Sex-Based Differences in Mortality and Metabolic Disease in Subadult Skeletal Assemblages of Tell Hisban

by

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Heterogeneity in morbidity and mortality risk results from various underlying host-level and societal factors. Identifying these risk factors in ancient communities is often hindered by the invisibility of some variables in the archaeological record and the methodological limitations of skeletal analysis. These both have impacted the complete understanding of the causes of infant mortality in the 19th century AD at the site of Hisban, Jordan, particularly the effects of sex on the risk of dying with active metabolic disease. This project uses dental proteomics to estimate the sex of 16 infants (up to 3 years of age) dying with (n=10) and without (n=6) skeletal lesions indicative of vitamin C deficiency, in addition to 8 modern control samples of known sex. Identification of sexually dimorphic amelogenin proteins (AMELY and AMLEX) can indicate the biological sex of the infant, which, in this cultural and temporal context, may align with their gender identity. With this information, we have been able to determine that more males than females (2:1) were found to have died overall, regardless of whether they showed signs of active scurvy or not, indicating there is no evidence of a sex-based mortality risk for those with indicators of active scurvy at the time of death. However, male infants, in general, had a higher mortality risk than female infants. These results inform future analysis of these infants' diet and

weaning patterns using incremental $\delta^{13}C$ and $\delta^{15}N$ analysis of dentin. Since metabolic diseases are closely tied to diet and socio-cultural practices, this project was an excellent opportunity to understand this community's biological and social determinants of infant morbidity and mortality.

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TABLE OF CONTENTS

LIST OF TABLES v
LIST OF FIGURES vii
CHAPTER ONE 1
Introduction 1
CHAPTER TWO 4
Background 4
Introduction 4
Historical and Archaeological Background 5
Sex-Based Differences in Infant Mortality Risk 9
Scurvy as a Factor in Increased Mortality Risk 12
The Developmental Origins of Health and Disease 23
Sex Estimation in Subadult Skeletal Remains 25
New Methods for Estimating the Sex of Infants and Children 29
CHAPTER THREE 33
Materials and Methods 33
Materials 33
Methods 34
Peptide Extraction from Enamel 34
NanoLC-MS/MS Analysis 35
Protein Identification 36
CHAPTER FOUR 37
Results 37
CHAPTER FIVE 45
Discussion 45
Evidence for Sex-Based Differences in Morbidity at Hisban 49
Scurvy and the Mother-Infant Dyad 50
Limitations and Sampling Bias 54
Summary 58
CHAPTER SIX 61
Conclusion 61
Future Research 62
REFERENCES 65
APPENDIX: Peptide IDs and Spectral Count for Tell Hisban Samples 01-24 82

LIST OF TABLES

Table 1. Percentage of regions worldwide (n=193) where male age-specific mortality was higher than female age-specific mortality in 2009. (World Health Organization, 2012) 11

Table 2. Daily Vitamin C Requirements (Popovich et al., 2009) 13

Table 3. The vitamin C content of a range of foods (Brickley et al., 2020) 14

Table 4. Age estimates of the subadults in the Hisban assemblage determined by Perry and Edwards (2021) 33

Table 5. Protein identification for modern controls and Hisban dental samples 40

Table 6. Descriptions of scorbutic lesions in the maxillae and mandibulae from Hisban selected for proteomic analysis (Individuals 19-24 did not display any skeletal lesions in these elements) 43

Table 7. Ages of Subadults from Tell Hisban with and without pathology by sex 44

Table 8. The Vitamin C Content of Foods Known to be Consumed by the Bedouin 53

Table A1. Peptide IDs and spectral count for Tell Hisban sample #01 81

Table A2. Peptide IDs and spectral count for Tell Hisban sample #02 81

Table A3. Peptide IDs and spectral count for Tell Hisban sample #03 83

Table A4. Peptide IDs and spectral count for Tell Hisban sample #04 85

Table A5. Peptide IDs and spectral count for Tell Hisban sample #05 98

Table A6. Peptide IDs and spectral count for Tell Hisban sample #06 89

Table A7. Peptide IDs and spectral count for Tell Hisban sample #07 90

Table A8. Peptide IDs and spectral count for Tell Hisban sample #08 92

Table A9. Peptide IDs and spectral count for Tell Hisban sample #09 94

Table A10. Peptide IDs and spectral count for Tell Hisban sample #10 94

Table A11. Peptide IDs and spectral count for Tell Hisban sample #11 95

Table A12. Peptide IDs and spectral count for Tell Hisban sample #12 95

Table A13. Peptide IDs and spectral count for Tell Hisban sample #14 96

Table A14. Peptide IDs and spectral count for Tell Hisban sample #15 96

Table A15. Peptide IDs and spectral count for Tell Hisban sample #16 97

Table A16. Peptide IDs and spectral count for Tell Hisban sample #17 98

Table A17. Peptide IDs and spectral count for Tell Hisban sample #18 98

Table A18. Peptide IDs and spectral count for Tell Hisban sample #19 99

Table A19. Peptide IDs and spectral count for Tell Hisban sample #20 99

Table A20. Peptide IDs and spectral count for Tell Hisban sample #21 100

Table A21. Peptide IDs and spectral count for Tell Hisban sample #22 100

Table A22. Peptide IDs and spectral count for Tell Hisban sample #23 100

Table A23. Peptide IDs and spectral count for Tell Hisban sample #24 101

LIST OF FIGURES

Figure 1. Factors affecting ascorbic acid levels in the human body (Halcrow et al., 2014) 17

Figure 2. Typical sites for hemorrhagic lesions in scurvy (Brickley et al., 2020) 20

Figure 3. Sequence alignment of AMELY and AMELX isoforms generated using Clustal Omega (Madeira, 2022) 37

Figure 4. Sequence Inclusion List for Mass Spectrometry Data Acquisition 39

CHAPTER ONE

Introduction

This study focuses on identifying sex-based mortality risks of infants buried in the Late Ottoman Period (1808-1918 CE) cemetery at Tell Hisban. The archaeological site of Tell Hisban is located approximately 20 km south of the capital city of Amman, Jordan, on the highland plateau known as the Madaba Plain, which was seasonally occupied by semi-nomadic agropastoral Bedouin tribes during the Ottoman Period. Previous analysis of a late 19th century skeletal sample from the site found high rates of metabolic disease, including vitamin D (rickets) and vitamin C deficiency (scurvy), in non-adult (subadult) individuals dying between 6 months and 3 years of age (Perry and Edwards, 2021), something rarely documented in other Ottoman Period skeletal assemblages (e.g., Sadvari, 2009; Rose and Khwaleh, 2012; Eakins, 1993) or earlier periods of occupation at the site (Grauer and Armelagos, 1998).

This study aims to determine how sex-based mortality risk could be linked to biological and social factors influencing health outcomes. Biological sex can affect morbidity and mortality through multiple pathways, and population-level studies have found that male infants and children tend to have a "higher biological risk" than females (Chen et al., 1981). This "innate frailty" (Bhatia, 1984) may be attributed to the effects of sex hormones (i.e., estrogen and testosterone) on the immune system (Klein and Roberts, 2010), as males are more susceptible to a wide variety of diseases caused by viruses, bacteria, fungi, and parasites (Klein, 2000; Wells, 2000; Owens, 2002; Pennell et al., 2012). Additionally, cultural behaviors and preferences such as differential treatment and access to resources may be responsible for health disparities, resulting in higher sex-specific mortality rates, especially among females (United Nations, 2011).

Secondarily, this study seeks to determine if there is a difference in mortality risk by biological sex in those dying with scurvy (vitamin C deficiency) in infancy. Typically, exploring sex-based morbidity and mortality in the skeletal remains of subadults is hindered by methodological limitations in skeletal sex estimation before puberty. Here, the biological sex of the infants at Hisban was estimated by measuring amelogenin proteins using the dental enamel proteomic method. This method works because, during tooth development, amelogenin peptides become trapped in the tooth's enamel matrix and remain in the tooth enamel long after the individual has died. If the AMELY peptide associated with Y chromosomes is present in a sample, the individual is likely male; if only AMELX peptides associated with X chromosomes are present, the individual is likely female. This data was then used to calculate the ratio of males to females dying in infancy to identify any differences in mortality risk by sex, taking into consideration not only the female:male sex ratio at birth of 1.07:1.00 (CIA Fact Book, 2021) but also the intrinsic increased risk of male mortality during infancy (United Nations, 2011). In addition, the sex ratio of infants dying with and without scurvy was calculated to clarify morbidity patterns within the sample.

These results were then contextualized within the historical and cultural data on this community, potential causes and physiological effects of vitamin C deficiency (scurvy), and how this reflects infant and maternal health. Metabolic diseases such as the scurvy observed at this site may serve as an important proxy of population-wide nutritional status impacted by biological factors (e.g., sex hormones and immune reactions) and cultural behaviors (e.g., subsistence patterns and weaning practices). Additionally, I explore the impacts of the mother-infant-nexus on infant health as one possible factor in the male:female infant mortality ratio at Tell Hisban. While this study focuses on only subadults and is only one piece in a larger project to study the

unique skeletal assemblage interred at Tell Hisban, I hypothesize that vitamin C deficiency in infants under 3 years of age was not evenly distributed between male and female subadults and that any differences in mortality ratio result from biological and social determinants.

CHAPTER TWO

Background

Introduction

The site of Tell Hisban is located in modern Jordan, just south of the capital city of Amman. This study focuses on the Late Ottoman Period (1808-1918 CE), during which many semi-nomadic agropastoral Bedouin tribes became more sedentary due to large-scale *Tanzimat* land use and agricultural reforms. The individuals represented in the skeletal assemblage from Tell Hisban likely belong to the Bedouin tribes who seasonally inhabited and farmed the region. Archaeological excavations in 1998, 2001, and 2004 unearthed the skeletal remains of a minimum of 52 individuals from a commingled context from the ruins of a 14th century structure. Of these commingled remains, 32 individuals were under the age of 15 years, with a high percentage (62.5% of the subadults) under the age of 3 years. Analysis performed by Perry and Edwards (2020, 2021) revealed skeletal indicators of metabolic disease, including vitamin C deficiency (scurvy) and vitamin D deficiency (rickets). Skeletal assemblages from nearby sites in Jordan and Israel (e.g., Eakins, 1993; Sadvari, 2009; Rose and Khwaleh, 2012) do not exhibit the same degree of metabolic disease, making investigation into Tell Hisban a unique opportunity to examine the occurrence of scurvy in this region during the late Ottoman Era.

Scurvy and other metabolic diseases develop due to many extrinsic factors (e.g., nutrition and cultural behaviors) and intrinsic factors (e.g., hormone levels and conditions limiting absorption) that directly affect the mortality of a given population. Furthermore, extrinsic mortality is an age-specific risk of death determined by factors and stressors that are not under the control of the individual (e.g., violence, famine, disease) (Machluf and Bjorklund, 2015; Quinlan, 2007). This is opposed to intrinsic mortality, which is the sum of the effects of internal factors that result in aging (Koopman et al., 2015). For this reason, metabolic diseases such as

scurvy can be an important proxy for population-wide nutritional status. An investigation into the pattern of vitamin C deficiency in subadults will lead to a better understanding of the morbidity and mortality of infants in Tell Hisban at the end of the Late Ottoman Period. Accurate data on the age and sex estimation is difficult to obtain in the case of commingled remains, even more so for subadults for whom skeletal sexual dimorphism is unreliable. Therefore, this project will utilize proteomic analysis of dental enamel to identify sexually distinct proteins that can indicate the chromosomal sex of the individual. In addition, previous analysis of the sample population found high rates of metabolic disease, including vitamin D and vitamin C deficiency, in subadults dying between 6 months and 3 years of age. Therefore, identifying the sex of the infants of Tell Hisban could reveal a differential risk for scurvy due to sex, which further illuminates the biological and social determinants of infant morbidity and mortality at Hisban.

Historical and Archaeological Background

The Ottoman Period cemetery of Tell Hisban lies approximately 20 miles east of the Jordan River, between the modern cities of Amman and Madaba. The archaeological site sits atop a hill (tell) on the northern edge of the modern-day Hisban, with expansive views of the surrounding plains and village below. The site was a major administrative center during the Mamluk Period (1253-1516 CE), and buildings from this period can still be seen on the top of the tell. Within the ruins of a 14th century Mamluk Period governor's residence resides a 5.8 m x 1.5 m room containing commingled (intermingled) human remains (Walker, 2001; Perry and Edwards, 2020, 2021). Various grave goods thought to be personal effects (e.g., pendants, worked and seed beads, rings, bracelets, shell necklaces, a hairpin, and coins) were recovered alongside the excavation of the human remains. Of the multiple coins recovered, a single copper Ottoman fils had a legible date, providing a *terminus post quem* of 1876 CE (Walker, 2001).

However, due to the commingled nature of the remains, personal effects could not be positively associated with any specific burials.

A minimum of 52 individuals (32 subadults and 20 adults) represented by well-preserved skeletal remains were recovered from the context (Perry and Edwards, 2020, 2021). Thirty-two subadults comprise 61.5% of the assemblage, and of those, 62.5% died under the age of 3 years. It is suspected that the commingling of the remains resulted from repeated use of the room for burial, where earlier burials were moved aside to accommodate new ones. Although the graves were not oriented toward the direction of Mecca, the burials at Tell Hisban were thought to be initially prepared using Muslim practices (Walker, 2001). Due to their seasonal movements, this cemetery likely contains only a fraction of the annual deaths. Ethnographic details of Bedouin burial practices describe cemetery locations near permanent water sources, allowing for ritualistic washing of the dead and refreshment for the living (Hobbs, 1989). The burial place is commemorated through repeated visitation and places a general acknowledgment of ownership of the land (Hobbs, 1989).

Hisban and its extensive underground cave and cistern system have been intermittently inhabited since the Late Bronze Age (Carroll, 2008; Walker, 2001). While there is evidence of Roman (106 BCE – 324 CE), Byzantine (117-1516 CE), Umayyad (640 -705 CE), and Abbasid (750 – 969) structures atop Tell Hisban, and much of the visible architecture dates to the Mamluk Period (1253-1516 CE). At the time of the Mamluk Period, named for a slave-soldier dynasty that ruled between Egypt and Syria, Hisban served as the capital for the southernmost district of Damascus and provided a rest stop on the postal route from Damascus to al-Kerak as well as for travelers and their animals bound for Egypt (Al-Bakhit, 1979; Russell, 1989; Walker, 2001). As the administrative importance of Hisban increased, so did the population size and fortifications.

However, near-constant warfare and ransacking punctuated the later part of the Mamluk Era (Al-Bakhit, 1979; Al-Maqrizi, 1837). Whether for this reason or another, by the early Ottoman Period of the 1500s, the area was primarily inhabited by nomadic pastoralist tribes (Russell, 1989). The Ottoman Empire (1516-1918) viewed itself as a leader of the Islamic world and, therefore, was responsible for bringing order and "civilization" to its subjects. As a result, they attempted to establish control over its frontiers and initiate regulations on the semi-nomadic tribes of Jordan (Carroll, 2008). The development of better transportation and trade expansion enabled further administrative control of the region. Hisban remained under Ottoman control throughout WWI until the Modern Arab Period began in 1918 (Abujaber, 1989).

Throughout the Ottoman Period, the site of Tell Hisban served as a seasonal encampment for agropastoral nomads, most notably the ‘Ajarma and the ‘Adwān (Russell, 1989). While we cannot be certain of the tribal identity of the individuals interred at the cemetery at Tell Hisban, the lack of permanent settlement at the site suggests they were likely semi-nomadic agropastoralists of Bedouin descent. The only distinguishing features of Hisban noted by early European travelers of the 17th and 18th centuries were the Bedouin tent encampments and the ruins of a “castle” (Chesney, 1868). During that time, various degrees of mobility and low-intensity cultivation existed among the pastoralist groups of Jordan. These nomadic tribes seasonally camped in Hisban to farm the land, grazed their livestock, and utilized the nearby springs. For example, the semi-nomadic 'Adwān tribe grazed their flocks of sheep and goats in the hills surrounding Hisban while camping near its spring in the warm months and farmed the Jordan Valley in the winter months. (Geraty and LaBianca, 1985; Russell, 1989; Walker, 2001). Nearby, the allied ‘Ajarma tribe reared sheep and cattle between Madaba and Amman (Geraty and LaBianca, 1985; Russell, 1989; Walker, 2001). Additionally, the expansive nomadic Beni

Sakhr tribe raised horses and camels on the plains to the east of Amman and the Arabian Desert and are often depicted as raiders and frequent adversaries of the ʻAdwān (Geraty and LaBianca, 1985; Russell, 1989; Walker, 2001).

The ʻAdwān, ʻAjarma, and Beni Sakhr tribes belong to a larger population of Arabic-speaking people known as Bedouin, who subsisted on producing grazing animals such as sheep, goats, and camels. Traditionally, the Bedouin lived in woven tents and migrated seasonally throughout Arabia and North Africa following the availability of grazing land and water (Lancaster, 1997). For many, animal husbandry and small-scale agriculture were relied upon for daily food consumption. Herding animals were kept for every household to supply meat and dairy products such as milk, butter, and yogurt (Abujaber, 1989). As agropastoralists, most of their agriculture employed "dry farming," meaning that flood irrigation was not feasible, and growers relied on seasonal rainfall to water their crops. The Bedouin relied on the produce of farming communities as seasonal cultivation plots organized by tribal affiliations (Carroll, 2008). During the 19th century, Ottoman officials sought to register the land around Hisban as part of the *Tanzimat* reforms. Although the land was also historically inhabited by the 'Adwān, it was officially assigned to the ʻAjarma (Russell, 1989), and like much of Jordan, the sheep and goat pastureland utilized by the tribe was converted to farmland (Rogan, 1999). Overtime, the landowners fell into debt, and the farmland was sold to a merchant, Nabulsi, from al-Salt. Although the ʻAjarma no longer held legal claims to the land, the tribes continued to farm the land on behalf of the new owner (Carroll et al., 2006; Russell, 1989).

By the end of the 19th century, effort was directed towards the large-scale production of cereals. Wheat accounted for nearly two-thirds of cereal production due to its high demand and market value, followed by barley, which is used for animal feed and millet. Millet was often used

instead of wheat for bread during times of scarcity. Additionally, any surplus of daily essentials of vetches, lentils, chickpeas, sesame, fenugreek seeds, and farik were sold to other clans and friendly tribes (Abujaber, 1989). Thus, the 19th century saw a shift throughout Jordan in subsistence patterns from foraging, animal husbandry, and small-scale farming to cereal-based agriculture as global grain demands increased (Abujaber, 1989). This agricultural intensification of Jordan may have impacted local communities' morbidity and mortality patterns. The increased dependence on cereals (e.g., wheat, barley, millet) and decreased consumption of dietary diverse foods that were sufficient sources of vitamin C may have led to metabolic deficiencies among the Bedouin, especially women and children. This study focuses on sex (and gender) and sex-based differences of scurvy as a potential factor contributing to mortality rates among Hisban subadults under 3 years old to better understand the differential impacts of this substance shift.

Sex-Based Differences in Infant Mortality Risk

Accurate sex estimation is crucial because sex-based differences often emerge as significant factors in heterogeneity in morbidity and mortality across the life course. The average sex ratio at birth is 107 males to 100 females (CIA Fact Book, 2021), and the overall male:female ratio in the global population ranges between 1.01 to 1.06 males for every female (Alkema et al., 2014; CIA Fact Book, 2021; Jacobsen et al., 1999). The difference in the male:female ratio between births and the general population reflects the greater chance of males dying under five years of age than females, even when controlling for access to medical resources (United Nations, 2011). Additionally, females have less frailty regarding many perinatal conditions and infectious diseases in early infancy and have a lower risk of being born with congenital anomalies (United Nations, 2011). Infant males living in extreme conditions such as famines and epidemics fare even worse in terms of mortality (Zarulli et al., 2018). This

outcome has been referred to as “innate frailty” (Bhatia, 1984) or “higher biological risk” (Chen et al., 1981). However, in many cultural contexts, girls receive fewer resources and experience differential treatment when compared to boys, resulting in greater frailty and higher mortality for females (United Nations, 2011).

Demographic data from the WHO identifies that this tendency for greater morbidity of males than females across the life courses exists in numerous countries around the globe (Table 1) (World Health Organization, 2012). Males are more susceptible to a wide variety of diseases caused by viruses, bacteria, fungi, and parasites (Klein, 2000; Wells, 2000; Owens, 2002; Pennell et al., 2012) and have higher incidences of tuberculosis, meningitis, respiratory infections, and hepatitis than females (Rustgi, 2007; Falagas et al., 2007; Oren et al., 2011; Case et al., 2012; Mobarak, 2012). Furthermore, males have a higher risk of morbidity and death from some degenerative diseases such as cardiovascular disease, diabetes, cancers, and cirrhosis of the liver (Lopez, 1984; Pilote et al., 2007; Kalra et al., 2008; Silbiger and Neugarten, 2008), in addition to external causes of death, such as violence, accidents, and suicide (World Health Organization, 2008). Females have less frailty regarding many perinatal conditions and infectious diseases in early infancy and have a lower risk of being born with congenital anomalies (United Nations 2012), and female infants are less likely to die from birth trauma, intrauterine hypoxia, and birth asphyxia (Waldron, 1998). That is not to say that males suffer disproportionally from all diseases. Females are more severely affected by toxoplasmosis and malaria (Roberts et al., 2001; Vlassoff and Bonilla, 1994), and mortality rates from measles and influenza are higher for females (Klein et al., 2011). Certain degenerative diseases, including chronic obstructive pulmonary disease, arthritis, and autoimmune disorders, affect females more frequently (Case and Paxton, 2005; Fairweather et al., 2008).

Table 1. *Percentage of regions worldwide (n=193), where male age-specific mortality was higher than female age-specific mortality in 2009. (World Health Organization, 2012)*

Age category (Years)	Number of regions with higher male mortality by age category (out of 193)	Percentage of regions with higher male mortality in each age category (%)
<1	180	93.26
1-4	128	66.32
5-9	146	75.65
10-14	153	79.27
15-19	167	86.53
20-24	173	89.64
25-29	163	84.46
30-34	165	85.49
35-39	177	91.71
40-44	188	97.41
45-49	190	98.45
50-54	189	97.93
55-59	189	97.93
60-64	187	96.89
65-69	186	96.37
70-74	189	97.93
75-79	182	94.30
80-84	184	95.34
85-89	179	92.75
90-94	169	87.56
95-99	165	85.46

There are multiple potential causes for this natural biological condition of frailty among males. One significant factor in sex-based health and disease disparities is the effect of sex hormones on the immune response (Klein and Roberts, 2010). The appearance of sex hormones is regulated by the hypothalamic-pituitary-gonadal axis (HPG axis), comprised of the hormone-producing hypothalamus, pituitary, and gonadal glands. The activation of the HPG axis occurs in

three phases: during the second trimester of fetal development, after birth between 1 and 6 months (mini-puberty), and at puberty (Lanciotti et al., 2018) Generally, the female sex hormone estrogen enhances immune competence, while the male sex hormone androgen reduces it, and therefore, females tend to mount a stronger immune response (Ahmed et al., 2010). Estrogen enhances immune function by upregulating pro-inflammatory cytokines, increasing the activity of interferon-producing dendritic cells, enhancing B cell antibody production, and CD_4+ T cell differentiation (Ahmed et al., 2010). Androgens suppress inflammatory responses by limiting the size of the thymus, limiting macrophage activity, and reducing the function of CD_4+ T cells (Ahmed et al., 2010). This difference in hormonal composition may explain why we see a higher mortality rate among Hisban infant males. As discussed above, males tend to be more susceptible to infections caused by viruses, bacteria, fungi, and parasites (Klein, 2000; Wells, 2000; Owens, 2002; Pennell et al., 2012), likely because of their suppressed inflammatory responses. While males and females may be equally exposed to infectious agents, more females can respond with an effective immune response than their male counterparts, decreasing their likelihood of severe illness and death. Based on these data, we expect the infants at Hisban to follow this general pattern of slightly higher male mortality than females, and any variation from this would indicate the presence of extrinsic impacts of sex on mortality risk at the site.

Scurvy as a Factor in Increased Mortality Risk

Metabolic diseases such as scurvy are just one of many factors in morbidity and mortality, exacerbated by differential access to dietary resources at the individual and population levels. Ascorbic acid, commonly known as vitamin C (VC), is a water-soluble nutrient required to synthesize hydroxyproline, an amino acid that creates and maintains collagen (Magiorkinis et al., 2011; Popovich et al., 2009). Unlike most animals, the human body cannot synthesize

ascorbic acid; therefore, it must be obtained through dietary sources (Anthony, 2011; Olmedo et al., 2006). Adults require approximately 45 mg-60 mg/day, and children require at least 15 mg/day to meet their daily metabolic requirements and prevent vitamin deficiency (Table 2). An individual's somatic stores of ascorbic acid range from 1500-2500 mg. Should stores drop below 350 mg, they will develop clinical vitamin C deficiency or scurvy (Olmedo et al., 2006; Popovich et al., 2009). Scurvy can develop in several ways: reduced intake of VC, increased physical requirements for VC, malabsorption of the vitamin, and genetic predisposition to lower VC levels. (Halcrow et al., 2014). Regardless of the cause, deficiency can occur in as little as 30-60 days (Anthony, 2011; Fain, 2005; Popovich et al., 2009).

Table 2. *Daily Vitamin C Requirements (Popovich et al., 2009)*

AGE GROUP	**RECOMMENDED DAILY VITAMIN C REQUIREMENT**
INFANTS	40-50 mg/day
CHILDREN (1-3 YEARS)	15 mg/day
PREGNANT WOMEN	85 mg/day
BREASTFEEDING WOMEN	120 mg/day
ADULTS	45-60 mg/day

Scurvy is closely linked to the levels of ascorbic acid in foods consumed and how these levels are affected by food preparation (Brickley et al., 2020). Foods with the highest concentration of VC are fresh fruits, vegetables, wild plants, and human breast milk (Follis et al., 1950). Conversely, meats, fish, grains, eggs, and dairy products contain minimal VC (Table 3) (Brickley et al., 2020; Fain, 2005; Popovich et al., 2009). The level of VC is the highest in raw foods. However, cooking makes nutrients more digestible (Abbey and McDonald, 1976). Because VC is sensitive to heat, ultraviolet rays, and exposure to oxygen, cooking and preservation methods can reduce food's ascorbic content by 20-40% (Fain, 2005; Popovich et al., 2009). Diet is socio-culturally determined. Therefore, the susceptibility to scurvy is highly

***Table 3**. The vitamin C content of a range of foods (Brickley et al., 2020)*

Food source	Raw vitamin C content mg/100g	Stored vitamin C content mg/100g	Cooked vitamin C content mg/100g
Angelica (Angelica sylvestris)	14-100	nd	0
Apple, crab	8	nd	nd
Apple, dessert	6	3.9	0.2 (boiled)
Avocado	10	nd	nd
Banana	8.7	7 (dried)	6.1 (boiled) 6.4 (fried)
Bean/grain sprouts	13.2-38.7	nd	6.1-35.6
Bilberry/whortleberry (Vaccinium myrtillus)	3	nd	nd
Birch leaves (Betula sp.)	460	nd	nd
Black crowberry (Empetrum nigrum)	30	nd	nd
Blackberry (Rubus sp.)	21	nd	2.8 (canned)
Breadfruit	29	nd	6.1 (boiled)
Cereals (un-sprouted)	0	0	0
Cloudberry (Rubus chamaemorus)	158	nd	nd
Corn, sweet, yellow (maize)	6.8	0 (cornmeal, cornflower, dried tortillas)	5.5 (boiled) 2.6 (canned)
Cranberries (Vaccinium sp.)	14	0.2 (dried)	1 (canned)
Eggs	0	0	0
Fig	2	1.2 (dried)	1 (canned) 4.4 (stewed)
Fish flesh (cod, char)	0-1 4 (liver)	3.5 (dried)	0 (boiled liver oil) 4 (liver smoked)
Goosegrass (Galium apanne)	78	nd	nd
Honey	0.5	na	0
Liver, beef	1.3	na	0.7 (fried)
Liver, lamb	0-4	na	4 (braised)
Liver, seal	18-35	na	14-30 (boiled)
Liver, chicken	17.9	na	27.9
Meat	0-2.3	0 (cured)	0-0.5 (boiled)
Millett (raw)	0	nd	nd
Milk, cow (mature)	2 (raw)	1.9 (evaporated)	0 (pasteurized)
Milk, goat/sheep	1.3-3	0 (cheese)	nd
Milk, guinea pig	12	nd	nd
milk, camel (mature)	3-6	nd	nd
Milk, human (colostrum)	7	nd	nd
Milk, human (transitional, 10th day postpartum	6	nd	nd
Milk, human (mature)	4-5	nd	nd
Mustard	70 (greens)	nd	nd

Nettle juice	160	nd	nd
Nuts, hazel	6.3	6.3 (dried)	2 (blanched) 3.8 (blanched)
Onions	4.8-27 (depending on variety and plant part)	4.5 (2 months) 23.4 (powdered)	5.2 (boiled) 1.8 (sauteed)
Orange	53.2	nd	nd
Parsley	133	125 (dried)	nd
Parsnip	17	nd	13 (boiled)
Peppers, sweet green	80.4	nd	74.4 (boiled) 177 (sauteed) 46.5 (canned)
Peppers, hot green	242.5	2-6	34.2 (canned)
Potatoes	5.7-19.7 (depending on variety)	8 (8-9 months) 3.8 (flour)	7.4 (peeled/boiled) 5.2 (boiled in skin)
Raspberries	26.2	nd	8.7 (canned)
Rose hips	426-7000	nd	nd
Rowanberries (Sorbus sp.)	80	nd	nd
Sauerkraut	14.7	10-15 (month)	nd
Scurvy grass (Cochleare officinalis)	200	nd	nd
Seal flesh	0.27-3	0 (dried)	0.5-252 0 (oil)
Spruce pine needles	65-200	<0.5 (fermented infusion)	Nd
Sweet potatoes	2.4-30	nd	12.8 (boiled)
Tomatoes, red	13.7	39 (sun-dried)	18.2 (stewed) 12.6 (canned)
Turnip, greens	60	nd	27.4 (boiled) 15.5 (canned)
Turnip, root	21	nd	11.6 (boiled)
Watercress (Nasturtium officinale)	43	nd	nd

*na, not applicable; nd, no data available.

variable within and between cultures (Brickley et al., 2020). However, any given diet is rarely completely devoid of VC (Follis et al., 1950).

Although scurvy is classified as a rare condition today, sub-clinical (lower than usual) cases are on the rise, particularly for lower-income groups and low to middle-income countries (Rowe and Carr, 2020). Those at the greatest risk of scurvy are individuals with restricted

ascorbic acid intake, e.g., low socioeconomic status, picky eaters, those suffering from neglect, restrictive diets, and infants breastfed by mothers with insufficient VC levels (Figure 1) (Popovich et al., 2009). Additionally, individuals with medical conditions that limit the absorption of VC, e.g., ESRD (end-stage renal disease), Crohn's disease, colitis, and Celiac disease, are at an elevated risk of developing VC deficiency (Fain, 2005; Popovich et al., 2009). Those who have undergone significant traumas and illnesses may have an increased requirement of VC (Berger, 2009), as do newborns born with insufficient levels of VC (Popovich et al., 2009), infection by pathogens such as *Helicobacter pylori* (Lahner et al., 2012) or *Plasmodium falciparum* (Raza et al., 2010), and chronic conditions such as anemia (Clark et al., 1992), and diabetes (Jacob, 1999). Lastly, individuals born with the haptoglobin Hp2-2 polymorphism are genetically predisposed to have impeded levels of ascorbic acid and require increased dietary intake to maintain a healthy level of VC (Delanghe et al., 2007; Langlois et al., 1997). It is also important to note that natural and ecological effects such as natural disasters, crop diseases, drought, earthquakes, major storms, and pests can severely impact food systems, thus affecting nutritional intake and leading to various metabolic conditions, including scurvy (Brickley et al., 2020).

A lack of ascorbic acid directly impacts the creation and maintenance of collagen, the primary structural protein of bone, connective tissues, and blood vessel walls. As a result, early symptoms of scurvy are tiredness, lethargy, weight loss, irritability, depression, and musculoskeletal pain and weakness (Cutforth, 1958; Akikusa et al., 2003). Eventually, the weakened blood vessel walls rupture, resulting in hemorrhaging, bruising, and the development of petechiae (small red spots) on the face, trunk, and limbs (Fain, 2005; Popovich et al., 2009). In addition, bone growth and mineralization will be reduced, particularly in those actively

Figure 1. *Factors affecting ascorbic acid levels in the human body (Halcrow et al., 2014)*

Reduced intake (Popovich et al., 2009)
- Low socioeconomic status
- Picky eaters
- Food fads and restrictive diets
- Poor dentition making chewing difficult
- Eating disorders
- Mental disabilities
- Neglect of infants and children
- Cognitive disorders
- Religious practices
- Infants breastfed by mothers with inadequate vitamin C levels

Increased requirement
- Major trauma (Berger, 2009; Long et al., 2003)
- Burns (Berger, 2009; Naidu, 2003)
- Critical illness (Berger, 2009; Luo et al., 2008; Schorah et al., 1996)
- Inflammatory conditions (Holley et al., 2011)
- Anemia (Clark et al., 1992; Oeffinger, 1993)
- Diabetes (Jacob, 1999, Sauberlich, 1994)
- Infections, e.g., Heliobacter pylori (Lahner et al., 2012; Long et al., 2003; Naidu, 2003), Plasmodium falciparum (Egwunyenga et al., 2004; Isamah and Asagba, 2003; Raza et al., 2010)

Malabsorption
- Newborns who have not absorbed enough vitamin C through fetal tissues (Popovich et al., 2009)
- Cancer patients with nausea and diarrhea (Popovich et al., 2009)
- Gastrointestinal disease, e.g., Whipple's disease, Celiac disease, Crohn's disease (Fain, 2005; Popovich et al., 2009).

Genetic predisposition
- Individuals with the haptoglobin Hp2-2 polymorphism have lower levels of ascorbic acid (Delanghe et al., 2007; Langlois and Delanghe, 1996; Langlois et al., 1997)

undergoing rapid bone growth and development (Brickley et al., 2020). One of the most recognizable signs of scurvy is red, inflamed, and bleeding gums caused by the degeneration of the gingiva (Bartley et al., 1953). Tooth loss in prolonged cases of scurvy is most common among individuals with a history of gingivitis (Pangan and Robinson, 2001), especially with single-rooted teeth, e.g., incisors and canines (Danzeiser, Wols, and Baker, 2004).

The severity of the signs and symptoms of scurvy depends on the individual's age and the duration of the deficiency (Léger, 2008). Hemarthrosis, joint pain, and swelling caused by blood

pooling in soft tissue frequently appear in adults and may lead to limping or inability to walk (Ratanchu-Ek et al., 2003). Infants characteristically adopt a “frog-like position” where they lie on their back with limbs semi-flexed and externally rotated to lessen the pressure from painful joints (McLaren, 1992). They may also develop pseudo-paralysis due to limb pain and rib deformities (Barlow, 1894).

The diagnosis of scurvy is primarily based on clinical presentation, dietary history, ascorbic acid serum levels, and radiographic findings on long bones (Popovich et al., 2009). Because VC deficiency often hinders the ability to metabolize folate and iron (Pangan and Robinson, 2001), scurvy often co-occurs with folate deficiency and anemia (Brickley et al., 2020). The range of initial symptoms of scurvy means it is frequently misdiagnosed in its early stages (Popovich et al., 2009). Once diagnosed, treatment aims to introduce the maximum dose of VC to the body to quickly increase the somatic stores (McKenna and Dawson, 1993; Glouberman, 2009; Larralde et al., 2007). A dramatic symptom improvement is seen within 24 hours after reintroducing VC to the diet, with pain subsiding in 2-4 days and gingival lesions recovering in 2-3 weeks. After initial treatment, five servings of fruit and vegetables daily are recommended to maintain healthy VC levels (Popovich et al., 2009).

Although scurvy can be quickly resolved with treatment, there are several ways in which it can be fatal. First, because VC maintains immune function by neutralizing or destroying pathogens (Jacob and Sotoudeh, 2002), scurvy patients are at a higher risk of infection (Bartley et al., 1953; Shaik-Dasthagirisab et al., 2013). Additionally, due to the hemorrhagic nature of the condition, scurvy can be fatal due to blood loss (Griffith et al., 1898; Follis et al., 1950; Milgram, 1990). Lastly, undiagnosed scurvy consequences include seizures, hemopericardium,

cardiomegaly, congestive heart failure due to anemia, and cerebral, conjunctival, and intraocular hemorrhage (Popovich et al., 2009).

Since ascorbic acid directly impacts the creation and maintenance of collagen – an essential bone component – the scurvy effects are often visible in the skeleton. Scurvy reduces osteoblastic activity, arresting the deposition of the osteoid framework for bones during growth and development in children and bone remodeling in adults (Ortner and Erickson, 1997; Ortner et al., 2001; Aghajanian et al., 2015). The skeletal symptoms of scurvy appear more dramatically in children because impaired bone formation results in low mineralization of newly deposited osteoid in growth areas (Brickley et al., 2020). This deposition can manifest in metaphyseal fractures visible in X-rays (Milgram, 1990).

Significant hemorrhaging occurs due to blood capillary fragility at sites where blood vessels are close to the skin or exposed to frequent muscle stress (Jaffe, 1972). In addition, abnormal cortical bone porosity generally develops in these locations due to increased vascularization and an overabundance of white blood cells and macrophages at the hemorrhage site (Stark, 2014).

Abnormal new bone formation and abnormal porosity due to scorbutic hemorrhaging cause the formation of "mixed lesions" to develop in specific regions of the skull and axial skeleton (Brickley and Ives, 2008; Ortner et al., 2001; Ortner and Erickson, 1997). The most typical sites for hemorrhagic lesions cranially are the greater wings of the sphenoid, maxilla, mandible, zygomatic bones, the cranial vault (Ortner and Erickson, 1997), orbit walls (McNab, 2014), and scapula, long bones, and ilia post-cranially (Figure 2) (Brown and Ortner, 2011). Vascular impressions may also appear on ectocranial surfaces of the occipital, frontal, and parietal bones (Brown and Ortner, 2011) due to rupturing of ectocranial vasculature and new

Figure 2. *Typical sites for hemorrhagic lesions in scurvy (Brickley et al., 2020)*

Lesion Location	Comments
Cranial/mandibular	
Sphenoid	The external surface, the greater wing, is strongly diagnostic. Generally bilateral and symmetric. Lesions also found on lesser wing and foramen rotundum
Orbital Walls	The superior wall/roof (frontal) is most often affected. Alterations also seen on the lateral wall (zygomatic). Lesions may show left/right asymmetry
The posterior surface of the maxilla	
Zygomatic bone, medial surface/postero-medial surface of the maxillary zygomatic process	
Coronoid process of mandible, medial surface	
Inferior surface, palatine processes of maxillae	
Maxillary and/or mandibular alveolar process	Care is needed to distinguish porosity linked to dental eruption in sub-adults and lesions associated with dental pathology in both sub-adults and adults.
Infraorbital foramen of maxilla	
Cranial vault	The ectocranial surface is more often affected than endocranial
Post-cranial	
Scapulae, supra- and infra-spinous fossae	Lesions of the supra-spinous process observed more constantly
Long bones	Especially metaphysis in sub-adults, but care is needed as some porosity is part of normal growth PNBF noted at linea aspera of the femur
Ilia	Care is needed as some porosity is part of normal growth

vessel formation (Lewis, 2004). The periosteal lesions are retained in the skeleton throughout active scurvy and quickly resolve with the reintroduction of ascorbic acid. Once the lesions are remodeled, little evidence of past illness is discernable (Parfitt, 2002, 2004; Ortner, 2003).

When examining skeletal remains, there are three methods to determine the possible presence of scurvy: macroscopic, microscopic, and radiographic. Macroscopic lesions specify pathognomic abnormal porosity and can be visually discerned without specialized equipment (Brickley et al., 2020). Microscopic and radiographic methods are best used as supporting

evidence and provide additional information on lesions that arise from impaired collagen growth (Brickley et al., 2020). Observable radiographic features for infantile scurvy, e.g., Pelkins' spur, Wimburger's line, Frankle's line, and osteopenia (Popovich et al., 2009) may suggest scurvy, but their absence does not prove that scurvy was not present (Joffe, 1961; Akikusa et al., 2003). While no characteristic histologic features are associated with scurvy alone (Follis et al., 1950), key features may be observed. In both adults and subadults, metaphyseal fractures and osteopenia within the cortical bone may be found. Microfractures in the trabecular bone and broken or irregularly ossified cartilage columns in subadults may also be visible (Brickley et al., 2020).

Maxillae and mandibulae are ideal for this project for three specific reasons: (1) mixed lesions present on the mandible and maxilla are diagnostic (although not pathognomic) indicators of scurvy (Snoddy et al. 2018), (2) the associated dentition provides reliable age range estimation for subadults (AlQahtani et al. 2010), and (3) proteomic analysis of the dental enamel in the associated teeth can provide sex estimation for each of the individuals. The skeletal assemblage was evaluated for a suite of macroscopic bony lesions (e.g., abnormal new bone formation, abnormal porosity, and mixed lesions) that can be linked to the hemorrhagic effects of scurvy – bleeding because of minor muscle actions such as mastication, speech, or suckling (Ortner and Erickson, 1997; Ortner et al., 1999, 2001, Snoddy et al., .2018). The maxillae and mandibulae of 16 subadults with embedded dentition and scorbutic lesions and 6 individuals that did not display any skeletal lesions in these elements were selected as test samples for this study (Table 6).

The presence of abnormal cortical porosity and subperiosteal new bone formation at the anterior surface, infraorbital foramina, posterior surface, and palatal surface of the maxilla is diagnostic of active scurvy, as is abnormal cortical porosity and subperiosteal new bone

formation on the medial surface, coronoid process, and the mylohyoid line of the mandible (Snoddy et al., 2018). While these diagnostic features have been observed in numerous archeological individuals who exhibit other diagnostic characteristics of scurvy, caution is needed when evaluating subadults due to the typical porosity associated with dental eruption (Ortner and Erickson, 1997; Ortner et al., 1999; Brickley and Ives, 2006; Geber and Murphy, 2012; Halcrow et al., 2014). Another concern is confirmation bias, a tendency to interpret new data to support pre-existing expectations, especially if an analyst is looking for disease (Snoddy et al., 2017). Therefore, Snoddy et al. (2018) recommend that the diagnostic value is based on the strength of the association between macroscopic osseous lesions, clinically observed features, and anatomical relationships.

The proper application of diagnostic criteria for identifying active scurvy is meaningful beyond the individual's health. The presence of vitamin C deficiency, along with other metabolic diseases, in a population provides important contextual information on past people. For example, Geber and Murphy (2012) examined 970 individuals from 19th century burials in Ireland to explore the etiology and impact of scurvy at the Kilkenny City workhouse. The individuals would have been inmates during the Great Irish Famine (1845-1852), a time of widespread starvation and disease. The potato blight significantly affected access and availability to the primary food source for many Irish. The potato is typically very high in VC (30 mg per 100g), with levels comparable to citrus fruit (Crawford, 1988). However, prolonged storage and cooking reduce VC levels, contributing to VC deficiency. The skeletal remains were analyzed microscopically using standard osteological methods (Buikstra and Ubelaker, 1994), and lesions were examined for diagnostic criteria and clinical features consistent with scurvy (Brown and Ortner, 2011; Ortner et al., 1999). The authors found a definitive diagnosis of scurvy in adults

and juveniles based on porotic lesions on the greater wings of the sphenoid and porous bone formation on the maxillae and mandible. Following analysis, 52% of the individuals examined were likely suffering from scurvy (16% definite, 11% probable, and 21% possible).

Additionally, adult males at the Kilkenny workhouse site were 1.7 times more frequently diagnosed with scurvy than adult females, likely due to sex-based differences in biological metabolism and inadequacies in their diet (Geber and Murphy 2012). The authors concluded that the exceptionally high prevalence of scurvy was likely due to famine and would have significantly impacted mortality in the population. Geber and Murphy (2012) and numerous others have observed that scurvy may develop due to many extrinsic and intrinsic factors (Halcrow et al., 2014; Brickley et al., 2016; Klaus, 2017). Further investigation into the demographics of scurvy-prone populations is essential to provide a more holistic view of the inhabitants.

The Developmental Origins of Health and Disease

Another factor impacting morbidity and mortality across the life course is the residual impact of stress while *in utero* and through early infancy. The DOHaD (Developmental Origins of Health and Disease) hypothesis highlights the link between stress during earlier periods of growth on disease and health outcomes later in life. Early life is considered a highly susceptible period, and it has been suggested that poor care, nutrition, and environmental circumstances adversely affect developmental trajectories (Barker, 2012). The impact of several social and environmental factors, including parental diet (Ng et al., 2010), infant care (Weaver et al., 2004), and social status (Borghol et al., 2012), have been explored. The effects of malnutrition on different gestational ages have also been linked to metabolic problems later in life (Barker et al., 1993). Furthermore, it has been suggested that immune function and disease susceptibility are

programmed early in life and affected by intrauterine development suppression (Gowland, 2015; McDade, 2003, 2012).

Investigating the developmental origins of health and disease in adults can also rely on tissues that form during childhood and are retained unchanged into adulthood, such as in the teeth for evidence of delayed or inhibited childhood growth. For example, adult individuals with skeletal markers of developmental stress that occurred early in life, such as linear enamel hypoplasias in the teeth and shorter-than-expected stature, had a higher risk of excess death during famine than under normal conditions (DeWitte and Yaussy, 2020).

Because the lives of mothers and their infants are so closely intertwined, it is reasonable to scrutinize the effect of this "mother-infant nexus" (Gowland and Halcrow, 2020) on health and disease outcomes. Additionally, analysis of infant skeletal remains may provide significant information about past health and can further the investigation of the "mother-infant nexus." Since the pregnant body prioritizes the developing fetus in times of nutritional stress, diverting resources away from the mother and towards the infant's needs, nutritional deficiencies and growth disruption in the infant may reflect the health status of their mothers (Chávez et al., 2000).

One known factor of childhood growth and health disruption is socio-economic, as social status mediates access to resources (i.e., nutritious food and better living and working conditions), therefore improving physiological and psychological well-being (Hodson and Gowland, 2020; Floud et al., 1990; Mays et al., 2009). The first 1,000 days of life are understood to be a period of heightened susceptibility to adversity and developmental plasticity (Barker et al., 2002), suggesting that fetal, perinatal, and infantile remains reflect the impacts of social inequalities most acutely (Hodson and Gowland, 2020).

A study conducted by Hodson and Gowland (2020) sought to examine the relationship between fetal/infant and maternal health in relation to social inequality in post-medieval (16th-19th century) London. The skeletal remains of 136 infants and fetuses from four post-medieval cemeteries (Broadgate, St. Bride's Lower, Cross Bones, and St. Thomas') representing the cheapest burial places and the poorest individuals in London were analyzed for evidence of growth disruption and pathological lesions. The authors found that remains of all ages had pathological skeletal changes (new bone formation (NBF), lytic lesions, metaphyseal expansion, and morphological changes), indicating that the pre-and postnatal environment was perilous and suggested that the maternal health was very poor and that the ability to care for offspring adequately continued to be severely compromised throughout infancy, ultimately leading to the death of the infant (Hodson and Gowland, 2020). Because infant health outcomes rely on the maternal ability to supply nutrients and antibodies through the placenta in utero and later via breast milk, infants are closely tied to maternal health. Analysis of fetal, perinatal, and infantile growth and health status provides an important approximation of maternal and community health (Baxter, 2005; Lewis, 2007).

Sex Estimation in Subadult Skeletal Remains

The difficulties in identifying the sex of skeletonized individuals before they have reached puberty hinder assessing what role that sex (and gender) may play in morbidity and mortality. Sex-based differences can easily be identified in adults who display distinguishing levels of sexual dimorphism in their skeleton. Sexual dimorphism is the difference between the body size, shape and composition, rate of development, and behavior of males and females of a species. It results from intrinsic factors like hormone levels and extrinsic factors, including nutrition and cultural behaviors (Stinson, 2012). While the primary sexual characteristics of the

genitalia begin to differentiate early *in utero*, secondary sex characteristics (i.e., body size and pelvic morphology) start to develop at puberty (Bogin, 1999).

The osteological approach to adult sex estimation uses morphological assessments and metric analysis of sexually dimorphic skeletal features to estimate sex (Buikstra and Ubelaker, 1994; Stevenson, 2009; Walker, 2005, 2008). The pelvis is visually assessed to distinguish male skeletal remains from that of females. Unlike the male pelvis, which follows a preadolescent pattern, the female pelvis is adapted for childbirth and continues to grow and develop until the completion of puberty (Moore, 2013). When assessing the sex of a subadult pelvis as young as 12 or 13, if female morphological traits are present, it can be reasonably assumed that the individual is likely female. However, if the characteristics appear masculine, the subadult pelvis could be either male or female (Buikstra and Ubelaker, 1994).

Like the pelvis, skulls can be visually assessed for sex estimation. Generally, male skulls are more robust, with a higher degree of rugosity observed at muscle insertion sites compared to the typically more gracile female skulls (Buikstra and Ubelaker, 1994). Several limitations should be considered when assessing skulls. First, sex-linked variations in cranial morphological features are population-specific, which must be considered, especially when working with ancient populations (Buikstra and Ubelaker, 1994). Additionally, assessment should be limited to adults aged between 20-55 years, as masculinization of the skull occurs in older (post-menopausal) females, and secondary sex characteristics may not be fully developed in younger males (Krogman and Işcan, 1986; Buikstra and Ubelaker, 1994). Craniometric analysis can also be performed to quantify sexual dimorphism. While not commonly applied in bioarchaeological contexts, forensic anthropologists often employ craniometric analysis using discriminant function analysis through programs such as FORDISC (Moore, 2013). Sex estimation from the

post-cranial skeleton can be performed using additional metric analysis of measurable sexually dimorphic traits. Excluding the pelvis, the skeleton of female adults maintains prepubescent gracility, unlike the skeletons of adult males, which have more robust features at muscle insertion sites (Stewart, 1979; Bass, 197). Metric analysis of specific features (i.e., femoral head diameter, scapular height, and size of talus and calcaneus) involves less subjectivity and lower observer error and has only recently become the standard in both forensic and bioarcheological contexts (Moore, 2013; Spradley and Jantz, 2011).

Identifying sex-based health differences in subadults is limited by the lack of sexually dimorphic osteological indicators that do not emerge until puberty. As a result, sexually dimorphic skeletal features cannot be evaluated with any certainty in subadults (Mays and Cox, 2000). The two major obstacles to researching sex estimation methods are the lack of a large, known skeletal sample of subadults and the fact that the smaller the bone, the greater the possibility for measurement error (Moore, 2013). In addition, size differences due to sexual dimorphism are unreliable in the sex estimation of subadults because of the variability of the rate at which males and females mature. Female subadults typically develop faster than males, but males will be larger on average than females of the same age group (Scheuer and Black, 2004).

The pelvis is the most sexually dimorphic bone in infancy, mirroring the later sexual dimorphism of the pelvis found in adults (Weaver, 1980). Infant males have longer ilia, ischia, and femoral necks, whereas infant females have a longer pubis and wider sciatic notch (Saunders, 2000). However, the smaller degree of sexual dimorphism for fetal remains and the substantial overlap in the shape characteristics of the sciatic notch of males and females results in inaccurate estimations using these techniques (Weaver, 1980; Holcomb and Konigsberg, 1995). The vertebrae may be the best opportunity for subadult sex estimation during the childhood

growth period (Moore, 2013). According to Gilsanz et al. (1997), the first lumbar vertebra (L1) may be used to estimate sex between ages 2 and 12 years. Their study found that the L1 correlated more with sex than body weight and was 11% smaller in females than males (Gilsanz et al., 1997). The T1 also has been suggested as a sexually diagnostic vertebra with an accuracy of 90% (Garoufi et al., 2020), compared to the 75%-85% accuracy exhibited by metric analysis of measurements on the articular surfaces and vertebral foramen or cervical vertebrae in subadults (Marino, 1995). Like many other methods, vertebral sex determinations are highly population-specific. Therefore, they should be utilized only in cases where other, more accurate predictors of sex (i.e., the skull and pelvis) are not available for analysis (Garoufi et al., 2020; Rozendaal et al., 2020). However, due to the commingled nature of the Hisban subadult remains, sex estimations based on post-cranial elements (e.g., pelvic morphology and vertebral size) cannot be reliably associated with the cranial elements used in this study.

As previously noted, sex estimation is one of several biological parameters allowing researchers to study and understand past populations' responses to the environment and disease (Agarwal and Wesp, 2017). Due to the methodological issues mentioned above, most studies must analyze adults whose sex can be estimated using typical osteological techniques to understand sex-based differences in morbidity and mortality. For instance, DeWitte (2017) determined that adult males were at a greater risk of death during the medieval Black Death in London than their less-frail female counterparts. This increased mortality risk can be exacerbated by persistent famine, such as that seen in the survivorship of individuals during Medieval Europe (Klein, 2000).

Like sex-based differences in mortality in adulthood, neonates, infants, and children also can have differential morbidity and mortality by sex. While O'Donnell et al. (2022) found no

differences between subadult males and females regarding overall mortality in a study of modern (and known sex) American juveniles, males were found to have a higher frequency of cribra orbitalia (CO) and porotic hyperostosis (PH) lesions than females. This largely stems from age-related variation in the age and duration of red-to-yellow bone marrow conversion, which is slowed in females (see also Kugel et al., 2001; Okada et al., 1989), and bone turnover rates, which is more rapid in males (see Debono et al., 2011). Therefore, the appearance and timing of bone lesion formation in childhood can vary depending on biological sex, which means that intrinsic sex-based differences in morbidity may be confounded by different patterns of lesion formation in males and females (O'Donnell et al., 2022).

While it is possible to estimate the biological sex of a skeletonized individual, researchers should not assume that biological sex will directly correlate with ascribed gender, i.e., males will participate in male roles, and females will participate in female roles established by their culture. These gender-based differences may include variations in foods consumed, social behaviors, occupations, and healing practices. Therefore, differences in these areas can impact an individual's health and overall well-being over prolonged periods (Zuckerman and Crandall, 2019).

New Methods for Estimating the Sex of Infants and Children

As discussed earlier, the health of infants and children can reflect the overall population's health. Data derived from the skeletal remains of deceased children are impacted by mortality bias when comparing them directly to adults, as these are the individuals who did not survive childhood. However, they can clarify heterogeneity in the experiences of those who did not survive to adulthood. As noted above, biological sex can influence morbidity and mortality risk in children and adults, but estimating the sex of subadults using skeletal remains is fraught with

methodological issues. The importance of evaluating sex in infants and children to illuminate differences in morbidity and mortality or mortuary treatment has led to molecular and proteomic innovations in sex estimation. The advent of DNA testing using archeological samples (Hagelberg et al., 1989; Horai et al., 1989) made it possible to identify the sex of subadult individuals reliably, this method has its drawbacks, including the overall expense, the level of skeletal and DNA preservation required for dependable results, and the possibility of ancient or modern contamination (Buonasera et al., 2020).

The proteomic analysis of dental enamel for sex estimation is a less expensive technique that utilizes a material less susceptible to diagenesis (Stewart et al., 2017). Amelogenin is a protein secreted by ameloblasts during dental formation and plays an essential role in enamel biosynthesis (Kwak et al., 2016). Two amelogenin variants, AMELX and AMELY, are coded by the genes on the X and Y chromosomes, respectively. Additionally, these proteins are enamel-specific, and thus their presence in enamel is likely biogenic rather than due to contamination (Stewart et al., 2016; Parker et al., 2019). In addition, these enamel peptides remain stable over archaeological time (Parker et al., 2019). Froment et al. (2020) confirmed that proteomic methods will successfully and efficiently estimate sex in individuals up to 5000 years old. The results of 11 Neolithic teeth of French origin and 2 present-day teeth were compared using shotgun nanoLC-MS/MS analysis. The sex of each of the 13 teeth was successfully estimated (4 females, 9 males) and revealed randomly degraded proteins. Minimal protein degradation was present in both the ancient and the present-day samples, suggesting that much of the protein fragmentation occurs from tissue proteolysis at the time of tooth death and not in the subsequent time between death and analysis (Froment et al., 2020).

Using proteomics for sex estimation can also be applied with deciduous dentition, allowing analysis of very young infants and children. Gowland et al. (2021) examined dental samples from subadult individuals ranging from 40 gestational weeks to 19 years of age to verify if amelogenin peptide material used to determine sex is present in the crowns of developing (i.e., not completely mineralized) teeth in addition to fully developed teeth. Using the minimally destructive acid etching method developed by Stewart et al. (2017), the authors examined 18th-19th-century cemetery remains, resulting in sex estimation from 39 individuals and one inconclusive result. It was concluded that sufficient peptide material from most developing crowns could effectively determine sex in subadult individuals (Gowland et al., 2021).

Dental proteomics lends itself well to sex determination in subadults, sexually ambiguous skeletal remains, and commingled collections where the lack of preservation of the skeleton prevents either osteological analysis or genomic testing. For these reasons, proteomics is considered by some to be the future of molecular sex estimation for bioarchaeological contexts (Hodgkins et al., 2021; Rebay-Salisbury et al., 2020). One example of proteomics used in bioarchaeological site analysis and interpretation is provided by Hodgkins et al. (2021) in the case of an infant hunter-gatherer burial from the Ligurian Alps. The 8,262-7,961 BCE individual was determined to be 40-50 days old at the time of death. Proteomic analysis determined the sex of the infant to be female, which was confirmed by additional DNA analysis. The presence of shell ornaments, beads, and faunal remains with the burial led the authors to suggest that the female infant had reached personhood and social group membership (Hodgkins et al., 2021).

Therefore, estimating the sex of infants at Hisban will serve to identify any sex-based mortality differences in addition to variations in early-life factors that could have had long-term impacts on adult health. In addition, since maternal health is a primary variable in the health

outcomes of infants, the health status and mortality risk of female infants could impact not only population fertility but also whether females' early life experiences may have led to particularly harmful circumstances as adults. Furthermore, it will reflect cultural factors like gender-based differential access to resources that could increase the risk for metabolic disease in infants dying before age 3 years. For instance, specialized diets in pregnant and nursing mothers, such as those identified via isotopic analysis from another Ottoman-era site in Jordan (Gregoricka and Ullinger, 2022), may contribute to a greater risk of metabolic disease in women and increased metabolic disease in neonates and infants. Because children depend on their carers for their nutrition, including breastfeeding, it stands to reason that the health of nursing mothers may significantly impact their child's well-being. Furthermore, if sex-based dietary differences occurred in infancy, this could compound or counteract a maternal deficiency, resulting in sex-based differences in metabolic disease and, potentially, variation in mortality and the risk of dying with active metabolic disease in infancy. Accurate identification of biological sex in this subadult sample, combined with the evidence of vitamin C deficiency and historical context, should give insight into the sex-based mortality risks and help determine how biological and social factors influenced health outcomes for 19th century Tell Hisban infants.

CHAPTER THREE

Materials and Methods

Materials

The Hisban assemblage consists of a commingled sample of 52 individuals (32 subadults and 20 adults) represented by well-preserved skeletal remains recovered from the Tell Hisban site. The 32 subadults comprise 61.5% of the assemblage, and of those, 62.5% died under the age of 3 years (Table 4). Teeth selected for proteomic analysis were limited to individuals under the age of 3 years as indicated by their dental eruption stage following AlQahtani et al. (2010).

Table 4. *Age estimates of the subadults in the Hisban assemblage determined by Perry and Edwards (2021)*

AGE	NUMBER (%)
BIRTH – 3.9 MONTHS	4 (12.5%)
4.0 - 7.9 MONTHS	4 (12.5%)
8.0 – 11.9 MONTHS	6 (18.8%)
1.0 - 1.9 YEARS	6 (18.8%)
2.0 – 2.9 YEARS	4 (12.5%)
3.0 – 4.9 YEARS	2 (6.3%)
5.0 – 9.9 YEARS	5 (15.6%)
10 – 14.9 YEARS	1 (3.1%)
15 – 17.9 YEARS	0 (0.0%)
TOTAL SUBADULTS	**32 (100 %)**

The skeletal assemblage had been evaluated for macroscopic evidence of skeletal lesion development indicative of vitamin C deficiency, including within the mandible and maxilla (Perry and Edwards, 2021). Twenty maxillae and 16 mandibulae of individuals under the age of 3 years were evaluated for the presence of scorbutic lesions and dental elements. Of these, 10 mandibulae and 6 maxillae were selected as they had sufficient dental enamel for sampling. In the end, 10 teeth (6 mandibular and 4 maxillary) associated with infants with scurvy and 6 teeth, all mandibular, from those without were selected for proteomics analysis (Table 5).

Care was taken to not oversample an individual by selecting mandibular and maxillary teeth likely from different individuals based on the age at death and contextual data. Additionally, to ensure the accuracy of our methods in identifying peptides belonging to both AMLEX and AMLEY, modern samples of known sex (n= 8) were examined alongside archaeological samples. The 8 control samples were donated from 4 individuals in varying quantities: 1 tooth was provided by male #1, male #2 provided 4 teeth, female #1 provided 1 tooth, and 2 teeth were provided by female #2. All control samples were complete deciduous crowns that had lost their root through resorption before natural exfoliation.

Methods

In preparation for proteomic analysis, each tooth was extracted from the alveolar bone using dental instruments, then photographed, bagged, and assigned a sample i.d. number. Care was taken to note the presence of dental calculus for sampling, but none was observed. The less destructive acid etching method for proteomic analysis was chosen for this project as opposed to a more destructive method (Parker et al. 2019, 2021; Froment et al. 2020; and Buonasera et al. 2020). A more conservative approach was preferred to prevent the total destruction of dental samples regardless of the risk of decreased peptide sensitivity for simple acid etching. The dental samples and 8 donated control samples were processed and underwent proteomic analysis by Dr. Tonya Zeczycki at the Brody School of Medicine's Mass Spectrometer Core. Dr. Zeczycki performed the peptide extraction from the enamel, nanoLC-MS/MS analysis, and protein identification for this project and provided the following information.

Peptide Extraction from Enamel

Methods used for peptide extraction are based on established protocols (Parker, 2019; Stewart, 2017, 2016). All samples were treated with a 3% hydrogen peroxide solution (~0.5 mL) for 5 min, removed from the solution (~0.5 mL) for 5 min removed from the solution, and

flushed thoroughly ~2 mL of sterile filtered (0.2 micron), ultrapure water. Acid etching was used to extract proteins from the enamel. Samples were first etched in glass sample vials 1 min with ~200-300 µL of a 10% HCl solution. After discarding this initial solution, samples were etched with an additional 400 µL of 10% HCl solution for 5 min. After 5 min, the teeth were removed from the acid etching solution and immediately washed with ultrapure water to prevent further sample destruction. Acid solutions were dried to completeness under a stream of N_2 overnight. After drying, the extracted peptides were resuspended in acidified water (0.1% formic acid, 500 µL) and desalted using solid phase extraction (Sep-PaK C18, Waters). Samples were applied to pre-conditioned columns using a positive pressure vacuum manifold and washed twice with acidified water (0.1% formic acid, 3 mL), twice with water:methanol:acetic acid mixture (50:45:5% + 0.1% formic acid v/v , 3 mL) and again with acidified water (0.1%, v/v 3 mL) before eluting with water:acetonitrile:formic acid (50:50:0.2%, v/v, 3 mL). Samples were dried to completeness overnight under a stream of N_2 and reconstituted in loading buffer (water:acetonitrile:formic acid; 98:2:0.1%, v/v, 50 µL).

NanoLC-MS/MS Analysis

Peptides were analyzed by nanoLC-MS/MS using an UltiMate 3000 RSLCnano system coupled to a Q Exactive PlusHybrid Quadrupole-Orbitrap mass spectrometer (ThermoFisher) via nanoelectrospray ionization. Peptides (4 µL injections) were first passed through an Acclaim PepMap 100 trapping column (20 mm × 0.075 mm, ThermoFisher) at 5 µL/min. For analytical separation, an effective linear gradient of 10-30% acetonitrile (+0.1% formic acid) over 60 min at a flow rate of 0.3 µL/min was used with a 2 µm EASY-Spray PepMap RSLC C18 column (75 µm × 250 mm, ThermoFisher) at a column temperature of 55 °C. Mass spectra were acquired in positive mode over a 300-2000 m/z scan range. MS1 were collected at a resolution of 70K with

an AGC target of $1e^6$ ions and a maximum injection time of 50 ms. Data dependent acquisition was used to collect MS2 spectra on the top 15 most abundant precursor ions with a charge >1, an isolation window of 1.5 m/z, a fixed first mass of 140 m/z, and a normalized collision energy 27. MS2 spectra were acquired at 17.5K resolution, a maximum injection time of 100 ms, an AGC target of $1e^5$, and dynamic exclusion enabled for 20 sec. The minimum AGC target for MS2 scans was $2e^3$ with an intensity threshold of $2e^4$.

Protein Identification

For protein identification, raw data were searched against the canonical human proteome (UP000005640, 20,593 proteins, accessed June 2023) supplemented with the AMELX_HUMAN isoforms (Q99217-1-3) and AMELY_HUMAN isoforms (Q99218-1-2) using FragPipe (Kong, 2017; Yu, 2020). Contaminant and decoy sequences were added as described (Kong, 2017). Precursor and fragment mass tolerances were 20 ppm; fragment charges were set from 1-6, and a minimum of 2 matched fragments were used for peak assignments after deisotoping. Nonspecific protein digestion was invoked with peptide length and mass range restricted to 4-25 amino acid residues and 500-5,000 Da, respectively. Variable modifications (no more than 3 per peptide) were Met and Trp oxidation (+15.99), N-term Acetylation (+42.01), and pyro-Glu/pyro-Gln formation at the peptide N-terminal (-17.06 and -18.01). The top 300 peaks were used for spectral processing. False discovery rates were set at 1%, and matches between runs were not used. Spectral counts were used to estimate relative protein abundances. Proteins identified with at least 0.98 probability and with at least 2 identifying peptides were considered a high probable inference and used for analysis.

CHAPTER FOUR

Results

Acid-etching was used to extract proteins and peptides from tooth enamel, which were then subjected to bottom-up mass spectrometry analysis to determine the relative amounts of amelogenin. Amelogenin has both X- and Y- chromosome (AMELX and AMELY, respectively) expression; each isoform differs in its primary amino acid sequence due to splice variations. Because of their unique sequences, the sex-specific protein isoforms AMELY (UniProt ID Q99218-1 and Q99218-2) and AMELX (UniProt ID Q99217-1, Q99217-2, and Q99217-3) can be distinguished from each other using mass spectrometry. The isoforms of each AMELY and AMELX (AMELY-1, AMELY-2, and AMELX-1-3) are due to splice variations. The canonical isoforms of each form are highlighted in green in the sequence alignment in Figure 3.

Figure 3. *Sequence alignment of AMELY and AMELX isoforms generated using Clustal Omega (Madeira, 2022)*

```
sp|Q99217-1|AMELX_HUMAN      MGTWILFACLLGAAFAMPLPPHPGHPGYINFSYE--------------VLTPLKWYQS-I    45
sp|Q99218-2|AMELY_HUMAN      MGTWILFACLVGAAFAMPLPPHPGHPGYINFSYENSHSQAINVDRIALVLTPLKWYQSMI    60
sp|Q99217-2|AMELX_HUMAN      MGTWILFACLLGAAFAMP----------------------------VLTPLKWYQS-I    29
sp|Q99217-3|AMELX_HUMAN      MGTWILFACLLGAAFAMPLPPHPGHPGYINFSYENSHSQAINVDRTALVLTPLKWYQS-I    59
sp|Q99218-1|AMELY_HUMAN      MGTWILFACLVGAAFAMPLPPHPGHPGYINFSYE--------------VLTPLKWYQSMI    46


sp|Q99217-1|AMELX_HUMAN      RPPYPSYGYEPMGGWLHHQIIPVLSQQHPPTHTLQPHHHIPVVPAQQPVIPQQPMMPVPG    105
sp|Q99218-2|AMELY_HUMAN      RPPYSSYGYEPMGGWLHHQIIPVVSQQHPLTHTLQSHHHIPVVPAQQPRVRQQALMPVPG    120
sp|Q99217-2|AMELX_HUMAN      RPPYPSYGYEPMGGWLHHQIIPVLSQQHPPTHTLQPHHHIPVVPAQQPVIPQQPMMPVPG    89
sp|Q99217-3|AMELX_HUMAN      RPPYPSYGYEPMGGWLHHQIIPVLSQQHPPTHTLQPHHHIPVVPAQQPVIPQQPMMPVPG    119
sp|Q99218-1|AMELY_HUMAN      RPPYSSYGYEPMGGWLHHQIIPVVSQQHPLTHTLQSHHHIPVVPAQQPRVRQQALMPVPG    106
                             **** ******************:***** ***** ************ : ** :*****


sp|Q99217-1|AMELX_HUMAN      QHSMTPIQHHQPNLPPPAQQPYQPQPVQPQPHQPMQPQPPVHPMQPLPPQPPLPPMFPMQ    165
sp|Q99218-2|AMELY_HUMAN      QQSMTPTQHHQPNLPLPAQQPFQPQPVQPQPHQPMQPQPPVQPMQPLLPQPPLPPMFPLR    180
sp|Q99217-2|AMELX_HUMAN      QHSMTPIQHHQPNLPPPAQQPYQPQPVQPQPHQPMQPQPPVHPMQPLPPQPPLPPMFPMQ    149
sp|Q99217-3|AMELX_HUMAN      QHSMTPIQHHQPNLPPPAQQPYQPQPVQPQPHQPMQPQPPVHPMQPLPPQPPLPPMFPMQ    179
sp|Q99218-1|AMELY_HUMAN      QQSMTPTQHHQPNLPLPAQQPFQPQPVQPQPHQPMQPQPPVQPMQPLLPQPPLPPMFPLR    166
                             *:**** ******** *****:******************:***** **********::


sp|Q99217-1|AMELX_HUMAN      PLPPMLPDLTLEAWPSTDKTKREEVD    191
sp|Q99218-2|AMELY_HUMAN      PLPPILPDLHLEAWPATDKTKQEEVD    206
sp|Q99217-2|AMELX_HUMAN      PLPPMLPDLTLEAWPSTDKTKREEVD    175
sp|Q99217-3|AMELX_HUMAN      PLPPMLPDLTLEAWPSTDKTKREEVD    205
sp|Q99218-1|AMELY_HUMAN      PLPPILPDLHLEAWPATDKTKQEEVD    192
                             ****:**** *****:*****:****
```

After mass spectrometry analysis, peptides were searched against the human proteome containing all isoforms of AMELY and AMLEX. Peptide and protein identification was determined at a 1% false discovery rate, and matching between runs was not used to minimize false positive identifications. Proteins identified with at least 98% probability and having a minimum of two identifying peptides were used for further analysis. Spectral counts were used to estimate relative protein abundances, allowing for a relative estimation of each sample's sex-specific amelogenin protein forms.

The resulting data are located in the appendix (Tables A1 – A23). Each table includes the AMELY peptides identified in each sample, the start and end location on the amino acid chain, and the spectral count for each peptide. The total spectral count was calculated by combining the spectral count for each peptide containing either AMELX or AMELY. For each table, the peptides unique to AMELY are indicated in red, those unique to AMELX are indicated in blue, and the peptides in both proteins remain black. The sex was determined for each sample by the presence of the peptides unique to AMELY, as it is sex-linked explicitly to the Y chromosome present in chromosomally male individuals (Figure 4). The two dimorphic peptides are clearly differentiated between males and females, with both AMELY and AMELX found in male samples and only the non-unique AMELY peptides and unique AMELX peptides found in female samples. AMELY is expressed in much lower amounts than AMELX, and although the peptides may not be visible, it does not mean that the individual is female. In these instances, sequences are reviewed for AMELX peptides in regions where AMELY peptides should be different, and the individual is identified as a “probable” female.

***Figure 4**. Sequence Inclusion List for Mass Spectrometry Data Acquisition*

HSQAINVDR	**SMIRPPY**SS
SYENS**HSQAINVDR**	YQ**SMIRPPY**
ENS**HSQAINVDR**	YQ**SMIRPPY**S
S**HSQAINVDR**	MPLPPHPGH**PGYINF**
LR**PLPPILPD**L	YQ**SMIRPPY**SS
LR**PLPPILPD**LH**LEAWPATDKTK**	**SMIRPPY**SSYGYEPMG
PLPPILPDLH**LEAWPATDKTK**	SYEVLTPLKWYQ**SMIRPPY**
R**PLPPILPD**LH**LEAWPATDKTK**	WYQ**SMIRPPY**S
DLH**LEAWPATDKTK**	YEVLTPLKWYQ**SMIRPPY**
LEAWPATDKTK	YEVLTPLKWYQ**SMIRPPY**S
LEAWPATDKTKQEEVD	**PGYINF**SYEVLTPLKWYQ**SMIRPPY**

Since the sex was correctly assigned to each of the 8 known control samples, we can be relatively confident in the sex determination for the 15 test samples with adequate peptide levels. However, a limitation of this study is that this method was used on archaeological subadults of unknown sex. Therefore, aDNA is the only other method that can be employed to corroborate the results. However, the control samples do indicate that the methods used here should successfully identify the individual's sex.

AMELX and AMELY peptides were correctly identified in each control sample and 93.8% (15/16) of the Hisban samples (Table 5). However, sex determination could not be confidently made for sample #13 due to its very low peptide abundance. Therefore, the sex determination for sample #13 is indeterminate. AMELY (male) specific peptides were identified

Table 5. *Protein identification for modern controls and Hisban dental samples*

Sample	Context	Tooth	Scorbutic Lesions	Bone	Age at death	AMELX	AMELY	Proteomic-derived sex estimation	Known sex
01	Control1	lm1	N/A	mandible	N/A	•	•	male	male
02	Control2	li1	N/A	mandible	N/A	•	•	male	male
03	Control3	ui2	N/A	maxilla	N/A	•	•	male	male
04	Control4	uc1	N/A	maxilla	N/A	•	•	male	male
05	Control5	lc1	N/A	mandible	N/A	•	•	male	male
06	Control6	lm2	N/A	mandible	N/A	•		female	female
07	Control7	lm2	N/A	mandible	N/A	•		female	female
08	Control8	lm1	N/A	mandible	N/A	•		female	female
Hisban Samples									
09	H01 FLDL Sq2 Loc3 PB13 B1 282a	rc^1	Yes	mandible	7.6-12.9 mos	•	•	male	N/A
10	H98 FLDL Sq2 Loc3 PB16 B22 73c	rc_1	Yes	mandible	1-2 years	•	•	male	N/A
11	H01 FLDL Sq5 Loc24 1&2	lc^1	Yes	maxilla	1-2 years	•		female	N/A
12	H98 FLDL Sq2 Loc3 PB16 B22 72b	ri_2	Yes	mandible	7.6-12.9 mos	•		female	N/A
13	H98 FLDL Sq2 Loc3 B4 43c	li^1	Yes	maxilla	5.3-9.7 mos			indeterminate	N/A
14	H98 FLDL Sq2 Loc3 PB13 B1 101b	lc^1	Yes	maxilla	2.3-6.7 mos	•		female* probable	N/A
15	H98 FLDL Sq2 Loc3 PB12 B5 136	lm_2	Yes	mandible	5.3-9.7 mos	•	•	male	N/A
16	H?? FLDL Sq2 Loc3 PB13 B11 241y	lc^1	Yes	maxilla	5.3-9.7 mos	•	•	male	N/A
17	H98 FLDL Sq2 Loc3 PB17 B23 222	lc^1	Yes	maxilla	1-2 years	•	•	male	N/A
18	H98 FLDL Sq2 Loc7 B28 14	LM^1	Yes	maxilla	2-3 years	•	•	male	N/A

19	H98 FLDL Sq2 Loc3 PB19 B25 167a	rc^1	No	mandible	1-2 years	•		female	N/A
20	H?? FLDL Sq2 Loc3 PB13 B11 240m	li_2	No	mandible	5.3-9.7 mos	•	•	male	N/A
21	H01 FLDL Sq2 Loc3 PB44 B1 284b	rc_1	No	mandible	5.3-9.7 mos	•	•	male	N/A
22	H98 FLDL Sq2 Loc3 PB15 49	lc_1	No	mandible	2.3-6.7 mos	•		female* probable	N/A
23	H98 FLDL Sq2 Loc3 B21 119	lm_1	No	mandible	7.6-12.9 mos	•	•	male	N/A
24	H98 FLDL Sq5 Loc2 68	rc_1	No	mandible	7.6-12.9 mos	•	•	male	N/A

in 66.7% of assigned samples, meaning that males were more represented than females (male/female sex ratio = 2:1), with 10 males identified among the 15 individuals. There were pathological lesions on 62.5% (10/16) of the samples. Of those individuals, 1 was identified as indeterminate, 3 were identified as females and probable females and 6 were identified as male, giving a male/female sex ratio of 2:1. For individuals without pathological lesions, 2 were identified as females and probable females, and 4 were identified as males, with a male/female sex ratio of 2:1. In total, 10 individuals displayed indicators of metabolic stress (Table 6), including specified areas of porosity, new woven bone, and mixed lesions over large areas of the maxilla and mandibular bones. The 2:1 male:female ratio in infants dying under the age of 3 years at Hisban indicates that male infants have a higher risk of mortality at this age. However, the results of a 2:1 male/female sex ratio in individuals with and without scurvy indicate no sex-based difference in the chance of dying with active vitamin C deficiency in the sample.

Age at death ranges based on the dental development and eruption for the Hisban subadults (AlQahtani et al., 2010) identified age-specific patterns in dying with active scurvy in the sample (Table 7). Most of the infants sampled here died between 5.4 and 9.7 months of age (midpoint: 7.5 months), followed by 1-2 years (midpoint: 1.5 years). This pattern somewhat follows the breakdown of the total sample, of which 50.0% of children under the age of 15 were between 5 months and 2 years old (Perry and Edwards, 2021). In addition, these were the ages at which children, both males and females, were more likely to die with active scurvy. The youngest age category (2.5 – 6.7 months, midpoint 4.5 months) consisted only of females, while males comprised most of the older age categories.

***Table 6**. Descriptions of scorbutic lesions in the maxillae and mandibulae from Hisban selected for proteomic analysis (Individuals 19-24 did not display any skeletal lesions in these elements)*

Sample Number	Accession Number	Evidence of Metabolic Disease
09	H01 FLDL SQ LOC3 PAIL 16 B22 MANDIBLE #282A	New bone formation along the mental eminence and mandibular ramus and porosity around the mental foramen.
10	H98 FLDL 2 SQ2 LOC3 PAIL 16 B2 MANDIBLE #73C	Significant new bone formation over large portions of the mandible, including porosity above the mandibular foramen (bilateral).
11	H01 FLDL SQ5 LOC 24 MAX #1 (LEFT)	New bone formation inferior to the margin of the nasal aperture, inferior to the zygomatic process, mixed lesions along the posterior of the maxilla, maxillary sinus, and porosity on the palatine process.
11	H01 FLDL SQ5 LOC 24 MAX #1 (RIGHT)	New bone formation inferior to the margin of the nasal aperture, inferior to the zygomatic process, and along the alveolar processes. Mixed lesions along the posterior maxilla and maxillary sinus and porosity on the palatine process.
12	H98 FLDL SQ2 LOC PAIL 16 B22 MANDIBLE #72B	Porosity along the mental eminence, new bone formation along the inferior margin of the mandible, mixed lesions superior to the mandibular foramen.
13	H98 FLDL SQ2 LOC 3 B4 MAX 43C (LEFT)	Mixed lesions over a large area of the maxilla.
14	H98 FLDL2 SQ2 LOC 3 PAIL 13 B1 MAX #101b (LEFT)	Mixed lesions along alveolar processes, anterior of the maxilla, zygomatic process, and left palatine.
15	H98 FLDL 2 SQ2 LOC 3 PAIL 12 B5 MANDIBLE #136	Porosity and new bone formation over large areas of the anterior left mandible.
16	H?? FLDL SQ2 LOC 3 PAIL 13 B11 MAX #241Y (LEFT)	New bone formation along the alveolar process, fontal process, orbital floor, and maxillary sinus.
17	H98 FLDL SQ2 LOC 3 PAIL 17 B23 MAX #222 (LEFT)	Mixed lesions on the anterior maxilla, alveolar processes, zygomatic process, and palate.
18	H98 FLDL SQ2 LOC 7 B28 MAX #14 (LEFT)	Mixed lesions inferior to the nasal spine, porosity surrounding the infraorbital foramen, and new bone formation along the alveolar process.

***Table** 7. Ages of Subadults from Tell Hisban with and without pathology by sex*

Age at death (midpoint)	# of females		# of males		# of indeterminate sex		Total
	With scurvy	Without scurvy	With scurvy	Without scurvy	With scurvy	Without scurvy	
4.5 months	1*	1*	0	0	0	0	**2* (12.5%)**
7.5 months	0	0	2	2	1	0	**5 (31.3%)**
10.5 months	1	0	1	2	0	0	**4 (25.0%)**
1.5 years	1	1	2	0	0	0	**4 (25.0%)**
2.5 years	0	0	1	0	0	0	**1 (6.2%)**
Total	**3**	**2**	**6**	**4**	**1**	**0**	**16 (100%)**

*Probable females

Several challenges arose when sampling the partially developed deciduous teeth using the acid etching method proposed by Stewart et al. (2017). One of the advantages of using this method is that the technique is minimally destructive compared to other methods that utilize bulk sampling (Parker et al. 2019, 2021; Froment et al. 2020; and Buonasera et al. 2020), allowing any remaining enamel to be used in additional studies. While many of the dental samples were already fragmented while still embedded in the alveolar bone of the maxillae and mandibulae, or the samples fragmented during the extraction process, it did not impact the amount of tooth enamel to sample. Additionally, several incompletely mineralized tooth crowns were sampled. Gowland et al. (2021) determined that it was possible to retrieve sufficient peptide material from incompletely developed perinatal teeth to estimate sex using the acid etching method. However, this study generally recovered the largest quantities of peptides from completely mineralized teeth. Regardless of the condition of the sample, adequate amelogenin was achieved in 23 of 24 of the dental samples. The acid etching worked well for this project, providing reliable sex estimations for the subadults exhumed from Tell Hisban.

CHAPTER FIVE

Discussion

The ratio between males and females in the sample of infants from Hisban suggests that males at the site had a slightly higher than expected mortality risk following birth than females. Assuming a normal sex ratio at birth of 107 males to 100 females (CIA Fact Book, 2021), males at Hisban were 1.87 times as likely as females to die before the age of 3 years. As noted previously, males tend to have greater innate frailty than females, resulting in an overall decrease in the number of males in relation to females across the general population. However, the infant males at Hisban experienced other factors besides the intrinsic differential that increased their mortality risk compared to females. Other studies have found variation in sex ratio can be affected by many factors, including environmental impacts, warfare casualties, sex preferential practices, and differential health outcomes in certain diseases (James, 1987a; James, 1987b), any of which could have played a role in the higher male infant mortality risk at Hisban.

The 2:1 male:female mortality ratio of the Hisban sample is much higher than contemporary intrinsic morbidity and mortality risk for boys under the age of 5 years. The global male:female mortality ratio for children <5 years is estimated to be 1.1:1, and Jordan currently sees a 1.2:1 mortality ratio for the same age group (CME Info, 2023). Unfortunately, understanding this disparity is not assisted by documentary evidence from the period, such as censuses. While there are numerous demographic records from the Ottoman Era, their primary foci were taxable households, landholdings, and draftable men for military service (Karpat, 1978). Women and children were likely undercounted, so we cannot accurately contextualize the notably higher male infant mortality rate at Hisban within its broader political and environmental context using documentary data.

The endocrinological factors that impact the differential risk of morbidity and mortality mentioned in the background likely played significant roles in the increased male mortality risk seen here, which will be explored further below in the context of scurvy. Cultural factors such as preferential treatment of infants by sex may have impacted morbidity and mortality. However, preferential treatment of male infants and children in traditional Bedouin tribes of the Negev and Sinai (Abu-Rabia, 2010) and isotopic data on evidence for weaning practices in adults from the Ottoman Periods sites of Khirbet al-Mudayna in Jordan (Gregoricka and Judd, 2016) and Tell el-Hesi in Israel (Gregoricka and Ullinger, 2022) would suggest male infants would have *lower* mortality risk than females. Abu Rabia (2020) attributes the societal preference for males to their lineal importance within a patriarchal system and their power to defend the family's honor. The birth of a daughter is seen as the birth of a future bride who will leave the family (and require a dowry), but someone in the meantime who can help with domestic chores such as gathering firewood and tending to livestock.

The increased value of sons over daughters also impacted breastfeeding practices amongst some Bedouin tribes. Not only were the cries of male infants addressed more quickly through feeding, but males were also weaned later than females (Abu-Rabia, 2010). The process of weaning is not a singular event. Instead, it is a gradual transition from a diet composed exclusively of human breast milk to one that contains an increasingly greater proportion of complementary liquids or solid foods (Millard, 2000). The gradual shift from a diet of exclusive breastfeeding to one of solid foods allows children to continue receiving the immunity-boosting benefits of breast milk while taking advantage of the advanced nutrients their growing bodies need through complementary foods. Abu-Rabia (2010:43) notes that males in traditional Bedouin communities are breastfed from birth until they are 1-2 years old, while females are breastfed

from birth until 6 months to 1 year old. A mother without surviving sons may begin weaning her infant daughter after less than six months to try to conceive again.

This timeline of exclusive breastfeeding has been confirmed through $\delta^{15}N$ and $\delta^{13}C$ analysis of skeletal remains from other Ottoman Period Bedouin cemeteries in the region. However, the presence of altered weaning schedules by sex is not as clear. Examination of bone collagen from the skeletal remains (7 subadults and 15 adult males and females of varying ages) of Ottoman Period Bedouin buried at Khirbat al-Mudayna in Jordan suggests that infants were exclusively breastfed until approximately six months to one year before gradually weaning onto foods consumed by the adult community (Gregoricka and Judd, 2016). Likewise, analysis of bone collagen by Gregoricka and Ullinger (2022) found that $\delta^{15}N$ values among infants from the Ottoman Period Tell el-Hesi in Israel suggest that supplementing breastfeeding with other foods (i.e., weaning) continued up to two years of age before the child isotope values eventually level out and become equal to adult values. Additionally, while sex could not be estimated for the infants, there were no identifiable dietary differences among the infants at Tell el-Hesi that could be attributed to sex-based complementary feeding patterns (Gregoricka and Ullinger, 2022).

Other maternal-related factors besides weaning practices can also impact not only the sex ratio at birth, which can then result in a skewed infant mortality sex ratio in the form of higher female mortality. It is suggested that the natural human sex ratio at birth can be impacted by paternal age, high parity, birth order, race, and physical and psychological stress (Pongou, 2013; Alkema et al., 2014; Zeitlin et al., 2002). For example, high parity (the number of previous pregnancies beyond 20 gestational weeks) raises women's gonadotropin levels, increasing the likelihood of female births in later pregnancies (Adibi et al., 2015; James and Rostron, 1985). While it is not clear that birth order or higher parity results in higher mortality risk for male vs.

female infants, higher parity does result in poorer birth outcomes and increased infant mortality (Aliyu et al. 2005). Ethnographic accounts by Dickerson (1949:506) state that early 20th century Bedouin women of nearby Kuwait and Saudi Arabia tended to have a very high parity rate, giving birth up to 15 times in their lifetime with only 3-4 children surviving to adulthood. If the Bedouin women at Hisban had similarly high parity rates, the male:female birth ratio of 1.07:1 may be closer to 1:1. If that is the case, one would expect greater representation of female infants in the sample due to their higher-than-normal numbers. That males are twice as likely to be in the sample indicates an even more significant than expected mortality risk.

Therefore, the evidence outlined above indicates we should see poorer outcomes for female infants from high-parity mothers and lower male infant mortality risks due to possible preferential treatment. However, that is not consistent with the skeletal record at Tell Hisban. One factor that has been dismissed as a probable cause for the discrepancy is the impact of warfare on infant mortality rates. Although male infants do not participate in violent conflicts, that does not protect them from acts of violence, including infanticide (Mays, 1993; Turner, 1993; Turner and Turner, 1995) or ritualized killing (Castro et al., 1991) during warfare. No evidence of these acts of violence occurring at Hisban or the surrounding area exists in the historical record, regardless of the history of disputes and warring Bedouin tribes. Therefore, further exploration of the causes of male mortality risk in Hisban infants should include investigating skeletal lesions related to disease and possible dietary differences in these infants using $\delta^{15}N$ and $\delta^{13}C$ values. While stable isotope analyses are beyond the purview of this study, the presence of scurvy in those dying in infancy was also assessed to identify a possible cause of increased male mortality at Hisban.

Evidence for Sex-Based Differences in Morbidity at Hisban

The only paleopathological evidence of physiological stress and morbidity visible in the mandibulae and maxillae from Hisban was abnormal bone formation and porosity consistent with scurvy (Perry and Edwards, 2021). The 2:1 ratio of male to female infants dying with scurvy does not deviate from the overall ratio of male:female mortality in infants, suggesting that male and female infants were equally likely to die with scurvy. The appearance and timing of childhood bone formation should be considered regarding the 2:1 male/female mortality rate in this sample. Age-related red-to-yellow marrow conversion and bone turnover rates vary between biological sexes and may impact subadult lesion development. After birth, bone marrow converts from vascular, red (hematopoietic) tissue to fatty, yellow (adipose) marrow that remains throughout adulthood (Valderrabáno and Wu, 2019). Hyperplastic lesions, including commonly recorded cribra orbitalia and porotic hyperostosis, caused by metabolic deficiencies (i.e., anemia and scurvy), are likely to develop before red-to-yellow marrow conversion begins (Brickley, 2018). Typically, cranial bones, such as those used in this study, start the red-to-yellow marrow conversion between 1 and 2 years and are completed by 15 to 19 years (Foster et al., 2004). Males tend to begin conversion earlier, suggesting that males may develop lesions earlier than their female counterparts (O'Donnell, 2019; Watts, 2013). Conversely, females undergo red-to-yellow marrow conversion more slowly, suggesting a later age window for developing lesions (Kugel et al., 2001; Okada et al., 1989).

Additionally, studies suggest that bone turnover is more rapid in males (Debono et al., 2011) as they experience a heightened osseous response to infection, resulting in a higher predisposition to skeletal lesions (O'Donnell, 2019). Furthermore, because vitamin C maintains immune function by neutralizing or destroying pathogens (Jacob and Sotoudeh, 2022), low

ascorbic acid levels can increase infection risk and, therefore, a higher mortality rate. While the increased mortality risk in males may not necessarily be linked with scurvy, the intrinsic sex-based differences may influence the appearance of skeletal lesions in growth patterns.

However, it is important to remember that this sample only includes Hisban infants *dying* with active scurvy. Clinical manifestations of scurvy typically occur between 6-10 months of chronic vitamin C deficiency (Jaffe, 1972). However, scurvy can develop in as little as 2-4 months during periods of rapid growth (Tamura et al., 2000). Because rapid growth mainly occurs during infancy and early childhood, skeletal evidence of scurvy should be expected to be highest for this age group (Ortner et al., 1999). Additionally, clinical resolution of scurvy following treatment occurs rapidly, meaning that most lesions would only be present in subadults suffering from active scurvy at the time of death (Snoddy et al., 2018). Therefore, this sample does not include those who suffered from scurvy in infancy but survived and lived into adulthood or those who died before skeletal lesions could develop. Regardless, as an element of mortality in infancy, scurvy did not seem to discriminate by sex.

Scurvy and the Mother-Infant Dyad

It also appears that, regardless of sex, maternal health may have largely impacted the chance of developing scurvy in infancy. The youngest infants dying with scurvy were still within the critical first months after birth, a period when endogamous factors related to maternal health and the *in-utero* environment play a significant role in infant morbidity and mortality. Because all nourishment before weaning (which typically starts around 6 months of age) is provided to the infant through human breast milk, instances of scurvy in breastfeeding infants can result from maternal malnutrition. During pregnancy and breastfeeding, a female's daily vitamin C requirements increase to compensate for the fetus's needs (120mg/day for pregnant females and

80mg/day for breastfeeding females (Popovich et al., 2009). Breastfeeding is not unidirectional but rather a dynamic process between the infant, the lactating person, and the breast milk, which adapts depending on the infant's nutritional needs (Bode et al., 2020). One possible factor for inadequate vitamin C levels in the mothers of Hisban is the typically high parity among 20th century Bedouin women. Numerous nutritionally draining pregnancies followed by lengthy periods of breastfeeding stress a woman's body and prevent the mother from recharging nutritionally (Merchant et al., 1990). If the mother was experiencing nutritional stress, the infant would be nutritionally disadvantaged at birth. Therefore, an infant's early-life vitamin C store levels should be higher than the mother's at birth (de Vries, 2018; Salmenperä, 1984; Lund and Kimble, 1943). The development of scurvy in infants at Hisban suggests maternal malnutrition prevented adequate early life stores of vitamin C.

However, most of the infants dying with scurvy died during the expected period of weaning, around 6 months to 2 years of age. According to Abu Rabia (2010), male and female infants traditionally had different weaning periods, which could affect different periods of frailty between sexes. According to ethnographic descriptions, females begin the weaning period around 6 months to one year of age, while males start weaning from breast milk around 1-2 years of age (Abu-Rabia, 2010). Stable isotope studies identify that the weaning period ends on average at around 2 years of age.

Understanding the individual risks for weaning children requires nuance and is dependent on the health of the mother and their infant. While we might expect to see an increased mortality risk for infants being weaned as young as 6 months, in actuality, an earlier start to weaning and the introduction of supplementary foods may have been beneficial if their mother was nutritionally deficient. Adding supplemental foods could help bridge the nutritional gap between

the mother's breast milk and the daily requirements needed to keep the infant healthy. Therefore, if the mother was suffering from malnutrition, the preferential practice of weaning males later may have been more detrimental to the infant's health than the risk of infection or exposure to food and fluid contamination.

The ages at which Hisban infants died with active scurvy do not differ significantly between males and females, including during their respective weaning periods. The 6 month to 2 year age range during which females are typically weaned is dominated by males at 3:1. If males indeed began weaning no earlier than 12 months, the increased risk for males during this period could be attributed to nutritional stress placed the infant due to maternal malnutrition and the lack of supplementary foods. Interestingly, the 1-2 year age range is equally distributed 1:1, and the 2-3 year range has only 1 male representation. It is clear from this limited sample that the mortality risk of the infant diminished the farther into the weaning period they survived. I am hopeful that future analysis of $\delta^{15}N$ incremental dentin may provide more information on the weaning practices at Hisban and allow for greater insight into the nutritional status of its inhabitants.

Because we see increased evidence of scurvy in infants depending on their sex-specific weaning period (3:1 for females aged 6 -12 months, and 3:0 for males aged 1-3 years), we cannot rule out that the foods used to supplement breast milk during the weaning period could have been deficient in vitamin C. Ethnographic and stable isotopic evidence of historic Bedouin diets indicates that adults consumed a mixed C_3/C_4 diet, likely including C_3 fruits, vegetables, and cereals (barley and wheat) alongside C_4 resources such as millet, sorghum, and proteins (milk, butter, yogurt, and meat) from animals that consumed C_4 plants (Table 8) (Abujaber, 1989;

***Table 8**. The Vitamin C Content of Foods Known to be Consumed by the Bedouin (Abujaber, 1989; Lancaster, 1997; Gregoricka and Ullinger, 2022)*

Food	Ascorbic Acid	Source
Cereals	0 mg/100g	Brickley et al., 2020
Chickpeas	4 mg/100g	Wallace et al., 2016
Eggs	0 mg/100g	Brickley et al., 2020
Lamb's liver	0-4 mg/100g	Brickley et al., 2020
Lentils	1.5 mg/100g	fdc.nal.usda.gov, 1986
Meat	0-2.3 mg/100g	Brickley et al., 2020
Millett	0 mg/100g	Brickley et al., 2020
Goat/sheep milk	1.3-3 mg/100g	Brickley et al., 2020
Camel milk	3-6 mg/100g	Brickley et al., 2020
Breast milk (colostrum)	7 mg/100g	Brickley et al., 2020
Breast milk (transitional)	6 mg/100g	Brickley et al., 2020
Breast milk (mature)	4-5 mg/100g	Brickley et al., 2020

Lancaster, 1997; Gregoricka and Ullinger, 2022). In some cases, such as at Tel el-Hesi, adult males may have had greater access to animal products than adult females (Gregoricka and Ullinger, 2022). Additionally, pregnant or nursing mothers (based on fetal and perinatal isotope results) at Tel el-Hesi may have consumed a diet with more C_4 resources than most adults (Gregoricka and Ullinger, 2022). This greater reliance on C_4 food sources is also seen in the childhood diet between 2 and 5 years, possibly from millet gruel or milk from an animal consuming C_4 sources (Gregoricka and Ullinger, 2022). Limited contemporary ethnographic accounts detail the importance of wheat and milk consumption in addition to animal protein, native plants, and herbs (Palmer, 2002). Even so, the exact contribution of vitamin C-rich foods to the Hisban diet may never be conclusively identified. However, isotopic analysis of the Hisban infants included in this study would confirm that no identifiable dietary differences between males and females can be attributed to sex-based complementary feeding patterns.

Limitations and Sampling Bias

Interpreting the health status of a past population from ancient skeletal remains is complicated. Three significant conceptual problems confound the interpretation of skeletal material from archaeological contests: demographic nonstationarity, selective mortality, and hidden heterogeneity (Wood et al., 1992). Demographic nonstationarity suggests that slight variations (e.g., migration or changes in fertility) significantly impact the age-specific mortality distribution far more than considerable modifications in mortality (Wood et al., 1992). Secondly, selective mortality cautions that skeletal samples are inherently biased, as they represent the dead, not the living. The presence of bone lesions in skeletons does not directly represent the disease frequency among the living population and, therefore, cannot accurately express the risk of disease or death at a given age in the entire population (Grauer, 2018). Lastly, the concept of hidden heterogeneity asserts that the population from which the skeletal samples are from comprises an unknown mix of individuals, each with various underlying frailties or susceptibility to disease and death. It cannot be assumed that everyone was equally susceptible to contracting, presenting, or dying of a particular disease (Wood et al., 1992). Interpreting the presence of disease or "stress" within a population from the frequency of skeletal lesions can be misleading (Wood et al., 1992; DeWitte & Wood, 2008). We are reminded that stress markers and lesions do not always mean identical levels of frailty. For a stress marker to be present in the skeletal record, the individual must have been healthy enough to survive the initial cause long enough for the marker to develop.

Additionally, some individuals may show no evidence of disease because they were too frail and died before skeletal lesions could form (Wood et al., 1992). Therefore, some of the individuals within the sample could have died with scurvy (or something else) but showed no

signs of lesions. Recent studies have found that factors such as bone marrow conversion and bone turnover rates (O'Donnell, 2019) differ between male and female children. In general, because the growth rate of new bone is highest between infancy and puberty, children are most likely to exhibit lesions in response to environmental stressors during this period. If they recover, they are more likely to undergo rapid reparative growth. Zierk and colleagues (2017) demonstrated that alkaline phosphatase levels surge after birth and peak at approximately 20 days of life, decreasing until about 4 years of age. Levels surge again at ages 10-12 years in females and 13-15 years in males. Therefore, skeletal lesions are most likely to develop because of increased osteoblastic activity until age 4, and evidence of these lesions may be obliterated by new bone formation during pubescent growth spurts (Brickley and Mays, 2019). This suggests that the birth to 4 years window is the best opportunity to identify any sex-based skeletal lesions caused by metabolic disease in the subadults from Tell Hisban.

Additionally, during childhood growth and development, bone formation markers (e.g., osteocalcin, bone-specific alkaline phosphatase) are significantly higher in healthy males than in females, making them more likely to mount skeletal reactions to stress and disease (Debono et al., 2011; Krall et al., 1997). It is also suggested that infant males have a higher risk of an increased inflammatory reaction and osteoclastic activity due to bacterial infections. (Gamaletsou et al., 2012; Valerio et al., 2017). Therefore, male children might be more predisposed to develop childhood skeletal lesions than females (O'Donnell et al., 2022).

The 2:1 ratio demonstrated by this sample supports the assertion that male infants have a higher mortality risk than females. However, males are less likely to develop skeletal lesions in response to stress and disease (scorbutic lesions) in the maxilla or mandible than females. On the other hand, taking into consideration that females are less likely to develop a visible osseous

response to physiological stress, they might have a *higher* risk of dying with active scurvy than males, and some scorbutic females have not developed notable mandibulae and maxillary lesions related to this condition. Regardless of whether males and females have the same risk of dying with scurvy, its presence indicates poor nutrition. It has a synergistic relationship with disease, which may not be visible in skeletal remains but exacerbates the already increased morbidity and mortality risks for males at Tell Hisban.

Sampling bias also may impact the results. The samples chosen for this project are a subset of a larger sample of subadults from Tell Hisban. Of the 20 maxillae and 16 mandibulae of individuals under the age of 3 years, 10 mandibulae and 6 maxillae were selected based on the presence of scorbutic lesions, creating a relatively small sample size of 16 individuals. Because it is unclear precisely how vitamin C deficient one must be or how long it takes for scorbutic lesions to develop, there is no way of knowing how many, if any, individuals without lesions were suffering from metabolic disease. Therefore, we cannot definitively say that 10 of the 6 sample individuals died *of* active metabolic disease, only that those individuals died *with* signs of active metabolic disease on their maxillae and mandibulae.

Due to the commingled nature of this site, we cannot positively associate any post-cranial elements with the cranial elements. Therefore, a possibility exists that some individuals who did not show maxillary and mandibular evidence of scurvy exhibited other indicators elsewhere in their skeleton. However, we are reminded that stress markers and lesions do not always directly reflect levels of frailty. For skeletal lesions to be present, the individual must have been healthy enough to survive the initial cause long enough for the marker to develop. Some individuals may show no evidence of disease because they were too frail and died before skeletal lesions could form (Wood et al., 1992). Additionally, because the individuals interred at Tell Hisban likely

belonged to the semi-nomadic agropastoral Bedouin tribes who seasonally inhabit the area, the cemetery itself was possibly used seasonally. This suggests that the dead interred at Tell Hisban is only one subsample of all deaths of those associated with the cemetery.

While care was taken to estimate the physiological age of all samples using dental development and eruption following AlQahtani et al.'s (2010) methods, there will always be uncertainty in the chronological age of the subadult individuals. Although the sequence of age-related biological changes is well-documented and largely considered universal, variations depend on sex, genetics, and population (Ríos and Cardoso, 2009). Therefore, despite the reliability of modern dental development and eruption standard references for subadults (e.g., AlQahtani et al., 2010; Moorrees et al., 1963; Ubelaker, 1989), their accuracy for archaeological populations depends on the standard's applicability to past populations. Additionally, while age estimation based on dental eruption and development is the least sensitive to physiological stress, undernutrition and chronic illnesses may ultimately slow dental eruption and stunt or delay skeletal growth (Bogin, 2001; Cardoso, 2006; Mays, 1999). Typically, multiple age estimation methods would be employed for a single individual to verify physiological age. However, in this commingled context, dental development and eruption are the only age estimation methods that can be utilized.

It is important to note that physiological age markers are not equivalent to chronological age but an estimate of the physiological status of an individual (Kemkes-Grottenthaler, 2002). Furthermore, chronological age may have little to do with the social position or abilities of the individual. Social age considers the timing of significant events or rites of passage in a life cycle and groups those who share the same life stage into an "age set" (Sofaer, 2011). Membership to a social age may depend on individual changes (e.g., a girl's first menstruation, reassigning her as

a child to a woman of marriageable age), or it may rely on experiences of another person with whom they affiliated (e.g., becoming a grandparent) (Counts and Counts, 1985). These social ages may be represented in the archeological record through the presence of grave goods with individuals of a certain age and sex (Gowland, 2006). However, the link between chronological and social age may not be synchronous; therefore, interpretation can be problematic. Relying on an assumed chronological age may limit the interpretation of the participants of an age category and their role in their community.

Depending on the population, membership to age sets may come with access to different resources or behaviors that may increase or mitigate mortality risks. For example, a boy's first haircut, sometime between 3 and 5 years of age, marks the ceremonial transition from an intermediate state to male status for Bedouin of the Negev and Sinai. Until then, the male child's hair is left uncut to appear as a girl since they are less vulnerable to the evil eye (Abu-Rabia, 2010). Circumcision of males between 5 and 6 years marks another shift in social age and is accompanied by great feasting and celebration (Cole, 1975; Dickson, 1949). This shift into personhood may have come with increased access to C_4 animal proteins typical of adult males (Gregoricka and Ullinger, 2022), giving members of this age a nutritional advantage over the uninitiated.

Summary

The higher male mortality observed at Hisban is likely caused by several factors that result in the innate frailty of male children due to the effects of sex hormones on immune responses. The female sex hormone estrogen enhances immune competence, while the male sex hormone androgen reduces it (Ahmed et al., 2010). Therefore, while males and females may be equally exposed to infectious agents, females tend to mount a stronger immune response to a

wide variety of diseases caused by viruses, bacteria, fungi, and parasites (Klein, 2000; Wells, 2000; Owens, 2002; Pennell et al., 2012), decreasing their likelihood of severe illness and death. Based on these data, we expected the infants at Hisban to follow this general pattern of slightly higher male mortality than females, and any variation from this would indicate the presence of extrinsic impacts of sex on mortality risk. Despite the increased mortality of males, the likelihood of a male infant dying with scurvy is the same as that of females among the subadult population at Hisban. Both female and male skeletal samples exhibited a mortality risk of 3:2 for those with evidence of active scurvy and those without. This supports the assertion that while there was no sex-based difference in the kinds of supplemental foods provided for the infants at Hisban, there was likely a difference in the timing of weaning and the introduction of supplementary foods. Additionally, the earlier conversion red-to-yellow marrow conversion (O'Donnell, 2019; Watts, 2013), paired with a heightened osseous response and more rapid bone turnover (Debono et al., 2011), results in a higher predisposition for males to form skeletal lesions than their female counterparts.

As nutritional deficiencies of ascorbic acid typically cause scurvy, the diet was likely a critical factor in the occurrence of vitamin C deficiency in the infants of this study. Infants rely on breast milk for nutrition during the earliest life stages, and any ill health or malnourishment the mother suffers can impact the infant's well-being (Chávez et al., 2000). For example, high-parity mothers with continuous pregnancies and prolonged periods of breastfeeding may struggle to recover nutritionally, which can lead to nutritional deficiencies in the mother and their child. Remarkably, the adult females in the sample do not show evidence of scurvy or other dietary deficiencies (Perry and Edwards, 2020), suggesting that we have to rely on the pathological lesions on infant skeletal remains to indicate maternal health at Hisban.

The age of the subadults of this study (~4.5 months to ~2.5 years) should be accounted for when evaluating their mortality risk. During the period immediately after birth, infant mortality is often related to endogenous factors, such as congenital abnormalities, childbirth complications, low birth rate, and prematurity (Lewis, 2007). However, in the post-natal period, exogenous environmental factors (e.g., malnutrition, poisoning, trauma, and infectious disease) pose the greatest mortality risk to infants (Lewis and Gowland, 2007). Because the subadults in this study are a part of the delayed post-natal (newborn), infant, and toddler categories, their mortality depends on exogenous factors.

Identifying the foods used to supplement breast milk during the weaning period could explain the high frequency of scorbutic lesions in the subadult population from Tell Hisban. A diet lacking in vitamin C, possibly caused by increased dependence on nutritionally deficient cereals (e.g., wheat, barley, millet), may be to blame. While ethnographic and isotopic evidence of historic Bedouin diets indicates that adults consumed a mixed diet, including fruits, vegetables, cereals (barley, wheat, millet, sorghum), and proteins (milk, butter, yogurt, and meat), the childhood diet was less diverse. Children between 2 and 5 years primarily ate complementary foods such as millet gruel and animal milk (Gregoricka and Ullinger, 2022). Consequently, this limited diet, and lessening amounts of nutrient-rich breast milk would have made the weaning period risky for infants to develop nutritional deficiencies like scurvy.

CHAPTER SIX

Conclusion

The individuals interred at the cemetery at Tell Hisban lived during the Late Ottoman period, a time of *Tanzimat* reform and the settlement of the semi-nomadic tribes of Jordan. Proteomic sex estimation was done to better understand the demographics of the subadult individuals interred at Tell Hisban. NanoLC-MS/MS spectroscopy was used to identify sexually dimorphic amelogenin proteins (AMELY and AMELX) and determine the biological sex of the unknown samples. Males were found to have a higher overall mortality rate (2:1) than females, and there appears to be no increased mortality risk by sex for subadults who died with active scurvy. Analysis of sex estimation combined with age estimation provided insight into how the relationship between the two may be a factor in the pattern of scurvy in the skeletal remains of the subadults of Tell Hisban.

Male and female infants at Hisban had the same chance of dying with scurvy, but reasons for the relatively high prevalence of this metabolic disease at Hisban remain elusive. Scurvy is a metabolic disease caused by a diet deficient in ascorbic acid (vitamin C), an essential component of the collagen found in bone and other connective tissues. Vitamin C deficiency causes abnormal new bone formation in subadults and fragile blood vessels, frequently co-occurring with anemia. For the individuals from Tell Hisban, scurvy may stem from maternal malnutrition and Bedouin weaning practices. There was an increased prevalence of dying with scurvy during the weaning period as children transitioned from exclusively breastfeeding to solid foods. Likely, the common supplemental foods (i.e., cereals and animal proteins) deficient in vitamin C were heavily relied upon. By the end of the Ottoman Period, much of the land surrounding Hisban had been converted from seasonal sheep and goat pastureland to farmland to sustain the agricultural intensification of cereal production, specifically wheat, following the *Tanzimat* reforms. A shift

away from a diverse diet provided by foraging, animal husbandry, and small-scale agriculture to an increased dependence on cereals deficient in vitamin C may have led to metabolic deficiencies among the Bedouin, especially women and children (Edwards, 2019).

Metabolic diseases such as scurvy (vitamin C deficiency) can be an important proxy for population-wide nutritional status as cultural behaviors, including substance practices and biological factors such as sex-specific hormone levels and genetic predisposition. However, what must be considered is that morbidity, as expressed by skeletal remains, does not directly represent the disease frequency among the living population and, therefore, cannot truly determine the risk of disease or death at a given age. Three significant conceptual problems confound the interpretation of skeletal material from archaeological contests: demographic nonstationarity, selective mortality, and hidden heterogeneity (Wood et al., 1992). It cannot be assumed that the demographics of the population remained static throughout the Late Ottoman Period, nor that all individuals at risk for scurvy died with visible skeletal lesions, or that there are those more susceptible to the disease because of variation or frailty (Wood et al., 1992). Therefore, interpreting the health status of a past population from ancient skeletal samples remains difficult.

Future Research

To better understand the pattern of metabolic disease at Tell Hisban, further research is needed to identify additional factors of increased mortality in individuals under the age of 3 years. Nitrogen stable isotopic analysis ($\delta^{15}N$) of incremental samples of dental dentin can substantiate the weaning periods of females aged 6-12 months and males aged 12-24 months among the inhabitants of Tell Hisban. Infants have the same isotopic ratio as their mothers at birth because the mother is the source of all nutrition during pregnancy (Jay et al., 2008).

Following birth, the trophic level will rise until exclusive breastfeeding ends. At this point, the trophic level will decrease when weaning begins and supplementary feeding occurs. The individuals will be considered fully weaned when the $\delta^{15}N$ value reaches + 1 SD of the female mean (Beaumont et al., 2015). Therefore, the individual's weaning period can be estimated because of this predictable trend.

While we cannot be certain what foods the Bedouin of 19th century Hisban consumed daily due to a lack of ethnographic accounts specific to this population, we do know that many other Bedouin tribes herded goats and sheep, in addition to engaging in small-scale agriculture, and relied on diets high in cereals and milk products (Abujaber, 1989; Palmer. 2002). The goats and sheep were kept primarily for their milk rather than meat, as the animals were only slaughtered for special occasions (Abu-Saad et al., 2001). As land increasingly became used for cereal agriculture in the Late Ottoman Era, there would have been a shift away from foraging and small-scale agriculture. It remains unknown why the subadult skeletal remains from Tell Hisban exhibit such a high frequency of scorbutic lesions compared to other Ottoman period sites. However, differences in their diet caused by an increased dependence on cereals deficient in vitamin C and away from other foods that may have provided sufficient vitamin C may have negatively impacted the health of mothers and their young children. Further examination of the diets of individuals from Tell Hisban compared to surrounding sites is required to attempt any definitive conclusions about this Bedouin population's nutritional status and overall health.

$\delta^{13}C$ values derived from bone can either reflect the collagen component of an individual's diet and thus offer insight into the proteins consumed during life or the mineral component, hydroxyapatite, that reflects their whole diet (i.e., carbohydrates, fats, and protein) (Ambrose and Norr, 1993). Carbon isotope values differ between C_3 and C_4 resources depending

on how the atmospheric carbon fixes during photosynthesis (Craig, 1954). C_4 plants (e.g., sorghum, millet, and other tropical grasses) typically range from -14% to -9%, while C_3 plants (e.g., cereals, legumes, and other temperate plants) typically range from -35% to -20% (DeNiro, 1985; Gregoricka and Ullinger 2022). Additionally, trophic levels increase by approximately +5% between plants and consumer bone collagen (van der Merwe and Vogel, 1978) and up to +1% for secondary consumers (van Klinken et al., 2000). Therefore, an individual's diet can be approximated by comparing the $\delta^{13}C$ levels to that of local faunal remains.

While this study focuses on only one piece in a larger project to study the unique skeletal assemblage interred at Tell Hisban, examining the subadult sample has offered insight into possible factors impacting infant morbidity and mortality. Continued analysis, including stable nitrogen isotopic analysis ($\delta^{15}N$) of incremental samples of dental dentin, will continue to reveal critical information that will help us better reconstruct the lives and deaths of the Bedouin interred at the 19th Century cemetery at Tell Hisban.

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APPENDIX: Peptide IDs and Spectral Count for Tell Hisban Samples 01-24

The following tables depict each result from the mass spectrometry analysis of samples #01-#24 from the Tell Hisban site (tables 10-32). Each table includes the identifying AMELY peptides identified in each sample, the start and end location on the amino acid chain, and the spectral count for each peptide. The total spectral count was calculated by combining the spectral count for each peptide containing either AMELX or AMELY. For each table, the peptides unique to AMELY are indicated in red, those unique to AMELX are noted in blue, and the peptides in both proteins remain black.

Table A1. *Peptide IDs and spectral count for Tell Hisban sample #01*

SAMPLE #01				
AMELY/AMELX Peptides ID				
Start	**End**	**Peptide**	**Protein**	**Spectral Count**
17	**31**	**MPLPPHPGHPGYINF**	**AMELY**	**3**
17	**40**	**MPLPPHPGHPGYINFSYEVLTPLK**	**AMELX**	**1**
32	**50**	**SYEVLTPLKWYQSIRPPYP**	**AMELX**	**1**
33	**50**	**YEVLTPLKWYQSIRPPYP**	**AMELX**	**1**
Total Peptide Spectral Count				
Q99217-1		**AMELX_HUMAN**	**AMLEX-1**	**3**
Q99218-2		**AMELY_HUMAN**	**AMLEY-2**	**3**
Sex Assignment				**MALE**

Table A2. *Peptide IDs and spectral count for Tell Hisban sample #02*

SAMPLE #02				
AMELY/AMELX Peptides ID				
Start	**End**	**Peptide**	**Protein**	**Spectral Count**
157	**170**	**DLTLEAWPSTDKTK**	**AMELX**	**2**
157	**171**	**DLTLEAWPSTDKTKR**	**AMELX**	**4**

24	**34**	**KWYQSIRPPYP**	**AMELX**	**1**
160	**171**	**LEAWPSTDKTKR**	**AMELX**	**1**
155	**171**	**LPDLTLEAWPSTDKTKR**	**AMELX**	**1**
19	31	LPPHPGHPGYINF	AMELX, AMELY	3
19	40	LPPHPGHPGYINFSYEVLTPLK	AMELX, AMELY	2
165	**187**	**LRPLPPILPDLHLEAWPATDKTK**	**AMELY**	**3**
158	**171**	**LTLEAWPSTDKTKR**	**AMELX**	**1**
17	24	MPLPPHPG	AMELX, AMELY	1
17	25	MPLPPHPGH	AMELX, AMELY	1
17	26	MPLPPHPGHP	AMELX, AMELY	2
17	27	MPLPPHPGHPG	AMELX, AMELY	4
17	28	MPLPPHPGHPGY	AMELX, AMELY	1
17	30	MPLPPHPGHPGYIN	AMELX, AMELY	5
17	31	MPLPPHPGHPGYINF	AMELX, AMELY	10
17	40	MPLPPHPGHPGYINFSYEVLTPLK	AMELX, AMELY	2
148	**171**	**MQPLPPMLPDLTLEAWPSTDKTKR**	**AMELX**	**1**
18	31	PLPPHPGHPGYINF	AMELX, AMELY	1
167	**187**	**PLPPILPDLHLEAWPATDKTK**	**AMELY**	**1**
149	**171**	**QPLPPMLPDLTLEAWPSTDKTKR**	**AMELX**	**3**
27	**34**	**QSIRPPYP**	**AMELX**	**1**
166	**187**	**RPLPPILPDLHLEAWPATDKTK**	**AMELY**	**1**
32	40	SYEVLTPLK	AMELX, AMELY	1
32	**50**	**SYEVLTPLKWYQSIRPPYP**	**AMELX**	**9**
46	**55**	**TALVLTPLKW**	**AMELX**	**1**
46	**56**	**TALVLTPLKWY**	**AMELX**	**1**
46	**64**	**TALVLTPLKWYQSIRPPYP**	**AMELX**	**1**
159	**171**	**TLEAWPSTDKTKR**	**AMELX**	**1**
19	**34**	**VLTPLKWYQSIRPPYP**	**AMELX**	**2**
25	**34**	**WYQSIRPPYP**	**AMELX**	**1**
33	40	YEVLTPLK	AMELX, AMELY	1
33	42	YEVLTPLKWY	AMELX, AMELY	1
33	**50**	**YEVLTPLKWYQSIRPPYP**	**AMELX**	**4**
33	**50**	**YEVLTPLKWYQSMIRPPY**	**AMELY**	**1**

26	**34**	**YQSIRPPYP**	**AMELX**	**1**
Total Peptide Spectral Count				
Q99217-1		**AMELX_HUMAN**	**AMLEX-1**	**72**
Q99218-2		**AMELY_HUMAN**	**AMLEY-2**	**39**
Sex Assignment				**MALE**

Table A3. *Peptide IDs and spectral count for Tell Hisban sample #03*

SAMPLE #03				
AMELY/AMELX Peptides ID				
Start	**End**	**Peptide**	**Protein**	**Spectral Count**
174	**187**	**DLHLEAWPATDKTK**	**AMELY**	**1**
157	**170**	**DLTLEAWPSTDKTK**	**AMELX**	**2**
157	**171**	**DLTLEAWPSTDKTKR**	**AMELX**	**5**
157	**175**	**DLTLEAWPSTDKTKREEVD**	**AMELX**	**5**
31	40	FSYEVLTPLK	AMELX, AMELY	1
37	44	GYEPMGGW	AMELX, AMELY	1
29	40	INFSYEVLTPLK	AMELX, AMELY	2
177	**187**	**LEAWPATDKTK**	**AMELY**	**1**
177	**192**	**LEAWPATDKTKQEEVD**	**AMELY**	**1**
160	**171**	**LEAWPSTDKTKR**	**AMELX**	**2**
160	**175**	**LEAWPSTDKTKREEVD**	**AMELX**	**2**
45	**54**	**LHHQIIPVLS**	**AMELX**	**2**
19	28	LPPHPGHPGY	AMELX, AMELY	1
19	29	LPPHPGHPGYI	AMELX, AMELY	1
19	31	LPPHPGHPGYINF	AMELX, AMELY	4
19	40	LPPHPGHPGYINFSYEVLTPLK	AMELX, AMELY	3
165	**175**	**LRPLPPILPDL**	**AMELY**	**1**
165	**187**	**LRPLPPILPDLHLEAWPATDKTK**	**AMELY**	**2**
158	**171**	**LTLEAWPSTDKTKR**	**AMELX**	**1**
158	**175**	**LTLEAWPSTDKTKREEVD**	**AMELX**	**2**
17	24	MPLPPHPG	AMELX, AMELY	1
17	26	MPLPPHPGHP	AMELX, AMELY	1
17	27	MPLPPHPGHPG	AMELX, AMELY	4

17	28	MPLPPHPGHPGY	AMELX, AMELY	3
17	29	MPLPPHPGHPGYI	AMELX, AMELY	1
17	30	MPLPPHPGHPGYIN	AMELX, AMELY	4
17	31	MPLPPHPGHPGYINF	AMELX, AMELY	20
17	32	MPLPPHPGHPGYINFS	AMELX, AMELY	3
17	40	MPLPPHPGHPGYINFSYEVLTPLK	AMELX, AMELY	5
148	**171**	**MQPLPPMLPDLTLEAWPSTDKTKR**	**AMELX**	**2**
21	31	PHPGHPGYINF	AMELX, AMELY	1
18	31	PLPPHPGHPGYINF	AMELX, AMELY	3
167	**187**	**PLPPILPDLHLEAWPATDKTK**	**AMELY**	**1**
20	31	PPHPGHPGYINF	AMELX, AMELY	2
39	**54**	**QAINVDRTALVLTPLK**	**AMELX**	**1**
149	**171**	**QPLPPMLPDLTLEAWPSTDKTKR**	**AMELX**	**1**
134	**147**	**QPLPPQPPLPPMFP**	**AMELX**	**1**
55	**75**	**QQHPPTHTLQPHHHIPVVPAQ**	**AMELX**	**1**
45	**54**	**RTALVLTPLK**	**AMELX**	**1**
28	**40**	**SIRPPYPSYGYEP**	**AMELX**	**1**
28	**41**	**SIRPPYPSYGYEPM**	**AMELX**	**1**
28	**42**	**SIRPPYPSYGYEPMG**	**AMELX**	**2**
28	**43**	**SIRPPYPSYGYEPMGG**	**AMELX**	**1**
28	**44**	**SIRPPYPSYGYEPMGGW**	**AMELX**	**3**
44	**59**	**SMIRPPYSSYGYEPMG**	**AMELY**	**1**
38	**54**	**SQAINVDRTALVLTPLK**	**AMELX**	**1**
32	45	SYENSHSQAINVDR	AMELX, AMELY	1
32	40	SYEVLTPLK	AMELX, AMELY	1
32	41	SYEVLTPLKW	AMELX, AMELY	1
32	43	SYEVLTPLKWYQ	AMELX, AMELY	1
32	**50**	**SYEVLTPLKWYQSIRPPYP**	**AMELX**	**6**
32	**50**	**SYEVLTPLKWYQSMIRPPY**	**AMELY**	**1**
159	**171**	**TLEAWPSTDKTKR**	**AMELX**	**1**
159	**175**	**TLEAWPSTDKTKREEVD**	**AMELX**	**4**
21	**34**	**TPLKWYQSIRPPYP**	**AMELX**	**2**
43	**54**	**VDRTALVLTPLK**	**AMELX**	**1**

33	40	YEVLTPLK	AMELX, AMELY	1
33	41	YEVLTPLKW	AMELX, AMELY	1
33	43	YEVLTPLKWYQ	AMELX, AMELY	2
33	**50**	**YEVLTPLKWYQSIRPPYP**	**AMELX**	**5**
33	**52**	**YEVLTPLKWYQSIRPPYPSY**	**AMELX**	**1**
33	**53**	**YEVLTPLKWYQSIRPPYPSYG**	**AMELX**	**1**
33	**51**	**YEVLTPLKWYQSMIRPPYS**	**AMELY**	**3**
26	**34**	**YQSIRPPYP**	**AMELX**	**1**
26	**35**	**YQSIRPPYPS**	**AMELX**	**1**
26	**36**	**YQSIRPPYPSY**	**AMELX**	**1**
26	**37**	**YQSIRPPYPSYG**	**AMELX**	**1**
26	**40**	**YQSIRPPYPSYGYEP**	**AMELX**	**1**
26	**44**	**YQSIRPPYPSYGYEPMGGW**	**AMELX**	**1**
42	**51**	**YQSMIRPPYS**	**AMELY**	**1**
Total Peptide Spectral Count				
Q99217-1		**AMELX_HUMAN**	**AMLEX-1**	**133**
Q99218-2		**AMELY_HUMAN**	**AMLEY-2**	**82**
Sex Assignment				**MALE**

Table A4. *Peptide IDs and spectral count for Tell Hisban sample #04*

SAMPLE #04				
AMELY/AMELX Peptides ID				
Start	**End**	**Peptide**	**Protein**	**Spectral Count**
157	**170**	**DLTLEAWPSTDKTK**	**AMELX**	**1**
157	**171**	**DLTLEAWPSTDKTKR**	**AMELX**	**3**
157	**175**	**DLTLEAWPSTDKTKREEVD**	**AMELX**	**2**
161	**175**	**EAWPSTDKTKREEVD**	**AMELX**	**1**
31	40	FSYEVLTPLK	AMELX, AMELY	1
29	40	INFSYEVLTPLK	AMELX, AMELY	1
29	**41**	**IRPPYPSYGYEPM**	**AMELX**	**1**
177	**187**	**LEAWPATDKTK**	**AMELY**	**1**
177	**192**	**LEAWPATDKTKQEEVD**	**AMELY**	**1**
160	**175**	**LEAWPSTDKTKREEVD**	**AMELX**	**2**
45	**54**	**LHHQIIPVLS**	**AMELX**	**2**
19	31	LPPHPGHPGYINF	AMELX, AMLEY	2

19	40	LPPHPGHPGYINFSYEVLTPLK	AMELX, AMELY	1
158	**171**	**LTLEAWPSTDKTKR**	**AMELX**	**1**
158	**175**	**LTLEAWPSTDKTKREEVD**	**AMELX**	**1**
17	23	MPLPPHP	AMELX, AMELY	1
17	24	MPLPPHPG	AMELX, AMELY	4
17	25	MPLPPHPGH	AMELX, AMELY	1
17	26	MPLPPHPGHP	AMELX, AMELY	2
17	27	MPLPPHPGHPG	AMELX, AMELY	5
17	28	MPLPPHPGHPGY	AMELX, AMELY	4
17	29	MPLPPHPGHPGYI	AMELX, AMELY	1
17	30	MPLPPHPGHPGYIN	AMELX, AMELY	6
17	31	MPLPPHPGHPGYINF	AMELX, AMELY	14
17	32	MPLPPHPGHPGYINFS	AMELX, AMELY	1
17	35	MPLPPHPGHPGYINFSYEN	AMELX, AMELY	1
17	40	MPLPPHPGHPGYINFSYEVLTPLK	AMELX, AMELY	2
23	31	PGHPGYINF	AMELX, AMELY	2
21	31	PHPGHPGYINF	AMELX, AMELY	1
21	40	PHPGHPGYINFSYEVLTPLK	AMELX, AMELY	1
18	28	PLPPHPGHPGY	AMELX, AMELY	1
18	31	PLPPHPGHPGYINF	AMELX, AMELY	1
18	40	PLPPHPGHPGYINFSYEVLTPLK	AMELX, AMELY	1
20	31	PPHPGHPGYINF	AMELX, AMELY	1
39	**54**	**QAINVDRTALVLTPLK**	**AMELX**	**1**
55	**65**	**QQHPPTHTLQP**	**AMELX**	**1**
28	**36**	**SIRPPYPSY**	**AMELX**	**1**
28	**41**	**SIRPPYPSYGYEPM**	**AMELX**	**1**
28	**42**	**SIRPPYPSYGYEPMG**	**AMELX**	**1**
28	**43**	**SIRPPYPSYGYEPMGG**	**AMELX**	**1**

28	**44**	**SIRPPYPSYGYEPMGGW**	**AMELX**	**2**
44	**53**	**SMIRPPYSSY**	**AMELY**	**1**
38	**52**	**SQAINVDRTALVLTP**	**AMELX**	**1**
32	45	SYENSHSQAINVDR	AMELX, AMELY	1
32	40	SYEVLTPLK	AMELX, AMELY	1
32	41	SYEVLTPLKW	AMELX, AMELY	1
32	**50**	**SYEVLTPLKWYQSIRPPYP**	**AMELX**	**4**
46	**54**	**TALVLTPLK**	**AMELX**	**1**
159	**170**	**TLEAWPSTDKTK**	**AMELX**	**1**
159	**171**	**TLEAWPSTDKTKR**	**AMELX**	**1**
159	**175**	**TLEAWPSTDKTKREEVD**	**AMELX**	**2**
21	**34**	**TPLKWYQSIRPPYP**	**AMELX**	**2**
43	**54**	**VDRTALVLTPLK**	**AMELX**	**1**
116	**132**	**VQPQPHQPMQPQPPVHP**	**AMELX**	**1**
33	45	YENSHSQAINVDR	AMELX, AMELY	1
33	40	YEVLTPLK	AMELX, AMELY	2
33	41	YEVLTPLKW	AMELX, AMELY	1
33	43	YEVLTPLKWYQ	AMELX, AMELY	2
33	**50**	**YEVLTPLKWYQSIRPPYP**	**AMELX**	**5**
33	**53**	**YEVLTPLKWYQSIRPPYPSYG**	**AMELX**	**1**
26	**33**	**YQSIRPPY**	**AMELX**	**1**
26	**34**	**YQSIRPPYP**	**AMELX**	**1**
26	**35**	**YQSIRPPYPS**	**AMELX**	**1**
26	**36**	**YQSIRPPYPSY**	**AMELX**	**1**
26	**37**	**YQSIRPPYPSYG**	**AMELX**	**1**
26	**40**	**YQSIRPPYPSYGYEP**	**AMELX**	**1**
26	**41**	**YQSIRPPYPSYGYEPM**	**AMELX**	**1**
26	**42**	**YQSIRPPYPSYGYEPMG**	**AMELX**	**1**
26	**43**	**YQSIRPPYPSYGYEPMGG**	**AMELX**	**1**
26	**44**	**YQSIRPPYPSYGYEPMGGW**	**AMELX**	**1**
42	**50**	**YQSMIRPPY**	**AMELY**	**1**
42	**51**	**YQSMIRPPYS**	**AMELY**	**1**
42	**52**	**YQSMIRPPYSS**	**AMELY**	**1**
Total Peptide Spectral Count				
Q99217-1		**AMELX_HUMAN**	**AMLEX-1**	**116**
Q99218-2		**AMELY_HUMAN**	**AMLEY-2**	**70**
Sex Assignment				**MALE**

Table A5. *Peptide IDs and spectral count for Tell Hisban sample #05*

SAMPLE #05				
AMELY/AMELX Peptides ID				
Start	**End**	**Peptide**	**Protein**	**Spectral Count**
157	**171**	**DLTLEAWPSTDKTKR**	**AMELX**	**3**
157	**175**	**DLTLEAWPSTDKTKREEVD**	**AMELX**	**3**
19	29	LPPHPGHPGYI	AMELX, AMELY	1
19	30	LPPHPGHPGYIN	AMELX, AMELY	1
19	31	LPPHPGHPGYINF	AMELX, AMELY	2
19	40	LPPHPGHPGYINFSYEVLTPLK	AMELX, AMELY	2
165	**187**	**LRPLPPILPDLHLEAWPATDKTK**	**AMELY**	**3**
158	**171**	**LTLEAWPSTDKTKR**	**AMELX**	**1**
158	**175**	**LTLEAWPSTDKTKREEVD**	**AMELX**	**1**
17	24	MPLPPHPG	AMELX, AMELY	1
17	27	MPLPPHPGHPG	AMELX, AMELY	2
17	29	MPLPPHPGHPGYI	AMELX, AMELY	1
17	30	MPLPPHPGHPGYIN	AMELX, AMELY	3
17	31	MPLPPHPGHPGYINF	AMELX, AMELY	14
17	32	MPLPPHPGHPGYINFS	AMELX, AMELY	2
17	40	MPLPPHPGHPGYINFSYEVLTPLK	AMELX, AMELY	5
26	**50**	**PGYINFSYEVLTPLKWYQSMIRPPY**	**AMELY**	**1**
21	31	PHPGHPGYINF	AMELX, AMELY	2
18	31	PLPPHPGHPGYINF	AMELX, AMELY	2
167	**187**	**PLPPILPDLHLEAWPATDKTK**	**AMELY**	**1**
20	31	PPHPGHPGYINF	AMELX, AMELY	1
122	**132**	**QPMQPQPPVHP**	**AMELX**	**1**
112	121	QPQPVQPQPH	AMELX, AMELY	1
55	**73**	**QQHPPTHTLQPHHHIPVVP**	**AMELX**	**1**
45	**54**	**RTALVLTPLK**	**AMELX**	**1**
28	**36**	**SIRPPYPSY**	**AMELX**	**1**

28	**40**	**SIRPPYPSYGYEP**	**AMELX**	**1**
28	**42**	**SIRPPYPSYGYEPMG**	**AMELX**	**1**
28	**44**	**SIRPPYPSYGYEPMGGW**	**AMELX**	**2**
32	40	SYEVLTPLK	AMELX, AMELY	1
32	**50**	**SYEVLTPLKWYQSIRPPYP**	**AMELX**	**4**
19	**34**	**VLTPLKWYQSIRPPYP**	**AMELX**	**1**
33	41	YEVLTPLKW	AMELX, AMELY	1
33	43	YEVLTPLKWYQ	AMELX, AMELY	1
33	44	YEVLTPLKWYQS	AMELX, AMELY	1
33	**50**	**YEVLTPLKWYQSIRPPYP**	**AMELX**	**4**
33	**50**	**YEVLTPLKWYQSMIRPPY**	**AMELY**	**1**
33	**51**	**YEVLTPLKWYQSMIRPPYS**	**AMELY**	**1**
26	**34**	**YQSIRPPYP**	**AMELX**	**1**
26	**35**	**YQSIRPPYPS**	**AMELX**	**1**
42	**50**	**YQSMIRPPY**	**AMELY**	**1**
Total Peptide Spectral Count				
Q99217-1		**AMELX_HUMAN**	**AMLEX-1**	**71**
Q99218-2		**AMELY_HUMAN**	**AMLEY-2**	**52**
Sex Assignment				**MALE**

Table A6. *Peptide IDs and spectral count for Tell Hisban sample #06*

SAMPLE #06				
AMELY/AMELX Peptides ID				
Start	**End**	**Peptide**	**Protein**	**Spectral Count**
157	**175**	**DLTLEAWPSTDKTKREEVD**	**AMELX**	**1**
29	**37**	**IRPPYPSYG**	**AMELX**	**1**
19	31	LPPHPGHPGYINF	AMELX, AMELY	3
17	28	MPLPPHPGHPGY	AMELX, AMELY	1
17	29	MPLPPHPGHPGYI	AMELX, AMELY	1
17	30	MPLPPHPGHPGYIN	AMELX, AMELY	3
17	31	MPLPPHPGHPGYINF	AMELX, AMELY	11
17	40	MPLPPHPGHPGYINFSYEVLTPLK	AMELX, AMELY	2

21	31	PHPGHPGYINF	AMELX, AMELY	2
149	**158**	**QPLPPMLPDL**	**AMELX**	**1**
134	**147**	**QPLPPQPPLPPMFP**	**AMELX**	**1**
45	**54**	**RTALVLTPLK**	**AMELX**	**2**
28	**36**	**SIRPPYPSY**	**AMELX**	**1**
33	43	YEVLTPLKWYQ	AMELX, AMELY	1
33	**50**	**YEVLTPLKWYQSIRPPYP**	**AMELX**	**1**
33	**51**	**YEVLTPLKWYQSIRPPYPS**	**AMELX**	**1**
26	**35**	**YQSIRPPYPS**	**AMELX**	**1**
Total Peptide Spectral Count				
Q99217-1		**AMELX_HUMAN**	**AMLEX-1**	**34**
Q99218-2		**AMELY_HUMAN**	**AMLEY-2**	**24**
Sex Assignment				**FEMALE**

Table A7. *Peptide IDs and spectral count for Tell Hisban sample #07*

SAMPLE #07				
AMELY/AMELX Peptides ID				
Start	**End**	**Peptide**	**Protein**	**Spectral Count**
157	**171**	**DLTLEAWPSTDKTKR**	**AMELX**	**2**
34	**50**	**EVLTPLKWYQSIRPPYP**	**AMELX**	**1**
29	**42**	**IRPPYPSYGYEPMG**	**AMELX**	**1**
160	**175**	**LEAWPSTDKTKREEVD**	**AMELX**	**2**
19	30	LPPHPGHPGYIN	AMELX, AMELY	2
19	31	LPPHPGHPGYINF	AMELX, AMELY	4
158	**175**	**LTLEAWPSTDKTKREEVD**	**AMELX**	**1**
17	23	MPLPPHP	AMELX, AMELY	1
17	24	MPLPPHPG	AMELX, AMELY	2
17	26	MPLPPHPGHP	AMELX, AMELY	2
17	27	MPLPPHPGHPG	AMELX, AMELY	4
17	28	MPLPPHPGHPGY	AMELX, AMELY	4
17	29	MPLPPHPGHPGYI	AMELX, AMELY	1

17	30	MPLPPHPGHPGYIN	AMELX, AMELY	7
17	31	MPLPPHPGHPGYINF	AMELX, AMELY	19
17	32	MPLPPHPGHPGYINFS	AMELX, AMELY	1
17	40	MPLPPHPGHPGYINFSYEVLTPLK	AMELX, AMELY	4
21	31	PHPGHPGYINF	AMELX, AMELY	3
18	28	PLPPHPGHPGY	AMELX, AMELY	1
18	30	PLPPHPGHPGYIN	AMELX, AMELY	1
18	31	PLPPHPGHPGYINF	AMELX, AMELY	2
20	31	PPHPGHPGYINF	AMELX, AMELY	1
164	**175**	**PSTDKTKREEVD**	**AMELX**	**1**
149	**158**	**QPLPPMLPDL**	**AMELX**	**1**
134	**147**	**QPLPPQPPLPPMFP**	**AMELX**	**1**
45	**54**	**RTALVLTPLK**	**AMELX**	**1**
28	**34**	**SIRPPYP**	**AMELX**	**1**
28	**35**	**SIRPPYPS**	**AMELX**	**1**
28	**41**	**SIRPPYPSYGYEPM**	**AMELX**	**1**
28	**42**	**SIRPPYPSYGYEPMG**	**AMELX**	**1**
32	45	SYENSHSQAINVDR	AMELX, AMELY	1
32	40	SYEVLTPLK	AMELX, AMELY	1
32	**50**	**SYEVLTPLKWYQSIRPPYP**	**AMELX**	**3**
32	**53**	**SYEVLTPLKWYQSIRPPYPSYG**	**AMELX**	**1**
159	**175**	**TLEAWPSTDKTKREEVD**	**AMELX**	**1**
21	**34**	**TPLKWYQSIRPPYP**	**AMELX**	**1**
21	**45**	**TPLKWYQSIRPPYPSYGYEPMGGWL**	**AMELX**	**1**
19	**34**	**VLTPLKWYQSIRPPYP**	**AMELX**	**1**
33	40	YEVLTPLK	AMELX, AMELY	1
33	43	YEVLTPLKWYQ	AMELX, AMELY	1
33	**50**	**YEVLTPLKWYQSIRPPYP**	**AMELX**	**5**
33	**52**	**YEVLTPLKWYQSIRPPYPSY**	**AMELX**	**1**
33	**53**	**YEVLTPLKWYQSIRPPYPSYG**	**AMELX**	**1**
33	**57**	**YEVLTPLKWYQSIRPPYPSYGYEPM**	**AMELX**	**1**
26	**35**	**YQSIRPPYPS**	**AMELX**	**1**
26	**37**	**YQSIRPPYPSYG**	**AMELX**	**1**

Total Peptide Spectral Count			
Q99217-1	AMELX_HUMAN	AMLEX-1	96
Q99218-2	AMELY_HUMAN	AMLEY-2	63
Sex Assignment			FEMALE

Table A8. *Peptide IDs and spectral count for Tell Hisban sample #08*

SAMPLE #08				
AMELY/AMELX Peptides ID				
Start	End	Peptide	Protein	Spectral Count
157	171	DLTLEAWPSTDKTKR	AMELX	5
157	175	DLTLEAWPSTDKTKREEVD	AMELX	1
29	36	IRPPYPSY	AMELX	1
160	175	LEAWPSTDKTKREEVD	AMELX	1
19	30	LPPHPGHPGYIN	AMELX, AMELY	2
19	31	LPPHPGHPGYINF	AMELX, AMELY	5
19	40	LPPHPGHPGYINFSYEVLTPLK	AMELX, AMELY	3
19	43	LPPHPGHPGYINFSYEVLTPLKWYQ	AMELX, AMELY	1
158	171	LTLEAWPSTDKTKR	AMELX	1
158	175	LTLEAWPSTDKTKREEVD	AMELX	1
17	23	MPLPPHP	AMELX, AMELY	2
17	24	MPLPPHPG	AMELX, AMELY	5
17	25	MPLPPHPGH	AMELX, AMELY	2
17	26	MPLPPHPGHP	AMELX, AMELY	4
17	27	MPLPPHPGHPG	AMELX, AMELY	5
17	28	MPLPPHPGHPGY	AMELX, AMELY	6
17	30	MPLPPHPGHPGYIN	AMELX, AMELY	7
17	31	MPLPPHPGHPGYINF	AMELX, AMELY	37
17	40	MPLPPHPGHPGYINFSYEVLTPLK	AMELX, AMLEY	7
17	41	MPLPPHPGHPGYINFSYEVLTPLKW	AMELX, AMELY	1

133	**156**	**MQPLPPQPPLPPMFPMQPLPPMLP**	**AMELX**	**1**
21	31	PHPGHPGYINF	AMELX, AMELY	2
21	40	PHPGHPGYINFSYEVLTPLK	AMELX, AMELY	1
22	**34**	**PLKWYQSIRPPYP**	**AMELX**	**1**
18	31	PLPPHPGHPGYINF	AMELX, AMELY	2
149	**156**	**QPLPPMLP**	**AMELX**	**1**
149	**157**	**QPLPPMLPD**	**AMELX**	**1**
149	**158**	**QPLPPMLPDL**	**AMELX**	**2**
134	**146**	**QPLPPQPPLPPMF**	**AMELX**	**3**
134	**147**	**QPLPPQPPLPPMFP**	**AMELX**	**2**
134	**148**	**QPLPPQPPLPPMFPM**	**AMELX**	**4**
45	**54**	**RTALVLTPLK**	**AMELX**	**1**
28	**34**	**SIRPPYP**	**AMELX**	**1**
28	**35**	**SIRPPYPS**	**AMELX**	**1**
28	**36**	**SIRPPYPSY**	**AMELX**	**1**
28	**44**	**SIRPPYPSYGYEPMGGW**	**AMELX**	**1**
32	40	SYEVLTPLK	AMELX, AMELY	2
32	43	SYEVLTPLKWYQ	AMELX, AMELY	1
32	**50**	**SYEVLTPLKWYQSIRPPYP**	**AMELX**	**8**
32	**52**	**SYEVLTPLKWYQSIRPPYPSY**	**AMELX**	**1**
159	**175**	**TLEAWPSTDKTKREEVD**	**AMELX**	**2**
43	**54**	**VDRTALVLTPLK**	**AMELX**	**2**
116	**132**	**VQPQPHQPMQPQPPVHP**	**AMELX**	**2**
25	**34**	**WYQSIRPPYP**	**AMELX**	**1**
33	45	YENSHSQAINVDR	AMELX, AMELY	1
33	43	YEVLTPLKWYQ	AMELX, AMELY	1
33	**50**	**YEVLTPLKWYQSIRPPYP**	**AMELX**	**7**
33	**51**	**YEVLTPLKWYQSIRPPYPS**	**AMELX**	**1**
33	**52**	**YEVLTPLKWYQSIRPPYPSY**	**AMELX**	**1**
33	**53**	**YEVLTPLKWYQSIRPPYPSYG**	**AMELX**	**2**
33	**57**	**YEVLTPLKWYQSIRPPYPSYGYEPM**	**AMELX**	**1**
26	**34**	**YQSIRPPYP**	**AMELX**	**1**
26	**35**	**YQSIRPPYPS**	**AMELX**	**1**
26	**36**	**YQSIRPPYPSY**	**AMELX**	**1**
Total Peptide Spectral Count				
Q99217-1	**AMELX_HUMAN**		**AMLEX-1**	**158**
Q99218-2	**AMELY_HUMAN**		**AMLEY-2**	**97**
Sex Assignment				**FEMALE**

Table A9. *Peptide IDs and spectral count for Tell Hisban sample #09*

SAMPLE #09				
AMELY/AMELX Peptides ID				
Start	**End**	**Peptide**	**Protein**	**Spectral Count**
37	**45**	**HSQAINVDR**	**AMELY**	**1**
19	27	LPPHPGHPG	AMELX, AMELY	1
17	25	MPLPPHPGH	AMELX, AMELY	2
17	27	MPLPPHPGHPG	AMELX, AMELY	1
17	31	MPLPPHPGHPGYINF	AMELX, AMELY	3
18	27	PLPPHPGHPG	AMELX, AMELY	2
180	**187**	**PSTDKTKR**	**AMELX**	**4**
180	**191**	**PSTDKTKREEVD**	**AMELX**	**1**
179	**187**	**WPSTDKTKR**	**AMELX**	**2**
Total Peptide Spectral Count				
Q99217-1		**AMELX_HUMAN**	**AMLEX-1**	**16**
Q99218-2		**AMELY_HUMAN**	**AMLEY-2**	**10**
Sex Assignment				**MALE**

Table A10. *Peptide IDs and spectral count for Tell Hisban sample #10*

SAMPLE #10				
AMELY/AMELX Peptides ID				
Start	**End**	**Peptide**	**AMELY/AMELX**	**Spectral Count**
31	**40**	**FSYEVLTPLK**	**AMELX**	**1**
29	**40**	**INFSYEVLTPLK**	**AMELX**	**2**
19	28	LPPHPGHPGY	AMELX, AMELY	1
17	24	MPLPPHPG	AMELX, AMELY	1
17	25	MPLPPHPGH	AMELX, AMELY	1
17	26	MPLPPHPGHP	AMELX, AMELY	1
17	27	MPLPPHPGHPG	AMELX, AMELY	1
17	31	MPLPPHPGHPGYINF	AMELX, AMELY	4
17	**40**	**MPLPPHPGHPGYINFSYEVLTPLK**	**AMELX**	**1**
71	**82**	**QQHPPTHTLQPH**	**AMELX**	**2**
32	**45**	**SYENSHSQAINVDR**	**AMELY**	**1**
32	**40**	**SYEVLTPLK**	**AMELX**	**1**
Total Peptide Spectral Count				
Q99217-1		**AMELX_HUMAN**	**AMLEX-1**	**16**

Q99218-2	**AMELY_HUMAN**	**AMLEY-2**	**10**
Sex Assignment			**MALE**

Table A11. *Peptide IDs and spectral count for Tell Hisban sample #11*

SAMPLE #11				
AMELY/AMELX Peptides ID				
Start	**End**	**Peptide**	**AMELY/AMELX**	**Spectral Count**
17	26	MPLPPHPGHP	AMELX, AMELY	1
17	31	MPLPPHPGHPGYINF	AMELX, AMELY	1
18	27	PLPPHPGHPG	AMELX, AMELY	1
71	**82**	**QQHPPTHTLQPH**	**AMELX**	**1**
71	**83**	**QQHPPTHTLQPHH**	**AMELX**	**2**
Total Peptide Spectral Count				
Q99217-1		**AMELX_HUMAN**	**AMLEX-1**	**6**
Q99218-2		**AMELY_HUMAN**	**AMLEY-2**	**3**
Sex Assignment				**FEMALE**

*Protein ID for AMELY based on overlapping peptides with AMELX. No unique AMELY peptides were found.

Table A12. *Peptide IDs and spectral count for Tell Hisban sample #12*

SAMPLE #12				
AMELY/AMELX Peptides ID				
Start	**End**	**Peptide**	**AMELY/AMELX**	**Spectral Count**
17	31	MPLPPHPGHPGYINF	AMELX, AMELY	1
71	**82**	**QQHPPTHTLQPH**	**AMELX**	**1**
33	**40**	**YEVLTPLK**	**AMELX**	**1**
Total Peptide Spectral Count				
Q99217-1		**AMELX_HUMAN**	**AMLEX-1**	**3**
Q99218-2		**AMELY_HUMAN**	**AMLEY-2**	**1**
Sex Assignment				**FEMALE**

*Protein ID for AMELY based on overlapping peptides with AMELX. No unique AMELY peptides were found.

Table A13. *Peptide IDs and spectral count for Tell Hisban sample #14*

SAMPLE #14				
AMELY/AMELX Peptides ID				
Start	**End**	**Peptide**	**AMELY/AMELX**	**Spectral Count**
71	**83**	**QQHPPTHTLQPHH**	**AMELX**	**1**
Total Peptide Spectral Count				
Q99217-1		**AMELX_HUMAN**	**AMLEX-1**	**1**
Q99218-2		**AMELY_HUMAN**	**AMLEY-2**	**0**
Sex Assignment				**FEMALE**

*Cannot be unambiguously assigned, most likely female.

Table A14. *Peptide IDs and spectral count for Tell Hisban sample #15*

SAMPLE #15				
AMELY/AMELX Peptides ID				
Start	**End**	**Peptide**	**AMELY/AMELX**	**Spectral Count**
173	**184**	**DLTLEAWPSTDK**	**AMELX**	**1**
29	**40**	**INFSYEVLTPLK**	**AMELX**	**1**
19	**40**	**LPPHPGHPGYINFSYEVLTPLK**	**AMELX**	**1**
17	26	MPLPPHPGHP	AMELX, AMELY	2
17	27	MPLPPHPGHPG	AMELX, AMELY	2
17	28	MPLPPHPGHPGY	AMELX, AMELY	1
17	30	MPLPPHPGHPGYIN	AMELX, AMELY	1
17	31	MPLPPHPGHPGYINF	AMELX, AMELY	6
17	**40**	**MPLPPHPGHPGYINFSYEVLTPLK**	**AMELX**	**3**
43	**52**	**QSIRPPYPSY**	**AMELX**	**1**
43	**53**	**QSIRPPYPSYG**	**AMELX**	**1**
43	**57**	**QSIRPPYPSYGYEPM**	**AMELX**	**1**
43	**58**	**QSIRPPYPSYGYEPMG**	**AMELX**	**1**
43	**60**	**QSIRPPYPSYGYEPMGGW**	**AMELX**	**4**
44	**60**	**SIRPPYPSYGYEPMGGW**	**AMELX**	**1**
32	**45**	**SYENSHSQAINVDR**	**AMELY**	**1**
32	**40**	**SYEVLTPLK**	**AMELX**	**1**
175	**184**	**TLEAWPSTDK**	**AMELX**	**1**
41	**48**	**WYQSIRPP**	**AMELX**	**1**
41	**50**	**WYQSIRPPYP**	**AMELX**	**2**
41	**51**	**WYQSIRPPYPS**	**AMELX**	**2**
41	**52**	**WYQSIRPPYPSY**	**AMELX**	**1**
41	**53**	**WYQSIRPPYPSYG**	**AMELX**	**1**
41	**58**	**WYQSIRPPYPSYGYEPMG**	**AMELX**	**3**

41	59	WYQSIRPPYPSYGYEPMGG	AMELX	1
41	60	WYQSIRPPYPSYGYEPMGGW	AMELX	4
33	40	YEVLTPLK	AMELX	2
42	51	YQSIRPPYPS	AMELX	1
42	52	YQSIRPPYPSY	AMELX	1
42	53	YQSIRPPYPSYG	AMELX	1
42	56	YQSIRPPYPSYGYEP	AMELX	1
42	57	YQSIRPPYPSYGYEPM	AMELX	1
42	58	YQSIRPPYPSYGYEPMG	AMELX	1
42	59	YQSIRPPYPSYGYEPMGG	AMELX	1
42	60	YQSIRPPYPSYGYEPMGGW	AMELX	2
Total Peptide Spectral Count				
Q99217-1		AMELX_HUMAN	AMLEX-1	55
Q99218-2		AMELY_HUMAN	AMLEY-2	13
Sex Assignment				MALE

*Protein ID for AMELY based on overlapping peptides with AMELX. No unique AMELY peptides were found.

Table A15. *Peptide IDs and spectral count for Tell Hisban sample #16*

SAMPLE #16				
AMELY/AMELX Peptides ID				
Start	**End**	**Peptide**	**AMELY/AMELX**	**Spectral Count**
34	45	ENSHSQAINVDR	AMELY	2
85	99	IPVVPAQQPVIPQQP	AMELX	1
19	28	LPPHPGHPGY	AMELX, AMELY	2
152	163	LPPQPPLPPMFP	AMELX	1
17	24	MPLPPHPG	AMELX, AMELY	1
17	25	MPLPPHPGH	AMELX, AMELY	2
17	26	MPLPPHPGHP	AMELX, AMELY	2
17	27	MPLPPHPGHPG	AMELX, AMELY	2
17	31	MPLPPHPGHPGYINF	AMELX, AMELY	1
18	27	PLPPHPGHPG	AMELX, AMELY	3
180	187	PSTDKTKR	AMELX	3
180	191	PSTDKTKREEVD	AMELX	3
102	113	PVPGQHSMTPIQ	AMELX	1
71	82	QQHPPTHTLQPH	AMELX	1
36	45	SHSQAINVDR	AMELY	2
181	191	STDKTKREEVD	AMELX	3
33	40	YEVLTPLK	AMELX	1
Total Peptide Spectral Count				
Q99217-1		AMELX_HUMAN	AMLEX-1	27
Q99218-2		AMELY_HUMAN	AMLEY-2	17
Sex Assignment				MALE

***Table A16**. Peptide IDs and spectral count for Tell Hisban sample #17*

SAMPLE #17				
AMELY/AMELX Peptides ID				
Start	**End**	**Peptide**	**AMELY/AMELX**	**Spectral Count**
19	28	LPPHPGHPGY	AMELX, AMELY	1
17	24	MPLPPHPG	AMELX, AMELY	1
17	31	MPLPPHPGHPGYINF	AMELX, AMELY	3
71	**83**	**QQHPPTHTLQPHH**	**AMELX**	**1**
36	**45**	**SHSQAINVDR**	**AMELY**	**1**
33	**50**	**YEVLTPLKWYQSIRPPYP**	**AMELX**	**2**
Total Peptide Spectral Count				
Q99217-1		**AMELX_HUMAN**	**AMLEX-1**	**8**
Q99218-2		**AMELY_HUMAN**	**AMLEY-2**	**6**
Sex Assignment				**MALE**

***Table A17**. Peptide IDs and spectral count for Tell Hisban sample #18*

SAMPLE #18				
AMELY/AMELX Peptides ID				
Start	**End**	**Peptide**	**AMELY/AMELX**	**Spectral Count**
34	**45**	**ENSHSQAINVDR**	**AMELY**	**1**
61	**70**	**LHHQIIPVLS**	**AMELX**	**1**
19	27	LPPHPGHPG	AMELX, AMELY	1
17	24	MPLPPHPG	AMELX, AMELY	2
17	25	MPLPPHPGH	AMELX, AMELY	1
18	27	PLPPHPGHPG	AMELX, AMELY	2
180	**191**	**PSTDKTKREEVD**	**AMELX**	**2**
36	**45**	**SHSQAINVDR**	**AMELY**	**2**
181	**191**	**STDKTKREEVD**	**AMELX**	**2**
32	**40**	**SYEVLTPLK**	**AMELX**	**1**
182	**191**	**TDKTKREEVD**	**AMELX**	**1**
33	**40**	**YEVLTPLK**	**AMELX**	**1**
42	**50**	**YQSIRPPYP**	**AMELX**	**1**
42	**51**	**YQSIRPPYPS**	**AMELX**	**1**
42	**53**	**YQSIRPPYPSYG**	**AMELX**	**1**
Total Peptide Spectral Count				
Q99217-1		**AMELX_HUMAN**	**AMLEX-1**	**17**
Q99218-2		**AMELY_HUMAN**	**AMLEY-2**	**9**
Sex Assignment				**MALE**

Table A18. *Peptide IDs and spectral count for Tell Hisban sample #19*

SAMPLE #19				
AMELY/AMELX Peptides ID				
Start	**End**	**Peptide**	**AMELY/AMELX**	**Spectra Count**
17	31	MPLPPHPGHPGYINF	AMELX, AMELY	1
71	**82**	**QQHPPTHTLQPH**	**AMELX**	**1**
71	**83**	**QQHPPTHTLQPHH**	**AMELX**	**2**
Total Peptide Spectral Count				
Q99217-1		**AMELX_HUMAN**	**AMLEX-1**	**4**
Q99218-2		**AMELY_HUMAN**	**AMLEY-2**	**1**
Sex Assignment				**FEMALE**

*Protein ID for AMELY based on overlapping peptides with AMELX. No unique AMELY peptides were found.

Table A19. *Peptide IDs and spectral count for Tell Hisban sample #20*

SAMPLE #20				
AMELY/AMELX Peptides ID				
Start	**End**	**Peptide**	**AMELY/AMELX**	**Spectral Count**
34	**45**	**ENSHSQAINVDR**	**AMELY**	**1**
19	31	LPPHPGHPGYINF	AMELX, AMELY	1
152	**163**	**LPPQPPLPPMFP**	**AMELX**	**1**
17	25	MPLPPHPGH	AMELX, AMELY	1
17	27	MPLPPHPGHPG	AMELX, AMELY	1
17	31	MPLPPHPGHPGYINF	AMELX, AMELY	2
18	27	PLPPHPGHPG	AMELX, AMELY	1
71	**83**	**QQHPPTHTLQPHH**	**AMELX**	**2**
33	**40**	**YEVLTPLK**	**AMELX**	**1**
33	**50**	**YEVLTPLKWYQSIRPPYP**	**AMELX**	**1**
42	**50**	**YQSIRPPYP**	**AMELX**	**1**
Total Peptide Spectral Count				
Q99217-1		**AMELX_HUMAN**	**AMLEX-1**	**12**
Q99218-2		**AMELY_HUMAN**	**AMLEY-2**	**7**
Sex Assignment				**MALE**

Table A20. *Peptide IDs and spectral count for Tell Hisban sample #21*

SAMPLE #21				
AMELY/AMELX Peptides ID				
Start	**End**	**Peptide**	**AMELY/AMELX**	**Spectral Count**
37	**45**	**HSQAINVDR**	**AMELY**	**1**
19	27	LPPHPGHPG	AMELX, AMELY	2
17	24	MPLPPHPG	AMELX, AMELY	4
17	25	MPLPPHPGH	AMELX, AMELY	3
17	26	MPLPPHPGHP	AMELX, AMELY	2
17	27	MPLPPHPGHPG	AMELX, AMELY	3
17	31	MPLPPHPGHPGYINF	AMELX, AMELY	2
18	27	PLPPHPGHPG	AMELX, AMELY	2
74	**82**	**PPTHTLQPH**	**AMELX**	**1**
71	**82**	**QQHPPTHTLQPH**	**AMELX**	**1**
71	**83**	**QQHPPTHTLQPHH**	**AMELX**	**2**
36	**45**	**SHSQAINVDR**	**AMELY**	**2**
182	**191**	**TDKTKREEVD**	**AMELX**	**1**
33	**40**	**YEVLTPLK**	**AMELX**	**1**
Total Peptide Spectral Count				
Q99217-1		**AMELX_HUMAN**	**AMLEX-1**	**24**
Q99218-2		**AMELY_HUMAN**	**AMLEY-2**	**3**
Sex Assignment				**MALE**

Table A21. *Peptide IDs and spectral count for Tell Hisban sample #22*

SAMPLE #22				
AMELY/AMELX Peptides ID				
Start	**End**	**Peptide**	**AMELY/AMELX**	**Spectral Count**
71	**82**	**QQHPPTHTLQPH**	**AMELX**	**1**
Total Peptide Spectral Count				
Q99217-1		**AMELX_HUMAN**	**AMLEX-1**	**1**
Q99218-2		**AMELY_HUMAN**	**AMLEY-2**	**0**
Sex Assignment				**FEMALE**

*Cannot unambiguously assign, most likely female.

Table A22. *Peptide IDs and spectral count for Tell Hisban sample #23*

SAMPLE #23
AMELY/AMELX Peptides ID

Start	End	Peptide	AMELY/AMELX	Spectral Count
173	184	DLTLEAWPSTDK	AMELX	1
171	184	LPDLTLEAWPSTDK	AMELX	1
19	28	LPPHPGHPGY	AMELX, AMELY	1
17	24	MPLPPHPG	AMELX, AMELY	1
17	26	MPLPPHPGHP	AMELX, AMELY	2
17	27	MPLPPHPGHPG	AMELX, AMELY	3
17	30	MPLPPHPGHPGYIN	AMELX, AMELY	1
17	31	MPLPPHPGHPGYINF	AMELX, AMELY	3
164	184	MQPLPPMLPDLTLEAWPSTDK	AMELX	1
71	82	QQHPPTHTLQPH	AMELX	1
43	52	QSIRPPYPSY	AMELX	1
32	40	SYEVLTPLK	AMELX	1
175	184	TLEAWPSTDK	AMELX	1
41	50	WYQSIRPPYP	AMELX	2
41	51	WYQSMIRPPYS	AMELY	1
33	41	YEVLTPLKW	AMELX	1
33	50	YEVLTPLKWYQSIRPPYP	AMELX	3
42	50	YQSIRPPYP	AMELX	1
42	51	YQSMIRPPYS	AMELY	1
Total Peptide Spectral Count				
Q99217-1		AMELX_HUMAN	AMLEX-1	25
Q99218-2		AMELY_HUMAN	AMLEY-2	13
Sex Assignment				FEMALE

Table A23. *Peptide IDs and spectral count for Tell Hisban sample #24*

SAMPLE #24				
AMELY/AMELX Peptides ID				
Start	End	Peptide	AMELY/AMELX	Spectral Count
17	31	MPLPPHPGHPGYINF	AMELX, AMELY	2
18	27	PLPPHPGHPG	AMELX, AMELY	2
71	79	QQHPPTHTL	AMELX	1
71	82	QQHPPTHTLQPH	AMELX	1
71	83	QQHPPTHTLQPHH	AMELX	1
36	45	SHSQAINVDR	AMELY	1
181	191	STDKTKREEVD	AMELX	1
Total Peptide Spectral Count				
Q99217-1		AMELX_HUMAN	AMLEX-1	8
Q99218-2		AMELY_HUMAN	AMLEY-2	5