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STABLE ISOTOPE RATIOS AS A PROXY FOR  
HUMAN GEOGRAPHIC PROVENANCE:  
BIOGEOGRAPHICAL EVIDENCE FROM  
THE 2H, 18O, AND 13C SIGNATURES IN  
MODERN HUMAN TEETH

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STABLE ISOTOPE RATIOS AS A PROXY FOR HUMAN GEOGRAPHIC  
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 $^{13}\text{C}$  ISOTOPIC SIGNATURES IN MODERN HUMAN TEETH

by

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B.S., Ohio University, 1987  
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A Dissertation  
Submitted in Partial Fulfillment of the Requirements for the  
Doctor of Philosophy

Department of Anthropology  
in the Graduate School  
Southern Illinois University Carbondale  
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DISSERTATION APPROVAL

STABLE ISOTOPE RATIOS AS A PROXY FOR HUMAN GEOGRAPHIC  
PROVENANCE: BIOGEOGRAPHICAL EVIDENCE FROM THE  $^2\text{H}$ ,  $^{18}\text{O}$ , AND  
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By

Anastasia Holobinko

A Dissertation Submitted in Partial

Fulfillment of the Requirements

for the Degree of

Doctor of Philosophy

in the field of Anthropology

Approved by:

Susan Ford, Chair

Izumi Shimada

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Wolfram Meier-Augenstein

Steven Symes

Graduate School  
Southern Illinois University Carbondale  
August 19, 2015

## AN ABSTRACT OF THE DISSERTATION OF

ANASTASIA HOLOBINKO, for the Doctor of Philosophy degree in PHYSICAL ANTHROPOLOGY, presented on August 19, 2015, at Southern Illinois University Carbondale.

TITLE: STABLE ISOTOPE RATIOS AS A PROXY FOR HUMAN GEOGRAPHIC PROVENANCE: BIOGEOGRAPHICAL EVIDENCE FROM THE  $^2\text{H}$ ,  $^{18}\text{O}$ , AND  $^{13}\text{C}$  SIGNATURES IN MODERN HUMAN TEETH

MAJOR PROFESSOR: Dr. Susan Ford

The positive identification of a decedent is paramount to a forensic investigation in which human remains have been recovered and must be identified. Due to increasing global mobility in the world's populations, it is not inconceivable that an individual might die far away from his or her home. Pinpointing an individual's geographic origin may contribute to definitive forensic identification of contemporary human skeletal remains in cases where dental records and other means of identification are either unavailable to law enforcement personnel or do not yield immediate results.

Stable isotope analysis of biogenic tissues such as tooth enamel and bone mineral has become a well-recognized and increasingly important method for determining the provenance of human remains, and it has been used successfully in bioarchaeological studies as well as forensic investigations. Both  $^{18}\text{O}$  and  $^2\text{H}$  stable isotope signatures are well established proxies as environmental indicators of climate (temperature) and source water and are therefore considered reliable indicators of geographic life trajectories of animals and humans. Similarly,  $^{13}\text{C}$  and  $^{15}\text{N}$  abundance data have distinguished dietary preferences in ancient human populations, and have been used to qualify  $^2\text{H}$  and  $^{18}\text{O}$  geolocational data that may be consistent with more than one location. Few if any studies have systematically investigated the multi-isotopic

signatures in human tooth enamel and dentin from living individuals. Since  $^{18}\text{O}$  abundance values obtained from tooth enamel of late-erupting molars are a source of information on geographic origin of an individual during adolescence when crown formation takes place, it was hypothesized that: 1) the stable isotope abundance of  $^2\text{H}$ ,  $^{18}\text{O}$ , and  $^{13}\text{C}$  in human tooth enamel and dentin is consistent with self-reported residential history and dietary preferences data, and 2) the isotopic variability evident between individuals with shared residential history and nutrient intake is quantifiable, and indicative of intra-individual variability.

Two pilot studies were conducted to: 1) evaluate the feasibility of extracting and measuring the  $^2\text{H}$  composition of human tooth enamel and its suitability as a proxy for human geographic provenance, and 2) compare the isotopic abundance of isotopic abundance of  $^2\text{H}$  and  $^{13}\text{C}$  in human crown dentin collagen obtained from archaeological and modern teeth with the  $^{18}\text{O}$  and  $^{13}\text{C}$  isotopic composition of the corresponding tooth enamel carbonate. A protocol for preparing tooth enamel for  $^2\text{H}$  analysis was successfully devised and implemented; however, no correlation was observed between tooth enamel  $^2\text{H}$  abundance values as measured plotted against source water  $^2\text{H}$  abundance values. While unexpectedly low rates of hydrogen exchange within mineral hydroxyl groups were documented, and it was possible to analyze tooth enamel for its  $^2\text{H}$  isotopic composition, the seemingly fixed nature of  $^2\text{H}$  abundance in tooth enamel means that the  $^2\text{H}$  isotopic signature of tooth enamel cannot be used as an indicator of geographic provenance. Conversely, positive correlations between collagen  $^2\text{H}$  abundance values of primary dentin with  $^2\text{H}$  abundance values for source water and

also with enamel  $^{18}\text{O}$  abundance values suggests that primary dentin collagen  $^2\text{H}$  values are linked to the isotopic composition of source water.

Third molar tooth enamel was sampled from 10 living volunteers undergoing routine tooth extractions at University of Toronto affiliated dental clinics in Ontario, Canada. The mixed-sex group of patients was given questionnaires in which they provided detailed residential history and answered questions pertaining to dietary preferences (e.g., vegetarian) prior to donating all four third molars. Enamel was drilled from the crown of two third molars from each subject, chemically cleaned, and subjected to an acid digest before being analyzed for its  $^{18}\text{O}$  and  $^{13}\text{C}$  composition using Isotope Ratio Mass Spectrometry. Herewith, isotope abundance values are presented using the delta notation as delta values in per mil (‰).

Mean  $\delta^{13}\text{C}_{\text{VPDB}}$  values for all samples ranged from -9.47 ‰ to -11.31 ‰ (pooled mean = -10.37 ‰), which suggested a persistent  $\text{C}_4$  plant dietary influence at the time the sampled tooth enamel was forming and is consistent with the typical North American diet. While inter-subject variation contributed the largest proportion of total  $\delta^{13}\text{C}$  variability, differences were not significant. The pooled mean  $\delta^{18}\text{O}_{\text{VSMOW}}$  value for enamel samples was 24.39 ‰, while individual subject mean  $\delta^{18}\text{O}_{\text{VSMOW}}$  values ranged from 23.76 ‰ to 25.18 ‰. Marked offsets (0.01 ‰ - 0.51 ‰) in mean  $\delta^{18}\text{O}_{\text{VSMOW}}$  values for each pair of third molars were observed. While subject variation was significant ( $p=0.0034$ ), neither diet nor sex significantly influenced the oxygen (or carbon) isotope data.

Following conversion of  $\delta^{18}\text{O}_{\text{VSMOW}}$  values to  $\delta^{18}\text{O}_{\text{Phosphate}}$  values, drinking water values were calculated using the Daux et al. (2008) equation, and compared to their

corresponding regional estimated annual average  $\delta^{18}\text{O}$  values in precipitation retrieved from the Online Isotopes in Precipitation Calculator (OIPC) (Bowen 2014). Despite observed correlations between the drinking water  $\delta^{18}\text{O}_{\text{Water}}$  values and  $\delta^{18}\text{O}_{\text{OIPC}}$  values of four subjects, no statistically significant correlations were evident between the two limited data sets. However, when the isotopically similar  $\delta^{18}\text{O}$  values of Toronto area residents were averaged and combined with the remaining Canadian data and the  $^{18}\text{O}$  data from 5 enamel samples analyzed in the  $^2\text{H}$  enamel pilot study, strong positive correlations were evident between  $\delta^{18}\text{O}_{\text{Phosphate}}$  values and  $\delta^{18}\text{O}_{\text{OIPC}}$  values ( $R^2 = 0.87$ ). Moreover, an equally strong linear relationship was observed between modeled annual precipitation  $^{18}\text{O}$  and calculated source water  $^{18}\text{O}$  ( $R^2 = 0.87$ ).

Based on the  $\delta^2\text{H}$ ,  $\delta^{18}\text{O}$ , and  $\delta^{13}\text{C}$  values measured in tooth enamel and dentin, it is possible to infer and confirm geographic provenance and dietary intake. However, while enamel carbonate  $\delta^{13}\text{C}$  values were consistent with self-reported dietary intake information and residential history, as could be expected vegetarians could not be distinguished from those who consumed meat without determining C/N isotopic ratios in dentin collagen. Further, the presence of strong linear relationships between the  $^{18}\text{O}$  composition of enamel carbonate and modeled source water  $^{18}\text{O}$  in the merged dataset illustrates the importance of considering site-specific isotopic complexities and using multi-isotope data obtained from multiple tissues when investigating the geographic origins of humans in an archaeological or forensic context. It is not possible to quantify intra-individual isotopic variability without sampling from larger, geographically diverse populations and controlling for as many variables as possible. The construction of regional databases containing  $^{18}\text{O}$  and  $^2\text{H}$  isotopic data obtained from a variety of

environmental and human and faunal tissue samples, and the application of such data to individual cases in which geographic origins are desired, is recommended provided the following caveats are considered: 1) whenever possible,  $\delta^{18}\text{O}_{\text{Phosphate}}$  values should be used when comparing  $\delta^{18}\text{O}$  values in tooth enamel with those in precipitation in order to avoid the unmitigated error associated with the application of carbonate conversion equations to enamel phosphate  $\delta^{18}\text{O}$  data, 2) the continuous consumption of food sourced elsewhere is liable to affect the overall enamel carbonate  $\delta^{18}\text{O}$  values, 3)  $\delta^{18}\text{O}$  values in precipitation are not inclusive of the variety of postprecipitation and hydrological processes unique to a particular location, nor are they an accurate representation of the isotopically mixed nature of tap water sourced from distant reservoirs. Hence, extrapolations should be made with caution. Enamel sequential microsampling methods may be advisable for narrowing down a geographic timeline if the overlapping of isotopic signals at various points along the tooth's longitudinal plane can be quantified and validated. While standardization of analytical methodology is critical to appropriate interpretations of the data, stable isotope profiling is not a standalone method and should be used in conjunction with other lines of evidence in determinations of human provenance.



## DEDICATION

This dissertation is dedicated to my husband, Carlos Alberto Calabrese, and my mother, Leni Holobinko. Without their continuous encouragement, assistance, and appreciation of my educational aspirations, this work would not have been possible. I also dedicate this work to the memory of my father, Andrew Holobinko, who valued family, hard work, and education above all else.

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Few doctoral students earn a Ph.D. without the support and assistance of many individuals and entities along the way. In the following paragraphs I acknowledge those individuals and organizations who have been instrumental in helping me achieve this degree. I offer a sincere apology to anyone I have neglected to recognize herein.

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## PREFACE

This dissertation is submitted for the degree of Doctor of Philosophy at Southern Illinois University Carbondale. The research described herein was conducted under the supervision of Dr. Susan M. Ford in the Department of Anthropology, Southern Illinois University Carbondale, between January 2009 and October 2014. All projects and associated methods were approved by the Southern Illinois University Carbondale Human Subjects Committee. Ethics approval for the sampling of Canadian material was granted by the McMaster University Human Research Ethics Board, Hamilton, Ontario.

This work is to the best of my knowledge original, except where acknowledgements and references are made to previous work. Portions of this work have been published elsewhere. Permission to reproduce the following works is provided in Appendix A.

Versions of Chapter 3 and Chapter 4 have been published as review papers in the following publications:

Holobinko A. 2012a. Theoretical and methodological approaches to understanding human migration patterns and their utility in forensic human identification cases. *Societies* 2:42-62.

Holobinko A. 2012b. Forensic human identification in the U.S. and Canada: a review of the law, admissible techniques, and the legal implications of their applications in forensic cases. *Forens Sci Int* 222:394.e1-394.e13.

A version of Chapter 5 has been published as Holobinko A, Meier-Augenstein W, Kemp HF, Prowse T, Ford SM. 2011.  $^2\text{H}$  stable isotope composition of human tooth enamel: a new tool for forensic human provenancing? *Rapid Commun Mass Spectrom* 25:910-916. I was responsible for the early stages of concept formation, and

contributed to manuscript composition and edits. The majority of the manuscript was developed by Meier-Augenstein W, and all data collection and analyses were conducted by Meier-Augenstein W and Kemp HF at the Stable Isotope Forensics Laboratory, James Hutton Institute (formerly Scottish Crop Research Institute), Dundee, UK. The final manuscript was disseminated to Ford S and Prowse TP for their approval prior to publication. Prior to manuscript preparation, this material was presented in poster format by Holobinko A at the 2010 European Geosciences Union General Assembly in Vienna, Austria.

A version of Chapter 6 has been presented as a poster by this author at the 2011 European Geosciences Union General Assembly, Vienna, Austria, and at the 2011 ISOSCAPES Meeting, West Lafayette, Indiana. (Holobinko A, Meier-Augenstein W, Kemp H, Prowse T, Schwarcz H. 2011.  $^2\text{H}$  stable isotope analysis of human tooth dentine: a pilot study). Holobinko A, Meier-Augenstein W, and Kemp H were all involved in the early stages of concept formation. Data collection and analyses were conducted by Meier-Augenstein W and Kemp H at the Stable Isotope Forensics Laboratory, James Hutton Institute, Dundee, UK.

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## CHAPTER 1

### INTRODUCTION

In the United States approximately 40,000 to 60,000 sets of stored unidentified human remains await provenance (Ritter 2007; Schmitt 2006). Most of these individuals are victims of violent crimes, whose bodies have been recovered by law enforcement personnel over the last 50 years (Hargrove 2005). While personal identification methods such as DNA and estimations of sex, age, and ancestry have been used to establish individual identities, there are circumstances in which these methods are ineffectual. In such cases where comparison with missing persons reports and DNA is not feasible - or even possible because geographic origins are unknown - the use of stable isotope analysis is a viable option for determining provenance and has been used successfully in a small number of forensic cases to determine regional origins (Rauch et al. 2007; Meier-Augenstein and Fraser 2008; Font et al. 2015).

The purpose of this research was twofold: (1) to determine if measured stable hydrogen ( $^2\text{H}/^1\text{H}$ ) and oxygen ( $^{18}\text{O}/^{16}\text{O}$ ) isotopic ratios tell the same biogeographical story about an individual (i.e. do both stable isotope signatures within the tooth enamel of adult living persons with known life histories exhibit identical or similar signature patterns regarding geographic residence at a particular developmental stage in an individual's lifetime); (2) to investigate the utility of carbon ( $^{13}\text{C}/^{12}\text{C}$ ) ratio analysis as a dietary qualifier when  $^2\text{H}$  and  $^{18}\text{O}$  isotopic data are consistent with more than one

location. This research is important because pinpointing an individual's geographic origins may contribute to definitive forensic identification of contemporary human skeletal remains in cases where dental records and other means of identification cannot be used by law enforcement personnel to establish a positive identification. For example, odontological assessment and DNA profiling may be impossible if the remains are sufficiently deteriorated (or mutilated). Alternately, these and other means of identification may be available but unable to produce meaningful results if comparisons with antemortem records or existing DNA profiles (victim or family) cannot be made. The results of this research are expected to contribute to the refinement of existing techniques as well as the development of a multi-faceted technique to facilitate the identification of unidentified human remains for which no tentative identification exists, or when other identification techniques are deemed inappropriate.

In many cases the identity of a deceased individual is known or suspected at or around the time of death. The identification of decedents who have been transported to a morgue may only require, in some cases, supplemental documentation (e.g., medical and/or dental records) to confirm what is already known or suspected. Well preserved skeletonized remains, while lacking the recognizable physical features of a fleshed body, can still offer DNA for analytical purposes provided there is reason to suspect the skeleton is that of a known missing person and that biological material from a relative is available for comparison; the presence of a distinctive skeletal anomaly also can confirm identification. However, the identification of some decedents is not always judicious; months, years, or even decades may pass before identification is made. Some decedents will never be identified.

Undocumented illegal immigrants may account for a large number of unidentified human remains recovered along international borders; Baker (2007) cited approximately 3,600 such cases along the U.S.-Mexico border between 1995 and 2005. Similarly, the remains of persons believed to be indigent can pose problems determining provenance; if an individual's place of origin or recent residence cannot be determined, then it is difficult to obtain medical and/or dental records that may be used for identification. Finally, the increasing global mobility evident in the world's population contributes to the problem of more individuals dying farther away from their "home communities" (Mack 1995:512); the increasing degree of international travel and intra- and intercontinental relocation can render missing persons reports useless. In the United States alone, over 600,000 entries are entered into the Federal Bureau of Investigation's National Crime Information Center's (NCIC) missing person database each year (FBI-NCIC 2014). As of December 31, 2013, the NCIC missing person database contained 84,136 active missing person records.

#### Isobiological Profiling: Stable Isotopes as Geolocational Tracers

Stable isotopes are mass dependent, non-radiogenic variants of particular chemical elements (Hoefs 2004). While isotopes behave similarly in chemical reactions, their reaction rates differ according to their respective atomic weights. These differences explain the varying abundance of naturally occurring isotopes in organic and inorganic compounds, and are the basis for stable isotope analysis. Primary development of stable isotope work occurred in the geochemical community (Fogel et al. 1997); anthropological application of these techniques began with archaeological dietary

reconstruction studies in the late 1970s (DeNiro and Epstein 1978a, 1978b; van der Merwe and Vogel 1978).

Stable isotope levels collected from various human tissues, including teeth, bone, hair, and finger nails, indicate that individuals possess isotopic “signatures.” Variation in stable isotope composition occurs with comparative predictability such that bioarchaeological and zoological research has successfully confirmed the utility of carbon ( $^{13}\text{C}/^{12}\text{C}$ ) and nitrogen ( $^{15}\text{N}/^{14}\text{N}$ ) isotope ratio analysis in dietary and habitat reconstructions (carbon  $\delta^{13}\text{C}$  and nitrogen  $\delta^{15}\text{N}$ , van der Merwe 1982; Klepinger 1984; DeNiro 1986; van der Merwe et al. 1990; Vogel et al. 1990). Similarly,  $^{13}\text{C}/^{12}\text{C}$  and  $^{15}\text{N}/^{14}\text{N}$  ratio analysis of hair, fingernails, feathers, and other biological materials with high turnover rates has assisted in establishing the migratory patterns of animals (e.g., Hobson 1990; van der Merwe et al. 1990; Vogel et al. 1990; Alisauskas and Hobson 1993; Smith et al. 1996) and recent movements of humans (e.g., Bol and Pfeleger 2002; Fraser et al. 2006; Bol et al. 2007; Rauch et al. 2007; Meier-Augenstein and Fraser 2008; Thompson et al. 2010). The analysis of strontium isotope ratios ( $^{87}\text{Sr}/^{86}\text{Sr}$ ) in geological bedrock and consumed foodstuffs has been incorporated into animal and ancient human migration studies (Price et al. 1994a, 1994b; Chamberlain et al. 1997; Budd et al. 2004; Evans et al. 2006) and dietary reconstructions (Chamberlain et al. 1997; Kennedy et al. 1997; Harrington et al. 1998).  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios also have been examined as isotopic indicators of the natal origins and migration patterns associated with modern human skeletal remains (Beard and Johnson 2000; Juarez 2008). Hydrogen ( $\delta^2\text{H}$ ) and oxygen ( $\delta^{18}\text{O}$ ) values are indicators of climatic change (Dansgaard 1964; Fricke et al. 1998) and food and water intake (Estep and Dabrowski 1980; Estep

and Hoering 1980; Schimmelmann et al. 1993; Sharp et al. 2003). More recently, oxygen ( $^{18}\text{O}/^{16}\text{O}$ ) and hydrogen ( $^2\text{H}/^1\text{H}$ ) isotope ratios have been studied as geolocational indicators of water sources (Ehleringer et al. 2008), and have proven useful in forensic case studies (Meier-Augenstein and Fraser 2008).

The aforementioned research suggests  $^{13}\text{C}$ ,  $^2\text{H}$ , and  $^{18}\text{O}$  isotope analysis is a suitable method for the differential identification of multi-regional variation in dietary patterns and water sources, and is subsequently appropriate for establishing geographic profiles. Despite the growing number of isotopic studies, as of this writing no studies have systematically compared the above isotopic compositions measured in human third molar tooth enamel from living individuals to assess their collective efficacy in linking the demographic data of known individuals with geographic origins.

Sampling from living volunteers is advantageous as isotopic data from biological tissues can be compared with self-documented residential and dietary records. If two or more third molars can be simultaneously obtained from the same individual, as might be the case in a forensic context if these biological materials are present, isotopic values representing the respective residence locations can be compared for the same individual (i.e. the intraindividual variability of stable isotope signatures can be assessed).

Stable isotope ratios can be measured in a variety of biological materials; bone, teeth, hair, and fingernails have been the most utilized due to their temporal significance. Since bone remodels approximately every 5 to 30 years depending on its nature and function (Ambrose 1993), its isotopic signature reflects the isotopic composition of food and water consumed within the corresponding time frame. Tooth

enamel is highly mineralized and formed during infancy or childhood (Hillson 1996), thus providing a relatively permanent  $^{13}\text{C}$  isotopic record of an individual's diet up to the point of tooth eruption. However, physiological processes such as pregnancy, breastfeeding, and weaning (e.g., Fogel et al. 1989, 1997; Herring et al. 1998) can alter the isotopic signal of tissues formed at or around birth and must be taken into consideration when evaluating corresponding isotopic delta values of teeth such as the incisors, premolars, and first molars (Bryant et al. 1996a). Conversely, hair and fingernails remodel much more rapidly, and thus are representative of fairly recent time periods. Generally, isotopic data obtained from an entire fingernail represents a period of approximately 6 months prior to the sampling date (Runne and Orfanos 1981). A sample of hair 1 cm in length will provide isotopic data consistent with the diet and location an individual has consumed and occupied, respectively, within the last month (Yoshinaga et al. 1996; Bol et al. 2007). By analyzing these different tissues, isotopic values representing an individual's life history can be obtained and used to create an isobiological profile.

While stable isotope analysis has become an increasingly common investigational implement in globally diversified studies of human origins and movement, little work has been conducted in determining the efficacy of a suite of stable isotopes in determinations of provenance. At the time of this project's inception, no systematic studies examining stable hydrogen isotopes in tooth enamel were available in the literature; a systematic development of the appropriate sampling and analytical protocol for  $^2\text{H}$  isotopic analysis has the potential for providing new methodology. Further, the systematic comparison of the same three isotopic ratios from human teeth with the

theoretical isotope ratios determined from existing life history data), and the evaluation of individual metabolic rates on isotopic variability within and between teeth that develop during similar temporal periods, may provide the necessary precision for further refinement of existing techniques currently applied in contemporary cases of forensic significance.

This study analyzed third molar teeth from living volunteers in conjunction with participating dentists. The project was designed to address the following objectives and test the corresponding hypotheses:

Objective 1: Develop an innovative sampling and analytical protocol for the  $^2\text{H}$  isotopic analysis of tooth enamel by:

- a) Determining the appropriate tooth enamel sample amounts necessary to generate a viable  $^2\text{H}$  signal on mass spectrometry analytical instrumentation.

H1: It is expected that while bioapatite hydrogen may be present in minute quantities, the abundance of  $^2\text{H}$  stable isotopes in tooth enamel will be sufficient for isotopic ratio analysis.

H2: Further, it is hypothesized that the amount of powdered tooth enamel required for  $^2\text{H}/^1\text{H}$  ratio analysis will be equal to that required for  $^{18}\text{O}/^{16}\text{O}$  ratio analysis.

- b) Determining the proper environmental conditions in which to prep the enamel samples for  $^2\text{H}/^1\text{H}$  ratio analysis.

H3: Since both hair and enamel powder are extremely hygroscopic materials, it is hypothesized that the protocol for preparing tooth enamel closely

emulates that of hair (i.e., equal care must be taken to maintain the powdered enamel samples under conditions of extreme desiccation).

- c) Straw-polling 25% of all enamel samples to validate preparatory methodology, analysis, and results.

Objective 2: Determine if the stable isotopic composition of human tooth enamel provides similar data related to geographic provenance and dietary intake as that obtained from human crown dentin by measuring the isotopic abundance of  $^2\text{H}$ , and  $^{13}\text{C}$  in human dentin collagen obtained from archaeological and modern teeth using continuous-flow isotope ratio mass spectrometry (IRMS) and comparing these findings with the  $^2\text{H}$ ,  $^{18}\text{O}$  and  $^{13}\text{C}$  isotopic composition of the corresponding tooth enamel.

H4: Significant correlations will be evident between the  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  values within tooth dentin. Similarly,  $\delta^{18}\text{O}_{\text{dentin}}$  and  $\delta^2\text{H}_{\text{dentin}}$  values will be correlated with  $\delta^{18}\text{O}$  values in enamel.

H5: A correlation will be evident between the  $\delta^{13}\text{C}$  values measured in tooth enamel and dentin.

Objective 3: Determine if the isotopic ratios of hydrogen and oxygen are consistent with an individual's reported residence by:

- a) Systematically comparing  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  values within the enamel of the third molar of living individuals with self-reported geolocal and dietary data. As third molar crown formation is initiated during adolescence (~7-12 years), and is completed between ~10-18 years, this tooth is suitable for use in the determination of residential patterns during the aforementioned temporal periods (Smith 1991).



H6: Significant correlations will be evident between the  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  values within the third molar tooth enamel of the study population.

- b) Determining the isotopic ratios of carbon in enamel carbonate to see if variability in diet can be detected in an individual's life history. For example, differences in  $\delta^{13}\text{C}$  values can distinguish Europeans from North Americans, which may be potentially useful in the forensic provenancing of unidentified individuals (Meier-Augenstein and Fraser 2008).

H7: Variation in  $\delta^{13}\text{C}$  values is indicative of the dietary variability represented in the study sample.

Objective 4: Determine if one or all of the isotopes are consistent with an individual's self-reported residential data by:

- a) Determining variation within teeth in the relative abundance of the three isotopes.

H8: The isotopic ratios of  $^{18}\text{O}/^{16}\text{O}$  and  $^2\text{H}/^1\text{H}$  within the tooth enamel of a third molar are expected to be consistent with the isotopic ratios corresponding to self-reported place of residence during adolescence.

- b) Systematically comparing the  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values within human teeth with published, mapped theoretical  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values.

H9: The stable isotopic composition of  $^2\text{H}$  and  $^{18}\text{O}$  in third molar enamel is consistent with an individual's reported residence during adolescence.

H10: The enamel carbonate  $\delta^{18}\text{O}$  values will be as precise an isotopic indicator of provenance as  $\delta^{18}\text{O}$  values obtained from the phosphate component of tooth enamel.

- c) Determining the isotopic ratio of  $^{13}\text{C}/^{12}\text{C}$  in tooth enamel carbonate to see if the dietary preferences of an individual can be detected isotopically. This information may be potentially useful in creating a forensic isotopic profile (e.g., if a person was a vegetarian or consumed meat).

H11: The isotopic ratio of  $^{13}\text{C}/^{12}\text{C}$  in tooth enamel carbonate is consistent with an individual's self-reported dietary preferences.

Objective 5: Determine intra-individual isotopic variability by systematically comparing  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  values in a third molar with that of another third molar from the same individual. Any existing variability in isotopic ratios will be a result of an individual's unique physiological processes (e.g., metabolic rate). The significance of determining intra-individual variability lies in the degree of certainty with which an isotopic proxy extracted from a tooth confirms the geographic history of a person. In other words, does the isotopic data from two third molars from the same individual give us the same answer (e.g., specific location or within a 100 mile radius), and thus facilitate the development of a unique isobiological profile?

H12: While it is expected that the delta values in each of two third molars will be similar due to a shared developmental time frame, it is hypothesized that the  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  values in each third molar from the same individual will not be identical.

#### Current Research Expectations: A Systematic Approach to Resolving Problems of Provenance

While the field of stable isotope forensics may be most known for its contributions to

the analysis of materials such as explosives (e.g., Benson et al. 2009), drugs (e.g., Ehleringer et al. 1999, 2000; Meier-Augenstein 2010), synthetic materials (e.g., Carter et al. 2005), and food (e.g., Rossmann 2001; Sieper et al., 2006), recent research has focused on isotopic analyses of modern human tissues and issues of provenance. Most of the work generated in this area is derived from individual case studies (Rauch et al. 2007; Meier-Augenstein and Fraser 2008; Font et al. 2015). Methodological studies exhibiting significant sample sizes (Mützel (Rauch) et al. 2009, n=111; Thompson et al. 2010, n= 46; Ehleringer et al. 2008, n=65) have been characterized by sampling, experimental, and statistical inconsistencies (e.g., bulk hair clippings unaccompanied by donor information, potential confounds resulting from inadequate mitigation of exchangeable hydrogen and isotopic data that has not been scale-corrected). For example, in order for  $^2\text{H}/^1\text{H}$  ratio data to suggest a significant linkage between the sample and source water, hydrogen exchangeability must be addressed and scale correction methods must be applied to the data; these two experimental components have not been universally applied to published work on the stable isotope analysis of human tissues (Meier-Augenstein 2010; Meier-Augenstein et al. 2013).

Few studies have been conducted in which a particular panel of stable isotopes is systematically measured in the same type of tissue from each individual sampled. Studies that have utilized a particular panel include bioarchaeological investigations in which the composition of one or more stable isotopes is measured in biological material available from viable skeletal remains (Evans et al. 2006); rarely is ethnographic data available for corroboration. More current work includes the longitudinal study by Fraser et al (2006), in which modern hair and fingernail samples were solicited from volunteers

who provided personal diet and travel habit information. The authors noted significant differences in the isotopic ratio delta values of  $^{18}\text{O}/^{16}\text{O}$  and  $^{15}\text{N}/^{14}\text{N}$  between hair and fingernails from the same individual, thus leading them to infer the possibility of intra-individual isotopic variability caused by the disparate metabolic rates of different tissues as a likely influence on the overall isotopic composition of a particular human tissue. Ostensibly no studies to date have examined the stable isotope ratios of late erupting permanent molars from living individuals with known life histories. While absolute comparisons between demographic data pertinent to this research (e.g. birth place, most recent residence/place of death) and  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  values of the water and food consumed by a particular individual are not possible, the predicted isotope delta values generated from models developed by Bowen et al. (2007) allow the comparison of measured isotope ratios with theoretical compositions. After an exhaustive literature survey, no systematic studies of  $\delta^2\text{H}$ ,  $\delta^{18}\text{O}$ , and  $\delta^{13}\text{C}$  values measured in the third molars of living individuals with self-reported residential data have been compared with the theoretical data on isotopic distribution maps.

This dissertation will review stable isotope methodology and its applications in the identification and understanding of human migration patterns, past and present. Since this research analyzes samples from living individuals of known geographic origins, the medico-legal value of such data within the broader context of forensic human identification techniques and their judicial implications will also be examined before presenting and evaluating the results from two pilot studies and the main dissertation research.

## CHAPTER 2

### STABLE ISOTOPE ANALYSIS: A REVIEW OF THE METHODOLOGY AND ITS APPLICATION IN STUDIES OF HUMAN MIGRATION AND GEOGRAPHIC ORIGINS

#### Introduction

Studies of human migration and geographic origins are important to anthropologists representing all four sub-disciplines. For example, linguistic anthropologists rely on geographic origin and migration data in their evaluation of the uniqueness or hybridization of a language. Similarly, archaeologists want to know if cultural artifacts can be linked with their representative historic or prehistoric population. Stable isotope analysis is a well-recognized method utilized in such research endeavors. However, stable isotope analysis perhaps has been of the most use to physical anthropologists interested in bridging the gaps in the human biogeographical story.

Stable isotope analysis of human biological material has become a fairly common tool in bioarchaeological research efforts and to some extent in forensic investigations. Although stable isotopes were first discovered in 1913, Urey's 1946 European lecture series marked the introduction of light stable isotopes to the geochemistry community (Sharp 2007). Prior to the emergence of stable isotope analysis as a valuable analytical tool in the archaeological dietary reconstruction studies of the 1970s (DeNiro and

Epstein 1978a, 1978b; van der Merwe and Vogel 1978), stable isotope analysis focused primarily on the determination of the elemental abundance of various stable isotopes and understanding the variation therein (Katzenberg 2000:306). The method subsequently became a seminal analytical technique used in paleoclimate reconstructions, the provenancing of ancient mineral artifacts, botanical studies, and wildlife migration. This chapter will review stable isotope methodology and evaluate its utility in studies of human migration and geographic origins with particular emphasis on bioarchaeological and forensic applications.

### Stable Isotope Analysis

Hydrogen, oxygen, carbon, nitrogen, and sulfur are biologically important elements that vary in their isotopic forms (Katzenberg and Krouse 1989). While isotopes behave similarly in chemical reactions, reaction rates of chemical bonds differ according to the respective atomic weights of the individual isotopes engaged in a chemical bond. These differences explain the varying abundance of naturally occurring isotopes in a given substrate, and are the basis for stable isotope analysis. Primary development of stable isotope work occurred in the geochemical community (Fogel et al. 1997); anthropological application of these techniques began with archaeological dietary reconstruction studies in the late 1970s (DeNiro and Epstein 1978a, 1978b; van der Merwe and Vogel 1978).

Stable isotope levels collected from various human tissues, including bone, teeth, hair, and finger nails, indicate that individuals possess isotopic “signatures”; variation in

stable isotope composition occurs with comparative predictability such that inferences regarding diet reconstruction (carbon  $\delta^{13}\text{C}$  and nitrogen  $\delta^{15}\text{N}$ , van der Merwe 1982; Klepinger 1984; DeNiro 1986) and the natal origin of unidentified human remains may be formulated (Katzenberg and Krouse 1989). Hydrogen ( $\delta^2\text{H}$ ) and oxygen ( $\delta^{18}\text{O}$ ) isotope values are indicators of climatic change (Dansgaard 1964) and food and water intake (Estep and Dabrowski 1980; Estep and Hoering 1980; Schimmelmann et al. 1993; Sharp et al. 2003). More recently, oxygen ( $^{18}\text{O}$ ) and hydrogen ( $^2\text{H}$ ) stable isotopes have also been studied as geolocational indicators of water sources (Ehleringer et al. 2008). Strontium isotope ratios have been studied with respect to geochemical applications (bedrock, sediment, and soil composition) (Pye 2004), delineating the origins of migratory wildlife (Chamberlain et al. 1997), distinguishing terrestrial versus marine food webs (Chamberlain et al. 1997; Kennedy et al. 1997; Harrington et al. 1998), and more recently as a geolocational indicator of the natal origins and migration patterns associated with human skeletal remains (Beard and Johnson 2000). The above investigations comprise a fraction of the research illustrating the effectiveness of stable isotope analysis as a method for differential identification of multi-regional water sources and geological substrates and the subsequent determination of human migration patterns and geographic origins in a bioarchaeological and forensic context.

### Geolocational Isotopic Indicators

Although structurally similar, the variability in mass in isotopes of the same element – and the corresponding difference in thermodynamic properties – produces a

differential in their respective reaction rates (Urey 1947). For example, the mass differential exhibited by the hydrogen isotopes results in differential bond strength when these isotopes bond to other elements. These differences in bond strength are responsible for fractionation, a process in which isotopes are partitioned unequally between two substances or tissues (Hoefs 2004). Fractionation is an important facilitator of heterogeneity in the biological processes of living organisms (Hoefs 2004). In the absence of fractionation many important inferences based on isotopic analyses could not be made.

The abundance of oxygen and the geographic variability within its isotopic ratios is a useful indicator of points of origin. While  $^{16}\text{O}$  is by far the most abundant (99.63%) of its three isotopes (Sharp 2007), it is commonly depleted in comparison to  $^{18}\text{O}$  (0.1995%). Subsequently, oxygen stable isotope values are typically reported as a ratio of  $^{18}\text{O}/^{16}\text{O}$  and represented by  $\delta^{18}\text{O}$  notation. Similarly, hydrogen has two stable isotopes, protium ( $^1\text{H}$ ) and deuterium ( $^2\text{H}$ ) (Sharp 2007). Hydrogen stable isotope values are reported as a ratio of  $^2\text{H}/^1\text{H}$  and expressed as  $\delta^2\text{H}$ . The  $\delta^2\text{H}$  notation will be observed from this point onward.

According to the latest International Union of Pure and Applied Chemistry (IUPAC) guidelines and recommended terms of stable isotope ratio measurements and reporting results thereof (Coplen 2011), the delta ( $\delta$ ) notation as a value representing stable isotope abundance of the heavier isotope (h) of a given element (E) in a given sample (S) relative to a specified international reference material (Ref) is defined as:

$$\delta^h\text{E}_{\text{Ref}} = (\text{R}_S - \text{R}_{\text{Ref}})/\text{R}_{\text{Ref}} = \text{R}_S/\text{R}_{\text{Ref}} - 1$$



where "R" denotes the abundance ratio ( $^h\text{E}$ )/( $^l\text{E}$ ) of the heavier isotope of element E over the lighter isotope of element E.

Since  $\delta$ -values thus calculated are numerically less than zero with significant numbers in the second or third decimal, they may be stated as per mil (‰) values as a representation of the scientific notation of presenting such numbers as multiples of  $10^{-3}$ . For example, with  $R_S = 0.01101296$  and  $R_{\text{Ref}} = 0.0112372$

$$\delta^h\text{E}_{\text{Ref}} = (0.980045 - 1) = -0.01995$$

$$\delta^h\text{E}_{\text{Ref}} = -0.01995 = -19.95 \times 10^{-3} = -19.95 \text{ ‰}$$

In the above example the minus sign signifies the isotopic abundance of  $^h\text{E}$  in the sample S is less than its isotopic abundance in the reference material Ref. Conversely,  $\delta^h\text{E}_{\text{Ref}}$  values with a positive sign signify an isotopic abundance of  $^h\text{E}$  in the sample S higher than that in the reference material Ref.

While imbibed water is considered the most important source of oxygen input (Longinelli and Peretti Paladino 1980; Longinelli 1984), other biologically available forms of oxygen include food (water and organic matter, and derived metabolic water) and atmospheric oxygen (Luz et al. 1984; Luz and Kolodny 1985). Consumption of drinking water and the water component in food become the primary contributors of oxygen isotopes to body water in mammals (Longinelli and Peretti Paladino 1980). Historically, drinking water has been obtained from surface waters, near-surface ground-waters, and collected from precipitation. The isotopic values of precipitation vary

systematically with geographic location (Budd et al. 2004), and affected by environmental variables such as temperature, distance from the ocean, latitude, elevation, and humidity (Yurtsever and Gat 1981; Ayliffe and Chivas 1990). The characteristic fractionation exhibited by oxygen and hydrogen in these kinetic processes requires comparison with a standard when analyzed. The  $\delta^{18}\text{O}$  values of carbonate require calibration against a calcite standard, VPDB (Vienna Pee Dee Belemnite) (Meier-Augenstein 2010). Both  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  values in all other materials are measured according to the VSMOW standard (Vienna Standard Mean Ocean Water). Because the  $\delta^{18}\text{O}$  value on the PDB scale is 30.91‰ higher than that on VSMOW, conversion equations are often employed when simultaneously reporting carbonate and water oxygen isotope data (Hoefs 2004; Sharp 2007).

The replacement of normal hydrogen ( $^1\text{H}$ ) with  $^2\text{H}$  results in an enriched ("heavy") water molecule which is ~9.05% more dense than normal water (Sharp 2007:6). The mass differential between normal water and heavy water produces variation in vapor pressures (Faure and Mensing 2005). Since "light" water molecules ( $^1\text{H}_2^{16}\text{O}$ ) will evaporate faster than heavier water molecules ( $^2\text{H}_2^{18}\text{O}$ ) (Gat 1984), water vapor subsequently is depleted in  $^2\text{H}$  and  $^{18}\text{O}$  relative to the body of liquid water left behind such as the surface layer of seawater. Condensation (e.g., in the form of vapor clouds) contains higher levels of  $^1\text{H}$  and  $^{16}\text{O}$  in comparison to the originating pool of water. As observed by Craig (1961), the strong association between the isotopic values of  $^{18}\text{O}$  and  $^2\text{H}$  in precipitation, expressed by the equation  $\delta\text{D} = 8\delta^{18}\text{O} + 10$ , forms the Global Meteoric Water Line (GMWL) and is based on global precipitation data. The Local Meteoric Water Line (LMWL), i.e., the linear values derived from precipitation samples

reflecting a single location or a set of multiple sites, can be significantly different from the GMWL (Craig 1961). While hydrological processes can cause water samples to plot off of the GMWL, generally the charting of precipitation samples allows them to be sourced and climatically characterized.

The isotopic composition of meteoric water is largely determined by geography and climate;  $\delta^{18}\text{O}$  values of environmental water directly reflect the corresponding area's climatological attributes (Dansgaard 1964). Generally,  $\delta^{18}\text{O}$  values decrease in the presence of falling temperatures, increasing elevation and latitude, and locations that are progressively farther from the sea (Yurtsever and Gat 1981). Relative humidity also has a marked influence on  $^{18}\text{O}/^{16}\text{O}$  isotopic ratios. Due to its lower atomic mass,  $^{16}\text{O}$  is preferentially lost during evaporation; subsequently the relative amount of  $^{18}\text{O}$  in surface water of rivers and lakes is enriched in more arid climates.

Plants have only one hydrogen precursor pool available for the biochemical production of macronutrients: water (Meier-Augenstein 2010). While fractionation affects  $\delta^2\text{H}$  values generated during lipid and terpenoid biosynthesis,  $\delta^2\text{H}$  values of carbohydrates remain relatively static, and thus are strongly correlated with  $\delta^2\text{H}$  values of source water (Fry 2006). The  $\delta^2\text{H}$  values of plant tissue reflect those of groundwater and precipitation, and are depleted in  $^2\text{H}$  relative to water source (Estep and Hoering 1980). As the measured  $\delta^2\text{H}$  in environmental water can fluctuate considerably relative to factors also affecting  $\delta^{18}\text{O}$  values,  $\delta^2\text{H}$  values found in plant tissue are functional indicators of regional variation in hydrogen isotopic ratios (Epstein et al. 1976). In contrast, animals rely on both food and water for their organically bonded hydrogen isotopic tissue content (Estep and Dabrowski 1980). Thus, a significant percentage of

water in the human body composition is derived directly from the ingestion of water or the indirect consumption of atmospheric moisture, plants, and other animals.

Oxygen and hydrogen isotope levels of food and water are incorporated in human bone, teeth, hair and other tissues over time, and thus can be associated with an individual's place of residence when the particular tissue was formed. The  $^{18}\text{O}$  and  $^2\text{H}$  isotopic composition of water is incorporated into bone and teeth during the mineralization process (White et al. 2004b). The  $^{18}\text{O}$  isotope composition of the mineral component of these two biological materials is regulated by body water as the biogenic phosphate and  $\delta^{18}\text{O}_{\text{bw}}$  equilibrate within the body's temperature-controlled physiological environment (White et al. 2002). Individual physiological mechanisms (e.g., body size, metabolic rate, and degree of water dependency/conservation ability) determine input/output levels of oxygen usage as well as isotopic value retention. For example, high metabolic rates, whether due to increased activity levels or particular illness, may affect  $\delta^{18}\text{O}$  values for body water and thus phosphate (White et al. 2004a). The established relationship between the isotopic content of these biological materials and the ingested food and water sources therefore can be expected to reflect local climatic variables (Luz et al. 1990, deer; Fricke et al. 1998). Despite the internal and external environmental influences, intrapopulational variation in  $\delta^{18}\text{O}$  values is generally low in humans (1 ‰) (Longinelli 1984), although more variation is expected in groups exhibiting geographic mobility. The above observations, based on Longinelli's correlation linking  $\delta^{18}\text{O}$  values of human bone phosphate and source water, have been confirmed and refined by Daux et al. (2008). While work conducted by Luz et al. (1984) suggested similar parallels between  $\delta^{18}\text{O}$  values of bio-apatite and source water,

sampling differences between the two earlier studies have raised questions of reliability (Meier-Augenstein 2010). Longinelli's bone samples were obtained from people who had resided in Central Europe (e.g., temperate climate), while the samples analyzed by Luz et al. (1984) came from individuals who had lived in the East and Northeast Africa (e.g., more arid climates).

The incorporation of  $^2\text{H}$  and  $^{18}\text{O}$  into biological material that experiences continual remodeling (e.g., bone, hair, dentin) or that which has become biochemically inert (e.g., the  $\delta^{18}\text{O}$  values in enamel from a fully erupted first molar) has facilitated the tracking of seasonal changes in temperature and humidity as well as paleoclimatic reconstructions (Fricke et al. 1998; Kirsanow et al. 2008). Additionally, the  $^2\text{H}/^1\text{H}$  ratios of avian feathers have been used successfully in the identification and tracking of migratory routes (Hobson and Wassenaar 1996).

Variation in the abundance of strontium isotopes is particularly significant ( $^{87}\text{Sr} = 7.0\%$ ;  $^{84}\text{Sr} = 0.56\%$ ;  $^{86}\text{Sr} = 9.86\%$ ;  $^{88}\text{Sr} = 82.58\%$ ) (Faure and Mensing 2005). The abundance of  $^{87}\text{Sr}$  is a product of the initial distribution of  $^{87}\text{Rb}$  (Rubidium) and the rate of its decay to  $^{87}\text{Sr}$ . Current abundances of  $^{87}\text{Sr}$  vary greatly, and are normally expressed as a ratio of  $^{87}\text{Sr}$  to  $^{86}\text{Sr}$  ( $^{87}\text{Sr}/^{86}\text{Sr}$ ). The long half-life of radiogenic  $^{87}\text{Sr}$  has resulted in minute differences in  $^{87}\text{Sr}/^{86}\text{Sr}$  over geological time periods, and can be measured precisely, with negligible error (Pye 2004). Significant variation is observed in global  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios; this variation is due to differences in both the age of the earth's outer layer and its composition for any particular location (Beard and Johnson 2000). These differences produce isotopic signatures unique to the geographic area in which the strontium sources are located.

Strontium, an earth metal, is found in rock formation, soil, sediment, food, and drinking water (Pye 2004; Hedges et al. 2005; Price et al. 1994b; Beard and Johnson 2000; Katzenberg 2000). Differential environmental effects (e.g., mineralization, leaching, and surface precipitation) can significantly affect primary and secondary  $^{87}\text{Sr}$  isotope compositions of soil, sediment, and subsequently food and drinking water (Pye 2004). For example, the  $^{87}\text{Sr}$  isotope composition in soil and stream water from the same region can differ significantly.

Strontium isotope composition of skeletal tissue is directly linked to the amount of  $^{87}\text{Sr}$  consumed in the form of dietary sources. Traditionally,  $^{87}\text{Sr}$  isotope studies of human skeletal tissues have focused on skeletal elements such as bone and teeth, both of which are “calcium-bearing phases” (Beard and Johnson 2000:1051). The structural similarities of calcium (Ca) and strontium facilitate the ability of strontium to partially replace calcium in the metabolic processes of plants and animals. Subsequently, strontium can substitute for calcium in the hydroxyapatite crystals of bone mineral, and is incorporated into human tissue via the calcium pathway (Åberg et al. 1998). The  $^{87}\text{Sr}$  isotope composition of an individual’s bones or teeth consequently reflects the dietary  $^{87}\text{Sr}$  isotope compositions during the period of time in which these elements were forming or experiencing exchange-facilitated transition (Beard and Johnson 2000), and thus can be associated with an individual’s place of residence when the particular tissue was formed.

The  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of soil and water are the same  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios observed in plants, and so forth up the food chain. While most stable isotopes are subject to fractionation,  $^{87}\text{Sr}/^{86}\text{Sr}$  isotope ratios do not fractionate. The progress of  $^{87}\text{Sr}/^{86}\text{Sr}$

isotopes through a food chain will remain constant at each point along the composition chain, although abundance at each point varies (Herz and Garrison 1998). The static nature of  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios thus makes them good evidence of biological and environmental variables. The utility of  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio analysis in discriminating between the meat, vegetable, and marine and freshwater components of an individual's diet (Toots and Voorhies 1965 meat and vegetables; Koch et al. 1992 and Åberg 1995 marine and freshwater) can thus indicate coastal/inland dietary patterns. However, using Sr/Ca ratios to infer diet requires consideration of variations in Sr/Ca ratios of bedrock or soil sources and the existing intravariation observed among various skeletal elements; human tooth enamel typically contains less strontium than bone (Price et al. 1994a; Ezzo et al. 1997; Grupe et al. 1997), although ratios between both elements remain the same (Herz and Garrison 1998). As Sr/Ca ratios may change over the lifetime of an individual (a possible indication of relocation to a geographic area with a different dietary isotope composition), it is also important to sample identical skeletal elements when making comparisons between individuals or populations (Beard and Johnson 2000).

The abundance of  $^{87}\text{Sr}$  in a sample is reported as a ratio ( $^{87}\text{Sr}/^{86}\text{Sr}$ ) instead of as a delta value. NBS 987 is the recognized lab standard with certified values for three absolute abundance ratios of strontium (Faure 2001).

### Isotopic Diet Markers

Diet markers such as  $^{13}\text{C}$  and  $^{15}\text{N}$  can offer qualifying information when geolocational data obtained from  $^2\text{H}$  and  $^{18}\text{O}$  are consistent with more than one location. As described

elsewhere (Bender 1968), plant species can be classified according to their respective environmentally specific photosynthetic pathways. Each of the three types of pathways is characterized by the initial product each generates during the process of converting carbon dioxide into organic compounds (i.e., carbon fixation). Two of the three types of photosynthetic pathways exert important influences on the  $^{13}\text{C}$  isotopic compositions of plants (Bender 1968). Each pathway demonstrates unique metabolic processes associated with different rates of isotopic fractionation, which are then reflected in the  $^{13}\text{C}$  composition of plant tissues. During the process of photosynthesis, environmental carbon dioxide ( $\text{CO}_2$ ) and water are converted into glucose by the plant. Following this conversion the relative amounts of  $^{12}\text{C}$  and  $^{13}\text{C}$  incorporated into the plant tissue will differ from the original  $^{13}\text{C}$  composition of the  $\text{CO}_2$ . The alteration of isotopic ratios during the photosynthetic metabolization of  $\text{CO}_2$  is an example of mass discrimination resulting in isotopic fractionation; plants exhibit preferential uptake of  $^{12}\text{C}$  to  $^{13}\text{C}$ , thus they are enriched in  $^{12}\text{C}$  and depleted in  $^{13}\text{C}$  compared to the  $^{13}\text{C}$  scale reference point VPDB (Bender 1968).

Most plants that habitually grow in temperate climate zones (e.g., wheat, rice, and barley) follow the Calvin-Benson pathway of photosynthesis or pathway of  $\text{C}_3$  carbon fixation (Schwarcz and Schoeninger 1991). The slow rate of  $\text{CO}_2$  uptake in  $\text{C}_3$  plants leads to  $\delta^{13}\text{C}$  values ranging from -20 ‰ to -35 ‰ (van der Merwe 1982); most  $\text{C}_3$  plants have  $\delta^{13}\text{C}$  values on the order of -26 ‰. More drought-resistant plants such as sugar cane, maize, and millet follow the Hatch-Slack pathway of photosynthesis or pathway of  $\text{C}_4$  carbon fixation (Schwarcz and Schoeninger 1991); the more rapid carbon dioxide uptake demonstrated by  $\text{C}_4$  plants leads to  $\delta^{13}\text{C}$  values ranging between



-9 ‰ and -16 ‰. Intermediate offsets of approximately -16 ‰ are useful in distinguishing between C<sub>3</sub> and C<sub>4</sub> plants in dietary reconstruction studies (van der Merwe 1982; MacFadden et al. 1999). Since the differential <sup>13</sup>C isotope uptake observed in plants also occurs during the digestive processes of biological organisms (Durrance 1986), it is possible to estimate proportions of C<sub>3</sub> and C<sub>4</sub> plants in the diet of an individual by examining the associated  $\delta^{13}\text{C}$  value (Schwarcz and Schoeninger 1991). The use of <sup>13</sup>C isotope analysis has been successful in detecting the introduction of maize, a C<sub>4</sub> plant, in archaeological populations (e.g., Schwarcz et al. 1985; Katzenberg et al. 1985; Finucane et al. 2006; Gil et al. 2009). Forensic applications of <sup>13</sup>C isotope analysis are more recent. Meier-Augenstein and Fraser (2008) were able to determine that an unidentified murder victim did not recently come from North America or Africa due to an absence of C<sub>4</sub> plant-derived <sup>13</sup>C/<sup>12</sup>C ratios in sampled hair and nails.

Carbon isotopes are also useful in gauging the amount of marine foods an individual organism consumes (Schoeninger and DeNiro 1984; Larsen et al. 1992). Marine mammals and fish exhibit  $\delta^{13}\text{C}$  values that are enriched by approximately 6 ‰ over terrestrial animals that consume C<sub>3</sub>-plant based foods, and depleted by roughly 7 ‰ when contrasted with terrestrial animals feeding on C<sub>4</sub>-plant based resources (Schoeninger and DeNiro 1984).

Joint  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  analyses provide the most accurate indication of a dietary reliance of human populations on marine food sources (Schoeninger and DeNiro 1984); the  $\delta^{15}\text{N}$  values of coastal populations are enriched over those of inland populations (Schoeninger et al. 1983). An offset of ~10 ‰ between the approximated  $\delta^{15}\text{N}$  value of atmospheric nitrogen (0 ‰) and that of soil nitrogen (10 ‰) (Mariotti 1983, atmosphere;

Shearer and Kohl 1989, soil) facilitates the subsequent distinction between nitrogen- and non-nitrogen fixing plants (e.g., those that incorporate atmospheric nitrogen into their tissues and those who rely on the uptake of soil nitrogen, respectively). In turn, the disparity in  $\delta^{15}\text{N}$  values also allows a quantifiable differentiation within and between feeding strategies. For example, herbivores generally exhibit a trophic level shift of approximately +3 to +3.4 ‰ from the plants they consume, and herbivores relying on nitrogen-fixing plants (e.g., legumes) as their primary food source can be distinguished from those who do not. Similarly, carnivores exhibit an additional trophic level increase of approximately +3 to +3.4 ‰ (Schoeninger and DeNiro 1984). Generally, a progressive increase of approximately +3.4 ‰ in  $\delta^{15}\text{N}$  values is noted between each successively higher trophic level (DeNiro and Epstein 1981; Fry 2006). These increases occur in both terrestrial and marine food webs, with ocean systems exhibiting up to 5 to 6 trophic levels.

Nitrogen isotope ratios have also been used to determine age at weaning (Fogel et al. 1989; Richards et al. 2002; Fuller et al. 2006), and have been studied extensively in archaeological populations to examine cultural and temporal patterns of breastfeeding and weaning (Katzenberg et al. 1993; Wright and Schwarcz 1999; Dupras and Tocheri 2007; Prowse et al. 2008).

Atmospheric nitrogen (Air) is the scale reference for nitrogen isotopic analysis. While the  $\delta^{15}\text{N}$  value of Air is constant at 0 ‰ almost everywhere on earth, laboratory reference materials required for scale normalization of measured  $\delta^{15}\text{N}$  values are distributed by the International Atomic Energy Agency (IAEA) and National Institute Standards and Technology (NIST) (Sharp 2007). Working standards to supplement

international reference materials may be prepared by each individual laboratory.

Sulfur stable isotopes are also of biological relevance to provenance studies.  $^{34}\text{S}$  and  $^{32}\text{S}$  are the two most abundant sulfur stable isotopes, and thus are the most commonly measured (Sharp 2007). The corresponding ratio,  $^{34}\text{S}/^{32}\text{S}$ , is expressed as  $\delta^{34}\text{S}$  and reported against the international standard Vienna Cañon Diablo Troilite (VCDT) meteorite. Sulfur isotopic compositions of human tissue are a direct reflection of dietary  $\delta^{34}\text{S}$  values (Macko et al. 1999; Richards et al. 2003; Richards et al. 2001), which in turn are a reflection of environmental  $^{34}\text{S}$  abundance and influenced by numerous variables, including local underlying bedrock, atmospheric deposition, proximity to the sea, and soil microbial activity (Richards et al. 2003). Terrestrial environments exhibit the most variability in  $\delta^{34}\text{S}$  values (Sharp 2007), with vegetation  $\delta^{34}\text{S}$  values averaging approximately 2-6 ‰ (Meier-Augenstein 2010) within a range of -22 to +22 ‰ (Peterson and Fry 1987).

Collagenous and keratinous tissues are especially useful for  $^{34}\text{S}/^{32}\text{S}$  ratio analyses as these protein-rich compounds contain large amounts of sulfur relative to other types of biological materials (Meier-Augenstein 2010). Due to the potential for significant variation in  $\delta^{34}\text{S}$  values, it is possible to distinguish between food sources (Privat et al. 2007; Richards et al. 2003; Richards et al. 2001) as long as  $\delta^{34}\text{S}$ ,  $\delta^{13}\text{C}$ , and  $\delta^{15}\text{N}$  values are obtained from the same sample material and interpreted in relation to one another (Bol and Pflieger 2002; Bol et al. 2007; Meier-Augenstein 2010). Problems may arise when an individual's  $\delta^{34}\text{S}$  values indicate coastal origins when in fact they have only consumed food items grown or reared in a coastal environment. Similarly, anthropogenic sources of  $^{34}\text{S}$  (e.g., atmospheric emissions) may influence the  $\delta^{34}\text{S}$

values of tissue from modern humans (Meier-Augenstein 2010).

### Diagenesis

Select skeletal material is susceptible to post-mortem contamination or alteration, also known as diagenesis. Diagenetic interaction between the burial environment and biological material occurs in the form of leaching and contamination (Sandford 1992) and can be caused by a variety of intrinsic (e.g., bone density, microstructure, and size) and extrinsic (e.g., soil pH, temperature, microorganismal activity, precipitation) mechanisms.

While the impermeable nature and heavy mineralization of tooth enamel makes this biological material extremely resistant to diagenetic effects (Hillson 1996), bone is more susceptible to postmortem modification; its porous structure and highly variable composition permit significant environmental interaction (Sandford 1992). While bone appearance and integrity can be affected, sample preparation methods are designed to mitigate some of the diagenetic effects. Another technique for handling diagenetic effects is Fourier Transform Infrared Spectrometry (FTIR analysis) (Shemesh 1990). Vibrational “peaks” of bone carbonate and bone phosphate are measured, and a corresponding crystallinity index is generated. Higher than normal crystallinity indices (approximately 3 to 3.5) indicate significant postmortem alteration of the sample.

Hair is durable; the cuticle covering the hair shaft is considerably resistant to degradation (Lubec et al. 1987; Macko et al. 1999). The insolubility of hair to a variety of fluids contributes to its hardness, yet these characteristics do not make it immune to diagenetic effects.

## Important Biological Reservoirs

Bone and Teeth. On average, bone remodels approximately every ten to thirty years (Ambrose 1993) due to the fact that different skeletal components remodel at different rates (e.g., ribs remodel approximately every five years while load-bearing bones such as the femur turn over approximately every 20-25 years) (Frost 1990; Pearson and Lieberman 2004; Hedges et al. 2007; Meier-Augenstein and Fraser 2008). The isotopic composition of bone may change over an individual's lifetime as stable isotopes are constantly incorporated into continually remodeling bone tissue.

Bone is composed of water, an inorganic mineral component (hydroxyapatite), and an organic matrix (collagen) (Schwarcz and Schoeninger 1991). Analytically available stable isotopes  $^{18}\text{O}$ ,  $^{13}\text{C}$ , and  $^{87}\text{Sr}$  are present in bone mineral while  $^2\text{H}$ ,  $^{15}\text{N}$ ,  $^{18}\text{O}$  and  $^{13}\text{C}$  are accessible in collagen. Sample preparation methods for the isotopic analysis of bone carbonate are generally based on the technique developed by Lee-Thorp (1989, 1991) and modified by others (Garvie-Lok et al. 2004:764; Henton et al. 2010). Powdered bone samples are cleaned with low molarity sodium hypochlorite ( $\text{NaOCl}$ ) and acetic acid, rinsed multiple times, and dried over a powerful desiccant (e.g., phosphorus pentoxide) prior to analysis by acid digest. Archaeological samples may require extended immersion or further treatment to mitigate the effects of diagenetic contaminants before carbonate  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  values can be obtained. Strontium isotope analysis requires an acid-wash of the apatite to remove not only soluble carbonates but those contaminants most likely to be effected through postmortem

alteration (Ezzo 1991; Price et al. 1992). Vennemann et al. (2002) concluded that the preferred method for measuring the abundance of  $^{18}\text{O}$  in tooth enamel phosphate is isolating the phosphate for precipitation as silver phosphate ( $\text{Ag}_3\text{PO}_4$ ); the compound's low degree of hygroscopicity and resistance to environmental contamination makes it an ideal substance for  $^{18}\text{O}$  analysis. Contrary to the relatively short time period required for carbonate isolation, preparing silver phosphate from bio-apatite can take six days (Stephan (2000) with some modifications by Meier-Augenstein (2010)). The isotopic analysis of collagen, the organic component of bone, requires dissolution of bone mineral before the collagen can be extracted; this may be accomplished by subjecting the powdered sample to lengthy incubations in sodium hypochlorite and hydrochloric acid (Hedges et al. 2007) or repeated rinses in increasing molarities of hydrochloric acid if larger chunks of archaeological material are used (Longin 1971; Chisholm et al. 1982).

Although the bi-composite nature of tooth enamel differs from bone in its representative percentages (~ 96% inorganic and 4% organic), preparation and analysis of dental enamel samples follow the same general procedures used on bone. Teeth are useful for isotopic studies because of their lower rates of remodeling (i.e., dentin) and ability to survive conditions under which bone would normally degrade. While dentin and cement are highly organic in constituency (Hillson 1996) and consequently more susceptible to diagenesis, tooth enamel is non-cellular, heavily mineralized, and more resistant to degradation. Because enamel does not remodel and thus is not predisposed to high levels of mineral exchange with the environment, enamel samples represent small, closed systems (Price et al. 2002), and thus reflect

the entire diet of an individual (van der Merwe 1982; Harrison and Katzenberg 2003) as well as geographic movement. With the exception of nitrogen (abundant primarily in dentin collagen), isotopic signatures contained within tooth enamel subsequently represent the isotopic compositions of all dietary sources consumed by an individual through late adolescence, including those food sources received during tooth formation while *in utero* and during childhood and adolescence.

Crown development of the permanent first molar begins *in utero* (Hillson 1996). Enamel from this tooth thus is potentially suitable for determining place of birth provided the isotopic signal alteration caused by physiological processes such as pregnancy and breastfeeding are taken into consideration (Bryant et al. 1996a). Crown enamel from the permanent second and third molar forms during middle childhood and adolescence, respectively. Therefore, these two teeth are ideal for use in the determination of residential patterns during those temporal periods.

Hair and Nails. Hair is an extremely durable material, characterized by its resistance to chemical and microbial degenerative effects (Lubec et al. 1987; Macko et al. 1999) and ability to remain intact for thousands of years if environmental conditions are favorable (Bonnichsen et al. 2001). Since human hair grows approximately 1 cm per month (Yoshinaga et al. 1996), isotopic analysis of hair offers cumulative information regarding an individual's diet and geographic background that can be narrowed down to a particular point in time (White et al. 1999; Roy et al. 2005).

Hair keratin as a proxy for other human tissues in isotopic analyses should be employed cautiously due to its growth rate and highly variable metabolic turnover

(O'Connell and Hedges 1999). While most of the body's proteins remodel throughout an individual's lifetime, keratin does not undergo this reabsorption process. Unlike bone protein (collagen), which reflects nutrient intake over long periods of time (see Stenhouse and Baxter 1979), hair provides a snapshot of more recent dietary changes. Stable carbon and nitrogen isotope analyses of ancient human hair have supported the theory of hair as a seasonal indicator of dietary variation (White 1993; White and Schwarcz 1994). Most research involving strontium isotopic analysis of human hair appears to have focused on radioactive  $^{90}\text{Sr}$  as a measurement of radioisotope exposure and subsequent content within human tissue, although some studies have examined  $^{87}\text{Sr}/^{86}\text{Sr}$  in mammalian hair (human and animal) (White et al. 1999; Roy et al. 2005; Font et al. 2012; Vautour et al. 2015).

Similarly, fingernails can be analyzed for short-term variation in diet and current or recent geolocational data. Roughly 6 months of growth is reflected in an entire adult human nail (Fuller et al. 2006); recent geographic locations may subsequently be determined for a specific individual. Fingernails are especially useful in obtaining dietary information for modern infants, as nails exhibit metabolic stasis, resistance to degenerative forces, and relatively quick synthesis (Fuller et al. 2006). While the amino acid composition of nail keratin ( $\beta$ -sheet) differs from that of hair keratin ( $\alpha$ -helix), the two different proteins are biochemically similar in their structure (see Goldsmith 1991). Thus, isotopic ratio data obtained from fingernails is comparable to that measured in hair with few differences (O'Connell et al. 2001).

Sample preparation for hair has to follow a strict protocol designed to mitigate the effects of hydrogen exchange. Fingernail samples follow similar procedure with little



variation. Generally, hair samples are cut into very small pieces (i.e. homogenized), cleaned, dried down in a drying oven for 12 hours at 40°C, and subsequently stored in a desiccator for 3 days (Landwehr et al. 2011). Following this period matching sets of hair subsamples are equilibrated, in separate sealed containers, with a water sample of a particular known isotopic composition and a second water sample of a particular but different known isotopic composition, respectively, for 4 days. Both sets of subsamples are dried down under vacuum for 7 more days until introduction to the analytical instrumentation.

### Analytical Instrumentation

Stable isotope ratio measurements are performed by an isotope ratio mass spectrometer (IRMS) comprised of five basic components: an inlet system, ion source, a flight tube, an ion collector assembly, and an electronic recording system (Hoefs 2004; Sharp 2007). Before the isotopic abundance of light stable isotopes (e.g.,  $^2\text{H}$ ,  $^{13}\text{C}$ ,  $^{18}\text{O}$ ) in a given compound or material can be measured, the sample must be converted to a gas isotopically representative of the sample material (gas source). Generally, the instrument operates by ionizing the gas sample, accelerating it over a potential, and then separating the resulting stream of ions based on their mass to charge ratio. For the resulting isotope ratios to be meaningful they must be compared with the isotope ratio of a reference material. Thus, the compositions of two gases, generated from the sample and a corresponding reference, are measured relative to each other. Heavy stable isotopes such as strontium are analyzed as aqueous solutions or as “gas” through laser ablation, and require measurement against NIST specified standards that

are generally used for thermal ionization mass spectrometry (TIMS) or for inductively coupled plasma mass spectrometry (ICP-MS) calibration adjustments (Herz and Garrison 1998).

### Bioarchaeological Applications

Ericson (1985) was the first to illustrate the potential utility of strontium isotopes in studies investigating residence patterns by examining strontium isotope ratios in human tooth enamel and bone. While his biogeochemical model concentrated on tracing childhood and marital residence patterns, his results can also be effectively applied to determining natal origins, provided, as the model stipulates, the appropriate biological tissue is sampled and that significant isotopic variation between the underlying bedrock of different areas exists.

Stable isotope studies investigating human origins began to proliferate in the early 1990's. Sealy et al. (1991) confirmed the link between geological strontium and dietary strontium when they demonstrated the isotopic ratio differences between marine and terrestrial food sources. A later study by Sealy et al. (1995) compared bone, enamel, and dentin  $^{87}\text{Sr}/^{86}\text{Sr}$  values from five unknown individuals whose pre- and historic skeletal remains were recovered from a South African site. Significant variation in  $^{87}\text{Sr}$  isotope composition between tissues was noted in two of the historic individuals, implying that their residence history included some degree of relocation.

Much work investigating the effectiveness of strontium isotopes in determining geographical origins and migration patterns has been conducted on European

prehistory and pre-Columbian samples (Price et al. 1994a; Evans et al. 2006; Slovak et al. 2009, Peru). Price et al. (1994a) analyzed  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of human bones and teeth from individuals believed to belong to the Bell Beaker period, a period distinguished by the seemingly random geographical distribution of characteristic material culture. Significantly different  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios in femoral bone and first permanent molar (M1) enamel samples from two archaeological sites suggested that several of the individuals spent their childhood in an area other than the general burial site. Although the sample size was small, leading to the interpretation that the disparate  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios were an isolated incident and not evidence of considerable mobility during the prehistorical period, the study contributed significantly to future related efforts. Grupe et al. (1997) expanded upon the Price et al. (1994a) pilot study by measuring  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios in bone and enamel from 69 skeletons (mixed age and mixed sex) from a Bell Beaker anthropological collection. Comparisons of the isotopic data with area geological differences revealed larger  $^{87}\text{Sr}/^{86}\text{Sr}$  values in enamel, which was indicative of residential mobility. The percentage of migrant individuals varied according to individual sites; approximately 23% of the individuals were distinguished as immigrants based on this ratio differential. While the data are highly suggestive of variation in residence patterns within the Bell Beaker period, questions regarding the extent of relocation and frequency of movement remained unanswered. Without supporting ethnographic data and analysis of tissues representative of a person's lifetime, this situation may be unavoidable.

Archaeological samples from the American Southwest have also been subjected to  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio analysis to determine mobility trends (Price et al. 1994b; Ezzo et al.

1997). Price et al. examined bone and M1 enamel from known, complete 14<sup>th</sup> century skeletons at Grasshopper Pueblo and Walnut Creek, Arizona, two geographical areas characterized by a particularly diverse geological profile. Expectations that local dietary sources would be reflected in bone samples were confirmed by homogeneous  $^{87}\text{Sr}/^{86}\text{Sr}$  values, whereas significant variation in tooth enamel sample values suggested some individuals had emigrated at a later stage in life. Related work by Ezzo et al. (1997) has provided continuity to the original pilot study (Price et al. 1994b) via examination of skeletal material from the same site.  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio analysis of bone and M1 tooth enamel from 69 individuals indicated that approximately one-half of the individuals sampled were likely to have been non-local residents of the pueblo. Although further analysis supported the occurrence of immigration on some level throughout the area's occupation, preferential sampling procedures may have resulted in nonrepresentative  $^{87}\text{Sr}/^{86}\text{Sr}$  values and inflated evidence of immigration.

While  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios depend solely on local geology since they reflect the underlying bedrock of a given area,  $\delta^{18}\text{O}$  values of drinking water are subject to alteration by climatic variables. Consequently, the analysis of both  $^{18}\text{O}$  and  $^{87}\text{Sr}$  isotope data can allow the placement of further constraints on the geographic origins of individuals (Evans et al. 2006). More recent studies have studied  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios in conjunction with  $^{18}\text{O}/^{16}\text{O}$  ratios to increase the robusticity of the data and resulting inferences.

Budd et al. (2004) studied  $^{18}\text{O}$  and  $^{87}\text{Sr}$  in permanent tooth enamel to test the validity of isotopic signatures retained in skeletal tissue as indicators of geographic origin and early medieval immigration patterns. While significant variation present in

some of the 53 samples permitted definitive inferences regarding movement patterns, similarities between local and regional strontium isotope signatures complicated efforts to further distinguish individuals originating from the general area. However, significant variation was observed in  $\delta^{18}\text{O}$  values between groups. Some groups exhibited  $\delta^{18}\text{O}$  values consistent with local origins yet suggestive of marked regional mobility;  $\delta^{18}\text{O}$  values obtained from other groups substantially deviated from local  $^{18}\text{O}$  abundance values. The tentative yet strongly suggestive nature of the evidence from this study and others (Evans et al. 2006) supports the use of  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios from skeletal remains as complementary, not independent, data. Isotopic geological homogeneity continues to be a cause of concern in strontium isotope studies. Thus, while most isotopic studies investigating geolocational patterns have traditionally focused on stable strontium isotope analysis, the use of oxygen stable isotopes - and more recently hydrogen - has become increasingly prevalent in similar efforts within the last decade.

Origin and migration studies utilizing stable oxygen isotope techniques emerged in the early 1990's. Schwarcz et al. (1991) analyzed human skeletal material from the remains of soldiers buried in an Ontario, Canada military cemetery with the goal of determining geographic origins of six individuals.  $\delta^{18}\text{O}$  values from the remains were comparable, but inconsistent with Ontario regional values. Results instead were indicative of provenience in lower latitudes. While Schwarcz and colleagues were unable to corroborate their postulation that the soldiers were from the Northeastern United States, their original hypothesis (i.e., some of the soldiers interred in Snake Hill were not Canadian citizens but instead U.S. military) was supported.

A similar investigation into the provenience of skeletal remains was conducted by

Keenleyside et al. (1997).  $^{18}\text{O}/^{16}\text{O}$  ratio analysis of bones and bone fragments associated with an 1845 lost arctic naval expedition revealed significant similarities between the  $\delta^{18}\text{O}$  values of the ~400 intact and fragmented samples (MNI = 11) and modern precipitation  $\delta^{18}\text{O}$  values from western Europe. Although the results were not unequivocal, oxygen isotope data combined with measured lead elemental levels reasonably suggested the remains were those of the arctic expedition crew, as suggested by local Inuit accounts.

Several oxygen isotope studies analyzing human skeletal material from the Mexican Teotihuacan archaeological sites Tlajinga, Oaxaca (Monte Alban), and Tlailotlacan have employed  $^{18}\text{O}/^{16}\text{O}$  ratio analysis to answer questions of human origins and migration (White et al. 1998, 2000, 2002, 2004b). The 1998 study examined  $\delta^{18}\text{O}$  values in human bone phosphate to determine the material's utility in determining geolocational patterns.  $\delta^{18}\text{O}$  values in 11 Tlajingan archaeological bone samples were compared with those measured in the tooth enamel of living individuals from the same area. The uniformity observed in the archaeological bone isotope ratios in conjunction with the minute variation in enamel isotope ratios suggested all 11 individuals (children and adults) were local inhabitants. The extremely low site variation in  $\delta^{18}\text{O}$  values, which is expected in a static population, was used as a geographical baseline against which to compare datasets from other sites. Further research conducted by the same research group suggested isotopically distinct populations, and the influence of differential dietary characteristics as opposed to different water consumption sources (White et al. 1998).

Phosphate oxygen isotope analysis has not always produced consistent results and

interpretations. Based on archaeological material evidence, White et al. (2000) hypothesized that 31 individuals interred at the Mayan city of Kaminaljuyú, Guatemala had immigrated from Teotihuacan, an adjacent region, and achieved prestigious status prior to death. Oxygen isotopic data obtained from first and third molar enamel phosphate did not corroborate their assertion. However, as the authors noted, isotopic data from the first and third molars are indicative of childhood migration patterns, and cannot account for adult transient residence patterns. Further, preferential, non-random sampling procedures were employed due to poor preservation of the material; both teeth were not available from the majority of individuals sampled.

Comparisons of isotopic ratios between archaeological samples and known, like-for-like material can facilitate valuable inferences regarding geographic origins and migration patterns. However, taphonomic and environmental influences may alter original signatures, thus raising a question of legitimacy in the results. While the migration hypothesis extended by Hoogewerff et al. (2001) to explain the geographic origins of "Ötzi", the Tyrolean Iceman, appeared to be supported by multi-elemental isotopic and trace element data and comparison with known values corresponding to a collection of pre-industrial historic skeletal material, documented taphonomic processes affecting the remains may have altered the original trace element abundances.

An ongoing bioarchaeological field project on the Mediterranean coast near Rome, Italy, has been the basis for several recent studies by Prowse et al. (2004, 2005, 2007). They have successfully attempted to reconstruct paleodiet and migration patterns by analyzing the  $^{18}\text{O}/^{16}\text{O}$  ratios of individuals interred in Isola Sacra near Rome, Italy. The work by Prowse and colleagues (2007) demonstrates the inferential utility of oxygen

isotope analysis. Researchers measured  $^{18}\text{O}/^{16}\text{O}$  ratios in the permanent tooth enamel (first and third molar pairs) of 61 interred individuals (male and female). Dentition samples were compared against deciduous controls obtained from modern Roman children. Two-thirds of the Isola Sacra first molars possessed  $\delta^{18}\text{O}$  values comparable to those from two children whose  $\delta^{18}\text{O}$  values indicate migration from North Africa either as children or while *in utero* when their mothers migrated. This observation, in particular, suggests that although Rome may have been the geographic origin for the majority of the subjects, some of the individuals may have been born in other places with similar isotopic signatures. The remaining first molars expressed  $\delta^{18}\text{O}$  values that suggested the individuals were born in a region outside of Rome. Third molar data supports the migration of a significant number of individuals to the Roman region as children, a finding that directly contradicts the historical assumption that unmarried adult males comprised the majority of migrants. These conclusions are in alignment with the multi-elemental isotopic analysis (strontium and oxygen) conducted by Evans et al. (2006, southern England) and Budd et al. (2004, Bavaria).

Due to the significant correlations between oxygen and hydrogen isotopes and climate variables, these two isotopic systems are frequently analyzed together to strengthen the resulting data. However, few of the growing number of geographic origins and migration studies employing  $^{18}\text{O}$  isotope analysis have included  $^2\text{H}$  analysis. This may be due to hydrogen exchangeability issues associated with extracting and measuring the  $^2\text{H}$  composition of various biological materials; it is well known that hydrogen bound to oxygen and nitrogen easily exchanges with environmental hydrogen



(DeNiro and Epstein 1981). Protocols designed to mitigate hydrogen exchange are stringent, with equilibration phases lasting seven or more days (Landwehr et al. 2011).

Following the seminal work of Cormie et al. (1994a, 1994b, 1994c), most of the work involving the hydrogen isotopic analysis of bone has been performed on animal samples to establish bone as a proxy for climate variables such as humidity and precipitation. A recent study by Reynard and Hedges (2008) demonstrated unequivocally that  $\delta^2\text{H}$  values differ between animal species, suggesting a trophic level effect that can be traced to various food webs. While their investigation of  $\delta^2\text{H}$  values as environmental indicators was of secondary importance, they hypothesized that variability in the hydrogen isotope ratios of bone collagen from different individuals could assist in the broad determination of geographic origins for those individuals. Their findings did not support this contention; uniformly low  $\delta^2\text{H}$  values observed in humans and cattle from a single area instead were suggestive of short-term climate fluctuations and not human migration from a nearby area whose species exhibited differential isotopic ratios.

Analytically available  $^2\text{H}$  is also accessible in tooth enamel (Holobinko et al. 2011a). However, the  $^2\text{H}$  abundance in tooth enamel appears to be fixed, thus rendering it inappropriate as an indicator of geographic provenance (see Chapter 5).

Dentin collagen is a biologically available source of  $^2\text{H}$ , but only within the last several years has it been investigated as an indicator of seasonality (Kirsanow et al. 2008; see Tuross et al. 2008). Again, the focus of such studies appears to be on the utility of isotopic data in reconstructing paleoenvironmental conditions. Kirsanow et al. (2008) measured  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values in dentin collagen sequentially sampled from

fossilized ovicaprid permanent mandibular premolars and molars. Significant isotopic trends were observed between the  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values within individual teeth. Comparison of the dental collagen isotopic variation with published annual ranges of the isotopic variation within regional meteoric precipitation indicated a correlation between the published data and dentinal  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values. Although these findings supported the authors' argument that hydrogen and oxygen isotopic measurements of dentin collagen are reliable indicators of seasonality, samples were not properly equilibrated and data were not scale-corrected based on a 2-point end member correction. More recent research has examined  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values in dentin collagen extracted from modern and archaeological late-erupting tooth samples. Holobinko et al. (2011b) measured the isotopic abundance of  $^2\text{H}$  and  $^{13}\text{C}$  in human dentin collagen obtained from archaeological and modern teeth using continuous-flow isotope ratio mass spectrometry (IRMS) and compared these findings with the  $^{18}\text{O}$  and  $^{13}\text{C}$  isotopic composition of the corresponding tooth enamel (see Chapter 6).

The number of studies focusing on the  $^2\text{H}$  analysis of hair and fingernails is steadily increasing. These biological materials are suitable for making inferences regarding the last several months of an individual's life, so isotopic data obtained from keratin is more useful in determining recent movements or recent residence as opposed to birth place and childhood migration patterns. Despite the apparent constraints of the method, hair is an advantageous material for archaeological studies in that it provides a temporal component to the stable isotope signatures; one strand of hair may be measured along its entire length to assess the isotopic ratios incorporated at weekly or monthly intervals (White 1993).

While ancient human hair samples have been examined with regard to dietary reconstruction (see White 1993, but see also Macko et al. 1999), few studies have assessed the utility of hydrogen isotope analysis of human hair in determining recent residence patterns (Fraser et al. 2006; Fraser and Meier-Augenstein 2007; Ehleringer et al. 2008). Sharp et al. (2003) investigated this issue in their work on archaeological hair samples. An ancient sample measuring 20cm in length was obtained from the Mount Aconcagua Inca mummy (a male child) from Argentina (~370 yr BP) (see Fernandez et al. 1999). Seasonal variations were evident in the hair sample; hydrogen, carbon, and nitrogen isotope ratios differed according to their sampled location along the length of the hair. The  $\delta^2\text{H}$  values suggested the boy had been transported to his high-altitude resting place less than a week prior to his sacrificial death. Dietary evidence suggested by  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values corroborated the  $\delta^2\text{H}$  data (see also Wilson et al. 2007).

### Forensic Applications

While the application of stable isotope analysis to medico-legal investigations involving the identification of human skeletal remains was recognized in the late 1980s (Katzenberg and Krouse 1989), the first accredited use of the technique for this purpose did not occur until approximately 2005 (Meier-Augenstein and Fraser 2008). While there have been relatively few studies concerned primarily with determining the geographic origins (or most recent residence) and tracing the recent movements of modern individuals (Pye 2004), this appears to be changing. The technique continues

to generate more interest as its utility and supplemental value in supporting other forms of identification continues to gain recognition.

$^{18}\text{O}/^{16}\text{O}$  and  $^2\text{H}/^1\text{H}$  ratio analyses are germane to forensic provenance studies because their distribution in meteoric precipitation, which is consumed by humans in the form of drinking water and water contained in food, varies geographically – and predictably – depending on the climatic variables previously discussed. Similarly, the  $^{87}\text{Sr}$  composition in nutritional resources is dependent upon an area's geological characteristics. However, an intrinsic dilemma concerning analysis of the above stable isotopes is the potential influence of globalization of food and water supplies and the ease with which nutritional commodities are transported internationally; whereas ancient human tissue may reflect geographically specific isotopic ratios at the time the resources were ingested, ratios in modern human tissue are potentially subject to greater convolution as a result of the “global supermarket effect” (Nardoto et al. 2006). While both of the above problems can blur interpretation of isotopic data, the problems are not insurmountable, as evidenced by the following forensic isotopic studies of human provenance.

Meier-Augenstein et al. (2005) measured the isotopic composition of scalp hair ( $^{13}\text{C}$  and  $^{15}\text{N}$ ) and bone apatite ( $^{18}\text{O}$ ) to ascertain whether or not a murder victim was from the local area (Ireland) or from another part of the country. Bone  $\delta^{18}\text{O}$  values indisputably indicated the deceased was likely from Eastern Europe or the northern part of the United States. While victim identification was not possible, analysis of hair  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  precluded North America as a geographical origin, subsequently suggesting an Eastern European country as the victim's point of origin.

Similar multi-elemental work was conducted by Meier-Augenstein and Fraser (2008). Notably, this particular isotope study led to the positive identification of a severely mutilated body and the eventual arrest of the perpetrators.  $\delta^{18}\text{O}$ ,  $\delta^2\text{H}$ ,  $\delta^{13}\text{C}$ , and  $\delta^{15}\text{N}$  values were measured in hair and fingernail samples;  $\delta^{18}\text{O}$  values were obtained from femoral bone.  $\delta^{15}\text{N}$  values indicated the victim had partaken in a protein-enriched diet; the lack of  $\text{C}_4$  plant derived carbon sources in  $\delta^{13}\text{C}$  values excluded North America and Africa as recent origins.  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  values confirmed the likelihood that the victim had spent the past 7 months in the general Dublin area. However, highly significant cortical bone apatite  $\delta^{18}\text{O}$  values ruled out Dublin as the point of origin, instead suggesting the victim hailed from an equatorial region. Corroborative DNA evidence coupled with the isotope data eventually led to the confirmation of Kenya as the deceased's geographic origins. In absence of the apatite isotopic data, authorities may have erred in their inferences of provenance and subsequently restricted their search to the local area.

Stable isotope analysis of modern human biological materials may be of exceptional value in cases where non-invasive sampling techniques are prudent. Fraser et al. (2006) collected hair and fingernail clippings from area volunteers of varying ethnic backgrounds and geographic origins. Individuals were queried for dietary intake and recent travel in order to examine the variability of isotopic signals in human hair and nails. While variability between isotopic compositions and biological tissues was noted, researchers also discovered a significant degree of intra-individual natural variability of stable isotope signatures. Causal factors underlying this variability require additional

investigation, and should be considered when interpreting isotopic data regarding a person's origins or recent movements between geographical locations.

$^{87}\text{Sr}$  has been studied with regard to modern human provenance. Juarez (2008) analyzed  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios in human tooth enamel to determine geographic origins for Mexican immigrants who perished during U.S.-Mexico border crossing attempts. Based on comparisons with control samples donated from Mexican-born individuals, she identified three distinct geographical regions within the data set. While attributing the marked differences between the regions to differential levels of geological strontium, she acknowledged the potential bias associated with such inferences due to a small sample size ( $n = 19$ ), the impact of global food consumption on  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios, and cultural and personal variation in food consumption.

The Juarez (2008) study ostensibly illustrates the utility of  $^{87}\text{Sr}/^{86}\text{Sr}$  analysis in a forensic context, yet the same concerns expressed by Juarez were also noted by Rauch et al. (2007). Rauch and colleagues measured the stable isotope ratios of light and heavy elements in scalp hair, teeth, and occipital bone from a male murder victim presenting dental evidence indicative of eastern European origins. The isotopic composition of  $^2\text{H}$ ,  $^{13}\text{C}$ ,  $^{15}\text{N}$ ,  $^{87}\text{Sr}$ , and  $^{206}\text{Pb}$  were measured in the tissues and soil samples from the burial site. Hair  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values indicated high consumption of animal meat and  $\text{C}_4$  plants, which was not totally consistent with unpublished isotopic data for the local German population. While strontium isotope data were determined to be of no additional assistance in determining origins due to the homogeneity present in the  $^{87}\text{Sr}/^{86}\text{Sr}$  values of Germany and Europe,  $^{206}\text{Pb}/^{204}\text{Pb}$  values differed significantly between hair and bone, and were largely responsible for determining the country of

origin, Romania, and eventually leading to the identification of the deceased. While  $^2\text{H}/^1\text{H}$  ratios were measured in the biogenic tissues, resulting  $\delta^2\text{H}$  values were not provided, nor addressed by the authors.

Sulfur isotope analysis has become evident in studies of modern human provenance. The same research team (Mützel (Rauch) et al. 2009) analyzed the isotopic composition of  $^2\text{H}$ ,  $^{13}\text{C}$ ,  $^{15}\text{N}$ , and  $^{34}\text{S}$  in hair samples from 111 volunteers from 13 countries. Residents of Costa Rica and Brazil could be distinguished from Europeans by  $\delta^{13}\text{C}$  values, and Australians by  $\delta^{34}\text{S}$  and  $\delta^2\text{H}$  values. Nitrogen isotope ratios provided additional discrimination between countries. Multi-elemental isotopic analyses of the hair of modern humans and animals has illustrated the value of measuring  $\delta^{34}\text{S}$  values to confirm dietary status and origins (Bol and Pflieger 2002; Bol et al. 2007). Similar work by Thompson et al. (2010) supports the utility of  $^{34}\text{S}/^{32}\text{S}$  ratio analysis in forensic investigations. In combination with  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  data, dietary  $\delta^{34}\text{S}$  values can further distinguish geographically distinct populations.

## Conclusions

Particularly,  $^{18}\text{O}/^{16}\text{O}$ ,  $^2\text{H}/^1\text{H}$ , and  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio analyses can yield valuable information concerning an individual's geolocational patterns, information that can prove extremely useful in a forensic and bioarchaeological context, since human remains often are discovered in areas in which the deceased did not reside. Depending upon the biological material sampled, the isotope selected for analysis, and the degree of environmental isotopic variation, isotope abundance values may offer recent locational

"snapshots" rather than pointing more toward overall migration patterns. For these reasons relying upon one stable isotope as an indicator of geographic origins, especially  $^{87}\text{Sr}$  or  $^{34}\text{S}$  due to regional geological homogeneity ( $^{87}\text{Sr}$ ) and the lack of accurate faunal or human baseline data ( $^{87}\text{Sr}$  and  $^{34}\text{S}$ ), is not recommended.

Determination of an individual's movement patterns requires a broader scope of examination, and would appear to be best studied by analyzing the isotopic composition of at least several elements. Diet markers such as  $^{13}\text{C}$ ,  $^{15}\text{N}$ , and  $^{34}\text{S}$  can offer qualifying information when geolocational data obtained from  $^2\text{H}$  and  $^{18}\text{O}$  are consistent with more than one location. Meier-Augenstein et al. (2005) excluded Northern Ireland – the location of a murder victim's body – as the deceased's place of residence, yet it was not until application of  $^{13}\text{C}$  and  $^{15}\text{N}$  isotopic analysis that researchers were able to narrow down a point of origin to two possible Eastern European countries. Prowse et al. (2007) reported potentially wide variations in points of origin for their samples (First to Third centuries AD), and recommended further  $^{87}\text{Sr}$  isotopic analysis to help resolve this issue. While modern biological  $^{87}\text{Sr}/^{86}\text{Sr}$  values appear to be relatively homogeneous, delta values in ancient material can exhibit significant variation, thus facilitating a more accurate determination of human origins and migration patterns. Comprehensive studies designed to establish detailed points of reference based on faunal and human isotopic data are necessary.

While stable isotope analysis has become a well-recognized and commonly utilized method for determining provenance of human remains in bioarchaeological contexts, it is perhaps most effective when corroborated by ethnographic accounts (Keenleyside et al. 1997; Prowse et al. 2007) and other lines of evidence (Shimada et al., 2004, Prowse et



al. 2010, mtDNA; Meier-Augenstein and Fraser 2008, DNA). Rapidly emerging forensic applications of the method appear promising, particularly when used in conjunction with similar complementary analytical techniques.

However, the method's efficacy in both contexts may be limited by type, availability, and preservation of biological material, analytical instrumentation considerations, natural variability between individuals (even within the same individual), and a researcher's awareness of the method's relevance or value. If the identification of historical movement patterns is in question, analysis of hair and nail keratin are unsuitable sampling materials. Similarly, if the goal is determination of recent travels using dietary  $\delta^{34}\text{S}$  values, keratinous materials are ideal provided the following two requirements are met: relatively large sample amounts required to offset the low relative abundance of  $^{34}\text{S}$  found in organic compounds; dedicated analytical instrumentation (Meier-Augenstein 2010). Finally, in addition to being affected by travel and the consumption of non-local foods, fluctuations in isotopic values are influenced by biological processes such as different growth rates of tissues, differences in metabolic rates, and differences in tissue (compound) specific trophic level shift. Further research into intra- and inter-individual variability is vital to the reduction of marginal error observed in isotope analysis studies.

The significant amount of literature published on bioarchaeological and forensic applications of stable isotope analysis supports the method's utility in such investigations. However, while the method has already proven a useful ally in the linkage of human remains to a point of origin or migratory route, further systematic research is necessary before its potential can become fully realized.

CHAPTER 3  
THEORETICAL AND METHODOLOGICAL APPROACHES TO UNDERSTANDING  
HUMAN MIGRATION PATTERNS AND THEIR UTILITY IN FORENSIC HUMAN  
IDENTIFICATION CASES

Introduction

Studies of human migration have traditionally focused on the evolutionary expansion of modern humans as they populated first the African continent and subsequently spread into Europe, Asia, and, more recently, Australia and the Americas. Diffusion theorists have investigated migration as a mechanism for maintaining cultural continuity and the development of markedly diverse human cultures. More recently, a considerable emphasis has also been placed on the causes and impact of current individual and familial migration events within the last 50 to 60 years. Tracing individual movement patterns is important from a historical, economic, and social perspective. However, it is also an important consideration in the case of forensic human identification. While the identity of a decedent often is known or suspected at or around the time of death, in many cases identification of human remains cannot be facilitated without the application of technological methods (e.g., DNA profiling) or comparisons with missing persons reports. If geographic origins or recent residence cannot be determined, then it will be difficult to obtain medical and/or dental records that may be

used for identification. The increasing degree of international travel and intra- and intercontinental relocation makes it more likely that the residence of an unidentified individual will not immediately be known, thus precluding DNA comparisons with presumed relatives and rendering missing persons reports useless.

The following is a cursory review of various theoretical approaches to the study of modern human migration, and includes an examination of several of the methods, intuitive and deductive, employed in the detection of human movement patterns before considering the tactical utility of the aforementioned concepts in determining forensic human identification (Holobinko 2012a).

### Theoretical Approaches

Why do human migration studies matter? The answer to this question frequently depends on who is asking the question. For example, the anthropologist studies migration to explain biological changes and the transmission of cultural and linguistic traits at the populational level (Keita 2010), whereas historians analyze cycles of continuity and change to understand migration processes and their implications, and also to correct misinterpretations (Lucassen et al. 2010). Geneticists study human dispersal patterns to determine and explain genetic variation between populations (Cox and Hammer 2010). Epidemiologists may examine particular migratory patterns to determine if health-related changes in a population are attributable to some extent to genetic predispositions evident in the source population (Keita 2010); social policy makers are interested in the potential policy implications of migratory behavior. The

following briefly explores the theoretical underpinnings of modern human movement patterns, including diasporic migration.

### Modern Theories of Human Movement Patterns

Studies of human migration have been dominated by researchers in three areas: anthropology, sociology, and history (Lucassen et al. 2010). While the anthropological approach to migration has culled data from numerous subdisciplines (e.g., archaeology, ethnology, cultural anthropology) into theoretical frameworks based on climatology, genetics, and cultural systems such as marriage and kin, these models have tended to focus on early, non-literate societies (Lucassen et al. 2010). The sociological approach to analyzing contemporary migration patterns has generated a considerable number of economic, demographic, social, and policy-oriented theoretical models, most of which overlap to some extent. These theories address the main concerns of the scientist: who is migrating, why are they migrating, where are they going, and what are the repercussions of the move (Clark 1986)? Numerous models, many of which are based on economic principles, have been developed to address internal migration and international migration patterns. Several of the more prominent theories are discussed below.

Internal Migration Theory. Most of the historical classic models of migration revolve around the economic forces affecting an individual's decision on where to establish residency (Fix 1999). Generally, migration followed a path from areas in which wages were low and labor was in demand to areas in which income and opportunities

exceeded the originating area. Ravenstein (1885) conducted the first systematic study of human migration and subsequently established economic motivation as the backbone of the entire concept. Lewis (1982) deemed the tenets of Ravenstein's study the "laws" of migration: migration is generally followed by a countermigratory event; most individuals migrate short distances; migrants relocating longer distances typically choose larger cities as their destination; preferential migration is evident in females and individuals (cf., sex differential (Seielstad et al. 1998); migration exhibits directionality (i.e., rural residents migrate more frequently than do their urban counterparts); young adults are more likely than families to make international moves. Despite the fact that Ravenstein's conclusions were based on observations of Western societies and thus skewed by the significant influence of industrialization on primarily short distance migration (Grigg 1977; Fix 1999), his work identified important aspects of internal or regional migration and laid the groundwork for future theoretical models (e.g., Lee 1966).

Central to many models of migration are two groups of causal factors affecting one's decision to migrate: push and pull factors (Lee 1966). Push factors are relative to the home country or region, while pull factors are associated with the potential destination. Typical push factors include but are not limited to: lack of jobs or few opportunities; adverse environmental conditions (e.g., famine, natural disasters); poor infrastructure, including inadequate medical care; lack of political or religious freedom. Some pull factors enticing migrants into other areas are increased job opportunities; better living conditions; freedom of speech; improved infrastructure and security. This partial list of causal factors applies not only to internal migration but international moves as well.

Recent studies of internal migration have categorized the migrant and motivating factors in terms of human capital, producers, and consumers (Bodvarsson and van den Berg 2009). Many economists who study migration employ a micro-level approach based on a labor-flow model, which essentially assumes a migrant will choose the destination offering the highest income in order to maximize his or her utility. Sjaastad (1962) first made the connection between migration and investment in human capital with a reductive model that identifies a migrant's ability to earn income as human capital and considers the act of migration an investment in one's own capital. In other words, the decision to relocate is solely influenced by the upfront costs and potential uncertainties associated with migration weighed against actual or perceived benefits (i.e., income potential). Notably, Sjaastad's (1962) model assumes that migration "costs" are synonymous with distance traveled, and that greater distances unequivocally result in more uncertainty regardless of beneficial kinship and migrant networks that may be of assistance to the migrant (Yap 1977; Taylor 1986).

The problem with the above economic internal migration model and closely related international migration models (e.g., Borjas 1987, 1991) is that they often assume that all variables reach ideal proportions (e.g., Sjaastad's model assumes 100% probability of the migrant securing employment in the destination) and remain constant between conditions in the migrant's home and potential locations destination (e.g., uneven distribution of amenities in both locations). Further, they fail to account for the multitude of non-economic causal factors (e.g., social and natural forces). Empirical data suggests that income maximization is not the sole deciding factor in the decision to migrate (e.g., Greenwood 1997). Additional modeling flaws include the inability to

account for multiple migrations, household migration decisions, uncertainty resulting from insufficient labor market information on a destination, remitting portions of one's income to family remaining in the home location, and how an individual's place in their life cycle affects if, when, and how many times they migrate (Polachek and Horvath 1977).

Theoretical shortcomings in human capital-investment models gave rise to equilibrium models of migration. These models acknowledge the diachronic nature of a continuum of income, prices, supply, demand, amenities, and utility functions (Bodvarsson and van den Berg 2009) while preserving the importance of the migrant's role as consumer. Consumption varies significantly within and between countries, especially developing vs. developed; amenities contributing to a higher quality of life (e.g., many of the causal factors identified in Lee's 1962 push/pull model) are strong motivators. Thus, equilibrium models are particularly suited to international migration studies. Household production migration models follow the same concept as the equilibrium or consumption models, although they identify the household as the migratory unit and consider variables relating to household production, consumption, and risk (see Becker 1965; Lancaster 1966; Willis 1973), simultaneously removing from the equation all individual influence or preference.

International Migration Theory. Borjas (1987, 1991) presented a two-stage international model designed to address the wide variation evident between workers and the associated conditions in source and destination countries. Instead of assuming all variables are held constant and at ideal levels, his model acknowledges the range of

personal (i.e., worker) and economic (e.g., wage differential) characteristics evident in both countries. The first stage of Borjas' (1987) model is based on several dichotomous assumptions that predict: (1) a directly proportionate relationship between migration rate and the destination country's mean income rate, (2) an inversely proportionate relationship between migration rate and source country's mean income rate, (3) a migration rate inversely proportionate to the relative costs of migration, and (4) less-skilled workers are more likely to migrate when skill transferability between the source and destination countries is low/unlikely. The second stage of the Borjas (1991) model builds upon its fundamental assumptions by incorporating predictions concerning the relationship between migration rate and mean level of education in the source country (e.g., notable disparities in mean education level within a source country result in smaller numbers of individuals migrating abroad). While the Borjas model has been used for years, others have expanded upon it as more current issues affecting immigration (e.g., explicit immigration restrictions) have become apparent (Hatton and Williamson 2005; Clark et al. 2007).

The internal and international immigration models discussed above are explicit, in that they generally assume that the migration decision and the act itself is synchronic and irreversible; none of the models account for the variability involved in temporary migration, multiple migrations, or involuntary migration (Bodvarsson and van den Berg 2009). Despite the focus on permanent immigration, historically immigrants have returned to their original homelands after spending a period of time in their destination countries (e.g., the post-World War I large net outflow of immigrants from the U.S.). Reasons for these return migrations vary. For example, an improved quality of life in the



source country may become possible, the support system offered by family relationships in the migrant's native region may become a primary motivating factor, or the destination country has implemented immigration policies making it impossible for the migrant to remain there past a certain date. Further, an immigrant may choose to return to the source country after coming to the conclusion that the decision to migrate was based on inaccurate knowledge of the destination country or simply an error in judgment (Bodvarsson and van den Berg 2009).

The increasing globalization of world markets over the past decade has had a tremendous impact on immigration; temporary immigration rates have increased markedly (Bodvarsson and van den Berg 2009). It is now common for multinational companies to move personnel between various international locations. Most countries issue visas without expiration dates to relocated multinationals and their family members. Temporary immigration also results from country-specific immigration policies. Generally, high-income destination countries make it easier to obtain work permits or temporary resident visas than permanent resident visas. The issuance of such temporary immigration documents is not new; numerous countries have developed work programs designed to offer immigrants temporary resident status in exchange for a specified—or unspecified—labor period. For example, the large number of Americans drafted into service during World War II prompted the U.S. government to offer Mexican immigrants temporary work visas in exchange for performing mostly farm-related labor duties (i.e., Bracero Program). Similarly, some western European countries (e.g., West Germany, France) exhibiting labor shortages in the 1960's instituted guest worker programs in which immigrants from areas such as southern

Europe and South Africa were able to relocate temporarily to the destination countries. Many migrants wishing to become permanent residents participated in these guest worker programs to gain entry, remaining in the countries until they were eventually granted permanent status. More recently, this trend has been observed in the Persian Gulf states (e.g., Dubai), which have hired temporary workers from densely populated countries such as Pakistan and India. However, the destination countries have imposed restrictions on the temporary visas to ensure foreign-born workers do not remain in their country permanently (Bodvarsson and van den Berg 2009).

Temporary immigration models are very similar to conventional models with notable exceptions. Dustmann (2001) assumes higher wages overseas, but speculates that consumption (e.g., amenities) is preferentially associated with the source country. As long as the immigrant maintains close ties to the native country, immigration rates are lower and the length of stay is decidedly temporary. If conditions in the source country deteriorate while simultaneously improving in the destination country, immigration rates increase and the stay abroad is extended. While empirical data supports Dustmann's (2001) predictions, the model is flawed in its failure to accommodate discrete variables such as significant family events, loss of employment, or changes in immigration policies (Bodvarsson and van den Berg 2009). Mexican immigrants to the U.S. consistently list source country population density and a persistently weak economy as primary causal factors for emigration (Card and Lewis 2007), yet their established networks in the destination country often eclipse income and prices as the primary determinants of duration (Richter et al. 2006).

Individuals leaving their home countries as a result of intolerable conditions (e.g., political, social, environmental) or those who are forcibly removed are considered involuntary immigrants. These individuals are often referred to as asylum seekers or refugees; their immigration concerns are not factored into any of the above explicit or implicit models (Bodvarsson and van den Berg 2009). Over the past 20 years relevant immigration issues have arisen. During the 1990's Europe experienced an influx of refugees a result of war and ethnic cleansing in the Balkans; currently Africa and the Middle East are exhibiting high rates of emigration. Because it is conceivable that most refugees will improve their conditions by immigrating, most of the theoretical work in this area concerns the enslaved migrant (Bodvarsson and van den Berg 2009).

Between 1500 and the late 19th century approximately 11 million Africans were enslaved and transported to the Western Hemisphere by slave traders from various European countries (see Curtin 1969; Eltis 1989; Klein 1999). "Human trafficking" is the current term most commonly applied to forced immigration of this type (Bodvarsson and van den Berg 2009). The basic labor-flow model of immigration may be most applicable to analyses of immigration characterized as slavery or human trafficking since it fundamentally concerns the shifting of labor resources from one country to another. Of course, in this model the enslaved individual has no ownership over his or her own human capital; all costs and returns are borne and received by the slave owners. Thus, the trafficking of humans can be quite profitable for the source country or "owner" provided the gains outweigh the costs; this was the case for some of the source countries (e.g., Holland, Portugal, England) exporting slaves during the Atlantic slave trade [emphasis added]. Essentially, provided all other variables are held constant,

forced international immigration (i.e., slavery) is more likely to occur if the costs of enslavement and international transport are relatively inexpensive, slave labor in the destination country generates a profit for the owner (i.e., income gained substantially surpasses maintenance costs), and slave labor is less expensive than alternative forms of labor (Bodvarsson and van den Berg 2009).

### Diasporas

While the term 'diaspora' is perhaps most familiar in relation to the Jewish and Trans Atlantic slave trade experiences (Lilley 2004), and has been studied with equal frequency in the two aforementioned contexts (e.g., Gilroy 1987; Gilroy 1993; Yelvington 2001), the term is generally associated with coerced dispersal from one's homeland. Communities developing as a result of diasporic migration are characterized by what is typically an involuntary dispersal from the home community, yet these communities maintain a constant link, spiritual or otherwise, with the original homeland (Lilley 2004). Involuntary immigration may result either from direct persecution or more indirect economic and/or political pressures (Bodvarsson and van den Berg 2009).

Theoretical models developed to explain Imperial and trade diasporas are concerned primarily with the accompanying expressions of change exhibited within the original and host communities (Eckardt 2010). Diasporas can be differentiated from migration based on numerous characteristics identified by Cohen (2008), not all of which are applicable to each diaspora event. Generally, however, diasporas can be distinguished based on the following: dispersal, forced or voluntary, from one's homeland into at least two foreign regions; expansion from a homeland for purposes of

work, trade, or other pursuits; a collective spiritual connection to the homeland, frequently involving myth-like properties; a strong group ethnic consciousness; difficulty assimilating into the host community; shared empathy and responsibility between the members of various diasporic settlements; the frequent coordination of a collective return movement to the homeland despite general satisfaction with the current way of life. While the term has perhaps been most applied to the Jewish and African experiences, Brubaker (2005) and others (e.g., Walden 2005; Weiner 2010) have noted the term's expansion to include the Armenian and Greek diasporas and, more recently, long distance nationalists (e.g., Irish, Kurds), labor migrants, and the New Orleans and U.S. Gulf Coast residents who were evacuated or displaced as a result of Hurricane Katrina.

Many cultural studies of diaspora groups focused primarily on the retention and transmission of cultural traits within migrant communities (e.g., Gilroy 1987; Gilroy 1993; Kahn 1994; Yelvington 2001). The same cultural focus was noted in early archaeological studies on African diasporas, which comprised the bulk of these initial diaspora studies (Eckardt et al. 2010); Webster (2010) provides a thorough review of the African diaspora, an event in which over 10 million Africans were enslaved and transported by force to the Western Hemisphere during the 400 year-long Atlantic slave trade (Bodvarsson and van den Berg 2009). Recent diaspora work has placed more of a bioarchaeological emphasis on health status and lifestyle of the immigrants (see Blakey 2001).

The introduction of trade and colonial diaspora research has brought to the forefront the unique attributes of these corresponding communities. Cohen (2008:83) has

described trade diasporas as a collection of "spatially dispersed" groups that are interconnected yet distinct from their homeland and the current communities in which they live. Archaeological investigations of Chinese trade diaspora communities appear to support Cohen's claim; studies conducted by Voss (2005) and Diehl et al. (1998) simultaneously demonstrated cultural continuity and affiliation with the homeland and few attempts at assimilation into the host community. Colonial diasporas concern the migratory experiences of colonizers, and have largely been ignored by archaeologists (Eckardt 2010). Cohen (2008:69) has coined the term "Imperial diaspora" to describe the British Empire and other colonial expansions as groups marked by a strong ethnic identity and consistent deference to the homeland's social and political institutions.

### Evidence of Migration

Numerous approaches designed to detect evidence of migration in the archaeological record have been developed. These techniques yield findings which can be loosely classified as either indirect (i.e., primarily inferential) or direct (i.e., quantifiable). Most of the following methods have been primarily, if not entirely, applied to prehistoric human migration studies. Nonetheless, they illustrate the type of evidence that may be present in a forensic context and the quandary posed by subjective interpretation of evidentiary material during the course of a medico-legal investigation.

### Archaeological and Bioarchaeological Evidence

Archaeologists have numerous implements in their tool kits with which to detect evidence of human migration: fossils representative of humans at various points during their evolutionary continuum have been recovered; material culture has been used to distinguish groups; burial ritual evidence yields insight into the mortuary practices—and social structure—of the parent community; skeletal remains can be cultural indicators. Since the above methods require speculation based on sometimes incomplete specimens, interpretations may be biased by subjective analyses. Consider, for example, the dilemma arising from the conflicting fossil hominid evidence supporting each of the theories explaining the initial expansion of anatomically modern humans. Strict proponents of the three models all claim, based on their determinations of "evidence", that their respective theory best explains incredibly complex migration patterns occurring within an extremely broad spatial context (Goldstein and Chikhi 2002) [emphasis added].

When written records are unavailable for consultation and comparison, archaeologists often must rely on stylistic variation in material culture to distinguish between groups, based on the assumption that groups of people subscribing to particular cultural norms will leave behind traces of their unique identity (Blom 2005). However, differentiating between ethnic groups using material culture is not always unambiguous (Jones 1997; Smith and Schreiber 2005). For example, Andrushko et al. (2009) acknowledged the crucial role of migration in the development of Andean social customs. Various Andean ethnic groups have been successfully distinguished based on ceramic style, yet these stylistic variations can be due to regional, temporal, or status-

based differences (see Andrushko et al. 2009). Further, some symbols of identity (e.g., some Andean apparel items) never make it into the archaeological record (Cock 2002). Another complication is the dynamic nature of ethnic identity, which may not always be evident in the material assemblages left behind (Ericksen 1992; Jones and Graves-Brown 1996; Bernardini 2005). Indirect representations of identity also fail to discriminate between the internalized nature of identity and the external labels affixed by others (Nagel 1994; Barth 1998).

The material culture of Africans enslaved in the Americas has been studied for several decades (Webster 2010). Slave-made ceramics, motifs, and architectural elements have been exemplified as strategies by which slaves and their descendants maintained and adapted their cultural traditions. While critics of this work have characterized the artifacts as lacking in specificity (see Perry and Paynter 1999), the more thorough studies have incorporated ethnographic evidence in their analysis of New World material culture believed to represent West African practices (e.g., Handler (1996), burial position). Fennell's (2007) analysis of the use of 'core' symbols within diasporic cultures combines anthropological theory with archaeological data to describe group identities.

Burial rituals and treatments are known to vary among individuals and to exhibit change over time (Cannon 2005:41). Historical trends in mortuary patterns have been characterized in terms of social and political contexts (Buikstra and Charles 1993; Parker Pearson 1993). The structure and complexity of a society can be inferred from the burial treatment afforded to its citizens; the more complex a society, the more variability is evident in its mortuary rituals (Binford 1971). Binford (1971) and Saxe



(1971) arrived at similar conclusions based solely on ethnographic data. O'Shea's (1984) analysis of prehistoric mortuary variability incorporates archaeological data and statistical analysis and arrives at similar conclusions that can realistically be applied to archaeological settings: mortuary populations tend to reflect the demographic and physiological characteristics of their living populations (1984:34).

While mortuary archaeology has uncovered material evidence of mortuary practices, it may also provide information on aspects completely unrelated to manner of death or mortuary treatment (i.e., preparation of the deceased) (Charles 2005). These clues can be present in the form of grave goods which may be authenticated, thus constituting direct evidence; other clues are more implicit and subject to interpretation (e.g., what does it *mean* to find a particular artifact associated with a particular burial?) (Charles 2005). Pearce (2010) was critical of using mortuary analysis – and epigraphic evidence – as the basis for making inferences regarding human migration. Based on his analysis of several examples from various Late Roman cemeteries, the identification of specific origins at the individual or community level was implausible based on the significant variation in cultural elements present in the burials. While the problems appear to be mostly empirical (e.g., insufficient data on burial practices within a region, small sample sizes), speculation is also a problem; to what degree of probability can one identify group boundaries from artifacts, and thus infer migration has occurred?

Although mortuary analysis does not necessarily include a thorough examination of skeletal material (e.g., determine evidence of pathology or other geographically pertinent identifying characteristics), geolocational information may be obtained from the remains. Craniofacial robusticity and dental complexity and traits (e.g., incisor

shoveling, three-rooted mandibular molars) are known to exhibit populational and temporal variation (see Larsen 1997:311).

Bioarchaeological analyses have been incorporated into mortuary archaeology, thus facilitating the formation of biocultural interpretations of skeletal and associated burial treatment data (Knudsen and Stojanowski 2008; Prowse et al. 2010). While the physical characteristics of human skeletal remains can function as indicators of ancestry, health, occupation, and lifestyle, caution must be employed when offering interpretations of skeletal material. For example, mortuary and bioarchaeological analyses of structured ancient burials may yield a trove of information suggestive of populational demographics, but what knowledge or evidence indicative of migration can we glean from the analysis of the single modern burial of a homicide victim? Depending on the degree of trauma evident in the remains and/or taphonomic influences, the evidence may tell us more about the individual(s) responsible for the burial than the identity of the deceased. And what sweeping generalizations can be inferred from the remains of one individual, particularly considering the extent to which international relocation is observed within all populations? While subjectivity is inherent in the above approaches, standardization of methods and logical evaluations based on multiple lines of hard evidence are paramount in order to posit feasible interpretations that are inclusive of associated material culture.

## Biomolecular and Biogeochemical Evidence

DNA. The concept of DNA profiling as a method of positive identification emerged in 1985 when Alex Jeffreys discovered significant individual variation between individuals in particular regions of DNA (Gill et al. 1985; Jeffreys et al. 1985a; Jeffreys et al. 1985b). While early use of the DNA technique was applied primarily to questions of paternity (Jeffreys et al. 1985a), its utility in problems of human identification became unmistakable when DNA analysis positively identified skeletal remains as those of Josef Mengele (Jeffreys et al. 1992).

Several types of DNA profiling have been used in human migration studies. Nuclear DNA (nuDNA) and mitochondrial DNA (mtDNA) are both accessible in a cell, but different genetic information is available from each type of DNA (Butler 2005; Komar and Buikstra 2008). Nuclear DNA represents the combined parental genetic material (i.e., one copy of nuDNA inherited from each parent), whereas the non-recombinant nature of mtDNA means it is maternally inherited and, theoretically, is traceable back to a single female ancestral type (Fix 1999). Similarly, the non-recombinant properties of Y chromosome DNA means it is passed from a male to his male offspring, and thus has been of interest in genetic studies due to its potential to trace paternal lineage (Cox and Hammer 2010).

The extraordinary differential in number of copies per cell between nuDNA and mtDNA (2 and more than 1000, respectively) contributes to the unique identifying capabilities of each: nuDNA allows the positive identification of an individual while mtDNA confirms familial relationships. Due to the significantly higher number of mtDNA

copies per cell, the analysis of mtDNA is a viable option in cases where STR analysis of biological samples is impractical (e.g., severely decomposed or skeletonized remains) (e.g., Loreille et al. (2010) or nuDNA simply cannot be recovered (Bender 2000; Budowle et al. 2003; Goodwin and Hadi 2007; Decorte 2010). The likelihood that a mtDNA profile can be generated under these circumstances makes it an especially useful technique when applied to historical investigations (e.g., Coble et al. 2009, Romanov children) and modern forensic cases (e.g., O'Reilly 2007, the "Adam" case); mtDNA can be compared to any maternal relative if no immediate relative is available for identification of the body or a nuDNA comparison (Goodwin and Hadi 2007; Decorte 2010).

Mitochondrial DNA and Y chromosome DNA studies have been useful in proposing explanations for the major human migration events. Forster (2004) examined recent mtDNA studies in conjunction with archaeological and climatological data, concluding that paleoclimate played a major role in the chronology of prehistoric migrations and demographic expansions. Schurr (2004) has described major geographic trends in the distribution of mtDNA variation in the Americas. The mtDNA lineages present in Native American populations can be attributed to five founding groups (see Schurr 2004), all of which are differentially distributed at the continental level; it is probable that the distribution reflects original settlement patterns and regional interpopulational variation. Most of the Y chromosome genetic variation observed in Native American populations appears to be linked with similar distribution patterns present in Siberian populations, suggesting two different populations histories within the New World (i.e., a secondary expansion of ancient Asian populations) (e.g., Bianchi et al. 1997; Bianchi et al. 1998).

Conversely, previous analyses of Neandertal mtDNA sequences suggested that Neandertals and modern humans did not interbreed. However, the recent sequencing of an entire composite Neandertal genome from three individuals presents clear evidence of gene flow between Neandertals and modern humans (Green et al. 2010).

Until recently mtDNA and Y chromosome DNA were the two preferred genetic markers for studying human history (Cox and Hammer 2010). However, these two types of DNA are necessarily sex-biased and therefore cannot account for most of the human genetic variation factor of demographic reconstructions (Cox and Hammer 2010); mtDNA and Y chromosome DNA are just two tiny components of what is otherwise an enormous human genome. Nevertheless, both types of DNA analysis are useful in determining individual human identity provided biological evidence from potential relatives is available for comparison, or haplotype databases are sufficiently large enough to account for significant variation in haplotype frequencies within a particular population (Goodwin and Hadi 2007). While positive identification is facilitated only by nuclear DNA, ancestral haplotype markers are linked to geographic provenance, and can correlate with life history evidence (e.g., isotope markers from permanent teeth) to provide a general identity profile.

Stable Isotope Profiling. Hydrogen, oxygen, carbon, and nitrogen are biologically important chemical elements that vary in their isotopic forms (Katzenberg and Krouse 1989). Variation in the stable isotope composition of various biological material occurs with comparative predictability such that inferences regarding diet reconstruction from  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values (van der Merwe 1982; Klepinger 1984; DeNiro 1986) and the natal

origin of unidentified human remains may be formulated (Katzenberg and Krouse 1989). Hydrogen ( $\delta^2\text{H}$ ) and oxygen ( $\delta^{18}\text{O}$ ) isotope abundance values of meteoric water (e.g., preserved in ice cores) are indicators of climatic change (Dansgaard 1964) while  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values of animal and human tissue are linked to food and water intake (Estep and Dabrowski 1980; Estep and Hoering 1980; Schimmelmann et al. 1993; Sharp et al. 2003; Fraser et al. 2006; Fraser and Meier-Augenstein 2007).  $^{18}\text{O}/^{16}\text{O}$  and  $^2\text{H}/^1\text{H}$  isotope ratio analyses are germane to migration studies because their distribution in meteoric precipitation, which is consumed by humans in the form of drinking water and water contained in fruit and vegetables, varies geographically—and predictably—depending on climatic variables (Ehleringer et al. 2008). Similarly, the  $^{87}\text{Sr}$  composition in nutritional resources is dependent upon an area's geological characteristics, and thus can be useful as a geolocational indicator of the geographic origins associated with human skeletal remains (Beard and Johnson 2000; Rauch et al. 2007; Juarez 2008), provided the impact of global food consumption on measured strontium ratios (i.e., "the supermarket effect") is negligible (Nardoto et al. 2006).

Ericson's (1985) seminal work was the first to illustrate the potential utility of strontium isotope ratios in investigating childhood and marital residence patterns. Subsequent stable isotope studies investigating human origins began to proliferate in the early 1990's. Much work investigating the effectiveness of strontium isotopes in determining geographical origins and migration patterns has been conducted on European prehistory samples (e.g., Price et al. 1994a; Grupe et al. 1997; Budd et al. 2004; Evans et al. 2006), those from the American Southwest (Price et al. 1994b; Ezzo et al. 1997), South America (e.g., Andrushko et al. 2009) and diaspora communities

(e.g., Schroeder et al. 2009; Killgrove 2010). For example, Price et al. (1994a) have noted significant variation in  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios from two archaeological sites believed to belong to the Bell Beaker period, an era distinguished by the seemingly random geographical distribution of characteristic material culture. While results from this study and another (Grupe et al. 1997) are suggestive of variation in residence patterns within the Bell Beaker period, questions regarding the extent of relocation and frequency of movement are likely to remain unanswered without supporting ethnographic data and analysis of tissues representative of a person's lifetime. Similarly, preferential sampling procedures may yield inaccurate  $^{87}\text{Sr}/^{86}\text{Sr}$  values and inflate evidence of immigration (Ezzo et al. 1997).

Since  $\delta^{18}\text{O}$  values in drinking water are subject to alteration by climatic variables, the analysis of both  $^{18}\text{O}$  and  $^{87}\text{Sr}$  isotope data can allow the placement of further constraints on the geographic origins of individuals (Evans et al. 2006). More recent work examining isotopic signatures as indicators of geographic origin and immigration patterns has studied  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios in conjunction with  $^{18}\text{O}/^{16}\text{O}$  ratios to increase the robustness of the data and resulting inferences, e.g., the Americas (Schwarcz et al. 1991; White et al. 1998; White et al. 2000); early medieval (Budd et al. 2004; Evans et al. 2006); African diaspora (Schroeder et al. 2009); Imperial Rome (Killgrove 2010).

Origin and migration studies utilizing stable oxygen isotope techniques emerged in the early 1990's with Schwarcz et al.'s (1991) demonstration that human skeletal remains recovered in Canada were actually those of U.S. military personnel. Studies analyzing human skeletal material from several Mexican Teotihuacan archaeological sites have employed  $^{18}\text{O}/^{16}\text{O}$  ratio analysis of human tooth enamel samples from living

and archaeological samples to establish baseline isotopic data and confirm isotopically distinct populations (White et al. 1998; White et al. 2000; White et al. 2002; White et al. 2004b). Similar work by Prowse et al. (2004, 2005, 2007, 2010) has demonstrated the utility of oxygen isotope analysis in reconstructing the paleodiets and migration patterns of individuals interred in a Roman cemetery; childhood immigration from areas outside of Rome, possibly including North Africa has been strongly supported. More recently, Bell et al.'s (2009) multi-isotopic analysis of skeletal remains recovered from the *Mary Rose*, a 16th century sunken British warship, has suggested that a significant number of the ship's crew may have been non-natives. Particularly, the  $^{18}\text{O}/^{16}\text{O}$  isotope ratio data imply that these individuals may have originated from lower latitudinal regions in which English was not spoken. Bell et al.'s findings lend support to the theory that a language disparity between the crew members and the ship's officers resulted in a critical navigational error, which ultimately led to the sinking of the *Mary Rose*.

Due to the significant correlations between oxygen and hydrogen isotopes and climate variables, these two isotopic systems are frequently analyzed together to strengthen the resulting data. However, few of the growing number of geographic origins and migration studies employing  $^{18}\text{O}$  isotope analysis have included  $^2\text{H}$  analysis, presumably due to hydrogen exchangeability issues associated with extracting and measuring the  $^2\text{H}$  composition of various biological materials (DeNiro and Epstein 1981; Holobinko et al. 2011a; Landwehr et al. 2011). Following the seminal work of Cormie et al. (1994), most of the work involving the hydrogen isotopic analysis of collagen extracted from bone and teeth has been performed on animal samples to establish bone collagen as a proxy for climate variables such as humidity and precipitation (e.g.,



Reynard and Hedges 2008). However, the number of studies focusing on the  $^2\text{H}$  analysis of hair and fingernails is steadily increasing. These biological materials are suitable for making inferences regarding the last several months of an individual's life, so isotopic data obtained from keratin is more useful in determining recent movements or recent residence as opposed to birth place and childhood migration patterns; strands of hair may be measured along their entire length to assess the isotopic ratios incorporated at weekly or monthly intervals (White 1993; Sharp et al. 2003; Meier-Augenstein 2010).

### Discussion and Conclusions

While climate variability over the past two million years is presumed to have influenced human migration patterns (see Goldstein and Chikhi 2002), it seems doubtful at this juncture that global climate change is having a similar impact on modern migration patterns. What is clear is that people migrate for many different reasons. Undoubtedly many if not most people choose relocation as a means of improving their financial situations. While this assumption is accommodated by the complex economic migration models (e.g., labor-flow), Lee's (1966) push/pull causal factors may have more practical value in forensic contexts since the factors are clearly defined and correlate with amenities important to many individuals (e.g., social programs, climate). Similarly, recent research trends in migration studies of modern societies have focused on more contemporary themes (e.g., the effect of globalization on migration patterns) (Cushing and Poot 2004; Lucassen et al. 2010).

There are problems, however, with applying any migration theory to forensic investigations in which the identity of a deceased is unknown. It is this author's opinion that it is highly unlikely an initial assumption regarding identification would be based on migration theory. If identification documents are not present on the decedent, then other clues on the body (e.g., unique body modification) or those in the near vicinity giving some indication of identification are likely to be sought. If an item believed to be associated with the body is recovered, then perhaps the significance of the item can facilitate theories regarding the person's recent whereabouts.

In such cases where identity may be suspected but not verifiable, pinpointing an individual's geographic origins and possible residence just prior to death may contribute to definitive forensic identification. Perhaps a distinctive material item associated with the body can be traced back to the individual (e.g., hotel key card) or an activity in which he or she was a participant (e.g., ticket stub from a sporting event). Clothing can indirectly suggest an individual's origins; a body clad in winter apparel and recovered in a tropical climate might indicate that the individual was a very recent visitor to the area. If there are no material clues associated with the remains, then perhaps fingerprints may be retrieved and checked against a computerized database. While fingerprints are no longer considered irrefutable proof of identity in the United States (i.e., *U.S. v. Llera Plaza et al.* 2002), they remain a valuable tool for law enforcement in the U.S., and are considered a valid form of identification by other countries (see Chayko and Gulliver 1999). DNA may also be sampled from the individual and results checked against a database (e.g., CODIS) or compared to a relative's sample if the remains are believed to be those of a missing person for whom a report has been made and the relative is

available for comparison. Notably, DNA analysis of unidentified human remains is conducted primarily to establish a linkage between the deceased and an alleged perpetrator of the crime (Komar and Buikstra 2008). While DNA analysis can be performed on skeletonized remains, there are situations in which this method is not feasible and alternate techniques must be considered. Physical characteristics such as age, sex, ancestry, and stature may be ascertained from the remains and compiled into a forensic anthropological profile, which can then be compared against a missing persons report(s).

It is clear that some of the methods used to study migration in ancient populations are inherently flawed due to their subjective nature. For example, one of the problems in interpreting identity from material culture is the responsive nature of ethnic identity and the inadequacy of static assemblages in identifying past groups and their movements (Eriksen 1992; Jones and Graves-Brown 1996; Bernardini 2005). While a forensic investigator examining items found with a body has the advantage of evaluating the materials in a current context, he or she still must inject meaning into the objects if their significance is not immediately known.

Stable isotope profiling can provide investigators with data suggestive of geographic origins and recent residence. Notably, isotope studies, particularly multi-elemental studies, have led to the identification of bodies recovered many miles away from their homeland (Rauch et al. 2007; Meier-Augenstein and Fraser 2008; Font et al. 2015); corroborative DNA evidence in conjunction with isotopic data can further narrow law enforcement efforts to establish identification. Stable isotope data acquired during a homicide investigation in the Republic of Ireland prevented the imprudent search of

thousands of immigrant records for a fingerprint match (Meier-Augenstein and Fraser 2008). Consequently, DNA cross-matching analysis facilitated the identification of the victim (a Kenyan immigrant who unlawfully applied for asylum by claiming Somalian origins) and the apprehension and conviction of his killers. If regional origins can be identified, then the chances of identification are greatly improved and the incorporation of migration theory into the investigation is more feasible. However, there are cases in which stable isotope analysis and DNA evidence are insufficient to establish a positive identification (e.g., O'Reilly 2007; Schwarcz 2007). For example, stable isotope profiling of a child's torso recovered in London, England, revealed that the boy had most likely originated from West Africa; these findings supported haplotype data obtained from DNA evidence (i.e., mtDNA and Y chromosome analysis) (O'Reilly 2007). Despite multiple lines of evidence suggesting the child may have been a victim of human trafficking and subsequent ritual murder, the remains were not identified until years later when the child's former caretaker came forward with information concerning the case.

Much speculation surrounds the historical and current migratory events mentioned previously, yet little if any attention has been placed on the significance of migration as it relates to forensic investigations. For example, the applicability of diaspora characteristics to contemporary human movement patterns makes this type of migration event pertinent to forensic contexts. It is clear that significant diversity is evident among diaspora communities (Hodge 2005) and interactions between diasporas and homelands and host communities are complex (Lilley 2004); no longer can these groups of individuals be characterized simply by forced dispersal and victimization. More attention to migration behavior associated with involuntary relocation and

temporary migration patterns would provide a more comprehensive understanding of these events as they have occurred in the past and as they evolve in contemporary societies. Further, closer scrutiny of the relationship between human migration patterns and the perception of such by law enforcement agencies handling forensic cases in which potential immigrants – documented or undocumented – are involved is advisable.

In conclusion, while most of the human migration theories used by researchers studying past populations are helpful in positing theories of group identification, they are of more limited value to the forensic investigator. The modern context of forensic casework necessitates a holistic approach in which identification of the decedent is paramount to the investigation.

CHAPTER 4

FORENSIC HUMAN IDENTIFICATION IN THE U.S. AND CANADA: A REVIEW OF  
THE LAW, ADMISSIBLE TECHNIQUES, AND THE LEGAL IMPLICATIONS OF THEIR  
APPLICATION IN FORENSIC CASES

Introduction

The definitive and early identification of human remains is of the utmost importance regardless of the circumstances under which the remains were discovered (e.g., crime scene, mass disasters). The use of expedient and established identification methods may become particularly significant when the context of the incident precludes readily available documentation (e.g., driver's license, passenger manifests) that is useful for initial and often general identification of remains. While identification methods such as distinguishing physical characteristics, forensic odontology, and DNA profiling are a few of the options available for reasonably immediate identification, the aforementioned may not be particularly useful in criminal cases where general identification of a deceased is virtually impossible due to extreme mutilation or skeletonization, the absence of medical or dental records, or relocation of a body to a region far from its origins (e.g., no maternal or immediate relative is available to submit a tissue sample for comparison), respectively.

Forensic human identification techniques are successful if they lead to positive personal identification. However, the strongest personal identification is of no use in the prosecution – or vindication – of an accused if the associated evidence and testimony is ruled inadmissible. For example, DNA evidence can become contaminated and subsequently repudiated. Similarly, fingerprints, generally considered an acceptable method of verifying identity, have been judicially challenged as a method of positive identification (*USA v. Plaza, Acosta and Rodriguez* 2002). This previously published review (Holobinko 2012b) examines the U.S. and Canadian legal rulings regarding the admissibility of expert evidence and testimony, and subsequently explores four established methods of human identification (i.e., DNA profiling, forensic anthropology, forensic radiography, forensic odontology) and the legal implications of their application in forensic cases. Stable isotope profiling, an analytical technique in which the isotopic similarity (i.e., provenance) of two substances can be compared, is also examined for its utility as a complementary method in determinations of forensic human identification.

### Evidentiary Guidelines

Despite differences between the United States and Canada judicial systems – criminal courts in Canada emulate British systems (Komar and Buikstra 2008) – multiple similarities exist. The following section reviews the Canadian and U.S. evidentiary guidelines and legal rulings pertinent to felonious crimes in which the positive identity of a victim (or accused) is in question.

## Evidence

Testimony, documents, and physical evidence presented in a court of law with the intention of proving or disproving an assumption are all forms of evidence (Komar and Buikstra 2008). Evidence is either observable and verifiable (e.g., blood spatter) or subject to individual perceptions (e.g., motive). Objective and subjective evidence can be further classified as circumstantial (i.e., speculative in nature), material (e.g., eyewitness testimony based on personal observation), and physical (e.g., shoe impressions left at a crime scene). Many forms of evidence categorized as one type may share characteristics indicative of another. For example, videotape from a surveillance camera provides an objective account of an event, while eyewitness testimony regarding the same incident may be subjective. Physical evidence, such as blood spatter at a crime scene, is inherently objective and analyzed using scientific methodology. However, evaluation of the data may include subjective interpretations.

Categorically, any material with evidentiary value can be placed into one of two groups (Komar and Buikstra 2008). The evidence may be identified as belonging to a particular group in which all members share similar characteristics (i.e., class evidence) (e.g., 12 gauge shotguns). Subsets within a class are created based on the number of traits shared by their members (e.g., a Remington brand 12 gauge shotgun). Conversely, an item may possess characteristics so distinct that it can be distinguished from other similar items in the same class (e.g., a 12 gauge Remington shotgun with uniquely modified barrel characteristics). In this case, when the evidence can be positively identified and traced back to a single source, it is considered individual evidence. Data generated by the individualized forensic human identification techniques



reviewed here are examples of individual evidence, and have been used to establish positive identification. In many forensic cases this implies the introduction of inculpatory evidence (i.e., that which implies or establishes guilt) to support the prosecution's argument against an accused. However, "positive identification" is not synonymous with victim identification. In some cases physical evidence has been introduced to exclude an accused as the perpetrator of a crime (i.e., exculpatory evidence).

### Chain of Custody

The evidentiary value of an item is subject to the interpretation of that evidence by those individuals responsible for presenting it to a judge and jury. The collection and preservation of such evidence (i.e., continuous chain of custody) goes directly to its admissibility in court (Komar and Buikstra 2008). Maintaining the integrity and security of evidence from its initial discovery to final disposition is crucial, and requires painstaking documentation. Since evidence is often transferred to other individuals for temporary retention or processing, each transfer must be well-documented; investigators must be aware of the exact location of evidence at all times. For example, while the body of a homicide victim may be taken to a morgue for storage, a sample of the victim's blood may be transported to another facility for toxicological screening. A subsample of the tube consequently may be transferred to a different lab for DNA profiling. Access to evidence is restricted to designated individuals in order to prevent unauthorized personnel from tampering with the evidence. A breach in any of the above protocol is equivalent to a break in the chain of custody (evidence). Such violations can have unforeseen and extensive legal ramifications; criminal cases involving strong

evidence against a defendant (e.g., State of California v. Orenthal James Simpson) have been lost because of questionable evidence collection and the resulting inadmissibility of that evidence (Aaseng 1995; Schuetz and Lilley 1999).

Chain of custody procedures often are specific to evidence type. For example, evidence continuity guidelines specific to forensic anthropological investigations have been outlined by Melbye and Jimenez (Melbye and Jimenez 1997), while blood samples are subject to different collection, preservation, and transport protocol (Schiro 2009).

### Expert Witness Testimony

The testimony provided by an individual to a court of law can be offered orally, under oath, or in a sworn written affidavit (Komar and Buikstra 2008). The three types of witness testimony, material, fact, and expert, vary according to the type of evidence to which witness is provided, and the degree to which testimony can be compelled by a judge. Eyewitnesses and character witnesses, generally public citizens, qualify as material witnesses; testimony from a witness to a crime can be compelled by the court. Fact witnesses include law enforcement and other individuals involved in the processing of crime scenes and evidence. Testimony from these individuals during the course of criminal proceedings is considered a professional obligation, and generally does not warrant the issuance of a court order. Expert witnesses possess qualifications specific to a particular area of knowledge. While material and fact witness testimony is based on direct knowledge or observation of an event or individual, expert witness

testimony is based on observation and experience, and is the only type of testimony beyond the realm of judicial coercion.

While changes in rules of evidence have effected concordant changes in some areas of expert witness testimony (Auxier 1989), generally the testimony of experts must adhere to several common principles (Auxier and Prichard 2001). First, testimony should be free of jargon; clear presentation of complex material to the layperson, whether judge, attorney, or juror, is essential. Second, the factual information presented in court should be accurate, objective, based on sound scientific principles, and free from bias. Without these fundamental components of expert testimony and the effective communication of such, the credibility of the data – and expert witness – may be compromised. Auxier and Prichard (2001) suggested that expert witnesses prepare extensively before testifying in court: impartiality and integrity during scientific analysis and subsequent testimony should be maintained at all times; reference and presentation materials should be kept nearby so they are easily producible if required; testimony should concern the scientific methods actually employed during analysis of the facts.

The qualifications of an expert witness vary considerably depending upon the methodologies employed. For example, a medical examiner in the United States may qualify as an expert witness provided he or she is in possession of a valid medical license and certification from a licensing board in forensic pathology (Komar and Buikstra 2001). Other professionals are required to have a Ph.D., M.D. or another terminal degree. These credentials often vary according to jurisdiction, and are determined by the presiding judge (Graham 2003). While opposing counsel may

question the training and knowledge possessed by a witness, only a judge may qualify the witness as an expert. United States and Canadian rules of evidence acknowledge the authority of the judge as “gatekeeper” in the final determination of which methods and theories may be introduced as admissible evidence, and which qualifications justify expert status. While the admissibility of evidence and expert testimony is a less complicated issue for the Canadian courts (Rogers and Allard 2004), recent decisions in Canadian law imply a significant influence by more stringent American rulings (i.e., *Daubert v. Merrell Dow Pharmaceuticals, Inc.*) (Glancy and Bradford 2007).

## Forensic Law

### U.S. Rulings

The Federal Rules of Evidence (FRE), implemented by the U.S. Congress in 1974, established the original standards for federal evidentiary matters (Gold et al. 1993). In particular, FRE 702 is one of three FRE concerning testimony by experts. Criteria for witness qualification and admissibility of scientific evidence are based on: the expert’s possession and demonstration of relevant skills, education, or experience; the judge’s ultimate authority on decisions regarding admissibility and reliability of the expert witness testimony; the testimony’s derivation from scientific data or knowledge which has been consistently applied to the facts of the case using reliable scientific methodology (Graham 2003).

While it appears that FRE 702 restricts expert witness testimony to particular scientific disciplines (Bruce 2006), its original intent may have been to avoid

introduction of subjective testimony in which no justification is offered for a particular method (Brautbar 1999). In other words, the function of FRE 702 was to encourage “intellectual rigor” in the employment of methodology and similarly apply this approach to the analysis of evidence related to the criminal proceedings. Thus, the rule focuses on scientific methodology and not agreement between various expert testimony (Brautbar 1999).

Subsequent to FRE 702, the federal appeals court case *Frye v. United States* (293 F.2s 1013, 1923) was responsible for establishing the first standard by which the admissibility of most scientific evidence in U.S. Federal Courts was judged (Gold et al. 1993). While FRE 702 addressed the qualification of scientific evidence and expert testimony, the Frye rule required that the scientific knowledge or test upon which the testimony or evidence was based should be generally accepted within the field from which it was derived (Masten and Strzelczyk 2001).

For most of the twentieth century, expert witness testimony and scientific evidence was subjected to the Frye standards, which included the judiciary authority to restrict introduction of pseudoscientific methods or principles (Masten and Strzelczyk 2001). However, Huber (1991) noted a significant number of personal injury liability cases in which the Frye criteria were not satisfied and judgment errors regarding admissibility facilitated disproportionate penalties and punitive damages. As long as the proposed expert witness was sufficiently qualified and willing to testify with a “reasonable degree of scientific certainty,” courts seldom looked farther into the details of the scientific evidence (Masten and Strzelczyk 2001). Not only did the admission of factually unsubstantiated expert testimony or evidence result in enormous liability judgments

(Huber 1991), in some cases DNA evidence was excluded due to the technique's recent development and implementation (Masten and Strzelczyk 2001).

Daubert v. Merrell Dow Pharmaceuticals (No. 92–102 509 US 579, 1993) had a significant impact on the admissibility of scientific testimony and evidence in the United States. The use of Benedectin, a morning sickness drug manufactured by Merrell Dow, was prohibited in 1983 after the filing of numerous lawsuits alleging the drug caused birth defects (Gold et al. 1993). Prosecutorial testimony was based on in vitro studies, animal research, chemical analyses, and a novel epidemiologic analytical method. Epidemiologic testimony was ruled inadmissible because it was based on methodology that had not been subjected to rigorous review in the relevant scientific community. Further, the supporting data derived from this analytical method were not statistically significant according to previously established standards. Ultimately, Daubert was dismissed by the court based on the drug's ostensible safety record and the plaintiff's failure to produce evidence justifying their assertion (i.e., the Frye precedent). The case was appealed, and the original trial court's decision was upheld by a federal appeals court (Gold et al. 1993) and the U.S. Supreme Court, which ruled that the "general acceptance" requirement of the Frye test conflicted with FRE 702 (Auxier and Prichard 2001); the ambiguous nature of "general acceptance" conflated genuine issues of fact and was incompatible with the FRE.

The Daubert decision was intended to clarify some of the ambiguous tenets of FRE 702 and the subsequent Frye ruling by requiring scientific evidence to meet reliability and relevance standards. Hence, the Court stated that the expert testimony must have evidentiary reliability, and required the theoretical or methodological underpinnings of

the testimony to be scientifically valid (Auxier and Prichard 2001). Specifically, the trial judge as “gatekeeper” determines whether the proposed expert opinion meets the reliability standard by judging it according to the following criteria: Has the theory or method already been tested, or can it be tested? Has the method or theory been peer-reviewed and published in a corresponding scientific journal? Is the error rate for the technique known or potentially determinable? Finally, is the method or theory accepted within the scientific community from which it has developed (i.e., the Frye test) (Brautbar 1999; Auxier and Prichard 2001; Komar and Buikstra 2008)? If potential expert witness testimony is to be introduced as evidence in a criminal court case for clarification purposes, then it must address evidentiary matters (Auxier and Prichard 2001). If this relevancy requirement is not met, then the testimony is of no use to the judge in his or her determination of fact regardless of the method’s reliability or proven utility. However, evidence meeting the reliability and relevance criteria is not guaranteed admissibility; FRE 403, one of the three FRE on which the Daubert decision is based, prohibits the admission of scientific evidence in cases where its potentially prejudicial, confusing, or misleading nature outweighs its probative value.

It is important to note that the Daubert ruling applies only to U.S. federal courts observing the FRE (Masten and Strzelczyk 2001). Indeed, while most states have adopted the Daubert evidentiary standards which amended FRE 702, some states adhere strictly to Frye, while the remaining few apply their own unique tests on a case-by-case determination to reflect a balance of relevance and materiality with judicial prejudice (Kaufman 2006). As evidence admissibility criteria vary among states, so does the application of Daubert to specific techniques. For example, DNA is admissible

under both Frye and Daubert (Rogers and Allard 2004); reliability goes to the proper performance of protocols and probability estimates. Conversely, evidence and expert testimony regarding handwriting, firearms/toolmarks, and latent prints, all of which are grounded in widely accepted techniques, fail Daubert (Tierney 2010). Regardless, the courts have admitted such evidence, qualifying the methods as “technical” knowledge under FRE 702. Further, experts are permitted to demonstrate association, but are prohibited from linking their observations with an “identification” as stated in FRE 403 (e.g., U.S. v. Hines 1999, handwriting; U.S. v. Green et al. 2005, firearms; U.S. v. Llera Plaza et al. 2002, fingerprints) (Tierney 2010).

Ostensibly Daubert was meant to clarify the convoluted nature of the application of FRE 702 and the Frye test. Instead, much confusion, misinterpretation, and misapplication of Daubert criteria to proffered evidence and expert testimony has arisen (Brautbar 1999). These quandaries can be traced, in part, back to the relative inexperience of judges and juries in evaluating scientific evidence and expert testimony (Kaufman 2001).

### Canadian Rules of Evidence

Regulated by the Canada Evidence Act (R.S., 1985, c. C-5) (Canada Department of Justice 2011), the Canadian counterpart to the U.S. FRE concerning expert witness testimony can be found in Kelliher (Village of) v. Smith ([1931] S.C.R. 672). The Supreme Court of Canada declared that in order for testimony to be considered “expert” it must pertain to subject matter that an average person, without the assistance



of the appropriate expert, cannot comprehend sufficiently enough to form correct judgments about the facts of the case.

In the above decision, Smith (plaintiff), a councilman for the village Kelliher (defendant), was severely injured when the chemical fire extinguisher he was operating on municipal property exploded due to poor maintenance practices (Kelliher (Village of) v. Smith ([1931] S.C.R. 672). The jury found that the injuries incurred by the plaintiff occurred as a result of the defendant's negligence (i.e., poor record of inspections and failure to maintain the equipment in proper working order). Plaintiff's contributory negligence was limited to his role as a village councilman, and not his operation of the fire extinguisher. Upon appeal, the Supreme Court upheld the original ruling. In his commentary, Lamont JJ stated that although the contended testimony was admissible because it was deemed expert, it was his opinion the jury were just as qualified as the expert witnesses to form a correct judgment about the plaintiff's operation of the fire extinguisher [emphasis added].

The Regina v. Mohan ([1994] 2 S.C.R. 9) decision arose from a case in which a pediatrician was accused of sexually assaulting four of his female patients (Rogers and Allard 2004; Glancy and Bradford 2007). Defense counsel offered expert testimony from a psychiatrist who intended to testify that perpetrators of such crimes belonged to a select group(s) of individuals exhibiting sexually deviant tendencies, and that the defendant did not possess the characteristics indicative of membership in that class(es) of individuals (Glancy and Bradford 2007). While the trial judge ruled the proffered testimony inadmissible because it went to character, the Ontario Court of Appeal disagreed, noting that opinion evidence regarding the likelihood of an individual's

membership in a particular group characterized by abnormal proclivities was relevant and should be judged based on its relevancy, not its sufficiency. Sopinka JJ elaborated on the Court's decision – and the Kelliher v. Smith ruling – by stating that the proffered expert evidence must not only be outside the experience and knowledge of the trier of fact and therefore necessary for proper consideration of the facts of the case, it must also be relevant (i.e., meeting a basic threshold of reliability and scientific consensus) (Bruce 2006). The development of and scientific consensus supporting a standard profile for an offender committing a particular type of offense satisfied the relevancy criteria. However, the Mohan Court found the defense expert evidence lacking in standardization, and ruled it unreliable and thus inadmissible (Glancy and Bradford 2007).

The Mohan decision, characterized by Sopinka JJ's ruling, established four governing factors of evidence admissibility: necessity, relevance, absence of exclusionary rule (i.e., evidence has not been illegally or inappropriately obtained), and proper qualifications of the witness (Rogers and Allard 2004; Glancy and Bradford 2007). While Canadian guidelines regarding the admissibility of expert evidence and testimony are generally less complicated than the U.S. FRE, they are not without issue. Similar to Daubert, Mohan made significant progress in defining the legal criteria available to judges in their analysis of a case; however, it has left many matters unresolved (Bruce 2006; Glancy and Bradford 2007). For example, the Canadian Supreme Court did not adequately explain "reliability" standards (Komar and Buikstra 2008). As a result, lower courts have been provided with little direction in accurately identifying the legally required characteristics of expert testimony. While new theories

and methods are to be subjected to intense scrutiny, ultimate determinations of reliability are left to the trier of fact. Further, terms crucial to the Mohan decision (e.g., “novel scientific theory,” “special knowledge,” “basic threshold”) were left undefined, and caused considerable confusion in subsequent cases (e.g., *R. v. McIntosh* [1997] 117C.C.C. (3d) 385 (Glancy and Bradford 2007)).

While the Daubert and Mohan rulings differ in their fundamental composition, additional discrepancies also are evident. Canadian relevance criteria require evidence to be evaluated *prima facie* (i.e., on first appearance) before being subjected to further tests of essentialism (e.g., does its potential for prejudicial bias outweigh its probative value) and reliability (Komar and Buikstra 2008), a term previously established as vague in nature. Further, experts in Canada are permitted, at the discretion of the trial judge, unlimited discourse on the “ultimate issue” before the court (e.g., was the defendant insane at the time he allegedly committed the crime?). Additionally, the U.S. exclusionary rule appears to be used in a less restrictive manner while use of the Canadian exclusionary rule has expanded considerably, presumably due to the 1982 enactment of the Canadian Charter of Rights and Freedoms (Nasheri 1996). Prior to 1982 the exclusionary rule was rarely used in Canada while the opposite was observed in the U.S.

Despite notable differences in evidence admissibility criteria, it appears that U.S. law is influencing recent Canadian decisions. In *Regina v. J. L.-J.* (2000), the trial judge excluded defense expert testimony on what he declared was a “novel” science (Rogers and Allard 2004; Glancy and Bradford 2007). While penile plethysmography is a component of phallometric testing, a valid method routinely forming the basis of expert

testimony in Canadian sentencing hearings, but not at the trial level, the trial judge declared the testimony unreliable for exculpatory purposes. Upon appeal, the Supreme Court reviewed the Mohan decision and issued the following statements. The expert evidence satisfied the subject matter necessity requirement, and was acceptable/met the Mohan “novel science” criteria. However, the Justice rejected the “general acceptance” concept found in Frye. Although noting that Daubert should be interpreted in the context of the U.S. FRE, the Justice agreed that the evidence met the reliable-foundation test outlined in Daubert (Glancy and Bradford 2007). However, due to other insufficiencies in the expert evidence and testimony, the Supreme Court concurred with the original trial judge that the expert opinion was not scientifically grounded. The consideration of Daubert principles, while not specifically outlined in the judiciary conclusions, appears to have been incorporated into Canadian law in that the Court now “recognizes those principles for acceptance of novel science” (Glancy and Bradford 2007:354. Patry et al. (2007) proposed that R. v. J.-L.J. extended Mohan by incorporating the admissibility principles from Daubert: testing of the technique; peer review and publication; known or potential error rates; general acceptance. Court deliberations based on Daubert and Mohan rulings suggest the Canadian Courts are moving toward more rigorous legal evaluations (Glancy and Bradford 2007).

### Personal Identification

In many cases the identity of a decedent is known or suspected at or around the time of death; most individuals carry some form of identification (e.g., driver’s license).

The identification of decedents who have been transported to a morgue may only require, in some cases, supplemental documentation (e.g., medical and/or dental records) to confirm what is already known or suspected. Anecdotal identifications, or those made through visual recognition or with the assistance of personal artifacts, are valuable methods of identification (Komar and Buikstra 2008). However, they carry no scientific credibility, and thus can be prone to error. There are also many circumstances in which identification cannot – or should not – be made anecdotally. Severe mutilation or decomposition, the emotive state of the deceased's friends or relatives, and changes in physical attributes of the decedent due to the passage of time or aging are all factors which may facilitate a misidentification (Black et al. 2010).

The medicolegal authority responsible for identifying unknown individuals in most jurisdictions is the coroner or medical examiner (Murphy et al. 1980; Weedn 1998; Rogers and Allard 2004; Blythe and Woodforde 2007). As there are different methods of establishing identification, there are also different identification categories (Komar and Buikstra 2008; Wiersema et al. 2009). Collectively these three categories form a hierarchy; each category is characterized by particular attributes that correspond with a level of certainty. The process of identification is progressive, and based on a series of attribute inclusion and exclusion. At the base of the identification hierarchy is tentative identification (i.e., confirmatory, directed), which implies suspected identity based on circumstantial materials (Komar and Buikstra 2008). The body of a deceased received by morgue personnel might be tentatively identified based on procurement of a driver's license from the decedent's wallet, provided the decedent's facial features have not been rendered unrecognizable. A decedent with no associated artifacts suggesting

identity would be assigned a designation of John Doe, Jane Doe, or simply Doe if the sex is indeterminable. Presumptive identification requires the inclusion of additional identification attributes such as those characterizing the decedent or place of death. A positive identification requires the successful demonstration of individualization (i.e., an expert's confirmation of a particular set of characteristics unique to the deceased) (Rogers and Allard 2004; Komar and Buikstra 2008). Fingerprints, nuclear DNA, and medical/dental records have all been accepted as methods of positive identification, although not all meet the admissibility criteria described previously. In the U.S. and Canada fingerprinting has an established history as a means of identification (Moore 2006). However, the admission of fingerprint evidence can be challenged (e.g., Page et al. 2011a; Page et al. 2011b). In 2002 the Eastern District Court of Pennsylvania ruled latent print evidence inadmissible as a method of positive identification (*U.S. v. Llera Plaza et al* 2002). Although the order was later vacated, the presiding judge initially concluded that the method employed to match unknown fingerprints retrieved from a scene to reference samples obtained from a suspect did not meet Daubert admissibility criteria (*U.S. v. Llera Plaza et al* 2002). While judicial gatekeepers have admitted forensic identification evidence (e.g., latent print evidence) failing to meet Daubert minimum standards (e.g., *State v. Pope* 2008, see Cole 2010), latent print testimony claiming absolute individualization is generally unsupported. Fingerprint evidence introduced in U.S. criminal proceedings may be used only to infer the probability of identity and not as a method of positive identification (*U.S. v. Llera Plaza et al* 2002; Moriarity and Saks 2005; SWGFAST 2009). Conversely, the Canadian courts generally have not questioned the validity of fingerprint evidence as a method of establishing

identity despite the technique's lack of standardization, U.S. legal influence, and the strict Mohan criteria (see Chayko and Gulliver (Robichaud 2009)). Although a number of methods have a proven utility in the development of a positive identification, only DNA and hard tissue techniques will be reviewed here.

## DNA

The concept of DNA (deoxyribonucleic acid) profiling as a method of positive identification emerged in 1985 when Alex Jeffreys discovered significant individual variation between individuals in particular regions of DNA (Gill et al. 1985; Jeffreys et al. 1985b; Jeffreys et al. 1985b). When the technique was first introduced the unique patterns of the polymorphic regions facilitated the designation of "fingerprint." Currently this pattern is referred to as a "profile" (Goodwin and Hadi 2007). DNA profiling has two forensic human identification applications: the positive identification of decedents and the analysis of biological material to confirm or exclude identity of an accused (Komar and Buikstra 2008)). While early use of the DNA technique was applied primarily to questions of paternity (Cole 2010), it was successfully used in the 1988 prosecution of a rape case in the U.K. (Jeffreys et al. 1992). Its utility in problems of human identification became unmistakable when DNA analysis positively identified skeletal remains as those of Josef Mengele (Jeffreys et al. 1992).

One strand of nuclear DNA consists of a randomly ordered series of four nucleotides. Each nucleotide is composed of one of four nitrogenous bases (adenine (A), guanine (G), thymine (T), or cytosine (C)), a sugar, and a phosphate group (Goodwin and Hadi 2007; Brown 2010). DNA in its double-stranded form resembles a

helical ladder, two linear sugar–phosphate strings linked by complementary nitrogenous bases (A–T or G–C) that form base pairs. It is the random ordering of nucleotides on each strand that contributes to a unique DNA profile. Since the number of base pairs is equivalent to the number of nucleotides present in DNA, and since the human genome (23 chromosomal pairs) consists of approximately three billion base pairs, individualization between different human DNA profiles is highly likely (Butler 2005).

Depending on the type of DNA utilized and method of polymorphic analysis, there are several types of DNA profiling possible. Nuclear DNA (nuDNA) and mitochondrial DNA (mtDNA) are both accessible in a cell, but different genetic information is available from each type of DNA (Butler 2005; Komar and Buikstra). Nuclear DNA represents the combined parental genetic material (i.e., one copy of nuDNA inherited from each parent). While there are just two copies of nuDNA in each cell, its tremendous number of base pairs allows it to uniquely identify an individual or monozygotic twins; genetic variation increases as the degree of genetic relationship decreases (Black et al. 2010). Conversely, the number of copies (>1000) of mtDNA in a single cell exceeds that of nuDNA, but fewer base pairs (16,569) are present. The most significant characteristic with regard to using mtDNA as a method for establishing human identification is that mtDNA is not unique to an individual but rather that individual's maternal lineage. While the contribution of mtDNA is undeniably valuable as a method of identification, it does not constitute positive identification.

Virtually all DNA (~99.5%) is identical between individuals (Goodwin and Hadi 2007). How, then, is it possible to construct a DNA profile unique to each person? This can be accomplished by analyzing polymorphisms, those regions of the genome which



differ between individuals. Tandem repeats are a class of polymorphisms whose characteristics make them particularly suited for forensic human identification purposes. The first of these to be used for DNA profiling were the variable number tandem repeats (VNTRs) (Goodwin and Hadi 2007; Decorte 2010); this hybridization method of DNA analysis generated relatively simple, straightforward profiles (Nakamura et al. 1987; Odelberg et al. 1989; Budowle et al. 1990; Wayne and Fourney 1990). The large quantities of genetic material required to perform VNTR analysis limited the technique to biological samples for which a large amount of well-preserved DNA was available. VNTR profiling was subsequently replaced with short tandem repeats (STRs) analysis (Goodwin and Hadi 2007). Short tandem repeats are relatively short (~100–350 bp) pieces of DNA exhibiting significant polymorphic variability. STR polymorphisms are ideal for DNA profiling because very little material is needed; small amounts of poor quality DNA obtained during forensic investigations are often the only samples available. Development of the polymerase chain reaction (PCR) technique permitted the use of even degraded genetic material (Holland et al. 1993; Lygo et al. 1994), since the enzymatic process amplifies an existing region of DNA by replicating it many times over (White et al. 1989; Komar and Buikstra 2008; Decorte 2010). The new techniques cannot, however, mitigate all degradation issues. The integrity of a DNA profile can be affected by the type of tissue sampled and the degree of decomposition present (Black et al. 2010). While analytically available DNA is present in teeth, bones, muscle, skin, and hair, a significant postmortem interval renders the soft tissues less likely to produce a good DNA profile.

The generation of a DNA profile using STR analysis can be characterized as follows (Goodwin and Hadi 2007; Black et al. 2010). Upon collection of the appropriate biological evidence, an appropriate extraction technique is selected based on the type of material submitted for analysis (Sambrook et al. 1989; Walsh et al. 1991; Scherczinger et al. 1997; Greenspoon et al. 1998). Following extraction (i.e., the separation of DNA from other cellular material), the amount of DNA is quantified to determine the optimal amount required for amplification (PCR). Target DNA identified from the original sample is denatured, renatured, and subsequently extended; the final copy obtained through PCR is identical to the original target sample. After the PCR reaction the DNA molecules are separated according to size using gel electrophoresis; fluorescent dyes are applied to the various PCR products to aid in the detection of PCR products from different loci. A profile is subsequently generated and then compared with a reference, if one is available. If the two match, then the generated profile is declared an inclusion, and the degree of similarity is calculated using probability estimates; if the two differ, then it constitutes an exclusion (Goodwin and Hadi 2007).

Due to the significantly higher number of mtDNA copies per cell, the analysis of mtDNA is a viable option in cases where STR analysis of biological samples is impractical (e.g., severely decomposed or skeletonized remains) (Loreille et al. 2010) or nuDNA simply cannot be recovered (Bender et al. 2000; Budowle et al. 2003; Goodwin and Hadi 2007; Komar and Buikstra 2008; Decorte 2010). The likelihood that a mtDNA profile can be generated under these circumstances makes it an especially useful technique when applied to historical investigations (Coble et al. 2009, Romanov children); mtDNA can be compared to any maternal relative if no immediate relative is

available for identification of the body or a nuDNA comparison (Goodwin and Hadi 2007; Decorte 2010). However, the fact that mtDNA is maternally inherited makes it an unsuitable method for establishing positive identification. Since mtDNA is not unique to an individual, interpretation is based on three categories of results: exclusion (two or more nucleotides differ), inconclusive (difference at one nucleotide position), or failure to exclude (no differences) (SWGDM 2003). Mitochondrial DNA evidence proffered in support of a positive identification exhibits much weaker probability estimates than those associated with nuDNA evidence (Komar and Buikstra 2008).

In 1998 the U.S. Federal Bureau of Investigation (FBI) introduced the Combined DNA Index System (CODIS), a three-tier (local, state, and national) database containing DNA profiles of: (1) convicted offenders, (2) arrestees, provided state laws permit sample collection, (3) samples collected from crime scene evidence, (4) missing persons for reference purposes, (5) samples voluntarily contributed by relatives of missing persons, (6) unidentified human remains (FBI 2010). As of July 2010, the convicted offender index contained 8,646,417 profiles; the forensic crime scene index profiles totaled 328,067. In total, 119,764 investigations have been assisted in some manner by CODIS. Similarly, Canada passed legislation in 1998 that allowed the creation of a National DNA Data Bank (NDDB) (RCMP 2006). The Data Bank contains DNA profiles generated from biological samples obtained from two sources: convicted offenders and crime scenes. No index for DNA profiles generated from samples donated by the relatives of missing persons is currently maintained by the NDDB. More than 231,000 samples have been received from convicted offenders; crime scene index

profiles total 63,514 (RCMP 2011). Over 18,000 investigations (primarily breaking and entering cases) have been aided by NDDB profiles.

Initial applications of DNA were used to determine paternity (Jeffreys et al. 1985a). As technology improved and investigators realized the utility of DNA profiling, forensic applications of the method grew considerably. DNA analysis has been used successfully not only as a method of victim and perpetrator identification but as a method of exonerating the wrongly convicted. Ten years after receiving a death sentence for rape and murder based on bite mark evidence, Ray Krone was released after exculpatory DNA testing indicated someone else had committed the crimes (Deitch 2009). In his review of forensic identification science, Saks (2010) espouses “the DNA model” as a research strategy that can facilitate the validation of data from other fields by reducing error risks. Instead of analysis based on the concept of “uniqueness”, DNA typing relies on objective data collection and the subsequent determination of probabilities concerning the likelihood of a particular profile being present in a particular population.

Indeed, there is no question as to the admissibility and validity of DNA profiling as a method of human identification. However, while DNA is admissible under Frye, Daubert, and Mohan standards, the reliability issue goes to proper laboratory protocol and probability estimates. DNA analysts working with forensic samples face a variety of difficulties: contamination from exogenous DNA introduced at the crime scene or during collection, preparation, or analysis; mixed samples (commingling of biological material within the same sample); sample quantity (i.e., insufficient quantity); PCR inhibition due to small sample size or degradation (Komar and Buikstra 2008). These analytical

challenges, in addition to the sometimes uncontrolled conditions under which forensic evidence is collected and the variability in laboratory practices, have led to doubts concerning potential error rates and the subsequent reversals of erroneous convictions (Lynch 2003).

Examiner bias has been responsible for interpretation difficulties and outright wrongful convictions. Thompson (1995) noted differential autorad (i.e., final product showing band patterns) scoring between various laboratories; discrepancies were due to disparities in skill levels and inherent observer bias. Giannelli (2008) reviewed the link between wrongful convictions, forensic science, and the need for crime lab regulation. He noted that while stringent DNA profiling regulations have developed gradually over the last two decades, numerous errors occurred that led to erroneous convictions; more than 200 convicts have since been exonerated with DNA testing. It is important to note that forensic evidence was presented in just 57% of these cases, and that it was the second leading type of evidence (following eyewitness identification at 79%) used in wrongful conviction cases. Pre-DNA serology of blood and semen evidence, which at the time lacked the discriminatory properties of DNA profiling, was the most frequently used forensic technique. Only three DNA cases were linked with a wrongful conviction. More than 25% of the 200 cases involved faulty lab analysis or testimony, and perjury.

### Forensic Anthropology

Stewart (1979) defined forensic anthropology as physical anthropology applied to the identification of skeletonized human remains in a medicolegal context. While the current definition of forensic anthropology has broadened to include subfields such as

forensic taphonomy, the individual identification of human remains continues to be a primary goal. The identification process begins with the creation of a biological profile, or osteobiography (Komar and Buikstra 2008). Data collection procedures begin with the identification of the remains as human or non-human (Snow 1982). Once the remains have been identified as human, then the anthropologist determines the minimum number of individuals present, and begins gathering information on distinguishing physical attributes. The data collected by the forensic anthropologist are compiled into a biological profile, which is then checked against a list of missing persons. A complete profile requires determination of the following attributes: sex, age, ancestry, stature, and unique physical (skeletal or other) features. The amount of physical data provided on a missing persons report varies between states. Florida, for example, requires the person filing the report to provide data on race, sex, and age. California supplements this suite of attributes with stature (height) and weight, while Texas allows the inclusion of “unique features” (Jantz and Ousley 2005; Komar and Buikstra 2008). Current reporting standards for Florida and California have been expanded to include additional identifying characteristics and pertinent information (e.g., availability of dental X-rays) (California Department of Justice 2011). Missing persons reporting protocol is similar among the Canadian provinces. Online missing persons reports for British Columbia, Ontario, Alberta, and Saskatchewan provide numerous details on the missing individual: sex, age, race, stature, weight, hair/eye color, and distinguishing features (e.g., unusual dental restorations, tattoos, clothing worn at time of disappearance) (AMPUHR 2011; MPUBU 2011; SACP 2011; VPD 2011). All of the aforementioned provinces had websites designated for missing

persons and unidentified human remains cases. Terminology use (i.e., “sex” vs. “gender”) differed between provincial websites.

Estimating the sex of skeletonized remains requires methods specifically developed to ascertain dimorphic characteristics present in various skeletal elements. Sex and gender are not synonymous; sex refers to an individual’s biological characteristics while gender implies social identity. Notably, an accurate determination of sex may contradict the decedent’s gender presentation (Walker and Cook 1998). The most important attributes for diagnosing sex in the postcranial skeleton develop in the pelvis during adolescence (Komar and Buikstra 2008). Numerous morphological features (e.g., shape and particular characteristics of the pubic bone) present on an intact adult pelvis can yield useful information regarding an individual’s sex (Rogers and Saunders 1994). Qualitative and quantitative methods have been developed to accurately evaluate these features (e.g., metric method (Washburn 1948), pubic region (Phenice 1969), dorsal pitting (Suchey et al. 1979), ventral arc (Sutherland and Suchey 1991)). While techniques for determining the sex of the subadult postcranial skeleton have been established, the most accurate estimations of sex occur after an individual has reached maturity (White and Folkens 2005). Subadult and fetal sex estimation methods will not be explored here.

While cranial features are considered a less accurate determinate of sex than pelvic characteristics (Komar and Buikstra 2008), the skull may be the only part of the skeleton available for evaluation. Five aspects of skull morphology are particularly useful in determining sex (see Walker (Buikstra and Ubelaker 1994)): nuchal crest, mastoid process, supraorbital margin, supraorbital ridge, and mental eminence. Male

crania usually are characterized by greater size and robusticity. For example, males display more prominent brow ridges, larger mastoid processes, and square chins (White and Folkens 2005). Generally, if postcranial material is available, then the pelvis should be used to estimate sex of the individual (and cranium). Sexually dimorphic postcranial skeletal elements (e.g., femoral proximal head) can also be assessed metrically (Komar and Buikstra 2008). Differences can be quantified by using either discriminant function analysis or single measurements compared to an established range with relatively high (83–93%) correct classification rates (France 1998).

Although subadult age can be estimated from long bone length (Ubelaker 1989) and epiphyseal closure (Stevenson 1924; McKern and Stewart 1957), there is general consensus that dental development methods, not skeletal maturation, produce the most accurate age estimates (e.g., Ubelaker 1989; Smith 1991; Saunders 2000). Tooth development is more closely associated with chronological age (White and Folkens 2005). Four distinct developmental periods can be observed in human dentition: the emergence of deciduous teeth during the second year of life; the eruption of two permanent incisors and the first permanent molar between the ages of 6 and 8 years; emergence of most permanent dentition with the exception of the third molars between 10 and 12 years; eruption of the third molars typically at approximately 18 years of age, although they may remain impacted (Hillson 1996). There are several ways to age developing teeth. For example, if the entire dentition is intact, it may be compared with a chart indicating mean stage of development (White and Folkens 2005). Each individual tooth may also be compared with established charts to determine its stage of formation. The assessment of attritional wear is also employed although highly variable



individualistic factors produce less reliable age estimates (Franklin 2010). Macroscopic and radiographic evaluations of root development and mineralization are among the preferred methods for estimating age from juvenile teeth (e.g., Franklin 2010; Reppien et al. 2006). Accuracy rates are affected by variability observed within the original reference populations.

Adult age may be inferred if the wisdom teeth are present and have erupted (Hillson 1996). Radiographic and macroscopic assessments of tooth developmental features comprise another group of adult aging techniques. Gustafson (1950) developed an aging method based on the evaluation of six dental structural characteristics (i.e., attrition, periodontosis, secondary dentin, cementum apposition, apical resorption, and translucency tooth root). Lamendin et al. (1992) further explored the relationship between age and root translucency and periodontosis by developing a technique in which measurements of these two dental features are taken and subjected to multiple regression analysis. The Lamendin method is particularly useful when applied to individuals over 40 years of age (Lamendin et al. 1992). The technique has since been modified according to sex and ancestry (e.g., Prince and Ubelaker 2002; González-Colmenares et al. 2007); accuracy rates may be correlated with source population data (González-Colmenares et al. 2007).

One of the more reliable methods of adult age estimation includes amino acid racemization in dentin, bone, or cartilage (see Schmeling et al. 2007). Error rates, largely age-dependent, are low under ideal conditions (e.g., post-mortem intervals not exceeding 20 years) (Griffin et al. 2008). Bone histological methods of aging also facilitate accurate estimations (Robling and Stout 2000). These generally destructive

techniques range from the measurement of histomorphological features (e.g., osteon size, type and density) to minimally invasive procedures including Thompson's (Thompson 1979) multiple linear regression technique.

Other useful aging methods include cranial suture closure analysis and the non-invasive evaluation of morphological changes in the face of the pubic symphysis, the auricular surface of the os coxae, and the sternal end of the fourth rib (see White and Folkens 2005). The known correlation between age and degree of cranial suture closure (Meindl and Lovejoy 1985) can provide valuable information to the forensic anthropologist when postcranial elements are unavailable for analysis. Krogman and İşcan (1986) have shown that the sphenoccipital synchondrosis is particularly useful in aging isolated crania; approximately 95% of individuals exhibit fusion at this location between 20 and 25 years of age. Despite the utility of cranial sutures as a skeletal indicator of age-at-death, it has been suggested that the technique is not well suited to forensic specimens due in part to the wide age range within closure categories (i.e., approximately 30 or more years) (e.g., Hershkovitz et al. 1997; Sahni et al. 2005).

Temporal modifications to the pubic symphyseal face were first analyzed by Todd (1920), whose 10 phase classification system was later expanded to account for sex and ancestral variation. Brooks (1955) subsequently modified the corresponding age ranges to reduce the inherent tendency for an observer to over-estimate age. McKern and Stewart (1957) further revised the aging method with their focus on three particular components of the pubic symphyseal face from an exclusively male sample. The most recent modification to the technique has resulted in a new series of standards that facilitate age estimations based upon comparisons between the comprehensive

pattern of the symphyseal face and six sex-specific and detailed reference age ranges (Brooks and Suchey 1990) generated from a large mixed sex modern sample.

Morphological changes to the auricular surface of the os coxae as an indicator of age was first examined by Lovejoy et al. (1958), who subsequently devised a system in which eight morphological phases with corresponding age ranges (early 20–60 or more years of age), descriptions, and archetypal photographs could be successfully used to estimate skeletal age. The main advantages of this method include its application to skeletal samples that have suffered post-mortem damage, and to individuals representative of both sexes and various ethnicities (see Franklin 2010); accuracy decreases, however, when the method is applied to samples not represented in the primarily American original reference populations. Buckberry and Chamberlain (2002) support the calculation of an age estimate based on a composite analysis of the various morphological features of the auricular surface. Subsequent review has suggested that a six phase system characterized by robust categories (i.e., no overlap between the mean ages) more accurately facilitates age predictions (Osborne et al. 2004).

The assessment of features associated with the costochondral junction of the right fourth rib has also been used to determine age. The subsequent comparison of these morphological characteristics with reference photographs or casts can allow the classification of a specimen into one of nine categories, each of which can be statistically delineated from the other (İşcan et al. 1984; İşcan et al. 1985). Yoder et al. (2001) demonstrated the method's application to forensic cases in which the fourth rib is missing or unidentifiable; an aggregate score generated from the evaluation of

multiple ribs, left or right, produces age estimates similar to those obtained from the right fourth rib.

The association of skeletal attributes with ancestry has been a controversial issue. However, missing persons reports typically include this information, and anthropologists also must communicate with law enforcement personnel and other individuals who acknowledge the racial categorization of people. Thus, ancestry determination is subsequently an important component of a biological profile. Prior to the introduction of the interactive discriminate function computer program FORDISC (Jantz and Ousley 2005), morphological or nonmetric traits were used to estimate ancestry; visual determinations of these traits still produce accurate determinations of ancestry (see Spradley and Jantz 2005). Rhine (1990) and Gill (1995) have compiled a list of nonmetric dental and cranial traits useful in determining ancestry (e.g., palate shape, width of nasal aperture, degree of prognathism).

FORDISC (Jantz and Ousley 2005) is the most commonly used method to assess ancestry (Komar and Buikstra 2008). Cranial (and/or postcranial) measurements and angles are entered and subsequently compared against a reference sample population. Ambiguous results are not uncommon; reference samples may present a mixture of biological, ethnic, and national groups. In particular, individuals of Hispanic descent are not well represented in the database due to issues such as misclassification (see Spradley and Jantz 2005). The term “Hispanic” is problematic, since its genetic connotations are unclear and it is broadly defined by the U.S. Census Bureau as anyone who is a native Spanish speaker from the western hemisphere (Ramirez and de la Cruz 2002).

Similarly problematic is the lack of comparative data upon which a biological profile is built. Sex determination typically is the first step in constructing a profile. As a result, “Hispanic” crania often are misclassified as female due to their smaller size and gracile nature (Spradley and Jantz 2005); postcranial skeletal components such as radii and humeri, if available, are better indicators of sex in Hispanic individuals than the cranium (Tise 2010). Spradley et al. (2008) have shown that neither cranial morphology nor metric data are objective enough to separate closely related groups of “Hispanic” individuals. While analysis of the os coxae is usually more than sufficient to estimate sex, the absence of the pelvis results in a marked decrease in accuracy of metric sex determination. Ancestry estimation accuracy rates of “Hispanic” individuals are low, possibly due to the multiple population affinities exhibited in morphological characteristics (i.e., Native American, European, and African) (Spradley and Jantz 2005).

Primary methods of stature estimation involved the use of long bone measurements and regression equations developed by Trotter (1970). However, these formulas were derived from small sample sizes, and measurement errors due to intra-observer and inter-observer bias have been noted by other researchers (Jantz et al. 1994). The introduction of FORDISC has permitted the use of a variety of long bones in the estimation of stature; the option of combining various skeletal elements allows the forensic anthropologist more flexibility if less postcranial material is available for analysis (Komar and Buikstra 2008).

While FORDISC continues to be a frequently used implement in the construction of a biological profile, the utility of the program in accurately determining sex, ancestry,

and stature of adult cranial and postcranial elements remains open for debate (Elliott 2008). Although identification can be facilitated if unidentified remains are exemplars of their source population, identification improves when the test specimen's source population is represented in the FORDISC reference groups sample, and when the unknown specimen is accurately sexed through cranial and postcranial morphological examination prior to using FORDISC to determine an individual's ancestry.

Rogers and Allard (2004) emphasized the need for North American forensic anthropological research to conform to standards established by the U.S. and Canadian rulings Daubert and Mohan, respectively. In particular, they suggested the application of standardized techniques to cranial suture pattern analysis as a means of improving the method's evidentiary value. In their report on the inadequacy of admissibility standards for forensic anthropological evidence, Christensen and Crowder (2009) noted that recent literature trends indicated the growing awareness and interest in the consideration of admissibility criteria when conducting analysis and preparing testimony. While similarly noting the problematic absence of standards for recovery and analysis of human remains between institutions and between individual researchers, they suggested a concentration of effort on issues of validation and error rates.

As forensic anthropological data have an established record of overlap with other identification methods, it is perhaps via this route that validation may be achieved. Individualized frontal sinus patterns are validated methods of identification provided proper antemortem (AM) and postmortem (PM) protocol has been followed (Besana and Rogers 2010). Watamaniuk and Rogers (2010) have investigated the potential for thoracic vertebral margin morphology as a method of positive personal identification.

Their results are promising; 87.5% of unknown radiographs were positively identified. Further research into the coupling of traditional anthropological methods with techniques satisfying Daubert and Mohan criteria should be explored.

### Forensic Radiography

Radiography is a technique in which objects (e.g., anatomical characteristics) are viewed using a specific range of electromagnetic radiation (Black et al. 2010). Forensic radiography is an important tool in the identification of human remains (Brogdon 1998), particularly in cases where decomposition, mutilation, or skeletonization prevent application of more commonly used methods of identification (e.g., DNA profiling or fingerprints). Radiographs can be particularly useful in the detection of injuries and the identification of pathological conditions and foreign objects (e.g., surgical implants) which can be used to personally identify an individual (Hines et al. 2007; Black et al. 2010).

Successful radiographic identification is dependent upon the comparison of morphological features or characteristics evident in both AM and PM radiographs from the same individual (Komar and Buikstra 2008). Radiographic comparisons are categorized in several ways. For example, unique features such as the permanent implantation of an orthopedic appliance usually are inscribed with serial or identification numbers which often can be traced back to the attending surgeon provided this information has been documented in AM records (Hines et al. 2007; Komar and Buikstra 2008). Evaluating the collective concordance of a suite of consistent features visible in one or more radiographs also can reliably support a positive identification,

provided at least 8 characteristics exhibit consistency between AM and PM documentation (Komar 2004). Finally, morphological features (e.g., frontal sinuses) can be grouped and classified according to their general description and variation therein. Classification systems have been developed for frontal sinus patterns (Christensen 2004; Cameriere et al. 2005), ectocranial suture patterns (Sekharan 1985; Rogers and Allard 2004), bone trabecular patterns (Mann 1998), and palatal rugae patterns (Limson and Julian 2004; Muthusubramanian et al. 2005). Due to the fairly recent validation of frontal sinus patterns and ectocranial sutures as methods of establishing identity, these two systems will be examined here.

The sinuses, pockets of air interspersed within some of cranial bones, become anatomically “fixed” once development is complete; however, subsequent pathological and physiological changes are possible (Brogdon 1998). The frontal sinuses form an open complex centrally located above the orbital rims and nasal area and within the lower frontal region. Individual variation is significant and well documented (Christensen 2004). While development is typically bilateral, unilateral development does occur (Brogdon 1998); approximately 5% of the population do not possess a frontal sinus. Ectocranial suture growth and development is affected primarily by genetic factors (Rogers and Allard 2004). Individual responses to various external stimuli determine eventual suture patterns. Sekharan (1987) has confirmed the uniqueness of cranial sutures in a comparison of 521 skulls and 100 radiographs. No duplications were noted, and bilateral differences were observed in all members of a subset examined for symmetry.



In order for frontal sinuses and cranial suture patterns to effectively establish identity of a decedent, two criteria should be met: patterns should remain fixed throughout a person's lifetime, and AM records for a missing person must be available for comparison with a decedent's PM data (Sekharan 1987; Rogers and Allard 2004). Since radiographs documenting the frontal sinuses and cranial suture patterns are requested infrequently (Brogdon 1998), the likelihood of procuring AM radiographs documenting these unique features is low. If AM radiographs of the frontal sinuses or cranial sutures are available, the orientation of PM radiographs must be identical for a valid comparison to be made; a minimum of four consecutive characteristics within a suture must be present and identical between AM and PM records for a positive identification to be sufficiently established (Rogers and Allard 2004).

While frontal sinus and cranial suture pattern research has demonstrated the utility of these methods in establishing positive identification, the scientific community remains divided on the admissibility issue. Besana and Rogers (2010) deemed both methods inadmissible under both Daubert and Mohan guidelines due to their associated lack of standardized measurements and known error rates. However, these shortcomings have not disallowed relevant expert witness testimony during court proceedings. Despite the fact that matters regarding evidentiary admissibility are subject to judicial authority, counsel for either the prosecution or defense may request a "Daubert hearing" on the admissibility of a particular forensic identification technique (see *U.S. v. Llera Plaza et al* 2002; Rogers and Allard 2004). In the case of Tennessee vs. Cosgrif comprehensive forensic anthropological expert testimony admitted by the trial judge included inculpatory frontal sinus evidence (Daubert Tracker 2011). Upon the defendant's appeal

that the frontal sinus testimony did not satisfy Daubert reliability criteria, conviction was upheld on the grounds that the expert witness had firmly established her qualifications and expertise; therefore, the entire testimony had been justifiably admitted.

Christensen (2004) emphasized the need for empirical data supporting the uniqueness of frontal sinus outlines. Her investigation quantified significant variability between the shapes of individuals' frontal sinus outlines; this constituted a considerable step forward in the standardization and validation of the technique. Nonetheless, the absence of standardized measurements and unknown error rates of the technique render them both "inadmissible" as per Daubert and Mohan rulings.

Generally, AM radiographs documenting "unique" features (e.g., injuries, pathological conditions) have long been used to identify human remains (Brogdon 1998). Komar (2004) reported on the successful use of multiple AM radiographs to confirm identification of postcranial remains as those belonging to an individual whose skull was found more than 66 miles away. Similarly, Burns (2009) positively identified human remains using radiographs of AM fractures and frontal sinus patterns. Sex estimation was inconclusive based on conflicting cranial and postcranial data obtained during the anthropological examination; AM radiographs facilitated a correct determination. In some cases, radiographic comparisons are more effective than DNA at establishing positive identification. Scott et al. (2010) has shown that the advantages of comparing AM skeletal features with AM radiographs can supersede supportive, yet inconclusive, DNA profiles. Provided AM and PM records meet the same standards, and radiograph interpreters are well-trained, this method of identification meets admissibility criteria (Komar and Buikstra 2008); accuracy rates can increase

significantly when identifications are made by experienced practitioners (Pretty et al. 2003).

### Forensic Odontology

Teeth are highly resistant to trauma and the effects of decomposition, fire, and other environmental influences (Black et al. 2010). Individual characteristics, whether congenital anomalies or dental restorations documented in AM radiographs, thus have the potential to uniquely identify a person. Forensic odontology (i.e., the examination, evaluation, and presentation of dental evidence) has played an important role in the identification of living and deceased individuals (Hardy 2007). Not only are odontological methods (e.g., dental radiography) useful in attempting to identify a single individual in a single case (Steadman and Konigsberg 2009), they have also been very effective in identifying victims of mass disasters (e.g., Morgan et al. 2006). Bitemark analysis, an area of forensic odontology, is more concerned with confirming or eliminating the identity of a perpetrator accused of a crime. While examination of bitemarks has led to the successful prosecution and conviction of violent offenders, the science behind the method has been questioned and convictions have been overturned on the basis of faulty bitemark analysis and testimony (Deitch 2009).

Forensic odontology for purposes of identifying a decedent involves the comparison of AM and PM dental records, including radiographs, in order to confirm an identification. The technique is not new, although identifications made during its very early uses relied on visual examination and not radiographs (e.g., Byers 2008, Parkman

murder). The process of making an identification through odontological examination follows protocol that parallels forensic anthropological procedures developed by Snow (1982) and illustrated by Taylor (1978). If the tooth specimen is loose, the taxonomic origins of the tooth are first determined (i.e., human or nonhuman). If the specimen is determined to be human, then its class (e.g., molar), number (e.g., first or second), and location (i.e., upper vs. lower, left vs. right) in the mouth are identified. Identifying dentition still implanted in the jaw normally would not be subject to the preceding criteria since an initial inspection would provide fairly obvious evidence regarding their origins (i.e., human vs. nonhuman) (Byers 2008). Generally, once the above criteria have been satisfied, the individual's ancestry, sex, and age would be determined, as would the likelihood of routine dental care and/or unique restorations, etc. The compilation of the previous attributes then facilitates a comparison between the remains and a missing person's report. Provided the missing person at some point during his or her lifetime received care from a dental practitioner, AM dental records including radiographs, if available, would be requested from the corresponding dentist, orthodontist, or oral surgeon and reviewed for their completeness and accuracy (Black et al. 2010). A satisfactory PM identification made using forensic odontological analysis requires the exemplary condition of AM records (Hardy 2007). AM documentation and radiographs must be legible, accurate, comprehensive, and validated; impediments to a proper analysis and comparison include reporting inaccuracies, lack of uniformity between practitioners in dental charts and charting, illegible or inadequate records.

While large databases and computer programs allow North American odontologists to calculate the objective frequency of the incidence of particular dental traits in the

population, the different possibilities of adult dental charting exceeds two billion possibilities (Hardy 2007). This suggests that the probability of two adults having exactly the same dentition is most likely zero. In North America, there is no definitive number of matching characteristics that must be reached before a positive identification can be established; evaluations are made on a case-by-case basis (Adams 2003a; Adams 2003b).

### Complementary Methods

It is clear that forensic human identification is based primarily on exclusionary principles, and a biological profile is more effective when constructed in layers. Even the extraordinarily robust probability estimates of DNA profiling are not necessarily sufficient to establish legally admissible positive identification evidence. Complementary methods are becoming increasingly important as law enforcement, legal counsel, and judiciaries request additional methodology to support a particular biological profile. Stable isotope profiling (SIP) is a unique method with demonstrated value in forensic cases where human remains are skeletonized or have been mutilated, and little if any identifying information can be ascertained from the body.

Data collected on the stable isotopic composition of various human tissues, including bone, teeth, hair, and finger nails, indicate that it is possible to compile a multi-variate stable isotope profile (SIP) (signature) for an individual; variation in stable isotope composition occurs with comparative predictability such that inferences regarding diet reconstruction (carbon  $\delta^{13}\text{C}$  and nitrogen  $\delta^{15}\text{N}$ , van der Merwe 1982; Klepinger 1984; DeNiro 1986) and the natal origin of unidentified human remains may

be formulated (Katzenberg and Krouse 1989). Hydrogen ( $\delta^2\text{H}$  or  $\delta\text{D}$ ) and oxygen ( $\delta^{18}\text{O}$ ) isotope values are indicators of climatic change (Dansgaard 1964) and food and water intake (Estep and Dabrowski 1980; Estep and Hoering 1980; Schimmelmann et al. 1993; Sharp et al. 2003).  $^{18}\text{O}/^{16}\text{O}$  and  $^2\text{H}/^1\text{H}$  ratio analyses are germane to forensic provenance studies because their distribution in meteoric precipitation, which is consumed by humans in the form of drinking water, varies geographically – and predictably – depending on climatic variables (Ehleringer et al. 2008). Similarly, the  $^{87}\text{Sr}$  composition in nutritional resources is dependent upon an area's geological characteristics, and thus can be useful as a geolocational indicator of the natal origins associated with human skeletal remains (Beard and Johnson 2000; Rauch et al. 2007; Juarez 2008), provided the impact of global food consumption on measured strontium ratios (i.e., “the supermarket effect”) is negligible (Nardoto et al. 2006).

What role does SIP play within the suite of analytical methods available to law enforcement? Despite regional geographic isotopic variability and evidence of global differences in dietary isotopic compositions, SIP is capable only of comparing two substances and determining the degree of isotopic similarity between their origins (Meier-Augenstein 2010). If variation is evident, then its significance must be determined. For example, are the differences significant enough to definitively pinpoint a geographic region and thus indicate where the person might have resided during childhood (e.g., isotopic data obtained from late erupting molars) or within the last several months (e.g., isotopic data obtained from hair or fingernails)? Notably, SIP cannot be used to make a positive identification (Meier-Augenstein 2010). However, it has been used to determine the dietary preferences and geographic trajectory of living

and deceased individuals. In this respect SIP may be useful in conjunction with other identifying markers as part of a biological profile. In particular, it provides a less invasive sampling method in cases where last known residence data are required and hair/fingernail clippings can be obtained and analyzed (Fraser et al. 2006).

Ehleringer and Matheson (2007) have thoroughly reviewed stable isotopes and the admissibility issues associated with this type of evidence. Stable isotope profiling is subject to the same evidentiary guidelines applied to DNA profiling and the hard tissue identification techniques described previously. As forensic applications of the technique are growing, practitioners would be well-advised to pay particular attention to the standardization of procedures and instrumentation and data interpretation; emphasizing these concerns during the early stages of the method's introduction to the legal system should reduce the likelihood of future legal challenges on the grounds of failure to satisfy reliability criteria.

While the application of stable isotope analysis to medico-legal investigations involving the identification of human skeletal remains was recognized in the late 1980s (Katzenberg and Krouse 1989), the first accredited use of the technique for this purpose did not occur until approximately 2005 (Meier-Augenstein and Fraser 2008). Notably, this multi-elemental ( $^{18}\text{O}$ ,  $^2\text{H}$ ,  $^{13}\text{C}$ , and  $^{15}\text{N}$ ) isotope study led to the positive identification of a severely mutilated body and the eventual arrest of the perpetrator once corroborative DNA evidence was obtained. A similar stable isotope study on human remains discovered in California determined the Oaxaca region of southern Mexico as the victim's likely origins (Schwarcz 2007). While DNA analysis (mtDNA and

nuDNA) confirmed the victim's ethnic ancestry, the combined stable isotope and DNA evidence was insufficient to establish the victim's identity (Ehleringer et al. 2010).

While there have been relatively few studies concerned primarily with determining the geographic origins (or most recent residence) and tracing the recent movements of modern individuals (Pye 2004), this appears to be changing. The technique continues to generate more interest as its utility and supplemental value in supporting other forms of identification continues to gain recognition.

### Conclusions

In the United States approximately 40,000–60,000 sets of stored unidentified human remains await provenance (Schmitt 2006; Ritter 2007). Most of these individuals are victims of violent crimes, whose bodies have been recovered by law enforcement personnel over the last 50 years (Hargrove 2005). Canadian national statistics are more elusive. However, Ontario, the most populated province (Statistics Canada 2010), retains approximately 108 sets of unidentified human remains; more than 350 individuals are listed as missing (Ontario's Missing Adults 2011). While personal identification can often be made based on a decedent's government-issued identification (e.g., driver's license) or confirmation by family members, there are circumstances in which these methods are ineffectual. In such cases where visual comparison with missing persons reports is not feasible, the use of DNA profiling, forensic anthropological profiling, forensic radiography, and forensic odontology have been used successfully to establish human identification.



Due to the legal ramifications of facilitating the misidentification of a victim or accused, practitioners from all fields in which human identification methods are derived must strive to meet the previously established legal admissibility criteria outlined in Daubert, Mohan, and R. v. J.-L.J. While the four techniques examined in this review are considered generally admissible as expert evidence or as the content of expert witness testimony, one or all may be excluded for reasons previously elucidated. If identification methods such as the above are subject to intense legal scrutiny, then experts employing more controversial techniques must be even more stringent in their pursuit of scientific validation. While “the DNA model” has been elevated as the definitive research strategy, standardization and regulation of lab protocol is ongoing. The need for these efforts has become increasingly apparent as more convictions are being overturned due to exculpatory DNA evidence. No longer can experts assume that generally admissible techniques and conclusions will remain so under the current evidentiary guidelines imposed by Daubert and Mohan. Indeed, Page et al. (2011a, 2011b) have echoed this concern with their timely and provocative analysis and evaluation of more than 500 cases in which the admissibility of identification evidence was challenged.

If “generally accepted” methods of identification remain subject to judicial discretion, what might this mean for complementary methods such as stable isotope profiling? Stable isotope profiling can yield valuable information concerning an individual’s geolocational patterns, information that can prove extremely useful in a forensic context since human remains often are discovered in areas in which the deceased did not reside. For this reason stable isotope profiling is gaining recognition for its utility in

determining the provenance of human remains. Nonetheless, it and other complementary methods must satisfy the same reliability criteria applied to conventional forensic identification techniques. While scientific evidence and testimony proffered in support of a positive identification is generally heard by both U.S. and Canadian courts, evidentiary admissibility is not guaranteed. Victim identifications are unequivocally more effective, practically and legally, when corroborated by multiple lines of evidence.

## CHAPTER 5

### $^2\text{H}$ AND $^{18}\text{O}$ ISOTOPE ANALYSIS OF HUMAN TOOTH ENAMEL: A PILOT STUDY

#### Introduction

Stable isotope analysis of biogenic tissues such as tooth enamel and bone mineral has become a well-recognized and increasingly important method for determining the provenance of human remains, and it has been used successfully in bioarchaeological studies as well as forensic investigations. In particular,  $^{18}\text{O}$  stable isotope signatures of bone and tooth enamel, respectively, are well-established proxies of climate (temperature) (D'Angela and Longinelli 1993; Ghosh and Brand 2003; Hedges et al. 2004; Cullen and Grierson 2006; Cullen and Grierson 2007) and geographic provenance (Longinelli 1984; D'Angela and Longinelli 1990; Bryant et al. 1996b; Barbour et al. 2001; Hoogewerff et al. 2001; Budd et al. 2004; Daux et al. 2008) and are therefore considered as indicators of geographic life trajectories of animals and humans. While the methodology for  $^2\text{H}$  analysis of human hair, fingernails, and bone collagen is currently used to determine human provenance, i.e. geographic origin and identify possible migration patterns, studies involving the analysis of  $^2\text{H}$  in tooth enamel appear to be nonexistent in the scientific literature. In an effort to develop an innovative sampling and analytical protocol for the  $^2\text{H}$  analysis of tooth enamel, this author and colleagues (Holobinko et al. 2011a) conducted a feasibility study, the details of which are presented below.

With the advent of instruments capable of on-line or continuous-flow  $^2\text{H}$  stable isotope analysis, methodologies and applications for the  $^2\text{H}$  analysis of bone collagen, human hair, and nails have been reported to determine geographic origin of ancient and modern man (Fraser et al. 2006; Fraser and Meier-Augenstein 2007; Ehleringer et al. 2008; Reynard and Hedges 2008). Given the widely acknowledged resilience of tooth enamel to diagenetic changes (Lee-Thorp 2008), and given the absence of scholarly materials concerning the  $^2\text{H}$  isotopic abundance of tooth enamel, we wondered if it would be possible to gain useful information from the  $^2\text{H}$  isotopic record locked into the hydroxyl groups of tooth enamel.

The apparent lack of published research in this area might be for the following reasons:

- a) Compared with the mineral calcium hydroxylapatite,  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ , carbonate ions in bio-apatite such as tooth enamel replace hydroxyl ions and even phosphate ions (Wopenka and Pasteris 2005; Chakraborty et al. 2006), yet only approximate weight percentage figures for the degree of carbonate incorporation have been published (Penel et al. 2005). For this reason bio-apatite is more accurately described chemically as carbonate-rich, hydroxyl-deficient apatite (Wopenka and Pasteris 2005) As the degree of carbonate substitution in tooth enamel is not precisely known, it could be suspected that the prospective yield of  $\text{H}_2$  could be too small to be reliably measured.
- b) Due to the theoretical possibility of hydrogen exchange in bio-apatite, potentially measurable  $\delta^2\text{H}$ -values may not convey any meaningful

information. However, in comparison with the pKa-values at 25°C of 14 or 15.5 for the dissociation of water ( $\text{H}_2\text{O} \leftrightarrow \text{OH}^- + \text{H}^+$ ) or of ethanol into ethoxide and a proton ( $\text{C}_2\text{H}_5\text{OH}/\text{C}_2\text{H}_5\text{O}^- + \text{H}^+$ ), respectively, the pKa-value for the dissociation of  $\text{OH}^-$  to yield  $\text{O}_2^- + \text{H}^+$  is 24, thus making the hydroxyl ion 108.5 times weaker a proton donor than ethanol, or 1010 times less likely to dissociate and, hence, less susceptible to H exchange than water.

Since no published protocols existed for sample preparation and analytical methods to obtain  $\delta^2\text{H}$ -values from the hydroxyl fraction of tooth enamel through isotope ratio mass spectrometric analysis, we designed a pilot study to establish the feasibility of measuring the  $^2\text{H}$  stable isotope composition of ground tooth enamel by continuous flow isotope ratio mass spectrometry (CF-IRMS) coupled-online to a high-temperature conversion elemental analyzer (TC/EA) and assess the utility of enamel  $\delta^2\text{H}$  values as an archaeological or forensic proxy for a person's geographic origin during late childhood when later erupting molars are forming. Specifically, we aimed to accomplish the following objectives:

- a) Determine the appropriate enamel sample amounts necessary to generate a viable  $^2\text{H}$  signal on the mass spectrometer analytical instrumentation. We hypothesized that the amount of powdered enamel required for measuring  $^2\text{H}$  composition would be equal to that required for determining the  $^{18}\text{O}$  composition of enamel.
- b) Determine the proper environmental conditions in which to prep the enamel samples for analysis. Since powdered tooth enamel is potentially as hygroscopic as ground hair, the protocol for preparing tooth enamel for  $^2\text{H}$

analysis was expected to be similar to that required for hair. Exceptional care is required to maintain the enamel powder samples under strict desiccation and in an environment free from fluctuations in ambient temperature and humidity.

## Materials and Methods

### Sample Preparation

Tooth samples, second or third permanent molars, were either archaeological specimens or had been donated by individuals undergoing dental treatment, i.e., tooth resection. No personal information was collected from living volunteers. The teeth were mechanically cleaned to remove any surface contamination and subjected to one of the following pre-treatment protocols. All preparation and subsequent analysis was performed by staff at the Stable Isotope Laboratory, James Hutton Institute (formerly Scottish Crop Research Institute), Dundee, UK.

Protocol B (Lee-Thorp et al. 1997; Lee-Thorp 2008). Approximately 35-40 mg of tooth enamel was sampled using a dental drill with a diamond-tipped drill bit. The collected powder was first washed with 1.5% NaOCl solution (40  $\mu$ L/mg sample for 30 min) followed by three rinses with deionized water. The powder was subsequently washed with 0.1M acetic acid (40  $\mu$ L/mg sample for 10 min) followed by three rinses with deionized water, and dried in a desiccator over phosphorus pentoxide ( $P_4O_{10}$ ) as drying agent.

Protocol S. The entire tooth was washed as described under protocol B and subsequently sampled using a dental drill with a diamond-tipped drill bit to yield a fine enamel powder, which was placed in a desiccator over self-indicating  $P_4O_{10}$  (Sicapent; Merck chemicals Ltd., Nottingham, UK) as drying agent.

Following initial cleaning, samples were stored in a non-evacuated desiccator over Sicapent for a minimum of 3 days. Samples used in this study were all prepared following the principle of identical treatment (Werner and Brand 2001) (i.e., subsamples of all materials were taken under identical conditions, weighed out into silver capsules, placed in well plates, and transferred into a desiccator). All samples prepared for this study were dried for at least 7 days prior to analysis in an evacuated desiccator over Sicapent as desiccant. To avoid transient  $^2H$  signals from atmospheric humidity interfering with  $^2H$  isotope analysis, the dried samples were transferred from the drying down desiccator into a Costech Zero-Blank autosampler (Pelican Scientific Ltd., Alford, UK) that was purged with dry helium and in which samples were kept isolated from the ambient atmosphere.

For the equilibration experiment, two subsets of a tooth sample prepared according to protocol S were weighed out into silver capsules. One subset was placed in a sealed desiccator containing water A ( $\delta^2H_{VSMOW}$  of water A = -54.1 ‰) while the other subset was placed in a sealed desiccator containing water B ( $\delta^2H_{VSMOW}$  of water B = -141.9 ‰). The samples were kept in these equilibration chambers for 4 days after which they were transferred into two separate desiccators containing Sicapent to be dried down

under vacuum for 7 days (Bowen et al. 2005). Once dried, samples were transferred for analysis into the Zero-Blank autosampler as described above.

Whenever sufficient sample amounts were available, tooth enamel samples were also analyzed for the  $^{18}\text{O}$  isotopic composition of their carbonate component. Sample preparation was carried out according to a protocol published elsewhere (Henton et al. 2010) but it briefly comprised a succession of washes with dilute NaOCl solution, dilute acetic acid, and water.

#### Bulk $^2\text{H}$ Isotope Analysis by TC/EA-IRMS

A Delta<sup>Plus</sup>XP isotope ratio mass spectrometer coupled to a high-temperature conversion / elemental analyzer (TC/EA) (both Thermo Scientific, Bremen, Germany) was used for  $^2\text{H}/^1\text{H}$  isotope ratio measurement of all samples. The samples were weighed into silver capsules and placed in an evacuated desiccator in the presence of  $\text{P}_4\text{O}_{10}$  as drying agent for 1 week before introduction into the TC/EA by means of a solid Costech Zero-Blank autosampler (Pelican Scientific Ltd.). The reactor tube was self-packed and comprised of an outer Alsint<sup>TM</sup> ceramic tube (SerCon, Crewe, UK) and an inner glassy carbon tube (Sigradur, HTW, Thierhaupten, Germany) filled with glassy carbon granulate, silver and quartz wool (SerCon, Crewe, Cheshire, UK). As a general rule, in this lab TC/EA reactors are cleaned and repacked every 100 samples to avoid non-identical conversion conditions due to carbonization (Wang and Sessions 2008) or silver build-up. The reactor temperature was set to  $1425^\circ\text{C}$  while the post-reactor gas chromatography (GC) column was maintained at  $85^\circ\text{C}$ . Helium (99.999% purity; BOC, Guildford, UK) was used as carrier gas and reactor head pressure was set to 1.45bar



corresponding to a measured flow rate of 90 mL/min. Data were processed using the instrument manufacturer's proprietary Isodat NT software (version 2.0; Thermo Scientific).

The working reference gas, H<sub>2</sub> (BOC), was calibrated against Vienna Standard Mean Ocean Water (VSMOW) using the international calibration material VSMOW ( $\delta^2\text{H}_{\text{VSMOW}}=0$  ‰; IAEA, Vienna, Austria) and checked against the international reference materials (RMs) Standard Light Antarctic Precipitation (SLAP) and Greenland Ice Sheet Precipitation (GISP). The H<sup>3+</sup> factor was determined on reference H<sub>2</sub> gas pulses of different signal size and peak area, and was found to be 4.89 ‰ / nA. A batch analysis typically comprised 10 samples run in triplicate, preceded and followed by a set of RMs (IAEA-CH-7,  $\delta^2\text{H}_{\text{VSMOW}} = -100.3$  ‰; IAEA). Batch runs also comprised triplicates of an RM (NBS-30, biotite,  $\delta^2\text{H}_{\text{VSMOW}} = -65.7$  ‰. IAEA) as well as an in-house standard (sodium benzoate,  $\delta^2\text{H}_{\text{VSMOW}} = -118.6 \pm 1.3$  ‰; Iso-Analytical, Crewe, UK) as acquisition quality controls. The precision of measurement as monitored by the RMs and lab standards was  $\pm 1.2$  ‰ or better. Each batch was also preceded and followed by a blank capsule for blank correction. The measured  $\delta^2\text{H}$ -values were two-endpoint normalized to the VSMOW scale using IAEA-CH-7 ( $\delta^2\text{H}_{\text{VSMOW}} = -100.3$  ‰) and coumarin ( $\delta^2\text{H}_{\text{VSMOW}} = +62.6 \pm 2.4$  ‰; Iso-Analytical) in analogy to the internationally accepted VSMOW / SLAP scale normalization method (Coplen 1994; Sharp et al. 2001; Sharp 2007) with NBS-30 assuming the role of GISP to monitor the quality of scale correction.

### <sup>18</sup>O Isotope Analysis of Enamel Carbonate by Gas-IRMS

Approximately 9 mg of sample were weighed into an Exetainer (Labco, High Wycombe, UK) and subjected to the procedure summarized below. Exetainers filled with 0.5 mg of NBS-19 ( $\delta^{13}\text{C}_{\text{VPDB}} = +1.95 \text{ ‰}$ ;  $\delta^{18}\text{O}_{\text{VPDB}} = -2.20 \text{ ‰}$ ; IAEA) or LSVEC ( $\delta^{13}\text{C}_{\text{VPDB}} = -46.60 \text{ ‰}$ ;  $\delta^{18}\text{O}_{\text{VPDB}} = -26.60 \text{ ‰}$ ; IAEA) were put through the same procedure contemporaneously to anchor and quality-control the sample results. Exetainers containing RMs or samples were flushed with zero-grade nitrogen (BOC) for 10 min to remove all traces of atmospheric  $\text{CO}_2$ . Once flushed, 0.8 mL of absolute (water-free) sulfuric acid (99.999%; Sigma-Adrich, Poole, UK) was added by injection through the septum of the Exetainer. Acid digestion was carried out by placing the prepared Exetainers into a thermostatically controlled heater block at 50°C for 6 h. The samples were allowed to cool at room temperature for at least 6 h prior to stable isotope analysis of the evolved  $\text{CO}_2$  using a proprietary arrangement of a gas isotope ratio mass spectrometer coupled to a Gilson autosampler (AP2003; Analytical Precision, Northwich, UK). The measured  $\delta^{18}\text{O}$  values were two-endpoint normalized using NBS-19 and LSVEC as the end members (Coplen et al. 2006). The reproducibility of the isotope analysis as monitored by the RMs was typically  $\pm 0.18 \text{ ‰}$ . The uncertainty of the sample measurements was typically  $\pm 0.50 \text{ ‰}$ , which is a composite of the aforementioned reproducibility and intra-sample variability.

## Results and Discussion

A total of 12 teeth representative of both archaeological and modern sources were examined. Initial work conducted on samples SC\_2M and SC\_3M to determine whether it was possible to obtain a measurable H<sub>2</sub> peak from the hydroxyl group in ground tooth enamel by high-temperature conversion at 1425°C showed that sample amounts of 1 mg or above per silver capsule yielded H<sub>2</sub> peaks of reproducible peak area and  $\delta^2\text{H}$ -values. Sample amounts of <0.5 mg that are typical for <sup>2</sup>H isotope analysis of organic material generated H<sub>2</sub> peaks that barely rose above baseline (Fig. 5.1).

Experiments were carried out using the above two samples to determine if mixing the sample material with additional carbon in the form of graphite would improve the H<sub>2</sub> yield. However, the addition of graphite to ground tooth enamel changed neither the H<sub>2</sub> yield in terms of peak area (see Fig. 5.2) nor the measured  $\delta^2\text{H}$ -values (see Table 5.1). Subsequent studies were carried out without the addition of graphite while using at least 1.2 to 1.5 mg of cleaned ground tooth enamel per silver capsule.

Measured  $\delta^2\text{H}$ -values for tooth enamel samples prepared using either of the two protocols (see Table 1) showed no significant difference ( $p=0.41$ ) after the data was subjected to single factor analysis of variance (ANOVA). Subsequent studies carried out to determine presence and magnitude of H exchange used tooth enamel samples prepared and cleaned according to protocol S (Table 5.2).

Similarly, a paired Student's t-test (one-tailed; unequal variance) revealed no significant difference between the  $\delta^2\text{H}$  values for enamel equilibrated with water A ( $\delta^2\text{H}_{\text{VSMOW}} = -54.1 \text{ ‰}$ ) or water B ( $\delta^2\text{H}_{\text{VSMOW}} = -141.9 \text{ ‰}$ ) ( $p=0.05$ ). While the resulting

p-value did not exceed the  $p < 0.05$  threshold of significance, it could be argued that either the difference in  $^2\text{H}$  isotopic composition between the waters used for the H exchange experiment was too small or the time allowed for H exchange to occur was too short. It is certainly conceivable that choosing waters with a difference in  $\delta^2\text{H}$  values of  $>200$  ‰ in conjunction with a longer equilibration time such as 4 weeks rather than 4 days might show a significant level of H exchange. While such an observation would indeed be of great interest to the fields of crystallography and material science, it would not have changed the outcome of this study with regard to using the  $\delta^2\text{H}$  values of tooth enamel as an archaeological or forensic proxy for a person's geographic origin as the results described below will show.

Stable  $^2\text{H}$  isotope analysis of enamel subjected to protocol B from molars of known geographic origin yielded  $\delta^2\text{H}$  values ranging from  $-110.4$  to  $-115.2$  ‰, while the associated uncertainties ranged from  $\pm 0.4$  to  $\pm 1.7$  ‰ (see Table 5.1). Linear regression analysis of tooth enamel  $\delta^2\text{H}$  values as measured plotted against source water  $\delta^2\text{H}$  values yielded an  $R^2$  of 0.01, thus indicating no correlation between the two data sets (see Fig. 5.3). Since it was not possible to collect and ship water samples from all locations, the calculated annual average  $\delta^2\text{H}$  values in precipitation were obtained using the Online Isotopes in Precipitation Calculator (OIPC) ([www.waterisotopes.org](http://www.waterisotopes.org)) based on work by Bowen et al. (Bowen and Wilkinson 2002). The OIPC-derived  $\delta^2\text{H}$  values for known locations in Scotland and Kent were in very good agreement with the measured  $\delta^2\text{H}$  values of local water samples.

In contrast to the  $^2\text{H}$  isotope data, analyzing the carbonate fraction of tooth enamel samples for  $^{18}\text{O}$  produced the expected results. In accordance with previously published

results, the tissue  $\delta^{18}\text{O}$ -values were positively correlated with the presumed  $\delta^{18}\text{O}$ -values for source water (Longinelli 1984; D'Angela and Longinelli 1990; Daux et al. 2008) (see Table 5.3). Using OIPC-derived  $\delta^{18}\text{O}$ -values for source water, linear regression analysis of the five data pairs gave  $\delta^{18}\text{O}$  (mineral) = 0.73  $\delta^{18}\text{O}$  (source water) + 22.83, which is relatively similar to the equations reported by Longinelli (1984) as  $\delta^{18}\text{O}$  (mineral) = 0.64  $\delta^{18}\text{O}$  (source water) + 22.37 or Daux et al. (2008):  $\delta^{18}\text{O}$  (mineral) = 0.65  $\delta^{18}\text{O}$  (source water) + 21.89. At the very least the results of the  $^{18}\text{O}$  analysis demonstrated that the findings of the  $^2\text{H}$  portion of this study were not an artifact of either samples or analytical method.

Even if the enamel  $\delta^2\text{H}$  data had shown a correlation with source water  $\delta^2\text{H}$  data, given the stark difference in the range of  $\delta^2\text{H}$ -values of 37 ‰ for source water compared with only 4.8 ‰ for the enamel  $\delta^2\text{H}$ -values, a 1 ‰ change in the  $\delta^2\text{H}$ -value of tooth enamel would therefore correspond to a change of almost 8 ‰ for source water. By comparison, depending on the population studied, a 1 ‰ change in the  $\delta^2\text{H}$ -value of modern human scalp hair has been found to correspond to changes in the  $\delta^2\text{H}$ -value for source water of 1.7 ‰ (Bowen et al. 2009), 1.8 ‰ (Fraser and Meier-Augenstein 2007), or 2.8 ‰ (Ehleringer et al. 2008), thus attributing to  $\delta^2\text{H}$ -values of hair a much higher degree of positive predictive value for geographic provenance.

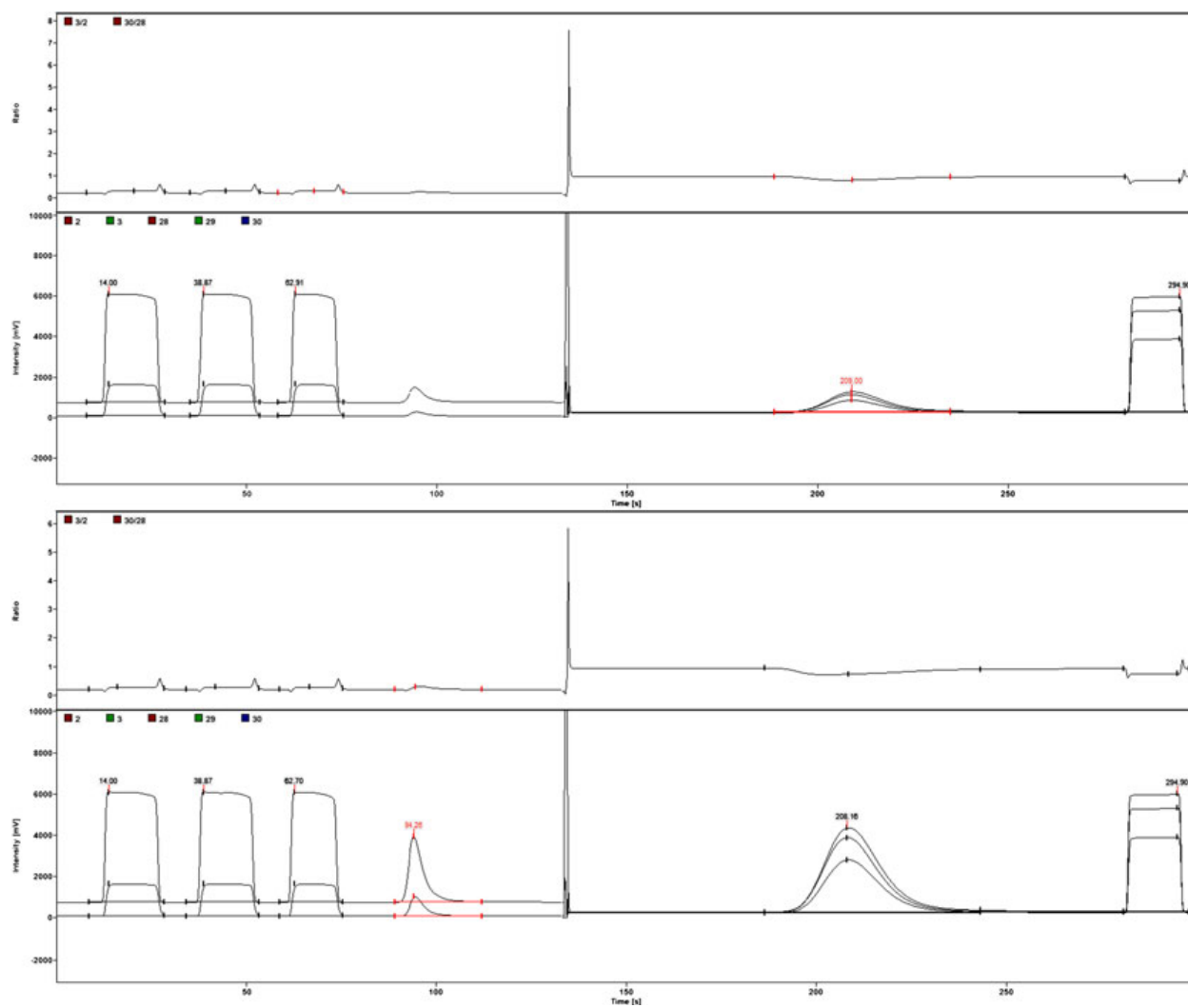
## Conclusions

While we have shown in this proof-of-concept study that it is possible to analyze tooth enamel for its  $^2\text{H}$  isotopic composition, the results presented above also indicate

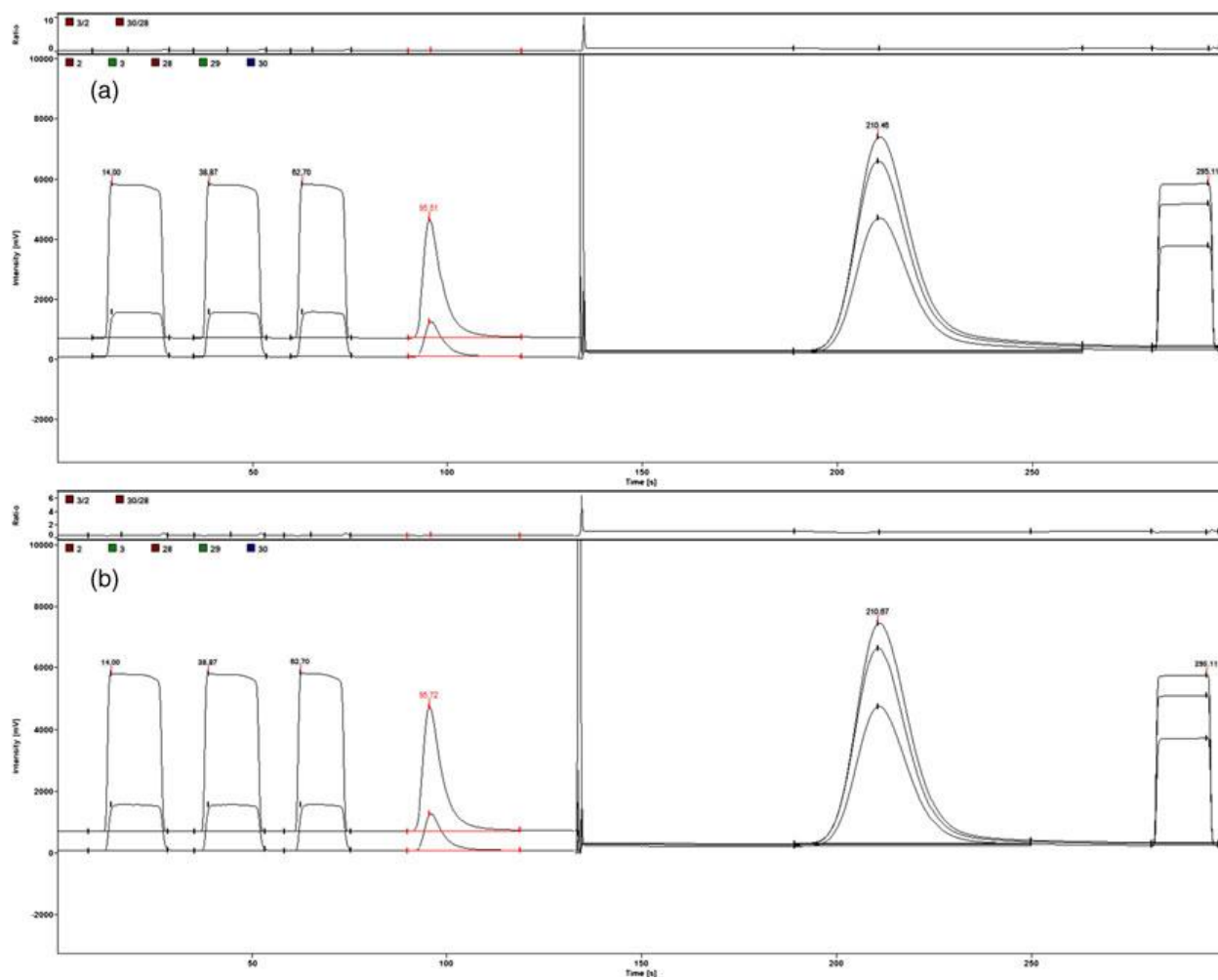
that the  $^2\text{H}$  isotopic signature of tooth enamel cannot be used as an indicator of geographic provenance. However, the seemingly fixed nature of  $^2\text{H}$  abundance in tooth enamel may contain information of interest to researchers in the fields of biogenic minerals, material science and crystallography.

### Acknowledgements

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**Fig. 5.1.** Difference in size and shape of H<sub>2</sub> peak (at ~94 s) when evolved from (a) 0.2 mg or (b) 1.0 mg of ground tooth enamel.



**Fig. 5.2.** Difference in size and shape of  $H_2$  peak (at  $\sim 95$  s) when evolved from 1.5 mg of tooth enamel (a) in the absence and (b) in the presence of powdered graphite.



TABLE 5.1. Comparison of  $\delta^2\text{H}$ -values of tooth enamel depending on sample treatment protocol.

Sample	Country of origin	$\delta^2\text{H}$ [‰], no pre-treatment	$\delta^2\text{H}$ [‰], pre-treatment B	$\delta^2\text{H}$ [‰], pre-treatment B plus graphite	$\delta^2\text{H}$ [‰], pre-treatment S
SC_2M <sup>a</sup>	France	-106.5 ± 3.4	-115.0*	-115.9*	
SC_3M <sup>a</sup>	France	-105.6 ± 3.7	-115.3*	-113.8*	
CLB1_2M <sup>b</sup>	Scotland, UK		-110.4 ± 1.6		
CLB3_2M <sup>b</sup>	UK, region not known				-107.9 ± 1.1
CLB4_2M <sup>b</sup>	Scotland, UK		-112.7 ± 0.8		
RM_2M <sup>a</sup>	Italy		-111.4 ± 1.0		-111.4 ± 0.3
IM_3M <sup>a</sup>	North Africa		-110.5 ± 0.4		-111.7 ± 0.5
JPUM_2M <sup>b</sup>	Not known				-104.3 ± 1.6
HFK_3M <sup>b</sup>	Kent, UK		-111.7 ± 1.7		-106.5 ± 0.8
CLM1_2M <sup>b</sup>	Not known		-111.3 ± 1.1		-108.6 ± 0.5
CLM3_2M <sup>b</sup>	Not known		-108.3 ± 0.5		-106.8 ± 0.5
CLM4_2M <sup>b</sup>	Not known		-109.7 ± 0.5		-108.5 ± 1.0

<sup>a</sup>Archaeological sample.

<sup>b</sup>Modern sample.

\*Samples for which  $\pm 1\sigma$  values are not given were only analyzed once due to limited sample amount.

TABLE 5.2. Comparison of  $\delta^2\text{H}$ -values of tooth enamel\* after H exchange and corresponding H exchange rates.

Sample	$\delta^2\text{H}$ enamel measured $\pm 1\sigma$ in [‰]	$\Delta\delta^2\text{H}$ enamel in [‰] (A-B)	$\delta^2\text{H}$ water	H exchange rate in %
CLB3_2M_A	-108.3 $\pm$ 1.9			
CLB3_2M_B	-110.6 $\pm$ 1.1	2.3		2.62
HFK_3M_A	-104.7 $\pm$ 1.2			
HFK_3M_B	-108.2 $\pm$ 1.9	3.5		3.99
CLM1_2M_A	-105.7 $\pm$ 1.4			
CLM1_2M_B	-108.7 $\pm$ 0.6	3.0		3.42
CLM4_2M_A	-103.1 $\pm$ 2.0			
CLM4_2M_B	-105.9 $\pm$ 0.3	2.8		3.19
JPUM_2M_A	-103.7 $\pm$ 1.6			
JPUM_2M_B	-104.9 $\pm$ 0.6	1.2		1.37
A			-54.1‰	
B		87.8	-141.9‰	

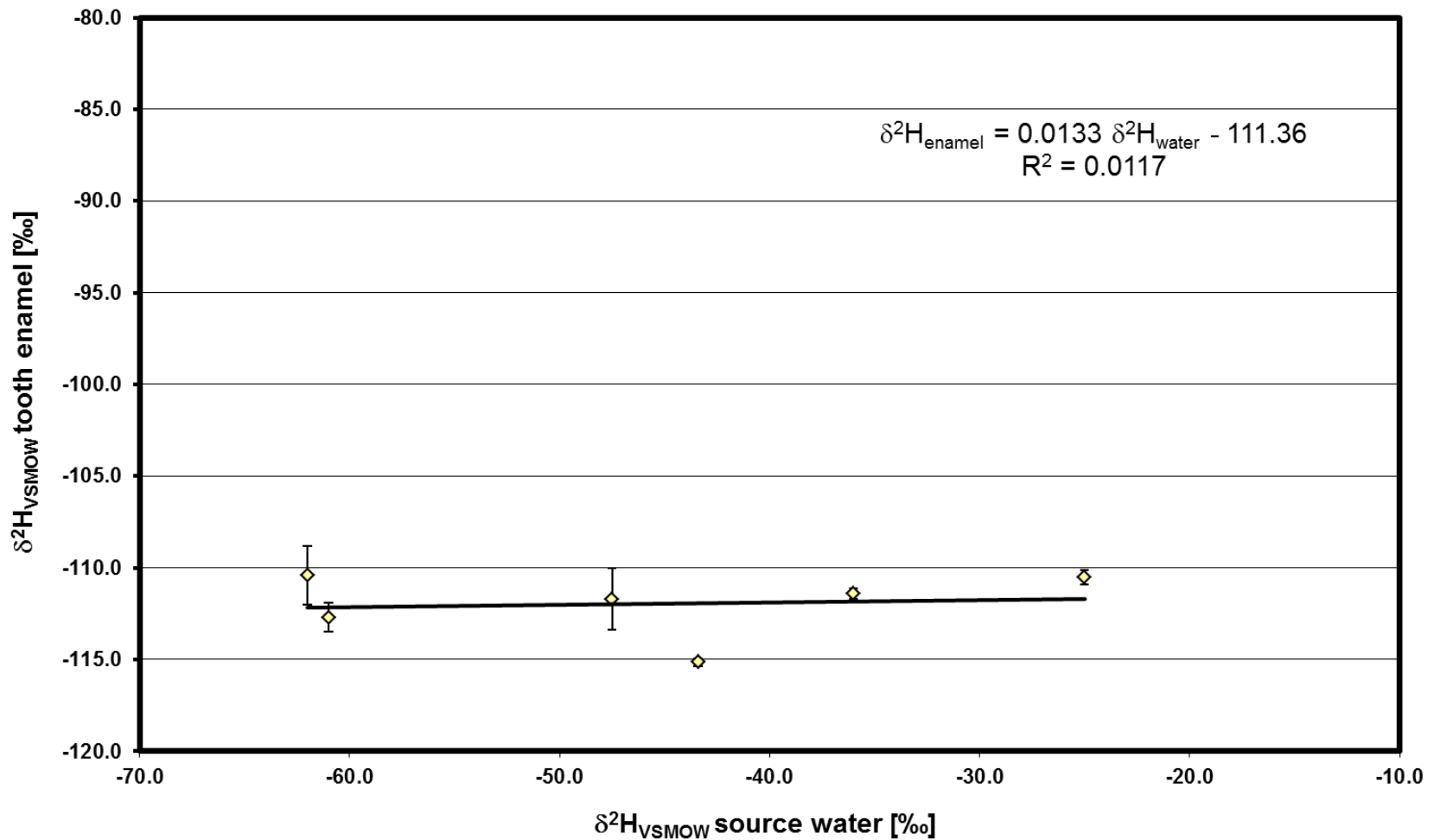
\*Tooth enamel samples were prepared according to protocol S.

TABLE 5.3. Comparison of  $\delta^2\text{H}$ -values of tooth enamel with  $\delta^{18}\text{O}$  (carbonate) values of tooth enamel from the same samples.

Sample	Country of origin	Tooth enamel $\delta^2\text{H}$ [‰], pre-treatment B	Mean tooth enamel $\delta^{18}\text{O}$ [‰] vs. VSMOW <sup>§</sup>	$\delta^{18}\text{O}$ [‰] source water calculated from enamel $\delta^{18}\text{O}$ <sup>#</sup>	$\delta^{18}\text{O}$ [‰] source water OIPC	$\delta^{18}\text{O}$ [‰] source water measured
CLB1_2M	UK, Scotland	-110.4 ± 1.6	14.91 ± 0.45	-10.74	-9.60	-9.70 ± 0.12
HFK_3M	UK, Kent	-111.7 ± 1.7	18.40 ± 0.71	-5.37	-7.90	-6.21 ± 0.16
RM_2M	Italy	-111.4 ± 1.0	18.71 ± 0.53	-4.89	-5.50	
SC_3M	France	-115.3	18.96 ± 0.13	-4.51	-5.80	
IM_3M	North Africa	-110.5 ± 0.4	19.49 ± 0.82	-3.69	-4.10	

<sup>§</sup>Phosphate  $\delta^{18}\text{O}$ -values were calculated from measured carbonate  $\delta^{18}\text{O}$ -values using the correlation equation of Iacumin et al. 1996.

<sup>#</sup>Source water  $\delta^{18}\text{O}$ -values were calculated using the correlation equation of Daux et al. 2008.



**Fig. 5.3.** Correlation plot of  $\delta^2\text{H}$ -values from tooth enamel<sup>#</sup> vs.  $\delta^2\text{H}$ -values of source water (calculated using the OIPC).  
<sup>#</sup> Samples SC\_2M and SC\_3M were 2<sup>nd</sup> and 3<sup>rd</sup> molars from the same individual and are therefore represented by one data point only. Although known to be of UK origin, sample CLB3\_2M is not shown here since the individual's place of residence during adolescence could not be verified.

## CHAPTER 6

### BULK STABLE ISOTOPE ANALYSIS OF HUMAN TOOTH DENTIN: A PILOT STUDY

#### Introduction

Stable isotope analysis of tooth enamel, bone, and hair has become a well-recognized and commonly implemented technique for determining provenance of human remains in bioarchaeological studies and, more recently, to facilitate the forensic identification of unidentified human remains (Lee-Thorp 2008; Meier-Augenstein and Fraser 2008). Both  $^{18}\text{O}$  and  $^2\text{H}$  stable isotope signatures are well established proxies as environmental indicators of climate (temperature) and source water and are therefore considered reliable indicators of geographic life trajectories of animals and humans (Hobson et al. 2004; Schwarcz and Walker 2006). Similarly,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  data have distinguished dietary preferences in ancient human populations, and have been used to qualify  $^2\text{H}$  and  $^{18}\text{O}$  geolocational data that is consistent with more than one location (Meier-Augenstein and Fraser 2008; Rauch et al. 2007). While studies focusing on the isotopic analysis of mammalian tooth dentin have emerged in the literature, few if any studies have systematically investigated the multi-isotopic signatures in human tooth dentin (Dupras and Tocheri 2007). Since  $\delta^{18}\text{O}$  values obtained from tooth enamel of late-erupting molars are a source of information on geographic origin of an individual during adolescence when crown formation takes place, we hypothesized that potentially

valuable information can be obtained from the stable isotopic composition of human crown dentin related to geographic provenance and dietary intake.

This study builds upon results obtained from a feasibility study conducted by Holobinko et al. (2011a), who determined that the abundance of  $^2\text{H}$  in tooth enamel, while measurable, is not an appropriate proxy for human provenance due to its seemingly “fixed” nature. Consequently, we measured the isotopic abundance of  $^2\text{H}$  and  $^{13}\text{C}$  in human dentin collagen obtained from archaeological and modern teeth using continuous-flow isotope ratio mass spectrometry (IRMS) and compared these findings with the  $^{18}\text{O}$  and  $^{13}\text{C}$  isotopic composition of the corresponding tooth enamel carbonate (Holobinko et al. 2011b). The results of this study were presented at the 2011 European Geosciences Union Assembly Meeting in Vienna, Austria.

## Materials and Methods

### Tooth Samples

Seven second or third permanent molars from humans of different geographic provenance were all mechanically cleaned to remove any surface contamination (Henton et al. 2010). Crown enamel and mantle dentin samples were collected using a dental drill with a diamond-tipped bit. Powdered enamel was cleaned and subjected to an acid-digest procedure as previously described (Henton et al. 2010; Holobinko et al. 2011a). Powdered primary dentin was demineralized for 72 h in 0.5 N HCl. Alkali-soluble contaminants were removed in 0.1 M NaOH for 24 h and residual lipids were dissolved in a 2:1:0:8 mixture of methanol, chloroform and water for 24 h. The collagen

pellet, isolated after repeated washes with deionized water and centrifugation, was freeze-dried to yield an amorphous white to yellowish material. All preparation and subsequent analysis was performed by staff at the Stable Isotope Laboratory, James Hutton Institute (formerly Scottish Crop Research Institute), Dundee, UK.

### Bulk $^2\text{H}$ , $^{18}\text{O}$ , $^{13}\text{C}$ , and $^{15}\text{N}$ Isotope Analysis

Stable isotope analysis of the various tissue samples by Continuous Flow – Isotope Ratio Mass Spectrometry (CF-IRMS) was carried out as described elsewhere (Henton et al. 2010; Holobinko et al. 2011a; Landwehr et al. 2011). To determine ‘true’ dentin collagen  $\delta^2\text{H}$  values, i.e.  $\delta^2\text{H}$  values of non-exchangeable H, all dentin collagen samples were subjected to a two-stage H exchange procedure described in detail in Landwehr et al. (2011), based on the protocol reported by Bowen et al. (2005). All measured stable isotope data were properly scale normalized to VPDB or VSMOW using two-point end-member correction.

## Results and Discussion

Comparing enamel  $\delta^{13}\text{C}$  values with dentin collagen  $\delta^{13}\text{C}$  values, both adjusted for trophic level isotopic shift, showed a consistent difference with dentin collagen  $\delta^{13}\text{C}$  values being slightly more negative than corresponding enamel  $\delta^{13}\text{C}$  values (Fig. 6.1). As to whether this difference is due solely to correspondingly minute changes in diet or is a reflection of diet change in conjunction with a very slow remodeling rate, it is not possible to definitively answer this question based on the currently available data.

However, a slope of 0.85 between enamel  $\delta^{13}\text{C}$  values and dentin collagen  $\delta^{13}\text{C}$  values with an  $R^2$  of 0.89 indicated a significant positive correlation between two substrates with very different biochemical properties (Fig. 6.1).

Dentin collagen  $\delta^2\text{H}$  values covered a range of 16.8 ‰ while the range for corresponding source water  $\delta^2\text{H}$  was 47.0 ‰ (Fig. 6.2). The slope of the linear regression line was 0.26, which is quite similar to those of 0.27 reported by Ehleringer et al. (2008) for the correlation between hair  $\delta^2\text{H}$  values and corresponding source water  $\delta^2\text{H}$  values in North Americans. One possible explanation for this shallow slope could be a remodeling rate of primary dentin that is slower than the formation rate of hair. By analogy, human hair is formed at a 10-30 times faster rate than human nails and this seems to be reflected by the fact that the correlation slope of 0.49 for  $\Delta\delta^2\text{H}$  (hair) /  $\Delta\delta^2\text{H}$  (water) reported for a largely European sample population is higher than the corresponding slope of 0.37 for  $\Delta\delta^2\text{H}$  (nails) /  $\Delta\delta^2\text{H}$  (water) (Fraser and Meier-Augenstein 2007).

Analysis of dentin collagen  $\delta^2\text{H}$  values with the matrix independent variable enamel  $\delta^{18}\text{O}$ , known to be well correlated with source water  $\delta^{18}\text{O}$  values, yielded a positive correlation between these two variables despite their dissimilar characteristics (Fig. 6.3). The correlation factor of 3.1 for enamel carbonate  $^{18}\text{O}$  and dentin collagen  $^2\text{H}$ , similar to the correlation factor for  $^2\text{H}$  and  $^{18}\text{O}$  within the same substrate (i.e., nail keratin, 3.84), suggests that the observed trend is valid or at least not coincidental.



## Conclusions

The positive correlation between collagen  $\delta^2\text{H}$  values of primary dentin with  $\delta^2\text{H}$  values for source water provides the first indication that collagen  $\delta^2\text{H}$  values of primary dentin may serve as a proxy for geographic provenance. Similarly, the positive correlation of collagen  $\delta^2\text{H}$  values from primary dentin with enamel  $\delta^{18}\text{O}$  values suggests that primary dentin collagen  $\delta^2\text{H}$  values are linked to the isotopic composition of source water.

The observed difference of about only 15% on average between corresponding enamel  $\delta^{13}\text{C}$  values and primary dentin collagen  $\delta^{13}\text{C}$  value could be interpreted as an indication that in healthy teeth the remodeling rate of primary dentin collagen is relatively slow. In support of this interpretation further corroborative data would be required from more recently formed tissues.

Preliminary findings suggest that multi-isotope signatures of human tooth dentin, but in particular the information contained in the  $^2\text{H}$  isotopic composition of dentin collagen may improve the quality or accuracy of a quantifiable dietary and geographical life trajectory of an unidentified individual.

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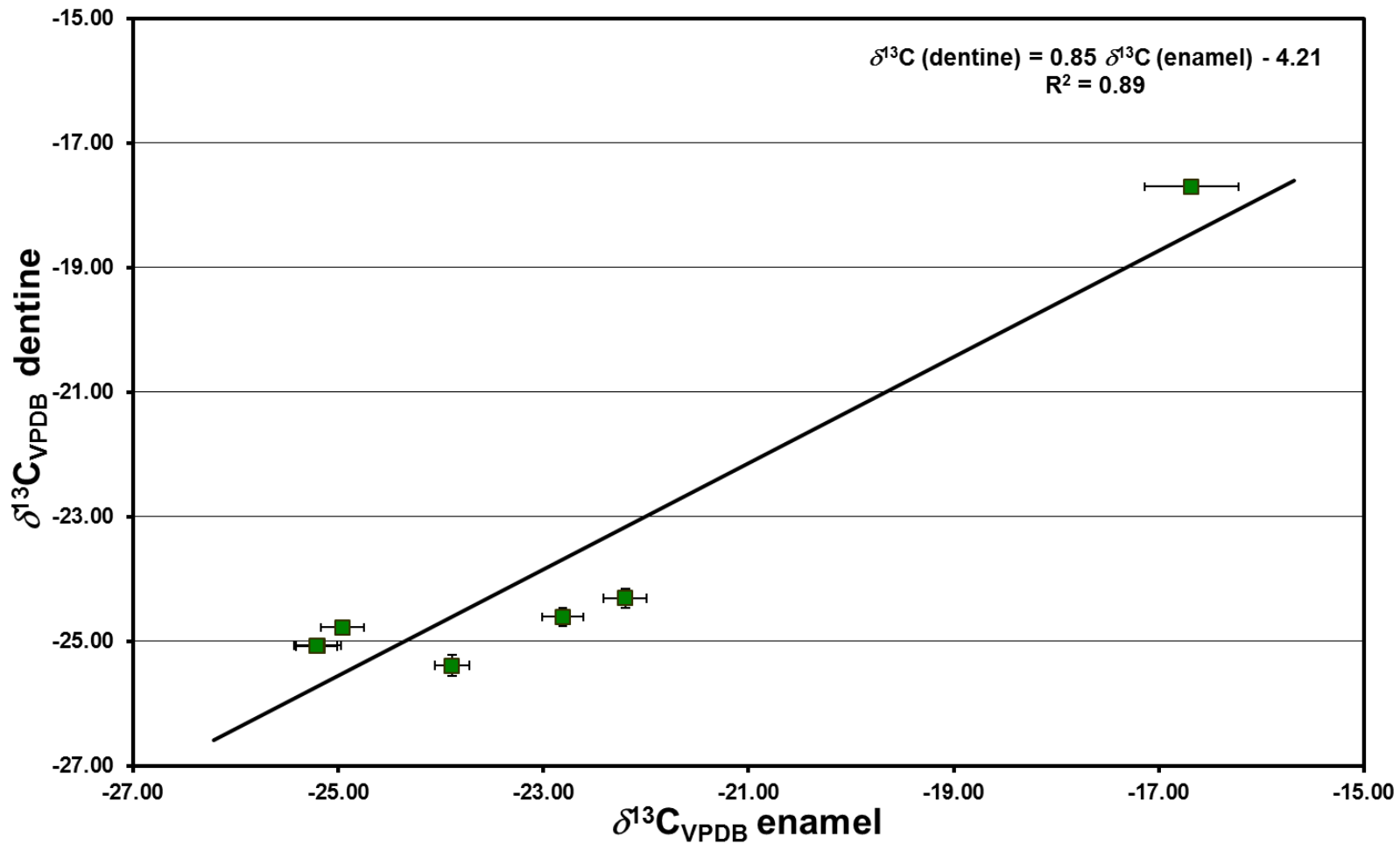


Fig. 6.1. Correlation plot of dentine  $\delta^{13}\text{C}$  values vs. enamel  $\delta^{13}\text{C}$  values.

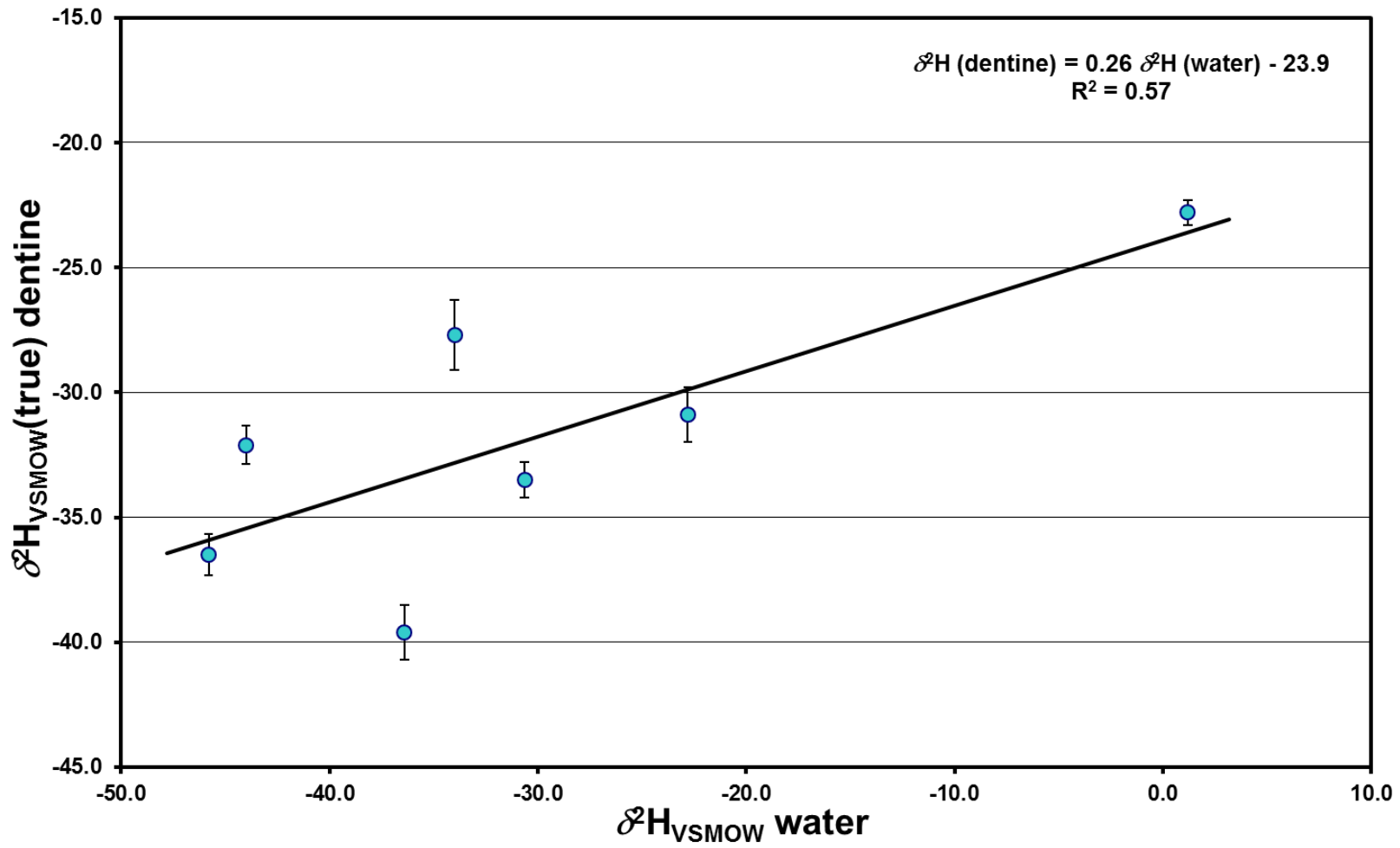


Fig. 6.2. Correlation plot of dentine  $\delta^2\text{H}$  values vs. water  $\delta^2\text{H}$  values.

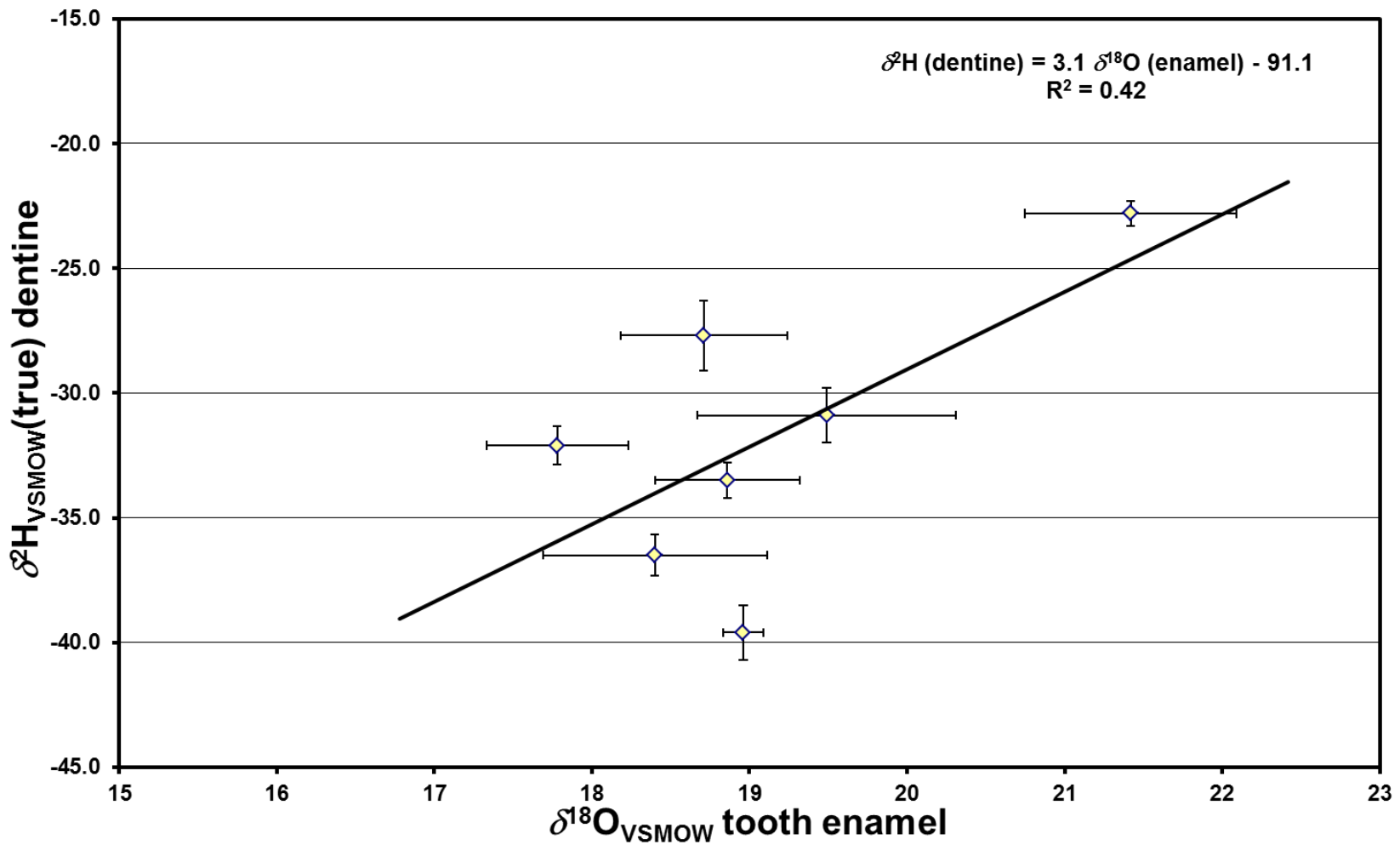


Fig. 6.3. Correlation plot between dentine  $\delta^2\text{H}$  values and enamel  $\delta^{18}\text{O}$  values.

## CHAPTER 7

### OVERVIEW OF MATERIALS AND METHODS

The primary goal of this doctoral research was to determine the collective efficacy of carbon, hydrogen, and oxygen isotope ratios in tooth enamel in determining geographic residence at a particular stage in an individual's lifetime. The three-fold methodology (inter-tooth analysis, intra-tooth analysis, and the systematic comparison of human isotopic delta values with mapped, theoretical isotopic values) employed to test and answer the research questions and corresponding hypotheses put forth in the Introduction is presented below. The cleaning and preparation procedures briefly outlined in this section are based on published protocols.

#### Pilot Studies

##### $^2\text{H}$ Isotopic Analysis of Tooth Enamel (Hypotheses 1, 2, 3, 6)

Prior to beginning the main project, two pilot studies were conducted to ensure a rigorous study design and increase the likelihood of a successful outcome. Since the  $^2\text{H}$  isotope analysis of tooth enamel was a significant, yet relatively unexplored, component of the dissertation research, the results from the corresponding pilot study identified practical problems in the overall experimental design (see Chapter 5). Briefly, while it

was possible to analyze the  $^2\text{H}$  isotopic composition of human tooth enamel, the measured  $\delta^2\text{H}$  values were not significantly different with regard to known differences in geographical provenance. Despite differential sample preparation protocols and hydrogen exchange equilibration studies, the  $^2\text{H}$  abundance in tooth enamel was not variable enough to be used as an indicator of geographic origins. Collectively, these results made it unnecessary to proceed with the testing of Hypothesis 6, which stated that a significant correlation would be evident between the  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values within third molar enamel of the Canadian volunteer study population. This finding further influenced the investigation of Hypotheses 8, 9, 10, and 12, which were revised to test strictly  $\delta^{18}\text{O}$  values within third molar enamel.

#### Bulk Stable Isotope Analysis of Tooth Dentin (Hypotheses 4, 5)

The second pilot study was originally designed to build upon the previous feasibility experiment by measuring the isotopic abundance of  $^2\text{H}$ ,  $^{13}\text{C}$ , and  $^{18}\text{O}$  in crown enamel and comparing the corresponding delta values to those obtained from mantle dentin, the layer of dentin most closely associated with crown enamel during tooth development and thus likely to yield similar isotopic data (see Chapter 6). While results from the tooth enamel  $^2\text{H}$  pilot study confirmed that  $^2\text{H}/^1\text{H}$  isotope ratios presently are unsuitable for use as a proxy for geographic provenance, in addition to test for a correlation between dentin  $\delta^2\text{H}$  and source water  $\delta^2\text{H}$  values the dentin pilot study was redesigned to test also for correlations between  $\delta^2\text{H}$  and  $\delta^{13}\text{C}$  values in dentin and  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  values in enamel carbonate.

A positive correlation was observed between the collagen  $\delta^2\text{H}$  values of primary dentin from teeth of this study population and  $\delta^2\text{H}$  values for source water (see Fig. 6.2). This could either be an indication of dentin remodeling albeit slowly or, if it does not, this correlation could show the volunteers did not change geographic location during childhood and early adolescence. Similarly, collagen  $\delta^2\text{H}$  values were positively correlated with enamel  $\delta^{18}\text{O}$  values, which are known to be well-correlated with source water  $\delta^{18}\text{O}$  values. However, the small yet consistent observed difference (~15% on average) between corresponding enamel  $\delta^{13}\text{C}$  values and primary dentin collagen  $\delta^{13}\text{C}$  value suggested that further corroborative data would be required from more recently formed tissues prior to formulating an appropriate explanation for the differential.

The results from both pilot studies suggested that the inclusion of  $^2\text{H}$  and  $^{13}\text{C}$  and  $^{18}\text{O}$  isotopic data from dentin collagen was within neither the financial nor temporal scope of the current project. Therefore, the isotopic abundance of two stable isotopes,  $^{18}\text{O}$  and  $^{13}\text{C}$ , was measured in the tooth enamel samples obtained from the study participants.

## Canadian Volunteer Cohort Study

### Sampling Procedure

All third molar tooth samples obtained for this study were contributed by the University of Toronto, Ontario, Canada. Dr. Gajanan Kulkarni, Department of Pediatric and Preventative Dentistry, Faculty of Dentistry, coordinated sample acquisition at the University of Toronto School of Dentistry and at their affiliated Parkway Dental Clinic.



Adult patients already scheduled for routine tooth extractions by local dentists were asked to participate in a brief survey and donate their extracted teeth for isotopic analysis. At the time patients were notified a late-erupting (i.e., second or third molar) tooth extraction would be necessary, their participation in this study was requested. Querying patients before the actual extraction allowed them to consider participating prior to their actual enrollment in the study. Only individuals of legal consenting age were permitted to participate. Participants were also asked to donate scalp hair samples and fingernail clippings at the time of their tooth extraction since analysis of these materials was initially considered during the project's planning stages.

Written patient consent was obtained prior to or on the date of extraction (See APPENDIX B). Patients were also required to complete a brief, anonymous Participant Questionnaire and answer questions pertaining to residential and dietary patterns (see APPENDIX C). No personal information was collected on study participants. Although each patient was required to print and sign his or her name on the consent form prior to the extraction procedure, the consent form was immediately separated from the completed Participant Questionnaire and stored in a different location to assure complete anonymity. All documentation pertinent to this phase of the research was previously approved by the Hamilton Health Sciences/Faculty of Health Sciences Research Ethics Board and the Southern Illinois University Carbondale (SIUC) Human Subjects Committee.

All other teeth (i.e., incisors, pre-molar, first molar) utilized for test sample purposes (see below) were extracted from cadavers supplied by the Southern Illinois University Carbondale Department of Anatomy. Human Subjects Approval was not required for

this component of the study as all donors previously authorized the use of their physical remains for research purposes. All extractions followed previously established protocol (see Kruger 1984a,1984b), and were performed under the supervision of Cydney A. Griffith, Associate Professor of Mortuary Science, in the School of Allied Health prior to the embalming procedure.

### Study Sample

A total of 18 patients donated their extracted third permanent molars to this project. While complete sets of 4 third molars from each patient were desired, only 10 of the above 18 patients' teeth met the viability criteria (i.e., sufficient crown enamel and absence of dental caries or restorations). Consequently, the third molars from those 10 individuals were used in the following systematic evaluation of isotopic ratio relationships. Two of the four third molars from each individual were used in this research. The remaining two teeth were retained for future validation studies. The demographic data for these donors are presented in Table 7.1. The subject pool consisted of six females and 4 males ranging in age from 16 to 27 years. While all 10 individuals resided in the province of Ontario at the time of sampling, provincial residential history during the ages of six to 18 years varied among study participants. Nine individuals were raised in Ontario; one subject originated from Montreal, Quebec. Two females were self-declared vegetarians; none of the individuals self-identified as adhering to a vegan diet. All ten individuals indicated that they had refrained from non-local travel during the 6 months prior to their sampling date (i.e., extraction

appointment).

## Dental Protocol

### Tooth Sampling

Dentition. At least two late erupting teeth (i.e., third molars) were obtained from each living adult human volunteer with known lifetime residential and dietary data. Sampling a late erupting second or third molar (M2 or M3) is critical since the respective crown development occurs during middle childhood and adolescence (Hillson 1996). Late-erupting molars therefore provide information on geographic origins between the approximate ages of 8 and 15 years. Alveolar location of the second or third molar (i.e., maxillary versus mandibular) has no influence on its developmental timeline. Dentin develops concurrently with enamel formation. While some patients had second molars extracted, only third molars were used in this study.

Intratooth Analysis. The isotopic data from the crown enamel of each third molar was analyzed to determine if oxygen and carbon stable isotopic ratios confirmed an individual's self-reported residence and dietary preferences during adolescence.

Intertooth Analysis. The isotopic data from the crown enamel of each third molar was compared to that of another third molar from the same individual to determine if teeth

forming at approximately the same time produce similar isotopic values (i.e., identify potential differences in remodeling rates).

### Extraction Methodology

Extraction of viable (i.e. sufficient crown/dentin preservation) third molars was performed by an oral surgeon affiliated with the University of Toronto, Ontario, Canada. All extracted teeth were previously designated for extraction, and all procedures were conducted at the clinic of the participating surgeon. The staff at each clinic was provided with a sampling kit (e.g., zip-top plastic bags, glass vials, individually packaged alcohol wipes), clinical staff instructions, and a binder containing copies of information letters, blank consent forms, and patient questionnaires.

Upon extraction the molars were placed in a clean, glass screw-thread sample vial labeled with the subject identifier number in accordance with established protocol (see APPENDIX D). Each vial was sealed and placed into a zip-top plastic bag. The bag was subsequently stapled to the corresponding Participant Questionnaire before being put aside for a later, pre-arranged collection date. Alcoholic saline (9:1 v/v; 0.9% NaCl and 100% ethanol) was later added to the vial to assist in eradicating any viable bacteria present on the teeth. The teeth were refrigerated in their glass vials until shipment to the James Hutton Institute, Scotland, for further processing.

### Enamel Procurement and Preparation

Initial Cleaning. Preparation of teeth for enamel procurement followed the previously

established methods of Lee-Thorp (2008) with modifications by Meier-Augenstein (2010) (See APPENDIX E). All teeth extracted underwent an initial cleaning process (i.e., light scrubbing with a soft-bristled toothbrush) to remove surface contaminants including blood and periodontal tissue. Each tooth was then soaked in 1.5% v/v sodium hypochlorite solution for 10 minutes, and rinsed thoroughly in deionized water for 5 minutes. Teeth were then soaked in 0.1M acetic acid for 10 minutes followed by another 5 minute rinse in deionized water before being left to air dry overnight.

Prior to further processing, two teeth from each set of 4 molars were selected for inclusion in this study. Each pair of teeth was photographed; sample characteristics were documented as appropriate. The remaining two teeth from each set of 4 molars were placed in storage for future analysis.

Procurement. Enamel was procured according to the following procedure. After air-drying, teeth were cleaned of any surface contaminants (including alveolar bone remnants) using a low-speed dental drill with a diamond drill bur. A Nouvag NM-300 dental drill fitted with a diamond bit was used to remove as much of the crown enamel layer as possible (i.e., a minimum of 80 mg dry sample) onto a square of aluminum foil. Exceptional care was taken to avoid contaminating the enamel with dentin as numerous subsamples of the same tooth enamel were run simultaneously to verify analytical reproducibility. Prior to chemical cleaning, each of the powdered enamel samples was weighed, divided equally into 2mL Eppendorf micro-centrifuge tubes, and stored in a non-evacuated desiccator over a strong drying agent (Sicapent or phosphorous pentoxide) to keep the powdered enamel isolated from ambient humidity. The enamel

was subsequently cleaned with sodium hypochlorite (NaOCl) according to the modified procedures originally outlined by Lee-Thorp (2008) before being prepared for isotopic ratio analysis (i.e., acid digest procedure) (see APPENDIX F).

Chemical Cleaning. The powdered enamel samples were chemically cleaned using a series of steps which included vortexing, mixing, centrifuging, aspiration and discard of supernatant, re-suspension of the resulting pellet, and two identical rinse sequences.

First, 0.04 mL/mg of 1.5% v/v NaOCl was added to each Eppendorf tube containing a powdered enamel sample. Following a brief vortex of the sample, the tube was placed on a rocker bed and left for 30 minutes to mix at room temperature. The sample was removed from the rocker bed, briefly vortexed, and centrifuged at 10,000 rpm for 5 minutes. After aspirating and discarding the supernatant, the pellet was re-suspended in 2 mL deionized water, vortexed for 10 seconds, and centrifuged at 10,000 rpm for 5 minutes [= rinse step one]. This rinse sequence was repeated twice.

After the final repetition the supernatant was aspirated and then discarded before the pellet was re-suspended in 0.04 mL/mg 0.1M acetic acid. Following a brief vortex, the sample was left on a rocker bed to mix for 10 minutes at room temperature, vortexed again, and then centrifuged at 10,000 rpm for 5 minutes. The sample was then subjected to a second rinse sequence identical to the first (see above). Following the final rinse sequence the supernatant was aspirated and then discarded.

Samples were placed in a non-evacuated desiccator over phosphorous pentoxide for 48 hours, and then transferred to an evacuated desiccator and dried with phosphorous pentoxide for a minimum of 7 days. After this final drying period samples

were weighed out (~8-10 mg) into 13 mL Exetainer tubes and stored in an evacuated desiccator until the acid digest procedure could be performed.

Sample Preparation for  $^{13}\text{C}/^{12}\text{C}$  and  $^{18}\text{O}/^{16}\text{O}$  Analysis. While  $^{18}\text{O}/^{16}\text{O}$  ratio analysis can be conducted on both mineral components of bioapatite (i.e. enamel carbonate and phosphate),  $^{13}\text{C}$  can be extracted only from carbonate. If both  $^{13}\text{C}$  and  $^{18}\text{O}$  are to be analyzed,  $\text{CO}_2$  from the enamel carbonate will yield delta values for both stable isotopes. Therefore, the powdered enamel was processed following established protocol for preparing enamel carbonate for  $^{13}\text{C}$  and  $^{18}\text{O}$  isotope analysis using an acid digest. For validation purposes powdered enamel samples were analyzed at two separate facilities (i.e., James Hutton Institute, Southern Illinois University Carbondale).

The James Hutton Institute Stable Isotope Laboratory, Scotland, utilizes a sulphuric acid digest procedure (after Henton et al. 2010) (see APPENDIX F). Briefly, each Exetainer (Labco, High Wycombe, UK) was flushed with gaseous nitrogen (N5.5 grade BOC #293679) for 8-12 minutes to remove traces of residual atmospheric  $\text{CO}_2$ . Using a 1 mL disposable syringe fitted with a 21G needle, 800  $\mu\text{L}$  sulphuric acid (99.999% purity) was injected through the septum of each Exetainer. A new syringe and needle was used for each Exetainer.

Exetainers were placed into a pre-heated heater block for 6 hours at  $50^\circ\text{C}$ . After 6 hours the heater block was switched off, and the samples were allowed to cool off while remaining in the heater block overnight. After the samples had cooled completely, they were transferred to the AP2003 Breath Gas Analyzer isotope ratio mass spectrometer for determination of  $^{13}\text{C}$  and  $^{18}\text{O}$  abundance and subsequent analysis.

Powdered enamel samples analyzed at the Mass Spectrometry Facility at Southern Illinois University Carbondale underwent an offline phosphoric acid digest (see APPENDIX G) prior to analysis for isotopic abundance. Each 20 ML Labco Exetainer containing approximately 5 mg of dried and cleaned powdered enamel was placed in a customized vial holder at a slight angle, and four drops (200  $\mu$ L) of 102% vacuum-degassed phosphoric acid was dropped using a Hamilton 0.5 mL syringe onto the inside wall of the vial close to the threaded neck. The vial tilt angle was adjusted to prevent the acid from traveling toward the bottom of the vial too quickly. The vials containing sample and acid were flush-filled offline with ultra-pure helium (>99.99995% He) for 5 minutes to remove atmospheric CO<sub>2</sub>, and then placed in the gas bench in a vertical position so the acid flowed toward the sample at the bottom of the vial. Exetainer tubes containing enamel samples, QC samples, and standards were left to react in the sample tray of the gas bench heating block for 24 hours at 25°C. Headspace CO<sub>2</sub> was analyzed at the same temperature.

Since the research samples were the first tooth enamel carbonates to be analyzed at the Mass Spectrometry Facility at SIUC, test samples consisting of enamel powder from cadaver teeth were drilled, cleaned, and subjected to phosphoric acidification in order to determine optimum amounts of sample and acid. Three bulk samples comprised of raw and cleaned enamel powder were drilled from incisors, a premolar, and a first molar using a Dremel drill with diamond-tipped drill bit. Test samples were cleaned using the previously described protocol (see APPENDIX E) and subjected to an experimental acid digest and IRMS analysis outlined in Appendix G.



## Enamel Isotopic Analysis

$^{13}\text{C}$  and  $^{18}\text{O}$  Isotopic Abundance. All powdered enamel samples were analyzed for  $^{13}\text{C}$  and  $^{18}\text{O}$  isotopic composition at the James Hutton Institute Stable Isotope Laboratory, Scotland and the Mass Spectrometry Facility at Southern Illinois University Carbondale. All enamel samples at either facility were analyzed along with blanks and exetainers containing QC materials and reference material laboratory standards for subsequent two-end-point normalization, or scale correction, a procedure employed to assist in the adjustment of measured  $\delta$  values to internationally accepted reference scales (see Meier-Augenstein 2010).

JHI Stable Isotope Laboratory staff transferred acidified samples to the AP2003 Breath Gas Analyzer isotope ratio mass spectrometer for determination of  $^{13}\text{C}$  and  $^{18}\text{O}$  abundance and subsequent analysis. Enamel samples, two internal acquisition quality controls (sodium bicarb-X:  $\delta^{18}\text{O}_{\text{VPDB}} = -12.38 \text{ ‰}$ ; sodium bicarb-H:  $\delta^{18}\text{O}_{\text{VPDB}} = -10.60 \text{ ‰}$ ), and 0.5 mg of the  $^{18}\text{O}$  international reference material NBS-19 (TS-limestone) ( $\delta^{13}\text{C}_{\text{VPDB}} = +1.95 \text{ ‰}$ ;  $\delta^{18}\text{O}_{\text{VPDB}} = -2.20 \text{ ‰}$ ; IAEA) or the international  $^{13}\text{C}$  reference material LSVEC (lithium carbonate) ( $\delta^{13}\text{C}_{\text{VPDB}} = -46.60 \text{ ‰}$ ;  $\delta^{18}\text{O}_{\text{VPDB}} = -26.60 \text{ ‰}$ ; IAEA) were put through the same acid digest procedure and subsequent analysis contemporaneously to anchor and quality control the sample results.

Each exetainer in the batch was sampled three times, and each aliquot of the  $\text{CO}_2$  contained therein was passed through a capillary into a gas sampling loop, and then ultimately admitted into the isotope ratio mass spectrometer (IRMS) for analysis. Once the sample gas was successfully received and analyzed, an aliquot of the internal reference gas ( $\text{CO}_2$ ) was introduced to the mass spectrometer as part of the same

analytical cycle.

The measured  $\delta^{18}\text{O}$  values were two-end-point normalized using NBS-19 and LSVEC as the end members. Reproducibility of the  $^{18}\text{O}$  isotope analysis as monitored by the reference materials (RMs) was typically  $\pm 0.18$  ‰. Uncertainty of the sample measurements was typically  $\pm 0.50$  ‰, which is a composite of the aforementioned reproducibility and intra-sample variability.

Dr. Mihai Lefticariu, Mass Spectrometry Facility, SIUC, determined the abundance of  $^{13}\text{C}$  and  $^{18}\text{O}$  in the enamel samples utilizing a Thermo Fisher Scientific Delta V Plus-IRMS coupled to a Thermo Fisher Scientific GasBench II system including autosampler. Enamel samples and exetainers containing 0.25 mg of the  $^{18}\text{O}$  international reference materials NBS-19 ( $\delta^{13}\text{C}_{\text{VPDB}} = +1.95$  ‰;  $\delta^{18}\text{O}_{\text{VPDB}} = -2.20$  ‰; IAEA) and NBS-18 (calcite) ( $\delta^{13}\text{C}_{\text{VPDB}} = -5.014$  ‰;  $\delta^{18}\text{O}_{\text{VPDB}} = -23.2$  ‰; IAEA), or the  $^{13}\text{C}$  reference material LSVEC ( $\delta^{13}\text{C}_{\text{VPDB}} = -46.60$  ‰;  $\delta^{18}\text{O}_{\text{VPDB}} = -26.60$  ‰; IAEA) were put through the same acid digest procedure (except only 40  $\mu\text{L}$  of acid were required for acidification) and subsequent analysis contemporaneously. Carrara marble powder (CM) ( $\delta^{13}\text{C}_{\text{VPDB}} = +2.16 \pm 0.05$  ‰;  $\delta^{18}\text{O}_{\text{VPDB}} = -1.76 \pm 0.05$  ‰; IAEA) was used for quality control measures.

Following acidification, exetainers were transferred to the temperature stabilized tray (81°C) and sampled by the instrument. One aliquot was taken from each exetainer and analyzed for  $\text{CO}_2$  abundance similar to the analytical procedure described above. Duplicate samples were run the next day. The average analytical precision was  $\pm 0.09$  ‰ for  $\delta^{13}\text{C}_{\text{VPDB}}$  and  $\pm 0.13$  ‰ for  $\delta^{18}\text{O}_{\text{VSMOW}}$ . The average difference between the original (first day) and the duplicate (second day) delta values was 0.06 ‰ for  $\delta^{13}\text{C}_{\text{VPDB}}$

and 0.04 ‰ for  $\delta^{18}\text{O}_{\text{VSMOW}}$ .

### Data Evaluation

Typically,  $^{18}\text{O}$  isotope analysis of tooth carbonate yields values on the VPDB scale, therefore requiring the conversion of  $\delta^{18}\text{O}_{\text{VPDB}}$  into corresponding  $\delta^{18}\text{O}_{\text{VSMOW}}$  values in order to calculate correlations between the  $\delta^{18}\text{O}$  values of tooth carbonate and the  $\delta^{18}\text{O}$  values of source water. This conversion is given by (Friedman and O'Neil 1977):

$$\delta^{18}\text{O}_{\text{VSMOW}} = 1.03086 \delta^{18}\text{O}_{\text{VPDB}} + 30.86$$

However, original  $\delta^{18}\text{O}$  values for the international reference material NBS-18 for  $^{18}\text{O}$  analysis of carbonate are reported by the SIUC Department of Chemistry and Biochemistry Mass Spectrometry Facility in accordance with VSMOW standards. Therefore, these values were converted back into VPDB values using the following formula suggested by O'Neil (1979), who reported deviating equations for VSMOW-VPDB scales:

$$\delta^{18}\text{O}_{\text{VPDB}} = 0.97006 \delta^{18}\text{O}_{\text{VSMOW}} - 29.94$$

Analytically available oxygen is present in the carbonate and phosphate fractions of human tooth enamel. If diagenesis has not affected changes to the bio-apatite, then  $\delta^{18}\text{O}$  from either fraction may be used to calculate corresponding  $\delta^{18}\text{O}$  values for source

water using the appropriate correlation equations. If carbonate values of  $^{18}\text{O}$  are to be used for this purpose, then  $\delta^{18}\text{O}_{\text{VPDB}}$  values must first be converted into  $\delta^{18}\text{O}_{\text{VSMOW}}$  values since  $\delta^{18}\text{O}_{\text{Phosphate}}$  values are traditionally reported on the VSMOW scale. Therefore, although phosphate  $\delta^{18}\text{O}$  values in the tooth enamel samples were not directly measured using mass spectroscopy, it is possible to estimate  $\delta^{18}\text{O}_{\text{Phosphate}}$  values by converting measured  $\delta^{18}\text{O}_{\text{VSMOW}}$  values using the formula:

$$\delta^{18}\text{O}_{\text{Phosphate}} = 0.98 \delta^{18}\text{O}_{\text{Carbonate}} - 8.5 \text{ (Iacumin et al. 1996)}$$

The original equation comparing  $\delta^{18}\text{O}_{\text{Phosphate}}$  values and  $\delta^{18}\text{O}$  values of source water was obtained using the bone apatite of individuals residing in a temperate climate (i.e., Central Europe) who died between the end of the 19<sup>th</sup> century and 1950 (Longinelli 1984). Work by Daux et al. (2008) has confirmed this equation while further refining it by including in their sample the tooth phosphate of modern individuals from diverse geographic areas and examining variance due to differences in the isotopic composition of drinking water as opposed to solid food water. Particularly, the  $^{18}\text{O}$  isotopic composition of foods which typically are cooked prior to consumption tends to be enriched due to the incorporation of  $^{18}\text{O}$ -enriched water during the cooking process. This leads to an increase in the  $\delta^{18}\text{O}$  component of a person's total water intake. Meier-Augenstein (2010:239) recommends utilizing both equations during the correlational analysis of phosphate and source water  $\delta^{18}\text{O}$  values. Measured  $\delta^{18}\text{O}_{\text{VSMOW}}$  values were converted into calculated  $\delta^{18}\text{O}$  phosphate values and compared using both equations:

$$\delta^{18}\text{O}_{\text{Phosphate}} = 0.64 \delta^{18}\text{O}_{\text{water}} + 22.37 \text{ (Longinelli 1984)}$$

$$\delta^{18}\text{O}_{\text{Phosphate}} = 0.65 \delta^{18}\text{O}_{\text{water}} + 21.89 \text{ (Daux et al. 2008)}$$

Calculated annual average  $\delta^{18}\text{O}$  values in precipitation were determined using the Online Isotopes in Precipitation Calculator (OIPC) (Bowen 2014), a program allowing users to calculate the estimated modern mean annual and monthly  $^{18}\text{O}$  isotope composition of precipitation at a specified location (Bowen and Wilkinson 2002; Bowen and Revenaugh 2003; Bowen et al. 2005) (Table 7.2). Users enter site location information including latitude, longitude, and altitude, and can request estimates of the 95% confidence intervals for estimated annual values, which are useful in determining the significance of any non-random sampling effects on the interpolated values. Three organizations provide the isotopic data available to OIPC users: the International Atomic Energy Association (IAEA), World Meteorological Organization (WMO), and the Global Network for Isotopes in Precipitation (GNIP).

### Statistical Analysis

Because the current research involved a small sample size (less than 20 individuals) and an unknown distribution of data, non-parametric statistical tests were seemingly the most appropriate methods for more complex quantitative analysis. However, the Wilcoxin test, for example, can be used only in cases in which both variables have been measured. The test is inappropriate for comparisons between the  $\delta^{18}\text{O}$  values measured in tooth enamel and interpolated annual average  $\delta^{18}\text{O}$  values in precipitation.

Similarly, the Mann-Whitney is unsuitable because the third molars from each subject are paired or matched.

The parametric analysis of variance method may also be suitable for analyzing data obtained under the above circumstances, provided all testing conditions and assumptions remain equal. An ANOVA's reduction in power resulting from small sample sizes may be offset by its exceptional ability to detect slight differences in the data. The delta values obtained from isotopic analysis were subjected to univariate descriptive statistics including means testing and standard deviation (e.g., a data point representing the  $\delta^{18}\text{O}$  value for tooth enamel would consist of a mean calculated from the duplicate analysis of one sample). The amount and significance of variation among subjects, molars, and subsamples was quantified using a 3-factor ANOVA with random effects (Model II). Linear regression analysis was employed to explore correlations between the tooth enamel  $\delta^{18}\text{O}$  values as measured and plotted against source water  $\delta^{18}\text{O}$  values. All tests used a minimum  $P < 0.05$  significance level, and were performed on SAS 9.3 (SAS Institute Inc., 2013-2014).

TABLE 7.1. Demographic data for 10 study participants from whom 4 third molars were obtained and subjected to isotopic ratio analysis. Nine subjects were born and raised in the province of Ontario, Canada (ON). One individual originated from Quebec, Canada (QC).

Subject Identifier Number	Sex	Age	Place of Birth	Residence at 6-10 years	Residence at 11-18 years	Dietary Preferences
SY4	F	20	Toronto, ON	Toronto, ON	Toronto, ON	Vegetarian
CL002	M	16	Toronto, ON	Toronto, ON	Toronto, ON	Meat
CL005	F	26	Richmond Hill, ON	Oakville/Scarborough, ON	Hamilton, ON	Vegetarian
CL006	M	27	Waterford, ON	Waterford, ON	Waterford, ON	Meat
SY007	F	22	Scarborough, ON	Scarborough, ON	Richmond Hill, ON	Meat
CL008	F	18	Toronto, ON	Markham, ON	Markham, ON	Meat
CL009	F	25	Montreal, QC	Montreal, QC	Montreal, QC	Meat
CL0010	F	18	Toronto, ON	Toronto, ON	Toronto, ON	Meat
CL0013	M	16	Toronto, ON	Toronto, ON	Toronto, ON	Meat
MCM1	M	20	Hamilton, ON	Hamilton, ON	Hamilton, ON	Meat

TABLE 7.2. Site data and corresponding OIPC interpolated mean annual  $^{18}\text{O}$  composition of precipitation. Isotopic fields calculated at 95% confidence interval (Bowen 2014; Bowen and Revenaugh 2003).

Subject Identifier Number	Latitude (N)	Longitude (W)	Elevation (m)	OIPC Isotopic Field (‰)
SY4, CL002, CL0010, CL0013	43.653226	-79.383184	118	-9.4 ± 0.3
CL005	43.467517	-79.687666	121	-9.3 ± 0.2/
	43.776426	-79.231752	159	-9.6 ± 0.3
	43.250021	-79.866091	155	-9.3 ± 0.3
CL006	42.931606	-80.289583	232	-9.2 ± 0.2
SY007	43.776426	-79.231752	159	-9.6 ± 0.3
	43.882840	-79.440281	231	
CL008	43.856100	-79.337019	190	-9.7 ± 0.3
CL009	45.508670	-73.553992	16	-10.7 ± 0.6
MCM1	43.250021	-79.866091	155	-9.3 ± 0.3



## CHAPTER 8

### RESULTS OF CANADIAN VOLUNTEER COHORT STUDY

The isotopic abundance of  $^{13}\text{C}$  and  $^{18}\text{O}$  was measured in the tooth enamel of 20 third molars donated by study participants residing in Ontario, Canada, at the time of sampling. The subject group consisted of males and females between the ages of 16 and 27 years with varying residential history and dietary preferences (Table 7.1). While tooth enamel samples were analyzed for their isotopic abundance at two different laboratories (Southern Illinois University Carbondale and the James Hutton Institute) for cross-validation purposes, for the sake of brevity only those results obtained from Southern Illinois University Carbondale are presented below.

#### Carbon Isotope Analysis

##### Detecting Regional Variability in Diet (Hypothesis 7)

Tooth enamel samples from 10 subjects (2 third molars from each individual) were analyzed for  $^{13}\text{C}/^{12}\text{C}$  isotopic ratio abundance using Gas Bench-IRMS (see APPENDIX H). The mean  $\delta^{13}\text{C}_{\text{VPDB}}$  (NBS-19, NBS-18, and LSVEC) values for all samples run in duplicate on two consecutive days ranged from -9.47 ‰ to -11.31 ‰ (Table 8.1, Fig. 8.1). The pooled mean  $\delta^{13}\text{C}$  value was -10.37 ‰ with a standard deviation (SD) of  $\pm 0.62$  and a 95% confidence interval of  $\pm 0.29$ . All values fell within the range of -9.0 ‰

to -16.0 ‰ (average of -12.0 ‰), which is indicative of a persistent C<sub>4</sub> plant dietary influence at the time the sampled tooth enamel was forming. The enriched  $\delta^{13}\text{C}$  values present in the dataset suggest a larger reliance on corn-based products, which is typical of a North American diet.

### Differentiating Between Dietary Preferences (Hypothesis 11)

Two females out of the ten individuals sampled were vegetarians. Both subjects (CL005, SY4), aged 26 and 20, lived in the same metropolitan region (Hamilton and the greater Toronto area, Ontario, Canada) during the period of time the sampled enamel formation was initiated and completed (Table 7.1, Table 7.2). Their mean  $\delta^{13}\text{C}$  values ranged from -9.92 ‰ to -11.16 ‰, respectively (Table 8.1, Fig. 8.1). The offset in mean  $\delta^{13}\text{C}$  values between each pair of third molars from each subject ranged from 0.02 ‰ (SY4, CL008) to 0.25 ‰ (CL006). Three individuals exhibited inter-molar offsets favoring carbon. The carbon and oxygen inter-molar offsets for a fourth individual (SY4, female, vegetarian) were equivalent.

In order to test for differences between vegetarian and non-vegetarian (meat-eater) dietary preferences, a linear mixed model was designed and fit to the data using restricted maximum likelihood (MLE) and the Kenward-Roger degrees of freedom method ( $p < 0.05$ ). The latter is particularly useful when attempting to reduce bias associated with small sample sizes (Bell et al. 2010). The model was constructed to include fixed Diet effects (meat-eater, vegetarian); random subject effects were nested within type of Diet (SAS v.9.3). Two random molar effects were contained within each of the 10 subjects; two measurements (i.e., subsamples) were taken for each molar.

Initially, molar-to-molar variation was allowed to vary by sex. However, application of a chi-square test (1df) based on the restricted MLE yielded results non-indicative of any influence of sex on molar-to-molar variation ( $p=0.5589$ ).

After the model was simplified by excluding any molar-to-molar variation due to sex, it became clear that inter-subject variation ( $\sigma^2_{\text{subject}} = 0.4133$ ) contributed the largest proportion of total  $\delta^{13}\text{C}$  variability. As expected due to the two molar samples from each subject constituting a matched pair, no evidence suggested intra-individual molar-to-molar variation ( $\sigma^2_{\text{molar}} = 0$ ;  $\sigma^2_{\text{subsample}} = 0.0256$ ). A Type III F-test of fixed Diet effects was conducted. Results indicated no evidence suggesting any difference between dietary preferences with respect to mean  $\delta^{13}\text{C}$  values ( $p=0.6633$ ). While one potential outlier was noted, no meaningful differences were observed between the results when the analysis was performed without this case so the data was retained.

## Oxygen Isotope Analysis

### Intra- and Inter-individual Variation in Residence Patterns (Hypotheses 8, 12)

Twenty tooth enamel samples were analyzed for  $^{18}\text{O}$  isotopic abundance using GasBench-IRMS (see APPENDIX G). All  $\delta^{18}\text{O}$  values were initially reported according to VSMOW standards (Table 8.1). The pooled mean  $\delta^{18}\text{O}_{\text{VSMOW}}$  value (NBS-19 and NBS-18) for enamel samples from ten modern individuals was 24.39 ‰ (SD  $\pm 0.39$ ; 95% c.i.  $\pm 0.18$ ). Individual subject mean  $\delta^{18}\text{O}_{\text{VSMOW}}$  values ranged from 23.76 ‰ to 25.18 ‰ (Table 8.1, Fig. 8.1). The difference in mean  $\delta^{18}\text{O}_{\text{VSMOW}}$  values for each pair of third molars varied widely; offsets ranging from 0.01 ‰ (CL0013) to 0.51 ‰ (CL009)

were observed. A mixed-sex group of 6 subjects presented with marked inter-molar oxygen offsets that favored oxygen by as much as 18 to 1 (i.e., 0.36 ‰ offset between molar mean  $\delta^{18}\text{O}$  values vs. 0.02 ‰ offset between mean molar  $\delta^{13}\text{C}$  values) in one female subject (CL008). Following conversion of  $\delta^{18}\text{O}_{\text{VSMOW}}$  values to  $\delta^{18}\text{O}_{\text{VPDB}}$  values (Table 8.1), bivariate plots were constructed for comparison of  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  values in tooth enamel (see Fig. 8.2, Fig 8.3).

A linear mixed model utilizing restricted MLE and the Kenward-Rogers degrees of freedom method was fit to the  $\delta^{18}\text{O}_{\text{VSMOW}}$  data. The model contained fixed Diet effects (meat-eater, vegetarian) and random subject effects nested within Diet type, initially identical to the carbon analysis. No gender differences with respect to molar-to-molar variation were noted ( $p=0.3668$ ). A subsequent refitting of the model to the data suggested that subject-to-subject variation ( $\sigma^2_{\text{subject}} = 0.1200$ ) appeared to be the largest source of total  $\delta^{18}\text{O}$  variability. Pairwise comparisons of the three variance components ( $\sigma^2_{\text{subject}}$ ,  $\sigma^2_{\text{molar}}$ ,  $\sigma^2_{\text{subsample}}$ ) confirmed the significance of subject variation ( $p=0.0034$ ). No difference between diet types with respect to mean  $\delta^{18}\text{O}$  values was detected ( $p=0.2544$ ).

#### Enamel Carbonate Isotopic Data as an Indicator of Provenance (Hypotheses 9, 10, 12)

Tooth enamel carbonate  $\delta^{18}\text{O}_{\text{VSMOW}}$  values were converted to their equivalent estimated enamel phosphate  $\delta^{18}\text{O}$  values using the equation developed by Iacumin et al. (1996) (Table 8.2). Since the calculated enamel phosphate  $\delta^{18}\text{O}$  values were derived from a linear transformation of the carbonate  $\delta^{18}\text{O}_{\text{VSMOW}}$  data, the two sets of data were necessarily correlated ( $R^2 = 1$ ). Drinking water  $\delta^{18}\text{O}$  values ( $\delta^{18}\text{O}_{\text{Water}}$ ) were

subsequently calculated from the enamel samples using equations by Longinelli (1984) and Daux et al. (2008) (Table 8.2). Individual subject mean  $\delta^{18}\text{O}_{\text{Water}}$  values ranged from -10.93 ‰ to -8.78 ‰ (Daux et al. 2008) and from -11.85 ‰ to -9.67 ‰ (Longinelli 1984); the pooled mean  $\delta^{18}\text{O}_{\text{Water}}$  values were -9.98 ‰ and -10.99 ‰, respectively. As expected, a slight offset between the two slopes was noted (see Fig. 8.4) due to the  $\delta^{18}\text{O}$  enrichment one would expect from consuming food cooked in water. Daux values were plotted against the phosphate values to illustrate the positive correlation between ingested water and tooth enamel phosphate  $\delta^{18}\text{O}$  values and utilized in the interpretation of multivariate statistical analyses (see below). Estimated annual average  $\delta^{18}\text{O}$  values in precipitation (i.e., source water) were calculated using the Online Isotopes in Precipitation Calculator (OIPC) (Bowen 2014), and ranged from  $-9.2 \pm 0.2$  ‰ to  $-10.7 \pm 0.6$  ‰ with 95% confidence intervals (Table 7.2, Table 8.2).

A qualitative comparison of the Daux drinking water values and annual average precipitation values suggested few positive correlations. The drinking water values of both third molars from each of the vegetarians, SY4 and CL005, fell within the corresponding range of precipitation values (Table 8.2). However, a differentiation was noted between molar values of the subject residing in Montreal at the time of enamel formation; the  $\delta^{18}\text{O}_{\text{Water}}$  value in CL009A correlated with the precipitation  $\delta^{18}\text{O}$  value while the  $\delta^{18}\text{O}_{\text{Water}}$  value of CL009B showed no correlation (Table 8.2). A similar discrepancy was observed in subject CL0010.

When Longinelli  $\delta^{18}\text{O}_{\text{Water}}$  values were compared against estimated annual OIPC  $\delta^{18}\text{O}$  values, weaker correlations were evident. Both molar  $\delta^{18}\text{O}_{\text{Water}}$  values (CL009A and CL009B) of the subject from Montreal were consistent with the  $\delta^{18}\text{O}_{\text{OIPC}}$  values

corresponding to Montreal location parameters. One of the two third molar  $\delta^{18}\text{O}_{\text{Water}}$  values from subject SY007 (SY007A) concurred with Scarborough, Ontario precipitation  $\delta^{18}\text{O}$  values.

A multivariate linear mixed model was fit to the data in order to statistically evaluate the above observed correlations between  $\delta^{18}\text{O}_{\text{Water}}$  values derived from Daux et al. (2008) (linear transformations of  $\delta^{18}\text{O}_{\text{VSMOW}}$  values) and estimated annual average  $\delta^{18}\text{O}$  values in precipitation. While the model contained fixed Diet effects (meat-eater, vegetarian) and random subject effects nested within type of Diet, the data supported neither additional random Molar effects nor additional random Subsample effects. Linear associations were weak to nonexistent. The estimated correlation between  $\delta^{18}\text{O}_{\text{VSMOW}}$  and  $\delta^{18}\text{O}_{\text{OIPC}}$  was -0.1772. Linear regression analysis of measured tooth enamel  $\delta^{18}\text{O}$  values plotted against annual average precipitation  $\delta^{18}\text{O}$  values is graphically illustrated in Fig. 8.5. Despite qualitative observations, no statistical correlation was evident between the two data sets ( $R^2 = 0.0058$ ).

Estimated monthly  $\delta^{18}\text{O}$  precipitation values were obtained from the OIPC by entering into the web site's online calculator the same location parameters used to calculate interpolated annual average  $\delta^{18}\text{O}$  values in precipitation. Predictably, estimated monthly  $\delta^{18}\text{O}_{\text{OIPC}}$  values exhibited tremendous variation (Table 8.3). More positive  $\delta^{18}\text{O}$  values in precipitation were typical during the late spring and summer months; monthly  $\delta^{18}\text{O}_{\text{OIPC}}$  values showed the heaviest depletion between the months of December and March. Comparisons between estimated monthly  $\delta^{18}\text{O}_{\text{OIPC}}$  values and the  $\delta^{18}\text{O}_{\text{Water}}$  values measured in tooth enamel indicated concordance between the majority of subjects and  $\delta^{18}\text{O}_{\text{OIPC}}$  values corresponding to the months of October and

November. While strong qualitative positive correlations were evident between enamel  $\delta^{18}\text{O}_{\text{Water}}$  values and OIPC estimated monthly  $\delta^{18}\text{O}$  precipitation values, statistical analyses were not performed as the absence of serial tooth enamel sampling precluded more precise comparisons between datasets.

The enamel carbonate  $\delta^{18}\text{O}$  values from archaeological and modern samples analyzed in the first pilot study (Table 5.3) were positively correlated ( $R^2 = 0.7854$ ) with the OIPC-derived  $\delta^{18}\text{O}$  values for source water (Fig. 8.6). These  $^{18}\text{O}$  enamel values and corresponding OIPC source water  $^{18}\text{O}$  values were combined with the Canadian volunteer data presented in Tables 7.1 and 8.1 to effectively increase the overall sample size as well as difference in geolocation and subsequently to assess the relationship between calculated  $\delta^{18}\text{O}_{\text{Phosphate}}$  values (Iacumin et al. 1996) and  $\delta^{18}\text{O}$  values in precipitation (Table 8.4). After averaging the  $\delta^{18}\text{O}_{\text{Phosphate}}$  and  $\delta^{18}\text{O}_{\text{OIPC}}$  data of the 8 Toronto volunteers and adding these averages together with the Waterford, ON (CL006), Montreal, QC (CL009), and pilot study sample data (N=5) from Table 5.3, linear regression analysis generated a correlation equation of  $^{18}\text{O}_{\text{enamel}} = 0.7792 \times ^{18}\text{O}_{\text{water}} + 23.131$  ( $R^2 = 0.872$ ) (Fig. 8.7), which differed only slightly from the two correlation equations offered by Daux et al. (2008):  $^{18}\text{O}_{\text{bone}} = 0.65(\pm 0.05) ^{18}\text{O}_{\text{water}} + 21.89(\pm 1.51)$ ;  $^{18}\text{O}_{\text{enamel}} = 0.518(\pm 0.1) ^{18}\text{O}_{\text{water}} + 21.53(\pm 3.55)$ , both of which generated an  $R^2$  value of 0.87.

The averaged Toronto data and pilot study sample data were converted into  $\delta^{18}\text{O}_{\text{Water}}$  values using the Daux et al. (2008) equation (Table 8.4). Calculated source water  $\delta^{18}\text{O}$  values for the amalgamated dataset containing all Canadian samples and 5 modern and archaeological pilot study samples were plotted against the estimated

annual OIPC  $\delta^{18}\text{O}$  values. The two variables were positively correlated given by a correlation equation of  $^{18}\text{O}$  calculated source water =  $1.1986 \times ^{18}\text{O}$  OIPC water + 1.9088 and exhibited a strong linear trend as shown by the  $R^2$  of 0.87 (Fig. 8.8). The slope of this correlation equation of 1.2 (rounded to the 1<sup>st</sup> decimal) is quite close to the theoretical slope of 1.0 one would expect for the case of source water oxygen being a one-to-one reflection of enamel carbonate oxygen without any biochemical mass discriminatory effects or the sum of all biochemical mass discriminatory effects being zero. A slope of approximately 1.2 suggests the involvement of a complex combination of processes in addition to mass discriminatory effects affecting the  $^{18}\text{O}$  composition of enamel carbonate. These processes most probably include mixing of different oxygen pools and different fluxes between pools.



TABLE 8.1. Reported  $\delta^{13}\text{C}_{\text{VPDB}}$  and  $\delta^{18}\text{O}_{\text{VSMOW}}$  mean values for modern M3 samples donated by study participants.  $\delta^{18}\text{O}_{\text{VPDB}}$  values were calculated using the O'Neil (1979) equation.

Subject ID Number	M3 Location	$\delta^{13}\text{C}_{\text{VPDB}}$ (‰)	$\delta^{18}\text{O}_{\text{VSMOW}}$ (‰)	$\delta^{18}\text{O}_{\text{VPDB}}^*$ (‰)
MCM1A	Mandibular	-10.23	24.22	-6.44
MCM1B	Mandibular	-10.39	24.19	-6.48
CL002A	Maxillary	-10.82	24.08	-6.58
CL002B	Maxillary	-10.78	23.86	-6.79
SY4A <sup>#</sup>	Mandibular	-11.14	24.75	-5.93
SY4B <sup>#</sup>	Mandibular	-11.16	24.73	-5.95
CL005A <sup>#</sup>	Mandibular	-10.08	24.57	-6.11
CL005B <sup>#</sup>	Mandibular	-9.92	24.66	-6.02
CL006A	Mandibular	-9.49	24.57	-6.11
CL006B	Mandibular	-9.74	24.27	-6.39
SY007A	Maxillary	-11.28	25.18	-5.51
SY007B	Maxillary	-11.31	24.90	-5.78
CL008A	Maxillary	-10.06	23.97	-6.68
CL008B	Maxillary	-10.08	24.33	-6.34
CL009A	Mandibular	-9.47	24.19	-6.48
CL009B	Mandibular	-9.65	24.70	-5.98
CL0010A	Mandibular	-9.95	24.51	-6.16
CL0010B	Mandibular	-9.99	24.60	-6.08
CL0013A	Mandibular	-10.97	23.76	-6.89
CL0013B	Mandibular	-10.86	23.77	-6.88

\*  $\delta^{18}\text{O}_{\text{PDB}} = 0.97006 \delta^{18}\text{O}_{\text{VSMOW}} - 29.94$  (O'Neil 1979)

<sup>#</sup> Vegetarian

TABLE 8.2. Oxygen stable isotope values for modern M3 samples donated by study participants. Reported  $\delta^{18}\text{O}_{\text{VSMOW}}$  mean values were converted to  $\delta^{18}\text{O}_{\text{phosphate}}$  and  $\delta^{18}\text{O}_{\text{water}}$  (ingested water) values. OIPC  $\delta^{18}\text{O}$  values are reported as interpolated mean annual values using 95% confidence intervals.

Subject ID Number	$\delta^{18}\text{O}_{\text{VSMOW}}$ (‰)	$\delta^{18}\text{O}_{\text{phosphate}^*}$ (‰)	$\delta^{18}\text{O}_{\text{water}^\dagger}$ (‰)	$\delta^{18}\text{O}_{\text{OIPC}^\ddagger}$ (‰)
MCM1A	24.22	15.24	-10.23	-9.3 ± 0.3
MCM1B	24.19	15.20	-10.29	
CL002A	24.08	15.10	-10.45	
CL002B	23.86	14.88	-10.78	-9.4 ± 0.3
SY4A <sup>#</sup>	24.75	15.75	-9.44	-9.4 ± 0.3
SY4B <sup>#</sup>	24.73	15.74	-9.46	
CL005A <sup>#</sup>	24.57	15.58	-9.71	-9.3 ± 0.2/ -9.6 ± 0.3
CL005B <sup>#</sup>	24.66	15.66	-9.58	-9.3 ± 0.3
CL006A	24.57	15.58	-9.71	-9.2 ± 0.2
CL006B	24.27	15.29	-10.16	
SY007A	25.18	16.18	-8.78	-9.6 ± 0.3
SY007B	24.90	15.90	-9.21	
CL008A	23.97	14.99	-10.61	-9.7 ± 0.3
CL008B	24.33	15.34	-10.07	
CL009A	24.19	15.20	-10.29	-10.7 ± 0.6
CL009B	24.70	15.71	-9.51	
CL0010A	24.51	15.52	-9.79	-9.4 ± 0.3
CL0010B	24.60	15.60	-9.67	
CL0013A	23.76	14.79	-10.93	-9.4 ± 0.3
CL0013B	23.77	14.79	-10.92	

\*  $\delta^{18}\text{O}_{\text{Phosphate}} = 0.98 \delta^{18}\text{O}_{\text{Carbonate}} - 8.5$  (Iacumin et al. 1996)

†  $\delta^{18}\text{O}_{\text{Phosphate}} = 0.65 \delta^{18}\text{O}_{\text{water}} + 21.89$  (Daux et al. 2008)

‡ OIPC  $\delta^{18}\text{O}$  values provided for residence at ages 6-10 years and 11-18 years

<sup>#</sup> Vegetarian

TABLE 8.3. Oxygen stable isotope monthly values for modern M3 samples donated by study participants. Reported  $\delta^{18}\text{O}_{\text{VSMOW}}$  mean values were converted to  $\delta^{18}\text{O}_{\text{phosphate}}$  (Iacumin et al. 1996) and  $\delta^{18}\text{O}_{\text{water}}$  (ingested water) (Daux et al. 2008) values. OIPC  $\delta^{18}\text{O}$  values are reported as estimated monthly values provided for residence at ages 6-10 years and 11-18 years.

Subject ID Number	$\delta^{18}\text{O}_{\text{water}}$ (‰)	$\delta^{18}\text{O}_{\text{OIPC}}$ (‰)											
		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec
MCM1A	-10.23	-15.5	-15.1	-10.9	-7.9	-	-6.1	-5.7	-5.9	-7.6	-9.7	-10.5	-13.6
MCM1B	-10.29					6.5							
CL002A	-10.45	-15.6	-15.2	-11.1	-8.1	-6.6	-6.2	-5.8	-6.0	-7.7	-9.8	-10.7	-13.8
CL002B	-10.78												
SY4A	-9.44	-15.6	-15.2	-11.1	-8.1	-6.6	-6.2	-5.8	-6.0	-7.7	-9.8	-10.7	-13.8
SY4B	-9.46												
CL005A	-9.71	-15.5	-15.1	-10.9	-7.9	-6.5	-6.1	-5.7	-5.9	-7.6	-9.7	-10.5	-13.6
CL005B	-9.58	-15.8	-15.3	-11.3	-8.2	-6.8	-6.3	-5.9	-6.1	-7.8	-9.9	-10.8	-14.0
CL006A	-9.71	-15.5	-15.1	-10.9	-7.9	-6.6	-6.1	-5.6	-5.8	-7.6	-9.7	-10.5	-13.5
CL006B	-10.16												
SY007A	-8.78	-15.8	-15.3	-11.3	-8.2	-6.8	-6.3	-5.9	-6.1	-7.8	-9.9	-10.8	-14.0
SY007B	-9.21	-16.0	-15.5	-11.5	-8.3	-6.9	-6.4	-6.0	-6.2	-7.9	-10.0	-11.0	14.2
CL008A	-10.61	-15.9	-15.4	-11.4	-8.3	-6.8	-6.4	-5.9	-6.1	-7.8	-10.0	-10.9	-14.1
CL008B	-10.07												
CL009A	-10.29	-17.0	-16.4	-13.0	-9.6	-7.9	-7.6	-7.0	-7.2	-8.7	-11.0	-12.0	-15.7
CL009B	-9.51												-13.8
CL0010A	-9.79	-15.6	-15.2	-11.1	-8.1	-6.6	-6.2	-5.8	-6.0	-7.7	-9.8	-10.7	
CL0010B	-9.67												
CL0013A	-10.93	-15.6	-15.2	-11.1	-8.1	-6.6	-6.2	-5.8	-6.0	-7.7	-9.8	-10.7	-13.8
CL0013B	-10.92												

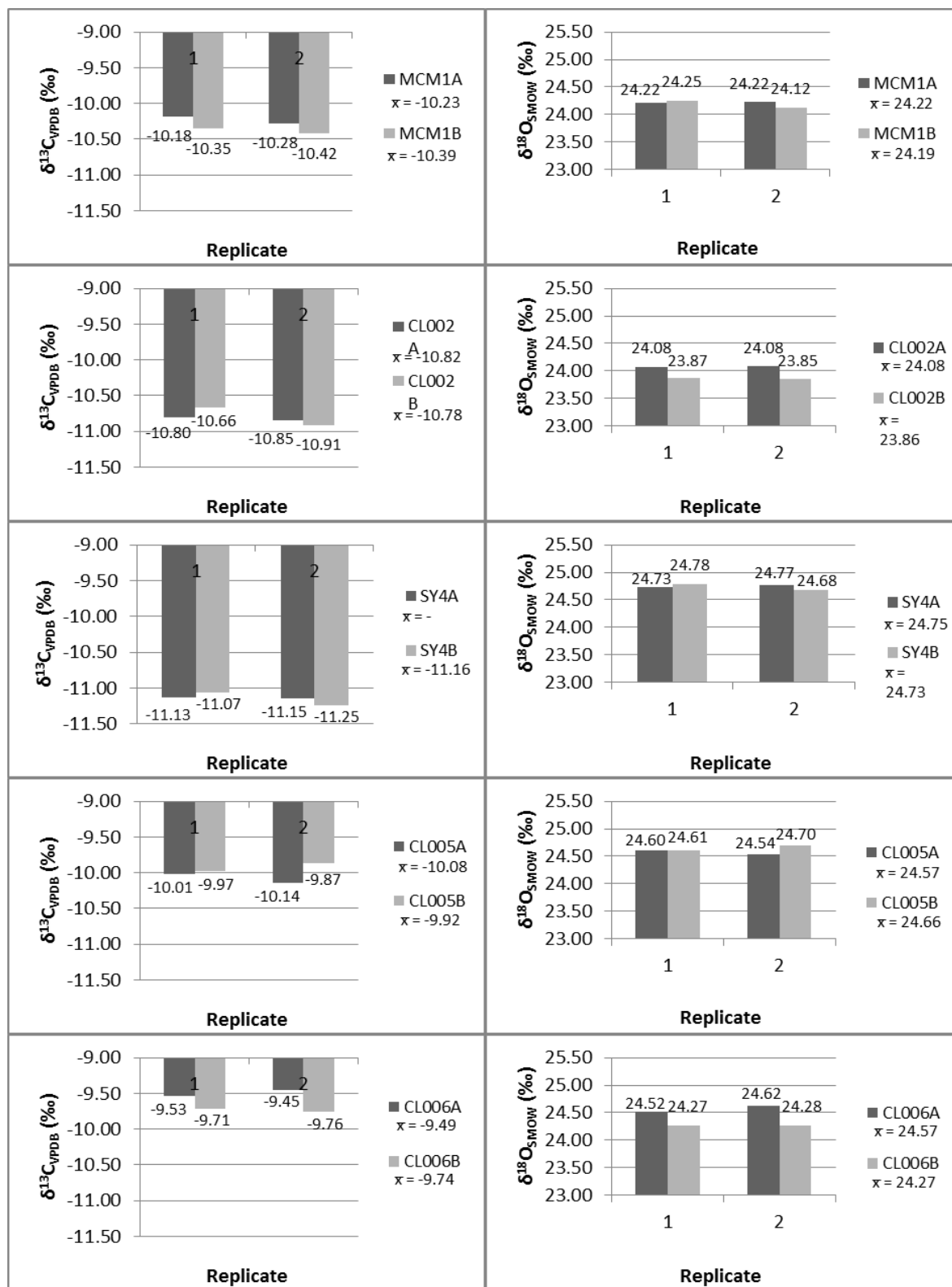
TABLE 8.4. Comparison of  $\delta^{18}\text{O}$  (carbonate) values of tooth enamel from 5 modern and archaeological samples previously analyzed for  $^2\text{H}$  abundance (see Chapter 5) and 10 modern Canadian samples. The 8 Toronto, Ontario samples are presented as an average.

Sample	Country of origin	Tooth enamel $\delta^2\text{H}$ [‰], pre-treatment B	Mean tooth enamel $\delta^{18}\text{O}$ [‰] VSMOW <sup>§</sup>	Mean tooth enamel S.D. $\delta^{18}\text{O}$ [‰]	$\delta^{18}\text{O}$ [‰] source water calculated from enamel $\delta^{18}\text{O}$ <sup>#</sup>	$\delta^{18}\text{O}$ [‰] source water OIPC
CLB1_2M	UK, Scotland	-110.4 ± 1.6	14.91	0.45	-10.74	-9.60
HFK_3M	UK, Kent	-111.7 ± 1.7	18.40	0.71	-5.37	-7.90
RM_2M	Italy	-111.4 ± 1.0	18.71	0.53	-4.89	-5.50
SC_3M	France	-115.3	18.96	0.13	-4.51	-5.80
IM_3M	North Africa	-110.5 ± 0.4	19.49	0.82	-3.69	-4.10
	Toronto, ON*		15.39			-9.45
CL006	Waterford, ON		15.44			-9.20
CL009	Montreal, QC		15.46			-10.70

<sup>§</sup>Phosphate  $\delta^{18}\text{O}$ -values were calculated from measured carbonate  $\delta^{18}\text{O}$ -values using the correlation equation of Iacumin et al. 1996.

<sup>#</sup>Source water  $\delta^{18}\text{O}$ -values were calculated using the correlation equation of Daux et al. 2008.

\*Averaged  $\delta^{18}\text{O}$  data from 8 Toronto subjects.



**Fig. 8.1.** Individual and mean  $\delta^{13}\text{C}_{\text{VPDB}}$  and  $\delta^{18}\text{O}_{\text{SNOW}}$  values for 20 modern M3 tooth enamel samples from 10 study participants.

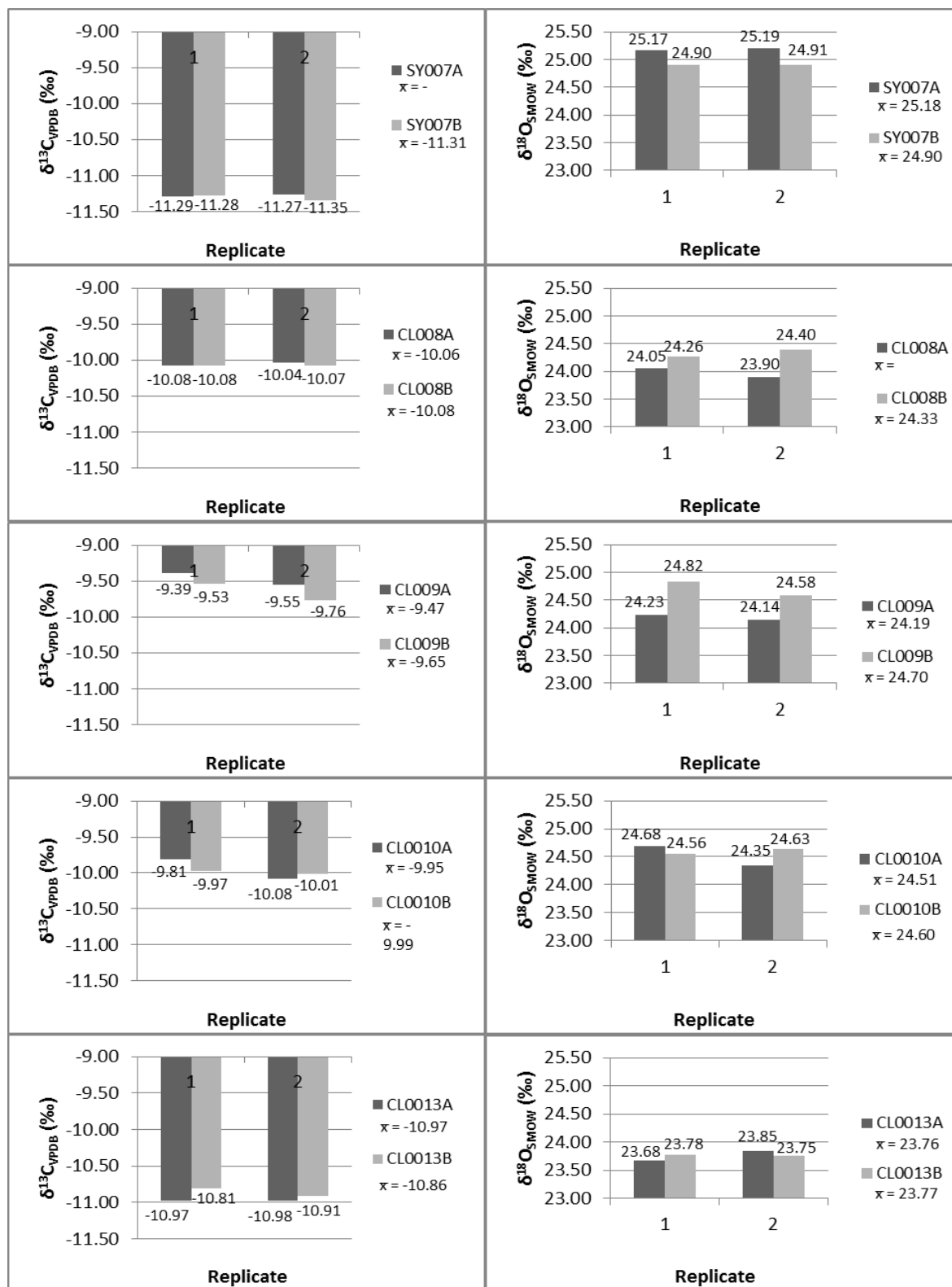
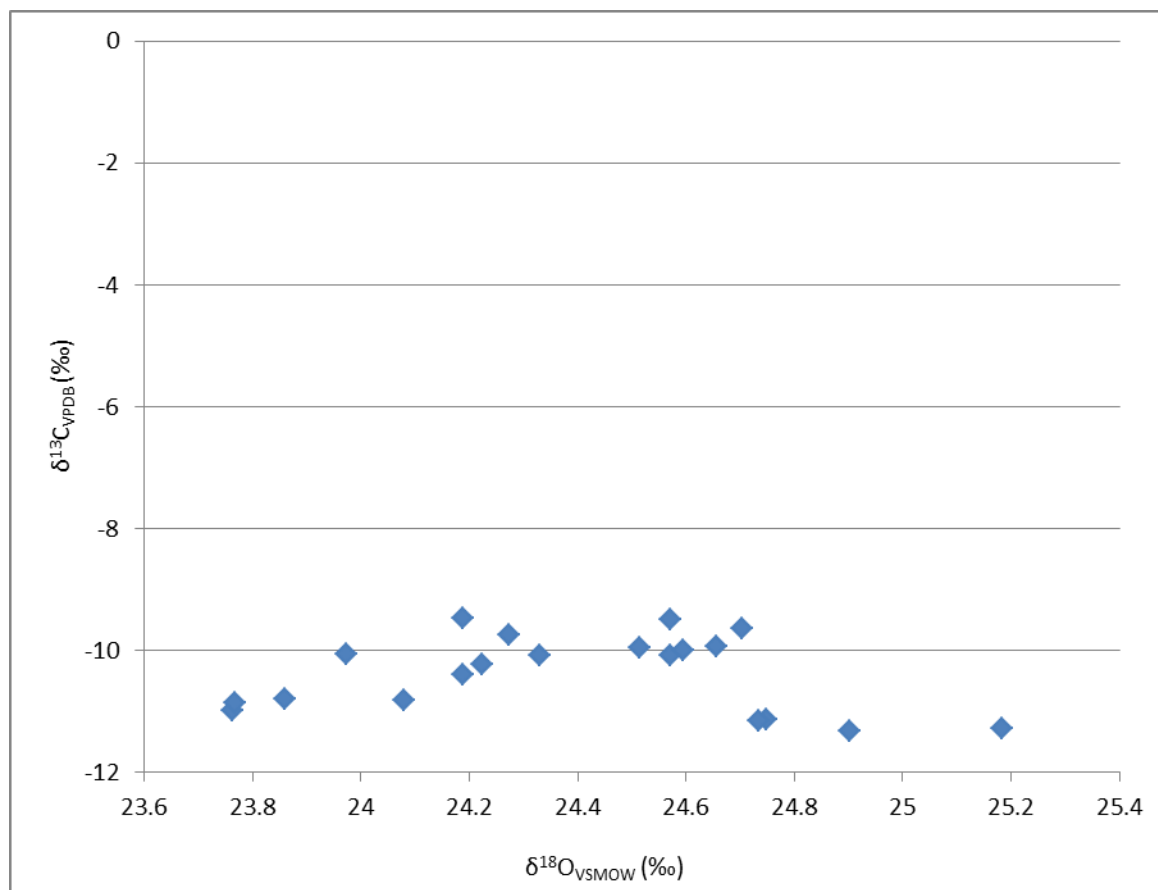
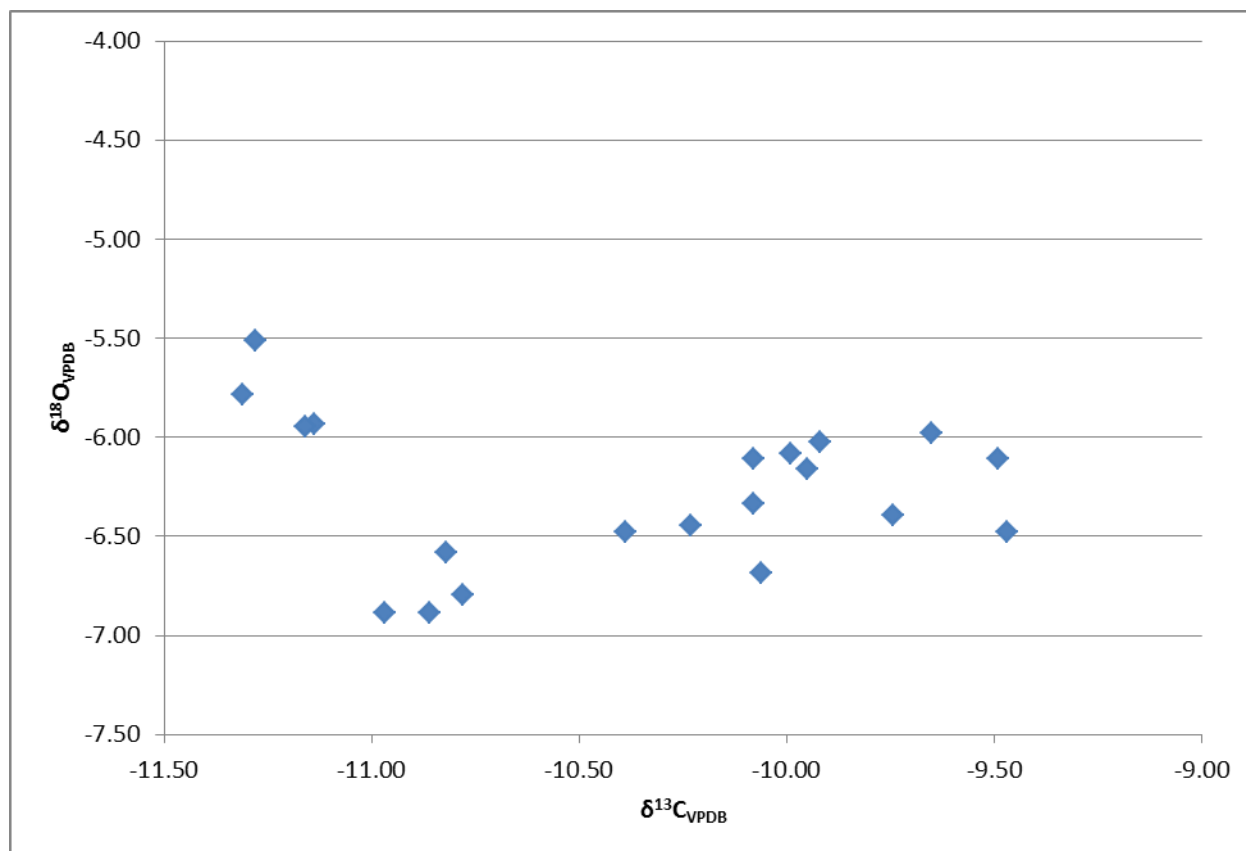


Fig. 8.1. Continued.

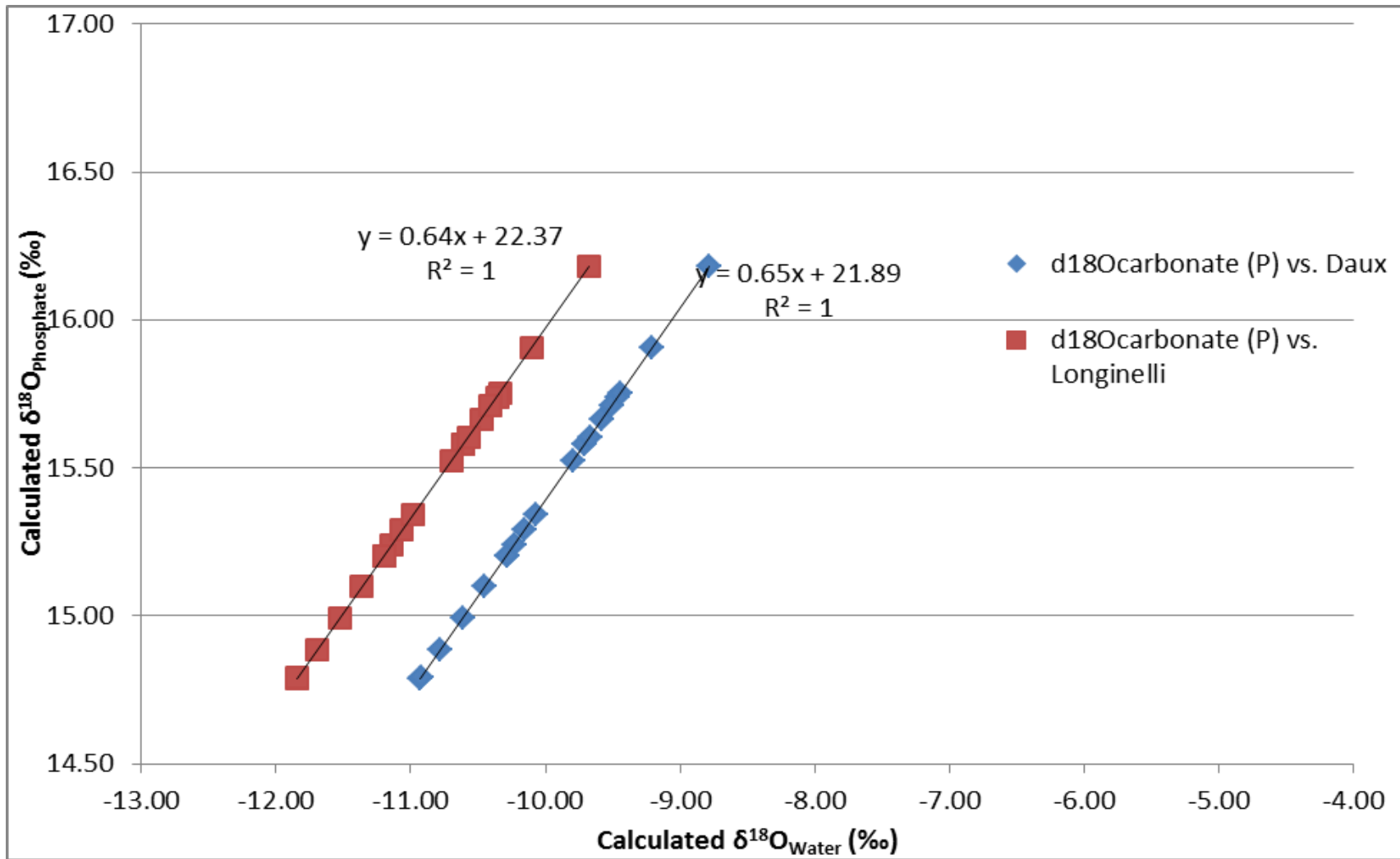


**Fig. 8.2.** Bivariate comparison of  $\delta^{13}\text{C}_{\text{VPDB}}$  and  $\delta^{18}\text{O}_{\text{VSMOW}}$  values measured in 20 third molars from 10 modern individuals with similar residence patterns during the time of enamel formation.

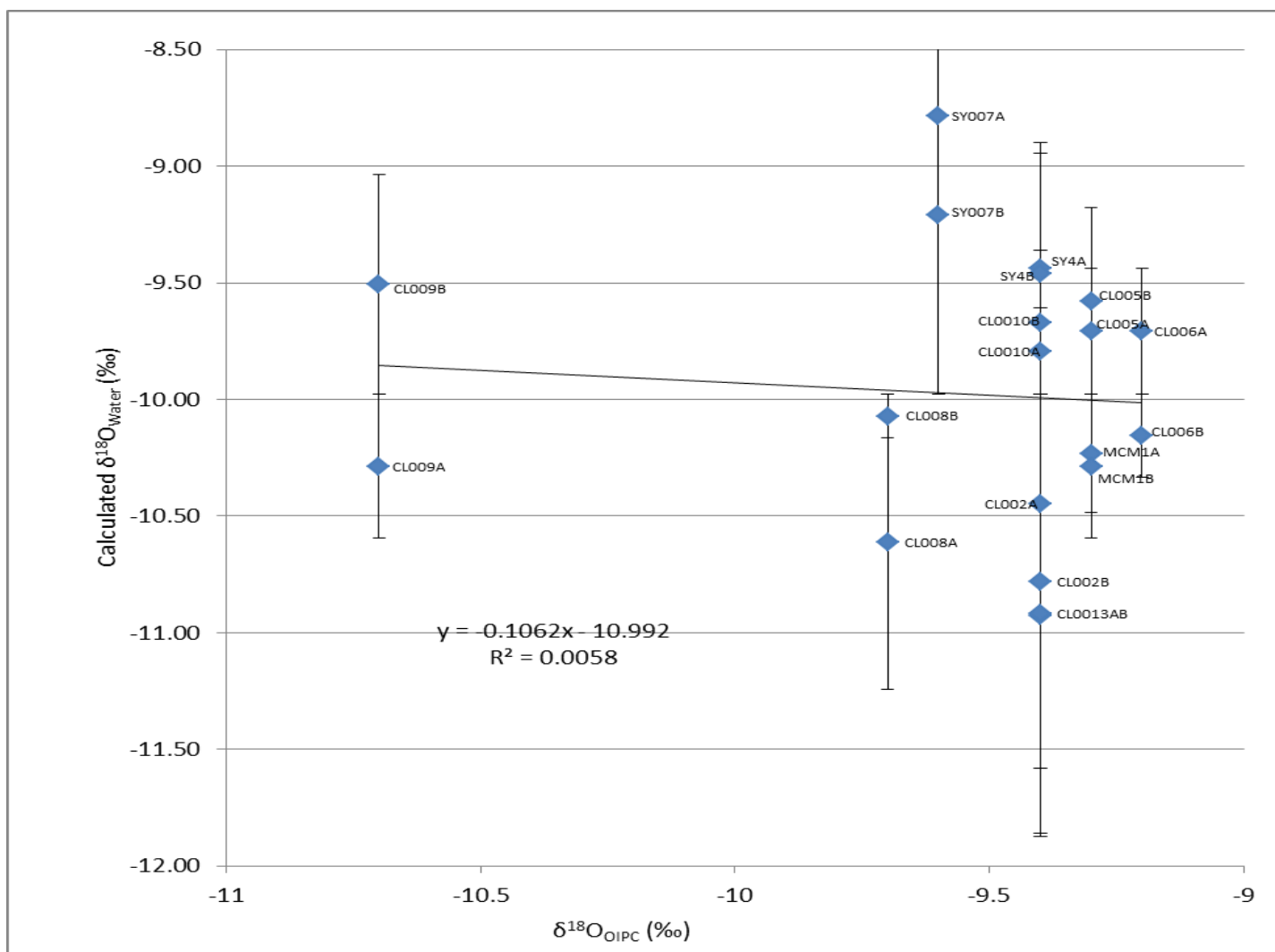


**Fig. 8.3.** Bivariate comparison of  $\delta^{13}\text{C}_{\text{VPDB}}$  and  $\delta^{18}\text{O}_{\text{VPDB}}$  values measured in 20 third molars from 10 modern individuals with similar residence patterns during the time of enamel formation.  $\delta^{18}\text{O}_{\text{VPDB}}$  values were converted from measured  $\delta^{18}\text{O}_{\text{SMOW}}$  values (O'Neil 1979).





**Fig. 8.4.** Tooth enamel  $\delta^{18}\text{O}$  drinking water values (Daux et al. 2008; Longinelli 1984) plotted against  $\delta^{18}\text{O}_{\text{Phosphate}}$  values calculated from enamel carbonate  $\delta^{18}\text{O}$  values. The offset between Daux et al. and Longinelli slopes can be attributed to enriched  $\delta^{18}\text{O}$  values arising from food preparation methods.



**Fig. 8.5.** Correlation plot of tooth enamel  $\delta^{18}\text{O}$  drinking water values (Daux et al. 2008) vs.  $\delta^{18}\text{O}$  values of precipitation (calculated using the OIPC) (Bowen 2014). Error bars represent amount of deviation between each molar sample mean  $\delta^{18}\text{O}_{\text{Water}}$  value and the collective sample mean  $\delta^{18}\text{O}_{\text{Water}}$  value of -9.98.

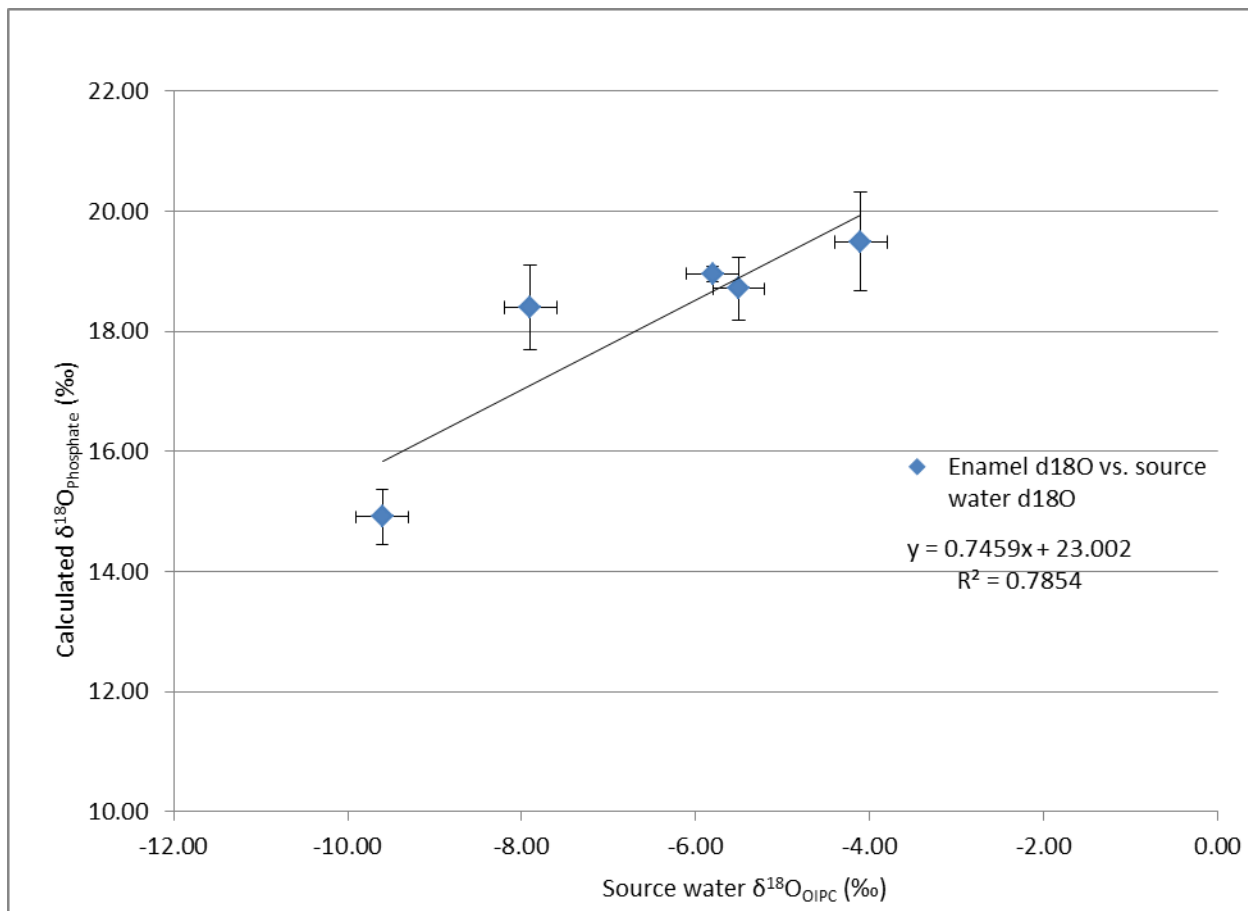


Fig. 8.6. Correlation plot of calculated tooth enamel  $\delta^{18}\text{O}_{\text{Phosphate}}$  values ( $^2\text{H}$  enamel pilot study archaeological and modern samples) vs. OIPC modeled water data. Error bars represent intra-individual uncertainties.

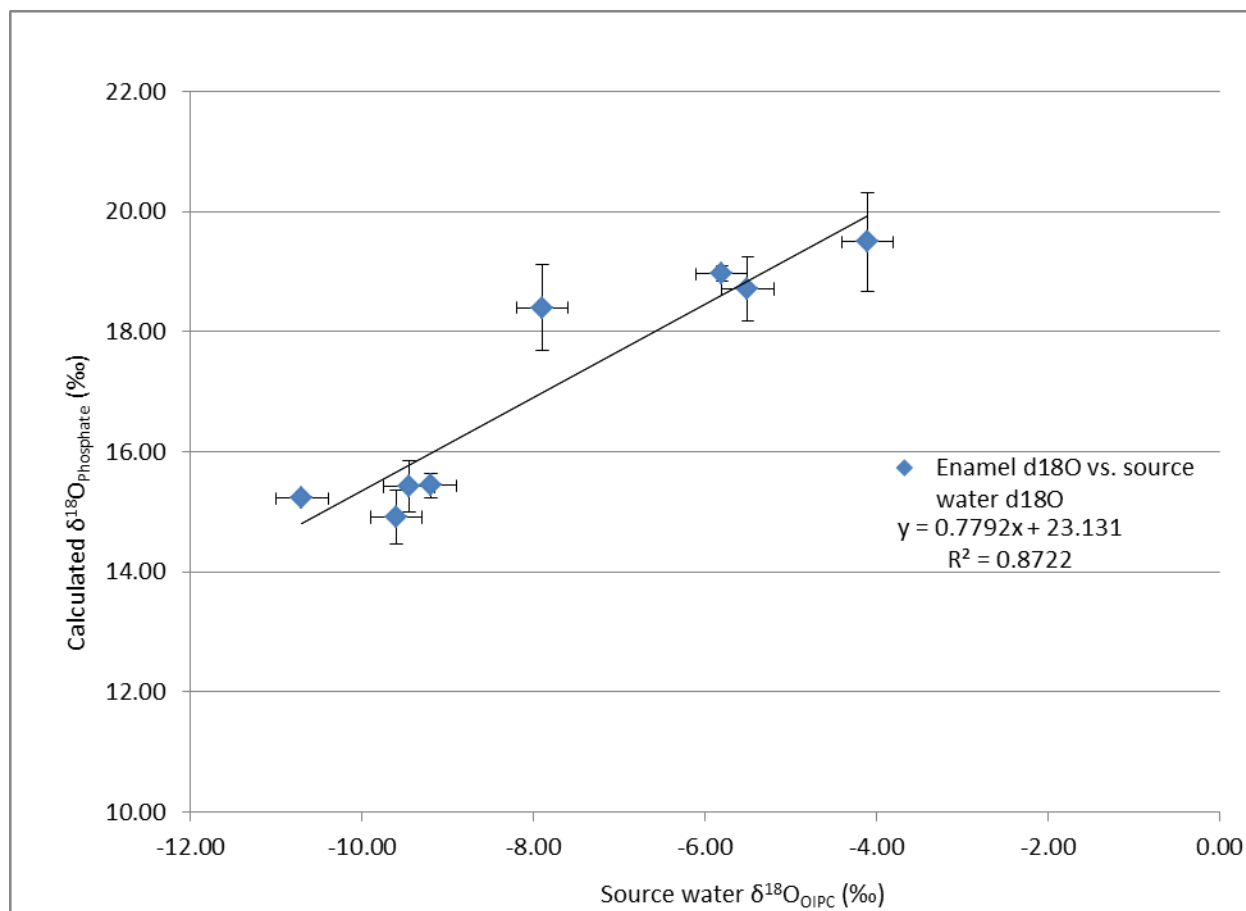


Fig. 8.7. Correlation plot of calculated tooth enamel  $\delta^{18}\text{O}_{\text{Phosphate}}$  values ( $^2\text{H}$  enamel pilot study samples and averaged Toronto data plus 1 Waterford and 1 Montreal sample) vs. OIPC modeled water data. Error bars represent intra-individual uncertainties for all samples except for the averaged Toronto group value (i.e., inter-individual standard deviation).

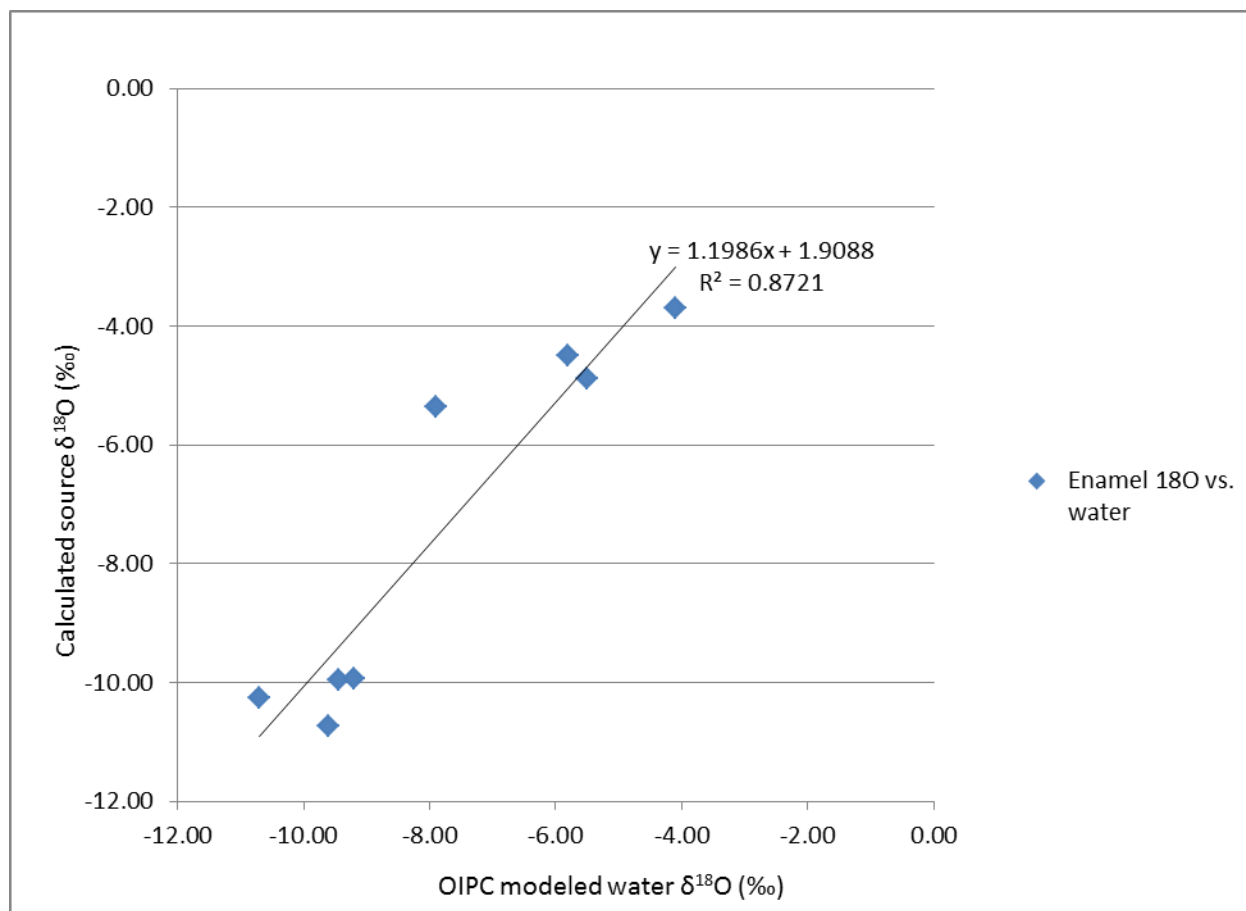


Fig. 8.8. Correlation plot of tooth enamel  $\delta^{18}\text{O}$  drinking water values (Daux et al. 2008) vs. modeled precipitation data ( $\delta^{18}\text{O}_{\text{OIPC}}$ ) for  $^{2}\text{H}$  enamel pilot study samples and averaged Toronto data plus 1 Waterford and 1 Montreal sample.

## CHAPTER 9

### DISCUSSION AND CONCLUSIONS

As discussed previously in the Introduction and Materials and Methods sections, the isotopic abundance of  $^{13}\text{C}$  and  $^{18}\text{O}$  in tooth enamel carbonate was measured and subsequently compared with an individual's self-reported residence during the time of enamel formation. The following section explores and interprets the results as they pertain to Objectives 3, 4, and 5 outlined in the Introduction: the likelihood that tooth enamel carbonate  $\delta^{18}\text{O}$  values and  $\delta^{13}\text{C}$  values are both consistent with residential history, isotopically detecting dietary variability, the collective efficacy of  $^{18}\text{O}$  and  $^{13}\text{C}$  isotopic data in confirming geographic origins, and interpreting and quantifying intra-individual variability.

#### Intra-Individual Variability

Sex of the individual and dietary preference had no significant influence on  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  values overall. Although subject-to-subject variation was the largest proportion of total  $\delta^{13}\text{C}$  variability, the  $\delta^{13}\text{C}$  enamel values did not significantly differ within or between subjects. However, between-subject variation in third molar  $\delta^{18}\text{O}_{\text{VSMOW}}$  values was statistically significant. Qualitatively, intra-individual variability was illustrated by the inter-molar offsets between mean oxygen and carbon isotopic values for each pair of

third molars from the same subject. More than half of the study subjects exhibited inter-molar offsets (or paired molar differences) that were greater for oxygen.

While the observed intra-individual inter-molar mean isotopic value offsets were not statistically significant, their relative homogeneity confirms a shared, similar residential and dietary history. Nevertheless, what can they tell us about intra-individual variability patterns? The  $^{18}\text{O}$  values exhibited the largest range in offsets between mean inter-molar values for one half of the subject pool. However, results for the remaining five subjects were mixed. Carbon exhibited the largest differential for 3 individuals while the remaining two samples generated offsets that were equivalent or exhibited little difference. Since tooth enamel was not sequentially sampled, and the dataset is small, it was not possible to quantify individual variation within each of the two third molars from the same subject in order to present a more comprehensive picture of intra-individual variability.

Sequential enamel sampling has been used to study seasonality of climate and birthing, the ecological behavior of archaeological and modern fauna (e.g., Balasse 2002; Stevens et al. 2011), and in the dietary variability in australopithecines (e.g., Lee-Thorp et al. 2010). While early efforts in this area predicted promising results (see Lee-Thorp 2002), more recent work (Lee-Thorp et al. 2010) has acknowledged the limitations of such sampling and questioned the validity of results obtained using relevant methodology (e.g., Chenery et al. 2012; Wright 2013). Wright (2013) employed enamel microsampling and homogenized whole tooth section sampling techniques to further qualify dietary and residential inferences pertaining to an archaeological sample of pre-molars and first molars (Wright and Schwarcz 1998, 1999). Inter-tooth

comparisons of  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values confirmed earlier results (Wright and Schwartz 1998). However, microsampling trends for oxygen were more difficult to interpret. Wide variability in oxygen values within a single tooth was attributed to site specific climatic variations (Wright 2013:129). But without knowing exact seasonal fluctuations for an area, distinctions between the isotopic signal dampening effects of the enamel maturation process or climatic irregularity are not plausible. As acknowledged elsewhere, the patterns of enamel maturation in modern human teeth are not well understood (Lee-Thorp 2010:3391). While a tooth's longitudinal isotopic record is certainly systematic, the degree of blending of the original isotopic signals at various points from crown to root produced by the concurrent processes of crown primary mineralization and enamel maturation has not been calculated. As illustrated in this dissertation research, comparisons between enamel values and expected drinking water values, and subsequently interpolated  $\delta^{18}\text{O}$  values in precipitation, are not necessarily straightforward. This observation has been confirmed by Wright (2013), who noted further discrepancies in the isotopic data presumably caused by artifacts of sample pretreatment methods and sampling location.

Although the oxygen-carbon offsets evident in this study sample were not as marked as those noted by Fraser et al. (2006) in their multi-isotope longitudinal study of hair and fingernails, the preferential oxygen offset ( $\delta^{15}\text{N}$  values were also noticeably more variable) was also observed in fingernails but not in hair from the same subject. It was hypothesized that this variability was attributable to the formation rate differential of the amino acid composing hair keratin. The alpha helical structure of hair keratin is ostensibly not as affected by biochemical processes because of its faster turnover and



less time exposed to environmental influences. Fingernails grow much more slowly, six months for an entire fingernail, as opposed to hair, which grows approximately 1 cm per month. Since enamel does not remodel, and is thus exposed to biochemical processes during its formation and maturation periods, it is possible that it might therefore be more affected by such processes, which could account for the observed variability. While the variability was actually small in comparison to that noted by Fraser et al. (2006), it is possible the trend might be more marked within a larger sample population. Contrary to the longitudinal study, all of the subjects participating in this study lived in the same area, so even less variability might be expected.

Since tooth enamel was not sequentially sampled, it is not possible to attempt to quantify the individual variation within each of two third molars from the same individual by comparing the inter-molar data between subjects to obtain a more comprehensive picture of intra-individual variability, neither is it prudent to infer patterns based on these results. While refining current sequential sampling methods may assist in the quantification of intra-individual variability based on third molar tooth enamel isotopic data, it is clear that intra-individual variability cannot be calculated based on isotopic data from one or two stable isotopes and one type of tissue, especially tooth enamel, from a small sample. Much larger sample sizes are necessary, and multi-isotopic data (i.e.,  $^{18}\text{O}$ ,  $^2\text{H}$ ,  $^{13}\text{C}$ ,  $^{15}\text{N}$ ,  $^{87}\text{Sr}$ ) from multiple tissue types, including hair and fingernails, and tooth enamel and dentin, is optimal.

## Confirming Residence Patterns During Adolescence

### Oxygen Isotopic Variation In Tooth Enamel

Influence of Food and Water. During the initial stages of this project's implementation an innovative sampling and analytical protocol for the  $^2\text{H}$  isotopic analysis of tooth enamel was developed and successfully tested (see Holobinko et al. 2011a). While it has been proven possible to extract and determine the isotopic abundance of  $^2\text{H}$  in human tooth enamel bioapatite, the "fixed" nature of this stable isotope in this matrix renders it unsuitable for use as a proxy for human provenance. However, it should be noted that this pilot study also revealed unexpected and very low rates of hydrogen exchange. The differences in  $\delta^2\text{H}$  values between the two exchange experiments performed were barely significant within the overall uncertainty of measurement despite the 4 day equilibration time; H exchange between the organic hydroxyl groups of a material such as keratin is known to occur within a matter of hours (Sharp et al. 2003).

Unlike  $^2\text{H}$ , analytically available  $^{18}\text{O}$  in the carbonate and phosphate components of human tooth enamel is a well-known indicator of climate and seasonality, and has been successfully used to provenance human remains retrieved from archaeological and forensic contexts. However, the results of the Canadian cohort study do not unequivocally support this generalization, although this may be due to the small differences in geographical provenance for this cohort of volunteers.

Despite qualitative observations suggesting correlations between  $\delta^{18}\text{O}$  values in precipitation ( $\delta^{18}\text{O}_{\text{OIPC}}$ ) and enamel drinking water  $\delta^{18}\text{O}$  values ( $\delta^{18}\text{O}_{\text{Water}}$ ), linear

associations were generally weak to nonexistent. While the residential data (i.e., city and province) supplied by all study participants could be categorized as isotopically similar, individual patterns were noted. Of the 10 study subjects queried, 9 listed cities in the province of Ontario, Canada, as their primary residence during the formative years (ages 6-18 years) for third molar enamel development, namely the initial deposition of enamel through completion of the mineralization process. At least one third molar from 4 of the 10 subjects demonstrated  $\delta^{18}\text{O}_{\text{Water}}$  values that corresponded to interpolated annual average  $\delta^{18}\text{O}_{\text{OIPC}}$  values. Two of the 4 individuals, both of whom were vegetarians, exhibited correlations between the  $\delta^{18}\text{O}_{\text{Water}}$  values of both third molars and  $\delta^{18}\text{O}_{\text{OIPC}}$  values. One of the above 4 subjects was from Montreal, Quebec; the remaining three subjects lived either in the metropolitan Toronto area or within 78 km in a southwest direction, during the years specified. Despite the absence of statistically significant correlations within the admittedly small sample, the divergent isotopic third molar data from several individuals can be used to illustrate the presence of potential associations and, concurrently, the lack thereof.

Conversely, merging the  $\delta^{18}\text{O}_{\text{VSMOW}}$  enamel (calculated phosphate as well as drinking water) and corresponding modeled OIPC water data from the Chapter 5 pilot study enamel samples with the corresponding data from the Canadian volunteer study (i.e., averaged Toronto  $\delta^{18}\text{O}$  values plus Waterford and Montreal data) yielded much stronger linear relationships between the two variables and generated a correlation equation that was in agreement with published work (Daux et al. 2008) given the limited sample (N=15). Averaging the Toronto data and including samples with isotopically distinct geographic origins (i.e., United Kingdom, Italy, France, North Africa) not only

increased the sample size, but allowed for a more rigorous analysis and holistic interpretation of the data trends.

A primary reason for the individual patterns observed in the Canadian cohort study population enamel  $\delta^{18}\text{O}$  values is twofold: isotopic systems are inherently complex, and subsequent comparisons between enamel  $\delta^{18}\text{O}_{\text{Water}}$  values and  $\delta^{18}\text{O}$  values in precipitation are not straightforward. First, assuming that the  $\delta^{18}\text{O}$  values of drinking or ingested water are disproportionately influenced by precipitation  $\delta^{18}\text{O}$  values is overly simplistic. The  $^{18}\text{O}$  composition of mammalian tooth enamel is determined primarily by the  $\delta^{18}\text{O}$  value of ingested water, and secondarily, by the oxygen molecules bound in food (Longinelli 1984; Luz et al. 1984; Ayliffe and Chivas 1990) and thirdly by metabolic water, that is water generated by the body's biochemical processes for which atmospheric oxygen is the precursor pool. The isotopic composition of meteoric water is subject to fluctuations resulting from variable climatic conditions (Dansgaard 1964) and various hydrological processes (Ehleringer et al. 2010). Similarly, the isotopic composition of oxygen molecules comprising food items also varies between and within nutrient categories (e.g., the  $\delta^{18}\text{O}$  values of plant cellulose are typically enriched in comparison to those of animal flesh; root tissue generally exhibits lower  $\delta^{18}\text{O}$  values compared to leaf tissue) (Epstein et al. 1977; Sternberg 1989; Yakir 1992; Tredget et al. 1993, Kohn 1996).

Results from a study examining isotopic variability in globally sourced bottled water brands would suggest that the increased consumption of bottled water has little to no effect on the  $^{18}\text{O}$  isotopic composition of an individual's tooth enamel, as generally the  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values of bottled water tend to be similar to those of natural local water

sources (Bowen et al. 2005). In other words, bottled water purchased and consumed in Ontario, Canada, is likely to have been regionally sourced. Two (SY4, CL0010) of the 10 subjects indicated regular consumption of Nestlé Pure Life Canada bottled water, which is bottled locally from rapidly renewing natural springs in both Ontario and British Columbia (Nestlé Waters Canada 2014). While it is unknown if the two individuals drank this brand of bottled water during the time of third molar enamel formation, their respective  $\delta^{18}\text{O}_{\text{VSMOW}}$  values were unremarkable compared to the subjects who presumably drank tap water or other beverages.

Chesson et al. (2010) noted regionality trends in  $\delta^{18}\text{O}$  values when examining the isotopic variability between beef sources and the location of U.S. supermarkets at which the meat was purchased (see also Chesson et al. 2008; O'Brien and Wooller 2007). While the  $^{18}\text{O}$  isotopic composition of beef purchased and consumed at fast food restaurants was not correlated with local tap water isotope values, significant correlations were noted with regard to beef purchased at supermarkets. Similarly, the island nation of Japan imports the majority of its food supplies, including beef, from countries such as Argentina, Australia, and the United States in order to provide its consumers with more economical alternatives to the expensive variety available within Japan (personal communication, Izumi Shimada). Although the staple protein component of most Japanese diets is of marine origin (e.g., fish, seaweed), the consumption of beef from international sources exemplifies the potential impact of the globalization of the country's food supplies. While some of the non-vegetarian study participants presumably ate fast food, including beef, on a regular basis, or even ate meat and other foods imported from an isotopically distinct region, it is not possible to

speculate as to the  $^{18}\text{O}$  isotopic composition of meat consumed by the non-vegetarian study participants or the impact the relevant  $\delta^{18}\text{O}$  values would have had on an individual's overall enamel  $^{18}\text{O}$  isotopic composition since subjects were not queried as to the type and source of meat they regularly included in their diet.

Influence of Carbonate- $^{18}\text{O}$  / Phosphate- $^{18}\text{O}$  Correlation. While the Iacumin (1996) equation linking  $\delta^{18}\text{O}_{\text{Carbonate}}$  values with those of  $\delta^{18}\text{O}_{\text{Phosphate}}$  produces a robust linear correlation in the absence of diagenetic alteration, another potential explanation for the overall lack of strong linear relationships between  $\delta^{18}\text{O}_{\text{Water}}$  values and precipitation  $\delta^{18}\text{O}$  values in the Canadian volunteer dataset may be associated with the application of carbonate conversion equations to enamel phosphate  $\delta^{18}\text{O}$  data when deriving drinking water values (e.g., Chenery et al. 2012).

Further complicating the issue is the lengthy and complicated sample preparation and analytical protocol for measuring phosphate enamel  $\delta^{18}\text{O}$  values (Chenery et al. 2012). Because phosphate is the primary source of oxygen within the tooth enamel, determining the abundance of  $^{18}\text{O}$  in the phosphate component of enamel is biochemically and isotopically advantageous. However, if structural carbonate is sufficiently preserved, as is typically the case with regard to enamel carbonate vs. bone apatite carbonate, the truncated and cost-effective methods of carbonate sample preparation and analysis can prove to be very beneficial, and yields rapid, accurate, and precise  $\delta^{18}\text{O}$  data (Sponheimer and Lee-Thorp 1999; Sharp et al. 2000; Henton et al. 2010; Chenery et al. 2012). Further, regression from carbonate oxygen data generates error rates no greater than that obtained from regressing phosphate oxygen

values. Pollard et al. (2011) has quantified substantial error rates in calculating  $\delta^{18}\text{O}$  drinking water values, between  $\pm 1\%$  and  $\pm 3.5\%$ , notably higher than those published in recent literature. Chenery et al. (2012) have shown that the use of  $\delta^{18}\text{O}$  carbonate values does not significantly increase the error rate for derived drinking water values; nonetheless, the original error rate associated with calculating drinking water values from phosphate values remains unmitigated. To alleviate some of the uncertainty associated with phosphate to carbonate conversions, Chenery et al. (2012) has recommended specific conversion equations for data from certain types of climates (e.g., Iacumin et al. (1996) and Metcalfe et al. (2009) for hot/arid climates, and Chenery et al. (2012) for cold, temperate and warm/humid).

Influence of Water- $^{18}\text{O}$  / Phosphate- $^{18}\text{O}$  Correlation. As mentioned above, the isotopic fractionation of oxygen between the time of its ingestion and subsequent incorporation into tooth enamel during the mineralization process creates a functional relationship between the oxygen chemically bound in water and food and the measured  $\delta^{18}\text{O}$  values of tooth enamel phosphate oxygen, and facilitates comparisons between phosphate  $\delta^{18}\text{O}$  values and the  $\delta^{18}\text{O}$  values of ingested water through the use of conversion equations (see Longinelli 1984; Luz et al. 1984; Daux et al. 2008). There is some controversy over the contextual applicability of these equations (see Pollard et al. 2011). The Luz equation, for example, is particularly appropriate for application to populations originating from arid climates while the Longinelli equation was derived from data obtained from temperate zones.

The Daux et al. (2008) equation for calculating drinking water values was chosen for

the Canadian dataset since its derivation is based on data sampled from geographically diverse regions, and it accounts for the  $^{18}\text{O}$  enrichment of the water of cooked food, a process that occurs when the cooking water evaporates (i.e., becomes  $^{18}\text{O}$  enriched) and  $\text{H}_2\text{O}$  molecules are subsequently exchanged between the cooking water and food water. The Daux ingested water values more closely corresponded to OIPC interpolated mean annual  $\delta^{18}\text{O}$  values for precipitation. However, Daux ingested water (drinking water plus the water in food items) values in tooth enamel are more positive (i.e.,  $^{18}\text{O}$  enriched) than source water (precipitation) by 1.05 ‰ to 1.2 ‰ (Daux et al. 2008). If the enrichment associated with using the Daux equation were eliminated from overall analysis, then the calculated  $\delta^{18}\text{O}_{\text{Water}}$  values in the dataset would be  $^{18}\text{O}$  depleted (i.e., more negative) and more in line with  $\delta^{18}\text{O}$  drinking water values calculated using Longinelli's (1984) equation derived from temperate data. Nevertheless, Longinelli  $\delta^{18}\text{O}_{\text{Water}}$  values are even more discordant with the interpolated annual average OIPC  $\delta^{18}\text{O}$  values in precipitation calculated from site parameter data provided by study participants. It is likely the vegetarians ate more vegetables than the non-vegetarians, but based on the information provided by participants it is impossible to determine the proportion of food cooked in water these individuals consumed; vegetative food sources may have been consumed in their raw form. The  $\delta^{18}\text{O}_{\text{VSMOW}}$  values and subsequently the  $\delta^{18}\text{O}_{\text{Water}}$  values of the vegetarians were enriched, but not significantly so when compared to other subjects whose  $\delta^{18}\text{O}$  values fell within the same confidence interval.

Although the dietary preferences and cooking habits corresponding to the 5 archaeological and modern pilot study samples were unknown,  $\delta^{18}\text{O}_{\text{Water}}$  values derived



from measured  $\delta^{18}\text{O}_{\text{VSMOW}}$  values (Daux et al. 2008) for these individuals and subsequently for all 15 subjects in the merged dataset were positively correlated with modeled source water  $\delta^{18}\text{O}_{\text{OIPC}}$  values. This observation supports the assertion that the 1.05 ‰ to 1.2 ‰ enrichment associated with the Daux equation does not create potentially unmitigated confounding artifacts in the analysis of enamel  $^{18}\text{O}$  data. While the error rates associated with drinking water conversion equations must be factored into one's interpretation of oxygen isotopic results, these findings suggest that isotopically similar data may be more sensitive to such error rates than a dataset characterized by isotopically distinct regions.

Influence of Modeled  $^{18}\text{O}$  Abundance in Precipitation. The Bowen Wilkinson (2002) interpolation scheme for estimating mean annual average  $\delta^{18}\text{O}$  values in precipitation has been shown to reduce the average error of estimates by 10-15% when compared to other methods tested (i.e., triangulation, inverse distance weighting, Cressman objective analysis) (Bowen and Revenaugh 2003). This would suggest that their method yields relatively accurate values. However, the Bowen Wilkinson interpolation model, like any model, is not without its limitations, perhaps the most significant of which is that the GNIP data set upon which their interpolations are based is not current, and is misrepresentative of all stations sampled. Precipitation data has been compiled over a period of only 40 years, and not all stations contributed data for all years. For example, the GNIP database includes data from 583 stations, yet  $\delta^{18}\text{O}$  values in precipitation are available from only 348 stations for one or more years. While Bowen and Revenaugh (2003) acknowledge this and other limiting factors in their analysis and

subsequent calculation of error rates, the lack of a linear relationship between the  $\delta^{18}\text{O}_{\text{Water}}$  values calculated from the enamel carbonate data of the 10 study subjects and the corresponding interpolated  $\delta^{18}\text{O}_{\text{IPC}}$  values may be partially attributed to the observable variation in monthly values. Although the confidence interval for the province of Ontario is considerably smaller and thus more precise than, for example, huge portions of the United States which lack site data, mean monthly values for Toronto and the surrounding areas ranged from -15.6 ‰ to -5.8 ‰. Similarly, Montreal exhibited a 10 ‰ difference between the months of July and January. Drinking water  $\delta^{18}\text{O}$  values exhibited marked concordance with monthly values, a strong contrast to the apparent overall lack of association with mean annual average values despite the model's interpolative advantages.

Moreover, the Bowen Wilkinson model doesn't take into account the regional postprecipitation hydrological processes (e.g., evaporation, chemical interactions with substrate materials) and water history that can alter the stable isotope ratio of the source water found in a particular area. While Bowen et al. (2007) noted a significant correlation between tap water isotope ratios and those of interpolated annual averages of local precipitation in the United States, they also found significant discrepancies between isotope ratios in many parts of the United States. Kennedy et al. (2011) quantified the temporal variation in U.S. tap water supplied by groundwater, surface water, and managed water. Tap water  $\delta^{18}\text{O}$  values were an average of 4 times lower than that of precipitation; the latter two water supplies produced the most variation. The predictive maps generated by the work of Kennedy et al. (2011) were used to successfully rule out geographic areas as the location of origin for human scalp hair

obtained from the remains of an unidentified female.

Provincial data indicates that almost 50% of Ontario residences derive their drinking water from Lake Ontario, a discharge area serving all municipalities in the province (Ontario Open Data 2014) and believed to be supplied by the Laurentian River System, an ancient river system with an origin approximately 200 km north of Lake Ontario (Sharpe and Russell 2004). While six of the Ontario communities described in the residential history provided by study participants are municipalities, more than 2.8 million Ontario residents rely on groundwater pumped from aquifers as a source of potable water. Montreal water treatment plants are supplied primarily by the St. Lawrence River, but some of this water is pumped directly into the multiple higher elevation reservoirs serving specific parts of the city (McGill University 2015). The above exemplifies the distinct regional hydrogeology found at many sites, and demonstrates how relationships between variables can be distorted or obscured when analyzing nonrandom samples with isotopically similar provenance. The  $^2\text{H}$  pilot study enamel samples were geographically diverse and were obtained from individuals whose drinking water (tap water, in some cases) came directly from local freshwater reservoirs fed by annually averaged precipitation. The corresponding calculated enamel phosphate and drinking water values for these samples were well correlated with measured and modeled source water  $\delta^{18}\text{O}$  values. The weak associations initially observed in the Canadian cohort  $^{18}\text{O}$  data were transformed into moderately strong positive correlations when the data from Canadians residing in urban environments were averaged, apportioned according to specific location (i.e., Toronto, Waterford, Montreal), and merged with the pilot study sample  $^{18}\text{O}$  data. This outcome is not

surprising when the isotopic complexities of the Ontario municipal water supply are considered, namely: tap water  $^{18}\text{O}$  is sourced from freshwater lakes with an  $^{18}\text{O}$  composition that is primarily linked to local precipitation  $^{18}\text{O}$ , but also influenced by the  $^{18}\text{O}$  of riparian water fueled by precipitation in Ontario's northern Georgian Bay region. Similarly, while the isotopically diverse  $^2\text{H}$  pilot study subject enamel  $^{18}\text{O}$  data was correlated with both measured tap water  $^{18}\text{O}$  data and modeled OIPC data, measured water values and modeled water values differed.

If we consider the likelihood that all localities, including Ontario, exhibit spatially explicit patterns of temporal variation in their  $\delta^{18}\text{O}$  values of tap water due to unique and isotopically complex hydrological and physical processes and seasonality, then it would be prudent for future isotopic studies to include the sampling of tap water from numerous sites across the respective country or regions. A database of tap water  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  values would facilitate robust comparisons between tooth enamel  $\delta^{18}\text{O}_{\text{Water}}$  values and the corresponding local area's tap water  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  values and interpolated OIPC  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  values in precipitation.

Simultaneously sampling dentin collagen and tooth enamel (from the same tooth) and hair or fingernails obtained from the same individual would further strengthen interpretations of isotopic data, particularly when a group of human tissue samples generate enamel  $\delta^{18}\text{O}_{\text{Water}}$  values that appear isotopically homogenous. As discovered during the implementation of the  $^2\text{H}$  dentin feasibility study (see Chapter 6), dentin collagen  $\delta^2\text{H}$  values were positively correlated with enamel  $\delta^{18}\text{O}$  values and corresponding source water  $\delta^{18}\text{O}$  values. The similarity in correlation factors between unmatched substrates (i.e., enamel carbonate  $^{18}\text{O}$  and dentin collagen  $^2\text{H}$ ) and that

within the same substrate (i.e., nail keratin) suggests a strong developmental and functional isotopic relationship between the two tissues and their link to establishing geographic provenance. Moreover, the similarity observed between the correlation between the above dentin collagen  $\delta^2\text{H}$  values and corresponding source water  $\delta^2\text{H}$  and the Ehleringer et al. (2008) hair  $\delta^2\text{H}$ /source water  $\delta^2\text{H}$  correlation for North Americans reinforces the view that living (biological and environmental) isotopic systems are complex and must be evaluated comprehensively.

Since the tooth enamel of the third molars obtained from the study subjects was not sequentially sampled, conjectures regarding correlations between monthly OIPC values and individual layers of enamel from one third molar are not possible. While enamel was preferentially drilled from the cusp of each tooth, this type of sampling yields more homogenized results and an imprecise or “blended” picture of an individual’s residential history at the time those enamel layers were forming. While intra-tooth isotopic studies performed on the archaeological enamel of varied species of herbivores has firmly established the link between dietary and seasonality shifts and temporal increments comprising apatite development/maturation (e.g., Balasse 2002), there is some question as to the successful application of sequential sampling methods to human tooth enamel and the interpretation of subsequent results. The generally poor understanding of enamel maturation patterns in modern human tooth crowns would suggest that while the method’s utility remains promising, the evaluation of results obtained from sequential sampling continues to be controversial (Lee-Thorp et al. 2010; Chenery et al. 2012; Wright 2013) and lacking the precision necessary to yield month-by-month time resolved data (personal communication, W. Meier-Augenstein); teeth

sample size requirements and variation in sampling methodology are primary concerns.

### Carbon Isotopic Variation In Tooth Enamel

The  $\delta^{13}\text{C}$  values in tooth enamel from the third molar provide information about the type of diet an individual consumed during adolescence. Individuals categorized as  $\text{C}_4$  plant diet consumers typically originate from regions such as North and South America, and some parts of Africa where diet is heavily influenced by  $\text{C}_4$  plants (i.e., corn). As expected, it was evident from the third molar enamel carbonate  $\delta^{13}\text{C}$  isotopic data that all of the individuals consumed a diet dominated by  $\text{C}_4$  plants, which is consistent with their collective North American residential history and with published  $\delta^{13}\text{C}$  data in enamel carbonate (Lee-Thorp et al. 1989).

While specific nutritional information (e.g., the proportion of leaf to root vegetables consumed) was not provided by the individuals who identified themselves as vegetarians, such dietary nuances could influence the total oxygen isotope composition of their tooth enamel carbonate during the given time frame of enamel formation.

Although two of the 10 subjects were self-reported vegetarians (i.e., non-meat-eating but consumers of other animal products), their  $\delta^{13}\text{C}$  values did not identify them as such because the composition of carbon stable isotopes in an individual's tooth enamel does not provide a complete picture of an individual's dietary habits. Further, the vegetarians may not have followed a vegetarian lifestyle during childhood, in which case their self-reported dietary profile would be misrepresentative of their nutritional regime at the time of crown mineralization. The  $^{13}\text{C}/^{12}\text{C}$  isotope ratios measured in tooth enamel will only provide information with regard to an individual's total diet  $^{13}\text{C}$

composition (Ambrose and Norr 1993; Tsiezen and Fagre 1993), whereas  $^{13}\text{C}$  and  $^{15}\text{N}$  isotopic data from collagen will determine the dietary proportion of protein component, and can further distinguish between marine and terrestrial protein sources. While none of the study participants identified themselves as vegans, further speculation with regard to the type (e.g., beans, milk), source (e.g., plant vs. animal), and quantity of protein consumed by the subjects was not possible without measuring the abundance of  $^{15}\text{N}$  and, subsequently, the C/N ratio in the collagenous protein of tooth dentin to ascertain the proportion of animal protein comprising the diet (van der Merwe and Vogel 1978; DeNiro 1985). Vegans can be distinguished from ovo-lacto vegetarians and omnivores based on their lower measured  $\delta^{15}\text{N}$  values in proteinaceous tissue (O'Connell and Hedges 1999; O'Connell et al. 2001). Since individuals following the latter two dietary preferences share nearly identical  $\delta^{15}\text{N}$  values, it is not possible to isolate the vegetarians or non-vegetarians who consumed large proportions of either fish and/or meat without  $^{15}\text{N}$  isotopic data from dentin collagen (see Meier-Augenstein 2010).

Generally,  $\delta^{13}\text{C}$  values can only be used to make broad inferences regarding a region of origin (e.g., North America vs. Europe), although differentiations between some intra-continental regions (e.g., South Africa) may be inferred. Thus, while carbonate  $\delta^{13}\text{C}$  values alone will not provide a comprehensive picture of a person's diet, and cannot distinguish vegetarians from non-vegetarians, they can be considered a sufficient qualifier when  $\delta^{18}\text{O}$  data is ambiguous. However, these qualifying determinations should be made on a case-by-case basis, and should always be offered in the context of comparison with a missing person report, if one is available. The

drinking water  $\delta^{18}\text{O}$  values of the two vegetarians in the study group were almost indistinguishable from those of the other study participants; three other individuals'  $\delta^{18}\text{O}_{\text{Water}}$  values fell within the same confidence interval as that associated with the vegetarians. Although calculated drinking water values were reasonably consistent with the participants' self-reported diet information after the large variation in monthly OIPC  $\delta^{18}\text{O}$  values was considered, explanations for the observed trend (e.g., absence of any significant enrichment of the  $\delta^{18}\text{O}_{\text{Water}}$  values) cannot be made without requesting from the subjects additional dietary information (e.g., proportion of raw vs. cooked vegetables consumed, type and source of meat/fish consumed). Also, complimentary testing of  $\delta^{18}\text{O}$  values from proteinaceous tissues with more rapid turnover (e.g., the keratin of hair and fingernail) would offer the advantage of incorporating a recently formed biological component to comprise an individual's comprehensive isotopic "profile." The strong positive correlation noted between enamel  $\delta^{13}\text{C}$  values and dentin collagen  $\delta^{13}\text{C}$  values measured in archaeological and modern teeth (see Chapter 6) is indicative of another research area worthy of exploration. Identifying the mechanisms or processes underlying the minor correlational difference between two substrates with dissimilar biochemical histories may clarify  $^{18}\text{O}$  data when evaluated in conjunction with  $\delta^{13}\text{C}$  values, or at least aid in determining if this differential is due to remodeling rates or minute changes in diet or both.

The tooth enamel  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  isotopic data were consistent with the subjects' self-reported dietary information and residential history (i.e., preferential  $\text{C}_4$  plant consumption characteristic of typical North American populations). Further interpretations of the data were precluded due to the absence of C/N ratios analysis in



dentin collagen. Future similar research efforts in this area would be advised to incorporate a multi-isotope, multi-tissue approach into their experimental design while refining the participant questionnaire for specificity (e.g., Meier-Augenstein and Fraser 2008; Font et al. 2015). Definitive conclusions concerning the various macronutrient components of an individual's diet at the time of enamel formation cannot be offered without determining at the minimum,  $\delta^{15}\text{N}$  values of corresponding dentin collagen; the inclusion of  $^{34}\text{S}/^{33}\text{S}$  isotope ratios is recommended.

### Conclusions

Based on the combination of  $\delta^2\text{H}$ ,  $\delta^{18}\text{O}$ , and  $\delta^{13}\text{C}$  values measured in tooth enamel and dentin in this dissertation research and its preliminary feasibility studies, it is possible to infer and confirm geographic provenance and dietary intake provided certain assumptions are met and analytical and interpretive limitations are taken into consideration.

While the  $^2\text{H}$  and  $^{18}\text{O}$  isotope analysis of human tooth enamel pilot study was the first such research effort to establish that  $\delta^2\text{H}$  values in the bioapatite of human tooth enamel are not suitable as a proxy for human provenance, the experiment also demonstrated an unexpected and remarkably low rate of hydrogen exchange (i.e., hydrogen in the mineral hydroxyl groups comprising the crystal lattice structure of bioapatite is not readily exchangeable). Conversely, the  $\delta^2\text{H}$  values in human crown dentin exhibited positive correlations with enamel carbonate  $\delta^{18}\text{O}$  values and the  $\delta^2\text{H}$  values of source water, an observation which confirms the likelihood that the  $^2\text{H}$  isotopic

signal in human crown dentin can be used to improve the accuracy of geographic provenance estimations, particularly when combined with the analysis of  $^2\text{H}/^1\text{H}$  ratios in hair or fingernail keratin from the same individual.

Meaningful data on human provenancing can be obtained quickly from measuring the abundance of both  $^{13}\text{C}$  and  $^{18}\text{O}$  in tooth enamel carbonate. The  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  carbonate values measured in two third molars from the same individual were in accordance with published carbonate data. The highly positive correlation between  $\delta^{18}\text{O}_{\text{Phosphate}}$  and  $\delta^{18}\text{O}_{\text{Carbonate}}$  facilitates the use of structural carbonate instead of phosphate, and allows the generation of carbon and oxygen isotope data simultaneously. Incorporating phosphate-carbonate conversion equations into data analysis allows the relatively simple substitution of carbonate values for phosphate values when the material sampled has not been altered by postmortem processes. However, if significant diagenetic modification is evident or suspected in samples exposed to protracted postmortem intervals or burial environments promoting diagenesis, the phosphate component of tooth enamel (or bone) should be analyzed regardless of the expensive and time-consuming pretreatment requirements unless diagnostic tests confirm sufficient carbonate preservation. Further, conversion equations should be chosen carefully to reflect the suspected climatic origins of one's population sample in order to reflect as accurately as possible the site-specific climatic variables.

Although the  $\delta^{18}\text{O}$  values in enamel carbonate and dentin determined in modern and archaeological late-erupting tooth samples of European and North African origins analyzed in the pilot studies correlated well with source water  $\delta^{18}\text{O}$  values, in the

Canadian cohort study inconsistencies were observed between the enamel carbonate  $\delta^{18}\text{O}$  values measured in a small Ontario, Canada population and the interpolated  $\delta^{18}\text{O}$  precipitation values corresponding to the subjects' reported residential history. However, combining  $^{18}\text{O}$  enamel (and corresponding  $^{18}\text{O}$  OIPC source water) data from the modern and archaeological enamel samples analyzed in the first pilot study with the Canadian volunteer data minimized the obfuscation created by isotopically homogenous samples by increasing the overall study population, producing positive linear correlations, and facilitating a robust and comprehensive analysis of the relationships between variables. When unique and complex regional postprecipitation hydrological processes and water history are considered during the evaluation of isotopic data, interpretive accuracy increases.

A primary  $\text{C}_4$  plant dietary component for all study participants was confirmed through analysis of enamel  $\delta^{13}\text{C}$  values, but vegetarian dietary preferences were undetected isotopically. It is not possible to determine someone's dietary preferences (e.g., vegan) based solely on  $\delta^{13}\text{C}$  values in tooth enamel or dentin. In order to determine the proportion of meat in an individual's diet, and to distinguish between marine vs. terrestrial protein sources,  $^{15}\text{N}/^{14}\text{N}$  isotope ratios and  $^{13}\text{C}/^{12}\text{C}$  isotope ratios in collagen, hair, and/or fingernails are required for such determinations.

Although this multi-phase research project examined the abundance of three stable isotopes in tooth enamel and dentin, the lack of definitive results with regard to correlations between the calculated drinking water  $^{18}\text{O}$  signal in enamel and source water (precipitation)  $\delta^{18}\text{O}$  values in the Canadian volunteer dataset illustrates the importance of using multi-isotope data obtained from multiple tissues when

investigating the geographic origins of humans in an archaeological or forensic context. The presence of confounding factors arising from individual differences in physiology, consumption, and shopping habits, and source water isotopic variability necessitate interpretive restraint when utilizing the approach in individual case work, but  $\delta^{18}\text{O}$  data can still provide useful information on an individual's geographic provenance.

Much of the multi-isotope work published on modern human remains recovered in a forensic context or on human tissue obtained from living individuals utilizes a small number of samples. Much larger and geographically diverse sample sizes are required for robust statistical analyses. In addition to the general dearth of human remains available for analysis, obtaining and generating data from the biological materials of living volunteers or from the deceased who have donated their remains for research purposes is further complicated by the limits imposed upon researchers by national and international Human Subject research regulatory committees. Even if initial approval to sample from the living or dead can be obtained, an extraordinary amount of time and funding is required to compile the type of study sample from which a populational database of relevant isotopic values can be constructed; analytical costs are often prohibitive unless sufficient funding has been secured. Thus, while the results from this research are very reproducible for two teeth from the same individual, caution should be used when extrapolating the individual patterns evident in smaller data sets to a larger population. As mentioned here and elsewhere, it is important to establish isotopic databases consisting of delta values measured in various environmental (e.g., tap water) and human and faunal tissues (e.g., enamel, dentin, bone, hair, nails) from area residents so that eventually direct comparisons between existing isotope precipitation

data or other isotopically mapped data and values obtained from unidentified human remains can be made, and answers can be posed to the question of intra-individual variability. In the interim, comparisons of  $\delta^{18}\text{O}$  calculated drinking water values with “mapped” interpolated  $\delta^{18}\text{O}$  values in precipitation (i.e., source water) should be extended, but with the caveat that correlations are not always evident or assumptions may not always be validated due to discrepancies caused by deficiencies in sample size, experimental design and analytical interpretations. Preferably, determinations with regard to geographic provenance based on direct comparisons of measured values of  $\delta^{18}\text{O}_{\text{Phosphate}}$  rather than on predicted drinking water  $\delta^{18}\text{O}$  values should be made. The theoretically mapped values in precipitation (and, for that matter, the geological maps of  $^{87}\text{Sr}/^{86}\text{Sr}$  distribution) (Beard and Johnson 2000) are useful for inferring continental and regional origins, but they are not particularly useful for refining estimates of geographic provenance unless environmental isotopic data varies significantly within and between areas.

Approaching the isolated forensic case from a comprehensive stable isotope perspective is suggested in order to effectuate identification of an individual. If multiple tissues cannot be analyzed, then the abundance of multiple stable isotopes, specifically  $^{18}\text{O}$ ,  $^2\text{H}$ ,  $^{13}\text{C}$ ,  $^{15}\text{N}$ , and  $^{87}\text{Sr}$ , should be determined in whatever tissue samples remain. Care should be taken to utilize two point end member scale corrections, particularly when analyzing  $^2\text{H}$ , in order to avoid the standardization errors that have confounded interpretations of  $^2\text{H}$  data. If isotopic data from tooth enamel only are available for analysis, then the resulting interpretation should be used solely for qualification purposes, and not as a definitive indicator of provenance regardless of context.

Increasing numbers of multi-isotope, multi-tissue longitudinal studies such as the work conducted by Fraser et al. (2006) are imperative in order to further the existing body of knowledge with regard to intra-individual isotopic variability and to validate data recorded in the recommended isotopic databases. While such studies are time-consuming and costly from an analytical perspective, they offer an invaluable opportunity to obtain comprehensive residential and precise dietary information from each participant. Explicitly worded participant questionnaires can assist in the identification of primary dietary macronutrients and cooking methods (including unique relationships between cultural practices and regional geology) (e.g., Tatsumi 2014), and in the clarification of isotopically distinct dietary components (locally sourced food vs. that which is purchased at a grocer, specialty import establishment, or restaurant). The research value of subjects' self-reported dietary habits cannot be overstated, but limitations inherent to the reporting process and the data generated therein (e.g., the assumption that occasionally consumed imported foods or beverages unduly influence the isotopic composition of a particular biological tissue) must be taken into account. Additional research analyzing multiple stable isotopes in cadaver tissue is also necessary, as the more invasive sampling methods required to obtain bone specimens cannot be applied to living volunteers. Determining the isotopic abundance of multiple stable isotopes in the bone, teeth, fingernails, and hair from the same individual will contribute toward the construction of a unique isotopic profile, which can then be used to convey that individual's biogeographical story, past or present.

Stable isotope profiling has a proven utility within the context of archaeological and forensic human provenancing. When used in conjunction with other lines of evidence,

particularly mitochondrial DNA and available contextual information, it can help further define regional areas of geographic origin. It can also hinder investigations if analytical methodology is not standardized or interpretations are extrapolated inappropriately. Clearly, stable isotope profiling is not a method of identification. However, it is possible that the data compiled from this study and future stable isotope projects may facilitate a reduction in both the number of unidentified human remains awaiting provenance and the number of missing persons, thus allowing another biogeographical chapter to be revealed.

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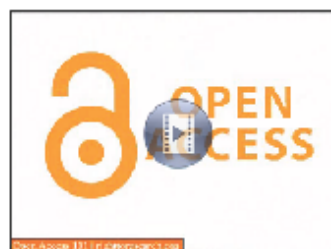
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## RE: including my publication in my dissertation

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## Appendix B. Ethical Research Consent Form/Information Letter.



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## ETHICAL RESEARCH INFORMATION/CONSENT FORM

### Stable Isotope/Human Provenance Study

**Investigator:** Anastasia Holobinko  
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**Purpose of the Study:**

You are invited to participate in a PhD research study concerning the stable isotope analysis of human teeth and hair. The purpose of this study is to determine if the stable isotope signatures in teeth and hair can effectively confirm geographic residence at various stages during an individual's lifetime. It is hoped that the results of this study help forensic investigators more accurately identify the geographic origins of unidentified individuals in forensic investigations.

**What will happen if you agree to participate?**

Should you agree to contribute to this study, we will ask you to complete a questionnaire so we can collect information concerning residence during childhood, adolescence, and at present, and dietary preferences for the corresponding time periods. In addition to the survey, which will take approximately 10 minutes to complete, you will be asked to donate extracted teeth to the project for subsequent isotopic analysis.

Teeth

If you have been given this survey by your dental clinician, you have already been informed that you will require a tooth extraction. Your scheduled procedure is in no way dependent upon your participation in this study; your treatment will not be affected one way or the other by your agreement or refusal to volunteer for this project.

## Appendix B. Continued.

The tooth sample(s) you provide will be used exclusively for stable isotopic analysis. **NO** DNA will be collected.

### Hair

Should you decide to participate in this study and also wish to donate a hair sample consisting of 1 lock of hair consisting of 50-100 individual strands, both your tooth sample and hair sample will be collected during your appointment for the routine tooth extraction. Hair must be at least 1 cm in length. While your doctor will be extracting your tooth, we ask that you take your own samples of hair. Instructions for hair sampling are provided on the questionnaire.

If you provide a hair sample to us, it will be used exclusively for stable isotope analysis. **NO** DNA will be collected. Since we are interested in collecting hair that has been cut, not pulled from the scalp, no root or follicle will be attached to the hair strands; DNA cannot be analyzed from only the hair shaft. Further, the isotopic analytical process destroys the hair, making DNA analysis impossible.

### Fingernails

Fingernails and hair have similar biochemical properties. If you decide to participate in this study and wish to donate fingernail clippings from several fingers in addition to **or** instead of a hair sample, the clippings will be taken during your appointment for the routine tooth extraction. Fingernails should be unpolished. Instructions for fingernail sampling are provided on the questionnaire.

### **What information about you may be collected?**

Specifically, the following information may be collected and used:

- Age
- Sex
- Dietary preferences
- History of residence from birth to age 18, and at present
- Travel history covering the last 6 months
- Use of hair dye
- Stable isotope analysis data

The findings obtained from analysis of your tooth (and hair/fingernails, if applicable) will be part of a large dataset that will eventually be incorporated into a PhD dissertation. While this work product will be available to the public, in no way can the resulting isotopic data from your tooth, hair, or fingernails be traced back to you.

### **What are the potential risks to you?**

There are no known risks associated with this study. Because the study is independent of your tooth extraction procedure, the absence of risk pertains to this study only.

### **Are there any benefits to you or others?**

There are no direct benefits, monetary or otherwise, to you should you desire to participate in this project. Similarly, there is no charge to you for your participation.

This study is the basis for a PhD research project. Therefore, the student conducting the research may



## Appendix B. Continued.

benefit in several ways: receiving a doctorate in Physical Anthropology, presenting the results at a scientific conference, or publishing the findings in a scientific journal.

It is hoped that the data obtained as a result of this study will assist forensic investigators in the identification of unidentified human remains.

### **What if you have more questions?**

If you have additional questions, or something is unclear, please contact Anastasia Holobinko at (905) 525-9140 x 24423 or [holobia@mcmaster.ca](mailto:holobia@mcmaster.ca).

### **Confidentiality**

We must have your name and signature on this consent form in order for you to participate in this study. However, your donated teeth (and hair/fingernails) will be identified only by number. We will not use your name or any other identifying information. The information we collect from your teeth, hair, or fingernails and survey responses cannot and will not in any way be linked to you. While we must retain your consent form to show your willing participation in this study, at no time will your consent form be physically associated with your questionnaire or tooth/hair/fingernail samples.

Your answers to the survey questions will be entered into a password-protected computerized spreadsheet. Your participation in this study and all information you provide to us will remain private unless you choose to tell someone you have participated in this project. Only the investigators listed above will have the authority to review information collected about you.

### **How can you find out what was learned in this study?**

The study is expected to conclude by approximately 1 September, 2012. A brief summary of the results can be made available to you if you wish to review it. If you would like to receive a copy of this summary, please let me know how you would like to receive it (via postal mail or email.)

### **Withdrawal from this study**

Your participation in this study is strictly voluntary. If you decide you do not want to be part of the study you may withdraw at any time until we create the password-protected database containing information collected from the sampled teeth; at this time we will be unable to trace any of the information back to you and therefore cannot withdraw you from the study. There is absolutely no penalty for withdrawing your consent.

If you withdraw from the study prior to the sampling of your teeth/hair/fingernails, neither your teeth nor hair/fingernails nor your survey information will be used. All materials will be destroyed.

If you wish to opt out of the study, for whatever reason, after donating your samples, please contact Anastasia Holobinko at (905) 525-9140 x24423 or [holobia@mcmaster.ca](mailto:holobia@mcmaster.ca).

You may be withdrawn from this study without your consent if the information you provide on the survey is incomplete, or there is not enough enamel/dentine on your tooth or teeth to provide a sufficient sample for analysis.

## Appendix B. Continued.

This study has been reviewed by the Hamilton Health Sciences/Faculty of Health Sciences Research Ethics Board and received ethics clearance. If you have concerns or questions about your rights as a participant or about the way the study is conducted, please contact the Office of the Chair of the HHS/FHS Research Ethics Board at (905) 521-2100, Ext. 42013.

**Thank you for taking the time to read this Information Letter.**

## Appendix B. Continued.

## CONSENT

**Signatures**

I have read the information presented above about a study being conducted by Anastasia Holobinko of McMaster University. I have had the opportunity to ask questions about my involvement in this study and to receive additional details should I request them. I understand that if I agree to participate in this study, I may withdraw from the study at any time prior to the isotopic analysis of my tooth/hair/fingernails and subsequent creation of the database. I agree to participate in this study. I will be given a signed copy of this form.

\_\_\_\_\_  
Signature of Participant

\_\_\_\_\_  
Printed name of Participant

\_\_\_\_\_  
Signature of Witness

\_\_\_\_\_  
Printed name of Witness

\_\_\_\_\_  
Signature of Person obtaining Consent

\_\_\_\_\_  
Printed name of Person obtaining Consent

Please check one:

\_\_\_\_\_ Yes, I would like to receive a summary of the study's results. Please send them to this email address \_\_\_\_\_ or to this mailing address \_\_\_\_\_

\_\_\_\_\_ No, I do not want to receive a summary of the study's results.



## Appendix C. Participant Questionnaire.

DATE: \_\_\_\_\_

**PARTICIPANT QUESTIONNAIRE****McMaster University Stable Isotope/Human Provenance Study****Instructions:**

Please answer the following questions as completely as possible. The information requested below is designed to assist us in identifying factors affecting the stable isotope composition of your teeth and hair, which in turn may be linked with specific geographic locations. Incomplete responses may result in the exclusion of your teeth and hair from the study. **Please do not include your name on this form.**

**Questions:**

1. Year of birth \_\_\_\_\_
2. Sex (M/F) \_\_\_\_\_

<b>Dental Staff Use Only</b>	
Subject Identifier	_____
Tooth position	_____
Date extracted	_____
Hair sample (Y/N)	_____
Nail sample (Y/N)	_____

3. Which dietary category would you associate with your childhood (until age 18 years)? Please circle only one category, unless you experienced a major shift in dietary habits. If this is the case, please indicate the ages at which each diet was followed.

Meat-eater (beef, poultry, pork, fish, etc.)

Vegetarian (no meat/fish, but perhaps eggs, cheese, etc.)

Vegan (no animal products whatsoever)

4. Do you rely primarily on bottled water for your water intake? (Please circle one.) If yes, please provide the brand name of the product you consume most often.

Yes                  No

5. Where have you resided from birth to age 18? (Please be as specific as possible, and continue your answers on the back of this form if necessary.)

	<b>From (Year)</b>	<b>To (Year)</b>	<b>City</b>	<b>State/Province</b>	<b>Country</b>
1.					
2.					
3.					
4.					
5.					

## Appendix C. Continued.

6. Where do you live currently?

City: \_\_\_\_\_  
 Province/State: \_\_\_\_\_  
 Country: \_\_\_\_\_

7. In the last 6 months have you remained in your local area without much travel except for commuting to/from work and occasional day trips? (Please circle one.) If No, please provide details on location (country/province/state) and general travel dates on the back of this page.

Yes            No

8. Do you dye your hair? (Please circle one.)

Yes            No

**Hair sampling instructions:**

- Hair must be at least 2.54 cm in length.
- Please cut 1 lock of hair, from the nape of the neck (or another area on the scalp, if you prefer), as near to the scalp as possible.
- The lock should consist of approximately 50-100 strands of hair.
- Please place your lock of hair in one of the aluminum foil pouches provided. Please ensure that the hair sample lays relatively flat and fully extended along the bottom of the foil pouch. (Note: If your hair length exceeds the length of the foil pouch, please cut off any excess from the natural end and discard.) Next, seal the hair sample inside by folding over the top edge of the foil pouch; place the foil pouch in one of the paper envelopes provided. Finally, place the paper envelope and foil pouch with its corresponding hair sample in one of the plastic bags provided and seal the bag.

**Fingernail sampling instructions:**

- Fingernails should be unpolished.
- Please use the nail clippers provided to trim the outer nail edges from as many fingernails (middle, ring, and index fingers, preferably) as possible.
- Please transfer your fingernail clippings to one of the glass storage vials provided. Seal the cap tightly and place the vial in one of the plastic bags provided.

## Appendix D. Dental Staff Instructions.

### McMaster University Stable Isotope/Human Provenance Study

#### **Dental Staff Instructions**

Thank you for your assistance with this research project. The purpose of this study is to determine if the stable isotope signatures in human tooth enamel, dentin, hair, and fingernails can be used collectively as a proxy for human geographic origins.

Patients already scheduled for routine tooth extraction will be asked to participate in a brief survey and donate their extracted teeth, a hair clipping (no root attached), and fingernail clippings for isotopic analysis. No DNA analysis will be performed on collected samples.

Participation in this study is limited to tooth, hair, and fingernail donation and completion of the demographic survey. Our goal is to obtain donated teeth and hair/fingernail samples from at least 20 individuals. Sample collection is expected to commence on June 1, 2011 and run until December 1, 2012.

If you would like additional information about this project or its investigators, please do not hesitate to contact the Principal Investigator conducting this research, Anastasia Holobinko (holobia@mcmaster.ca, 519.394.0203 or 440.339.1350).

#### **Included with this packet are the following materials:**

1. McMaster University Ethical Research Information/Consent Form
2. Participant Questionnaire
3. Subject I.D. Log
4. Binder
5. Permanent marker
6. Glass storage vials with lids (extracted teeth and fingernail clippings)
7. Plastic storage bags (hair and tooth/fingernail storage vials)
8. Scissors
9. Fingernail clippers
10. Manila envelopes
11. Bin for temporary storage of samples

## Appendix D. Continued.

**Instructions**

- ❖ At the time patients are notified a **late-erupting** tooth extraction will be necessary, please ask them if they would be willing to participate in this study. Querying patients before the actual extraction will allow them to consider participating prior to their actual enrollment in the study. Participating patients may donate teeth and hair **and/or** fingernails, or teeth only.
  - Minors will not be included in this study.
- ❖ Patient consent may be obtained prior to or on the date of extraction. If the patient wishes to withdraw consent prior to or on the date of extraction, please return the signed consent form to the patient for disposal.
- ❖ Completion of two forms are required of each patient at the time of intake:
  - *McMaster University Ethical Research Consent Form* (two original signed copies). The following signatures are required on the last page:
    - A dental staff member as the "Person obtaining Consent."
    - The participant (volunteer) as the "Participant" in front of a witness. Please allow the patient as much time as required to read the form.
    - A witness should sign on the last page of the form verifying the participant's signature.
    - Each participating patient must sign two original forms.
    - One original should be given to the patient before samples are obtained. The remaining original should be retained in the study document binder.
  - *Participant Questionnaire*.
    - The participant should complete the questionnaire on the date of extraction (prior to the procedure). Please ask the patient to read the form and answer all questions completely.
    - Hair samples, if provided by the participant, should be clipped, bagged, and labeled accordingly (see questionnaire for instructions).
    - Fingernail clippings may also be collected at this time, placed in a glass storage vial, and labeled accordingly (see questionnaire for instructions).
- ❖ Please retain all forms in the binder provided. Blank forms are in the front half of the binder. Completed forms should be placed in their corresponding section in the second half of the binder.
- ❖ Just prior to the tooth extraction each volunteer should be assigned a unique subject identifier number (SID #).
  - Please ensure that no numbers are used more than once. The first participant should be assigned the number \_\_-001; the second volunteer should be \_\_-002, etc. The first part of the SID # should consist of two characters identifying the doctor or clinic performing the procedure (e.g., WO-001 for Dr. Woods, Subject #1).
    - If a participant has previously had a tooth extracted in your clinic (e.g., "\_\_-001") and this is a second donation, please use the original SID # followed by "b." (e.g., \_\_-001b).

## Appendix D. Continued.

- All teeth extracted from the same individual should have the same base subject identifier number (SID #).
- ❖ Enter the SID # in two places:
  - The Subject I.D. Log
  - The participant's questionnaire in the gray box labeled "Dental Staff Use Only."
- ❖ If multiple teeth are being pulled from the same individual, more than one tooth may be donated.
- ❖ Write the **tooth** number (position) for all teeth donated and the extraction date in two places: (1) Subject I.D. Log, (2) on the participant's questionnaire in the gray box labeled "For Dental Staff Only."
- ❖ Write the SID # and tooth position number on the storage vial with a permanent marker. Please place only one tooth in each vial, and ensure the lid is sealed tightly.
- ❖ Using a permanent marker, write the SID # on a plastic bag. Place the tooth vial(s) in the plastic pouch, seal the bag, and staple it to the participant survey. The vial(s), plastic pouch, and questionnaire should all have the same SID #.
- ❖ If a **hair** sample is being donated, please write "Yes" or "Y" on the:
  - Subject I.D. Log
  - Participant's questionnaire in the gray box labeled "For Dental Staff Only."
- ❖ If no hair sample is collected, please enter "No" or "N" on the:
  - Subject I.D. Log
  - Participant's questionnaire.
- ❖ If a hair sample is being collected, please provide the participant with the designated scissors, an index card, bulldog clip, and plastic bag. Please ensure that the plastic bag is tightly sealed, and that the scissors are sterilized prior to their next use.
- ❖ Using a permanent marker, write the SID # on each plastic bag. Staple it to the participant survey. The vial(s), plastic pouches (teeth, hair), and questionnaire should all have the same SID #.
- ❖ If a **finger nail** sample is being donated, please write "Yes" or "Y" on the:
  - Subject I.D. Log
  - Participant's questionnaire in the gray box labeled "For Dental Staff Only."
- ❖ If no nail clippings are collected, please enter "No" or "N" on the:
  - Subject I.D. Log
  - Participant's questionnaire.
- ❖ If a nail sample is being collected, please provide the participant with the designated clippers and glass vial with screw-cap top. Please ensure the clippers are sterilized prior to their next use.
- ❖ Write the SID # on the storage vial with a permanent marker. Please ensure the lid is sealed tightly before placing it in the plastic bag provided.
- ❖ Using a permanent marker, write the SID # on the plastic bag. Staple it to the participant survey. The vial(s), plastic pouches (teeth, hair, fingernails), and questionnaire should all have the same SID #.



## Appendix D. Continued.

- ❖ Please place all participants' teeth, hair, fingernails, and surveys in the provided manila envelopes (one envelope per subject number). The envelopes should be placed in the designated storage container and retained until they are picked up by the Principal Investigator, Anastasia Holobinko. Her contact information is:

Anastasia Holobinko  
Department of Anthropology  
McMaster University  
CNH-#524  
1280 Main Street West  
Hamilton, Ontario L8S 4L9  
Phone: (519) 394-0203  
Fax: (519) 394-0211  
Email: [holobia@mcmaster.ca](mailto:holobia@mcmaster.ca)

- ❖ This isotope study is scheduled to end on December 1, 2012. At the completion of the study your clinic will be notified and all materials, including any remaining tooth samples and surveys, subsequently picked up by Anastasia Holobinko.

Appendix E. Procurement and Chemical Cleaning of Tooth Enamel for Stable Isotope Analysis. Adapted from Lee-Thorp (2008) with modifications by Meier-Augenstein (2010).

1. Photograph and document tooth characteristics.
2. Bulk clean the tooth as follows:
  - a. Place tooth in 1.5% v/v sodium hypochlorite for 10 minutes. Remove and rinse thoroughly with deionized water for 5 minutes.
  - b. Place tooth in 0.1M acetic acid for 10 minutes. Remove and rinse thoroughly with deionized water for 5 minutes.
  - c. Allow whole tooth to air-dry overnight.
  - d. Using either a *Dremel* multi-tool or a *Nouvag* NM-300 dental drill with a diamond-tipped bit remove as much of the enamel layer as possible (minimum of 80 mg dry sample is required) onto an aluminum foil square.
  - e. Record the weight of the enamel removed.
  - f. Divide the sample equally between two 2 mL *Eppendorf* micro-centrifuge tubes.
  - g. Powdered samples should be stored in a non-evacuated desiccator over phosphorous pentoxide unless they are to be chemically cleaned immediately.
3. Chemically clean the powdered enamel as follows:
  - a. Add 0.04 mL/mg 1.5% v/v sodium hypochlorite to each *Eppendorf* tube containing powdered sample.
  - b. Vortex sample briefly.

## Appendix E. Continued.

- c. Place on a rocker bed and leave for 30 minutes at room temperature.
- d. After 30 minutes, remove from rocker, vortex briefly and centrifuge at 10,000 rpm for 5 minutes.
- e. Aspirate and discard supernatant, re-suspend pellet in 2 mL deionized water, vortex for 10 seconds, and centrifuge at 10,000 rpm for 5 minutes [= rinse step one].
- f. Aspirate and discard supernatant, re-suspend pellet in 2 mL deionized water, vortex for 10 seconds, and centrifuge at 10,000 rpm for 5 minutes [= rinse step two].
- g. Aspirate and discard supernatant, re-suspend pellet in 2 mL deionized water, vortex for 10 seconds, and centrifuge at 10,000 rpm for 5 minutes [= rinse step three].
- h. Aspirate and discard supernatant.
- i. Re-suspend pellet in 0.04 mL/mg 0.1M acetic acid.
- j. Vortex sample briefly.
- k. Place on a rocker bed and leave for 10 minutes at room temperature.
- l. After 10 minutes, remove from rocker, vortex briefly, and centrifuge at 10,000 rpm for 5 minutes.
- m. Aspirate and discard the supernatant, re-suspend pellet in 2 mL deionized water, vortex for 10 seconds, and centrifuge at 10,000 rpm for 5 minutes [= rinse step one].

## Appendix E. Continued.

- n. Aspirate and discard the supernatant, re-suspend pellet in 2 mL deionized water, vortex for 10 seconds, and centrifuge at 10,000 rpm for 5 minutes [= rinse step two].
- o. Aspirate and discard the supernatant, re-suspend pellet in 2 mL deionized water, vortex for 10 seconds, and centrifuge at 10,000 rpm for 5 minutes [= rinse step three].
- p. Aspirate and discard supernatant.
- q. Place samples in a drying oven for 48 hours at  $T < 45^{\circ}\text{C}$ ), or if a drying oven is not available, place samples in a non-evacuated desiccator with phosphorus pentoxide for 48 hours.
- r. Transfer samples into an evacuated desiccator with phosphorus pentoxide for a minimum of 7 days.
- s. Weigh samples (~8-10 mg) into 13 mL Exetainer tubes and store in an evacuated desiccator prior to the acid digest procedure.

Appendix F. Preparation of Tooth Enamel for  $^{13}\text{C}/^{18}\text{O}$  Stable Isotope Analysis. Adapted from Henton et al. (2010).

1. Preparation of tooth sample:
  - a. Remove pre-weighed samples in Exetainers from storage desiccator.
  - b. Ensure both IAEA (NBS-19 and LSVEC) and internal acquisition quality controls (Bicarb-X and Bicarb-H) standards are available from the AP2003 Principal Operator.
  - c. Prepare a blank Exetainer to act as a “blank” sample and an Exetainer to which only sulphuric acid will be added to act as an “acid blank” sample.
2. Procedure:
  - a. Switch on the heater block and set the temperature control to 50°C. This will allow the heater block to pre-heat while the samples are being prepared.
  - b. Flush each Exetainer with gaseous nitrogen (N5.5 grade BOC #293679) for 8-12 minutes to remove traces of residual atmospheric  $\text{CO}_2$ . This can be achieved by placing a vent needle [21G] in the cap septa, while a second needle [19G] acts as a guide through which untreated fused silica capillary is fed. Ensure vent needle sits just under the septa and feed the fused silica to 1 cm off the base of the Exetainer.
  - c. Use a 1 mL disposable syringe fitted with a 21G to inject 800  $\mu\text{L}$  of sulphuric acid (99.999% purity) through the septum of each Exetainer. Ensure a new syringe/needle is used for each Exetainer.
  - d. Place the Exetainers into the pre-heated heater block for 6 hours at 50°C. After 6 hours switch the heater block off and allow samples to cool while

## Appendix F. Continued.

remaining in the heater block overnight. As an alternative samples can be removed from the heater block, placed in a rack, and left to cool overnight.

- e. After the samples have cooled completely, transfer them to the AP2003 Breath Analyzer for the determination of  $^{13}\text{C}$  and  $^{18}\text{O}$  isotopic abundance.
- f. If necessary, the samples can be left for a period of up to 14 days post-digest before being analyzed, provided the Exetainer lids have been tightly secured.

## Appendix G. Experimental Acid Digest and IRMS Analysis Protocol for Enamel Test Samples at SIUC Mass Spectrometry Facility.

1. Preparation of tooth sample (Day 1 and 2):
  - a. Day 1: Drilled approximately 66 mg of crown tooth enamel from a total of 4 teeth. Measured out 8 mg of powder onto each of 8 foil squares. Four of the 8 samples were cleaned according to the procedure described in Appendix D. The remaining 4 samples were left as is. All 8 samples were left at 60°C in a drying oven overnight.
  - b. Day 2: The four samples of raw enamel powder were removed from the drying oven and prepped for an experimental acid digest.
  - c. Ensure IAEA (NBS-19, NBS-18, and LSVEC) and internal acquisition quality control (Carrara marble) standards are available.
  - d. Prepare a blank Exetainer to act as a “blank” sample and an Exetainer to which only phosphoric acid will be added to act as an “acid blank” sample.
2. Procedure (Day 2):
  - a. Phosphoric acid was warmed until it reached a viscous stage via placement in an oven at 50°C - 60°C or 60°C - 70°C for 2-3 hours.
  - b. Switch on the heater block and set the temperature control to 25°C. This will allow the heater block to pre-heat while the samples are being prepared.
  - c. Using two needles (one to flush, one to vent), flush each blank Exetainer with ultra-pure Helium to remove traces of residual atmospheric CO<sub>2</sub>.

## Appendix G. Continued.

- d. Use a 500  $\mu\text{L}$  syringe to draw up warmed phosphoric acid. Inject 1 drop ( $\sim 20 \mu\text{L}$ ) of acid into the control Exetainer. Inject 1 drop of acid into each of the Exetainers containing NBS-19, NBS-18, LSVEC, and CM. Inject 3 drops into each Exetainer containing enamel samples EN1 and EN2.
- e. It was noted that too much acid was added to Exetainers containing NBS-19 and LSVEC (typically they should receive  $\sim 1$  drop each). Therefore, inject 4 drops into each Exetainer containing enamel samples EN3 and EN4. Screw all Exetainer lids on tightly, taking care to avoid allowing acid to touch the septum of Exetainer lids.
- f. Flush fill the Exetainers with ultra-pure Helium two at a time.
- g. Place the Exetainers into the pre-heated heater block at  $25^\circ$  to allow reaction to occur.
- h. After the acid digest is complete, engage the gas bench autosampler analytical instrument to begin the determination of  $^{13}\text{C}$  and  $^{18}\text{O}$  isotopic abundance.

Analytical results (11/27/2013)

Sample #	$\delta^{13}\text{C}_{\text{VPDB}}$	$\delta^{18}\text{O}_{\text{VSMOW}}$
E1	-10.45	25.49
E2	-10.34	25.79
E3	-10.14	24.25
E4	-10.43	23.75



Appendix H. Analytical Results for Tooth Enamel Carbonate Samples Run at the Southern Illinois University Carbondale Department of Chemistry and Biochemistry Mass Spectrometry Facility. Original  $\delta^{18}\text{O}$  values are reported in VSMOW.

Analytical method used: Gas Bench-IRMS

Instruments used: Thermo Delta V Plus IRMS, GasBench II

Analysis date: 12-11, 12-2013

Sample #	$\delta^{13}\text{C}_{\text{VPDB}}$	$\delta^{18}\text{O}_{\text{VSMOW}}$	Sample #	$\delta^{13}\text{C}_{\text{VPDB}}$	$\delta^{18}\text{O}_{\text{VSMOW}}$
MCM1A	-10.18	24.22	MCM1A	-10.28	24.22
MCM1B	-10.35	24.25	MCM1B	-10.42	24.12
CL002A	-10.80	24.08	CL002A	-10.85	24.08
CL002B	-10.66	23.87	CL002B	-10.91	23.85
SY4A	-11.13	24.73	SY4A	-11.15	24.77
SY4B	-11.07	24.78	SY4B	-11.25	24.68
CL005A	-10.01	24.60	CL005A	-10.14	24.54
CL005B	-9.97	24.61	CL005B	-9.87	24.70
CL006A	-9.53	24.52	CL006A	-9.45	24.62
CL006B	-9.71	24.27	CL006B	-9.76	24.28
SY007A	-11.29	25.17	SY007A	-11.27	25.19
SY007B	-11.28	24.90	SY007B	-11.35	24.91
CL008A	-10.08	24.05	CL008A	-10.04	23.90
CL008B	-10.08	24.26	CL008B	-10.07	24.40
CL009A	-9.39	24.23	CL009A	-9.55	24.14
CL009B	-9.53	24.82	CL009B	-9.76	24.58
CL0010A	-9.81	24.68	CL0010A	-10.08	24.35
CL0010B	-9.97	24.56	CL0010B	-10.01	24.63
CL0013A	-10.97	23.68	CL0013A	-10.98	23.85
CL0013B	-10.81	23.78	CL0013B	-10.91	23.75

## VITA

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Publications:

Holobinko A. 2012. Forensic human identification in the U.S. and Canada: a review of the law, admissible techniques, and the legal implications of their application in forensic cases. *Forens Sci Int* 222:394.e1-394e.13.

Holobinko A. 2012. Theoretical and methodological approaches to understanding human migration patterns and their utility in forensic human identification cases. *Societies* 2:42-62.

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Holobinko A, Waring G. 2009. Conflict and reconciliation behavior trends of the bottlenose dolphin (*Tursiops truncatus*). *Zoo Biol* 28:1-19.