A STUDY OF THE EFFECT OF ALTITUDE AND LOCAL BIOLOGIES UPON NUNOAN BREAST MILK CONTENT

By

Lauren Alexis Schafrank

In

Anthropology

Submitted to the

Department of Anthropology

University of Pennsylvania

Thesis Adviser: Professor Morgan Hoke, Ph.D.

2019

Abstract

As a readily available substance and primary source of nutrition for infants, breast milk serves as a reasonable window into adaptive human physiology and human growth differences in physiologically stressed environments. Prior studies in Tibet show a considerable buffering of breast milk composition at high altitude but imply that economic status and cultural differences in breastfeeding practices influence milkfat content significantly. With an elevation of over 4000 meters above sea level, relatively low levels of socioeconomic status, and variability in women's schedules resulting in high variability of breastfeeding patterns, Nunoa, Peru provides an excellent location for investigating altitudinal and sociocultural impact on the nutritional value of breast milk. A mid-feed, self-expressed collection of breast milk was completed for 23 mothers. The samples were aliquoted into sterile vials within 30 minutes of collection and then frozen prior to analysis, ensuring maximum preservation of the macromolecules. Participants completed a detailed survey assessing sociodemographic characteristics, diet, and infant feeding practices. For some mothers, observations of breastfeeding behavior occurred, providing additional evidence for feeding variability. Significant variability in breastfeeding patterns and composition was observed. Primarily, in comparison to prior observations in highland Tibet, Nunoan mothers fed more frequently and for shorter bursts, impacting total volume and overall feeding time. Furthermore, Nunoan mothers produced milk with lower lactose levels (6.01 ± .89 g/100mL compared with 7.25 ± 0.35 g/100mL in Tibetan High Nubris). Nevertheless, breast milk fat composition in high-altitude populations of Nunoa (4.56±.78 g/100mL) was similar to that observed previously in the Tibetan highlands, and represented higher breast milk fat content than that seen in other comparative milk composition studies at lower altitudes. Our results suggest that individual anatomical indices of lower trunk fat are not the strongest predictors of milk fat levels. Instead, the Nunoan data suggests that relative distribution of anatomical fat is a more reliable predictor of milk fat concentrations. Specifically, maternal waist-to-hip ratio may in fact be a more reliable measurement to predict milk fat rather than the general measurements of adiposity currently used. This indicator provides a new method for predicting breast milk composition and opens the door for future studies of breast milk content in physiologically stressed populations and elsewhere.

Acknowledgements

I want to thank the community of Nuñoa for allowing me to come into their district, perform my work, and for their committed participation in my study. To everyone who underwent and listened to my broken Spanish, as I tried to explain my work, provided me with breast milk samples, and shared their maternity care stories, I can not thank you enough for all you have done for me and my research. As a future physician, I have gained knowledge that I could have never obtained in the classroom, and for that I thank you. You have reminded me just how important it is to remain human in a field that too often separates the body from the soul. As I enter this field, I see now more than ever how important breaking this barrier down and making sure the doctor-patient relationship is first and foremost just that- a relationship. Thank you for these lessons and life experiences that I will work everyday to remember. I look forward to returning to the community of Nuñoa to try and improve the health system in the future. However, until then, I want to humbly and deeply thank everyone in the community for helping me with my endeavors. It was truly an honor to learn from, live in, and take part in the community activities of such a warm and vibrant community.

I would also like to thank my family. Without your love and continued support, my ability to undergo such a project would not have been possible. Mom and Dad, your faith in my ability to achieve whatever I set my mind to keeps me going even when all odds seem to be against me. Your unwavering support does not go unseen. I give you so much credit for always allowing me to chase me dreams, even when they take me to every remote corner of the world. You two are the reason for all of my successes and I want to take a moment to properly thank you for everything you have done for me. I can never thank you enough for all the selfless things you have done and continue to do, including indulging my adventurous spirit, putting up with my moodiness when things get tough, and of course just your unwavering love.

Of course, I can not go without a huge thank you to my fellow NAP and BAM Lab members. Clare, Fiona, Meg, Nora, and Peyton you guys were my rocks throughout this trip. You guys woke up with me at 5am with smiles and worked (often) 14 hour days in order to collect the necessary data for this project. You guys challenged me to think deeper, work harder, and of course kept me sane on days where things didn't go as planned. Your dedication to this work, and my personhood, was truly something I can never thank you enough for. I am forever grateful to this amazing group of women that I am lucky enough to call family now.

Lastly, but certainly not least, I want to thank my phenomenal advisor Dr. Morgan Hoke. Who knew finding your poster advertising for student volunteers on your trip to Peru would change my life forever. You gave me the ability to experience more than I ever knew life could offer and taught me how impactful research can be. Your devotion to research, the classroom, and your students does not go unseen. Whether in our BAM Lab group or in class, everyone who meets you admires your lighthearted humor that can make lectures on rabies and syphilis captivating and engaging. It is something I truly admire. Professors too often do not get the praise they deserve and your dedication that reaches well beyond the classroom into the community, not only in Philadelphia but also in the remote regions of South America, is something that deserves the highest of praise. You are the only professor that I've worked with who not only helps me with my studies but asks me about how I am doing. I want to wholeheartedly thank you for this experience and the opportunity to work with one of the very best in the field (the very best in my opinion). I look forward to our continued work and friendship over the years and hope that this is just the beginning of our work together.

Contents

1. Background

1.1 Introduction:

In light of our current understanding of adaptation to physiological stressors, it is evident that local biologies are produced through an interaction of environmental and sociocultural forces. As defined by Margaret Lock, "local biologies" refer to the impact of "evolutionary, environmental and individual variables" upon physiology (Lock 2001). It can be difficult and sometimes impossible to separate the physiological effects of any one ecological stressor from economic, cultural, and societal influences. For decades, researchers have examined the effect of high altitude on indigenous residents as well as migrant populations to these regions. Many of these studies have investigated the effect of high altitude on human growth rate and adult size, and have focused primarily upon the evolutionary and physiological adaptations to high altitude (see Frisancho, 2013 for review). Early investigations showed a pattern of reduced linear growth at high altitude within the Quechua population of Nunoa (Frisancho and Baker 1970; Frisancho 1969, 1970). This pattern of altered growth in Andean children was confirmed by later research (Bailey et al. 2007; Beall 1981; Beall et al. 1977; de Meer et al. 1993; Greksa et al. 1984, 1988; Greksa 2006; Haas 1972; Haas, Baker, and Hunt 1977; Haas et al. 1982; Hoff 1974; Mueller et al. 1978; Stinson 1980, 1982; Leonard et al. 1995; Mortola et al. 2000; Moore 2003). The implication of these studies was that hypoxia at high altitude led to generalized decreased growth of all organ systems, and that all available energy was redirected to the brain or maximizing oxygen transport rather than feeding musculoskeletal growth (Hoke 2017).

However, this pattern of reduced musculoskeletal growth attributed to high altitude is also seen in malnourished Andean populations living at lower altitudes and in other undernourished populations around the world (Hoff 1972; Hoke 2017). Furthermore, a 1989

Andean study at high altitude demonstrated that children of higher socioeconomic class and improved diet, compared to Andean children of 20 years before and their contemporary poorer peers, have reduced stunting of growth (Leonard 1989). That is, well-fed children of wealthier families living at high altitude are taller than children measured in that same area decades before, while poorer children are not. A more recent study in Nunoa by Hoke and Leatherman reinforce Leonard's conclusions, that in low-energy high-altitude environments, it is possible for the stunting of linear growth attributed to energy shifting to vital organs to be abated when energy/nutrition is no longer limited (Hoke and Leatherman 2018). These findings do not negate the importance of altitude-related hypoxia in growth but point toward the likelihood that socioeconomic factors, including poor nutrition, may interact and compound the effects of environmental stress upon human growth and physiology (Hoke 2017).

More recently, new physiological phenomena, such as breast milk composition, are being evaluated for the potential biological effects of high altitude. For most infants, breast milk is the primary source of nutrition; therefore, variations in its composition will likely have notable health and growth implications for the developing child (Ellison 2001; Finley 1985; Barbosa 1997). As an easily tested, readily available and largely ubiquitous substance in mothers, breast milk serves as a reasonable window into the adaptive physiology required of high-altitude residents. Given the metabolic burden of lactation already established in mothers living in oxygen-abundant lower altitudes, breast milk composition of those residing at high altitudes may be assumed to be compromised compared to lowland mothers (Ellison 2001; Barbosa 1997). Nevertheless, recent studies in Tibet reveal the strong buffering capacity of breast milk by demonstrating that high altitude alone exhibits little effect on the macronutritional adequacy of breast milk (Quinn 2016). This work further implies that secondary stressors may account for variabilities in breast milk observed elsewhere in high-altitude populations (ibid). Given Quinn's findings in Tibet that mothers may have the physiological capacity to preserve breast milk composition despite the stress of high altitude, it is possible that Andean mothers may buffer breast milk composition against high-altitude stress similarly, unless secondary stressors are present.

At an elevation of over 4000 meters, the rural community of Nunoa, Peru, was chosen to be used as representative of high altitude regions in this study of breast milk composition. Prior studies demonstrating the differential effect of socioeconomic factors upon childhood growth at high altitude suggest that breast milk composition variations at high altitude may similarly reflect socioeconomic differences (Leonard 1989; Hoke and Leatherman 2018). Given the stabilization of breast milk in prior studies, if breast milk composition proves to be altered in Nunoa, secondary stressors other than altitude must be considered. Such secondary stressors may include those which cause alterations in breastfeeding behavior including socioeconomic status and activities, nutrition, and local cultural practices. Several studies have already demonstrated that variability in feeding patterns, maternal nutritional status, and economic conditions influence breast milk fat content (Vitzthum 1992; Morbeck 1998; Jensen 1999; Brown 1986). The region of Nunoa provides an opportunity to study the potential cultural and economic impact upon the breast milk composition of mothers living at high altitude (Hoke 2017). Any variability in breast milk between high-altitude residents of different genetic background, varied birthplace, or contrasting economic status would generate significant implications regarding adaptive physiology.

The focus of this paper is to evaluate breast milk content in Nunoan mothers and to specifically analyze whether or not reported nutritional status and breastfeeding practices influence breast milk composition. I will first present a literature review of high-altitude physiological adaptation in general, leading into a comprehensive review of breast milk compositional variances, particularly those based on altitudinal and cultural variances of the mother's environment. After a brief cultural and economic snapshot of Nunoa, and a succinct discussion of local biologies, I will present and compare the breast milk composition data from my study of Nunoan mothers who will be categorized according to reported socioeconomic level and breastfeeding practices. I propose that the synergistic cultural, economic and nutritional stressors influencing breastfeeding practices among poorer mothers, adding to the underlying physiological altitudinal stress, will alter the composition of their breast milk.

1.2 Impact of High Altitude on Tibetan and Andean Physiology

In response to a physiological stressor such as high altitude, organisms will respond along three interacting pathways, that include genetic, functional/physiological and cultural/behavioral routes of adaptation (Leonard 2018). Genetic adaptations are heritable and involve natural selection. Functional adaptations involve biological changes that are non-heritable and are acquired at some time during the organism's lifetime, and some of these can only be acquired if the organism is exposed to the environmental stressor early in their lifetime or specifically during growth. Cultural adaptations are non-biological strategies to the environmental stressor that may involve behavioral changes. These cultural adaptations can be transmitted to future generations, like genetic adaptations, and may provide a beneficial response to the environmental stressor or, alternatively, induce further stressors to which a population must respond (Leonard 2018). These three categories of adaptation act interdependently; in other words a physiological adaptation may induce behavioral cultural responses that, in turn, influence further physiological response (see Figure 1 reproduced from Leonard, 2018).

Prior studies of the impact of high altitude stress on human physiology have primarily assessed alterations in growth patterns and respiratory responses to a hypoxic environment. Studies dating back as far as 1890 detail the disparate adaptations between Tibetan and Andean populations in their physiological response to low oxygen environments. Andeans demonstrate an elevation in hemoglobin concentration in an altitude-dependent manner beginning at heights of 1600 meters or more, while Tibetans respond with higher oxygen saturation levels and do not show hemoglobin increases until at least 4000 meters of altitude (Viault 1890; Beall 2006; Garruto et al. 2003; Frisancho 2013). Beall's work further reveals that there are two or possibly three different phenotypes for altitude-related hypoxic adaptation (Beall 2006). Using quantitative genetics to analyze intra-population differences in these phenotypic traits, Beall demonstrated that increased oxygen saturation has no apparent heritability among Andeans but has autosomal dominant inheritance among Tibetans. The lack of such an inheritance among Andeans, though present in Tibetans, implies there is a difference among separate populations in how they adapt to hypoxic environments (Beall 2006; Weiss 1993).

If Beall is correct, and genes exist for improved survival in high altitude environments, then one would expect natural selection to act upon this gene or genes, making them more common in high altitude populations. Subsequent infant survival studies by Beall in Tibet seem to support this (Beall 2007). Survival rates for infants born to mothers possessing a high probability for a high oxygen saturation gene based on quantitative genetic assessments were, in fact, three to four times greater than those born to mothers less likely to carry the gene (Beall, 2006). Andeans, by contrast, do not demonstrate population genetic differences in adaptation to high altitude (Beall, 2006). Nevertheless, Andeans do adapt to the low oxygen environment with increasing serum hemoglobin content that becomes evident at elevations of only 1600 meters.

This adaptation is qualitatively different than seen in Tibetans, who do not show hemoglobin increases until they are above 4000 meters of altitude (Beall 2006).

In addition to increased hemoglobin, Andean populations demonstrate other physiological adaptations to high altitude, specifically increased lung capacity and chest size. Frisancho and colleagues (2013) focused upon lung and chest volume growth relative to body growth in high altitude residents as compared to lowland inhabitants. Citing the lack of evidence for a genetic etiology for Andean adaptation to altitude in the Beall study, Frisancho et al. looked to further demonstrate that varying patterns of growth and development in Andeans exposed to high altitudes represent a primarily functional rather than genetic adaptation to low oxygen conditions. The authors point out collective studies that show natives to high altitude conditions have delayed overall body growth relative to growth in lung and chest volume at early ages indicating that energy was shifted away from linear growth to the development of larger chest and lung volumes. This same finding was seen in low-altitude urban natives who migrated to high altitude early in life. Yet, those individuals that migrated after the age of 10 did not show this chest volume adaptation. Thus, Frisancho et al. concluded that environmental influences (low oxygen) during early growth and development, rather than genetics, imparted long-lasting adaptive benefits for high-altitude dwelling Andeans. Furthermore, the earlier the age exposed to hypoxic environments, the greater the influence of the environment. The study concluded that exposure to high altitude at birth or during growth contributes to long-term functional improvement in those living in low oxygen conditions.

Frisancho et al. went on to explain that the lack of a genetic adaptation in Andeans, previously shown by lower infant mortality among high oxygen saturation genotypes in Tibetans by Beall in 2006 and 2010 studies, can be explained by the relative isolation of the Tibetan population compared to the Andeans. The more historically isolated Tibetan population allowed for natural selection to take place; whereas, Andean political and economic structures had the effect of mixing low and high-altitude populations, especially after the colonial invasion by the Spanish in 1532, thus blending the gene pool. Once racially-segregated populations mixed with their European invaders, primarily in urban spaces, creating *mestizo* populations. Furthermore, population movements during colonization, some imposed by Spanish authority and others as a fleeing response to imposed rule and resettlement, resulted in an alteration of the Andean gene pool (Gose 2008 as cited in Hoke 2017). In addition, many indigenous residents were wiped out by disease (Hoke 2017). During the colonial rule that spanned the 16th through early 19th centuries, laborers were also forced to leave the highlands near Nunoa to work in silver mines outside the region. More recently in the 1980s and 1990s, infighting between leftist Sendero Luminoso forces and the Peruvian military that often centered around peasant cooperatives in the area led to personal and food insecurity and the evacuation of many to safer lowland locales (Leatherman and Thomas 2008). All of these migrations may account for the relative lack of genetic influence upon Andean adaptation to high altitude compared to Tibetans. However, the lack of genetic etiology for oxygen saturation or hemoglobin level does not dismiss the possibility of genetic adaptation in the Andean case. A study by Moore et. al. (2004) reveals a genetic basis (single nucleotide polymorphisms) behind reduced vasoconstriction in pregnant Andeans with a longer generational history at high altitude compared to lowlanders or Europeans (Moore 2004).

The fact that Andeans and Tibetans react differently, both functionally and genetically, to the same severe physiological stress imposed by high altitude suggests that there may be elements induced by human intervention that influence the population differences. Accordingly, breast milk composition in Andean mothers may represent more than simply a physiological response to altitude. Breast milk composition differences noted between residents in Nunoa might then represent a variety of economic, sociocultural and health care disparities.

1.3 The Biochemistry of Milk Composition

The nutrient and biochemical composition of breast milk has a significant influence on the healthy development of children (Koletzko et al 2009; Riva et al. 2004). Previously considered to be uniform among different mothers, recent investigations of breast milk show considerable variability in macronutrient composition, especially fat content, even among women of the same population and background (Jensen 1999; Prentice 1995; Wojcik et al. 2009 as reported in Quinn, 2012). Possible influences on this variation have been proposed, yet the keys to variation seem to be independent of maternal characteristics and diet when food availability is adequate (Coward et al. 1984; Villalpando & del Prado 1999; Quinn, 2016).

Buffering of milk composition, particularly of fat content, is well documented (Prentice et al. 1994; Villalpando & del Prado 1999; Brown 1986; Quinn 2012; Jelliffe and Jelliffe, 1978; Quinn 2016; Barbosa 1997). A study of Filipino women revealed that, even in situations where maternal diet is considered suboptimal, diet and nutritional status of the mother does not significantly alter breast milk composition (Quinn 2012). Even vegetarian mothers have adequate fat totals in milk, though they may lack some specific fatty acids (Finley 1985). Likewise, maternal milk remains unaltered even under conditions of severe malnutrition, although volume may be compromised (Jelliffe and Jelliffe 1978). For mothers with low fat content in milk, the body seems to respond by adjusting production levels to compensate. For example, in the Otomi population of Mexico, indigenous women with lower breast milk fat content compensate with higher milk production rates (Barbosa 1997). Likewise, protein and lactose levels are equivalent and independent of maternal BMI and diet (Barbosa 1997). The stability of breast milk is even more impressive given the enormous metabolic cost of lactation that results in a depletion of maternal reserves and impacts "a woman's long-term energy balance" (Ellison 2001:94).

Several factors beyond maternal nutrition have been implicated in altering breast milk composition. For one, early life nutrition and growth history of the mother can alter milk composition (Quinn 2012). In addition, milk composition has been shown to change with length of feeding and over the course of the years of lactation (Quinn 2012). Thus, the cultural mode of lactation, namely the local patterns and practices of breastfeeding, may have significant impact on milk makeup.

1.4 The Effect of Altitude on Milk Composition

Given the energy demand of lactation, and further requirements imposed by the hypoxic stress of high altitude, one might hypothesize that the added burden of high-altitude existence may impact maternal reserves and breast milk composition, accordingly. Yet, as previously reviewed, remarkable genetically-influenced adaptations and environmentally-triggered physiological responses exist among mothers in low oxygen/high-altitude environments (Beall 2006; Frisancho 2013; Moore 2004). These responses include documented increases in oxygen saturation (Tibetans) and hemoglobin levels (Andeans), as well as reduced vasoconstriction during pregnancy (Andeans), increased uterine blood flow (Andeans) and greater uterine artery blood flow velocity (Tibetans) (Moore 2004). Given these established changes in physiology at high altitude, we might suspect there may be similar alterations in postnatal physiology to facilitate life at high altitude, and changes in breast milk composition might be one of these adaptations. Therefore, it is important to assess if such an adaptation occurs to sustain the nutritional value of breast milk, and thus the infant growing up at high altitude.

Quinn's study of Filipino mothers indicates that maternal physiology can provide a "buffer from short term variation" for breast milk (Quinn 2012: 538). Mothers in this study maintained normal breast milk and milk energy levels despite disparities in BMI and adequacy of diet. Likewise, a study of undernourished Gambian mothers showed significant maternal buffering of fetal growth (Johnson 2017). Yet, it was not until the Quinn et. al. 2016 study that breast milk composition was examined in high altitude populations. In Tibet, Quinn assessed whether or not milk fat content was impacted by altitude, and further examined if any such changes could be attributed purely to altitude variation or were rather due to "secondary predictors" reflecting individual characteristics of the infant and mother (Quinn 2016: 233).

The results showed these mothers produced milk with an exceptionally high fat content, as might be expected to serve the increased metabolic requirements of their developing infants at high altitude. Here, maternal adipose tissue likely serves as an important energy reserve for the added metabolic costs of lactation, particularly in producing high-fat milk (Quinn 2016). Thus, a negative nutritional status of the lactating mother, limited nutrition or food inadequacy, might impact breast milk composition in a high-altitude environment (Quinn 2016). Studies in Mexico have already shown the importance of maternal fat stores in maintaining milk volume and fat levels in malnourished populations (Villalpando 1992). The positive association of maternal fat reserves, measured by triceps skinfold thickness, with milk volume and fat content has also been demonstrated in Bangladesh (Brown 1986).

While Quinn's (2016) study found no connection between maternal BMI or diet and milk fat content, it contradicted a number of other studies (Brown 1986; Villalpando 1992; Butte et al. 1984; Prentice et al. 1994; Villalpando and del Prado 1999). Furthermore, Quinn's subjects had varied diets but were not reported to be malnourished. More limited food options and impoverished conditions facing some other mothers at high altitude would provide an added physiological burden that could compromise fat stores and milk content that might otherwise be buffered by healthier mothers or those in lowland environments. Thus, Quinn's work, which implies that high altitude on its own has minimal effect on the adequacy of breast milk nutrition, encourages further investigation into secondary stressors for variabilities observed elsewhere in high-altitude populations. Such secondary stressors might include cultural and economic variables, as well as variations in breastfeeding practices, similar to those seen in Nunoa.

1.5 Study Setting: Nunoa, Peru:

Living at altitudes that are for the most part above 4000 meters and in one of the most isolated districts of Peruvian Andes, the Nunoan population is subject to very distinct and intense natural physical stressors (Baker 1996). In addition to these stressors, the inhabitants must cope with difficulties associated with a long history of political economic turmoil and marginalization. The following discussion will provide a brief but detailed snapshot of the distinct geography, economic, and cultural aspects of this region to provide a better understanding of the potential secondary stressors that could accompany altitudinal stress and impact breast milk composition of Nunoan mothers.

Nunoa is one of nine districts that comprise the Melgar Province of the Puno Region in Peru. It nears the Eastern border of Bolivia and is approximately 280 kilometers southeast of Cuzco. The area is served by the Nunoa River, which in part emanates from the Quelccaya Glacier, the largest glacier in the tropics. Of the about 14,000 persons living in the district, the majority are herders with small attached farms that grow tubers, potatoes, and grains such as quinoa (The Nunoa Project, 2017). The grazing land is among the best in the Andean and

Bolivian Plateau (Altiplano), and Nunoan residents are particularly proud of their herds which may include alpaca, huacaya, llamas, sheep, cattle and horses (The Nunoa Project, 2017).

Historically, the indigenous people of this region are of Quechua descent (Leatherman and Brooke Thomas 2009). Pre-Columbian settlement in the Andes comprised of “kin-ordered social groups” that spread throughout the Altiplano to make use of the variety of agricultural opportunities available at different elevations (Spalding 1984, as cited in Hoke 2017). The relative wealth of the area fell into decline after invasion by the Spanish due to reordering of the settlement and labor taxation of the local community, requiring a proportion of community workers into forced labor in Spanish mines (Garrett 2005). This colonial incursion of Nunoa led to underdevelopment of the land that persists even today (Dell 2010). During the colonial era of this region, this long standing indigenous economic system was negatively impacted by the conquistadors, resulting in many inhabitants leaving this region for opportunities in Lima and other cities (Hoke 2017). This exodus encouraged acculturation between the Quechua people and the Colonial Hispanic population; although, the Nunoa region still retains a great deal of pastoral farmers today.

The acculturation of Andean Peru during colonization gradually led to striking economic partitioning of society. A notable period of “intermarriages between indigenous elites and Spanish colonists” was “responsible for the initial formation of a growing mestizo population who often demonstrated strong vitriol toward the indigenous population as a way of establishing themselves in the social order of colonial society” (Hoke 2017). By the late 19th century, this division resulted in “increasingly unequal distributions of capital and land ownership” throughout the Altiplano between laborers and those that had ascended to the upper class as hacienda owners (Hoke 2017: 61). Haciendas expanded with the growth of the international wool market and encroached on native farmland, forcing peasants into peonage (Leatherman 1996). This has resulted in a long lasting and multigenerational divide within the original indigenous population (Hoke 2017: 61).

The massive upheaval caused initially by colonization resulted in large changes for the social structure of Nunoa. It shifted the economic structure and gave rise to more opportunities in urban regions, enticing those better off and able to move away (Hoke 2017). Since the time of colonization "a number of well-to-do or better-off families from town have migrated to larger cities, both for economic opportunities and the amenities and comforts of life in a city" (Hoke 2017:73). Reform policies of the 1970s were initiated with the goal to eliminate the feudal structure of Peruvian farming and enhance peasant demands for land. While coastal farming workers saw benefits of the reforms, the highland residents of Nunoa failed to gain an equitable distribution of land and many were evicted from expropriated haciendas by large landowners whose assets were taken away (Luerssen 1994). By the early 1990s, the area had become a battleground between socialist Sendero revolutionaries and Peruvian counterinsurgency forces, resulting in further disruption of agropastoral production and increased food and economic insecurity (Leatherman and Thomas 2008).

With declining prices on alpaca fiber, and the only available schools located in the urban center, the town of Nunoa has now become "more and less indigenous" as herding class children necessarily move to the urban center, often shedding their traditional dress and culture (Hoke 2017: 74). This has left the herding area with a poorer population along with wealthier families who have "amassed enough resources to make ends meet" (Hoke 2017: 78). The result of this is the establishment of "three distinct microeconomic environments...the urban zone, the dairying zone, and the herding zone" (Hoke 2017:78). Given the potential impact of health care disparities, nutrition, and cultural differences in feeding practices between the poorer high-elevation herding population and those in the urban center, any study of the effect of altitude on breast milk composition must address such discrepancies.

1.6 The Concept of Local Biologies

In the late 1980s, ethnographer Margaret Lock conceptualized “local biologies,” a term that recognized that physical health and well-being is, at least in part, altered by evolutionary, environmental, sociocultural, economic, historical and other individual variables (Niewohner and Lock 2018). Likewise, the experience of biological processes “molds and contains the subjective experience of individuals and the creation of cultural interpretations” (Lock 1993a: 39). This concept derived from an ethnographic study of menopause in Japanese and North American women, which showed the relative lack of hot flashes and night sweats in Japanese women compared to Americans (Lock 1993b). This discrepancy was later shown to be based in different mechanisms of estrogen metabolism in Japanese and North American women (Melby 2015). Lock further concluded that established differences in Japanese rates of heart disease, breast cancer and osteoporosis could also be attributed to local biologies (Lock 1993b).

Other studies have demonstrated environmental epigenetics to give legitimacy to contentions that poverty, discrimination and social disadvantage can lead to enduring detrimental health effects (Niewohner and Lock 2018; Landecker 2011). Recent evidence implicates developmental and epigenetic mechanisms deriving from early environmental factors, such as maternal stress, in cardiovascular health disparities between races (Kuzawa 2008). Furthermore, epigenetic mechanisms, notably DNA methylation, have been correlated with exposures to air pollution and/or psychosocial stress, increasing the risk of diabetes, hypertension and premature birth in socially disadvantaged populations with disproportionately higher exposures (Vick

2017). Thus, an individual's experience in a given environment, and within a specific historical context, has the power to drastically impact their biology, even at the molecular level.

As this understanding applies to breast milk composition differences in high altitude populations, it has already been demonstrated that the oxygen-carrying adaptation to altitude is vastly different between Tibet and the Andes. As mentioned, Tibetans respond to altitude-related hypoxia through a genetically-mediated increase in red blood cell oxygen saturation (Beall 2006). Andeans, by contrast, do not demonstrate population genetic differences in adaptation to high altitude, and adapt to the low oxygen environment with increasing serum hemoglobin content (Beall 2006). Despite an equivalent altitudinal stress, the adaptive mechanisms are starkly different. Thus, a physiological response to altitude in not "universal." It is reasonable to assume then that breast milk alterations in response to physiological stress such as altitude may differ. Complicating this further are other potential stressors that may differ between *and within* populations experiencing high-altitude stress in the cultural, social and microeconomic environments.

1.7 The Potential Impact of Local Biologies on Breast Milk Composition in Nunoa

The variation in economic activity in Nunoa, already described by Hoke (2017), suggests the presence of variation in factors other than altitude, that may account for differing breast milk content between mothers in the highland herding region from those in the urban center (Hoke 2017). A 1992 study of Nunoan breastfeeding by Vitzthum reveals that economic status influenced breastfeeding duration and interval patterns; namely, poorer herding zone mothers weaned infants much earlier, with individual feeds being shorter and less regular (Vitzthum 1992; Morbeck 1998). More recent investigations that may reflect a change in the economic patterns in recent decades do not show a significant difference in the age of weaning across economic zones in Nunoa (Hoke 2017). Given the findings of Ballard and Morrow that demonstrate the duration of individual breastfeeds influences fat content, and that composition may vary even within individual feeds, it is critical that any analysis of breast milk alteration in Nunoa account for any variable that affects feeding patterns and individual feed duration (Vitzthum 1992; Ballard and Morrow 2013). If there are altitudinal disparities in breast milk content, differences in the duration, frequency and volume of feedings must be accounted for.

Quinn's study of Tibetan mothers accomplished this by regularly assessing "mid-feed" samples two to three minutes into a feed (Quinn 2016: 236). However, the comparative reliability of mid-feed samples will be compromised if regular feeding schedules are not followed by the mother at home. A study of altitude-induced differences in breast milk, such as that proposed for Nunoa, must ascribe to such regularity in samples and needs to account for differences in workload, nutrition, culture and socioeconomic status that might influence breastfeeding duration and patterns of feeding.

Beyond the influence of breastfeeding patterns, the political, cultural, social, and economic history of this region can have strong impact upon maternal physiology and thus breast milk composition, as per the concept of local biologies. From a political perspective, the clash between the Peruvian counterinsurgency forces and the Sendero Luminoso group during the late 1980s and early 1990s, led to unrest, deaths, and burning of farmlands in Nunoa (Hahn 2009). Some locals reported that their lifestyle was transported back fifty years in terms of infrastructure, while poverty and malnutrition became the norm (Fieldnotes Summer 2018). Many of today's Nunoan mothers were born in this era of substandard nutrition and increased social turbulence. Given Quinn et al.'s findings that milk composition variability at high altitude does not relate to maternal BMI or current diet, but may be impacted by historical issues such as maternal early life nutrition and growth patterns, the effect of this historical food deprivation must be considered in assessing breast milk composition in Nunoa today (Quinn et al. 2016).

Since the mid-1990s, in an attempt to bolster dwindling support, the Peruvian government paved roads in the area, allowing for the transport of scarce foodstuffs, like fish and fruit, and also the establishment of medical care outposts (Hahn 2009). Unfortunately, few in the Nunoan area can afford these food items and subsist on unenriched bread, noodles, and rice (Fieldnotes). A large portion of lactating Nunoan mothers skip meals or consume a minimal third meal often consisting of tea and bread. Furthermore, available health services are often not properly utilized based on a long standing distrust of medical institutions that resulted in part from excessive hysterectomies coerced by Fujimori's administration in the 1990s along with more recent ideologically-based programs that discourage contraception, impose fines for missing prenatal appointments, and instill severe penalties for abortion (Miranda 2004). Those that attend health centers and lactation classes often come in contact with urban culturally-detached physicians, filling their one-year requirement for accreditation, who provide advice (15 minute feeds per 1-2 hours) that is not consonant with indigenous working schedules and family responsibilities (Fieldnotes).

The indigenous lifestyle, particularly of highland farmers, is particularly stressful on lactating mothers, most of whom return to work in the immediate postpartum period and are also responsible for many other children as well as household and agricultural tasks. They often suffer from the dual burden of malnutrition. Many skip meals to feed their children or survive on starch-based diets with little nutritional value. It has been well established that adipose tissue is an important energy reservoir for lactating women in regions with a high degree of food insecurity (Quinn 2016). Given the high fat milk produced in Tibet by mothers at high altitude, and the established need for maternal fat stores to support high fat milk in this stressful environment, it would not be surprising to find that Nunoan mothers lack the adequate food stores to support the high energy milk needed by children of this region (Brown 1986; Quinn 2016).

1.8 A Study of the Effect of Altitude and Local Biologies upon Nunoan Breast Milk Content

Following Quinn's 2016 study in Tibet, which failed to show significant changes in breast milk nutrient composition due to altitude alone and suggested secondary factors for breast milk variations seen elsewhere, I enacted a study of breast milk in Nunoan mothers that considered the potential impact of cultural and economic factors. Samples were taken at mid-feed, following the protocol outlined by Quinn et al. (2016). In addition to gathering data on the long-term duration of lactation in high-altitude dwellers, I focused on the habitual duration of individual feeds and intervals between feeds in order to analyze the data for variability related to feed duration and daily cultural practices that impact breastfeeding patterns.

My hypothesis was that the general Nunoan population would demonstrate a buffering of milk composition at high altitude, but women who are malnourished or who suffer the greatest and persistent nutritional and sociocultural stress (food inadequacy, health disparities, psychological stress, e.g.,) would demonstrate altered breast milk composition. That is, significant differences in breast milk nutrient composition were predicted within the Nunoan population based on individual cultural, economic and nutritional conditions. In addition, I proposed that feeding patterns, perhaps induced by economic or social background, would further influence breast milk composition. In conclusion, this was designed to be an exploratory studying attempting to assess the feasibility of future milk analysis work in this region with three main objectives:

1. To assess the macromolecular composition of Nunoan maternal breast milk.

2. To examine the relation between maternal and infant characteristics in predicting breast milk composition, paying particular attention to household income, maternal BMI, maternal age, maternal triceps and subscapular fat, maternal waist and hip circumference, maternal waist-to-hip ratio, infant age, parity, and observed feeding time.

3. To compare the Nunoan data with the only other high altitude study of breast milk composition to date, conducted in Tibet by E.A. Quinn in 2016.

2. Methods

2.1 Research Methods and Study Participation Overview

This project employed fully informed and consenting lactating women and their infants from the Nunoa region of Peru. Breast milk samples from participating mothers were collected, immediately frozen, and shipped to the laboratory of Dr. Robin Miriam Bernstein of The University of Colorado Department of Anthropology for biochemical analysis.

2.2 Ethical Considerations

To ensure ethical practices, CITI certification was obtained for both IRB and Human Subjects Research background, and these guidelines were followed during data collection from Nunoan volunteers and throughout the research process. Professor Morgan Hoke, Ph. D., M.P.H. of the Department of Anthropology at the University of Pennsylvania, served as my thesis advisor/Investigation PI and assisted in the creation of an IRB proposal for research abroad by combining our projects together. Once in Nunoa, we obtained written consent from both the local municipal government and health center medical officials to conduct research. This background ensured that only ethical and approved research was conducted. IRB-approved recruitment forms, radio advertisements, and house-to-house recruiting methods were used to obtain participants. In addition to IRB approval, careful measures throughout the collection process were taken to ensure minimal risk to the participants.

Lactating women that showed interest in participation in the study were encouraged to meet with us at our laboratory, located at Giron Ramon Pizarro N° 360, at which time we answered questions and explained the purpose and type of research being performed, along with a description of the potential risks, benefits, and tests required of study subjects. Only after a thorough run through of our work would we ask the potential participant if they wanted to be a part of our study. The goal was to obtain samples from thirty mother-infant dyads for the analysis of breast milk composition.

2.3 Obtaining participants

The majority of my first two weeks in Nunoa consisted of getting to know the townspeople and familiarizing ourselves the community. After traveling to the district of Nuñoa, Peru, I required a few days to acclimate to the high altitude. I used this time to learn more about the area and people of Nunoa and to take classes in Spanish. Under the guidance of Dr. Hoke, I learned how to become part of the community and make myself better known. I attended local celebrations, including many commemorating the Year of the Campesino. Through direct participation in these festivities, I got to learn more about the community history and met many mothers who might later participate in the study. I also accompanied Dr. Hoke to the health center, where I became acquainted with the staff and medical officials. This was vital since their facility served to store the milk samples in a generator-supported, subzero (-20^{0}C) freezer.

The mother-infant dyad pairs provided researchers with five items to participate in our study: a 10 mL milk sample, anthropometric measurements, answers to a nutritional survey, a fecal sample, and a blood sample for hemoglobin and glucose levels. The fecal and blood samples were used for concurrent microbiome, inflammation, and nutritional studies. In order to be eligible for inclusion in this study, each woman had to have given birth to a single newborn. They also had to be currently breastfeeding, able to breastfeed freely, and willing to self-express 8-10 mL of their milk for analysis.

2.4 Collecting and Storing Samples

The devised collection process was non-invasive and was easy to administer even in the most rural of areas. The following steps were followed to obtain samples:

1. Mid-feed samples were collected via hand self-expression by mothers.
2. Surveys on nutrition and feeding were obtained.
3. Milk was aliquoted into sterile vials in a sterile field.
4. Milk was frozen within 30 mins of expression.

In the following subsections, each step of the milk collection process is broken down to describe the process in detail.

A **mid-feed sample** not only provides an extremely accurate read of milk fat composition, but causes minimal impact upon the nutrition the infant receives during a feed (Quinn, 2012). Therefore, for both the purpose of ease, preservation of behavioral norms, and minimization of risk for both infant and mother, the mid-feed method was chosen. The feed resumed immediately and relatively seamlessly after filling the collection container. Mothers were instructed to abstain from any feeding or lactating for at least one hour prior to the collection. Upon arrival, the mother started the feed as normal. Two minutes later, feeding was stopped to fill a small, sterile container using a self-expression method. Mothers self-expressed 8-10 mL of milk into a sterile collection container and then continued to feed as normal until completion of the feed (Figure 2). Each 8-10 mL sample that was collected was from a single breast, except for the occasional situation when single breast milk supply was insufficient and another mid-feed sample was then obtained from the other breast.

The **hand self-expression** method was chosen for multiple reasons. Firstly, it is non-invasive and easy to explain and demonstrate to lactating mothers. Most women in the Nunoan community are familiar with or have performed self-expression before for cultural healing practices, such as the topical application of breast milk for eye infections. More importantly, manual self-expression was chosen over pumps to minimize possible contamination between samples and to eliminate any chance of spreading infection. Pumps must be sterilized between every use to help prevent infection; however, even after proper sterilization, process pumps can still cause nipple infections (Miller 2013). The self-expression method minimizes this risk.

Infants were weighed before and after feeding to assess milk volume intake. Time stamps were used to note the start and completion of the feed, the beginning and end of the mid-feed sampling, along with any breaks that occurred during the feed. To eliminate inconsistency, since milk is incredibly variable in composition throughout the day, even across a single feed, the collection samples were only taken in the morning between the hours of 6am-10am. It has been demonstrated that milk expressed early in the day can contains more foremilk (lower in fat content than mid-feeds) and milk expressed later in the day is found to be higher in hindmilk (higher fat content than mid-feeds). Therefore, this was considered during the analysis process (Miller 2013, p. 3). This diurnal consistency, along with the regularity of mid-feed sampling, would later afford more accurate comparative analysis with prior breast milk assessments in high altitude regions of Tibet, which followed the same protocol (Quinn 2016).

At the completion of the feed, the sample was labeled with the donor ID number, date and time at which the collection began, and the mother then underwent a **survey**. This survey collected basic information about where and how the mother and child live, including details about maternal and infant nutrition, basic family economics, and breastfeeding practice customs.

Great care was taken to ensure only noninvasive questions were asked of the participants. This survey queried maternal and infant age, diet, approximate household income, and the time since the last feed. Basic questions were also asked to best define participant nutritional knowledge, specifically what mothers felt were healthy and unhealthy food choices for their infants and themselves. Once the collection process and information gathering was complete, samples were placed in the freezer, within 30 minutes of expression, and were stored there until shipped to the United States for analysis.

To preserve the integrity of milk biomolecular composition, 2ml **aliquots** from the sterile collection container were transferred into vials and frozen within 30 minutes from the collection start time to maintain true macromolecule composition.

Great care was taken to avoid contact with the inside or top of the container, lid or vials in order to maintain sample sterility. In addition, this process occurred in a sterile field to further minimize contamination risk. Lastly, at least half an inch of space was left above the milk sample in the aliquot vials to leave room for milk volume expansion during freezing.

This same collection process was undertaken by Dr. E.A. Quinn during her field collection of breast milk in a high□altitude adapted population of Tibetans (Quinn 2016). In a remote region similar to that in Nunoa, Dr. Quinn reported great success in breast milk collection using this technique. I was in contact with Dr. Quinn in the months leading up to the initiation of my study to best understand the nuances of this collection process. Therefore, with both Dr. Hoke and Dr. Quinn’s guidance I was confident in both the ethical and technical practices required to obtain the milk samples in Nunoa. My samples were obtained successfully with minimal risk and invasiveness. Mothers never had to disrobe at anytime or alter their normal breastfeeding practice in any way, increasing their comfort level throughout the process as much as possible. Furthermore, the information was collected without impingement on participant privacy. Great care was taken to ensure males were not present during collections unless the participant mother gave consent for male partner presence. Occasional sensitive moments presented when survey questions were asked about economic status and how often meals were skipped due to financial limitations. Overall, maximal effort was employed to reduce potential discomfort for the lactating mother.

2.5 Macromolecule Analysis Protocol

Analysis of milk samples took place in Dr. Robin Bernstein's laboratory and were analyzed by doctoral candidate Jennifer Washabaugh at The University of Colorado. Samples were analyzed for lipid, carbohydrate (specifically lactose), and protein content via the LactoScope FTIR (Delta Instruments B.V., Drachten, the Netherlands). The 2 mL aliquots were thawed for a minimum of 30 minutes before analysis. The sample was then diluted by a factor of 10 by adding 18mL of ddH2O and heating to 38.5 degrees Celsius in a warm water bath for a maximum of 20 minutes. Each sample was run in duplicate for reliability of breast milk macronutrient content. This method shows < 3% variance in fat (FAT), total protein (PRO), true protein (TRP), and lactose (LAC) when tested against reference methods (Smilowitz et al. 2014). Testing for protein looked at total protein, true protein and non-protein nitrogen. True protein was determined by taking the entire nitrogen content in milk minus the non-protein nitrogen (NPN) fraction (Carratù et al. 2003) and consists of protein, free amino acids, and peptides (Feng et al. 2016).

2.6 Data Analysis

In order to remain consistent with Quinn et al. (2016), and to ensure accurate comparisons between the Nunoan and Tibetan population data, the same statistical analyses used by Quinn et al. (2016) were run for the Nunoan milk samples. Thus, analysis protocol for predators of milk composition were run using Stata 15 (College Station, TX) and all milk macronutrients were log transformed in order to allow for linear regression analyses. To compare the Nunoan population data with the High Nubri and and Katmandu populations in Tibet, a Welch–Satterthause t-test was run.

3. Results

3.1 Descriptive characteristics

The sample group consisted of 23 mother-infant dyad pairs (see Table 1). Maternal-reported ages of mother and child participants averaged 28.27 years and 10.96 months, respectively. Anthropometric measurements revealed that mothers' height, weight, BMI, waist-to-hip ratio, and sum of maternal skinfolds were 150.14 cm, 61.64 kg, 27.34 (kg/m^2), 0.87, and 42.96mm, respectively.

Sociocultural, nutritional and economic characteristics were also self-reported. Nuñoan mothers, as seen in Table 2, reported average monthly income of 702.62 soles monthly (equivalent to 211.54 USD). 36.36 % took part in Juntos (a 2005 government implemented cash program that provides small monthly payments to poor families), and 81.82% took part in Vaso de Leche (a nationally funded social welfare program that offers daily food rations to vulnerable populations in Peru).

Bearing these sample characteristics in mind, the analysis was organized according to the three corresponding objectives for this study: to enumerate the mean macromolecule composition of Nunoan breast milk, to distinguish if there are any maternal and infant characteristics that can predict breast milk composition, and to compare the Nunoan data to that of Tibet.

3.2 Mean Macromolecule Composition of Nunoan Breast Milk

To assess the macromolecule composition means of the sums of each macromolecule category were calculated. Mean fat was 4.56 ± 0.78 (g/100mL), and ranged from a minimum of

2.33 g/100mL to a maximum of 5.47 g/100mL. Mean protein was 1.52 ± .35 (g/100mL), ranging from a minimum of 1.14 g/100mL to maximum of 2.73 g/100mL. Finally, mean sugar was 6.01 ± .89 (g/100mL), ranging from a minimum of 2.42 g/100mL to a maximum of 6.96 g/100mL (Table 3).

3.3 Relation Between Maternal and Infant Characteristics in Predicting Breast Milk Composition

The potential predictive factors of milk macromolecular composition examined included household income, maternal BMI, maternal age, maternal triceps and subscapular fat, maternal waist circumference, maternal hip circumference, maternal waist-to-hip ratio, infant age, parity, and observed feeding duration. To perform the statistical analysis for this section, log transformations of each macromolecule (fat, protein, and sugar) were used to assess if there are linear associations between macromolecule composition and specific predictive factors. The findings are as follows:

Milk fat: Infant age, tricep folds, subscapular folds, hip circumference, and feed duration had no significant impact upon milk fat content. Parity, maternal age, maternal BMI, maternal waist-to-hip ratio, and maternal waist circumference, when run as individual predictive factors against the log transformation of milk fat, were all significant predictors of milk fat composition. However, when run as multivariable regressions, the only factor to remain a significant predictor of milk fat composition was maternal waist-to-hip ratio (Table 4). Those with low waist-to-hip ratios generated higher fat content. Table 4 shows the three of 13 statistical models that display maternal and infant characteristics that proved significant in individual and multivariate analysis in predicting breast milk fat composition. Model 1 (maternal BMI and milk fat) shows increased maternal BMI results in less fat content, and trends toward significance (p =0.076). Model 2 (waist-to-hip ratio and milk fat) reveals a significant, negative correlation between waist-to-hip ratio and milk fat (p=0.006). Finally, Model 3 is a multivariable regression between parity, waist-to-hip ratio, maternal age, and maternal BMI and demonstrates the only statistically significant variable for predicting milk fat levels, when in tandem with multiple predictive factors, is maternal waist-to-hip ratio (p= 0.044).

Milk protein: Infant age, maternal age, BMI, tricep folds, subscapular folds, waist circumference, waist-to-hip ratio, and parity had no significant impact upon milk protein content. Hip circumference, and feeding interval(s), when run as individual predictive factors against the log transformation of milk protein, were significant predictors of milk protein composition. However, when run as multivariable regressions, the only factor to remain a significant predictor of milk protein composition was maternal hip circumference, which was a negative predictor for protein content (p=0.016).

Milk lactose: Maternal age, BMI, tricep folds, hip circumference had no significant impact upon milk lactose content. Subscapular fold thickness trends toward significance in predicting milk lactose. Thicker folds correlate with higher milk lactose content (p=0.085). Infant age, waist circumference, waist-to-hip ratio, parity, and feeding duration, when run as individual predictive factors against the log transformation of milk lactose, were significant predictors of milk protein composition. Table 5 shows 3 of the 13 regression models run to assess maternal and infant characteristics in predicting breast milk lactose composition. Model 1 (waist-to-hip ratio and milk lactose) reveals a significant positive correlation between waist-to-hip ratio and milk lactose (p=0.034). Model 2 is a multivariable regression between infant age and waist-to-hip ratio that demonstrates when both variables are present each predictor falls out of significance (p= 0.189; p= 0.260). Finally, Model 3 is a multivariable regression between log transformation of milk sugar, waist-to-hip ratio, baby age, and waist-to-hip x infant age, revealing that when the interaction between infant age and waist-to-hip ratio (WHR) are considered, they are highly predictive of milk lactose such that the older the child, and the lower the WHR, the lower the lactose content (Table 5).

3.4 Comparison of Nunoan Data with Other Reported Breast Milk Composition Studies

The results of the statistical comparison between Nuñoan and Tibetan populations are presented in Figure 3. In comparing the Nunoan macromolecule composition to the Tibetan High Nubri (rural inhabitants at altitude > 10000 ft) and Katmandu (urban population situated at 4600 ft) populations, we found no significant difference in feeding frequency (n/24hr) between the Nunoan population (11.9 feeds per day), and Tibetans who feed on average 8.6 (High Nubri), and 8.4 (Katmandu) times per day. There was no significant difference between milk fat composition of Nunoan and High Nubri populations. There was, however, a significant difference between milk fat composition of Nunoan and Katmandu populations (p=0.0024). Sugar composition was found to be significantly lower in the Nunoan population, having an average of 6.01 ± .89 g/100mL vs. the Tibetan populations that showed 7.25 ± 0.35 and 7.19 ± 0.55 g/100mL respectively (p=0.00, p=0.00). Nunoan breast milk shows an elevation in protein, compared to High Nubri milk, that is trending toward significance (p=0.068). The Nunoan is significantly higher in protein than Katmandu milk (p=0.0042).

4. Discussion

4.1 Predictive Factors for Fat Composition

Maternal waist-to-hip ratio is the most significant predictor of milk fat composition for Nunoan mothers. Small waist-to-hip ratios (small waist circumference to large hip circumference) result in higher milk fat levels. This factor was significant in predicting fat levels in all multivariable regression models (Table 4). Quinn et al. (2016) pointed out the influence of maternal fat reserves upon milk fat composition, noting that maternal "adipose tissue is preferentially deposited in the trunk and thighs," in both "well- and under-nourished populations" (Butte et al. 1984; Butte and Hopkinson 1998; Sohlström and Forsum,1995). However, the specifics of body type and the relative anatomical dimensions of fat distribution have not been addressed in these populations.

Waist-to-hip ratio (WHR) has been shown through many mortality and morbidity related studies as an indicator for adult health (de Koning et al. 2007; Perona et al. 2017; Barquera et al. 2007; Butovskaya et al. 2017; Chukwunyere and Anigbogu 2017). For instance, de Koning et al. (2007) showed a 0.01 increase in WHR correlates with a 5% increased risk of future cardiovascular disease (de Koning 2007). Even more relevant for our study is that high maternal WHR has been associated with childhood stunting (Barquera 2007).

Based on the prior studies noted in Quinn et. al. (2016) that demonstrate maternal fat mobilization to the trunk and thighs (Butte et al. 1984; Butte and Hopkinson 1998; Sohlström and Forsum 1995), it would be a reasonable expectation for increased hip circumference to predict higher breast milk fat content. However, the data obtained from Nunoan mothers indicate that hip size alone is a poor predictor of milk fat composition while low waist-to-hip ratio is a strong predictor of high milk fat content. Thus, the Nunoan findings support that higher adipose tissue in the lower regions results in higher fat compositions in breast milk, but the significant negative correlation between maternal waist-to-hip ratio and milk fat, notably suggests that maternal nutrition and overall health status may play a large role in milk fat composition.

4.2 Predictive Factors for Protein Composition and Lactose Composition

The only predictive factor of milk protein was maternal hip circumference with a minimal (low regression coefficient), negative correlation between hip circumference and milk protein composition [coefficient = - 0.018, p= 0.016*). This suggests that protein is much more variable from mother to mother than can be assessed by any one or grouping of predictive variables. A more in depth analysis of protein composition could examine the variability in maternal diet, particularly a thorough 24 hour intake assessment prior to milk collection, in order to assess the effects of distal and proximal dietary habits upon milk protein composition.

Predictive factors for lactose composition included increased maternal waist circumference, maternal waist-to-hip ratio, and infant age (p= 0.029, p= 0.034, 0.038). However, the most significant predictor of milk fat composition for Nunoan mothers was noted when the interaction between waist-to-hip and baby age are parsed out, showing that infant age alone has a significant negative correlation with milk lactose (p= 0.003). This was an expected relationship, as mothers with younger infants may not have fully recuperated their normal body habitus (i.e. uterus remains enlarged and skin has not retracted yet). Furthermore, it has been noted that there is a decrease in body fat through prolonged breastfeeding (McClure et al. 2012).

4.3 Differences between Nunoan and Tibetan populations:

This high altitude study confirmed elevated breast milk fat levels comparable to the high altitude Tibetan residents in the Quinn (2016) study, which represented higher fat levels than that previously documented in comparative studies of breast milk in lower altitude regions, such as Bangladesh, Pakistan, Ivory Coast, Australia, Guatemala, Egypt, Philippines and the Americas (Quinn 2016).

The statistically significantly lower levels of lactose composition and higher levels of protein in Nunoan milk samples, compared to the High Nubri resident samples obtained in Tibet, were surprising. Lactose levels in milk in the Nunoan study were best predicted by infant age, similar to the pattern noted in Tibet by Quinn (2016). However, the overall decrease in lactose composition found amongst Nunoan breast milk samples, relative to that in Tibet, is contrary to expectation. Given that foremilk is high in lactose and low in fat, while hindmilk is notably fattier, one would expect that the unanimous on-demand feeding pattern observed in Nunoa would have produced high lactose levels. That is, the typically short feeding burst intervals observed here should predominantly produce breast milk that should resemble foremilk. This finding of low lactose levels in Nunoa may then reflect specific circumstances in this region, such as dietary influences or a physiological adaptation to high altitude that differs from the Tibetan response. We have already noted distinct respiratory responses between Andeans and Tibetans to hypoxia at high altitude. Perhaps differing lactose content in breast milk reflects a unique pattern of breast milk buffering in Nunoa not seen in Tibet.

Alternatively, the differing lactose levels between Nunoa and Tibet could reflect the fact that the observed Nunoan mothers were, by comparison, shorter and stockier than the Tibetan mothers studied. Though maternal and infant age were similar in both populations, anthropometric maternal measurements were considerably different between the two populations. Most notably, Nunoan mean weight and BMI (61.64 kg and 27.34 (kg/m^2) was considerably higher than Tibetan figures (52.6kg and 21.7 kg/m^2). Given that the Nunoan results demonstrate a significant negative correlation between waist-to-hip ratio and lactose levels, future comparative studies of milk composition in these two high altitude populations will need to take anthropometric differences into account, or will need to control these variables.

5. Conclusions, Study Limitations, and Future Directions

Through this study, we were able to enumerate the macromolecular composition of breast milk in this region, determine that maternal waist-to-hip ratio is a significant predictor of milk fat, show that hip circumference was a significant predictor of milk protein, demonstrate the interaction between infant age and maternal waist-to-hip ratio as a significant predictor of milk sugar, and perform cross analyses with the High Nubri and Katmandu populations of Tibet which revealed similar high fat levels in the breast milk of rural highland residents in both settings.

It is clear that variability within and across regions is high in terms of predictive factors for milk macromolecule composition. However, it is also apparent that there are a few trends beginning to unfold as reliable breast milk composition predictors. Most notably, maternal waist-to-hip ratios may in fact be a more reliable measurement with which to predict both milk fat and sugar content than general or focal measurements of adiposity currently used for these predictions. This was an unexpected finding that may help to explain regional and individual differences in milk fat composition. To confirm the significance of WHR in this regard, further comparative studies looking at the effects of waist-to-hip ratio and the proportionality of maternal fat upon breast milk composition need to be performed. If the Nunoan results are paralleled in future research, then waist-to-hip ratio could become a primary predictor of milk fat composition.

Another future direction would be to investigate the evidence for the maternal depletion hypothesis through breast milk composition assays. Maternal Depletion Syndrome refers to the nutritional and physiological impact of successive lactations and pregnancies upon the developing child as well as the mother. The trending towards significant linear regressions run between maternal age and the logarithmic transformation of milk fat in this study, and between parity and the logarithmic transformation of milk fat, support the maternal depletion syndrome hypothesis. This supports the need for further analysis of maternal physiological stress and nutritional insufficiency ascribed to successive and numerous pregnancies. Potential outcomes of maternal depletion include, but are not limited to maternal anemia and inadequate pregnancy weight gain, as well as small infant birth weight and size (Winkvist et al. 1992). This can also result in poorer milk nutrition, as demonstrated through the two regression models examining the relationship between maternal age and parity with milk fat content in our study (Table 4). A deeper understanding and analysis of the impact of maternal age and parity upon breast milk composition may provide support for this hypothesis.

Using the Quinn (2016) protocol for a region with highly irregular and primarily on-demand feeding patterns posed a few limitations. Namely, Nunoan mothers fed more frequently and for shorter bursts, in comparison to Tibetan mothers. This on-demand feeding practice, observed unanimously across participants, impacted total volume and overall feeding time. Therefore, future studies should revisit the Quinn (2016) protocol to better fit this unique population feeding culture. The Nunoan population had very short feeding durations that made a pre-collection wait time of two minutes difficult to carry out, as many of the mothers ran out of milk or usually stopped feeding before this two minute interval was completed. Thus, a protocol that better fits this breastfeeding culture should be considered for future breast milk studies. However, an understanding of the milk composition in early periods of a feed must also be considered, since foremilk contains large quantities of protein, and hindmilk contains larger quantities of fat (Miller 2013). A new protocol could positively impact researcher ability to perform meaningful cross-population analyses.

References Cited:

Baker, P. T. (1996). Adventures in human population biology. *Annual Review of Anthropology, 25*, 1-18.

Bailey, S.M., Xu, J., Feng, J.H., Hu, X., Zhang, C., Qui, S. (2007). Tradeoffs between oxygen and energy in tibial growth at high altitude. *Am J Hum Biol 19*, 662–668.

Ballard, O. & Morrow, A.L. (2013). Human Milk Composition. *The Pediatric Clinics of North America. 60*(1), 49–74.

Barbosa, L., Butte, N. F., Villalpando, S., Wong, W. W., & Smith, E. O. (1997). Maternal energy balance and lactation performance of Mesoamerindians as a function of body mass index. *The American Journal of Clinical Nutrition, 66*(3), 575-583. doi:10.1093/ajcn/66.3.575

Barquera, S., Peterson, K. E., Must, A., Rogers, B. L., M Flores, M., Houser, R. Monterrubio, E. & Rivera-Dommarco, J. (2007). Coexistence of maternal central adiposity and child stunting in Mexico. *International Journal of Obesity 31*(4), 601–607.

Beall, C. (2007). Two Routes to Functional Adaptation: Tibetan and Andean High-Altitude Natives. *Proceedings of the National Academy of Sciences of the United States of America, 104*, 8655-8660.

Beall, C. M. (2006). Andean, Tibetan, and Ethiopian patterns of adaptation to high-altitude hypoxia. *Integrative and Comparative Biology,46*(1), 18-24. doi:10.1093/icb/icj004

Beall, C. M. (1981). Optimal Birthweights in Peruvian Populations at High and Low Altitudes. *American Journal of Physical Anthropology 56*(3): 209–216.

Beall, C. M., Baker, P. T., Baker, T. S. & Haas, J.D. (1977). The Effects of High Altitude on Adolescent Growth in Southern Peruvian Amerindians. *Human Biology 49*(2): 109–124.

Brown K.H., Akhtar N.A., Robertson A.D., & Ahmed M.G. (1986). Lactational capacity of marginally nourished mothers: relationships between maternal nutritional status and quantity and proximate composition of milk. *Pediatrics, 78*(5), 909–919.

Butovskaya, M., Sorokowska, A., Karwowski, M., Sabiniewicz, A., Fedenok, J., Dronova, D., Negasheva, M., Selivanova, E. & Sorokowski, P. (2017). Waist-to-hip ratio, body-mass index, age and number of children in seven traditional societies. *Scientific Reports*, *7*(1), 1622-9.

Butte, N. F., Garza, C., Stuff, J. E., Smith, E.O., & Nichols, B.L. 1984. Effect of maternal diet and body composition on lactational performance. *Am J Clin Nutr, 39*, 296–306.

Butte, N., & Hopkinson, J. (1998). Body composition changes during lactation are highly variable among women. *The Journal of Nutrition . 128*(2), 381S–385S.

Carratù B., Boniglia C., Scalise F., Ambruzzi A., & Sanzini E (2003). Nitrogenous components of human milk: Non-protein nitrogen, true protein and free amino acids. *Food Chemistry 81*(3),357–362.

Chukwunyere, C. & Anigbogu, C. (2017). Comparison of waist-to-hip ratio and body mass index in first trimester as surrogate markers for development of preeclampsia. *Endocrine Practice : Official Journal of the American College of Endocrinology and the American Association of Clinical Endocrinologists. 23*(4), 124.

Coward, W. A., Paul, A. A., & Prentice, A. M. 1984. The impact of malnutrition on human lactation: observations from community studies. *Fed Proc, 43*, 2432–2437.

de Koning, L., Merchant, A.T., Pogue, J., & Anand, S.S. (2007). Waist circumference and waist-to-hip ratio as predictors of cardiovascular events: meta-regression analysis of prospective studies, *European Heart Journal*, *28*(7), 850–856.

Dell, M. (2010). The persistent effects of Peru's mining mita. *Econometrica*, *78*(6), 1863-1903.

de Meer, K., Bergman, R., Kusner, J. S., & Voorhoeve, H. W. (1993). Differences in physical growth of aymara and quechua children living at high altitude in peru. *American Journal of Physical Anthropology, 90*(1), 59-75. doi:10.1002/ajpa.1330900105

Ellison, P. T. (2001). *On fertile ground*. Cambridge, MA: Harvard University Press.

Feng, P., Gao, M., Burgher, A., Zhou, T. H., & Pramuk, K. (2016). A nine-country study of the protein content and amino acid composition of mature human milk. *Food & nutrition research*, *60*, 31042.

Finley, D. A., Lönnerdal, B., Dewey, K. G., & Grivetti, L. E. (1985). Breast milk composition: Fat content and fatty acid composition in vegetarians and non-vegetarians. *The American Journal of Clinical Nutrition,41*(4), 787-800. doi:10.1093/ajcn/41.4.787

Frisancho, A. R. (2013). Developmental Functional Adaptation to High Altitude: Review. *American Journal of Human Biology*. doi:10.1002/jhb.22367

Frisancho A.R. (1969). Human growth and pulmonary function of a high altitude Peruvian Quechua population. Hum Biol 91:365–379.

Frisancho A.R. (1970). Developmental responses to high-altitude hypoxia. *Am J Phys Anthropol 32*(2), 401–407.

Frisancho A.R., Baker P.T. (1970). Altitude and growth: a study of the patterns of physical growth of a high-altitude Peruvian Quechua population. *Am J Phys Anthropol 32*(2), 279–292.

Garrett, D. T. (2005). *Shadows of empire : the Indian nobility of Cusco, 1750-1825.* Cambridge, UK: Cambridge University Press.

Garruto, R. M., C.-T. Chin, C. A. Weitz, J.-C. Liu, R.-L. Liu, and X. He. (2003). Hematological differences during growth among Tibetans and Han Chinese born and raised at high altitude in Qinghai, China. *Am. J. Phys. Anthropol. 122*, 171–183.

Greksa, Lawrence P. (2006). Growth and Development of Andean High Altitude Residents. *High Altitude Medicine & Biology 7*(2), 116–124.

Greksa, L. P., Spielvogel, H., Paredes-Fernandez, L., Paz-Zamora, M., & Caceres, E. (1984). The Physical Growth of Urban Children at High Altitude. *American Journal of Physical Anthropology 65*(3), 315–322.

Greksa, L. P., Spielvogel, H., Paz-Zamora, M., Caceres, E., & Paredes-Fernandez, L. (1988). Effect of Altitude on the Lung Function of High Altitude Residents of European Ancestry. *American Journal of Physical Anthropology 75*(1), 77–85.

Haas, J. D. (1972). Physical Growth and Development of Peruvian Infants at High and Low Altitude. *American Journal of Physical Anthropology 37*, 438–439.

Haas, J. D., Baker, P. T., & Hunt, E. E. (1977). The Effects of High Altitude on Body Size and Composition of the Newborn Infant in Southern Peru. *Human Biology 49*(4), 611–628.

Haas, J. D., Moreno-Black, G., Frongillo, E. A., ... & Luis-Hurtado, G. (1982). Altitude and Infant Growth in Bolivia: A Longitudinal Study. *American Journal of Physical Anthropology 59*(3), 251–262.

Hahn, R.A., & Inhorn, M.C. (2009). *Anthropology and Public Health.* London: Oxford University Press.

Hoff, C. (1974). Altitudinal Variations in Physical Growth and Development of Peruvian Quechua. *Homo 24*, 87–99.

Hoke, Morgan. (2017). Growing Babies, Growing Inequalities: A Biocultural Examination of Influences of Infant Growth in Nuñoa, Peru. Northwestern University, ProQuest Dissertations Publishing. https://proxy.library.upenn.edu:2072/docview/1914377876/fulltextPDF/959E1942FEDE4902PQ/1?accountid=14707

Hoke, M. K. and Leatherman, T. L. (2018). Secular trends in growth in the high-altitude district of Nuñoa, Peru 1964-2015. *Am J Phys Anthropol. 168*(1), 200-208.

Jelliffe, D. B., & Jelliffe, E. F. (1978). The volume and composition of human milk in poorly nourished communities. A review. *The American Journal of Clinical Nutrition, 31*(3), 492-515. doi:10.1093/ajcn/31.3.492

Jensen, R. G. (1999). Lipids in human milk. *Lipids, 34*(12), 1243-1271.

Johnson W., Darboe M.K., Sosseh F., Nshe P., Prentice A.M., & Moore S.E. (2017) Association of prenatal lipid□based nutritional supplementation with fetal growth in rural Gambia. *Matern Child Nutr. 13*(2), e12367.

Koletzko, B., von Kries, R., Closa, R., Escribano, J., Scaglioni, S., Giovannini, M., Beyer, J., Demmelmair, H., Anton, B., Gruszfeld, D., Dobrzanska, A., Sengier, A., Langhendries, J.P., Rolland Cachera, M.F., & Grote, V. (2009). Can infant feeding choices modulate later obesity risk? *Am J Clin Nutr 89*, 1502S–1508S.

Kuzawa, C., & Sweet, E. (2008). *The embodiment of race: Health disparities in the age of epigenetics.* ().Institute for Policy Research, Northwestern University. Retrieved from Policy File Index Retrieved from https://proxy.library.upenn.edu/login?url=https://proxy.library.upenn.edu:2072/docview/1820767917?accountid=14707

Landecker, H. (2011). Food as exposure: Nutritional epigenetics and the new metabolism. *Biosocieties, 6*(2), 167-194.

Leonard, W. R. (2018). Centennial perspective on human adaptability. *American journal of physical anthropology*, *165*(4), 813-833

Leatherman, T. L. (1996). A biocultural perspective on health and household economy in southern peru. *Medical Anthropology Quarterly, 10*(4), 476-495.

Leatherman, T. and Hoke, M. (2016). Critical Biocultural Anthropology: A Model for Anthropological Integration. In, *Companion to Contemporary Anthropology*. S. Coleman, S. Hyatt, and A. Kingsolver (Eds.). New York: Taylor and Francis.

Leatherman, T.L. and Brooke Thomas, R.B. (2009) 'Structural Violence, Political Violence and the Health Costs of Civil Conflict: A Case Study from Peru', in Hahn, R.A. and Inhorn, M.C. (eds.), Anthropology and Public Health: Bridging Differences in Culture and Society, 2nd ed., Oxford: Oxford University Press, 243–267.

Leonard, W. R. (2018). Centennial perspective on human adaptability. *American Journal of Physical Anthropology*, *165*(4), 813-833

Leonard, W. R., DeWalt, K. M. Stansbury, J. P., & McCaston, M. K. (1995). Growth Differences between Children of Highland and Coastal Ecuador. *American Journal of Physical Anthropology 98*(1), 47–57.

Leonard, W.R. (1989). Nutritional Determinants of High-Altitude Growth in Nuñoa, Peru. *American Journal of Physical Anthropology. 80*(3), 341–52.

Lock, M. M. (2001). The Contributions of Medical Anthropology to Anthropology and Beyond. *Medical Anthropology Quarterly, 15*(4), 478-492.

Lock, M. M. (1993a). *Encounters with aging : Mythologies of Menopause in Japan and North America*. London: University of California Press, p38-39.

Lock, M. (1993b). Cultivating the body: Anthropology and epistemologies of bodily practice and knowledge. *Annual Review of Anthropology, 22*(1), 133-155.

Luerssen, S.(1994). Landlessness, Health, and the Failures of Reform in the Peruvian Highlands. *Human Organization, 53*(4), 380-387

McClure, C. K., Catov, J., Ness, R., & Schwarz, E. B. (2012). Maternal visceral adiposity by consistency of lactation. *Maternal and child health journal*, *16*(2), 316-321.

Melby, M. K., Lock, M., & Kaufert, P. (2005). Culture and symptom reporting at menopause. *Human Reproduction Update, 11*(5), 495-512.

Miller, E. M., Aiello, M. O., Fujita, M., Hinde, K., Milligan, L., & Quinn, E. A. (2013). Field and laboratory methods in human milk research. *American Journal of Human Biology*, *25*(1), 1-11.

Miranda, J.J. (2004). Reproductive health without rights in Peru. *Lancet, 363*(9402), 68-69.

Moore, L., Shriver, M., Bemis, L., Hickler, B., Wilson, M., Brutsaert, T., . . . Vargas, E. (2004). Maternal Adaptation to High-altitude Pregnancy: An Experiment of Nature—A Review. *Placenta, 25*, S60-S71, doi:10.1016/j.placenta.2004.01.008

Moore, L. G. (2003). Fetal Growth Restriction and Maternal Oxygen Transport during High Altitude Pregnancy. *High Altitude Medicine & Biology 4*(2), 141–156.

Morbeck, M., Galloway, A., & Zihlman, A. (1998) *The Evolving Female: A Life History Perspective.*Princeton, Princeton University Press, December 9, 1996.

Mortola, J. P., Frappell, P. B., Aguero, L., & Armstrong, K. (2000). Birth Weight and Altitude: A Study in Peruvian Communities. *The Journal of Pediatrics. 136*(3), 324–329.

Mueller, W. H., Schull, V. N., Schull, W. J., Soto, P., & Rothhammer, F. (1978). A Multinational Andean Genetic and Health Program: Growth and Development in an Hypoxic Environment. *Annals of Human Biology. 5*(4), p.329-352.

Niewöhner, J., & Lock, M. (2018). Situating local biologies: Anthropological perspectives on environment/human entanglements. *Biosocieties,* doi:10.1057/s41292-017-0089-5

The Nunoa Project Website. http://www.nunoaproject.org/history.html. Published 2017. Accessed April 15, 2018.

Perona, J. S., Schmidt-RioValle, J., Rueda-Medina, B., Correa-Rodríguez, M., & González-Jiménez, E. (2017). Waist circumference shows the highest predictive value for metabolic syndrome, and waist-to-hip ratio for its components, in Spanish adolescents. *Nutrition Research (45)*, 38-45.

Prentice A. (1995). Regional variations in the composition of human milk. In: Jensen RG, editor. Handbook of Milk Composition. Academic Press, San Diego, CA. p 115–221.

Prentice, A. M., Goldberg, G.R., & Prentice, A. (1994). Body mass index and lactation performance. *Eur J Clin Nutr, 48*(Suppl 3), S78–S89

Quinn, E. A., Bista, K. D., & Childs, G. (2016). Milk at altitude: Human milk macronutrient composition in a high-altitude adapted population of tibetans. *American Journal of Physical Anthropology,159*(2), 233-243. https://www.ncbi.nlm.nih.gov/pubmed/26397954

Quinn, E. A., Largado, F., Power, M., & Kuzawa, C. W. (2012). Predictors of breast milk macronutrient composition in filipino mothers. *American Journal of Human Biology, 24*(4), 533-540. doi:10.1002/ajhb.22266 http://proxy.library.upenn.edu:2120/doi/10.1002/ajhb.22266/abstract

Riva, E., Verduci, E., Giovannini, M., & Agostoni, C. (2004). Early protein intakes and adiposity: reloaded or downloaded? *Acta Paediatr, 93*, 725–726.

Schafrank, L., *Fieldnotes Summer 2018*. Nunoa, Peru

Smilowitz, J. T., Lebrilla, C. B., Mills, D. A., German, J. B., & Freeman, S. L. (2014). Breast milk oligosaccharides: structure-function relationships in the neonate. *Annual review of nutrition*, *34*, 143-169.

Sohlström A, Forsum E. 1995. Changes in adipose tissue volume and distribution during reproduction in Swedish women as assessed by magnetic resonance imaging. *Am J Clin Nutr, 61*(2), 287–295.

Stinson, S. (1980). The Physical Growth of High Altitude Bolivian Aymara Children. *American Journal of Physical Anthropology 52*(3), 377–385.

Stinson, S. (1982). The Effect of High Altitude on the Growth of Children of High Socioeconomic Status in Bolivia. *American Journal of Physical Anthropology 59*(1), 61–71.

Viault, F. (1890). Sur l'augmentation considerable du nombre des globules rouges dans le sangchez les habitants des hautes plateaux de l'Amerique du sud. *CR Acad Sci Paris 111*, 918–919 cited in Bunn, H. F. (2013). Erythropoietin. *Cold Spring Harbor Perspectives in Medicine, 3*(3), a011619.

Vick, A.D. & Burris, H.H. (2017). Epigenetics and Health Disparities. *Current Epidemiology Reports 4*(1): 31–37.

Villalpando, S. F., Butte, N. F., Wong, W. W., Flores-Huerta, S., Hernandez-Beltran, M. J., Smith, E. O., & Garza, C. (1992). Lactation performance of rural mesoamerindians. *European Journal of Clinical Nutrition, 46*(5), 337.

Villalpando, S., & del Prado, M. 1999. Interrelation among dietary energy and fat intakes, maternal body fatness, and milk total lipid in humans. *J Mammary Gland Biol Neoplasia, 4*, 285–295.

Vitzthum, V. (1992). Infant nutrition and the consequences of differential market access in Nuñoa, Peru. *Ecology of Food and Nutrition,28*(1-2), 45-63. doi:10.1080/03670244.1992.999125

Weiss, K. M. (1993). *Genetic variation and human disease: principles and evolutionary approaches* (Vol. 11). Cambridge University Press.

Winkvist, A., Rasmussen, K. M., & Habicht, J. P. (1992). A new definition of maternal depletion syndrome. *American journal of public health*, *82*(5), 691-4.

Wojcik, K. Y., Rechtman, D.J., Lee, M. L., Montoya, A., & Medo, E. T. (2009). Macronutrient analysis of a nationwide sample of donor breast milk. *J Am Diet Assoc 109*, 137–140.

Tables

Table 1:

Table 1: Sample characteristics of mothers and infants

Variable	Mean(SD)
N	22
Maternal age (years)	28.27
Parity	2.32 (1.46)
Infant age (months)	10.96 (6.38)
Infant sex (% male)	45
Maternal Height (cm)	150.14(4.94)
Maternal Weight (kg)	61.64(10.90)
Maternal BMI (kg/m^2)	27.34(4.68)
Maternal Waist to Hip Ratio	.87(.07)
Sum of Maternal Skinfolds(mm)	42.96 (13.48)

* depicts the average biological demographic characteristics of study participants (mothers and infants) and the values are means (standard deviations) for mothers and infants

Table 2:

Table 2: Socioeconomic characteristics of participants

Variable	Participants
N	22
Participation in juntos (%)	36.36
Participation in vaso de leche (%)	81.82
Monthly household income	702.62 (589.29)

* depicts the average socioeconomic demographic characteristics of study participants (mothers and infants). Participation of Juntos and Vaso de Leche measured in percent participation and monthly household income reported in soles.

Table 3:

Table 3: Descriptive Characteristics of milk macronutrient and energy content

	N = 23	
	Mean	SD
Fat (g/100 mL)	4.56	.78
Sugar (g/100 mL)	6.01	.89
Protein (g/100 mL)	1.51	.35
True Protein (g/100 mL)	1.04	.30
Number of reported daily feedings	11.93	10.30
Average length of observed feed (seconds)	300.30	172.84

* depicts the average macromolecule composition of study participants breast milk and basic feeding practice averages. The average macromolecule composition of study participants breast milk and basic feeding practices. Macromolecule composition is measured in g/100mL, and feed length is reported in seconds

Table 4:

Table 4. Regression models testing for an association between predictors and milk fat composition

	Milk fat		
	Model 1	Model 2	Model 3
Parity			-0.048 (0.21)
Waist to hip ratio		-1.51 (0.006)**	-1.43(0.04)*
Maternal age			0.001 (0.89)
Maternal bmi	-0.016 (0.08)		0.003 (0.80)
Constant	1.95	2.82	2.73
R2	0.15	0.32	0.41

*p <0.05, **p<0.01, ***p<0.001
(model 1: linear regression log transformation of milk fat and bmi; model 2: linear reg log transformation of milk fat waist2hip; model 3: multivariable regression between the log transformation of milk fat , child quantity, waist2hip ratio, maternal age, and bmi)

Table 5:

Table 5. Regression models testing for an association between predictors and milk Sugar composition

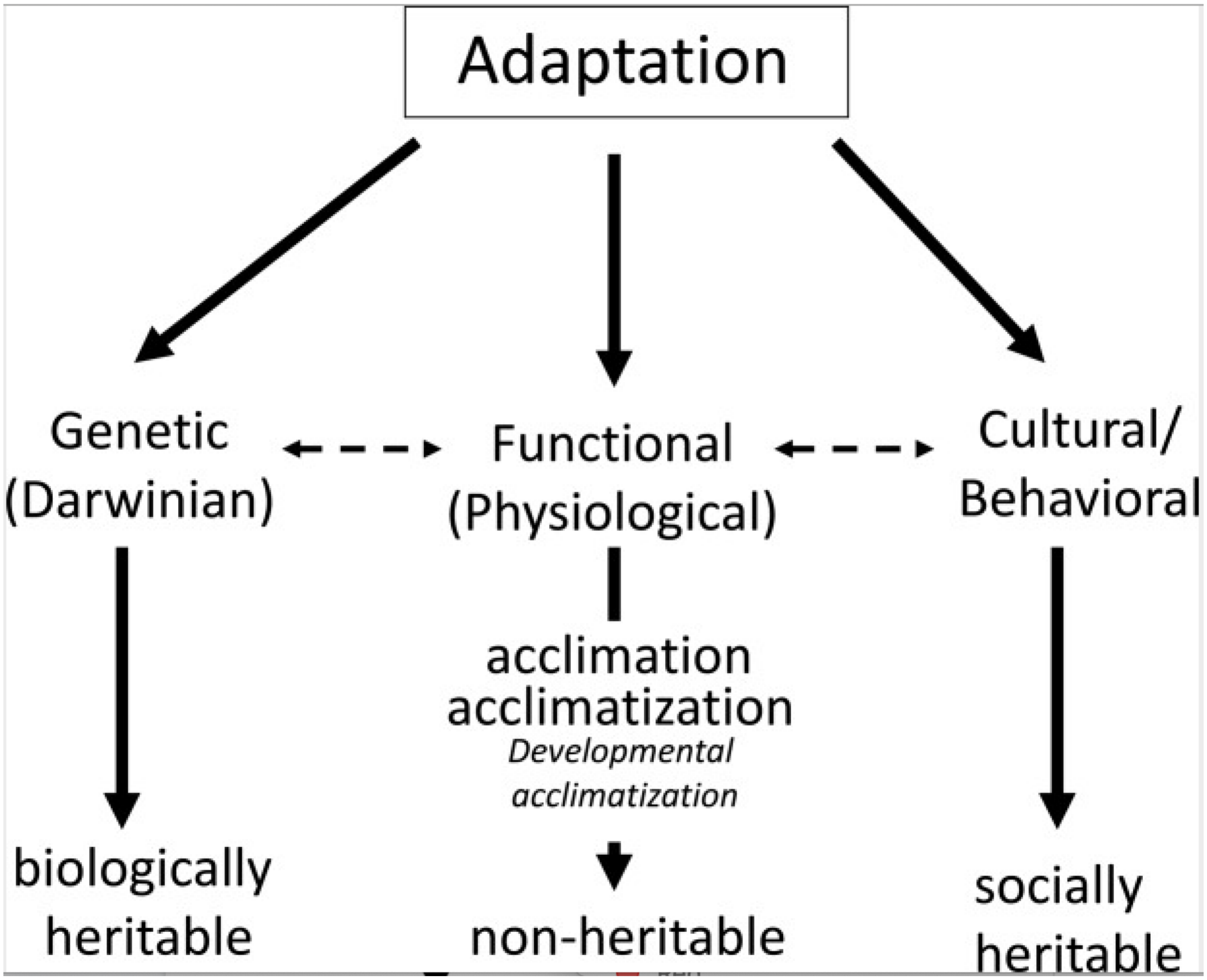
	Milk lactose		
	Model 1	Model 2	Model 3
Waist to hip (W2H)	1.29 (0.034)*	.10(0.93)	-.68(0.35)
Infant age		-.009(0.24)	-.25(0.003)**
Waist Circumference		.006(0.39)	
Waist-hip ratio x Infant age			.28(0.003)**
Constant	0.65	1.22782	2.43
R2	0.21	0.29	0.55

*p <0.05, **p<0.01, ***p<0.001

(model 1: linear regression log transformation of milk sugar and waist2hip; model 2: multivariable regression between the log transformation of milk sugar waist2hip, baby age, and waist circumference; model 3: multivariable regression between the log transformation of milk sugar, waist to hip ratio, baby age, and W2H x babyage)

Figures

Figure 1: Adaptation map showing the interconnections between the three types of adaptations from the Leonard et al. (2018) study on centennial perspective on human adaptability (Leonard 2018).

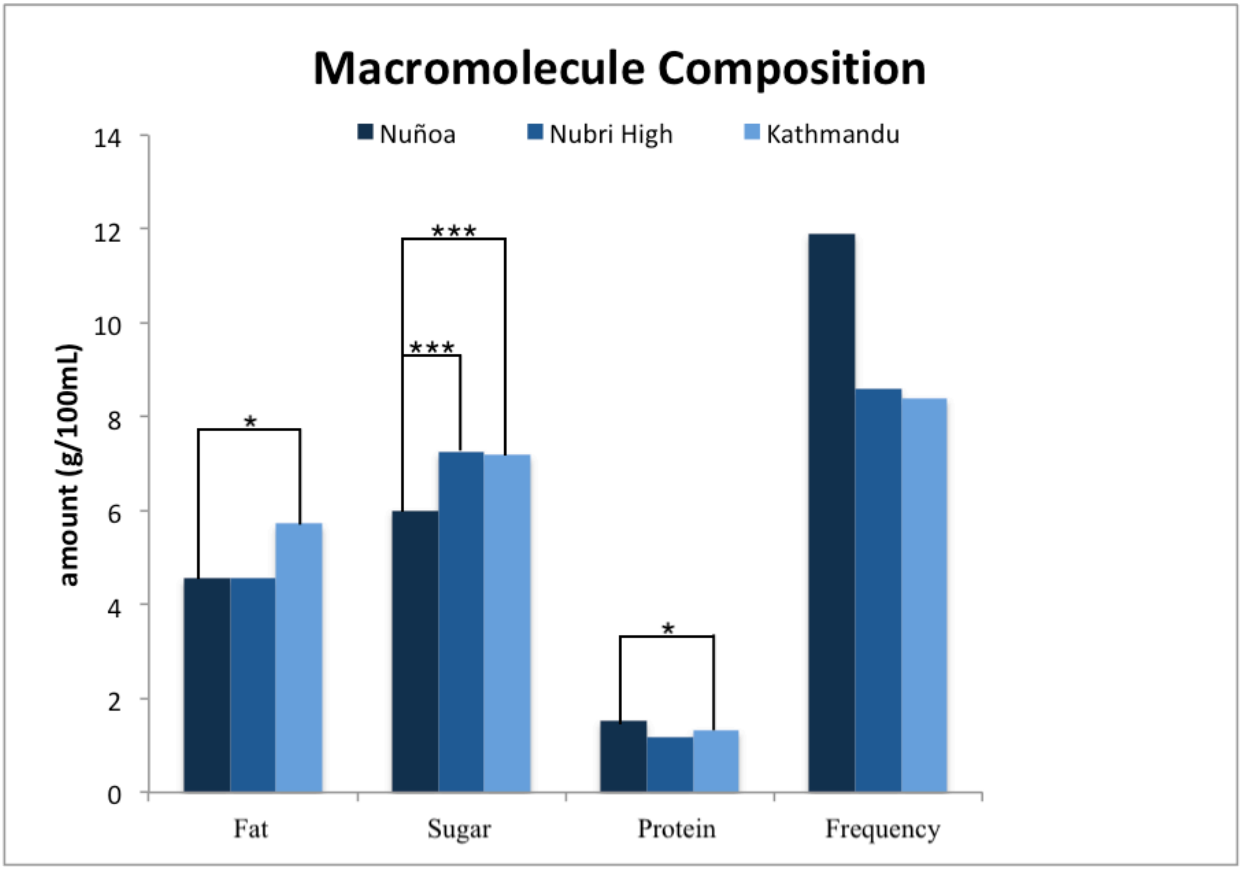


*depicting the different paths of adaptations and the interrelationships between them

Figure 2: Image of an early morning collection on the mountain side of Lekechani, Peru from my fieldwork in the summer of 2018.

*Depicting an example of a mid-feed, self-expressed collection (photo consented by participant)

Figure 3: A graph showing sample t tests with unequal variances were run comparing the fat, sugar, and protein content in the Nunoan, High Nubri, and Katmandu populations.



*depicting the average macromolecule breast milk composition of Nunoan, High Nubri, and Katmandu populations. Reveals a significant difference in milk fat between Nunoan and Katmandu populations, a significant difference in milk sugar between Nunoan and Katmandu and between Nunoan and High Nubri populations, a significant difference in milk sugar between Nunoan and Katmandu populations [*p<0.05;**p<0.01, ***p<0.001]