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Nutritional Stress & Early Development in the Neolithic Near East: A Comparison of Adult and Subadult Tooth Size

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By

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Abstract

The transition from hunting and gathering to farming during the Neolithic Revolution in the Near East came with a diet that relied heavily on domesticated crops. This change in diet could lead to a reliance on lower nutritional quality foods compared to the hunter-gatherer diet. Such a decline in nutritional quality could lead to an increase in nutritional stress among early agriculturalists. Stress could occur at various stages along the life course and could be related to the mother-infant dyad. Stress can be recorded in the dentition that form *in utero* and throughout infancy, childhood, and adolescence. Therefore, this study aims to evaluate size of the dentition of adults and subadults to evaluate potential changes in stress during the Neolithic in relation to diet. A comparison of adult and subadult teeth was performed between the populations of Çatalhöyük and Aşıklı Höyük, along with a comparison of mean tooth size between the populations of Çatalhöyük, Aşıklı Höyük, Boncuklu Höyük, and Tepecik-Çiftlik. Dietary isotopes were evaluated to identify any possible nutritional deficiencies that potentially caused a delay in enamel formation during early infancy. Results indicate Nevalı Çori and Çayönü Tepesi had the lowest meat consumption than more recent Neolithic Near East populations, indicating a gradual increase of animal protein consumption throughout the Neolithic period within the region. Canine size among subadults at Aşıklı Höyük was smaller than adults which could indicate maternal and infant stress. Overall Çatalhöyük mean tooth size was smaller than that of any other Neolithic site for which data were available. Smaller tooth size observed at Çatalhöyük may be due to experienced stress or could be related to dental wear.

Keywords: Neolithic, nutritional stress, DOHaD, tooth size

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INTRODUCTION

The mother infant relationship is unique in that the mother’s own health, activities, and nutritional status impact the health of her own child while simultaneously influencing her health through lactation (Moffat & Prowse, 2018). Since a mother’s offspring is highly reliant on her for their nutritional needs from the time of conception to the introduction of complementary foods, the examination of subadult remains may provide insight into a population’s adaptability to their own environment (Lewis, 2019). Taking a life course approach into the examination of subadult remains helps us identify early life experiences that have the potential to impact later stages in life (Mays et al., 2017). The Developmental Origins of Health and Disease hypothesis (DOHaD) states that disproportionate fetal growth and undernutrition during early infancy may lead to diseases such as cardiovascular disease, type 2 diabetes and hypertension later in life (Barker, 2003).

The DOHaD is mainly associated with health outcomes, such as cardiovascular disease and diabetes, illnesses that cannot be observed in the bioarchaeological record (Temple, 2019). However, examination of DOHaD by Armelagos et al. (2009) and Temple (2019) emphasizes the importance of examining teeth due to the influence of maternal stress on enamel formation. Although tooth size is strongly influenced by genetic factors (Kieser, 1990), environmental stress, such as malnutrition and disease, has been shown to influence dental development (Garn et al., 1980; Mountain et al., 2021). For example, Guagliardo (1982) identified subadults with smaller tooth size than their adult counterparts from the Averbuch site located in Tennessee. This stunting in tooth size was attributed to malnutrition or disease due to overpopulation and limited availability of resources. Stojanowski et al. (2007) conducted a similar study comparing the tooth sizes of adult and subadults at the Florida site of Mission San Luis de Apalachee and found significantly smaller subadult teeth compared to the adults. Like Guagliardo (1982), Stojanowski et al. (2007) concluded a reduction in tooth size was likely caused by the decline of quality in life related to diet, morbidity and generalized stress.

Through the early introduction of agriculture in the Near East, so too came a documented elevation of nutritional stress (Larsen, 2003). According to Larsen (2000), the transition to agriculture not only resulted in a change in skeletal size, but in tooth size. The early agricultural diet focused more on a limited variety of plant-based foods, causing a decrease in meat consumption. This increased reliability on crops, such as cereals and legumes, potentially created the risk of micronutrient deficiencies (i.e., vitamin B-12) that likely raised the nutritional stress within these early agricultural societies (Larsen, 2003). As the mother and infant share a unique bond that impacts one another's health, this study aims to explore differential survival of children and adults through an examination of tooth size and diet. Since the permanent dentition begins to form *in utero* throughout childhood and does not remodel, teeth from Near Eastern Neolithic sites were analyzed to identify potential stress throughout the life course. Moreover, isotopic data can inform our understanding of diets in the past. Therefore, isotopic data were also explored to further understand past diet and stress. The sites of interest include: Boncuklu Höyük, Aşıklı Höyük, Çatalhöyük, Tepecik-Çiftlik, Çayönü Tepesi, Nevalı Çori, Tell el-Kerkh. To identify possible maternal nutritional stress on early development, which could be associated with increased stress at a populational level, the following research hypotheses were investigated.

1. Throughout the Neolithic there was a dietary shift to rely more heavily on domesticated grains that would have led to an increase in nutritional stress.
2. Dependance on domesticated grains created enough nutritional stress to inhibit enamel growth during early development, resulting in smaller teeth.
3. Early developmental stress was connected to maternal nutritional health.

BACKGROUND

Neolithic background

Plant and animal domestication began in the Fertile Crescent of Southwest Asia over 10,000 years ago and resulted in significant changes to social complexity, population size, mobility, nutrition, and lifestyle (Larsen et al., 2015; Larsen et al., 2019). Childe (1936) explained the Neolithic Revolution as a time when humankind was able to produce nutritious grains that could easily be stored and a time of increased reproductivity, leading one to believe that it was a time of improved health and security. As research advanced over the past century, prehistorians argued that the transition to agriculture provided larger quantities of food for growing populations by producing low quality and less desirable foods (Cohen, 1984). The transition to agriculture created a diet often reliant on domesticated animals and limited crops. The study of human skeletal remains has advanced our understanding of nutritional stress through the observation of increased pathological conditions of the oral cavity, occlusal abnormalities, iron deficiency anemia, infection, and bone loss (Larsen, 2003). To further research the implications of early agricultural diets on early child development, information was gathered from following Neolithic Near East archaeological sites: Çayönü Tepesi, Nevalı

Çori, Boncuklu Höyük, Tell el-Kerkh, Aşıklı Höyük, Çatalhöyük, and Tepecik-Çiftlik (Fig. 1).

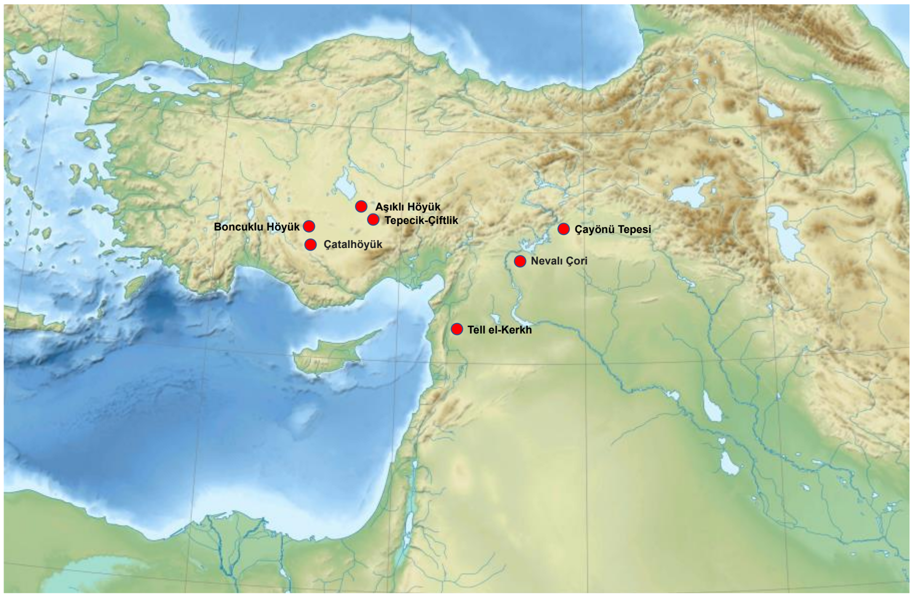

Figure 1. Locations of Çayönü Tepesi, Nevalı Çori, Boncuklu Höyük, Tell el-Kerkh, Aşıklı Höyük, Çatalhöyük, and Tepecik-Çiftlik.

Çayönü Tepesi

The site of Çayönü Tepesi (10,200 – 8,270 cal. BC) is located on the Ergani plain in southeastern Anatolia (Pilloud et al., 2020). The landscape was composed of woodland and forest steppe with little access to aquatic resources (Pearson et al., 2013). Plants consumed included wild nuts, peas, lentil, and bitter vetch. The importance of cereals (*Triticum* spp.) varies throughout the occupation with pulses an alternative (Pearson et al., 2013; Özbek, 1995). Pigs were the primary faunal assemblage (likely in states of protodomestication), followed by caprines (mostly sheep), cattle and red deer (Pearson et al., 2013; Pilloud et al., 2020). As can be seen throughout the archaeological record, the gathering of wild vegetation and hunting of wild game was still an important part of the Çayönü Tepesi diet and was in the early transition to an agricultural lifestyle.

The mortuary practices of Çayönü Tepesi's early aceramic phases contain intramural and extramural burials (Pilloud et al., 2020). The later aceramic phases primarily used a large public space to place their remains. This building is known as the "Skull Building" and contains remains of males and females of different ages. Human skeletal remains also included "decapitated" skeletons and burnt remains (Beyer-Honca, 1995). In much later phases, residents returned to burying their dead beneath the floors of their homes (Pilloud et al., 2020).

Nevalı Çori

Nevalı Çori is located in the upper Euphrates basin in southeast Anatolia and likely dates from 8,620 BCE to 8,200 BCE (Tobolczyk, 2016). The population consumed pulses such as lentil, field pea, grass-pea, bitter vetch, and horse bean. Nevalı Çori is one of the earliest known sites to display evidence of domesticated stock, which mainly included sheep and goats (Arbuckle & Hammer, 2019; Lösch et al., 2006). Wild animals were also included in their diet, which was composed of gazelle, hare, red deer, boar, and goat (Grupe & Peters, 2011). Research by Lösch et al. (2006) suggests that the Nevalı Çori population may have had difficulty procuring meat, causing them to rely heavily on a plant-based diet. Grupe and Peters (2011) have stated that early farmers may have taken advantage of C_4-plants, which are unsuitable for human consumption, by feeding it to their livestock, thus reducing resource competition between the domesticated animals and human occupants.

Inhumations have been found beneath the floors of homes and between buildings. Most inhumations were secondary burials. One skull was found to have a dagger beneath it and has been interpreted as a tool for defleshing. Other burials suggest some skulls may have been retrieved from interment after the decay of the soft tissue (Lichter, 2016).

Boncuklu Höyük

Boncuklu Höyük (8,500 – 7,500 cal. BC) is located on the Konya Plain of Central Anatolia (Pilloud et al., 2020). Its assemblage includes large amounts of wetland species in addition to large mammals, such as cattle and boar (Baird et al., 2018; Pilloud et al., 2020). Archaeological evidence has suggested that greater significance was placed on wetland exploitation (i.e., fish and waterfowl) and less importance placed on the semiarid woodland, such as almond and terebinth (Baird et al., 2018). The diet was comprised of cattle and boar as well as bird and fish. Domesticated crops included emmer and free-threshing wheat, barley, and einkorn wheat (Pilloud et al., 2020).

Boncuklu Höyük appeared to have removed the heads of their dead and buried them beneath the floors of their houses. Additional burials have also been uncovered in midden areas, along with bodies that have no skulls (Pilloud et al., 2020).

Tell el-Kerkh

Tell el-Kerkh (8,700 – 5,700 cal. BC) is in the south Rouj Basin in northwest Syria. The faunal assemblage consists of wild (gazelle and red deer) and domesticated (cattle, pigs, sheep, and goats) animals. A lake was located in the middle of the basin, which allowed for the gathering of aquatic animals (fallfish, catfish, and frogs) from either the lake or tributaries (Itahashi et al., 2018). Vegetation did not only include emmer grains, but also chickpea, faba bean, broad bean, and horse bean (Tanno & Willcox, 2006).

Types of burials at Tell el-Kerkh consist of primary inhumation, secondary burial, and cremation burial. All primary inhumation burials were in flexed positions (Tsuneki, 2011). Skulls and long bones were occasionally removed from the primary burial and placed in a shallow pit. There is evidences of collective burials and those who were cremated were done so in shallow cremation pits (Tsuneki, 2011)

Aşıklı Höyük

The Archaeological site of Aşıklı Höyük (8,200 – 7,300 cal. BC) is located on the bank of the Melendiz River in the Cappadocia region of Turkey (Pilloud et al., 2020). Plant macrofossils and ground stone artifacts have provided evidence of cereal grasses, pulses, hackberry, nuts, and seeds (Stiner et al., 2014). Aşıklı Höyük meat consumption levels stayed rather consistent while gradually transitioning to animal domestication (Itahashi et al., 2021; Stiner et al., 2014). Although small amounts of fresh water fish bones were found among the faunal assemblage, isotopic analysis indicates a primarily terrestrial diet (Itahashi et al., 2021).

Remains at Aşıklı Höyük were also found beneath the floors of houses. Most burials were single primary interments and were either in the flexed or extended position. Those that were not single interments were double burials (Esin et al., 1991).

Çatalhöyük

Çatalhöyük dates from approximately 7,100 cal. BC to 5,950 cal. BC and is located in Central Anatolia (Pilloud et al., 2020). Domesticated animals were composed of sheep, goat, and some cattle. Wild animals included aurochs, boars, deer, and hare. However, according to Pearson et al. (2015) cattle were the main source of meat due to their large size. Also, new research has provided evidence of milk consumption at

Çatalhöyük, which suggests milk was predominantly derived from sheep and goats (Hendy et al., 2018). Cultivated plants are comprised of emmer, einkorn, barley, and legumes (Richards et al., 2003). Although wild plants were likely a part of the Çatalhöyük diet, domesticated plants dominated and included wheat, barley, pea, lentil, and bitter vetch (Fairbairn et al., 2005).

Single interment burials beneath platforms form the majority of interments in Çatalhöyük, which were typically located beneath the central room of houses (Boz & Hager, 2013). Remains were placed in a flexed position and several primary burials were found to be missing a skull (Andrews et al., 2005; Pilloud et al., 2020). Interestingly, cut marks have been found on the atlas vertebra of one adult burial missing the cranium and mandible (Andrews et al., 2005). Also, a skull was uncovered which had been plastered and painted red (Boz & Hager, 2013).

Tepecik-Çiftlik

Tepecik-Çiftlik was occupied from approximately 7,000 cal. BC to 5,599 cal. BC and is located in Cappadocia, Turkey (Pilloud et al., 2020). The Tepecik-Çiftlik population relied on domesticated and wild animals, but increased consumption of wild animals towards the end of the Neolithic period. The archaeobotanical record is constructed of domesticated wheat, barley, pea, bitter vetch, and lentil (Kameray et al., 2017). Tepecik-Çiftlik contains intramural and extramural burials and there is evidence to suggest certain burial areas were reused. A large mass grave containing individuals of both sexes and all ages was found in level five (Pilloud et al., 2020).

METHODS

Data Collection

Since isotopic data derived from bone collagen reflects protein consumed, levels of δ^{15}N and δ^{13}C have been used to determine the source of dietary protein (Pearson et al., 2013). Therefore, data on dietary isotopes were derived from the literature to evaluate dietary changes. Nitrogen samples (δ^{15}N) were identified for Aşıklı Höyük (Itahashi et al., 2021; Pearson et al., 2010), Çatalhöyük (Richards et al., 2003), Çayönü Tepesi (Pearson et al., 2010), Tell el-Kerkh (Itahashi et al., 2018), and Nevalı Çori (Lösch et al., 2006). Carbon samples (δ^{13}C) were identified from Aşıklı Höyük (Itahashi et al., 2021), Çatalhöyük (Richards et al., 2003), Tell el-Kerkh (Itahashi et al., 2018), and Nevalı Çori (Lösch et al., 2006) (Table 1). The estimated-age-at-death for the individuals sampled range from fetus to adult. These samples were selected: 1) to gain a general overview of dietary differences among Neolithic populations, and 2) to identify and compare weaning patterns. It was assumed that populations who relied more heavily on plant-based diets would encounter greater rates of iron and vitamin B-12 deficiencies and therefore exhibit greater signs of nutritional stress. In addition to overall population diet, weaning patterns were also of interest because the introduction of solid foods aligns with an infant's decline in maternal nutritional reliance, meaning the end of the mother infant dyad and the beginning of independent food consumption. Boxplots were created using ggplot2 in the R statistical computing environment (R Core Team, 2021) using all available dietary isotopic data to visualize differences between each population. The purpose of the boxplots was to create a visual representation of change in diet over time throughout the Neolithic Near East and identify Neolithic populations who may have been at greater risk of dietary deficiencies. The visualization was also used to identify populations who may have experienced less nutritional stress and whose enamel defects may be caused by other sources, such as generalized stress and overpopulation. Infant and child $\delta^{15}N$ data were used to create a scatterplot to observe differences in weaning patterns between Çatalhöyük, Aşıklı Höyük, and Çayönü Tepesi. Only infant $\delta^{15}N$ data for these three sites were used because nitrogen isotope data for additional sites was not found in published literature.

Table 1. Total number of isotopic samples and age range for each site

Site	N	Ages	Type of Isotope	References
Çatalhöyük	50	Infant to adult	Nitrogen	(Richards et al., 2003)
	50	Infant to adult	Carbon	(Richards et al., 2003)
Aşıklı Höyük	68	Fetus to adult	Nitrogen	(Itahashi et al., 2021; Pearson et al., 2010)
	44	Child to adult	Carbon	(Itahashi et al., 2021)
Tell el-Kerkh	18	Child to adult	Nitrogen	(Itahashi et al., 2018)
	18	Child to adult	Carbon	(Itahashi et al., 2018)
Nevalı Çori	45	Infant to adult	Nitrogen	(Lösch et al., 2006)
	45	Infant to adult	Carbon	(Lösch et al., 2006)
Çayönü Tepesi	36	Perinatal to adult	Nitrogen	(Pearson et al., 2010)

Odontometric data for the left maxillary and mandibular permanent teeth from four Neolithic sites in the Near East (Çatalhöyük, Boncuklu Höyük, Tepecik-Çiftlik, and Aşıklı Höyük) were provided by Dr. Marin Pilloud of the University of Nevada, Reno (Table 2). Buccolingual and mesiodistal diameters of the crown were measured using the guidelines provided by Moorrees and Reed (1964) and Hillson (1996). Buccolingual and mesiodistal dimensions of the cervix were also measured using the standards specified by Hillson et al. (2005). Each measurement was recorded to the nearest 0.10mm using digital calipers. Only the permanent maxillary and mandibular central incisors, canines, first molars, and permanent mandibular lateral incisors were incorporated into this study because calcification of these teeth begin during early infancy (Uzuner et al., 2017)

(Table 3), This is a time when offspring are highly reliant on breastmilk, which may change in nutritional quality if the lactating mother is deficient in micronutrients, such as vitamin B-12, folate and iodine (Allen, 2005).

Table 2. Number of individuals from each site with odontometric data

Site	Total	Subadults (3 - 12 years)	Adolescents* (12 – 20 years)	Adults Males (20+)	Adult Females (20+)	Adult Indeterminate Sex (20+)
Çatalhöyük	310	69	44	74	85	38
Boncuklu Höyük	6	2	0	NA	NA	4
Tepecik-Çiftlik	36	10	9	NA	NA	27
Aşıklı Höyük	33	6	2	11	14	0
*Adolescent tooth measurements were only used for ANOVA tests comparing tooth sizes between populations.						

Table 3. Chronology of permanent tooth development (Uzuner et al., 2017)

Tooth	Calcification Begins		Crown Completed	
	Maxillary	Mandibular	Maxillary	Mandibular
Central	3 mo	3 mo	4 ½ yr	3 ½ yr
Lateral	11 mo	3 mo	5 ½ yr	4 yr
Canine	4 mo	4 mo	6 yr	5 ¾ yr
Third premolar	20 mo	22 mo	7 yr	6 ¾ yr
Fourth premolar	27 mo	28 mo	7 ¾ yr	7 ½ yr
First Molar	32 wk in utero	32 wk in utero	4 ¼ yr	3 ¾ yr
Second Molar	27 mo	27 mo	7 ¾ yr	7 ½ yr
Third Molar	8 yr	9 yr	14 yr	14 yr

Statistical analyses of mean tooth size within Neolithic populations in the Near East were conducted in the R statistical computing environment (R Core Team, 2021). First, Welch two-sample t-tests were run in base R to identify differences in mean tooth size between adults and subadults within Çatalhöyük and Aşıklı Höyük. There was not enough adult and subadult dental measurements for Tepecik-Çiftlik to run a Welch t-test and observe a possible reduction in subadult mean tooth size. The aim of this test was to evaluate if there was a difference in permanent tooth size between subadults (as the non-survivors) and the adults (as the survivors). Smaller tooth size among the subadults could indicate increased stress during childhood resulting in increased mortality.

Second, it was presumed that populations who consumed greater amounts of plant-based foods, such as cereals and legumes, may be more susceptible to higher nutritional stress resulting in smaller tooth dimensions in comparison to populations who consumed greater amounts of animal-based foods. To evaluate this hypothesis, the mean dental measurements between Çatalhöyük, Aşıklı Höyük, Boncuklu Höyük, and Tepecik-Çiftlik were compared using a one-way analysis of variance (ANOVA).

RESULTS

Subadult Variation in Isotope Values

Subadult $\delta^{15}N$ values by age are provided in Figure 2. A sudden decline in $\delta^{15}N$ between each site in Figure 2 indicates a decline in breastmilk consumption and the start of the weaning process. Aşıklı Höyük had the shortest period of exclusive breastfeeding (EBF), which ended at 12 months of age. Çatalhöyük's period of EBF seems to vary between 12 months and 2.5 years of age (Pearson, 2018). Çayönü Tepesi subadults also show a significant drop in $\delta^{15}N$ at ~2 years of age (Pearson et al., 2010), leading to the assumption of a much higher reliance of plant-based foods in comparison to Aşıklı Höyük and Çatalhöyük (Pearson et al., 2010).

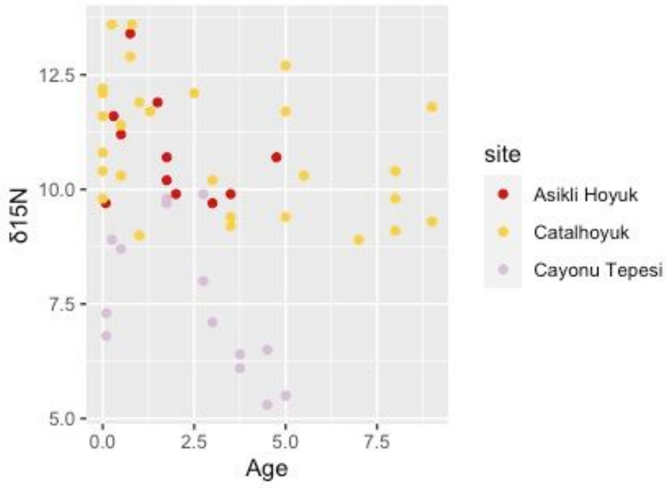

Figure 2. δ15N levels of subadults between the populations of Aşıklı Höyük, Çatalhöyük and Çayönü Tepesi. (Based on data from Pearson et al., 2010 & Richards et al., 2003)

Population Variation in Isotope Values

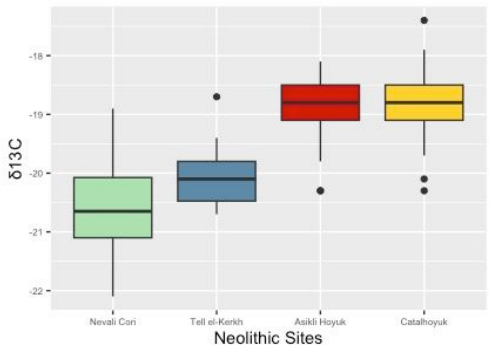

Figure 3. A comparison of δ13C levels between the populations of Nevalı Çori, Tell el-Kerkh, Aşıklı Höyük, and Çatalhöyük. Based on data from (Itahashi et al., 2021; Itahashi et al., 2018; Lösch et al., 2006; Pearson et al., 2010; Richards et al., 2003)

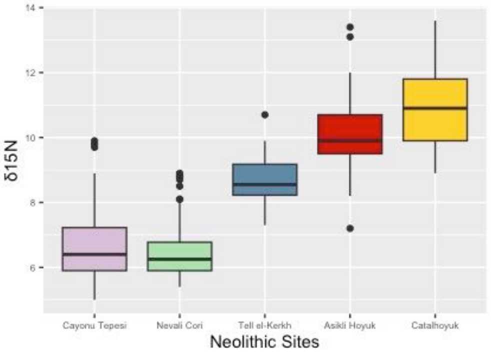

Figure 4. A comparison of δ15N levels between the populations of Çayönü Tepesi, Nevalı Çori, Tell el-Kerkh, Aşıklı Höyük, and Çatalhöyük. Based on data from (Itahashi et al., 2021; Itahashi et al., 2018; Lösch et al., 2006; Pearson et al., 2010; Richards et al.)

Changes in nitrogen and carbon isotopes between Near Eastern populations (adults and subadults) throughout the Neolithic period can be seen in Figures 3 and 4. Pre-pottery sites, such as Nevalı Çori, appear to have consumed far more plant-based protein in comparison to the later Neolithic sites of Aşıklı Höyük, and Çatalhöyük.

Odontometric Data of Subadults and Adults

In an examination of dental size differences of permanent teeth between subadults and adults in Aşıklı Höyük, only the buccolingual measurement of the maxillary canine was significantly different (Table 4 & 5). In this dimension, subadults displayed a significantly smaller mean measurement (7.65) compared to adults (8.28, p = 0.003). The mean dimensions of the maxillary first incisor, mandibular second incisor, mandibular canine, and mandibular first incisor were smaller within the subadult population in comparison to their adult counterparts but were not statistically significant. It should be noted that sex could not be determined among subadults; therefore, males and females were pooled together in the adult sample.

Table 4. Aşıklı Höyük T-test results for left maxillary dental metrics by age cohort.

	MAXILLARY						
	Subadult			Adult			
	N	Mean	SD	N	Mean	SD	P-value
I1_crn_bl	2	7.31	0.38	9	7.08	0.55	0.55
I1_crx_md	2	7.44	0.81	9	6.93	0.59	0.53
I1_crx_bl	2	6.17	0.60	10	6.45	0.34	0.63
C_crn_bl	2	7.65	0.09	13	8.28	0.59	0.003**
M1_crn_bl	4	11.37	0.65	6	11.36	0.41	0.98
M1_crx_md	3	8.09	0.52	9	7.78	0.57	0.44

Table 5. Aşıklı Höyük T-test results for left mandibular dental metrics by age cohort

	MANDIBULAR						
	Subadult			Adult			
I2_crn_bl	5	5.96	0.56	15	6.28	0.41	0.28
I2_crx_md	5	4.03	0.25	13	3.97	0.16	0.64
I2_crx_bl	5	5.71	0.64	15	6.08	0.37	0.28
C_crn_bl	6	7.37	0.53	20	7.63	0.53	0.31
C_crx_md	5	5.11	0.38	18	5.09	0.44	0.93
C_crx_bl	5	7.18	0.55	20	7.42	0.50	0.41
M1_crn_bl	6	10.3	0.55	17	10.37	0.52	0.73
**Significant at the α = 0.05 level.							

Tables 6 and 7 show the t-test results calculated between Çatalhöyük subadult and adult populations. Most mandibular and maxillary dentition resulted in a significant difference between mesiodistal dimensions, which demonstrated mean dimensions that were smaller among the adults than the subadult population. The smaller mesiodistal dimensions among the adult population is assumed to have been caused by dental wear, and not from a disruption in enamel formation caused by stressors. Pearson correlation coefficient tests were run between age category and tooth dimensions of Çatalhöyük mesiodistal measurements that were designated as significantly different in size. Correlation test resulted in a negative correlation, confirming that difference in size was likely due to dental wear, as there was a decrease in size with an increase in age. This difference of mesiodistal dimensions led to 15 out of 16 mesiodistal measurements of Çatalhöyük subadult means to be larger than the adult mean dimensions. Out of those 15 mesiodistal dimensions, 9 were significantly larger. There were no statistically significant differences between the buccolingual crown or cervical dimensions of any individual tooth. Just as the Aşıklı Höyük t-test, Çatalhöyük male and female adults were pooled together since sex was not estimated for the subadult sample.

Table 6. Çatalhöyük t-tests results for left maxillary dental metrics by age cohort

	MAXILLARY						
	Subadult			Adult			
Tooth	N	Mean	SD	N	Mean	SD	P-value
I1_crn_md	38	8.74	0.55	71	8.12	0.60	6.25E-07**
I1_crn_bl	43	7.06	0.55	91	6.98	0.35	0.34
I1_crx_md	38	6.63	0.62	88	6.37	0.55	0.03**
I1_crx_bl	38	6.38	0.50	91	6.27	0.40	0.22
I2_crn_md	36	6.40	0.72	73	6.24	0.44	0.26
I2_crn_bl	35	6.13	0.61	72	6.20	0.35	0.57
I2_crx_md	34	4.80	0.51	71	4.79	0.33	0.91
I2_crx_bl	32	5.80	0.58	73	5.76	0.36	0.70
C_crn_md	29	7.43	0.41	88	7.22	0.46	0.03**
C_crn_bl	30	8.14	0.72	111	8.07	0.56	0.63
C_crx_md	28	5.43	0.42	108	5.35	0.43	0.37
C_crx_bl	27	7.78	0.72	109	7.55	0.62	0.14
M1_crn_md	50	10.22	0.61	72	9.75	0.59	4.45E-05**
M1_crn_bl	58	11.26	0.62	108	11.23	0.54	0.76
M1_crx_md	56	7.78	0.51	104	7.67	0.56	0.24

M1_crx_bl	49	10.18	0.54	74	10.14	0.56	0.66
**Significant at the α = 0.05 level.							

Table 7. Çatalhöyük t-tests results for left mandibular dental metrics by age cohort.

	MANDIBULAR						
	Subadult			Adult			
Tooth	N	Mean	SD	N	Mean	SD	P-value
I1_crn_md	36	5.40	0.35	66	4.70	0.44	5.61E-14**
I1_crn_bl	36	5.81	0.48	63	5.89	0.38	0.36
I1_crx_md	36	3.60	0.27	66	3.52	0.26	0.14
I1_crx_bl	36	5.53	0.50	64	5.59	0.37	0.54
I2_crn_md	32	6.12	0.93	77	5.52	0.45	0.001**
I2_crn_bl	36	6.30	0.75	110	6.27	0.38	0.83
I2_crx_md	34	4.10	0.81	109	3.93	0.34	0.25
I2_crx_bl	33	6.07	0.59	111	5.98	0.40	0.42
C_crn_md	29	6.59	0.54	89	6.38	0.48	0.07**
C_crn_bl	30	7.72	0.73	134	7.57	0.55	0.30
C_crx_md	25	5.13	0.52	130	5.03	0.48	0.37
C_crx_bl	25	7.52	0.82	133	7.27	0.64	0.16
M1_crn_md	43	11.04	0.57	84	10.57	0.61	5.23E-05**
M1_crn_bl	53	10.30	0.70	134	10.38	0.50	0.44
M1_crx_md	50	8.87	0.53	127	8.69	0.56	0.04**
M1_crx_bl	41	8.87	0.49	81	8.75	0.57	0.18

**Significant at the α = 0.05 level.

Population comparisons of odontometrics

One-way ANOVA tests were employed to identify differences in mean tooth dimensions between the overall populations of Çatalhöyük, Tepecik-Çiftlik, Aşıklı Höyük, and Boncuklu Höyük (Tables 8 & 9). Overall population calculations included all permanent dentition of subadults, adolescents, and adults; sexes were pooled again as there were no sex estimates for the subadults. Boncuklu Höyük data were not available for each tooth but were incorporated into the maxillary first incisor and second incisor, as well as the mandibular first molar.

Each tooth showed a significant difference between populations (Tables 8 & 9). However, mesiodistal measurements appeared to be greatly affected by dental wear, especially among the Çatalhöyük adult population. Therefore, buccolingual dimensions were investigated further. The following buccolingual dimensions showed a significant statistical difference (α = 0.05) between populations (Figures 5-11): maxillary first incisor (crown), maxillary second incisor (crown and cervix), maxillary canine (cervix), maxillary first molar (crown), mandibular first molar (crown and cervix). Except for the first molar, the dentition of Çatalhöyük were the smallest.

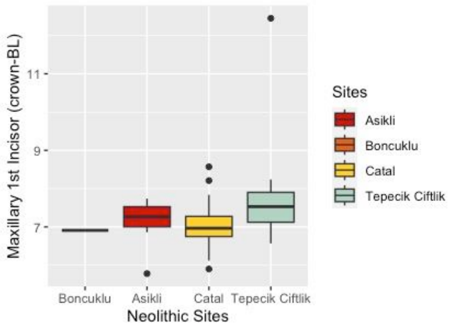

Figure 5. Comparison of the maxillary first incisor of the mean buccolingual crown dimensions between Boncuklu Höyük, Aşıklı Höyük, Çatalhöyük, and, Tepecik-Çiftlik populations.

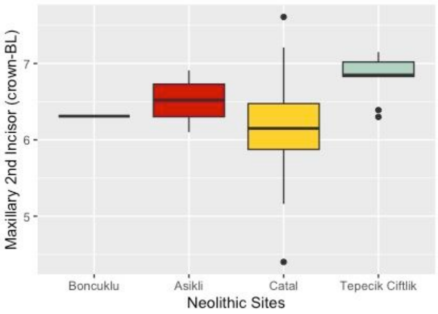

Figure 6. Comparison of the maxillary second incisor of the mean buccolingual crown dimensions between Boncuklu Höyük, Aşıklı Höyük, Çatalhöyük, and, Tepecik-Çiftlik populations.

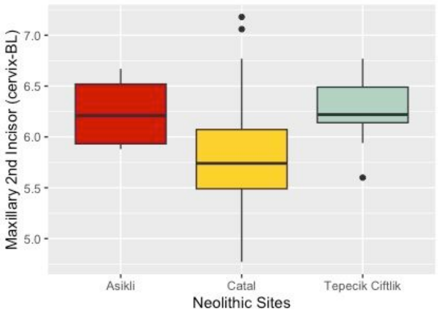

Figure 7. Comparison of the maxillary second incisor of the mean buccolingual cervix dimensions between Aşıklı Höyük, Çatalhöyük, and, Tepecik-Çiftlik populations.

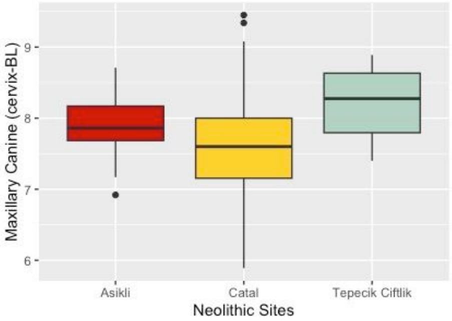

Figure 8. Comparison of the maxillary canine of the mean buccolingual cervix dimensions between Aşıklı Höyük, Çatalhöyük, and, Tepecik-Çiftlik populations.

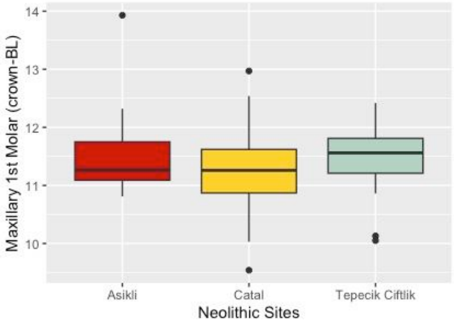

Figure 9. Comparison of the maxillary first molar of the mean buccolingual crown dimensions between Aşıklı Höyük, Çatalhöyük, and, Tepecik-Çiftlik populations.

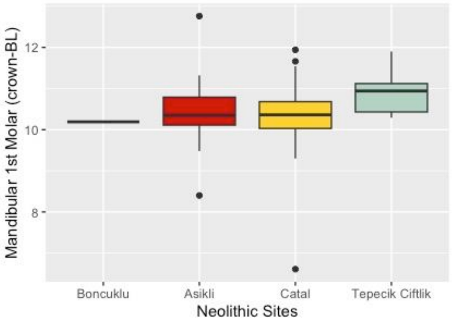

Figure 10. Comparison of the mandibular first molar of the mean buccolingual crown dimensions between Boncuklu Höyük, Aşıklı Höyük, Çatalhöyük, and, Tepecik-Çiftlik populations.

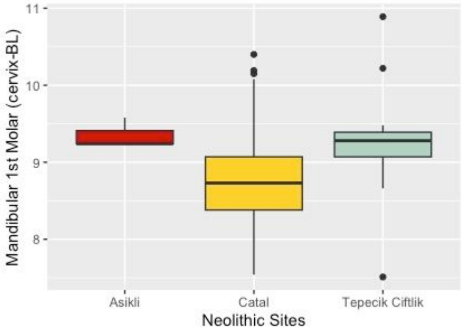

Figure 11. Comparison of the mandibular first molar of the mean buccolingual cervix dimensions between Aşıklı Höyük, Çatalhöyük, and, Tepecik-Çiftlik populations.

Table 8. ANOVA results comparing maxillary dental measurements of permanent teeth between populations of Çatalhöyük, Tepecik-Çiftlik, Aşıklı Höyük, and Boncuklu Höyük

Maxillary			
Tooth	Measurement	F-statistic	P value
I1*	Crown mesiodistal	7.90	6.68e-05**
I1*	Crown buccolingual	9.052	1.34e-05**
I1*	Cervix mesiodistal	5.119	0.002
I1*	Cervix buccolingual	1.65	0.18
I2*	Crown mesiodistal	4.324	0.006
I2*	Crown buccolingual	6.471	4.02e-04**
I2	Cervix mesiodistal	4.553	0.012**
I2	Cervix buccolingual	6.349	0.002**
C	Crown mesiodistal	6.246	0.003
C	Crown buccolingual	2.9	0.058
C	Cervix mesiodistal	4.262	0.016
C	Cervix buccolingual	4.479	0.013
M1	Crown mesiodistal	5.287	0.006**
M1	Crown buccolingual	3.114	0.046**
M1	Cervix mesiodistal	5.928	0.003**
M1	Cervix buccolingual	2.3	0.104

I, incisor; C, canine; M, molar
* Boncuklu Höyük data was not available for all calculations. Results with asterisk include Boncuklu Höyük dental measurements.
**Significant at the $\alpha = 0.05$ level.

Table 9. ANOVA results comparing mandibular dental measurements of permanent teeth between populations of Çatalhöyük, Tepecik-Çiftlik, Aşıklı Höyük, and Boncuklu Höyük

Mandibular			
Tooth	Measurement	F-statistic	P value
I1	Crown mesiodistal	4.496	0.013**
I1	Crown buccolingual	1.696	0.188
I1	Cervix mesiodistal	0.975	0.38
I1	Cervix buccolingual	0.876	0.419
I2	Crown mesiodistal	3.513	0.0325**
I2	Crown buccolingual	1.333	0.266
I2	Cervix mesiodistal	2.53	0.082
I2	Cervix buccolingual	1.193	0.305
C	Crown mesiodistal	6.94	0.001**
C	Crown buccolingual	2.374	0.095
C	Cervix mesiodistal	4.133	0.017**
C	Cervix buccolingual	1.361	0.259
M1*	Crown mesiodistal	1.109	0.347
M1*	Crown buccolingual	3.305	0.021**
M1*	Cervix mesiodistal	2.67	0.048**
M1*	Cervix buccolingual	3.93	0.010**

I, incisor; C, canine; M, molar
* Boncuklu Höyük data was not available for all calculations. Results with asterisk include Boncuklu Höyük dental measurements.
**Significant at the $\alpha = 0.05$ level.

Odontometric data of the permanent teeth were also divided by subadults for comparison across populations. Tables 10 and 11 show the one-way ANOVA results for the mean dimensions between subadult populations of Çatalhöyük, Aşıklı Höyük, Tepecik-Çiftlik, and Boncuklu Höyük. Once again, Boncuklu Höyük measurements were only available for the maxillary first incisor and second incisor, in addition to the mandibular first molar, and was therefore not incorporated into all ANOVA calculations.

A statistically significant difference (α = 0.05) was found between populations regarding the crown mesiodistal and buccolingual measurements of the maxillary first incisor. As seen in Figure 12, subadults from Tepecik-Çiftlik exhibit the largest mean dimensions, whereas Çatalhöyük and Boncuklu Höyük exhibit smaller mean dental dimensions. Also, the mean mesiodistal and buccolingual dimensions of the cervix of the maxillary first molar were also significantly different (Fig. 13), which demonstrated a significantly larger mean among Aşıklı Höyük subadults.

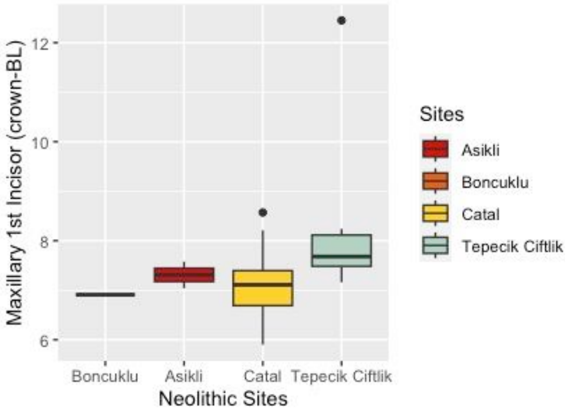

Figure 12. Comparison of subadult maxillary first incisor of the mean buccolingual crown dimensions between Boncuklu Höyük, Aşıklı Höyük, Çatalhöyük, and, Tepecik-Çiftlik populations.

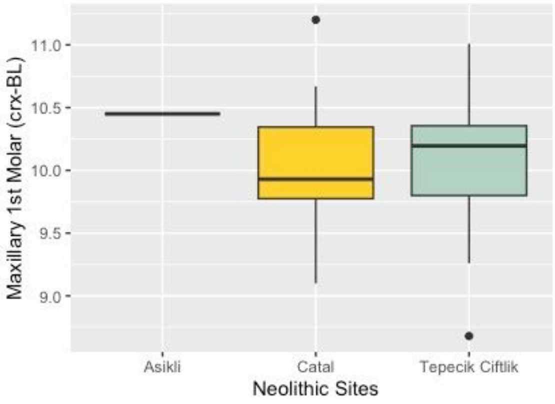

Figure 13. Comparison of subadult maxillary first molar of the mean buccolingual cervix dimensions between Aşıklı Höyük, Çatalhöyük, and, Tepecik-Çiftlik populations.

Table 10. ANOVA results comparing maxillary dental measurements of permanent teeth between subadults of Çatalhöyük, Tepecik-Çiftlik, Aşıklı Höyük, and Boncuklu Höyük*

Maxillary			
Tooth	Measurement	F-statistic	P-value
I1*	Crown mesiodistal	3.358	0.027**
I1*	Crown buccolingual	4.934	0.005**
I1*	Cervix mesiodistal	2.612	0.063
I1*	Cervix buccolingual	0.657	0.583
I2*	Crown mesiodistal	0.371	0.774
I2*	Crown buccolingual	1.716	0.18
I2	Cervix mesiodistal	0.393	0.678
I2	Cervix buccolingual	1.038	0.365
C	Crown mesiodistal	0.891	0.421
C	Crown buccolingual	0.893	0.42
C	Cervix mesiodistal	0.367	0.696
C	Cervix buccolingual	0.471	0.63
M1	Crown mesiodistal	0.77	0.468
M1	Crown buccolingual	0.12	0.887
M1	Cervix mesiodistal	4.293	0.018**
M1	Cervix buccolingual	3.394	0.041**

I, incisor; C, canine; M, molar

* Boncuklu Höyük data was not available for all calculations. Results with asterisk include Boncuklu Höyük dental measurements.

**Significant at the $\alpha = 0.05$ level.

*Table 11. ANOVA results comparing mandibular dental measurements of permanent teeth between subadults of Çatalhöyük, Tepecik-Çiftlik, Aşıklı Höyük, and Boncuklu Höyük**

Mandibular			
Tooth	Measurement	F-statistics	P-value
I1	Crown mesiodistal	0.409	0.667
I1	Crown buccolingual	1.633	0.208
I1	Cervix mesiodistal	0.809	0.453
I1	Cervix buccolingual	0.662	0.521
I2	Crown mesiodistal	0.033	0.968
I2	Crown buccolingual	0.734	0.486
I2	Cervix mesiodistal	0.133	0.876
I2	Cervix buccolingual	0.989	0.38
C	Crown mesiodistal	0.667	0.52
C	Crown buccolingual	0.782	0.465
C	Cervix mesiodistal	0.14	0.87
C	Cervix buccolingual	0.433	0.652
M1*	Crown mesiodistal	0.375	0.772
M1*	Crown buccolingual	0.54	0.657
M1	Cervix mesiodistal	0.391	0.678
M1	Cervix buccolingual	2.401	0.102

I, incisor; C, canine; M, molar

* Boncuklu Höyük data was not available for all calculations. Results with asterisk include Boncuklu Höyük dental measurements.

**Significant at the $\alpha = 0.05$ level.

DISCUSSION

Aşıklı Höyük subadults

The smaller subadult left maxillary canine of Aşıklı Höyük may not be as significant as it appears due to sexual dimorphism of the canine. There is approximately 3% to 7% difference in tooth size between males and females, with the canine being the most sexually dimorphic (Pilloud & Kenyhercz, 2016). Since the sex of the subadults is unknown, male and female adult measurements were not separated; therefore, if there are more females in the subadult sample it may be skewing the results. A majority of Aşıklı Höyük adults were female (61.5%, 8/13). Also, there were only two subadult canines to compare, this sample may be too small to imply that there was significant nutritional stress placed on the population.

There is a strong association between maternal vitamin B-12 deficiency and infant B-12 status at birth. Inadequate maternal intake of vitamin B-12 may further lower B-12 secretion in breastmilk and further expose infants to B-12 depletion (Allen, 2005). Symptoms caused by vitamin B-12 deficiency appear around four to seven months of age and include growth stunting, cerebral atrophy, and other developmental problems (Allen, 2012). That is also approximately the same age at which the maxillary canine begins calcification (Uzuner et al., 2017). Unlike Çatalhöyük, whose population did not see a significant difference in tooth size between their subadult and adult populations, the Aşıklı Höyük population consumed less animal sourced foods (ASF), which may account for an insufficient vitamin B-12 intake since almost all vitamin B-12 derives from ASF with the exception of fortified foods (Figure 4) (Allen, 2012).

While EBF refers to the period in which infants consume only breastmilk (Pearson, 2018; WHO, 2023), total breastfeeding (TBF) refers to an infant's consumption of breastmilk combined with complementary foods (Pearson, 2018). As demonstrated by Pearson (2018) and in Figure 2, Aşıklı Höyük appears not only to have the shortest EBF duration in comparison to Çatalhöyük and Çayönü Tepesi, but also the shortest TBF. Aşıklı Höyük subadults appear to have started weaning at 12 months of age with TBF ending at 2 years of age. The World Health Organization (2021) argues that TBF should last until two years of age. Complementary foods are foods especially made for infants to consume in additional to breastmilk (WHO, 2000) and should be much higher in nutrients than food consumed by adults. Most healthy term infants have iron stores that provide adequate amounts of iron until about six months of age. After six months, stores become depleted and complementary foods are needed due to the relatively low levels of iron in breastmilk (Miniello et al., 2021). For example, a six to eight month old who is breastfed requires nine times as much iron as an adult male (Dewey, 2013). Also, cereal-based diets typically do not contain adequate nutritional density for quickly growing infants. Porridges made from flour have low energy density. Cereals also contain phytate (antioxidant compounds found in whole grains, legumes, and nuts) which prevent iron and zinc absorption (Dewey, 2013). Therefore, smaller canines among Aşıklı Höyük subadults may be an indication of stress caused by early weaning and insufficient nutrients in available complementary foods.

The ANOVA results for the maxillary first incisor and maxillary first molar show the overall Aşıklı Höyük population as having larger teeth than Çatalhöyük, which may be caused by multiple variables. Although nutritional stress may still be a factor in tooth size reduction among the Çatalhöyük population, Brace et al. (1987) stated that tooth size reduction doubled from 1% per 2,000 years to 1% per 1,000 years and was caused by a change in food processing. Research conducted by Larsen et al. (2015) has used the stature estimates of Çatalhöyük adolescents and determined that childhood growth was normal and children were born into a fairly healthy environment. Also, Pinhasi and Meiklejohn (2011) state that the reduction in tooth size is a complex relationship between genetics, ontogenetic and environmental mechanisms and should be evaluated on a case-to-case geographical basis. Since Çatalhöyük was inhabited during the later Neolithic period, it would be advantageous to incorporate additional research related to diet and heredity before providing a possible conclusion for Çatalhöyük smaller tooth size.

Population comparisons

The ANOVA results revealing larger mean tooth dimensions among the Aşıklı Höyük and Tepecik-Çiftlik versus the Çatalhöyük and Boncuklu Höyük adult and subadult populations may speak to their difference in ASF consumption. Although isotopic values were not available for Tepecik-Çiftlik or Boncuklu Höyük, the archaeological record informs us that Boncuklu Höyük was an early Neolithic settlement that was in the earlier stages of agriculture. We also have evidence to suggest that the site relied on wetland resources as well as domesticated crops, such as wheat and barley (Baird et al., 2018; Pilloud et al., 2020). Boncuklu Höyük's plant assemblage is filled with nuts and seeds and has a high representation of wild cattle in addition to wild boar (Baird et al., 2018). Introducing domesticated crops into their diets may have decreased vitamin B-12 and iron consumption while simultaneously increasing their phytate consumption. However, like Çatalhöyük, multiple variables should be contemplated, and additional research is needed, especially since the amount of available tooth measurements for Boncuklu Höyük was much less than that of the other Neolithic sites. The archeological findings at Tepecik-Çiftlik suggest higher levels of meat consumption in the lower levels, but greater vegetable consumption in the upper level of the archaeological site (Kameray et al., 2017). Which level Tepecik-Çiftlik samples were taken from are unknown, however, their larger mean dimensions may indicate they lived during a period of higher ASF consumption. Since Tepecik-Çiftlik inhabitants increased plant and wild game consumption towards the end of the Neolithic period (Kameray et al., 2017), it may also appear that they adjusted subsistence strategies to better meet their nutritional needs. Further research is required to eliminate possible reasons for larger tooth size, including isotopic analysis of bone collagen and additional documentation of skeletal stress markers.

Phenotypic variation of the permanent tooth crown size is 56% - 92% affected by additive genetic variation, whereas 8% – 29% is influenced by environment (Hughes et al., 2016). Therefore, there is a possible 29% chance that the mean tooth size of Çatalhöyük was influenced by environmental factors, such as nutritional stress. With no difference between adult and subadult mean tooth size and a higher consumption of animal-based proteins it was assumed that Çatalhöyük mean population tooth size would be larger than Boncuklu Höyük, Aşıklı Höyük, and Tepecik-Çiftlik. As this is not the case, additional archaeological and biological contexts should be investigated as well as the possibility for additional stressors aside from dietary. Even the significant dental wear among the adult Çatalhöyük population should be considered seeing as several teeth showed significant amount of dental wear. In addition, the child ANOVA results only provided significant differences in population tooth size for the maxillary first incisor and maxillary first molar, with Çatalhöyük subadults having the smaller teeth (Fig. 12-13), which may indicate possible buccolingual wear among the adults and therefore impacting overall population size and its comparison to other populations. Additional research is needed and may include a comparison of frequency between other skeletal stress markers, such as linear enamel hypoplasia, between other Neolithic sites.

Except for early weaning practices and possible low-nutrient complementary foods, Aşıklı Höyük's ability to gradually transition from wild to domesticated animals may demonstrate a greater ability to adapt to the agricultural lifestyle than those of the other Neolithic archaeological sites. In both ANOVAs comparing overall population and subadult populations (Fig. 5-13), the mean size of the maxillary first molar and maxillary first incisor was larger than that of Çatalhöyük, which may reflect the nutritional well-being of the subadults' mothers since subadults would have relied exclusively on maternal nutritional resources during the initial development of these teeth. This may imply that the adult diet of Aşıklı Höyük provided greater nutritional density in comparison to Çatalhöyük or Boncuklu Höyük.

CONCLUSIONS

Comparisons between Çatalhöyük adult and subadult teeth suggest the dietary patterns in later Neolithic periods may not have created enough nutritional stress among mothers or children to disrupt enamel formation, demonstrating a possible adaptation to an agricultural diet. As seen in Figure 4, later Neolithic sites increased their reliance on ASFs, possibly decreasing the risk of iron and vitamin B-12 deficiencies. Smaller canine teeth among Aşıklı Höyük subadults may indicate weaning began too early and/or complementary foods were not nutritionally dense; therefore, creating high levels of nutritional stress during a time of high nutritional need. However, examining tooth size between Neolithic populations may indicate that Çatalhöyük was under greater amounts of generalized stress in comparison to other sites, such as Aşıklı Höyük or Tepecik-Çiftlik. To further understand the importance of the mother infant dyad in relation to nutritional stress within the Neolithic period additional research is suggested. Possible research may include the comparison of adult and subadult teeth of older Neolithic sites and the comparison of linear enamel hypoplasia frequency between Neolithic populations.

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