272
–0.978772429	–0.537261619	–0.470224285
–1.070904076	–0.546360276	–0.476390814
AVERAGE	AVERAGE	AVERAGE
–1.036746495	–0.531944477	–0.502255457
SD	SD	SD
0.050472555	0.017684403	0.050233958

545 – 2nd molar – 4th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.947266014	–0.367571045	–0.603631073
–0.931171499	–0.537424496	–0.399253489
–1.054931699	–0.626562102	–0.405207692
AVERAGE	AVERAGE	AVERAGE
–0.977789737	–0.510519214	–0.469364085
SD	SD	SD
0.067289821	0.131575122	0.116316728

545 – 2nd molar – 5th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.893550949	–0.314572996	–0.593934444
AVERAGE	AVERAGE	AVERAGE
–0.893550949	–0.314572996	–0.593934444
SD	SD	SD
———	———	———

545 – 2nd molar – 6th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.926238987	–0.54867732	–0.402227723
–0.907402298	–0.319336882	–0.591219615
AVERAGE	AVERAGE	AVERAGE
–0.916820642	–0.434007101	–0.496723669
SD	SD	SD
0.01331955	0.162168179	0.133637448

545 – 2nd molar – 7th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.888355718	–0.413382572	–0.445621766
–0.891589528	–0.527878891	–0.442276704
–0.822199091	–0.321370692	–0.513448643
AVERAGE	AVERAGE	AVERAGE
–0.867381446	–0.420877385	–0.467115704
SD	SD	SD
0.039162459	0.103457906	0.040160344

545 – 2nd molar – 8th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.947155547	–0.44105283	–0.538612979
–0.93723374	–0.573852524	–0.439220538
–0.928486652	–0.587506311	–0.343331178
AVERAGE	AVERAGE	AVERAGE
–0.937625313	–0.534137222	–0.440388232
SD	SD	SD
0.009340605	0.080902005	0.097646137

545 – 2nd molar – 9th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.91743512	–0.497054492	–0.421621756
–0.882249063	–0.455971149	–0.419115387
–0.922403847	–0.460256823	–0.437433105
–0.960472216	–0.520121331	–0.468186568
AVERAGE	AVERAGE	AVERAGE
–0.920640061	–0.483350949	–0.436589204
SD	SD	SD
0.032009644	0.030674855	0.022571843

545 – 2nd molar – 10th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.955194387	–0.382002614	–0.542899954
–0.988758402	–0.434911672	–0.585928418
–1.004921833	–0.56162189	–0.434119296
AVERAGE	AVERAGE	AVERAGE
–0.982958207	–0.459512059	–0.520982556
SD	SD	SD
0.025366048	0.092301979	0.078241815

545 – 3rd molar – 1st sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.876484047	–0.41337118	–0.467780459
AVERAGE	AVERAGE	AVERAGE
–0.876484047	–0.41337118	–0.467780459
SD	SD	SD
———	———	———

545 – 3rd molar – 2nd sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.920188596	–0.533061288	–0.409165303
–0.913512298	–0.463902847	–0.467786256
AVERAGE	AVERAGE	AVERAGE
–0.916850447	–0.498482068	–0.438475779
SD	SD	SD
0.004720855	0.048902403	0.041451273

545 – 3rd molar – 3rd sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.918605827	–0.415857735	–0.523792256
AVERAGE	AVERAGE	AVERAGE
–0.918605827	–0.415857735	–0.523792256
SD	SD	SD
———	———	———

545 – 3rd molar – 4th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.887162348	–0.374491392	–0.511347261
AVERAGE	AVERAGE	AVERAGE
–0.887162348	–0.374491392	–0.511347261
SD	SD	SD
———	———	———

545 – 3rd molar – 5th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.864262394	–0.475515544	–0.393582437
–0.890684803	–0.548833556	–0.322192894
AVERAGE	AVERAGE	AVERAGE
–0.877473598	–0.51217455	–0.357887666
SD	SD	SD
0.018683464	0.051843664	0.05048003

545 – 3rd molar – 6th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.892606739	–0.431854309	–0.480299955
–0.839408374	–0.447768391	–0.393436122
AVERAGE	AVERAGE	AVERAGE
–0.866007556	–0.43981135	–0.436868039
SD	SD	SD
0.037616925	0.011252956	0.061422005

545 – 1st molar – bulk sample

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.065408091	–0.596916239	–0.449190373
–1.048241851	–0.598798274	–0.467418457
–1.114998	–0.49988764	–0.624818277
–1.171039872	–0.602819729	–0.562861066
AVERAGE	AVERAGE	AVERAGE
–1.099921954	–0.574605471	–0.526072043
SD	SD	SD
0.055218008	0.049872707	0.082573776

545 – 2nd molar – bulk sample

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.964377763	–0.422431091	–0.526622306
–0.974279587	–0.550592575	–0.41477336
–0.940960745	–0.417417789	–0.550739166
–1.009616884	–0.520392748	–0.491747001
AVERAGE	AVERAGE	AVERAGE
–0.972308745	–0.477708551	–0.495970458
SD	SD	SD
0.028527058	0.067883712	0.059301359

545 – 3rd molar – bulk sample

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.931006196	–0.456397942	–0.464106039
–0.982929925	–0.589183328	–0.402148089
AVERAGE	AVERAGE	AVERAGE
–0.956968061	–0.522790635	–0.433127064
SD	SD	SD
0.036715621	0.093893446	0.043810886

D.3.2 LWHGP, Hoy, UK – Sheep #549

549 – 1st molar – bulk sample

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.090744557	–0.538767163	–0.562996876
–1.098697016	–0.628057194	–0.507146435
AVERAGE	AVERAGE	AVERAGE
–1.094720787	–0.583412179	–0.535071656
SD	SD	SD
0.005623238	0.063137587	0.039492225

549 – 2nd molar – bulk sample

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.994301234	–0.454278477	–0.510761592
–0.988320238	–0.488246553	–0.528892697
AVERAGE	AVERAGE	AVERAGE
–0.991310736	–0.471262515	–0.519827144
SD	SD	SD
0.004229202	0.024019057	0.012820627

549 – 3rd molar – bulk sample

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.990699929	–0.440698507	–0.517093501
–0.9531074	–0.552946332	–0.405506217
–0.967875612	–0.490760403	–0.405506217
–0.965912234	–0.421892555	–0.525811141
AVERAGE	AVERAGE	AVERAGE
–0.969398794	–0.476574449	–0.463479269
SD	SD	SD
0.015637818	0.058626213	0.067036054

D.3.3 LWHGP, Hoy, UK – Sheep #552

552 – 1st molar – bulk sample

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.110478278	–0.528647378	–0.60749952
–1.088959266	–0.525653263	–0.662366443
AVERAGE	AVERAGE	AVERAGE
–1.099718772	–0.52715032	–0.634932981
SD	SD	SD
0.01521624	0.00211716	0.038796774

552 – 2nd molar – bulk sample

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.945854482	–0.434009742	–0.514470251
–0.97288355	–0.334495224	–0.628586115
AVERAGE	AVERAGE	AVERAGE
–0.959369016	–0.384252483	–0.571528183
SD	SD	SD
0.019112437	0.070367391	0.080692101

552 – 3rd molar – bulk sample

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.841766428	–0.384117324	–0.448948377
–0.956145368	–0.501103347	–0.464238185
AVERAGE	AVERAGE	AVERAGE
–0.898955898	–0.442610335	–0.456593281
SD	SD	SD
0.080878125	0.08272161	0.010811527

D.3.4 LWHGP, Hoy, UK – Sheep #668

668 – 1st molar – bulk sample

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.999946026	–0.454276393	–0.557175492
–1.049166196	–0.534093748	–0.494829037
–1.01333515	–0.493070638	–0.529108715
AVERAGE	AVERAGE	AVERAGE
–1.020815791	–0.493813593	–0.527037748
SD	SD	SD
0.025448503	0.039913864	0.031224779

668 – 2nd molar – bulk sample

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.068011907	–0.605640717	–0.473600634
–1.137443447	–0.582320386	–0.584590926
–1.073242795	–0.575370036	–0.451506361
–1.098476892	–0.495374214	–0.61881954
AVERAGE	AVERAGE	AVERAGE
–1.09429376	–0.564676338	–0.532129365
SD	SD	SD
0.031692654	0.047981012	0.08204284

668 – 3rd molar – bulk sample

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.127626276	–0.601030469	–0.535515844
–1.176767491	–0.55473288	–0.652517449
–1.148969833	–0.562131603	–0.575666039
AVERAGE	AVERAGE	AVERAGE
–1.1511212	–0.572631651	–0.587899778
SD	SD	SD
0.024641145	0.024870765	0.059452436

D.3.5 LWHGP, Hoy, UK – Sheep #799

799 – 1st molar – 1st sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.10931381	–0.430592813	–0.654143893
–1.070152196	–0.405849771	–0.635944843
–1.133631837	–0.569852941	–0.557810902
AVERAGE	AVERAGE	AVERAGE
–1.104365948	–0.468765175	–0.615966546
SD	SD	SD
0.032027757	0.088414404	0.051179689

799 – 1st molar – 2nd sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.139417269	–0.515772507	–0.644897251
–1.170643341	–0.645634157	–0.536146501
–1.197584326	–0.654806625	–0.604282035
AVERAGE	AVERAGE	AVERAGE
–1.169214979	–0.60540443	–0.595108596
SD	SD	SD
0.029109823	0.077758888	0.054952665

799 – 1st molar – 3rd sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.199547599	–0.582601627	–0.635629351
–1.133586634	–0.572099467	–0.554744616
–1.1904728	–0.466425412	–0.728214758
AVERAGE	AVERAGE	AVERAGE
–1.174535678	–0.540375502	–0.639529575
SD	SD	SD
0.035752009	0.064257573	0.086800814

799 – 1st molar – 4th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.184220688	–0.486487857	–0.707156548
–1.243417099	–0.635701552	–0.619797574
–1.144937071	–0.523832061	–0.579544856
–1.133673815	–0.629508799	–0.46183936
AVERAGE	AVERAGE	AVERAGE
–1.176562168	–0.568882567	–0.592084585
SD	SD	SD
0.049557348	0.075185887	0.101866955

799 – 1st molar – 5th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.14809251	–0.548063815	–0.626290562
–1.115375596	–0.532551336	–0.589249606
–1.169229979	–0.529761055	–0.632209199
–1.196172249	–0.696121249	–0.446281914
AVERAGE	AVERAGE	AVERAGE
–1.157217583	–0.576624364	–0.57350782
SD	SD	SD
0.034135917	0.080070411	0.086921621

799 – 1st molar – 6th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.136538868	–0.481246359	–0.654192568
–1.142128004	–0.684626235	–0.508297019
–1.143469054	–0.422743606	–0.653904679
AVERAGE	AVERAGE	AVERAGE
–1.140711975	–0.529538733	–0.605464755
SD	SD	SD
0.003675694	0.13745815	0.084149851

799 – 1st molar – 7th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.002284409	–0.414465777	–0.570468342
–0.956398794	–0.403401481	–0.551951676
–1.039352632	–0.568918617	–0.467980525
–1.059596613	–0.452610321	–0.595094192
AVERAGE	AVERAGE	AVERAGE
–1.014408112	–0.459849049	–0.546373684
SD	SD	SD
0.045373615	0.075706757	0.055168958

799 – 2nd molar – 1st sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.124017552	–0.52829842	–0.625619425
–1.059860826	–0.537084047	–0.526133353
–1.163783732	–0.658750944	–0.523117782
AVERAGE	AVERAGE	AVERAGE
–1.11588737	–0.574711137	–0.558290187
SD	SD	SD
0.052436319	0.072913056	0.058328322

799 – 2nd molar – 2nd sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.084114171	–0.569625196	–0.523409408
–0.967473842	–0.408549237	–0.557068581
–1.013176985	–0.525623575	–0.49527017
AVERAGE	AVERAGE	AVERAGE
–1.021588332	–0.501266003	–0.525249387
SD	SD	SD
0.058773333	0.083254637	0.030940266

799 – 2nd molar – 3rd sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.935131481	–0.445681939	–0.517324164
–0.960297735	–0.500723523	–0.424286533
–0.933587406	–0.470542869	–0.504536183
AVERAGE	AVERAGE	AVERAGE
–0.94300554	–0.47231611	–0.48204896
SD	SD	SD
0.014995367	0.027563604	0.050430711

799 – 2nd molar – 4th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.031944739	–0.516890844	–0.542126312
–0.95630143	–0.449908641	–0.504508074
–0.933372544	–0.51872458	–0.47658837
AVERAGE	AVERAGE	AVERAGE
–0.973872904	–0.495174688	–0.507740919
SD	SD	SD
0.051581847	0.039212268	0.032888356

799 – 2nd molar – 5th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.912326946	–0.512270368	–0.405822782
–0.928898707	–0.466204749	–0.467650074
–1.05316568	–0.580796081	–0.473632888
–0.919338361	–0.42235158	–0.479761791
AVERAGE	AVERAGE	AVERAGE
–0.953432423	–0.495405695	–0.456716884
SD	SD	SD
0.066834849	0.067738552	0.034287817

799 – 2nd molar – 6th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.851058985	–0.38821035	–0.467760172
–0.82219529	–0.34450763	–0.458329204
–0.894000532	–0.495883706	–0.424071145
–0.873848899	–0.484277062	–0.386928623
–0.86942439	–0.465883768	–0.405453504
AVERAGE	AVERAGE	AVERAGE
–0.862105619	–0.435752503	–0.42850853
SD	SD	SD
0.027035241	0.066076037	0.034315002

799 – 2nd molar – 7th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.925058848	–0.441042698	–0.458474025
–0.937380427	–0.388139023	–0.541928212
–0.912471006	–0.323705579	–0.609780634
AVERAGE	AVERAGE	AVERAGE
–0.924970094	–0.384295767	–0.536727623
SD	SD	SD
0.012454948	0.058762895	0.075787249

799 – 2nd molar – 8th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.852451831	–0.436418429	–0.390334572
–0.854196721	–0.410932173	–0.476703441
–0.889431957	–0.465950068	–0.408547305
AVERAGE	AVERAGE	AVERAGE
–0.86536017	–0.43776689	–0.425195106
SD	SD	SD
0.020865027	0.027533724	0.045527545

799 – 3rd molar – 1st sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.91079088	–0.512268015	–0.421263916
–0.838501725	–0.406310904	–0.417895848
–0.866669319	–0.371829124	–0.482831622
AVERAGE	AVERAGE	AVERAGE
–0.871987308	–0.430136014	–0.440663795
SD	SD	SD
0.036436811	0.073188096	0.036557218

799 – 1st molar – bulk sample

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.142437123	–0.529366004	–0.603440539
–1.151926277	–0.600189025	–0.565871458
–1.123969217	–0.578112209	–0.542472497
AVERAGE	AVERAGE	AVERAGE
–1.139444206	–0.569222413	–0.570594831
SD	SD	SD
0.014216802	0.036238742	0.030757247

799 – 2nd molar – bulk sample

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.968883337	–0.450811	–0.519592985
–0.981195181	–0.586367305	–0.358684494
–0.956248062	–0.405097281	–0.539628587
–0.916813849	–0.374791782	–0.536445752
AVERAGE	AVERAGE	AVERAGE
–0.955785107	–0.454266842	–0.488587955
SD	SD	SD
0.027905846	0.093445895	0.087047374

799 – 3rd molar – bulk sample

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.95667351	–0.453280995	–0.488794842
–0.903764826	–0.478866267	–0.40928202
AVERAGE	AVERAGE	AVERAGE
–0.930219168	–0.466073631	–0.449038431
SD	SD	SD
0.037412089	0.018091519	0.056224056

D.3.6 LWHGP, Hoy, UK – Sheep #800

800 – 1st molar – bulk sample

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.149692204	–0.547806472	–0.606574525
–1.136263317	–0.480951778	–0.665163089
–1.04480011	–0.490593109	–0.570563866
–1.156013059	–0.642465589	–0.502070265
AVERAGE	AVERAGE	AVERAGE
–1.121692173	–0.540454237	–0.586092936
SD	SD	SD
0.05191863	0.074132867	0.068245991

800 – 2nd molar – bulk sample

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.964441044	–0.444214282	–0.507532525
–0.917649168	–0.46000276	–0.439187935
–0.90891117	–0.42776518	–0.510230903
–0.956364267	–0.552720007	–0.375104239
AVERAGE	AVERAGE	AVERAGE
–0.936841412	–0.471175557	–0.458013901
SD	SD	SD
0.027636463	0.055933591	0.064309508

800 – 3rd molar – bulk sample

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.9372951	–0.466833009	–0.500961723
–0.975713347	–0.434993868	–0.533330026
AVERAGE	AVERAGE	AVERAGE
–0.956504223	–0.450913439	–0.517145874
SD	SD	SD
0.027165803	0.022513673	0.022887847

D.3.7 LWHGP, Hoy, UK – Sheep #2914

2914 – 1st molar – bulk sample

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.307783022	–0.70459182	–0.598347569
–1.352697261	–0.615486753	–0.733498603
AVERAGE	AVERAGE	AVERAGE
–1.330240142	–0.660039287	–0.665923086
SD	SD	SD
0.031759163	0.063006797	0.095566213

2914 – 2nd molar – bulk sample

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.190537516	–0.489449139	–0.737540828
–1.192375658	–0.586163766	–0.582353451
–1.194160172	–0.470208597	–0.718021726
AVERAGE	AVERAGE	AVERAGE
–1.192357782	–0.515273834	–0.679305335
SD	SD	SD
0.001811394	0.062141664	0.0845281

2914 – 3rd molar – bulk sample

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.078306105	–0.529073753	–0.53563854
–1.058833966	–0.454798353	–0.616689031
–1.099949924	–0.472525522	–0.588013196
AVERAGE	AVERAGE	AVERAGE
–1.079029999	–0.485465876	–0.580113589
SD	SD	SD
0.020567536	0.03879173	0.041098642

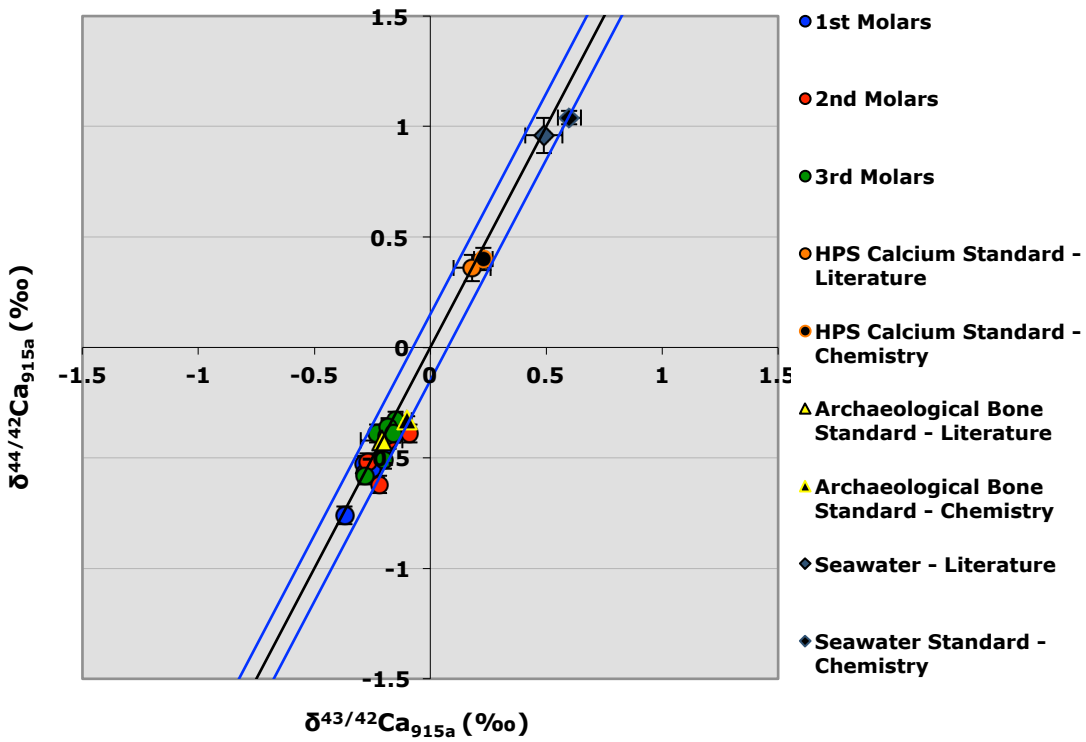

Figure D.5: Three-isotope plot of mean standards and Hoy sheep bulk enamel results.

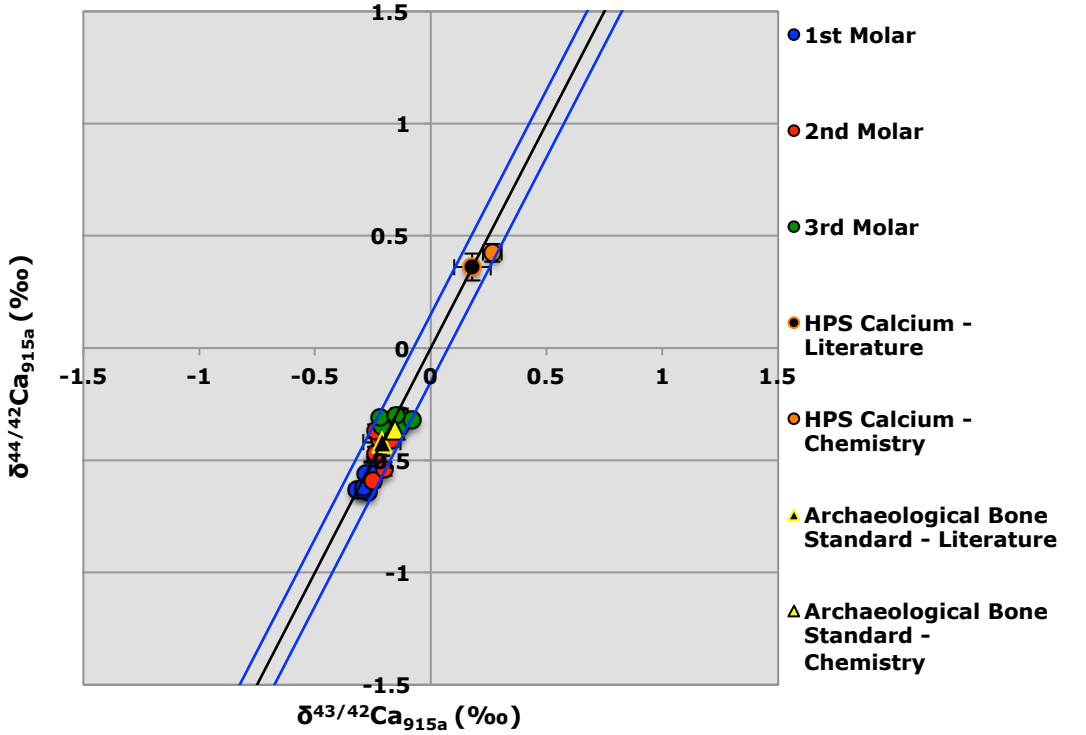

Figure D.6: Three-isotope plot of mean standards and Hoy sheep 545 incremental enamel sample results.

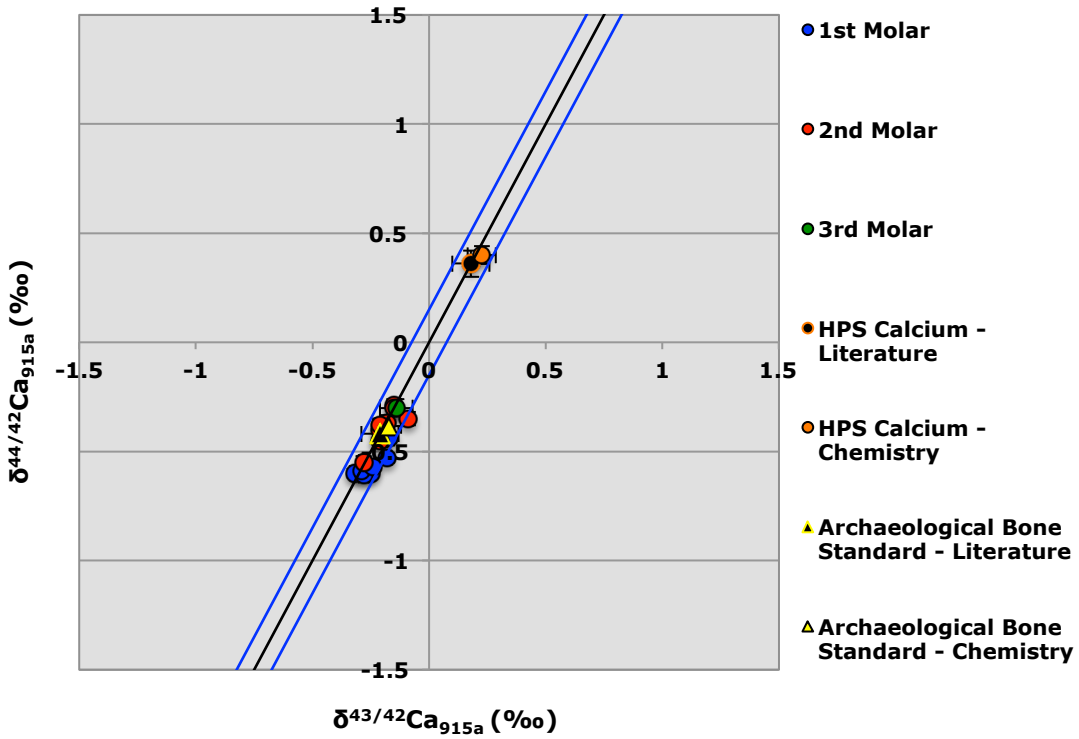

Figure D.7: Three-isotope plot of mean standards and Hoy sheep 799 incremental enamel sample results.

D.4 Archaeological sheep samples B29 – Tell Abu Hureyra, Syria (Standard Fisher07)

D.4.1 Standard sample clean (SC)

B29 – 1st molar – 1st sample segment (SC)

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.143127215	–0.493920187	–0.647545987
–0.984244959	–0.484831217	–0.495917975
–1.143127215	–0.493920187	–0.647545987
–0.984244959	–0.484831217	–0.495917975
–1.017771863	–0.519182173	–0.523578052
–1.01395325	–0.459537705	–0.582602498
AVERAGE	AVERAGE	AVERAGE
–1.04774491	–0.489370448	–0.565518079
SD	SD	SD
0.075233391	0.019294253	0.070987812

B29 – 1st molar – 2nd sample segment (SC)

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.025524157	–0.528475646	–0.514502142
–1.033878754	–0.500886891	–0.517532578
–1.061867292	–0.50538	–0.585547208
–1.005897376	–0.507550095	–0.532774952
–0.986092111	–0.413715116	–0.551783998
AVERAGE	AVERAGE	AVERAGE
–1.022651938	–0.49120155	–0.540428176
SD	SD	SD
0.028676957	0.044597127	0.029238764

B29 – 1st molar – 3rd sample segment (SC)

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.091022	–0.556063621	–0.548570153
–1.069451703	–0.631825148	–0.452463447
–1.057571457	–0.592683801	–0.470900225
–1.11116644	–0.64078272	–0.461507488
–1.032938632	–0.402196221	–0.616826659
AVERAGE	AVERAGE	AVERAGE
–1.072430046	–0.564710302	–0.510053594
SD	SD	SD
0.023706006	0.038927241	0.044120061

B29 – 1st molar – 4th sample segment (SC)

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.124061323	–0.519356366	–0.654050619
–1.036753481	–0.459533482	–0.588814994
–1.024794329	–0.4410812	–0.582389532
–1.116395637	–0.629572442	–0.467902218
–1.057098996	–0.408828866	–0.631844987
AVERAGE	AVERAGE	AVERAGE
–1.071820753	–0.491674471	–0.58500047
SD	SD	SD
0.045754578	0.086945751	0.071930001

B29 – 1st molar – 5th sample segment (SC)

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.108063225	–0.57659661	–0.508394713
–1.009116205	–0.461743092	–0.582385924
–1.091319387	–0.427222822	–0.634956033
AVERAGE	AVERAGE	AVERAGE
–1.069499606	–0.488520841	–0.575245556
SD	SD	SD
0.052959469	0.078204335	0.063582078

B29 – 1st molar – 6th sample segment (SC)

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.069747235	–0.625045959	–0.430811382
–1.084247915	–0.510206427	–0.576547534
–1.090978491	–0.514573455	–0.579699362
–1.072180685	–0.514660944	–0.501986254
–1.068581291	–0.528269298	–0.52657337
–1.073120189	–0.447967948	–0.665991382
AVERAGE	AVERAGE	AVERAGE
–1.076475968	–0.523454005	–0.546934881
SD	SD	SD
0.009033702	0.057225759	0.080055616

B29 – 1st molar – 7th sample segment (SC)

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.993031682	–0.468829718	–0.520742927
–0.957112256	–0.516964476	–0.455620403
–1.000938291	–0.519225114	–0.458613253
–1.042408832	–0.47759878	–0.535734746
AVERAGE	AVERAGE	AVERAGE
–0.998384582	–0.501764281	–0.488359672
SD	SD	SD
0.030317968	0.026466252	0.037242872

B29 – 2nd molar – 1st sample segment (SC)

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.099487238	–0.629101212	–0.498685144
–1.133740076	–0.489157019	–0.647301311
–1.144521014	–0.534949662	–0.594478778
–1.150113234	–0.649730809	–0.510850148
–1.120046849	–0.454528759	–0.687276712
AVERAGE	AVERAGE	AVERAGE
–1.129581682	–0.551493492	–0.587718419
SD	SD	SD
0.020363013	0.085491553	0.082679097

B29 – 2nd molar – 2nd sample segment (SC)

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.161033661	–0.590279453	–0.576118941
–1.146537769	–0.583318023	–0.563666433
–1.21241065	–0.523375127	–0.699673073
–1.211397372	–0.716348825	–0.520172152
–1.268021687	–0.57849646	–0.582086595
AVERAGE	AVERAGE	AVERAGE
–1.199880228	–0.598363578	–0.588343439
SD	SD	SD
0.048185548	0.071116472	0.066781646

B29 – 2nd molar – 3rd sample segment (SC)

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.095524002	–0.438668011	–0.662862948
–1.230900434	–0.530279901	–0.708958574
AVERAGE	AVERAGE	AVERAGE
–1.163212218	–0.484473956	–0.685910761
SD	SD	SD
0.095725593	0.064779388	0.032594529

B29 – 2nd molar – 4th sample segment (SC)

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.065168699	–0.413272475	–0.653471295
–1.029552713	–0.523557805	–0.535661759
–1.118760635	–0.578488492	–0.560421834
–1.068925815	–0.518831756	–0.538683013
AVERAGE	AVERAGE	AVERAGE
–1.070601966	–0.508537632	–0.572059475
SD	SD	SD
0.036681641	0.069041407	0.055383816

B29 – 2nd molar – 5th sample segment (SC)

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.046218404	–0.463753742	–0.609878798
–1.116231715	–0.495965687	–0.619275571
–1.138662349	–0.567043088	–0.600637791
AVERAGE	AVERAGE	AVERAGE
–1.100370823	–0.508920839	–0.60993072
SD	SD	SD
0.048219775	0.052849307	0.009318999

B29 – 2nd molar – 6th sample segment (SC)

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.159932653	–0.565070381	–0.628886004
–1.089842272	–0.509908768	–0.572980708
–1.131836162	–0.498237799	–0.625491568
–1.145677272	–0.498185183	–0.656310175
–1.157335002	–0.652043145	–0.486129818
AVERAGE	AVERAGE	AVERAGE
–1.136924672	–0.544689055	–0.593959655
SD	SD	SD
0.028570973	0.066087625	0.067396381

B29 – 2nd molar – 7th sample segment (SC)

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.128819005	–0.472952186	–0.616146761
–1.16133109	–0.406594644	–0.743466786
AVERAGE	AVERAGE	AVERAGE
–1.145075048	–0.439773415	–0.679806773
SD	SD	SD
0.022989516	0.046921868	0.090028853

B29 – 2nd molar – 8th sample segment (SC)

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.01198296	–0.470936336	–0.548328836
–1.141450282	–0.544108363	–0.600596882
–1.110300776	–0.463783555	–0.681089247
AVERAGE	AVERAGE	AVERAGE
–1.087911339	–0.492942751	–0.610004989
SD	SD	SD
0.067575232	0.044454813	0.066878368

B29 – 2nd molar – 9th sample segment (SC)

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.08187413	–0.571679153	–0.501566622
–1.169843343	–0.580830749	–0.59439044
AVERAGE	AVERAGE	AVERAGE
–1.125858737	–0.576254951	–0.547978531
SD	SD	SD
0.062203627	0.006471156	0.065636352

B29 – 2nd molar – 10th sample segment (SC)

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.090199842	–0.500110116	–0.544534238
–1.180899906	–0.622735117	–0.561012426
–1.159712665	–0.521703557	–0.582739745
AVERAGE	AVERAGE	AVERAGE
–1.143604137	–0.54818293	–0.562762136
SD	SD	SD
0.047447222	0.065460603	0.019162758

B29 – 2nd molar – 11th sample segment (SC)

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.108633287	–0.614822734	–0.504295796
–1.05409802	–0.47572974	–0.576440326
–1.135381008	–0.563043653	–0.554796198
–1.09702862	–0.540057315	–0.530066553
AVERAGE	AVERAGE	AVERAGE
–1.098785233	–0.54841336	–0.541399718
SD	SD	SD
0.033844092	0.057668543	0.031157939

B29 – 2nd molar – 12th sample segment (SC)

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.114301815	–0.580401693	–0.525960807
–0.994937316	–0.536815446	–0.479482777
–1.085559776	–0.629685295	–0.418437276
AVERAGE	AVERAGE	AVERAGE
–1.064932969	–0.582300811	–0.474626953
SD	SD	SD
0.062298233	0.046464042	0.053925983

B29 – 3rd molar – 1st sample segment (SC)

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.942783576	–0.507794998	–0.433866369
–0.940312925	–0.468827563	–0.468047251
–0.893769516	–0.383600284	–0.529741418
–0.970208068	–0.512119786	–0.46459478
AVERAGE	AVERAGE	AVERAGE
–0.936768521	–0.468085658	–0.474062455
SD	SD	SD
0.031706264	0.059593526	0.04017334

B29 – 1st molar – bulk sample (SC)

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.009216374	–0.598721351	–0.408395624
–1.024764673	–0.543636585	–0.507378647
AVERAGE	AVERAGE	AVERAGE
–1.016990524	–0.571178968	–0.457887135
SD	SD	SD
0.010994308	0.038950812	0.069991567

B29 – 2nd molar – bulk sample (SC)

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.074382159	–0.45189394	–0.628002735
–1.096844222	–0.55379256	–0.536219621
–1.137214339	–0.554697552	–0.577266859
AVERAGE	AVERAGE	AVERAGE
–1.102813574	–0.520128017	–0.580496405
SD	SD	SD
0.031838586	0.059094177	0.045976706

B29 –3rd molar – bulk sample (SC)

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.999446329	–0.516236202	–0.479524312
–1.069765521	–0.519351592	–0.557876598
–1.03155651	–0.498750825	–0.483541008
–1.062741646	–0.537743133	–0.499034477
AVERAGE	AVERAGE	AVERAGE
–1.040877501	–0.518020438	–0.504994099
SD	SD	SD
0.032228255	0.015971414	0.036244636

D.4.2 Ultra sample clean (UC)

B29 – 1st molar – 1st sample segment (UC)

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.135229773	–0.523221106	–0.621075579
–1.050432194	–0.398728678	–0.67991096
–1.080219941	–0.54637628	–0.540648032
–1.088426021	–0.495589256	–0.624392305
–1.136777985	–0.644825506	–0.496727806
–1.090419032	–0.310862932	–0.782001552
AVERAGE	AVERAGE	AVERAGE
–1.096917491	–0.486600626	–0.624126039
SD	SD	SD
0.033498406	0.117162547	0.101237245

B29 – 1st molar – 2nd sample segment (UC)

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.045033799	–0.500157884	–0.537242054
–1.027245649	–0.51639794	–0.503393243
–0.957329437	–0.465412051	–0.493762752
–1.082422854	–0.686218521	–0.400507931
–1.120390536	–0.554935123	–0.574079533
–1.037531706	–0.415245987	–0.65845871
–1.035855437	–0.471697028	–0.601479516
AVERAGE	AVERAGE	AVERAGE
–1.04368706	–0.515723505	–0.538417677
SD	SD	SD
0.050353551	0.087000143	0.083566008

B29 – 1st molar – 3rd sample segment (UC)

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.098676002	–0.551027701	–0.562270704
–1.024391991	–0.417243931	–0.605972088
–1.095494154	–0.516293199	–0.587129743
–1.044606112	–0.536840383	–0.478231166
–1.056684926	–0.421463792	–0.608442449
–1.063227754	–0.478942642	–0.586495704
–1.196961603	–0.570839318	–0.623656114
–1.08550959	–0.494863774	–0.561593809
–1.098676002	–0.551027701	–0.562270704
–1.024391991	–0.417243931	–0.605972088
–1.066235586	–0.435991188	–0.652299511
–1.00124154	–0.470111075	–0.558600272
–1.136211164	–0.515513742	–0.614037264
–1.204121405	–0.595929731	–0.589063265
AVERAGE	AVERAGE	AVERAGE
–1.083194016	–0.498439343	–0.576723972
SD	SD	SD
0.052627395	0.056960548	0.045366064

B29 – 1st molar – 4th sample segment (UC)

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.232961162	–0.692672173	–0.527108901
–1.241208109	–0.559148989	–0.654119558
–1.067659342	–0.425754218	–0.629525685
–1.177296229	–0.49106515	–0.695781823
AVERAGE	AVERAGE	AVERAGE
–1.179781211	–0.542160132	–0.626633992
SD	SD	SD
0.079955945	0.114168778	0.071764626

B29 – 1st molar – 5th sample segment (UC)

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.099177907	–0.488717991	–0.592915437
–1.107628362	–0.464897907	–0.629498355
–1.13868367	–0.640072204	–0.496422654
–1.019505196	–0.442590074	–0.555950691
AVERAGE	AVERAGE	AVERAGE
–1.091248784	–0.509069544	–0.568696784
SD	SD	SD
0.050755533	0.089343039	0.056772607

B29 – 1st molar – 6th sample segment (UC)

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.078717867	–0.407268243	–0.632534603
–1.131362129	–0.425699355	–0.688214177
AVERAGE	AVERAGE	AVERAGE
–1.105039998	–0.416483799	–0.66037439
SD	SD	SD
0.037225115	0.013032765	0.039371404

B29 – 1st molar – 7th sample segment (UC)

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.063657694	–0.525529679	–0.546549904
–1.11624073	–0.530093158	–0.59608447
–1.012339677	–0.474455413	–0.502871333
–1.120147636	–0.481690017	–0.633123534
–1.111917263	–0.571502314	–0.561994864
AVERAGE	AVERAGE	AVERAGE
–1.0848606	–0.516654116	–0.568124821
SD	SD	SD
0.04655856	0.039594593	0.0494157

B29 – 2nd molar – 1st sample segment (UC)

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.162747406	–0.526330369	–0.635814665
–1.217773215	–0.535796002	–0.669709297
–1.146954018	–0.556337186	–0.598069444
AVERAGE	AVERAGE	AVERAGE
–1.17582488	–0.539487853	–0.634531135
SD	SD	SD
0.037176671	0.015340293	0.035837169

B29 – 2nd molar – 2nd sample segment (UC)

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.087307046	–0.519879646	–0.54277535
–1.138794702	–0.521960271	–0.632232711
–1.116688222	–0.48955276	–0.625917961
AVERAGE	AVERAGE	AVERAGE
–1.114263323	–0.510464226	–0.600308674
SD	SD	SD
0.02582934	0.018139716	0.04992526

B29 – 2nd molar – 3rd sample segment (UC)

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.060073192	–0.478435883	–0.601673521
–1.076545984	–0.579478194	–0.499158252
–1.125901434	–0.487412346	–0.638494393
–1.086762999	–0.464258955	–0.638312376
AVERAGE	AVERAGE	AVERAGE
–1.087320902	–0.502396344	–0.594409636
SD	SD	SD
0.027972041	0.052264379	0.065819214

B29 – 2nd molar – 4th sample segment (UC)

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.095552741	–0.513142665	–0.579627489
–1.069650423	–0.465759742	–0.602398415
–1.047312447	–0.451869032	–0.587272044
–1.177583279	–0.534628733	–0.630221447
–1.205175823	–0.515874661	–0.685941307
–1.04430425	–0.43078525	–0.633402676
AVERAGE	AVERAGE	AVERAGE
–1.106596494	–0.485343347	–0.619810563
SD	SD	SD
0.068758863	0.041508182	0.039097378

B29 – 2nd molar – 5th sample segment (UC)

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.088419786	–0.426474622	–0.633674192
–1.052087642	–0.532413863	–0.515476723
–1.098841352	–0.52540131	–0.571222789
–1.092983846	–0.573977387	–0.512685072
–1.110770755	–0.520773325	–0.614971767
–0.963603247	–0.41685763	–0.55869043
–1.029583754	–0.462740205	–0.573751934
AVERAGE	AVERAGE	AVERAGE
–1.062327197	–0.494091192	–0.568638987
SD	SD	SD
0.052000184	0.059248908	0.0455799

B29 – 2nd molar – 6th sample segment (UC)

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.975280017	–0.405726273	–0.543598123
–0.989003597	–0.442492153	–0.527888013
–0.964331192	–0.32491998	–0.645718082
–1.062869748	–0.500215531	–0.574803712
–1.132155948	–0.520768525	–0.605622036
–1.074181455	–0.488350986	–0.56815183
–1.043596606	–0.520444727	–0.524569403
–1.043807054	–0.540992249	–0.4745113
–1.109130125	–0.513334024	–0.613915416
–0.990237457	–0.416544887	–0.558011495
–1.004674168	–0.43101958	–0.592966977
–0.980037284	–0.435525015	–0.586714183
AVERAGE	AVERAGE	AVERAGE
–1.030775388	–0.461694494	–0.568039214
SD	SD	SD
0.055435653	0.063219145	0.046234733

B29 – 2nd molar – 7th sample segment (UC)

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.075412015	–0.578587647	–0.481445704
–1.146400003	–0.511645701	–0.596165908
–1.110678544	–0.594500182	–0.543503579
–1.130660629	–0.561846911	–0.546278478
–1.146449228	–0.550926086	–0.589853282
AVERAGE	AVERAGE	AVERAGE
–1.121920084	–0.559501305	–0.55144939
SD	SD	SD
0.029860991	0.031457928	0.046002218

B29 – 2nd molar – 8th sample segment (UC)

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.122585153	–0.560115619	–0.590139086
–1.174983864	–0.534687876	–0.614773186
–1.151651273	–0.5763237	–0.556109594
–1.111249362	–0.575840497	–0.52777657
–1.030549135	–0.508870698	–0.487297671
–1.071952485	–0.511674004	–0.552640566
AVERAGE	AVERAGE	AVERAGE
–1.110495212	–0.544585399	–0.554789446
SD	SD	SD
0.052680661	0.030600815	0.045030749

B29 – 2nd molar – 9th sample segment (UC)

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.12488193	–0.507026932	–0.599235587
–1.104307659	–0.518644701	–0.562337085
–1.173551022	–0.497691266	–0.729820464
–1.098351043	–0.430745558	–0.658131651
–1.087222069	–0.492635786	–0.605092083
–1.140485227	–0.510990908	–0.617029391
–1.142183202	–0.576278532	–0.54020826
–1.144950767	–0.596976395	–0.533992338
AVERAGE	AVERAGE	AVERAGE
–1.126991615	–0.51637376	–0.605730857
SD	SD	SD
0.028844616	0.051341403	0.065068066

B29 – 2nd molar – 10th sample segment (UC)

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.000161224	–0.469448996	–0.533207678
–1.059121902	–0.431043425	–0.651959293
AVERAGE	AVERAGE	AVERAGE
–1.029641563	–0.45024621	–0.592583486
SD	SD	SD
0.041691495	0.02715684	0.083970072

B29 – 2nd molar – 11th sample segment (UC)

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.085754642	–0.50167301	–0.579692174
–1.047926909	–0.472584345	–0.558905539
AVERAGE	AVERAGE	AVERAGE
–1.066840776	–0.487128678	–0.569298857
SD	SD	SD
0.026748247	0.020568792	0.014698371

B29 – 2nd molar – 12th sample segment (UC)

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.976601541	–0.513331661	–0.465188401
–0.998975263	–0.419455353	–0.589937529
–1.044707768	–0.622289009	–0.42534035
AVERAGE	AVERAGE	AVERAGE
–1.006761524	–0.518358674	–0.49348876
SD	SD	SD
0.034714319	0.101510227	0.085870487

B29 – 3rd molar – 1st sample segment (UC)

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.086120352	–0.494779501	–0.601583964
–1.030346569	–0.532467862	–0.530793801
–1.144328014	–0.635043349	–0.51470953
AVERAGE	AVERAGE	AVERAGE
–1.086931645	–0.554096904	–0.549029098
SD	SD	SD
0.056995054	0.072590277	0.046218896

B29 – 1st molar – bulk sample (UC)

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.160652312	–0.507026932	–0.664439449
–1.157223636	–0.608308947	–0.577342116
–1.119958753	–0.456399972	–0.686537954
–1.15910008	–0.541448455	–0.599339795
–1.056159381	–0.49734748	–0.574147236
AVERAGE	AVERAGE	AVERAGE
–1.130618833	–0.522106357	–0.62036131
SD	SD	SD
0.044941223	0.056926978	0.051842372

B29 – 2nd molar – bulk sample (UC)

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.096844222	–0.55379256	–0.536219621
–1.074382159	–0.45189394	–0.628002735
–1.137214339	–0.554697552	–0.577266859
–1.08374539	–0.448889861	–0.65127992
–1.064128016	–0.410119349	–0.630084518
AVERAGE	AVERAGE	AVERAGE
–1.091262825	–0.483878652	–0.604570731
SD	SD	SD
0.028374549	0.066315427	0.046901855

B29 – 3rd molar – bulk sample (UC)

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.999446329	–0.516236202	–0.479524312
–1.069765521	–0.519351592	–0.557876598
–1.03155651	–0.498750825	–0.483541008
–1.062741646	–0.537743133	–0.499034477
AVERAGE	AVERAGE	AVERAGE
–1.040877501	–0.518020438	–0.504994099
SD	SD	SD
0.032228255	0.015971414	0.036244636

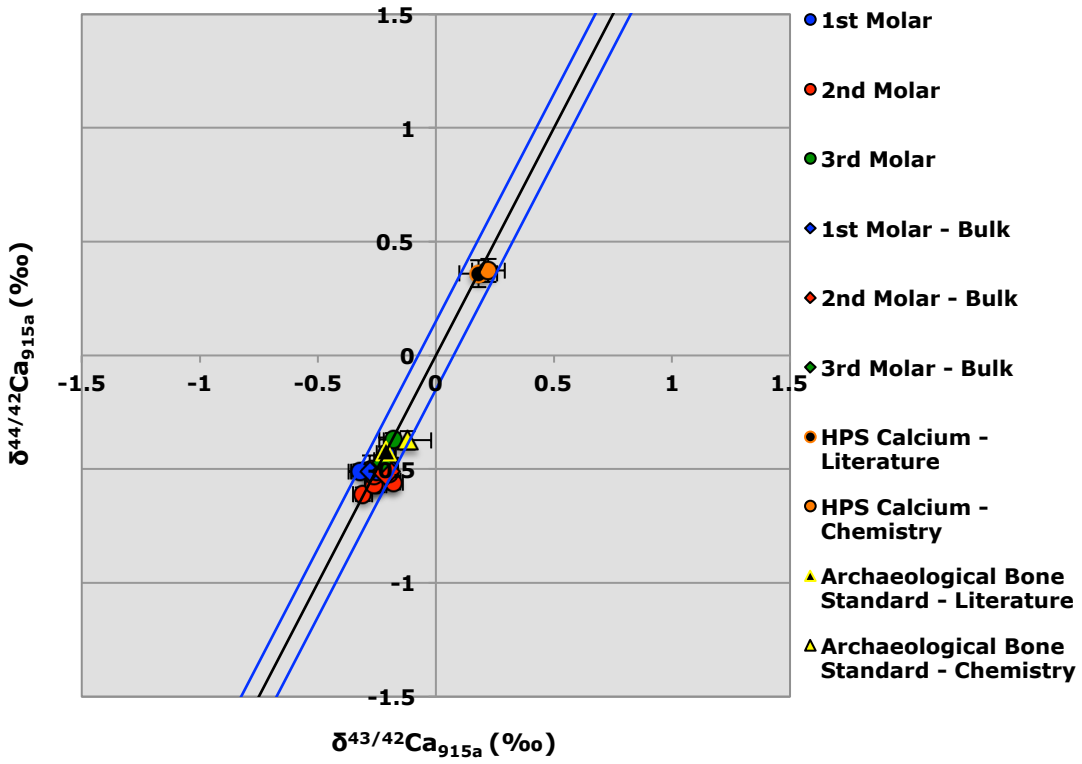

Figure D.8: Three-isotope plot of mean standards and Abu Hureyra sheep B29 incremental and bulk enamel sample results (standard clean method).

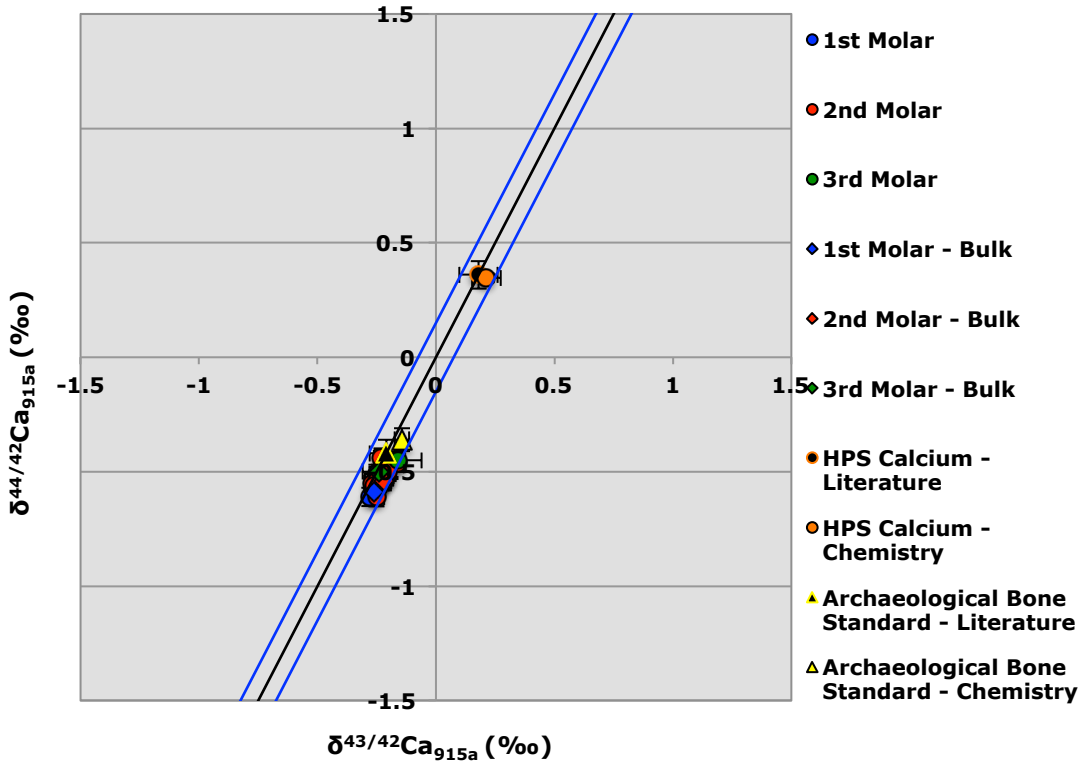

Figure D.9: Three-isotope plot of mean standards and Abu Hureyra sheep B29 incremental and bulk enamel sample results (ultra-clean method).

D.4.3 Archaeological sheep samples B59 – Tell Abu Hureyra, Syria (Standard Fisher07)

B59 – 1st molar – 1st sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.172506432	–0.693764846	–0.4707573
–1.179374462	–0.565075573	–0.619336938
–1.195174395	–0.62913588	–0.566319757
AVERAGE	AVERAGE	AVERAGE
–1.182351763	–0.629325433	–0.552137998
SD	SD	SD
0.01162357	0.064344846	0.0752982

B59 – 1st molar – 2nd sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.139140291	–0.67788509	–0.467838432
–1.15111476	–0.590377083	–0.551271331
–1.147191655	–0.484535064	–0.628337073
AVERAGE	AVERAGE	AVERAGE
–1.145815569	–0.584265746	–0.549148945
SD	SD	SD
0.006104686	0.096819778	0.080270368

B59 – 1st molar – 3rd sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.148788789	–0.533090685	–0.619513434
–1.254962223	–0.661600522	–0.560550268
–1.217690655	–0.507669018	–0.70905077
–1.213961409	–0.555690779	–0.634539371
–1.217770641	–0.697900788	–0.504454987
AVERAGE	AVERAGE	AVERAGE
–1.210634744	–0.591190358	–0.605621766
SD	SD	SD
0.038411005	0.083600708	0.077448244

B59 – 1st molar – 4th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.10347145	–0.418256193	–0.659841081
–1.198650345	–0.475401967	–0.764599359
–1.188327103	–0.443186072	–0.730446625
–1.209416947	–0.6914692	–0.507900328
AVERAGE	AVERAGE	AVERAGE
–1.174966461	–0.507078358	–0.665696848
SD	SD	SD
0.048434856	0.125133232	0.113883379

B59 – 1st molar – 5th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.118377243	–0.537567713	–0.551244015
–1.160700061	–0.64088281	–0.513966896
–1.149917061	–0.571763166	–0.566403892
–1.032608232	–0.431826534	–0.634700468
AVERAGE	AVERAGE	AVERAGE
–1.115400649	–0.545510056	–0.566578818
SD	SD	SD
0.058042697	0.087124838	0.050476927

B59 – 1st molar – 6th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.107690906	–0.468790933	–0.635062252
–1.152545846	–0.631784504	–0.501672241
–1.140753637	–0.601737229	–0.51403374
–1.117134224	–0.649721859	–0.507639353
AVERAGE	AVERAGE	AVERAGE
–1.129531153	–0.588008631	–0.539601897
SD	SD	SD
0.020706686	0.081906824	0.063840094

B59 – 1st molar – 7th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.979557434	–0.480264352	–0.511160334
–0.986048762	–0.484708704	–0.51409105
–1.02125287	–0.436384349	–0.60380303
AVERAGE	AVERAGE	AVERAGE
–0.995619688	–0.467119135	–0.543018138
SD	SD	SD
0.022435002	0.026709706	0.052661652

B59 – 2nd molar – 1st sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.24069125	–0.621063721	–0.626166312
–1.136475443	–0.568123009	–0.557966522
–1.081111879	–0.526401688	–0.532907008
–1.08339894	–0.519370688	–0.573026852
AVERAGE	AVERAGE	AVERAGE
–1.135419378	–0.558739777	–0.572516673
SD	SD	SD
0.074696526	0.046790299	0.039408868

B59 – 2nd molar – 2nd sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.043880039	–0.53824532	–0.523870192
–1.050936931	–0.478440285	–0.523857201
–0.991888179	–0.439216768	–0.576351016
–1.010666883	–0.407067801	–0.632232711
–1.083395855	–0.48493373	–0.613249915
–1.032544374	–0.393035684	–0.628932766
AVERAGE	AVERAGE	AVERAGE
–1.035552044	–0.456823265	–0.5830823
SD	SD	SD
0.035773858	0.054298242	0.049974212

B59 – 2nd molar – 3rd sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.082403878	–0.469267575	–0.582754196
–1.107940813	–0.503731987	–0.561036771
–1.09234207	–0.535564469	–0.582411182
–1.106063992	–0.615848446	–0.495570836
–1.04030394	–0.395513224	–0.647630262
AVERAGE	AVERAGE	AVERAGE
–1.085810938	–0.50398514	–0.573880649
SD	SD	SD
0.02750031	0.081366736	0.05451584

B59 – 2nd molar – 4th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.087301938	–0.501280795	–0.536090014
–1.141923101	–0.537832123	–0.625677559
–1.072438057	–0.515271829	–0.533177926
–1.069384519	–0.478405071	–0.57033042
–1.024895408	–0.441547809	–0.576447472
–1.055496917	–0.411493307	–0.635117342
–1.041086604	–0.432160065	–0.597873059
AVERAGE	AVERAGE	AVERAGE
–1.070360935	–0.473998714	–0.58210197
SD	SD	SD
0.037767818	0.046997095	0.040081986

B59 – 2nd molar – 5th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.082434751	–0.586534547	–0.505324508
–1.089001653	–0.453053743	–0.650791486
–1.107981167	–0.574638667	–0.473990911
–1.112011288	–0.599880024	–0.557537913
–1.070134415	–0.390887268	–0.684806287
–1.08889512	–0.441233247	–0.66584288
AVERAGE	AVERAGE	AVERAGE
–1.091743066	–0.507704583	–0.589715664
SD	SD	SD
0.015771375	0.08971297	0.089573801

B59 – 2nd molar – 6th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.076702581	–0.575021966	–0.499114924
–1.120574373	–0.657746459	–0.464928866
–1.141352439	–0.544941585	–0.607315048
–1.097929314	–0.473467436	–0.622682367
AVERAGE	AVERAGE	AVERAGE
–1.109139676	–0.562794362	–0.548510301
SD	SD	SD
0.027965763	0.076296431	0.078284211

B59 – 2nd molar – 7th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.03957664	–0.473848617	–0.567253655
–1.13048474	–0.58873312	–0.567186846
–1.101028244	–0.482740865	–0.594839765
–1.154870892	–0.526377489	–0.622616787
–1.083247576	–0.459928711	–0.629096487
AVERAGE	AVERAGE	AVERAGE
–1.101841618	–0.50632576	–0.596198708
SD	SD	SD
0.044316644	0.052337231	0.029417383

B59 – 2nd molar – 8th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.164054786	–0.586267545	–0.598021262
–1.150879112	–0.588448931	–0.542200217
AVERAGE	AVERAGE	AVERAGE
–1.157466949	–0.587358238	–0.57011074
SD	SD	SD
0.009316608	0.001542473	0.039471439

B59 – 2nd molar – 9th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.987926765	–0.540459736	–0.427710694
–1.097438266	–0.457619596	–0.656969141
–1.008452197	–0.508016781	–0.511198342
AVERAGE	AVERAGE	AVERAGE
–1.031272409	–0.502032038	–0.531959392
SD	SD	SD
0.058213089	0.041743084	0.116030706

B59 – 3rd molar – 1st sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.082013799	–0.516988233	–0.57623674
–1.08889822	–0.500882288	–0.569959421
–1.119792148	–0.634036747	–0.52027525
–1.171999454	–0.571920758	–0.609950554
–1.134860969	–0.52561875	–0.652852595
AVERAGE	AVERAGE	AVERAGE
–1.119512918	–0.549889355	–0.585854912
SD	SD	SD
0.036516848	0.053943037	0.049282749

B59 – 1st molar – bulk sample

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.134351836	–0.520829748	–0.655900006
–1.115269771	–0.582119051	–0.521344075
–1.182336182	–0.565358681	–0.629123782
AVERAGE	AVERAGE	AVERAGE
–1.14398593	–0.556102494	–0.602122621
SD	SD	SD
0.034555574	0.031675739	0.07122585

B59 – 2nd molar – bulk sample

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.132747182	–0.628573002	–0.532132117
–1.054473565	–0.451898087	–0.627959996
AVERAGE	AVERAGE	AVERAGE
–1.093610373	–0.540235544	–0.580046057
SD	SD	SD
0.055347805	0.12492803	0.067760543

B59 – 3rd molar – bulk sample

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.994692594	–0.48303845	–0.459119731
–1.061221815	–0.455015512	–0.595064682
–1.052840032	–0.574525098	–0.492841401
AVERAGE	AVERAGE	AVERAGE
–1.036251481	–0.50419302	–0.515675271
SD	SD	SD
0.036234229	0.062500177	0.070790513

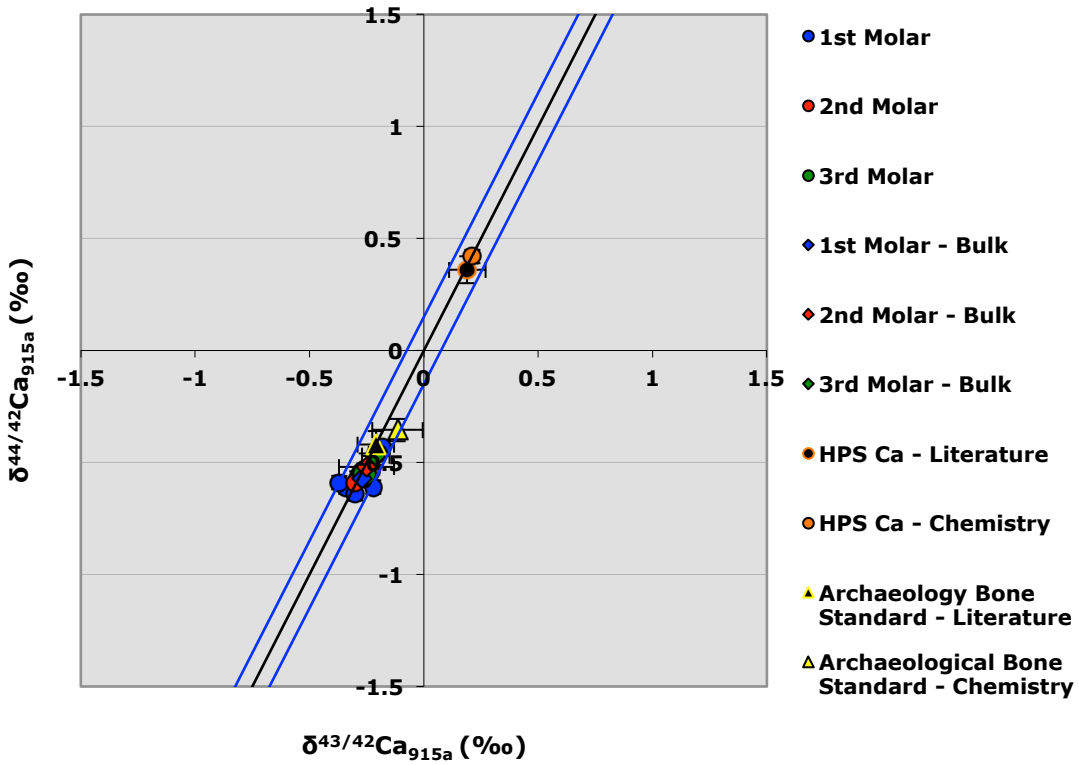

Figure D.10: Three-isotope plot of mean standards and Abu Hureyra sheep B59 incremental and bulk enamel sample results.

D.4.4 Archaeological sheep samples B252 – Tell Abu Hureyra, Syria (Standard Fisher07)

B252 – 1st molar – 1st sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.048152347	–0.466755726	–0.591501578
–1.012393984	–0.526842511	–0.517519748
–1.039354317	–0.441499073	–0.595223333
–1.047013021	–0.60219364	–0.405953573
–1.064480136	–0.632176642	–0.452508322
–1.048152347	–0.466755726	–0.591501578
–0.921522463	–0.404700039	–0.5203107
–0.917917638	–0.471516927	–0.421394444
AVERAGE	AVERAGE	AVERAGE
–1.012373282	–0.501555035	–0.511989159
SD	SD	SD
0.059014569	0.07940985	0.078066941

B252 – 1st molar – 2nd sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.081087257	–0.527305927	–0.536489005
–1.003069135	–0.508591983	–0.486708517
–1.082104501	–0.540387653	–0.53939439
–1.092257237	–0.490059612	–0.598069444
AVERAGE	AVERAGE	AVERAGE
–1.064629532	–0.516586294	–0.540165339
SD	SD	SD
0.041348937	0.021977178	0.045550803

B252 – 1st molar – 3rd sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.058192016	–0.604663033	–0.49866352
–1.010816737	–0.49671387	–0.517673754
–1.083516174	–0.583747012	–0.529695472
AVERAGE	AVERAGE	AVERAGE
–1.050841642	–0.561707972	–0.515344248
SD	SD	SD
0.036902886	0.057249846	0.01564658

B252 – 1st molar – 4th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.047260665	–0.510342481	–0.560925496
–1.048373312	–0.528648132	–0.551548053
–1.172553417	–0.519729005	–0.656704582
–0.986024668	–0.390975369	–0.566905717
AVERAGE	AVERAGE	AVERAGE
–1.063553015	–0.487423747	–0.584020962
SD	SD	SD
0.078289199	0.064731851	0.048866248

B252 – 1st molar – 5th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.960041996	–0.480962846	–0.452516737
–1.089232869	–0.462701857	–0.582931276
–1.01301821	–0.399974255	–0.629096487
–1.103661475	–0.568292935	–0.539183787
AVERAGE	AVERAGE	AVERAGE
–1.041488638	–0.477982973	–0.550932072
SD	SD	SD
0.067303031	0.069482951	0.075182501

B252 – 1st molar – 6th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.979853806	–0.480887594	–0.495905679
–1.079498361	–0.498929715	–0.597732334
–0.997912936	–0.579435556	–0.480496492
–1.010465227	–0.441472679	–0.551654032
–0.985696012	–0.540131792	–0.477193852
AVERAGE	AVERAGE	AVERAGE
–1.010685269	–0.508171467	–0.520596478
SD	SD	SD
0.040228922	0.053335215	0.052478434

B252 – 1st molar – 7th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.979792318	–0.446362833	–0.52997784
–0.943154295	–0.501308461	–0.442978176
–1.025404394	–0.487394417	–0.566817921
–1.020684543	–0.544936574	–0.499077791
–1.032653207	–0.416010665	–0.622813568
AVERAGE	AVERAGE	AVERAGE
–1.005218513	–0.472194398	–0.544846369
SD	SD	SD
0.036037021	0.04779507	0.068072271

B252 – 1st molar – 8th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.948736869	–0.413937744	–0.535824349
–0.937597073	–0.469153179	–0.483286347
–0.950788499	–0.409263142	–0.530076412
–0.958436879	–0.397588728	–0.579404793
AVERAGE	AVERAGE	AVERAGE
–0.94888983	–0.422485698	–0.532147975
SD	SD	SD
0.008608152	0.031862289	0.03932121

B252 – 1st molar – 9th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.927444229	–0.436944333	–0.492487246
–1.030936643	–0.436815751	–0.600782876
–1.003910116	–0.441753023	–0.554713672
–1.00639761	–0.475981338	–0.526945514
–1.125260457	–0.513104883	–0.619839833
AVERAGE	AVERAGE	AVERAGE
–1.018789811	–0.460919866	–0.558953828
SD	SD	SD
0.071067805	0.033441465	0.052242446

B252 – 2nd molar – 1st sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.029904419	–0.454355703	–0.57678711
–0.946404515	–0.505159516	–0.436298824
–1.001866726	–0.476327446	–0.530273665
AVERAGE	AVERAGE	AVERAGE
–0.994711473	–0.439137599	–0.559235346
SD	SD	SD
0.039262648	0.046697263	0.025268851

B252 – 2nd molar – 2nd sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.938442472	–0.362525486	–0.564642228
–0.951867019	–0.390086719	–0.570514331
–0.964818383	–0.455614412	–0.514805304
–0.870433425	–0.333122738	–0.544790441
AVERAGE	AVERAGE	AVERAGE
–0.931390325	–0.385337339	–0.548688076
SD	SD	SD
0.042040483	0.052307325	0.02512737

B252 – 2nd molar – 3rd sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.997565144	–0.507722669	–0.473861725
–1.005002235	–0.514724806	–0.452230799
–1.046801708	–0.406646955	–0.628364303
–0.95956993	–0.585941539	–0.35626547
–1.033549059	–0.420532076	–0.569962952
AVERAGE	AVERAGE	AVERAGE
–1.008497615	–0.487113609	–0.49613705
SD	SD	SD
0.033986885	0.073929112	#DIV/0!

B252 – 2nd molar – 4th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.065474093	–0.516560944	–0.575459439
–0.990473137	–0.500815845	–0.517853544
–0.872519353	–0.400574617	–0.490031883
–0.958377205	–0.528691316	–0.4436571
AVERAGE	AVERAGE	AVERAGE
–0.971710947	–0.486660681	–0.506750491
SD	SD	SD
0.079917266	0.058514326	0.055089277

B252 – 2nd molar – 5th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.953272571	–0.464111754	–0.501585257
–1.07913772	–0.602322055	–0.462036994
–1.020995378	–0.531747146	–0.511769139
–0.986901002	–0.392531737	–0.595515027
AVERAGE	AVERAGE	AVERAGE
–1.010076668	–0.497678173	–0.517726604
SD	SD	SD
0.053704327	0.089987989	0.056120127

B252 – 2nd molar – 6th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.903094135	–0.376173234	–0.502331812
–1.04382494	–0.508746527	–0.521056255
–0.958473766	–0.410577207	–0.564386586
AVERAGE	AVERAGE	AVERAGE
–0.968464281	–0.431832322	–0.529258218
SD	SD	SD
0.070895329	0.068795018	0.031830063

B252 – 2nd molar – 7th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.094192188	–0.554093365	–0.524188671
–0.911566592	–0.514584905	–0.514584905
–1.055254496	–0.550205694	–0.486922865
–1.068814556	–0.514970941	–0.539307455
–0.989846595	–0.468857734	–0.526733262
AVERAGE	AVERAGE	AVERAGE
–1.023934886	–0.520542528	–0.518347432
SD	SD	SD
0.073688256	0.03443623	0.019655946

B252 – 2nd molar – 8th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.97804593	–0.392927308	–0.569998265
–0.929481166	–0.380959417	–0.517956337
–0.943883761	–0.383762481	–0.517311344
–0.99571955	–0.32295718	–0.675985761
AVERAGE	AVERAGE	AVERAGE
–0.961782602	–0.370151597	–0.570312927
SD	SD	SD
0.030441001	0.031875336	0.07464857

B252 – 2nd molar – 9th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.974238078	–0.369563571	–0.606323084
–1.023934285	–0.51483201	–0.508650154
–1.023369068	–0.520102179	–0.514760605
AVERAGE	AVERAGE	AVERAGE
–1.007180477	–0.46816592	–0.543244614
SD	SD	SD
0.028530354	0.085432787	0.054712927

B252 – 2nd molar – 10th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.009340313	–0.56980057	–0.433721204
–1.036372696	–0.489483009	–0.545151558
–0.947740507	–0.457152834	–0.48008276
–1.068806367	–0.627178181	–0.467710911
AVERAGE	AVERAGE	AVERAGE
–1.015564971	–0.535903648	–0.481666608
SD	SD	SD
0.05133716	0.077107302	0.046641794

B252 – 2nd molar – 11th sample segment

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.919517289	–0.420764135	–0.492923907
–0.975907202	–0.489618029	–0.492725329
–0.915366339	–0.521576494	–0.427501348
AVERAGE	AVERAGE	AVERAGE
–0.936930277	–0.477319553	–0.471050194
SD	SD	SD
0.033818754	0.051519145	0.037714538

B252 – 1st molar – bulk sample

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–1.061834748	–0.453220511	–0.598648234
–1.099906252	–0.609171596	–0.477241174
–1.036399431	–0.407006022	–0.619966646
AVERAGE	AVERAGE	AVERAGE
–1.06604681	–0.489799376	–0.565285351
SD	SD	SD
0.031962246	0.105930353	0.076989944

B252 – 2nd molar – bulk sample

$\delta^{44/42}$Ca‰	$\delta^{43/42}$Ca‰	$\delta^{44/43}$Ca‰
–0.965078967	–0.457577507	–0.477371358
–1.008328681	–0.489510007	–0.529846468
–0.936488411	–0.390863901	–0.542469134
AVERAGE	AVERAGE	AVERAGE
–0.969965353	–0.445983805	–0.51656232
SD	SD	SD
0.036168545	0.05033462	0.034522189

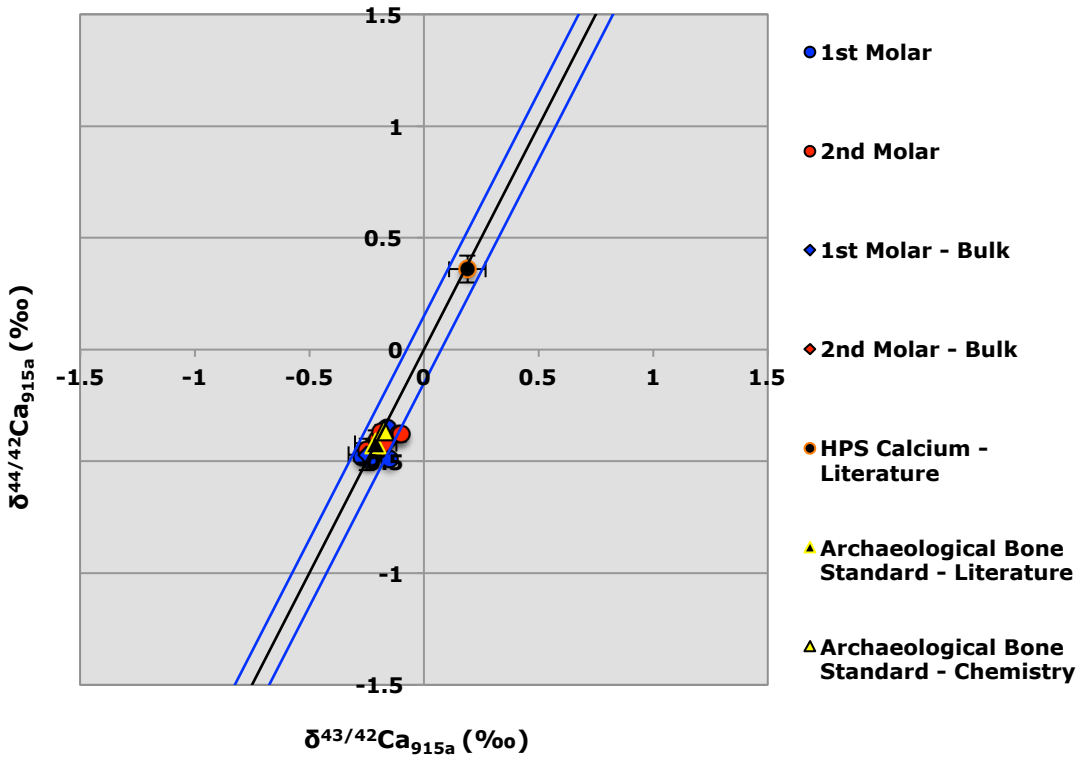

Figure D.11: Three-isotope plot of mean standards and Abu Hureyra Sheep B252 incremental and bulk enamel sample results.

APPENDIX E

CARBON AND OXYGEN ISOTOPE RESULTS FOR STANDARDS AND SAMPLES

Carbon apatite and oxygen isotope analyses were undertaken in the Department of Archaeological Science, University of Bradford. The international carbonate standards analysed were IAEA-CO-1 (CO-1), IAEA-CO-8 (CO-8) and IAEA-NBS-19 (NBS-19), as well as the in-house carbonate standards of Merck CaCO3 (Merck) and NOCZ and the dental enamel standard BES. The samples analysed at Bradford were the serial samples from sheep HF29 and HF30 from Highfield Farm, Yorkshire, and sheep B59 and goat B252 from Tell Abu Hureyra, Syria.

E.1 Standards

Table E.1: Standards, University of Bradford. Vienna Pee Dee Belemnite (VPDB) for carbonate measurements [1 standard deviation (SD)]

Standard	**$\delta^{13}C_{VPDB}$ ‰**	**SD**	**$\delta^{18}O_{VPDB}$ ‰**	**SD**
BES	–11.26	0.04	–5.51	0.06
	–11.59	0.06	–5.78	0.16
	–11.06	0.03	–5.36	0.07
	–10.86	0.06	–5.67	0.04
	–11.05	0.04	–5.58	0.09
Average	–11.17		–5.58	
Standard measurement	–11.10		–5.73	
Standard	**$\delta^{13}C_{VPDB}$ ‰**	**SD**	**$\delta^{18}O_{VPDB}$ ‰**	**SD**
CO-1	2.43	0.04	–2.37	0.09
	2.56	0.95	–2.51	0.24
Average	2.50		–2.44	
Standard measurement	2.49	0.03	–2.42	0.07

Standard	**$\delta^{13}C_{VPDB}$ ‰**	**SD**	**$\delta^{18}O_{VPDB}$ ‰**	**SD**
CO-8	–5.70	0.06	–22.58	0.10
Standard measurement	–5.76	0.03	–22.66	0.19
Standard	**$\delta^{13}C_{VPDB}$ ‰**	**SD**	**$\delta^{18}O_{VPDB}$ ‰**	**SD**
Merck	–35.58	0.06	–16.93	0.08
	–35.91	0.15	–17.41	0.26
	–35.58	0.06	–17.02	0.09
	–35.26	0.49	–17.06	0.06
	–35.28	0.12	–17.03	0.05
Average	–35.52		–17.09	
Standard measurement	–35.45		–17.03	
Standard	**$\delta^{13}C_{VPDB}$ ‰**	**SD**	**$\delta^{18}O_{VPDB}$ ‰**	**SD**
NBS-1	2.26	0.07	–2.20	0.08
Standard measurement	1.95		–2.19	
Standard	**$\delta^{13}C_{VPDB}$ ‰**	**SD**	**$\delta^{18}O_{VPDB}$ ‰**	**SD**
NOCZ	2.10	0.06	–1.68	0.08
	2.23	0.06	–1.68	0.06
	2.31	0.25	–1.67	0.08
Average	2.27		–1.68	
Standard measurement	2.090	0.12	–1.86	0.19

E.2 Carbon apatite and oxygen isotope sample measurements, University of Bradford

Table E.2 Highfield Farm lamb 29, first and second molar mean serial sample results

1st molar	$\delta^{13}C_{VPDB}$ ‰	SD	$\delta^{18}O_{VPDB}$ ‰	SD
HF29 M1-1	–17.56	0.07	–3.21	0.19
HF29 M1-2	–17.50	0.05	–2.54	0.04
HF29 M1-3	–16.30	0.04	–3.03	0.09
HF29 M1-4	–16.08	0.03	–3.21	0.07
HF29 M1-5	–16.41	0.04	–4.00	0.12
HF29 M1-6	–16.61	0.02	–4.22	0.07
HF29 M1-7	–16.70	0.03	–4.51	0.08

2nd molar	$\delta^{13}C_{VPDB}$ ‰	SD	$\delta^{18}O_{VPDB}$ ‰	SD
HF29 M2-1	–15.78	0.05	–4.09	0.06
HF29 M2-2	–16.16	0.02	–4.01	0.06
HF29 M2-3	–16.25	0.05	–4.64	0.05
HF29 M2-4	–16.37	0.02	–5.47	0.11
HF29 M2-5	–16.72	0.04	–5.87	0.11
HF29 M2-6	–16.91	0.05	–7.23	0.13

Table E.3: Highfield Farm lamb HF30, first and second molar mean serial sample results

1st molar	$\delta^{13}C_{VPDB}$ ‰	SD	$\delta^{18}O_{VPDB}$ ‰	SD
HF30 M1-1	–18.10	0.04	–3.31	0.10
HF30 M1-2	–16.795	0.08	–2.87	0.05
HF30 M1-3	–15.35	0.13	–3.30	0.50
HF30 M1-4	–15.92	0.07	–3.29	0.10
HF30 M1-5	–15.905	0.05	–3.91	0.10
HF30 M1-6	–15.93	0.12	–4.07	0.18
HF30 M1-7	–16.30	0.08	–4.43	0.24
HF30 M1-8	–16.71	0.13	–4.40	0.21

2nd molar	$\delta^{13}C_{VPDB}$ ‰	SD	$\delta^{18}O_{VPDB}$ ‰	SD
HF30 M2-1	–15.98	0.10	–4.09	0.13
HF30 M2-2	–15.89	0.09	–4.35	0.12
HF30 M2-3	–16.16	0.06	–5.12	0.17
HF30 M2-4	–16.20	0.05	–5.72	0.09
HF30 M2-5	–15.68	0.06	–5.62	0.11
HF30 M2-6	–15.34	0.26	–5.99	0.77
HF30 M2-7	–15.39	0.18	–7.02	0.86

Table E.4: Tell Abu Hureyra B59, first, second and third molar mean serial sample results

1st molar	$\delta^{13}C_{VPDB}$ ‰	SD	$\delta^{18}O_{VPDB}$ ‰	SD
B59 M1-1	–8.81	0.08	–2.52	0.12
B59 M1-2	–9.14	0.10	–1.86	0.12
B59 M1-3	–9.51	0.06	–2.21	0.12
B59 M1-4	–8.52	0.06	–3.50	0.11
B59 M1-5	–9.08	0.07	–4.28	0.15
B59 M1-6	–10.62	0.07	–4.02	0.10
B59 M1-7	———	——	———	——

2nd molar	$\delta^{13}C_{VPDB}$ ‰	SD	$\delta^{18}O_{VPDB}$ ‰	SD
B59 M2-1	–9.15	0.05	–5.28	0.12
B59 M2-2	–10.24	0.07	–4.45	0.11
B59 M2-3	–11.22	0.05	–2.49	0.08
B59 M2-4	–10.66	0.08	–0.18	0.15
B59 M2-5	–9.84	0.03	0.16	0.09
B59 M2-6	–8.44	0.06	0.18	0.07
B59 M2-7	–7.23	0.14	–0.81	0.25
B59 M2-8	–7.24	0.07	–2.84	0.19
B59 M2-9	–7.92	0.06	–4.57	0.18

3rd molar	$\delta^{13}C_{VPDB}$ ‰	SD	$\delta^{18}O_{VPDB}$ ‰	SD
B59 M3-1	–7.90	0.06	–3.29	0.15

Table E.5: Tell Abu Hureyra B252, first and second molar mean serial sample results

1st molar	$\delta^{13}C_{VPDB}$ ‰	SD	$\delta^{18}O_{VPDB}$ ‰	SD
B252 M1-1	–12.12	0.06	–0.35	0.08
B252 M1-2	–10.54	0.04	2.83	0.10
B252 M1-3	–10.28	0.05	1.36	0.13
B252 M1-4	–9.78	0.07	0.71	0.13
B252 M1-5	–9.87	0.05	0.02	0.11
B252 M1-6	–9.85	0.07	–1.31	0.21
B252 M1-7	–10.42	0.03	–2.69	0.06
B252 M1-8	–11.11	0.05	–4.14	0.07
B252 M1-9	–11.13	0.07	–3.72	0.11

2nd molar	$\delta^{13}C_{VPDB}$ ‰	SD	$\delta^{18}O_{VPDB}$ ‰	SD
B252 M2-1	–9.95	0.10	–2.35	0.18
B252 M2-2	–9.58	0.09	–2.34	0.15
B252 M2-3	–9.62	0.03	–3.51	0.08
B252 M2-4	–9.35	0.09	–3.56	0.12
B252 M2-5	–10.57	0.34	–5.03	0.53
B252 M2-6	–11.22	0.06	–4.54	0.07
B252 M2-7	–11.74	0.04	–5.12	0.06
B252 M2-8	–12.08	0.04	–4.02	0.06
B252 M2-9	–12.61	0.09	–3.18	0.20
B252 M2-10	–12.34	0.09	–1.34	0.15
B252 M2-11	–11.72	0.13	–2.08	0.13

APPENDIX F

STATISTICAL RESULTS

F.1 Modern results

Table F.1 One-way ANOVA: Amber, Coco, Fifi2010, Fifi2011, Helena, Jet

```
Source  DF       SS       MS     F      P
Factor   5  0.02192  0.00438  1.31  0.301
Error   19  0.06350  0.00334
Total   24  0.08542

S = 0.05781   R-Sq = 25.66%   R-Sq(adj) = 6.10%


                              Individual 95% CIs For Mean Based on
                              Pooled StDev
Level     N     Mean   StDev  -----+---------+---------+---------+----
Amber     4  -1.4074  0.0672             (------------*-----------)
Coco      6  -1.3811  0.0399                      (---------*---------)
Fifi2010  2  -1.4401  0.0437  (-----------------*-----------------)
Fifi2011  4  -1.4616  0.0107   (-----------*-----------)
Helena    5  -1.4339  0.0752         (-----------*-----------)
Jet       4  -1.3872  0.0755                 (------------*-----------)
                              -----+---------+---------+---------+----
                                -1.500    -1.450    -1.400    -1.350

Pooled StDev = 0.0578
```

Table F.2 Two-Sample T-Test and CI: 2010, 2011

```
Two-sample T for 2010 vs 2011 milk

       N     Mean   StDev  SE Mean
2010   7  -1.4357  0.0640    0.024
2011  17  -1.4013  0.0555    0.013

Difference = mu (2010) - mu (2011)
Estimate for difference:  -0.0344
95% CI for difference:  (-0.0970, 0.0283)
T-Test of difference=0 (vs not =): T-Value = -1.24 P-Value = 0.246  DF = 9
```

Table F.3 Two-Sample T-Test and CI: Fifi 2010, Fifi 2011

```
Two-sample T for Fifi 2010 vs Fifi 2011

           N     Mean   StDev  SE Mean
Fifi 2010  2  -1.4401  0.0437    0.031
Fifi 2011  4  -1.4616  0.0107   0.0054

Difference = mu (Fifi 2010) - mu (Fifi 2011)
Estimate for difference:  0.0215
95% CI for difference:  (-0.3770, 0.4199)
T-Test of difference=0 (vs not =): T-Value = 0.68  P-Value = 0.618  DF = 1
```

Table F.4 One-way ANOVA: HF29, M1, HF30, M1, HF33, M1, HF35, M1, HF44, M1, HF61, M1

```
Source  DF       SS       MS      F      P
Factor   5  0.14282  0.02856  20.16  0.000
Error   25  0.03543  0.00142
Total   30  0.17825

S = 0.03764   R-Sq = 80.12%   R-Sq(adj) = 76.15%


                              Individual 95% CIs For Mean Based on
                              Pooled StDev
Level     N     Mean   StDev  ---+---------+---------+---------+------
HF29, M1  6  -1.0721  0.0309                                (----*-----)
HF30, M1  5  -1.2408  0.0324  (-----*-----)
HF33, M1  6  -1.2352  0.0353    (----*----)
HF35, M1  7  -1.1953  0.0531           (----*----)
HF44, M1  4  -1.1124  0.0198                     (------*-----)
HF61, M1  3  -1.0938  0.0324                       (-------*------)
                              ---+---------+---------+---------+------
                              -1.260    -1.200    -1.140    -1.080
Pooled StDev = 0.0376
```

Table F.5 Two-Sample T-Test and CI: M1-Male, HF lambs, M1-Female, HF lambs

```
Two-sample T for M1-Male, HF lambs vs M1-Female, HF lambs

                      N     Mean   StDev  SE Mean
M1-Male, HF lambs    12  -1.1070  0.0252   0.0073
M1-Female, HF lambs  18  -1.2213  0.0455    0.011


Difference = mu (M1-Male, HF lambs) - mu (M1-Female, HF lambs)
Estimate for difference:  0.1142
95% CI for difference:  (0.0876, 0.1408)
T-Test of difference=0 (vs not =): T-Value = 8.81 P-Value = 0.000  DF = 27
```

Table F.6 One-way ANOVA: HF29, M2, HF30, M2, HF33, M2, HF35, M2, HF44, M2, HF61, M2

```
Source  DF       SS       MS     F      P
Factor   5  0.07486  0.01497  8.31  0.000
Error   21  0.03784  0.00180
Total   26  0.11270

S = 0.04245   R-Sq = 66.43%   R-Sq(adj) = 58.43%


                              Individual 95% CIs For Mean Based on Pooled
StDev
Level     N     Mean   StDev    -+---------+---------+---------+--------
HF29, M2  5  -1.1106  0.0275                     (----*-----)
HF30, M2  5  -1.2293  0.0369    (----*-----)
HF33, M2  5  -1.1873  0.0397          (----*-----)
HF35, M2  6  -1.1590  0.0576              (----*-----)
HF44, M2  3  -1.0550  0.0544                        (------*-------)
HF61, M2  3  -1.1282  0.0165                (------*------)
                                -+---------+---------+---------+--------
                              -1.260    -1.190    -1.120    -1.050
Pooled StDev = 0.0424
```

Table F.7 Two-Sample T-Test and CI: M2-Male, HF lambs, M2-Female, HF lambs

```
Two-sample T for M2-Male, HF lambs vs M2-Female, HF lambs

                       N     Mean   StDev  SE Mean
M2-Male, HF lambs     11  -1.1002  0.0430    0.013
M2-Female, HF lambs   16  -1.1898  0.0528    0.013


Difference = mu (M2-Male, HF lambs) - mu (M2-Female, HF lambs)
Estimate for difference:  0.0896
95% CI for difference:  (0.0514, 0.1278)
T-Test of difference = 0 (vs not =): T-Value = 4.84 P-Value = 0.000 DF = 24
```

Table F.8 Two-Sample T-Test and CI: M1-Other males, M1-HF61

```
Two-sample T for M1-Other males vs M1-HF61

                  N     Mean   StDev  SE Mean
M1-Other males   10  -1.0882  0.0331    0.010
M1-HF61           3  -1.0938  0.0324    0.019


Difference = mu (M1-Other males) - mu (M1-HF61)
Estimate for difference:  0.0056
95% CI for difference:  (-0.0625, 0.0738)
T-Test of difference = 0 (vs not =): T-Value = 0.26  P-Value = 0.810 DF = 3
```

Table F.9 Two-Sample T-Test and CI: M2-HF61, M2-Other males

```
Two-sample T for M2-HF61 vs M2-Other males

                 N     Mean   StDev  SE Mean
M2-HF61          3  -1.1282  0.0165   0.0095
M2-Other males   8  -1.0898  0.0459    0.016


Difference = mu (M2-HF61) - mu (M2-Other males)
Estimate for difference:  -0.0384
95% CI for difference:  (-0.0817, 0.0050)
T-Test of difference = 0 (vs not =): T-Value = -2.04 P-Value = 0.076 DF = 8
```

Table F.10 Two-Sample T-Test and CI: M1-Male, HF lambs, M2-Male, HF lambs

```
Two-sample T for M1-Male, HF lambs vs M2-Male, HF lambs

                     N     Mean   StDev  SE Mean
M1-Male, HF lambs   12  -1.1070  0.0252   0.0073
M2-Male, HF lambs   11  -1.1002  0.0430    0.013


Difference = mu (M1-Male, HF lambs) - mu (M2-Male, HF lambs)
Estimate for difference:  -0.0068
95% CI for difference:  (-0.0385, 0.0249)
T-Test of difference=0 (vs not =): T-Value = -0.45 P-Value = 0.656 DF = 15
```

Table F.11 Two-Sample T-Test and CI: M1-Female, HF lambs, M2-Female, HF lambs

```
Two-sample T for M1-Female, HF lambs vs M2-Female, HF lambs

                       N     Mean   StDev  SE Mean
M1-Female, HF lambs   18  -1.2213  0.0455    0.011
M2-Female, HF lambs   16  -1.1898  0.0528    0.013


Difference = mu (M1-Female, HF lambs) - mu (M2-Female, HF lambs)
Estimate for difference:  -0.0314
95% CI for difference:  (-0.0662, 0.0034)
T-Test of difference=0 (vs not =): T-Value = -1.85 P-Value = 0.075 DF = 29
```

Table F.12 One-way ANOVA: HF29 - Bone, HF30 - Bone, HF33 - Bone, HF44 - Bone, ...

```
Source  DF       SS       MS     F      P
Factor   6  0.11218  0.01870  5.87  0.001
Error   20  0.06373  0.00319
Total   26  0.17590

S = 0.05645   R-Sq = 63.77%   R-Sq(adj) = 52.90%


                                        Individual 95% CIs For Mean Based on
                                        Pooled StDev
Level              N     Mean   StDev   --+---------+---------+---------+---
----
HF29 - Bone        4  -1.1560  0.0334              (----*-----)
HF30 - Bone        5  -1.1410  0.0854                (----*----)
HF33 - Bone        4  -1.1053  0.0569                   (----*-----)
HF44 - Bone        2  -1.0493  0.0610                      (-------*-------)
Stillborn - bone   4  -1.2287  0.0616      (-----*-----)
Fifi - bone        3  -1.2503  0.0400    (------*------)
Helena - bone      5  -1.0732  0.0282                      (-----*----)
                                         --+---------+---------+---------+---
                                        -1.30     -1.20     -1.10     -1.00
```

Table F.13 One-way ANOVA: HF29 - Bone, HF30 - Bone, HF33 - Bone, HF44 - Bone

```
Source  DF       SS       MS     F      P
Factor   3  0.01810  0.00603  1.44  0.283
Error   11  0.04597  0.00418
Total   14  0.06407

S = 0.06465   R-Sq = 28.25%   R-Sq(adj) = 8.68%


                                Individual 95% CIs For Mean Based on
                                Pooled StDev
Level         N     Mean   StDev  -----+---------+---------+---------+----
HF29 - Bone   4  -1.1560  0.0334  (---------*---------)
HF30 - Bone   5  -1.1410  0.0854     (--------*--------)
HF33 - Bone   4  -1.1053  0.0569         (---------*---------)
HF44 - Bone   2  -1.0493  0.0610             (-------------*-------------)
                                -----+---------+---------+---------+----
                                  -1.190    -1.120    -1.050    -0.980

Pooled StDev = 0.0646
```

Table F.14 Two-Sample T-Test and CI: Fifi - bone, Helena - bone

```
Two-sample T for Fifi - bone vs Helena - bone

               N     Mean   StDev  SE Mean
Fifi - bone    3  -1.2503  0.0400    0.023
Helena - bone  5  -1.0732  0.0282    0.013


Difference = mu (Fifi - bone) - mu (Helena - bone)
Estimate for difference:  -0.1771
95% CI for difference:  (-0.2607, -0.0934)
T-Test of difference = 0 (vs not =): T-Value = -6.73 P-Value = 0.007 DF = 3
```

Table F.15 Two-Sample T-Test and CI: HF30, Unashed, HF30, Ashed

```
Two-sample T for HF30, unashed vs HF30, ashed

               N     Mean   StDev  SE Mean
HF30, unashed  4  -0.9850  0.0511    0.026
HF30, ashed    6  -1.1495  0.0792    0.032


Difference = mu (HF30, unashed) - mu (HF30, ashed)
Estimate for difference:  0.1645
95% CI for difference:  (0.0671, 0.2620)
T-Test of difference = 0 (vs not =): T-Value = 3.99  P-Value = 0.005 DF = 7
```

Table F.16 Two-Sample T-Test and CI: Male lambs - Bone, Female lambs - Bone

```
Two-sample T for Male lambs - Bone vs Female lambs - Bone

                     N     Mean   StDev  SE Mean
Male lambs - Bone    6  -1.1204  0.0667    0.027
Female lambs - Bone  9  -1.1252  0.0722    0.024


Difference = mu (Male lambs - Bone) - mu (Female lambs - Bone)
Estimate for difference:  0.0048
95% CI for difference:  (-0.0752, 0.0848)
T-Test of difference = 0 (vs not =): T-Value = 0.13 P-Value = 0.898 DF = 11
```

Table F.17 Two-Sample T-Test and CI: HF and Still lambs, Helena and Fifi

```
Two-sample T for HF and Still lambs vs Helena and Fifi

                     N     Mean   StDev  SE Mean
HF and Still Lambs  19  -1.1455  0.0784    0.018
Helena and Fifi      8  -1.1396  0.0965    0.034


Difference = mu (HF and Still lambs) - mu (Helena and Fifi)
Estimate for difference:  -0.0058
95% CI for difference:  (-0.0907, 0.0790)
T-Test of difference=0 (vs not =): T-Value = -0.15 P-Value = 0.883 DF = 11
```

Table F.18 Two-Sample T-Test and CI: Fifi - bone, Stillborn - bone

```
Two-sample T for Fifi - bone vs Stillborn - bone

                   N     Mean   StDev  SE Mean
Fifi - bone        3  -1.2503  0.0400    0.023
Stillborn - bone   4  -1.2287  0.0616    0.031


Difference = mu (Fifi - bone) - mu (Stillborn - bone)
Estimate for difference:  -0.0216
95% CI for difference:  (-0.1284, 0.0852)
T-Test of difference = 0 (vs not =): T-Value = -0.56 P-Value = 0.605 DF = 4
```

Table F.19 One-way ANOVA: HF29 - Bone, HF30 - Bone, HF33 - Bone, HF44 - Bone, Helena - bone

```
Source  DF       SS       MS     F      P
Factor   4  0.02748  0.00687  2.10  0.132
Error   15  0.04916  0.00328
Total   19  0.07664

S = 0.05725   R-Sq = 35.85%   R-Sq(adj) = 18.75%


                                  Individual 95% CIs For Mean Based on
                                  Pooled StDev
Level           N     Mean   StDev  ----+---------+---------+---------+-----
HF29 - Bone     4  -1.1560  0.0334  (--------*--------)
HF30 - Bone     5  -1.1410  0.0854     (-------*-------)
HF33 - Bone     4  -1.1053  0.0569         (--------*--------)
HF44 - Bone     2  -1.0493  0.0610              (-----------*-----------)
Helena - bone   5  -1.0732  0.0282               (-------*------)
                                  ----+---------+---------+---------+-----
                                   -1.190    -1.120    -1.050    -0.980

Pooled StDev = 0.0572
```

Table F.20 One-way ANOVA: 545-M1-Bulk, 545-M2-Bulk, 545-M3-Bulk

```
Source  DF       SS       MS      F      P
Factor   2  0.05232  0.02616  14.46  0.002
Error    8  0.01447  0.00181
Total   10  0.06679

S = 0.04253   R-Sq = 78.33%   R-Sq(adj) = 72.92%


                                Individual 95% CIs For Mean Based on
                                Pooled StDev
Level         N     Mean   StDev  ----+---------+---------+---------+-----
545-M1-Bulk   4  -1.0999  0.0552  (------*------)
545-M2-Bulk   4  -0.9723  0.0285                  (------*------)
545-M3-Bulk   3  -0.9410  0.0380                     (--------*-------)
                                ----+---------+---------+---------+-----
                                 -1.120    -1.050    -0.980    -0.910

Pooled StDev = 0.0425
```

Table F.21 One-way ANOVA: 549-M1-Bulk, 549-M2-Bulk, 549-M3-Bulk

```
Source  DF       SS       MS     F      P
Factor   2  0.02441  0.01221  8.11  0.012
Error    8  0.01204  0.00151
Total   10  0.03646

S = 0.03880   R-Sq = 66.96%   R-Sq(adj) = 58.70%


                                  Individual 95% CIs For Mean Based on
                                  Pooled StDev
Level        N     Mean   StDev   --+---------+---------+---------+-------
549-M1-Bulk  4  -1.0672  0.0526   (--------*--------)
549-M2-Bulk  3  -0.9690  0.0388                     (---------*----------)
549-M3-Bulk  4  -0.9694  0.0156                      (--------*--------)
                                   --+---------+---------+---------+-------
                                  -1.100    -1.050    -1.000    -0.950

Pooled StDev = 0.0388
```

Table F.22 One-way ANOVA: 552-M1-Bulk, 552-M2-Bulk, 552-Bulk-M3

```
Source  DF       SS       MS     F      P
Factor   2  0.06569  0.03284  8.90  0.023
Error    5  0.01845  0.00369
Total    7  0.08414

S = 0.06074   R-Sq = 78.07%   R-Sq(adj) = 69.30%


                                  Individual 95% CIs For Mean Based on
                                  Pooled StDev
Level        N     Mean   StDev  ---------+---------+---------+---------+
552-M1-Bulk  3  -1.0959  0.0126  (--------*--------)
552-M2-Bulk  3  -0.9161  0.0761                   (--------*--------)
552-Bulk-M3  2  -0.8990  0.0809                   (----------*----------)
                                  ---------+---------+---------+---------+
                                        -1.10     -1.00     -0.90     -0.80
Pooled StDev = 0.0607
```

Table F.23 One-way ANOVA: 668-M1-Bulk, 668-M2-Bulk, 668-M3-Bulk

```
Source  DF       SS       MS      F      P
Factor   2  0.04894  0.02447  19.52  0.001
Error    9  0.01128  0.00125
Total   11  0.06022

S = 0.03541   R-Sq = 81.27%   R-Sq(adj) = 77.10%


                                  Individual 95% CIs For Mean Based on
                                  Pooled StDev
Level        N     Mean   StDev   --+---------+---------+---------+-------
668-M1-Bulk  4  -1.0161  0.0228                            (------*-----)
668-M2-Bulk  4  -1.0943  0.0317                (------*-----)
668-M3-Bulk  4  -1.1725  0.0473   (------*-----)
                                   --+---------+---------+---------+-------
                                  -1.200    -1.140    -1.080    -1.020

Pooled StDev = 0.0354
```

Table F.24 One-way ANOVA: 799-M1-Bulk, 799-M2-Bulk, 799-M3-Bulk

```
Source  DF        SS        MS      F      P
Factor   2  0.076726  0.038363  40.51  0.000
Error    7  0.006629  0.000947
Total    9  0.083354

S = 0.03077   R-Sq = 92.05%   R-Sq(adj) = 89.78%


                                 Individual 95% CIs For Mean Based on
                                 Pooled StDev
Level        N     Mean   StDev  -----+---------+---------+---------+----
799-M1-Bulk  4  -1.1250  0.0311  (---*----)
799-M2-Bulk  4  -0.9558  0.0279                      (----*---)
799-M3-Bulk  2  -0.9302  0.0374                       (------*-----)
                                 -----+---------+---------+---------+----
                                   -1.120    -1.040    -0.960    -0.880
Pooled StDev = 0.0308
```

Table F.25 One-way ANOVA: 800-M1-Bulk, 800-M2-Bulk, 800-M3-Bulk

```
Source  DF       SS       MS      F      P
Factor   2  0.07244  0.03622  20.67  0.000
Error   10  0.01752  0.00175
Total   12  0.08996

S = 0.04186   R-Sq = 80.53%   R-Sq(adj) = 76.63%


                                 Individual 95% CIs For Mean Based on
                                 Pooled StDev
Level        N     Mean   StDev  ----+---------+---------+---------+-----
800-M1-Bulk  5  -1.1096  0.0525  (----*-----)
800-M2-Bulk  4  -0.9368  0.0276                       (-----*------)
800-M3-Bulk  4  -0.9858  0.0375                  (-----*------)
                                 ----+---------+---------+---------+-----
                                  -1.120    -1.050    -0.980    -0.910
Pooled StDev = 0.0419
```

Table F.26 One-way ANOVA: 2914-M1-Bulk, 2914-M2-Bulk, 2914-M3-Bulk

```
Source  DF       SS       MS      F      P
Factor   2  0.11229  0.05615  48.20  0.000
Error    7  0.00815  0.00116
Total    9  0.12045

S = 0.03413   R-Sq = 93.23%   R-Sq(adj) = 91.30%


                                  Individual 95% CIs For Mean Based on
                                  Pooled StDev
Level         N     Mean   StDev  ------+---------+---------+---------+---
2914-M1-Bulk  3  -1.3138  0.0362  (----*---)
2914-M2-Bulk  3  -1.1924  0.0018             (----*---)
2914-M3-Bulk  4  -1.0593  0.0429                        (---*---)
                                  ------+---------+---------+---------+---
                                     -1.30     -1.20     -1.10     -1.00

Pooled StDev = 0.0341
```

Table F.27 One-way ANOVA: 545-M1-Bulk, 549-M1-Bulk, 552-M1-Bulk, 668-M1-Bulk, ...

```
Source  DF       SS       MS      F      P
Factor   6  0.16913  0.02819  15.72  0.000
Error   20  0.03586  0.00179
Total   26  0.20499

S = 0.04234   R-Sq = 82.51%   R-Sq(adj) = 77.26%


                                  Individual 95% CIs For Mean Based on
                                  Pooled StDev
Level         N     Mean   StDev  ------+---------+---------+---------+---
545-M1-Bulk   4  -1.0999  0.0552                       (---*---)
549-M1-Bulk   4  -1.0672  0.0526                          (---*----)
552-M1-Bulk   3  -1.0959  0.0126                       (----*-----)
668-M1-Bulk   4  -1.0161  0.0228                                (---*----)
799-M1-Bulk   4  -1.1250  0.0311                    (---*----)
800-M1-Bulk   5  -1.1096  0.0525                      (---*---)
2914-M1-Bulk  3  -1.3138  0.0362  (----*----)
                                  ------+---------+---------+---------+---
                                     -1.30     -1.20     -1.10     -1.00

Pooled StDev = 0.0423
```

Table F.28 One-way ANOVA: 545-M1-Bulk, 549-M1-Bulk, 552-M1-Bulk, 799-M1-Bulk, 800-M1-Bulk

```
Source  DF       SS       MS     F      P
Factor   4  0.00733  0.00183  0.87  0.505
Error   15  0.03167  0.00211
Total   19  0.03900

S = 0.04595   R-Sq = 18.80%   R-Sq(adj) = 0.00%


                                  Individual 95% CIs For Mean Based on
                                  Pooled StDev
Level        N     Mean   StDev  ----+---------+---------+---------+-----
545-M1-Bulk  4  -1.0999  0.0552         (-----------*-----------)
549-M1-Bulk  4  -1.0672  0.0526                  (-----------*-----------)
552-M1-Bulk  3  -1.0959  0.0126        (-------------*-------------)
799-M1-Bulk  4  -1.1250  0.0311  (-------------*-----------)
800-M1-Bulk  5  -1.1096  0.0525        (----------*----------)
                                  ----+---------+---------+---------+-----
                                   -1.160    -1.120    -1.080    -1.040

Pooled StDev = 0.0460
```

Table F.29 One-way ANOVA: 545-M2-Bulk, 549-M2-Bulk, 552-M2-Bulk, 668-M2-Bulk, ...

```
Source  DF       SS       MS      F      P
Factor   6  0.19721  0.03287  23.97  0.000
Error   18  0.02469  0.00137
Total   24  0.22190

S = 0.03703   R-Sq = 88.88%   R-Sq(adj) = 85.17%


                                  Individual 95% CIs For Mean Based on
                                  Pooled StDev
Level         N     Mean   StDev  ----+---------+---------+---------+-----
545-M2-Bulk   4  -0.9723  0.0285                        (---*---)
549-M2-Bulk   3  -0.9690  0.0388                        (----*----)
552-M2-Bulk   3  -0.9161  0.0761                             (----*----)
668-M2-Bulk   4  -1.0943  0.0317             (---*--)
799-M2-Bulk   4  -0.9558  0.0279                          (--*---)
800-M2-Bulk   4  -0.9368  0.0276                           (---*---)
2914-M2-Bulk  3  -1.1924  0.0018  (----*---)
                                  ----+---------+---------+---------+-----
                                   -1.20     -1.10     -1.00     -0.90

Pooled StDev = 0.0370
```

Table F.30 One-way ANOVA: 545-M2-Bulk, 549-M2-Bulk, 552-M2-Bulk, 799-M2-Bulk, 800-M2-Bulk

```
Source  DF       SS       MS     F      P
Factor   4  0.00733  0.00183  1.10  0.398
Error   13  0.02167  0.00167
Total   17  0.02900

S = 0.04082   R-Sq = 25.28%   R-Sq(adj) = 2.29%


                                  Individual 95% CIs For Mean Based on
                                  Pooled StDev
Level        N     Mean   StDev  -----+---------+---------+---------+----
545-M2-Bulk  4  -0.9723  0.0285   (----------*----------)
549-M2-Bulk  3  -0.9690  0.0388  (------------*-----------)
552-M2-Bulk  3  -0.9161  0.0761              (------------*------------)
799-M2-Bulk  4  -0.9558  0.0279       (----------*----------)
800-M2-Bulk  4  -0.9368  0.0276            (----------*----------)
                                  -----+---------+---------+---------+----
                                    -1.000    -0.960    -0.920    -0.880

Pooled StDev = 0.0408
```

Table F.31 One-way ANOVA: 545-M3-Bulk, 549-M3-Bulk, 552-M3-Bulk, 668-M3-Bulk, ...

```
Source  DF       SS       MS      F      P
Factor   6  0.17596  0.02933  16.76  0.000
Error   16  0.02800  0.00175
Total   22  0.20396

S = 0.04183   R-Sq = 86.27%   R-Sq(adj) = 81.12%


                                  Individual 95% CIs For Mean Based on
                                  Pooled StDev
Level         N     Mean   StDev   --+---------+---------+---------+-------
545-M3-Bulk   3  -0.9410  0.0380                          (----*----)
549-M3-Bulk   4  -0.9694  0.0156                        (---*---)
552-M3-Bulk   2  -0.8990  0.0809                             (-----*-----)
668-M3-Bulk   4  -1.1725  0.0473   (----*---)
799-M3-Bulk   2  -0.9302  0.0374                          (-----*-----)
800-M3-Bulk   4  -0.9858  0.0375                      (---*----)
2914-M3-Bulk  4  -1.0593  0.0429              (---*----)
                                   --+---------+---------+---------+-------
                                  -1.20     -1.10     -1.00     -0.90

Pooled StDev = 0.0418
```

Table F.32 One-way ANOVA: 545-M3-Bulk, 549-M3-Bulk, 552-M3-Bulk, 799-M3-Bulk, 800-M3-Bulk

```
Source  DF       SS       MS     F      P
Factor   4  0.01270  0.00317  2.01  0.169
Error   10  0.01577  0.00158
Total   14  0.02847

S = 0.03972   R-Sq = 44.60%   R-Sq(adj) = 22.44%


                                  Individual 95% CIs For Mean Based on
                                  Pooled StDev
Level        N     Mean   StDev  ------+---------+---------+---------+---
545-M3-Bulk  3  -0.9410  0.0380          (---------*---------)
549-M3-Bulk  4  -0.9694  0.0156     (--------*--------)
552-M3-Bulk  2  -0.8990  0.0809               (-----------*-----------)
799-M3-Bulk  2  -0.9302  0.0374         (-----------*-----------)
800-M3-Bulk  4  -0.9858  0.0375  (--------*--------)
                                  ------+---------+---------+---------+---
                                     -1.000    -0.950    -0.900    -0.850

Pooled StDev = 0.0397
```

Table F.33 Two-Sample T-Test and CI: 545-M1-2, 799-M1-1

```
Two-sample T for 545-M1-2 vs 799-M1-1

          N     Mean   StDev  SE Mean
545-M1-2  3  -0.4670  0.0431    0.025
799-M1-1  3  -0.5344  0.0320    0.018


Difference = mu (545-M1-2) - mu (799-M1-1)
Estimate for difference:  0.0673
95% CI for difference:  (-0.0314, 0.1660)
T-Test of difference = 0 (vs not =): T-Value = 2.17 P-Value = 0.118  DF = 3
```

Table F.34 Two-Sample T-Test and CI: 545-M1-3, 799-M1-2

```
Two-sample T for 545-M1-3 vs 799-M1-2

           N     Mean   StDev  SE Mean
545-M1-3   4  -0.5408  0.0486    0.024
799-M1-2   3  -0.5992  0.0291    0.017


Difference = mu (545-M1-3) - mu (799-M1-2)
Estimate for difference:  0.0584
95% CI for difference:  (-0.0236, 0.1405)
T-Test of difference = 0 (vs not =): T-Value = 1.98 P-Value = 0.119  DF = 4
```

Table F.35 Two-Sample T-Test and CI: 545-M1-4, 799-M1-3

```
Two-sample T for 545-M1-4 vs 799-M1-3

           N     Mean   StDev  SE Mean
545-M1-4   5  -0.6362  0.0426    0.019
799-M1-3   3  -0.6045  0.0358    0.021


Difference = mu (545-M1-4) - mu (799-M1-3)
Estimate for difference:  -0.0317
95% CI for difference:  (-0.1039, 0.0405)
T-Test of difference = 0 (vs not =): T-Value = -1.13 P-Value = 0.310 DF = 5
```

Table F.36 Two-Sample T-Test and CI: 545-M1-5, 799-M1-4

```
Two-sample T for 545-M1-5 vs 799-M1-4

           N     Mean   StDev  SE Mean
545-M1-5   3  -0.6327  0.0636    0.037
799-M1-4   5  -0.6017  0.0442    0.020


Difference = mu (545-M1-5) - mu (799-M1-4)
Estimate for difference:  -0.0309
95% CI for difference:  (-0.1636, 0.1018)
T-Test of difference = 0 (vs not =): T-Value = -0.74 P-Value = 0.512 DF = 3
```

Table F.37 Two-Sample T-Test and CI: 545-M1-6, 799-M1-5

```
Two-sample T for 545-M1-6 vs 799-M1-5

           N     Mean   StDev  SE Mean
545-M1-6   4  -0.6320  0.0275    0.014
799-M1-5   4  -0.5872  0.0341    0.017


Difference = mu (545-M1-6) - mu (799-M1-5)
Estimate for difference:  -0.0448
95% CI for difference:  (-0.1011, 0.0116)
T-Test of difference = 0 (vs not =): T-Value = -2.04 P-Value = 0.097 DF = 5
```

Table F.38 Two-Sample T-Test and CI: 545-M1-7, 799-M1-6

```
Two-sample T for 545-M1-7 vs 799-M1-6

           N      Mean    StDev  SE Mean
545-M1-7   3   -0.6200   0.0110   0.0063
799-M1-6   3  -0.57071  0.00368   0.0021


Difference = mu (545-M1-7) - mu (799-M1-6)
Estimate for difference:  -0.04924
95% CI for difference:  (-0.07795, -0.02053)
T-Test of difference = 0 (vs not =): T-Value = -7.38 P-Value = 0.018 DF = 2
```

Table F.39 Two-Sample T-Test and CI: 545-M1-8, 799-M1-7

```
Two-sample T for 545-M1-8 vs 799-M1-7

           N     Mean   StDev  SE Mean
545-M1-8   5  -0.4921  0.0289    0.013
799-M1-7   4  -0.4444  0.0454    0.023


Difference = mu (545-M1-8) - mu (799-M1-7)
Estimate for difference:  -0.0477
95% CI for difference:  (-0.1202, 0.0248)
T-Test of difference = 0 (vs not =): T-Value = -1.83 P-Value = 0.142 DF = 4
```

Table F.40 Two-Sample T-Test and CI: 545-M2-3, 799-M2-1

```
Two-sample T for 545-M2-3 vs 799-M2-1

           N     Mean   StDev  SE Mean
545-M2-3   3  -1.0367  0.0505    0.029
799-M2-1   3  -1.1159  0.0524    0.030


Difference = mu (545-M2-3) - mu (799-M2-1)
Estimate for difference:  0.0791
95% CI for difference:  (-0.0546, 0.2129)
T-Test of difference = 0 (vs not =): T-Value = 1.88 P-Value = 0.156 DF = 3
```

Table F.41 Two-Sample T-Test and CI: 545-M2-4, 799-M2-2

```
Two-sample T for 545-M2-4 vs 799-M2-2

           N     Mean   StDev  SE Mean
545-M2-4   4  -0.9192  0.0327    0.016
799-M2-2   3  -1.0216  0.0588    0.034


Difference = mu (545-M2-4) - mu (799-M2-2)
Estimate for difference:  0.1024
95% CI for difference:  (-0.0596, 0.2645)
T-Test of difference = 0 (vs not =): T-Value = 2.72 P-Value = 0.113  DF = 2
```

Table F.42 Two-Sample T-Test and CI: 545-M2-6, 799-M2-4

```
Two-sample T for 545-M2-6 vs 799-M2-4

          N     Mean   StDev  SE Mean
545-M2-6  2  -0.9168  0.0133   0.0094
799-M2-4  4  -0.9813  0.0446    0.022


Difference = mu (545-M2-6) - mu (799-M2-4)
Estimate for difference:  0.0644
95% CI for difference:  (-0.0126, 0.1415)
T-Test of difference = 0 (vs not =): T-Value = 2.66 P-Value = 0.076  DF = 3
```

Table F.43 Two-Sample T-Test and CI: 545-M2-7, 799-M2-5

```
Two-sample T for 545-M2-7 vs 799-M2-5

          N     Mean   StDev  SE Mean
545-M2-7  3  -0.8674  0.0392    0.023
799-M2-5  4  -0.9534  0.0668    0.033


Difference = mu (545-M2-7) - mu (799-M2-5)
Estimate for difference:  0.0861
95% CI for difference:  (-0.0260, 0.1981)
T-Test of difference = 0 (vs not =): T-Value = 2.13 P-Value = 0.100  DF = 4
```

Table F.44 Two-Sample T-Test and CI: 545-M2-8, 799-M2-6

```
Two-sample T for 545-M2-8 vs 799-M2-6

          N      Mean    StDev  SE Mean
545-M2-8  3  -0.93763  0.00934   0.0054
799-M2-6  4   -0.9534   0.0668    0.033


Difference = mu (545-M2-8) - mu (799-M2-6)
Estimate for difference:  0.0158
95% CI for difference:  (-0.0919, 0.1235)
T-Test of difference = 0 (vs not =): T-Value = 0.47 P-Value = 0.672  DF = 3
```

Table F.45 Two-Sample T-Test and CI: 545-M2-9, 799-M2-7

```
Two-sample T for 545-M2-9 vs 799-M2-7

          N      Mean    StDev  SE Mean
545-M2-9  4   -0.9406   0.0688    0.034
799-M2-7  2  -0.93122  0.00871   0.0062


Difference = mu (545-M2-9) - mu (799-M2-7)
Estimate for difference:  -0.0094
95% CI for difference:  (-0.1206, 0.1019)
T-Test of difference = 0 (vs not =): T-Value = -0.27 P-Value = 0.806 DF = 3
```

Table F.46 Two-Sample T-Test and CI: 545-M2-10, 799-M2-8

```
Two-sample T for 545-M2-10 vs 799-M2-8

           N      Mean    StDev  SE Mean
545-M2-10  3   -0.9830   0.0254    0.015
799-M2-8   2  -0.93122  0.00871   0.0062


Difference = mu (545-M2-10) - mu (799-M2-8)
Estimate for difference:  -0.0517
95% CI for difference:  (-0.1201, 0.0166)
T-Test of difference = 0 (vs not =): T-Value = -3.26 P-Value = 0.083 DF = 2
```

F.2 Archaeological results

Table F.47 One-way ANOVA: B59-M1-B, B59-M2-B, B59-M3-B

```
Source  DF       SS       MS     F      P
Factor   2  0.01743  0.00871  5.39  0.056
Error    5  0.00808  0.00162
Total    7  0.02551

S = 0.04019   R-Sq = 68.33%   R-Sq(adj) = 55.66%


                              Individual 90% CIs For Mean Based on
                              Pooled StDev
Level     N     Mean   StDev  --------+---------+---------+---------+-
B59-M1-B  3  -1.1440  0.0346  (------*-------)
B59-M2-B  2  -1.0936  0.0553        (---------*--------)
B59-M3-B  3  -1.0363  0.0362                  (--------*-------)
                              --------+---------+---------+---------+-
                                   -1.140    -1.080    -1.020    -0.960

Pooled StDev = 0.0402
```

Table F.48 One-way ANOVA: B29-M1-B-1, B29-M2-B-1, B29-M3-B-1

```
Source  DF        SS        MS     F      P
Factor   2  0.010532  0.005266  6.00  0.037
Error    6  0.005264  0.000877
Total    8  0.015797

S = 0.02962   R-Sq = 66.67%   R-Sq(adj) = 55.57%


                                Individual 90% CIs For Mean Based on
                                Pooled StDev
Level       N     Mean   StDev  ----+---------+---------+---------+-----
B29-M1-B-SC  2  -1.0170  0.0110                      (---------*---------)
B29-M2-B-SC  3  -1.1028  0.0318  (-------*--------)
B29-M3-B-SC  4  -1.0409  0.0322                  (------*------)
                                ----+---------+---------+---------+-----
                                 -1.120    -1.080    -1.040    -1.000

Pooled StDev = 0.0296
```

Table F.49 One-way ANOVA: B29-M1-B-2, B29-M2-B-2, B29-M3-B-2

```
Source  DF       SS       MS     F      P
Factor   2  0.01790  0.00895  6.83  0.012
Error   11  0.01442  0.00131
Total   13  0.03231

S = 0.03620   R-Sq = 55.39%   R-Sq(adj) = 47.28%



Level       N     Mean   StDev
B29-M1-B-UC  5  -1.1306  0.0449
B29-M2-B-UC  5  -1.0913  0.0284
B29-M3-B-UC  4  -1.0409  0.0322

            Individual 90% CIs For Mean Based on Pooled StDev
Level          +---------+---------+---------+---------
B29-M1-B-UC     (------*-------)
B29-M2-B-UC               (------*------)
B29-M3-B-UC                         (-------*-------)
               +---------+---------+---------+---------
            -1.160    -1.120    -1.080    -1.040

Pooled StDev = 0.0362
```

Table F.50 Two-Sample T-Test and CI: B252-M1-B, B252-M2-B

```
Two-sample T for B252-M1-B vs B252-M2-B

           N     Mean   StDev  SE Mean
B252-M1-B  3  -1.0660  0.0320    0.018
B252-M2-B  3  -0.9700  0.0362    0.021


Difference = mu (B252-M1-B) - mu (B252-M2-B)
Estimate for difference:  -0.0961
95% CI for difference:  (-0.1848, -0.0074)
T-Test of difference = 0 (vs not =): T-Value = -3.45 P-Value = 0.041 DF = 3
```

APPENDIX G

CHAPTERS 5, 6 AND 7 $\delta^{44/42}Ca$, $\delta^{18}O$ AND $\delta^{13}C$ VALUE TABLES

Table G.1: Mean results for milk and feed samples from Highfield Farm, Yorkshire

Samples	Mean $\delta^{44/42}Ca_{915a}$ (‰)	SD	*n*	Samples	Mean $\delta^{44/42}Ca_{915a}$ (‰)	SD	*n*
Amber's milk	–0.84	0.07	4	Average milk	–0.85	0.01 SE	6
Coco's milk	–0.81	0.04	6	Grass	–0.38	0.05	5
Fifi 2010's milk	–0.87	0.04	2	Hay	–0.63	0.06	6
Fifi 2011's milk	–0.89	0.01	4	Course mix	–0.36	0.01	2
Helena's milk	–0.86	0.08	5	Sugar beet	–0.48	0.05	3
Jet's milk	–0.82	0.08	4	Average feed	–0.46	0.06 SE	4

Table G.2: Highfield Farm male (♂) and female (♀) first (M1) and second (M2) molar mean bulk enamel calcium isotope results

Sheep	Age at death	Teeth	$\delta^{44/42}Ca_{915a}$ (‰)	SD	*n*
HF29 ♂	7 months, 21 days	M1	–0.50	0.03	6
		M2	–0.54	0.03	5
HF30 ♀	7 months, 14 days	M1	–0.67	0.03	5
		M2	–0.66	0.04	5
HF33 ♀	7 months, 7 days	M1	–0.67	0.04	6
		M2	–0.62	0.04	5
HF35 ♀	7 months, 14 days	M1	–0.63	0.05	7
		M2	–0.59	0.06	6
HF44 ♂	7 months, 14 days	M1	–0.54	0.02	4
		M2	–0.49	0.05	3
HF61 ♂	5 months, 6 days	M1	–0.52	0.03	3
		M2	–0.56	0.02	3

Table G.3: Serial incremental $\delta^{44/42}Ca$ values for first (M1) and second (M2) molars from sheep HF29

Samples	$\delta^{44/42}Ca_{915a}$ (‰)	SD	*n*	Samples	$\delta^{44/42}Ca_{915a}$ (‰)	SD	*n*
HF29-M1-1	–0.60	0.02	3	HF29-M2-1	–0.56	0.03	3
HF29-M1-2	–0.58	0.04	4	HF29-M2-2	–0.58	0.08	12
HF29-M1-3	–0.56	0.08	4	HF29-M2-3	–0.49	0.07	3
HF29-M1-4	–0.58	0.04	4	HF29-M2-4	–0.47	0.03	4
HF29-M1-5	–0.60	0.04	6	HF29-M2-5	–0.44	0.05	3
HF29-M1-6	–0.57	0.02	3	HF29-M2-6	–0.38	0.08	5
HF29-M1-7	–0.50	0.04	5				

Table G.4: Serial incremental $\delta^{44/42}Ca$ values for first (M1) and second (M2) molars from sheep HF30

Samples	$\delta^{44/42}Ca_{915a}$ (‰)	SD	*n*	Samples	$\delta^{44/42}Ca_{915a}$ (‰)	SD	*n*
HF30-M1-1	–0.56	0.03	5	HF30-M2-1	–0.66	0.04	4
HF30-M1-2	–0.58	0.06	4	HF30-M2-2	–0.67	0.01	3
HF30-M1-3	–0.62	0.03	4	HF30-M2-3	–0.68	0.05	3
HF30-M1-4	–0.63	0.04	5	HF30-M2-4	–0.66	0.05	4
HF30-M1-5	–0.66	0.04	4	HF30-M2-5	–0.65	0.04	4
HF30-M1-6	–0.67	0.02	3	HF30-M2-6	–0.65	0.03	3
HF30-M1-7	–0.69	0.03	4	HF30-M2-7	–0.58	0.05	6
HF30-M1-8	–0.69	0.02	4	HF30-M2-8	–0.43	0.03	3

Table G.5: Serial incremental $\delta^{13}C$ and $\delta^{18}O$ values for first (M1) and second (M2) molars from sheep HF29

Samples	$\delta^{13}C_{VPDB}$ ‰	SD	$\delta^{18}O_{VPDB}$ ‰	SD	*N*
HF29 M1-1	–17.6	0.07	–3.2	0.19	9
HF29 M1-2	–17.5	0.05	–2.5	0.04	9
HF29 M1-3	–16.3	0.04	–3.0	0.09	9
HF29 M1-4	–16.1	0.03	–3.2	0.07	9
HF29 M1-5	–16.4	0.04	–4.0	0.12	9
HF29 M1-6	–16.6	0.02	–4.2	0.07	9
HF29 M1-7	–16.7	0.03	–4.5	0.08	9
HF29 M2-1	–15.8	0.05	–4.1	0.06	9
HF29 M2-2	–16.2	0.02	–4.0	0.06	9
HF29 M2-3	–16.3	0.05	–4.6	0.05	9
HF29 M2-4	–16.4	0.02	–5.5	0.11	9
HF29 M2-5	–16.7	0.04	–5.9	0.11	9
HF29 M2-6	–16.9	0.05	–7.2	0.13	9

Table G.6: Serial incremental $\delta^{13}C$ and $\delta^{18}O$ values for first (M1) and second (M2) molars from sheep HF30

Samples	$\delta^{13}C_{VPDB}$ ‰	SD	$\delta^{18}O_{VPDB}$ ‰	SD	*N*
HF30 M1-1	–18.1	0.04	–3.3	0.10	9
HF30 M1-2	–16.8	0.08	–2.9	0.05	13
HF30 M1-3	–15.4	0.13	–3.3	0.50	10
HF30 M1-4	–15.9	0.07	–3.3	0.10	19
HF30 M1-5	–15.9	0.05	–3.9	0.10	10
HF30 M1-6	–15.9	0.12	–4.1	0.18	9
HF30 M1-7	–16.3	0.08	–4.4	0.24	9
HF30 M1-8	–16.7	0.13	–4.4	0.21	9
HF30 M2-1	–16.0	0.10	–4.1	0.13	9
HF30 M2-2	–15.9	0.09	–4.4	0.12	9
HF30 M2-3	–16.2	0.06	–5.1	0.17	9
HF30 M2-4	–16.2	0.05	–5.7	0.09	9
HF30 M2-5	–15.7	0.06	–5.6	0.11	4
HF30 M2-6	–15.3	0.26	–6.0	0.77	10
HF30 M2-7	–15.4	0.18	–7.0	0.86	10

Table G.7: Highfield Farm bone samples. Male and female lambs, mature ewes and a stillborn lamb of unknown sex

Sheep	$\delta^{44/42}Ca_{915a}$ (‰)	SD	*n*	Sheep	$\delta^{44/42}Ca_{915a}$ (‰)	SD	*n*
HF29 ♂	–0.59	0.03	4	HF44 ♂	–0.48	0.06	2
HF30 ♀	–0.57	0.09	5	Helena ♀	–0.50	0.03	5
HF30 ♀, unashed	–0.42	0.05	4	Fifi ♀	–0.68	0.04	3
HF33 ♀	–0.54	0.06	4	Stillborn	–0.66	0.06	4

Table G.8: Mean Hoy sheep first (M1), second (M2) and third molar (M3) bulk enamel $\delta^{44/42}Ca$ values (1 standard deviation, SD)

Sheep	Age at death	Teeth	$\delta^{44/42}Ca_{915a}$ (‰)	SD	*n*
545	2 years, 10 months	M1	–0.53	0.06	4
		M2	–0.40	0.03	4
		M3	–0.37	0.04	3
549	1 year, 9 months	M1	–0.50	0.05	4
		M2	–0.40	0.04	3
		M3	–0.40	0.02	4
552	2 years, 11 months	M1	–0.53	0.01	3
		M2	–0.35	0.08	3
		M3	–0.33	0.08	2
668	2 years, 5 months	M1	–0.45	0.02	4
		M2	–0.52	0.03	4
		M3	–0.60	0.05	4
799	2 years, 7 months	M1	–0.56	0.03	4
		M2	–0.39	0.03	4
		M3	–0.36	0.04	2
800	2 years, 7 months	M1	–0.54	0.05	5
		M2	–0.37	0.03	4
		M3	–0.42	0.04	4
2914	3 years, 3 months	M1	–0.74	0.04	3
		M2	–0.62	0.01	3
		M3	–0.49	0.04	4

Table G.9: Serial incremental $\delta^{44/42}Ca$ values for first (M1), second (M2) and third (M3) molars from sheep Hoy 545 (1 standard deviation, SD)

Sample	M1 $\delta^{44/42}Ca_{915a}$ ‰	SD	*n*	M2 $\delta^{44/42}Ca_{915a}$ ‰	SD	*n*	M3 $\delta^{44/42}Ca_{915a}$ ‰	SD	*n*
545-1	–0.56	0.06	4	–0.59	0.02	3	–0.31	——	1
545-2	–0.47	0.04	3	–0.55	0.01	2	–0.35	0.01	2
545-3	–0.54	0.05	4	–0.47	0.05	3	–0.35	——	1
545-4	–0.64	0.04	5	–0.35	0.03	4	–0.32	——	1
545-5	–0.63	0.06	3	–0.32	——	1	–0.31	0.02	2
545-6	–0.63	0.03	4	–0.35	0.01	2	–0.30	0.04	2
545-7	–0.62	0.01	3	–0.30	0.04	3			
545-8	–0.49	0.03	4	–0.37	0.01	3			
545-9				–0.37	0.07	4			
545-10				–0.41	0.03	3			

Table G.10: Serial incremental $\delta^{44/42}Ca$ values for first (M1), second (M2) and third (M3) molars from sheep Hoy 799 (1 standard deviation, SD)

Sample	M1 $\delta^{44/42}Ca_{915a}$ ‰	SD	*n*	M2 $\delta^{44/42}Ca_{915a}$ ‰	SD	*n*	M3 $\delta^{44/42}Ca_{915a}$ ‰	SD	*n*
799-1	–0.53	0.03	3	–0.55	0.07	3	–0.34	0.06	5
799-2	–0.60	0.03	3	–0.43	0.04	3			
799-3	–0.60	0.04	3	–0.37	0.07	3			
799-4	–0.60	0.04	5	–0.40	0.07	4			
799-5	–0.59	0.03	4	–0.38	0.07	4			
799-6	–0.57	0.01	3	–0.29	0.03	5			
799-7	–0.44	0.05	4	–0.36	0.01	2			
799-8				–0.30	0.02	3			

Table G.11: Mean Tell Abu Hureyra sheep first (M1), second (M2) and third (M3) molar bulk enamel $\delta^{44/42}Ca$ values (1 standard deviation, SD; SC, standard clean; UC, ultra clean)

Animal	Age at death	Teeth	$\delta^{44/42}Ca_{915a}$ (‰)	SD	*n*
B59	12–24 months	M1	–0.57	0.03	5
		M2	–0.52	0.06	3
		M3	–0.47	0.04	3
B29 – SC	21–24 months	M1	–0.45	0.01	2
		M2	–0.53	0.03	3
		M3	–0.47	0.03	4
B29 – UC		M1	–0.56	0.04	5
		M2	–0.52	0.03	5
		M3	–0.47	0.03	4
B252	12–24 months	M1	–0.50	0.03	3
		M2	–0.40	0.04	3

Table G.12: Serial incremental $\delta^{44/42}Ca$ values for first (M1), second (M2) and third (M3) molars from Tell Abu Hureyra sheep B59 (1 standard deviation, SD)

M1 $\delta^{44/42}Ca_{915a}$ ‰	SD	*n*	M2 $\delta^{44/42}Ca_{915a}$ ‰	SD	*n*	M3 $\delta^{44/42}Ca_{915a}$ ‰	SD	*n*
–0.61	0.01	3	–0.57	0.07	4	–0.55	0.04	5
–0.58	0.01	3	–0.47	0.04	6			
–0.64	0.04	5	–0.52	0.03	5			
–0.60	0.05	4	–0.50	0.04	7			
–0.55	0.06	4	–0.52	0.02	6			
–0.56	0.02	4	–0.54	0.03	4			
–0.43	0.02	2	–0.53	0.04	5			
			–0.59	0.01	2			
			–0.46	0.06	3			

Table G.13: Serial incremental $\delta^{44/42}Ca$ values for first (M1), second (M2) and third (M3) molars from Tell Abu Hureyra sheep B29 – standard clean (SC) (1 standard deviation, SD)

M1 $\delta^{44/42}Ca_{915a}$ ‰	SD	*n*	M2 $\delta^{44/42}Ca_{915a}$ ‰	SD	*n*	M3 $\delta^{44/42}Ca_{915a}$ ‰	SD	*n*
–0.48	0.08	6	–0.56	0.02	5	–0.37	0.03	4
–0.45	0.03	5	–0.63	0.05	5			
–0.50	0.02	5	–0.59	0.1	2			
–0.50	0.05	5	–0.50	0.04	4			
–0.50	0.05	3	–0.53	0.05	3			
–0.51	0.01	6	–0.57	0.03	5			
–0.43	0.03	4	–0.58	0.02	2			
			–0.52	0.07	3			
			–0.56	0.06	2			
			–0.57	0.05	3			
			–0.53	0.03	4			
			–0.49	0.06	3			

Table G.14: Serial incremental $\delta^{44/42}Ca$ values for first (M1), second (M2) and third (M3) molars from Tell Abu Hureyra sheep B29 – ultra clean (UC) (1 standard deviation, SD)

M1 $\delta^{44/42}Ca_{915a}$ ‰	SD	*n*	M2 $\delta^{44/42}Ca_{915a}$ ‰	SD	*n*	M3 $\delta^{44/42}Ca_{915a}$ ‰	SD	*n*
						–0.52	0.06	3
–0.54	0.03	6	–0.61	0.04	3			
–0.47	0.05	7	–0.54	0.03	3			
–0.52	0.06	14	–0.52	0.03	4			
–0.61	0.08	4	–0.54	0.07	6			
–0.52	0.05	4	–0.49	0.05	7			
–0.54	0.04	2	–0.46	0.06	12			
–0.51	0.05	5	–0.52	0.05	9			
–0.54	0.03	6	–0.54	0.05	6			
			–0.56	0.03	8			
			–0.46	0.04	2			
			–0.52	0.03	7			
			–0.47	0.07	3			

Table G.15: Serial incremental $\delta^{44/42}Ca$ values for first (M1) and second (M2) molars from Tell Abu Hureyra goat B252 (1 standard deviation, SD)

M1 $\delta^{44/42}Ca_{915a}$ ‰	SD	*n*	M2 $\delta^{44/42}Ca_{915a}$ ‰	SD	*n*
–0.44	0.06	8	–0.42	0.04	3
–0.49	0.04	4	–0.36	0.04	4
–0.48	0.04	3	–0.44	0.03	5
–0.49	0.08	4	–0.40	0.08	4
–0.47	0.07	4	–0.44	0.05	4
–0.44	0.04	5	–0.40	0.07	3
–0.44	0.04	6	–0.45	0.07	5
–0.38	0.01	4	–0.39	0.03	4
–0.45	0.07	5	–0.44	0.03	3
			–0.45	0.05	4
			–0.37	0.03	3

Table G.16: Serial incremental $\delta^{13}C$ and $\delta^{18}O$ values for first (M1), second (M2) and third (M3) molars from sheep B59 (1 standard deviation, SD)

Samples	$\delta^{13}C_{VPDB}$ ‰	SD	$\delta^{18}O_{VPDB}$ ‰	SD	*n*
B59-M1-1	–8.81	0.08	–2.52	0.12	9
B59-M1-2	–9.14	0.10	–1.86	0.12	9
B59-M1-3	–9.51	0.06	–2.21	0.12	9
B59-M1-4	–8.52	0.06	–3.50	0.11	9
B59-M1-5	–9.08	0.07	–4.28	0.15	9
B59-M1-6	–10.62	0.07	–4.02	0.10	9
B59-M2-1	–9.15	0.05	–5.28	0.12	6
B59-M2-2	–10.24	0.07	–4.45	0.11	10
B59-M2-3	–11.22	0.05	–2.49	0.08	10
B59-M2-4	–10.66	0.08	–0.18	0.15	10
B59-M2-5	–9.84	0.03	0.16	0.09	3
B59-M2-6	–8.44	0.06	0.18	0.07	10
B59-M2-7	–7.23	0.14	–0.81	0.25	19
B59-M2-8	–7.24	0.07	–2.84	0.19	10
B59-M2-9	–7.92	0.06	–4.57	0.18	10
B59-M3-1	–7.90	0.06	–3.29	0.15	10

Table G.17: Serial incremental $\delta^{13}C$ and $\delta^{18}O$ values for first (M1) and second (M2) molars from goat B252 (1 standard deviation, SD)

Samples	$\delta^{13}C_{VPDB}$ ‰	SD	$\delta^{18}O_{VPDB}$ ‰	SD	*n*
B252-M1-1	–12.12	0.06	–0.35	0.08	9
B252-M1-2	–10.54	0.04	2.83	0.10	9
B252-M1-3	–10.28	0.05	1.36	0.13	9
B252-M1-4	–9.78	0.07	0.71	0.13	9
B252-M1-5	–9.87	0.05	0.02	0.11	9
B252-M1-6	–9.85	0.07	–1.31	0.21	9
B252-M1-7	–10.42	0.03	–2.69	0.06	3
B252-M1-8	–11.11	0.05	–4.14	0.07	13
B252-M1-9	–11.13	0.07	–3.72	0.11	10
B252-M2-1	–9.95	0.10	–2.35	0.18	10
B252-M2-2	–9.58	0.09	–2.34	0.15	19
B252-M2-3	–9.62	0.03	–3.51	0.08	13
B252-M2-4	–9.35	0.09	–3.56	0.12	10
B252-M2-5	–10.57	0.34	–5.03	0.53	9
B252-M2-6	–11.22	0.06	–4.54	0.07	6
B252-M2-7	–11.74	0.04	–5.12	0.06	6
B252-M2-8	–12.08	0.04	–4.02	0.06	6
B252-M2-9	–12.61	0.09	–3.18	0.20	9
B252-M2-10	–12.34	0.09	–1.34	0.15	9
B252-M2-11	–11.72	0.13	–2.08	0.13	9