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Amanda Veile

Candidate

Anthropology

Department

This dissertation is approved, and it is acceptable in quality
and form for publication:

Approved by the Dissertation Committee:

[Signature]

, Chairperson

Jane B. Lancaster

Michael Brown

Jeff W. King

Kevin Paul

THE EVOLUTIONARY ECOLOGY OF HUMAN INFANCY

BY

AMANDA VEILE

B.S., Anthropology, University of New Mexico, 2004

M.S., Anthropology, University of New Mexico, 2006

DISSERTATION

Submitted in Partial Fulfillment of the
Requirements for the Degree of

Doctor of Philosophy
Anthropology

The University of New Mexico
Albuquerque, New Mexico

July, 2011

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DEDICATION

To my daughter, Simone Michelle.
The world is a better place now because you are in it.

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The completion of this dissertation would not have been possible without my family, committee members, mentors and friends who have provided invaluable support over the course of many years. In particular, I would like to thank my daughter Simone. While she came along late in my graduate career, two years of motherhood has provided me as much data on the ecology of infancy as did two years of fieldwork. She has also helped me become an efficiency expert, and brought joy and meaning to my life in innumerable unexpected ways.

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ABSTRACT

Infancy is a time of profound energetic trade-offs, and in many South American native groups, infant growth is stunted and mortality by infectious disease is high. The goal of this dissertation was to explore the nature of human infancy from a life history theoretical perspective. Specifically, I investigated infant growth, feeding patterns, and thymic development in two South American native populations, the Tsimane of Bolivia and the Pumé of Venezuela. This broad goal is addressed through four specific goals: 1) to model the weaning transition using behavioral data collected in Tsimane communities where infants experience varying mortality rates, 2) to consider the relationship between infant feeding and growth patterns; 3) to compare infant body and thymus size in two South American native societies, and 4) to theorize how the thymus may be shaped by natural selection. Results suggest that infant feeding is a complex and varied process that is influenced more by infant growth than by perceptible mortality risk, and that trade-offs between investment in growth and cellular immune function vary between native communities inhabiting diverse ecologic settings. These findings illuminate the role of early postnatal conditions in shaping maternal behaviors and infant health outcomes; and underscore the pressing need to identify the mechanisms leading to the establishment of immunophenotypes in South American native populations.

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CHAPTER I

INTRODUCTION

1.1 Introduction

The purpose of this dissertation research was to explore the evolutionary ecology of human infancy by investigating the nature of the weaning transition, growth patterns, and thymic development in two South American native populations, the Tsimane of Bolivia and the Pumé of Venezuela. This dissertation includes the collection of original, empirical evidence that illuminates the processes underlying infant and child health outcomes in two contemporary South American native populations. This research also provides a lens on the life histories of our ancestors and conditions that shaped the biology of human disease resistance.

South American native communities provided a unique opportunity to observe infant development and maternal-infant interactions and health patterns in a setting where some life conditions resemble those experienced by our foraging human ancestors. In contemporary, industrialized societies, technology, sanitation and medicine have minimized the costs of infant care practices that do not adhere to the evolved biology of infancy (Ball, 2008, McKenna & McDade, 2005). The behaviors of contemporary forager and forager-horticulturalist women, in contrast, are often shaped in a high child-mortality context. They may therefore exhibit adaptive caretaking strategies, such as optimal feeding practices and protection from disease exposure, that maximize infant survival and benefit maternal fitness.

The thymus, a primary lymphoid organ, and its T-lymphocyte repertoire are involved in intra-cellular pathogen defense, stimulate antibody production, and coordinate immune responses across the human life course (Abbas & Lichtman, 2011; McDade, Beck, Kuzawa, & Adair, 2001). Much research has focused on the impacts of malnutrition and infection on the thymus in areas of the developing world where infants experience more nutritionally and pathogenically challenging

pre- and postnatal environments than those experienced by infants from privileged Western populations (Aaby, Marx, Trautner, Rudaa, Hasselbalch, Jensen & Lisse, 2002; Chevalier, Sevilla, Zalles, Sejas, Belmonte & Parent, 1994). Studies from the developing world underscore the relevance of the thymus for infant and child health outcomes and emphasize the need to better understand its developmental influences. As South American natives experience high levels of infant malnutrition and mortality, it is relevant to consider the factors influencing immune system differentiation in early postnatal life as well as the implausibility of a universal thymic growth trajectory in infancy.

1.2 Theoretical Background

Life history theory is an analytical framework invoked in studies of humans and other animals to explain inter- and intra-population variation in development, behavior, and reproduction. Fitness is maximized when energetic resources are allocated optimally to competing life history functions growth, survival, and reproduction (Gadgil & Bossert, 1970; Smith & Fretwell, 1974; Hill & Hurtado, 1996; Kaplan, Hill et al. 2000; Charnov, 2004). Individuals have a fixed energy budget at a given point in time, therefore trade-offs exist between competing life history functions and investment in one comes at a cost to another (Hill & Hurtado, 1996). When resources are scarce these energetic trade-offs become more pronounced and in risky situations, short-term survival mechanisms are favored at the expense of growth and reproduction (Tschirren & Richner, 2006). The scheduling and duration of key events (e.g.; development, reproduction, senescence), or life history strategies, are shaped by natural selection to produce the largest possible number of surviving offspring. Under this framework parenting strategies are seen as behavioral strategies shaped by natural selection, adaptive and flexible across socio-ecologic settings, and designed to increase fitness by maximizing reproduction and offspring survival.

The human life course is characterized by a prolonged lifespan, and an extended period of juvenile dependence, the subsidization of reproduction through the provisioning of females and their offspring by men, older post reproductive individuals and other kin, and the gradual development in the first seven years of life an exceptionally large brain (Kaplan, Hill et al. 2000). Compared to other social mammals, who exhibit just three life history phases, (infancy, juvenility, and adulthood), the human life history strategy includes well defined periods of, infancy, childhood, adolescence, adulthood, and a post-reproductive phase (Bogin & Smith, 1996). Infancy is the period of maternal provisioning of offspring through lactation and ends with weaning

(termination of lactation) (Bogin, 1998). Childhood is a time of prolonged nutritional dependence and learning (Hochberg, 2009), and the transition between infancy and childhood entails important changes in metabolism and immune function (Dettwyler, 2004).

In contrast to the abrupt transition from prolonged breastfeeding to nutritional independence observed in our closest living relatives, the great apes, the human infancy phase is short and weaning is accelerated (Kennedy, 2005). This is possible because other group members (fathers, grandparents and other extended kin) contribute to the provisioning of weaned children, allowing earlier cessation of breastfeeding and allowing mothers to resume ovulation and invest in future reproductive opportunities. The insertion of a childhood period seems to provide an adaptive reproductive function in that human interbirth intervals are shortened and reproductive rates increased (Humphrey 2010, Bogin, 1999a; Bogin, 1999b). Furthermore, energy-dense provisioned foods provide nutrients required to fuel human brain growth that continues until about seven years of age (Kennedy, 2005). Sellen (2009) argues that contemporary patterns of infant development and feeding probably emerged in the Pleistocene because these derived characteristics are unique to humans. There is little variation in infant developmental patterns with respect to alimentary, neurobehavioral and psychomotor development across contemporary human populations (*ibid*). Still substantial cross-cultural variation in infant feeding practices and immune function exist, particularly between industrialized and subsistence-based societies. These differences are driven by factors such as disease ecology, resource availability, and subsistence strategy. Ecological differences are further mediated by intricately intertwined cultural and demographic factors including household wealth, household composition (Howrigan, 1988), maternal education (Kazimi & Kazimi, 1979), maternal social support (Raphael & Davis, 1985) maternal experience (Dettwyler, 1987; Zeitlin, 1991), maternal illness or pregnancy (Cosminsky, Mhloyi et al. 1993) availability of all parents (Quinlan, 2003) and maternal workload (Van Esterik, 2002; Ghosh, Mascie-Taylor et al. 2006).

Human infancy is a time of pronounced energetic trade-offs, as infants must convert available resources to costly body and brain growth and maintenance, as well as to develop a complex adaptive immune system that will protect them from pathogens throughout the life course (McDade, 2005a). Mothers, as the primary caregivers of infants, are expected to employ adaptive infant care and feeding strategies that facilitate infant growth and survival. All mothers face constraints and trade-offs, particularly where resources are scarce. Infant care strategies must

therefore be flexible across diverse ecologic contexts and also should balance the needs of multiple dependent (current and future) offspring.

Infant Feeding and Infant Health

A successful transition from exclusive breastfeeding to nutritional independence facilitates optimal growth, development, and survival for infants (McDade & Worthman, 1998). Human milk has evolved as an adaptation to meet the complex demands of the developing infant that provides adequate macro-and micro-nutrients uniquely adapted to the energetic demands of a growing infant (ibid). Breast milk also provides passive immune factors for immunologically naive infants as well as bioactive hormones and growth factors that help regulate histological growth and differentiation and facilitate nutrient transfer in infants (Goldman, 1993; Grosvenor, Picciano et al. 1993; Lönnerdal, 2003). Breastfeeding has been shown to reduce respiratory and diarrheal morbidity in infants, which is particularly relevant in many rural and urban developing world regions where sanitation is substandard and medical care is infrequently available (Räihä, 1981; Popkin, Adair et al. 1990; Cunningham, 1995). Infant feeding practices should reflect infant mortality risk, which varies across the South American native communities in this study. It is expected that weaning should be delayed in communities where infant infectious disease mortality is high. Because lactation is energetically costly for mothers and may be diminishing returns to intensive nursing where infant mortality is low, we expect to see accelerated weaning in communities where infant mortality rates are low. This leads to the following general hypothesis:

H1. The timing and nature of the weaning transition will vary across communities experiencing different infant mortality rates; weaning will be extended in communities where infectious disease morbidity is high, and accelerated in communities where infectious disease morbidity is low.

Infant Feeding and Infant Growth

Growth faltering (defined as a child's growth curve moving downwards (de Onis, Wijnhoven et al. 2004) is frequently reported upon introduction of complementary foods (Lunn P.G., Northrop-Clewes C.A. et al. 1991). While this is sometimes provided as justification for delaying complementary feeding, the interactions between growth, immunity, and weaning are complex. In several mammalian models, including humans, in which offspring are weaned when

they achieve a certain threshold body size (Lee, 1996). As infant body size and growth vary substantially across societies, it is therefore not surprising that human weaning schedules vary greatly (Bogin, 1998). A relationship between weaning and infant body size has been found in studies of weaning and growth (Padmadas, Hutter et al. 2002), though the question of the directional causality of this relationship has only been explored in a handful of studies ((Marquis, Habicht et al. 1997; Fawzi, Herrera et al. 1998; Simondon & Simondon, 1998; Simondon, Simondon et al. 2001; Tracer, 2009), and it appears that infant feeding patterns are maternal investment strategies that are driven by (rather than drivers) of infant condition.

Maternal decision-making with respect to infant feeding is influenced by variation in growth and condition of individual infants. According to the threshold models of weaning, larger, faster-growing infants will achieve necessary threshold weights at earlier ages (Ellison, 2001). They also require more nutrients to fuel rapid growth, and thus will outstrip maternal energetic resources (breast milk energy) at younger ages and require additional nutrients from supplementary foods (Institute of Medicine, 1991; Jenkins & Haywood, 1985; Jenkins, Orr-Ewing & Heywood, 1984). There are therefore diminishing returns to prolonged intensive breastfeeding of fast-growing infants. By accelerating the weaning process in rapidly-growing infants, mothers are able to reduce the energetic burden of lactation. This benefits maternal fitness by allowing an earlier resumption of ovulation. This leads to the following general hypothesis:

H2. Large infants will experience an accelerated weaning process compared to smaller infants.

The Infant Feeding Study

Adaptive caregiving strategies should reflect trade-offs in maternal investment that balance infant mortality risk with the costs of lactation and other current and future reproductive investments (Ball & Panter-Brick, 2001). It has long been observed that infant caregiving practices in many industrialized countries do not reflect the evolved biology of infancy (Bruner, 1972; Barash, 1986; Ball, 2007). Levine observed that cross-cultural infant care practices reflect broader socio-cultural systems, which often differ vastly from infant development environments of human evolutionary history (1988). In contemporary, modernized societies, technology, sanitation and medicine have minimized the costs of certain infant feeding practices (such as short

breastfeeding durations or lack of co-sleeping) that may be maladaptive in less epidemiologically and nutritionally privileged settings.

Subsistence societies are defined here as those “in which food and other goods are largely produced by the household rather than purchased, and little or no surplus is generated for trade or cash” (Kramer & Greaves, 2007). Mothers in subsistence societies are observed to practice infant care giving strategies that maximize infant survival under conditions more closely resembling those under which our human ancestors probably evolved (in particular, poor sanitation, resource scarcity and high infant mortality). Such care giving practices are characterized by close maternal-infant proximity and frequent, on-demand breastfeeding for the first several months of the infant’s life (Dettwyler, 1987; Vitzthum, 1994).

Adaptive behavioral optima are difficult to determine, and cultural ideologies (ideas, beliefs and assumptions) regarding suitable infant feeding strategies also shape maternal decision-making (Cosminsky, Mhloyi et al. 1993). Retrospective interviews are often used to identify markers of the weaning transition (Wilson, Milner et al. 2006) but this approach can be problematic if mothers report cultural ideals, rather than actual behaviors (Vitzthum, 1994). Only observational studies can accurately ascertain breastfeeding structure by quantifying varied aspects of nursing behaviors (ibid); however such data are rare because it is extremely logistically difficult and time-consuming to collect. In this study, a large observational dataset is used to test the hypotheses regarding infant feeding, mortality and infant growth in a cohort of infants navigating the weaning transition in a forager-horticulturalist society. The relationship between observed and reported infant feeding practices is also explored.

Thymic Development in Infancy

Life history theory provides a framework from which to consider ecological factors underlying distinct immune profiles exhibited by contemporary human populations (Shell-Duncan, 1997; McDade, Beck et al. 2001a). Under this framework, immune function is a survival and repair mechanism whose development and maintenance is associated with both learning and energetic costs that are outweighed by the benefit of having an effective pathogen defense system (Sheldon & Verhulst, 1996; Read & Allen, 2000). As immunological memory is established most effectively in infancy; this period of unique life history-tradeoffs is of particular interest in investigations of developmental plasticity in immune function.

The thymus, a primary lymphoid organ, and its T-lymphocyte repertoire are essential to intra-cellular pathogen defense, stimulate antibody production, and coordinate immune responses across the human life course. Attempts to establish normal thymic development have met with methodological problems, and substantial variation has been reported in its developmental trajectory (Kendall, Johnson et al. 1980). Clearly, environmental factors influence thymic development in early infancy and continue to impact its trajectory over time.

Much research has focused on the impacts of malnutrition and infection on the thymus in areas of the developing world where infants experience more nutritionally and pathogenically challenging pre- and postnatal environments than those experienced by infants from privileged Western populations (Aaby, Marx et al. 2002; Chevalier, Diagbouga et al. 2002; Collinson, Moore et al. 2003). Studies from the developing world underscore the relevance of the thymus for infant and child health outcomes and emphasize the need to better understand its developmental influences. While well-nourished humans are capable of mounting adequate cellular immune responses, malnourished individuals have small thymuses and exhibit diminished cellular immunity (Koster, Palmer et al. 1987; Chandra, 1992; Shell-Duncan, 1997; Savino, 2002). As a particularly costly organ, the thymus should be sensitive to ecologic conditions and develop according to environmental cues of lifetime nutritional availability and disease exposure (George & Ritter, 1996; McDade & Worthman, 1999).

The Tsimane and Pumé are subsistence forager-horticulturalists and foragers and experience malnutrition and high levels of environmental pathogenicity. The Tsimane in general have more access to food resources. They inhabit an environment with more abundant game, practice more intensive agriculture, and have greater access to market foods. The Pumé experience greater resource scarcity, and particularly in the lean wet season, Pumé women have lower BMI than Tsimane adults (Walker, <http://anthropology.missouri.edu/people/walker.html>). It is expected that Pumé infants will also have both smaller body size and smaller thymus size than Tsimane infants, being born to smaller mothers and experiencing more pronounced seasonal resource scarcity. This is particularly true in the savanna Pumé communities. As the thymus is energetically expensive to maintain, and often observed to involute under conditions of malnutrition, we expect less well-nourished Pumé infants to also have smaller thymuses than Tsimane infants across infancy. Previous research has documented thymic growth when nutritional conditions are improved in infants and children undergoing immuno-nutritional rehabilitation (Chevalier, Sevilla,

Zalles, Sejas, Belmonte & Parent, 1994). If thymus size is directly linked to infant nutritional status, we should see a positive relationship between anthropometric indicators of nutritional status and thymus size in both Tsimane and Pumé infants.

This leads to the following hypotheses:

H1. *Pumé infants will have smaller body size than Tsimane infants.*

H2. *Pumé infants will also have smaller thymuses than Tsimane infants.*

H3. *Anthropometric measures will be positively correlated with thymus size in Tsimane and Pumé infants.*

The Infant Thymus Study

Many studies of thymic development in infancy are conducted within nutritionally and epidemiologically privileged populations. Such environments are recent and novel within the context of human evolution, and furthermore, are not even representative of the postnatal experience in many contemporary societies. Considerable flexibility is observed in growth and reproductive maturation patterns (Walker, Gurven et al. 2006; Kramer, 2008) and as plasticity exists in immune development as well, it is possible that a range of thymic developmental trajectories exist. Establishing norms based on well-nourished infants inhabiting sanitary, urban environments obscures the tremendous cross-cultural variation in selection pressures shaping infant development (McDade, 2003). In studying thymic size South American native infants, an attempt is made to provide a preliminary, albeit it imperfect lens on the conditions and selection pressures shaping the biology of cellular immune development. In this study ultrasonography is used to explore infant thymic size in South American native infants and to test the hypotheses regarding the relationship between infant anthropometry and thymus size. Comparisons of thymic size in South American native infants with thymic size in more nutritionally privileged urban populations are also provided.

1.3 Execution of the Research Project

The research protocols of this dissertation, including informed consent, were reviewed and approved by the University of New Mexico Institutional Review Board and the University of New Mexico Health Sciences Center Human Research Review Committee. Data collection was funded by an NSF Research Experience for Undergraduate Students Grant, and NSF Graduate Research Fellowship, and an NSF Research Experience for Graduate Students Grant.

Description of Research Projects

The datasets used in this dissertation were collected under the auspices of two larger projects, “The UNM-UCSB Tsimane Health and Life History Project” (Bolivia) and “Demographic Processes in Pumé Transitional Subsistence Economies” (Venezuela). By participating as a research assistant on these projects, I was able to gain valuable fieldwork experience and access to larger demographic, anthropometric, and behavioral datasets larger than could have been collected on my own. The projects are described here briefly.

UNM-UCSB Tsimane Health and Life History Project

“The UNM-UCSB Tsimane Health and Life History Project is a joint health and anthropology project aimed at understanding the impacts of ecology and evolution on the shaping of the human life course. The project focuses on health, growth and development, aging, economics and biodemography of small-scale populations of hunter-gatherers and horticulturalists. The project also combines biomedical and anthropological research with medical attention among Tsimane, an indigenous forager-farming group living in central lowland Bolivia in the Beni Department. Research with the Tsimane of Amazonian Bolivia began in 2001 under the joint directorship of Michael Gurven (Anthropology, University of California, Santa Barbara) and Hillard Kaplan (Anthropology, University of New Mexico).” (<http://www.unm.edu/~tsimane/>, 2011).

Demographic Processes in Pumé Transitional Subsistence Economies

“Karen Kramer and Russell Greaves, who have worked with the Pumé since 1992, began a new demographic project in 2005. The river Pumé (horticulturalists) and the savanna Pumé (foragers) are two groups of genetically related, but economically distinct native South Americans who live on the llanos of southwest Venezuela. The river Pumé live in permanent villages along

the major rivers that drain the llanos and have a mixed subsistence base of fish, manioc horticulture, animal husbandry, wild foods and occasional wage labor. In contrast, the Pumé who live in the savannas between these major rivers are mobile foragers, subsisting on hunting, fishing, wild root and mango collection, and seasonal bitter manioc cultivation. The current project combines reproductive history and anthropometric data to look at differences in growth and development trajectories, nutritional status, child mortality and age at first birth.” (http://www.fas.harvard.edu/~bioanth/kramer_projects.html, 2011).

Schedule of Data Collection

Data collection for my dissertation occurred in three phases, the first of which occurred when I was an undergraduate student. A detailed description of data collected, including sample sizes, is provided in the appendix section. Specific data collection methods and analyses are described in detail in Chapters III and IV. For a detailed list of the data collected, see Appendix A.

Phase 1-Tsimane Behavior and Infant Feeding Practices (January–July 2003, February–June 2005)

I spent six months in the field in which I monitored time allocation and resource use patterns and conducted structured interviews on infant feeding patterns and infant health among the Tsimane. This exploratory research culminated in a senior honor’s thesis and provided a foundation for future dissertation research.

Phase 2- Demography, Household Sanitation, Thymic Volume Estimates, and Infant Anthropometry (July 2006–February 2007)

I spent one month of intensive training in immuno-nutritional assessment and nutritional rehabilitation of malnourished children by Dr. Ricardo Sevilla at the Center for Immuno-Nutritional Rehabilitation in Cochabamba Bolivia. This training was followed by seven months of data collection in Tsimane villages in which I collected demographic and household sanitation data, mapped villages using GPS, constructed village censuses and conducted infant health checks and thymic volume assessments via portable ultrasound.

Phase 3 (Comparative) Demography, Infant Feeding, Thymic Volume, Estimates, And Infant Anthropometry March–May 2007

In a five-week period, I assisted in the collection of demographic and anthropometric data, monitored time allocation, and conducted infant health checks and thymic volume assessments via portable ultrasound in Pumé villages in Apure, Venezuela.

Scope of the Dissertation

The dissertation is organized as follows. In Chapter II, a brief historical and ethnographic description of the Tsimane and the Pumé study populations is provided. Detailed sections on maternal-infant relations and infant/child health provide a socio-ecological context for subsequent data analysis. Both inter- and intra-population variation relevant to infant health and development are described, particularly with respect to the impacts of recent acculturation. In Chapter III, the transition from exclusive breastfeeding through nutritional independence in Tsimane infants is modeled, and the role of infant body size in driving maternal decisions with respect to infant feeding is considered. In Chapter IV, Tsimane and Pumé infant anthropometric characteristics and thymic volume are compared. The South American native thymic volume measures are then compared to non-native urban populations. The nature of thymic development in infancy is discussed. In Chapter V, a summary of the findings of the dissertation are provided, along with tentative conclusions regarding the evolutionary ecology of human infancy and directions for future research.

CHAPTER 2

DESCRIPTION OF STUDY POPULATIONS

This chapter provides ethnographic descriptions of the Tsimane of Bolivia and the Pumé of Venezuela. These South American native groups provided a unique opportunity to research infants in a context that more closely resembles the conditions under which humans evolved than the urbanized settings in which infant development research often occurs. Like many contemporary South American natives, the Tsimane and Pumé remained isolated until relatively recent history and maintained a foraging lifestyle. They inhabit pathogen-dense environments and experience chronic malnutrition, profound infectious disease susceptibility, and high infant mortality rates (Kramer & Greaves, 2007, Gurven & Kaplan et al. 2007). This combination of environmental pressures was expected to impact maternal behaviors and infant health outcomes.

The Tsimane and Pumé are subsistence societies, and mothers in such societies are often observed to practice indulgent caregiving behaviors, characterized by close maternal-infant proximity and frequent, on-demand breastfeeding for the first several months of the infant's life (Dettwyler, 1987; Vitzthum, 1994). Despite maternal efforts, infant and child mortality is often high in these societies (Coimbra & Santos, 2004). The Tsimane and Pumé inhabit marginal, tropical environments associated with high rates of disease transmission and sanitation is poor in their communities (Kramer & Greaves, 2007; Gurven, Kaplan & Zelada Supa, 2007; Coimbra & Santos, 2004). In general, contemporary foragers experience frequent disease exposure across the life course and helminth infection is the norm (Gurven, Kaplan et al. 2008; Hurtado, Frey et al. 2008; Vasunilashorn, Crimmins et al. 2010).

South American natives exhibit high immunoglobulin titers, elevated C-reactive protein, and frequent infectious disease morbidity (McDade, Leonard et al. 2005b; Gurven, Kaplan et al. 2008). Differential investment in various immune system components is shaped by local ecologies, and the presence of such immunologic phenotype suggests a developmental pathway established early in the life course while memory is developing (Hurtado, 2003). Understanding developmental processes in infant immunity is relevant to understanding health outcomes in South American natives, which are influenced by a combination of factors including parasitic infection, trauma, and genetic homozygosity (Hurtado, Hill et al. 2001; Hurtado, Hurtado et al. 2004). These immunologic profiles may also reflect trade-offs within the immune system whereby investment is biased away from energetically expensive defenses toward less costly ones (McDade, 2005).

2.1 Choice of Study Populations

The Tsimane and Pumé are not replicas of our stone age past, nor are they primitive, isolated or uniform. Like most modern foragers each have a long if sporadic history of contact with colonizing forces. Their subsistence base, level of acculturation, and history of exposure to global forces are unique. These differences contribute to substantial differences in demographic profiles and subsistence strategies practiced by the two populations. While they may provide an imperfect lens on human evolutionary history, contemporary foragers and forager-horticulturalists present an opportunity to document the tremendous range of human behavior and development patterns across diverse and marginal ecological settings. Furthermore, this research is relevant to understanding health and disease patterns in contemporary South American native societies. As Hurtado and colleagues point out, “effective control of infectious diseases is probably the single most important public health initiative that could greatly improve the quality of life for contemporary South American native peoples” (Hurtado, Lambourne et al. 2005) A step toward achieving this important goal is improving our understanding of the behavioral and environmental factors influencing South American indigenous disease susceptibility, particularly in the period of infancy when survival is paramount and immunological memory is developing.

The Infant Feeding Study

Time allocation studies were conducted from 2002-2007 in ten Tsimane communities. The communities are grouped into three “types” based on geographic region and ranked by

acculturation based on a combination of demographic and sociocultural characteristics (see Appendix B and C for further detailed descriptions of each village). The Tsimane are an ideal population in which to study infant feeding study for several reasons. Women's subsistence tasks are compatible with infant care so they remain in close contact with infants throughout the day, facilitating observation of mother-infant interactions. Infants nurse frequently and for long durations, and bottle-feeding is not practiced. Infant mortality in the Tsimane is high compared to the Bolivian national average (Gurven & Kaplan et al., 2007). Most deaths in infancy and childhood are attributable to infectious disease (ibid), so it was expected that infant feeding behaviors would reflect the threat of infectious disease mortality. The Tsimane population size was appropriate to obtain a representative sample of infants at different stages of the weaning transition. Finally, Tsimane villages exhibit a continuum of acculturation, which allowed for examination of infant feeding practices in a relatively genetically homogenous population experiencing a range of environmental pressures.

The Infant Thymus Study

Tsimane and Pumé infant anthropometry and thymic volume were collected in 2006-2007 and the two populations are compared to examine how human immune function, and the thymus in particular, should be designed by natural selection to develop in infancy. The Tsimane and Pumé populations were chosen for the thymus study because they provided an opportunity to explore thymic development in a setting where high pathogen burden and resource scarcity influence the development of infant immunological defenses. Despite logistical challenges, the method of thymic ultrasonography in the field was successfully piloted, enabling comparison of infant thymic size in two genetically distinct groups of South American natives. Very little research has examined cellular immunity in infants from subsistence societies, and to my knowledge no study has examined infant thymic size in South American forager-horticulturalists.

2.2 The Tsimane of Bolivia

Bolivia is consistently ranked one of the poorest countries in Latin America, despite economic growth experienced since 2005 under the administration of Evo Morales Ayma (Parida, 2010). The country is unique in having an indigenous president and an indigenous majority

population (~60%) (WHO, 2005). The Tsimane ethnic group is among one of the largest and least acculturated lowland tribes (Huanca, 1999). Found in the forests and savannas of the Beni region of northeastern Bolivia, Tsimane share distant genetic affiliation with the Yuracare, Trinitario and Quechua ethnic populations, and little affiliation with the nearby Aymara who inhabit the highlands (Bert, Corella et al. 2001). The Tsimane language is considered an isolate that is most closely related to Mosotene, the language of a neighboring indigenous group that inhabits the southern and northern extremes of Tsimane territory (Chicchon, 1992; Huanca, 1999). Tsimane have remained relatively self-reliant and autarkic, even at the time of the Inca empire and in the face of colonizing powers from Europe (Nyberg, 2009; Ellis, 1996).

Beni is the second-largest of Bolivia's nine departments. Established in 1842, Beni lies on average 155 meters above sea level, and its numerous rivers are tributaries of the Amazon. Tsimane territory falls within its San Borja municipality. The regional center, San Borja, is located 230 km west of Beni's capital Trinidad and has a population of ~16,273 (INE, 2003). The region is typically warm and wet, with an average annual temperature of 26.8°C and seasonal periods of heavy rainfall occurring from November to April. Rainfall peaks in January and February and overland travel to San Borja becomes quite treacherous. The dry season occurs from May-October, and temperatures can be quite cool in June and July. Under favorable conditions, San Borja can be reached overland from La Paz and Santa Cruz, Bolivia's major cities, within 24 hours.

Tsimane History and Overview

The Tsimane are ~8000 forager-horticulturalists inhabiting 80-100 villages the Maniqui River system between San Borja and the town of San Ignacio de Mojos (VAIPO 1998; INE 2003). The Tsimane were exposed to Jesuit missionaries in the late 17th century, but remained semi-nomadic until the 1950's (Chicchon 1992), when Catholic and Protestant missions were created. The late 1970's began a period of rapid social and ecological change for the Tsimane when a road was constructed allowing access to Beni from the highland capital city of La Paz. The Tsimane forest gained attention for its vast mahogany reserves and was subsequently opened to commercial logging in the 1980's (Jones, 1990). Since that time, the logging industry has provided sporadic wage labor opportunities for Tsimane men and has decimated much of the forests that the Tsimane inhabit (Nyberg, 2009). The Beni Biosphere Reserve, located downstream from San Borja, was

established 1982 and was declared by UNESCO to be a protected area 1986. Commercial logging is prohibited inside the reserve and only Tsimane are permitted to reside there. Many Tsimane choose instead to reside in heavily logged areas located outside of the reserve.

Other global forces have profoundly impacted Tsimane society over the last four decades. Starting in the early 1970's the New Tribes mission created a system of bilingual schools with trained Tsimane teachers and an elected village chief in each village downstream from the Catholic mission. New Tribes missionaries are also responsible for "Radio Chimane", a local radio station providing news and Bible messages in the Tsimane language starting in 1993. The radio station has contributed substantially to communication and solidarity between Tsimane communities. Announcements are made that are relevant to the Tsimane communities and personal messages are broadcast for a small fee. For example, if a Tsimane man is out of the village working and his wife becomes sick, a message can be sent to him over the radio. Currently all but the most remote Tsimane villages have schools, but there is tremendous variation in the quality of instruction and regularity with which classes are held (Tanner, 2005). The majority of schoolteachers are Tsimane and the Tsimane language is spoken in all communities, though some communities have instructors who are "napos" (non-indigenous Bolivians) who contribute to the superior Spanish fluency of children in these villages. While many Tsimane are bilingual in Tsimane and Spanish, Spanish use is reserved for interactions with outsiders such as merchants or employers.

In 1989, a central representative organization, the Gran Consejo Tsimane, was founded with assistance of the New Tribes Mission. The 1990's was an important time for the Tsimane, as representatives began to interact with larger indigenous movements in Bolivia. The CPIB (Central de Pueblos Indigenas del Beni) was formed in the late 1980's and organized the "March for Territory and Dignity" from Trinidad to La Paz in 1990, which a handful of Tsimane attended. With the help of CPIB, the Tsimane were officially granted territory in the Tsimane forest which has met limited success in the face of commercial logging interests. In 2010, Jorge Añez Claros, the President of the Gran Consejo Tsimane, was elected mayor of San Borja, a testament to the growing political influence of the Tsimane and Bolivia's indigenous peoples as a whole.

Tsimane Subsistence Strategies

The Tsimane practice a combination of economic activities that blend traditional subsistence strategies with wage labor and market exchange. Still much of the diet comes from

slash-and-burn horticulture, fishing, hunting, and gathering. More than 80 species of cultivated plants have been documented in Tsimane fields and gardens, (Piland, 1991) but major crops are rice, plantain, and corn, and sweet manioc, which are used for consumption at the household level and/or sometimes sold at local markets. The typical diet consists of plantain, yucca, rice, meat, fish, foraged fruits and chicha (lightly fermented sweet manioc beverage). Rice and citrus fruits are not native to South America and were likely introduced by the Jesuits in the 17th century. Traditional diets are supplemented by market foods such as refined sugar and canned fish, which are purchased in San Borja or brought to villages by traveling merchants.

Hunting is practiced, particularly in less acculturated and more geographically remote communities, and usually using dogs. While shotguns and rifles are owned and shared in most communities, bow and arrow hunting is still practiced when ammunition is unavailable. Some of the animals commonly hunted and consumed by Tsimane are peccary, tapir, howler and capuchin monkey, paca, and armadillo. Fishing is another subsistence strategy practiced by men and occasionally by women and children. Common methods are hook and line or communal fishing using nets or barbasco, a method of communal fishing in which plants are used to poison fish. Tsimane men have also begun to engage in wage labor and regional market exchange. The most common forms of wage labor are for commercial logging companies or doing agricultural labor. Very few women engage in wage labor though they do occasionally sell or trade agricultural products and woven bags in town or to traveling merchants. Wage labor and market exchange occur to varying extents in different Tsimane communities and generally serve to augment, rather than replace, traditional subsistence practices.

Tsimane Marriage and Reproduction

Tsimane live in villages composed of extended household clusters, each of which typically contains three or four residences composed of consanguineal and/or affinal kin. Houses are constructed from plant materials, usually a palm roof supported by poles attached with rope made from tree bark. In less acculturated communities, houses are open, but it is becoming more common to construct walls from wood or mud. Adults within a group of kin-related households primarily perform subsistence tasks. Spouses engage in extensive cooperation and sex roles are well-defined. Women exert considerable domestic control and are responsible for providing childcare, processing and preparing food, and making *chicha* (homemade beer). Men acquire

game and fish and engage in wage labor. Both sexes collect forest foods, fetch firewood and water, and work in horticultural gardens.

Tsimane marriages are fairly monogamous and stable, though a small number of polygynous marriages exist (Winking & Kaplan et al. 2007, Winking & Gurven et al. 2011). The divorce rate is 20% and divorce is more common early in a marriage (ibid). Marriages are generally facilitated by kin and women do not face divorce restrictions. There are no formal rules of post-marital residence, however newly married couples often reside near the wife's natal kin for at least a few years. During this time the husband works with affinal kin in subsistence tasks, but bride service is not formally recognized. After several years the couple and their joint children may or may not relocate to live near the husband's natal family. Tsimane women marry, on average, by age 16 (Rucas, Gurven et al. 2006), reach menarche at 13.9 years and have their first birth by age 18.6 (Walker, Gurven et al. 2006).

The Tsimane are primarily a natural fertility population, with just a handful of women from acculturated villages reporting use of hormonal birth control methods (usually depo-provera injections). Most Tsimane women spend their entire reproductive lifespan in a cycle of pregnancy and lactation. Interbirth intervals (IBI) are quite short (~2 years) and total fertility rate (TFR or average number of children expected to be born to a female during her lifetime) is 8.5 (Kaplan, Gurven et al. 2010). This is an extremely high fertility rate, even among neotropical indigenous groups whose populations are observed to increase rapidly in the early stages of acculturation (McSweeney, 2005; Werner, 1983; Early & Peters, 1990; Picchi & College, 1994)

There are relatively few pregnancy taboos concerning diet and activity of Tsimane women. Pregnant women engage in hard physical labor well into the third trimester. Sexual intercourse with the mother is considered taboo in the last two trimesters until roughly six months after birth. Following birth, which is attended by female kin (and sometimes, fathers are present), Tsimane mothers and neonates spend a week or so resting in a mosquito net. Newborns are often painted with *bi* (*Genipa americana*), a black dye found in lowland South America, as it is believed the blackness renders the baby invisible from malevolent spirits that might otherwise cause harm (Gurven, 2007). It is common for infants to not receive proper names until about a year after birth. Young infants are worn close to the mother in slings or rocked in a hammock while mothers engage in household tasks, and are breastfed exclusively and more or less on-demand in the first few months. Older infants and small children are often cared for by older siblings and other

female kin while mothers participate in subsistence activities. Direct paternal care is rare in the first six years of a child's life (Winking, 2009).

Infant Feeding in the Tsimane

Data from exploratory interviews of 81 mothers of infants regarding complementary feeding practices are now presented. Interviews were conducted in two forest communities, one acculturated community, and two river communities in 2003 and in four bioreserve communities in 2006. Most Tsimane mothers begin the weaning process using complementary foods such as boiled or masticated plantain, though a small number of mothers reported feeding masticated meat, fish or boiled manioc. The ensuing period of mixed breastfeeding and complementary feeding is prolonged and varies across mother-infant pairs. At about 12 months of age, infants are observed to spend more time consuming solid foods than they spend nursing. Tsimane mothers often breastfeed throughout a portion of a subsequent pregnancy but most infants are weaned by the time their younger sibling is born. Breastfeeding can last for three years or more, but most often ceases by two years of age.

Quantitative results suggest that Tsimane infants begin to consume complementary foods between three and six months of age (mean=4.66 months). Early introduction of complementary foods, along with long breastfeeding durations, is frequently observed in subsistence societies (Sellen & Smay, 2001). Figure 2.1 provides a frequency diagram of infant age at introduction of complementary foods based on maternal reports, which range from less than one month to over nine months of age.

A breakdown of first infant foods as reported by mothers is provided in Figure 2.2. Plantain was the first infant food reported by 79% of mothers, perhaps because in Tsimane villages, plantain is the most accessible and easily acquired resource. Plantain is mainly composed of 60-90% simple carbohydrates which are highly digestible (FAO, 1990) and provide cellular energy for the high growth, immunologic, and activity demands of the infant. Another 19% of mothers reported giving infants meat or fish as their first food. Meat and fish vary in availability across Tsimane communities, depending on village location and time of year, and may be very important infant foods in upriver regions where anemia is common. Though less digestible than plantain, fish and meat are the best source of dietary protein within the local ecology. Protein is essential to the growth and maintenance of essential body tissues, as well as in the development of

hemoglobin and the production of antibodies. All mothers who reported fish or meat mentioned masticating it prior to feeding it to their infants. Boiling and mastication render foods soft for infants to easily digest. Mastication of weaning foods is considered to be a crucial behavioral adaptation (Pelto, Zhang & Habicht, 2010) as maternal saliva contains IgA antibodies such as those found in breast milk (Fitzsimmons, Evans, Pearce, Sheridan, Wientzen & Cole, 1994) which may confer additional antigenic protection to weanlings.

There was substantial variation in womens' responses regarding their infant feeding practices. Figure 2.3 reveals variation in the timing of introduction of complementary infant foods by community type based on maternal reports. Several mothers indicated that they had altered their infant feeding practices according to infant signals, mainly; the feeding occurred whenever the infant began expressing interest in foods or was "hungry". Women expressed flexibility in their infant feeding practices, for example, an older mother who introduced complementary foods prior to one month of age believed that her breast milk was inadequate to sustain an infant. She said she had exclusively breast-fed four infants, all of which died, but the two that she began feeding before one month of age had both survived.

Figure 2.1. Distribution of age at initiation of complementary feeding in 81 Tsimane infants

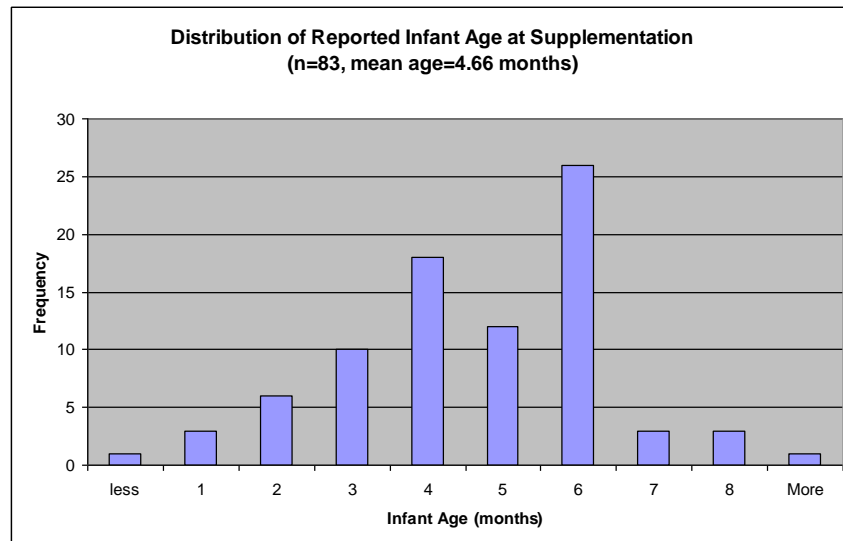


Figure 2.2. Breakdown of foods first introduced to 81 Tsimane infants as reported by mothers

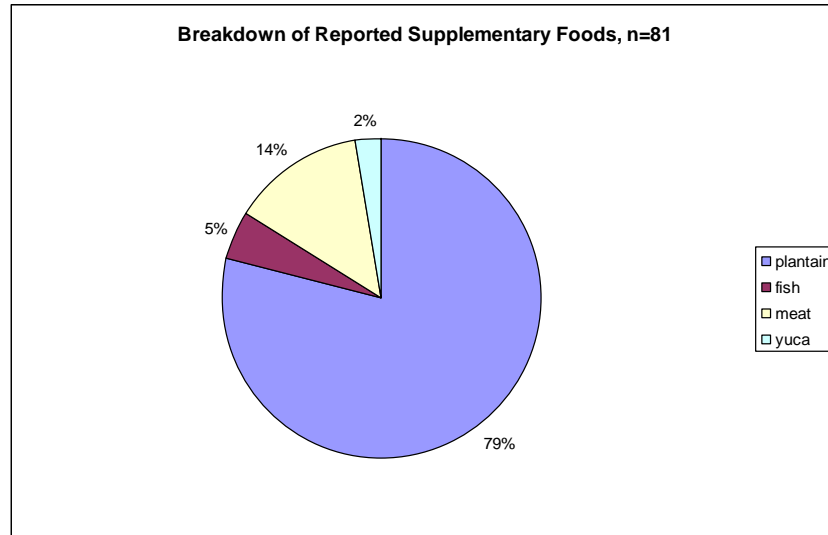
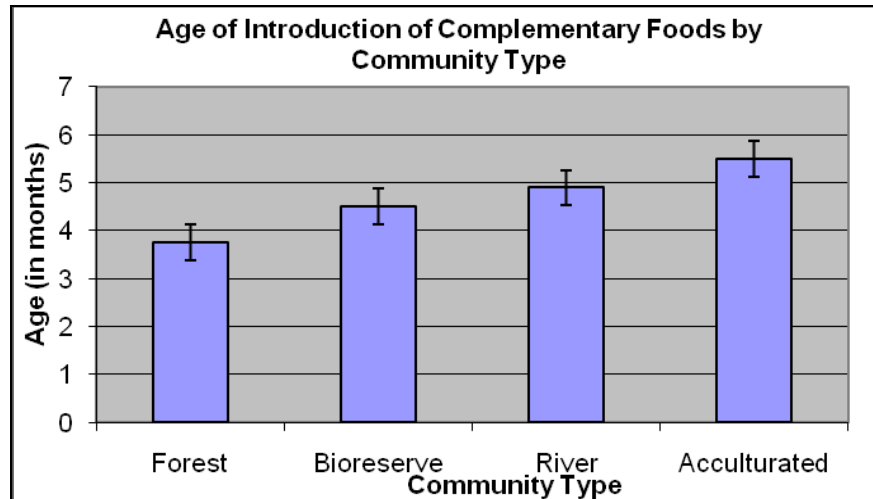


Figure 2.3. Reported age of introduction of complementary foods by community type (with standard error bars)



Infant/Child Health

The Tsimane infant mortality rate (IMR) is higher than the Bolivian national average, averaging around 12.6% from 1990-2002, though it varies considerably across communities (Gurven, Kaplan et al. 2007). The rate remains high despite the fact that Bolivian national infant

mortality rates have decreased steadily since access increased to modern medical facilities in the last decade, but remains higher than the Bolivian national average (Gurven, Kaplan et al. 2007). As in many tropical settings, household sanitation is poor in Tsimane villages. Only three of the communities studied had wells within walking distance of a handful of households, but all but one of them went through long periods of dysfunction due to lack of maintenance. Drinking water is obtained from rivers and creeks, and most Tsimane do not boil their drinking water. Water boiling is costly in terms of fuel, time, and energy, and the benefits of water boiling are not clear-cut in this setting as there are multiple routes to antigen exposure aside from drinking water.

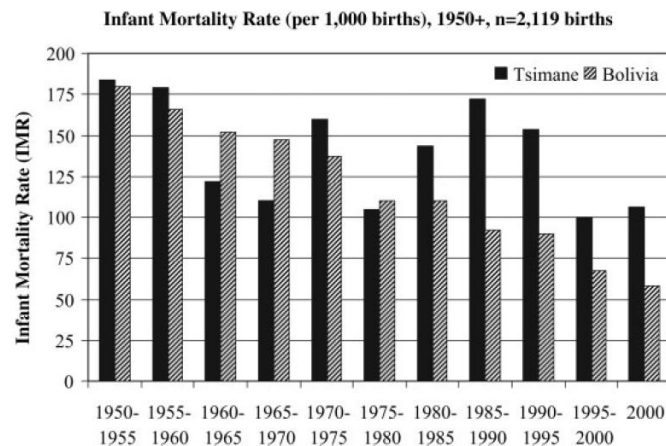
Most Tsimane households maintain hand-dug latrines, though in areas of low population density it is common to defecate on the edge of fields. Small children often defecate near houses, and this is usually carried away with a shovel by mothers or older siblings. Domestic pigs, dogs, and chickens roam freely in the villages and their feces litter pathways between houses, though individual yards are generally kept clean. During the rainy season, flooding is common and materials from latrines washes up and can be deposited in Tsimane living areas. In 2006, there was severe flooding in the downriver region which destroyed crops and houses. A medical team from San Borja came through in the aftermath to attend to the health needs of affected families, however the visits were hurried and many families were not seen.

While some of the villages had solar-powered electricity, it was only used to power ham radio communication. Only one village (Tacuaral del Maito) had a generator and refrigerator at the time of data collection, which was kept in the house of a non-Tsimane professor. Food such as fruit and charqui (dried and salted meat and fish) are hung in houses or separate kitchens or outside in the yard for storage, which attracts scavenging animals and flies. It is not uncommon for Tsimane to consume meat that has begun to rot. Another source of antigen exposure is chicha. There is potential for contamination at many points of the chicha preparation process, from unwashed hands, pots, and bowls, to the variable duration of the fermentation process during which flies may find their way into the batch. Chicha is widely shared by Tsimane adults and children alike and further contamination may occur as bowls of chicha are transferred between individuals. In this study chicha consumption was observed in infants as young as three month of age.

This combination of factors contributes to profound susceptibility to infections that are major causes of death in infancy and childhood. Figure 2.4 shows that Tsimane infant mortality

was substantially higher than the Bolivian national average from 1950-2000 and remains high despite declines at the national level. Tsimane children aged 2-15 exhibit elevated levels of C-reactive protein (CRP, which indicates a high infectious disease burden) (McDade, Leonard et al. 2005b). Parasitic infections are common in children; Tanner and colleagues report that 76% of 92 sampled children were positive for hookworm infection and 15% were positive for multiple-species infections *S.* (Tanner, Leonard, McDade, Reyes-Garcia, Godoy & Huanca, 2009). Gurven (2007) and colleagues estimated that 55% of deaths in infants <1 year, and 10.8% of deaths in children aged 1-5 years old were attributable to infectious disease, mainly gastro-intestinal or respiratory infections. Anemia is also prevalent among Tsimane children under age 10 (Lindsay KM, Aiello et al. 2003). Finally, Tsimane children exhibit growth stunting, which is characteristic of South American indigenous populations (Foster, Byron et al. 2005; Blackwell, Pryor et al. 2009; Godoy, Nyberg et al. 2010). Growth stunting reflects a combination of long-term mild malnutrition and micronutrient deficiency along with energetic trade-offs with chronic immunologic activation (McDade, Reyes-García et al. 2008).

Figure 2.4. Infant mortality rate per 1,000 births, from 1950 to 2000



Copied from “Mortality Experience of Tsimane Amerindians of Bolivia: Regional Variation and Temporal Trends” by Gurven and Kaplan et al., published in AJHB, 2007.

Tsimane Access to Medical Facilities

In San Borja, Tsimane may seek medical care at the main hospital or Horeb, a New Tribes Mission health clinic which has provided intermittent access to medicines in exchange for labor

since 1990. Still, delayed treatment of infants often results in death, as illness often occurs suddenly and progresses quickly. Parents who must travel long distances to hospitals may arrive too late for successful treatment. Malnutrition and dehydration underlie much infant disease and inhibit the efficacy of antibiotic treatments, and the infection-malnutrition cycle is extremely difficult to break in resource-scarce settings (Chandra, 1992; Chevalier, 1994). Cost of treatment at the San Borja hospital is free, but Tsimane often report being treated poorly by the staff. While Horeb is designed to meet the needs of Tsimane patients, the health post is seriously understaffed and at the time of the study lacked even basic medical equipment. Neither Horeb nor the San Borja hospital consistently staff Tsimane-speaking interpreters, so at the time of data collection many Tsimane were unable to communicate their needs effectively. When Tsimane die in hospitals rumors often spread that the death was intentional by hospital staff, contributing to a general distrust of doctors.

Vaccinations are available free at the hospital in San Borja to mothers and small children, and a team traveling from the hospital enters Tsimane villages to vaccinate children and provide health care to villagers. Demographic interviews revealed several waves of measles or rubella that killed a large number of small children over the past sixty years (Gurven, 2007). Immunization campaigns are fairly recent, confined to the past ten years. The efficacy of this system is unclear, but infant Tsimane mortality rates declined from 1995 and 2000, although they were still roughly twice the Bolivian national average (see Figure 2.4). As Tanner point out, the vaccine record-keeping system is disorganized and many children seem to receive only a partial course of vaccinations (Tanner, 2005). In general coverage in Tsimane communities is sporadic and generally limited to villages located in close proximity to San Borja. During community visits by campaign personnel, all present and willing Tsimane receive immunizations against measles, mumps, rubella (MMR), smallpox, polio, and more recently in the past years, yellow fever (Gurven, 2007). Often these visits are not announced to communities in advance and as a result many households are missed simply because they were out of the community or working in their agricultural fields when the vaccination team came.

Since 2001, the Tsimane Health and Anthropology Project's medical team made bi-annual or annual visits to Tsimane villages providing free medical care. The project has recently expanded its coverage to 88 Tsimane villages. Other sources of medical treatment are pharmacies in San Borja, where parasitic medications and antibiotics are available for purchase without a

prescription. Some Tsimane skip the doctor and purchase medicines directly from the pharmacy, or from traveling merchants who visit the villages, when they or their child are ill. In fact medication is obtained easily for many Tsimane communities and the concern is the observation that they are often misused (Tanner ,2005; Calvet-Mir, Reyes-García et al. 2008). Tsimane use of wild medicinal plants is well-documented. Reyes-Garcia has reported the existence of 169 plant species with one or many medicinal uses, though in a 15-month observation period she observed the use of only 39 species (Reyes-García 2001). In her study more species of medicinal plants were observed entering households in the less acculturated community Yaranda than the more integrated community San Antonio. In unstructured interviews, most Tsimane mothers reported using plant medicines to treat their infants, particularly for diarrhea or cough. Slight but positive associations are reported between parent ethnobotanical knowledge and indices of child health (McDade, Reyes-Garcia et al. 2007).

2.3 The Pumé of Venezuela

In contrast to Bolivia's indigenous majority, Venezuela's indigenous comprise only about 2% of the country's population (GBV, 2010). The current government administration of Hugo Chavez is generally supported by Venezuela's indigenous groups, especially since it began providing some tribes with land titles starting in 2005. While much international attention has focused on Amazonian tribes of Venezuela, lesser-known groups inhabit the southwestern plains of the state of Apure. The Pumé (called the Yaruro in the ethnographic literature prior to the 1980's) are one such ethnic group and are found along and between the Capanaparo, Cinaruco, and Riecito rivers near the border of Colombia. The Pumé share genetic heritage with Andean groups in neighboring Colombia and Ecuador (Salzano and Callegari-Jaques 1988) and their language is considered by some to be an isolate (Obregón Muñoz, 1981). It has also been classified as a member of the Chibchan language family that extends from Honduras to northern Colombia (Key, 1979).

The state of Apure has existed since 1864 and consists of low, flat savannas, called *llanos* or plains that are intersected by several rivers that form part of the western Orinoco river basin. The main urban center in Apure is its capital, San Fernando (pop. 497,100). Government administration and cattle ranching are the major economic activities of the region. The llanos inhabited by the Pumé and neighboring Hiwi experience a hyperseasonal climate pattern of

drought and fire from November to April followed by heavy rain and flooding from May through October. There are no permanent roads throughout most of territory but in dry the season, most villages can be reached by truck in a seven-or eight hour drive from San Fernando.

Pumé History and Overview

The presence of Pumé in the southern llanos was first documented by Spanish explorers in 1589. Jesuits forcibly combined Pumé to missions in 1739, and then following the Jesuit expulsion, Pumé inhabited Capuchin missions throughout the 18th and 19th centuries. The Venezuelan War of Independence led to a near abandonment of the llanos by all but indigenous Venezuelans that lasted for about 100 years. The 1930's saw a renewed interest in the region, and encroachment by neocolonial cattle ranchers (*criollos*) accelerated in the 1960's. Ranches still exist in the region and there have been sporadic periods of conflict between Pumé and *criollos* that commandeered and fenced much of Pumé foraging lands. Pumé still recognize the benefit of increased access to wages and market goods, and some communities have expanded their subsistence base. Resource acquisition activities range from hunting, fishing and foraging to slash-and burn horticulture, and occasional wage labor and market exchange. The local ecology of the llanos is extremely resource-scarce compared to that of the Tsimane, as plant diversity and large game are rare (Greaves, 1997).

Pumé population growth has been considerable at least for the last 30 years. A reliable census conducted in 1982 counted 3859 (OCEI, 1985) and another in 1992 counted 5400 (OCEI, 1995). Kramer and Greaves calculate a 3.15% population increase from 1982 to 2001 (Kramer & Greaves, 2007). Recent Pumé population estimates number 8000 (Greaves, personal communication). A handful of Pumé have migrated to the neighboring town of Achaguas or to San Fernando, however the vast majority reside in rural areas. Pumé communities vary with respect to acculturation and subsistence strategies are determined by geographic proximity to rivers and suitable agricultural land. The Pumé use the terms *savanna Pumé* to refer to mobile, central place foragers found in the interior far from major waterways, and *river Pumé* to refer to the sedentary forager-horticulturalist communities located along the major rivers. Gragson estimated in 1997 that 83% of the Pumé were river dwelling and only 17% resided in the interior (Gragson, 1997).

The savanna and river Pumé provide an opportunity to study a genetically homogenous native population in which subsistence strategies have only very recently diverged. They inhabit the same environment, experience similar disease exposures, and neither have access to medical care. Although the river communities have access to more market and horticultural foods, those still are very limited. Even small differences in settlement patterns and subsistence strategies have dramatically impacted fertility and infant mortality patterns (Kramer & Greaves, 2007). The five Pumé study villages are in close geographic proximity, situated within a 400 k² area and are no more than half a day's walk apart. The acculturated communities are Chainero, Chaparralito, and Rosario. The savanna communities are Doro Aná and Yagurí. Of the five Pumé study communities, only one had a school which began operating shortly before our visit in June 2007. Only 4% of savanna and 24% of river Pumé report speaking rudimentary Spanish and as of 2007 only 3% of river and no savanna Pumé had ever attended school. None of the communities have a health clinic, store, electricity, well water, or can be reached by permanent road.

Savanna Pumé

The Pumé who live in the savannas between the Capanaparo, Cinaruco, and Riecito rivers are mobile central-place foragers, subsisting on hunting, fishing, and wild root and mango collection. The soils of the llanos are highly acidic (Roa Morales, 1981) and of low nutritive quality (Medina, 1982), therefore savanna Pumé maintain only small gardens of bitter manioc and only in the wet season. Men and women specialize in sex-specific subsistence tasks. Women are the main providers of mangoes and roots and men provide most of the fish and all the hunted game, while both sexes participate in garden labor (see Figure 2.5) (Kramer, 2005). Figure 2.6 provides a breakdown of the foods that compose the savanna Pumé diet (based on each food contribution by weight) averaged over a three-year observation period (Kramer & Greaves, 2010). Wild roots and mangoes compose a large portion of the Pumé diet (35% and 25%, respectively), fish and meat are important protein sources, contributing 15% and 10% of dietary weight, and the contribution of cultivated manioc is minimal (10%) (ibid).

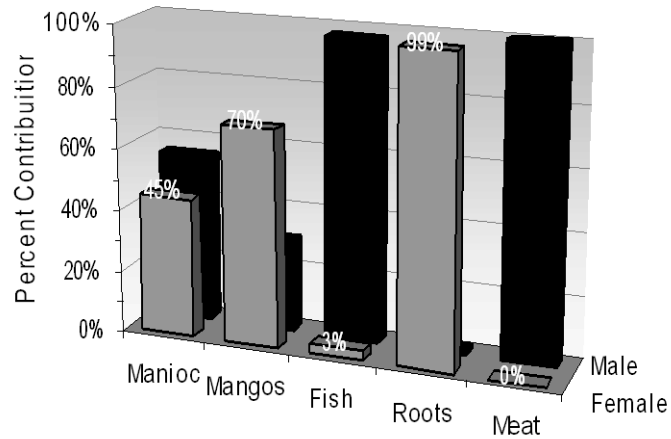
There is variation in wet and dry-season resource acquisition strategies. In the dry season fishing is a primary subsistence activity because fish become highly concentrated in contracted water sources. Barbasco, spear fishing and hook and line fishing are all practiced by Pumé men and boys. In the lean wet season when fish become dispersed, the subsistence base shifts to small

game hunting and root harvesting. Caiman, armadillo, small mammals and lizards and migratory birds (egrets, herons, spoonbills, and storks) are all hunted with bow and arrow. Even with the help of dogs, hunting returns are low due to the paucity and small size of game in the llanos. Cultivated bitter manioc and about ten species of wild roots are consumed by the savanna (Gragson, 1997), largely in the wet season. Mangoes are the only wild fruit that contribute substantially to the Pumé diet savanna, and both men and women travel great distances to reach them. Hunting, foraging and fishing returns are widely shared in the savanna communities (Kramer & Greaves, 2010).

Wage labor and animal husbandry have only recently been added to the savanna Pumé subsistence repertoire and are practiced sporadically. Only 47% of the savanna men report having engaged in wage labor in the past year (Kramer & Greaves, 2007). Dogs, chickens and pigs are becoming more common since Greaves reported their presence as early as the 1990's. However, adoption of these new subsistence strategies does not compensate for the pronounced resource scarcity experienced by the savanna Pumé.

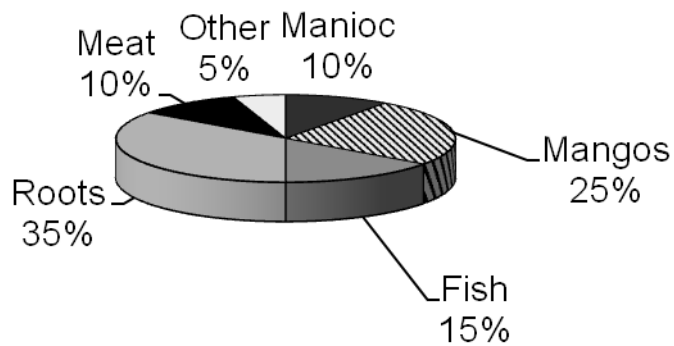
Savanna communities tend to consist of fewer than 50 individuals and rotate 5-7 time annually in response to the water table. Gragson observed that the savanna Pumé inhabit more substantial thatch multi-family dwellings in wet-season settlements and ephemeral nuclear-family houses in dry season settlements (Gragson, 1995). Houses are typically open and are built of poles tied together with vines and roofed with moriche palm fronds, though some dry season houses are little more than brush shades. Houses are inhabited for about six months out of a year and re-inhabited annually for 3-5 years or when housing materials deteriorate and become infested with insects (Gragson, 1995). Up to ten temporary camps consisting of conical huts are also used throughout the year, and their components eventually recycled into more substantial housing structures in settlements (ibid).

Figure 2.5. Proportional male and female contributions to principle food categories by weight in savanna Pumé communities



Copied from 2010 manuscript “Bilateral Kin Associations among Hunter-Gatherers: Postmarital Residence among Foragers” by Kramer and Greaves.

Figure 2.6. Savanna Pumé subsistence base calculated as annual contribution of each food by weight



Copied from 2010 manuscript “Bilateral Kin Associations among Hunter-Gatherers: Postmarital Residence among Foragers” by Kramer and Greaves.

River Pumé

The river Pumé were mobile until approximately 50 years ago, when they began to settle in permanent villages along the Capanaparo, Cinaruco and Riecito Rivers. These rivers are major transportation routes through the llanos, thus river historically have had more contact with outsiders to the region and were the focus of most early ethnographic study (Petrullo, 1939). Like the savanna Pumé, they have a mixed subsistence base of fish, manioc horticulture, and wild foods. The main crops cultivated by river Pumé are manioc and corn, and their garden return rates exceed those of savanna Pumé because floodplain soils are better suited to agriculture than the sandy savanna soils. Fish are larger and more abundant in the major rivers than the streams and seasonal lagoons of the savanna and river Pumé rely more extensively on animal husbandry.

Men in river communities are more likely to engage in wage labor than savanna men with 73% of river men reporting having engaged in wage labor in the past year (Kramer & Greaves, 2007). Some highly acculturated villages located nearer to the main highway also specialize in production of hammocks, water jugs and other pottery for trade and retail sale. There is a system of exchange in place between river and savanna dwelling Pumé, whereby savanna Pumé trade foraged materials (arrow canes, resin, fiber, weaving materials) for market goods (clothing, pasta, tools, cookware, soap) more accessible to river Pumé. Market foods such as pasta, rice and flour were distributed by government agencies in 2005-2007. While some savanna villagers did travel to these giveaways, they primarily benefited Pumé in the river communities.

River Pumé communities are typically larger than savanna communities. Most individuals inhabit single houses year-round, though they do occasionally travel to temporary foraging camps. The houses of river Pumé have larger gardens and are more substantial and dispersed than those of savanna Pumé. Many river Pumé houses have walls and some have corrugated roofs which are purchased for cash from wage labor. Overall they practice more intensive agriculture, enjoy more protein from fishing and domestic animals, and have increased access to cash and market goods when compared to savanna Pumé. As a result of these factors river Pumé experience less seasonal variance in food availability and are far better nourished.

Reproductive Environment

Despite differences in acculturation, mobility and resource availability, river and savanna Pumé inhabit a similar cultural and reproductive environment. Girls reach menarche at around 13 years of age and the majority marries by 15. Marriage prior to the onset of menses is not uncommon, although girls do not initiate conjugal relations until after menarche. There is no documented evidence of coercive marriage or sexual activity. Early pregnancy is encouraged and the average age at first birth is 15.5 (Kramer & Greaves, 2008). The teen birth rate is 195 births/1000 women aged 15-19 (ibid).

Serial monogamy was the most frequently observed Pumé marriage pattern, although a small proportion of adults (11%) marry polygynously. Polygyny is more common in the savanna communities, and sororal polygyny is the most common form of plural marriage. Many early marriages result in divorce and women face no divorce restrictions. The Pumé are matrilocal and out-marriage is very rare. Young couples often share a home with the wife's family or in a house just a few meters away, and young husbands contribute to provisioning their wife's family. Pumé share food extensively across matriline and rely on extended kin for childcare and other forms of allocare (Kramer & Greaves, 2007). In particular, young reproductive aged-women appear to be buffered from the energetic demands of foraging as they receive caloric contributions from older individuals (Kramer, Greaves et al. 2009).

Kramer and Greaves have examined Pumé fertility in great detail in both savanna and river communities. Modern forms of contraception are unavailable to women of both community types, and Pumé women exhibit reproductive profiles typical of natural fertility populations. The only exception is the timing of reproduction. Pumé age at first birth is earlier than seven other groups of South American foragers for whom data are available (Walker, Gurven et al. 2006). This is true not only in the river communities, but also in the nutritionally stressed savanna communities. TFR for women 40 and older is 7.41 for savanna women and 7.75 for river women (Kramer and Greaves 2007). These rates do not differ significantly from each other but are much higher than the Venezuelan national TFR of 2.7 (ibid). A comparison of period and cohort fertility rates suggests that reproductive patterns have not changed markedly for the savanna Pumé in recent decades, however the river Pumé are undergoing a fertility transition (Kramer and Greaves 2007). For Pumé mothers age 15-40, birth intervals are shorter for river-dwelling women (average 2.87 years) as compared to savanna women average of 3.1 years (ibid).

Maternal/Infant Relations and Child Health

During the first six months of a child's life, infants are breast fed on demand. Children are introduced supplemental foods at about six months and fully weaned by two and a half to three years old. Premasticated adult food is introduced first, followed by small portions of soft food such as mangos. By the age of two children are eating small whole fish with bones. If a mother becomes pregnant while breastfeeding, depending on age of the child, she may wean the child or continue to breast-feed through the pregnancy. Unlike many other child-development environments where mothers and babies are inseparable for the first six months, Pumé infants are held, cared for and occasionally nursed by others from the day they are born. Pumé live in dirt floor houses and brush shades, and children are permitted to crawl on the ground, although parents are vigilant. Child begins to walk at about a year old, and once mobile are free to roam throughout the village, though seemingly unsupervised; a related adult is always in close proximity.

The Pumé live in a malarial environment. Other significant diseases in the region include measles, yellow fever, Chagas disease, tuberculosis and other respiratory diseases (Lizarralde & Seijas, 1991). Infant mortality is high, especially for very young mothers (<14 years old) who have a fourfold risk of losing their firstborn compared to mothers who are 14 or older at first birth (Kramer, 2008). Infant mortality in river communities is 13.2%, (comparable to the Tsimane rate) and significantly higher than the Venezuelan national average of 1.96% (PRB 1996; Kramer & Greaves, 2007). In savanna communities, infant mortality is 34.6%, nearly three times the river rate (Kramer & Greaves, 2007). It is estimated that 10% of savanna children who survive infancy do not survive to reproductive maturity (Kramer, Greaves et al. 2009).

While cause of death is not known for a sample large enough for analysis, seasonal under nutrition, chronic parasite loads, disease exposure and opportunistic infection clearly have a synergistic effect on the high levels of morbidity and mortality among Pumé infants and children, and particularly those in the savanna. Infectious disease peaks in the lean wet season, a time when individuals are likely to be immunologically vulnerable due to inadequate caloric intake (Barreto & Rivas, 2007). Health care workers occasionally enter the more accessible river communities but rarely reach the savanna communities. There were vaccination teams in the savanna but children have not received vaccines in the last ten years (Kramer, Greaves et al. 2009).

There are marked differences in river and savanna Pumé communities, although they inhabit the same territory and share genetic heritage. Acculturation and increased food availability appeared to have relaxed constraints on childbearing as evidenced by more frequent births in the river communities. These shifting fertility patterns in river communities also contribute to higher child survivorship and recent rapid population growth. Table 2.1 provides data from Kramer and Greaves (2007) and summarizes some important demographic characteristics exhibited by the two community types.

Table 2.1. Summary of characteristics distinguishing variation between river and savanna Pumé communities.

Community type	acculturation	community size	TFR	IMR
river	higher	larger	7.75	13.2
savanna	lower	smaller	7.41	34.6

CHAPTER III

INFANT FEEDING PRACTICES AND CHILD HEALTH

Weaning human infants is best described as a process that is dynamic, complex, and incremental in nature. A successful transition from exclusive breastfeeding to nutritional independence facilitates optimal growth, development, and survival for infants (McDade & Worthman, 1998). Many contemporary biological models of weaning approach the process from an adaptationist perspective in which fitness maximization is a driving force of life history strategies (Sellen, 2001b). Optimal breastfeeding and weaning strategies are variable and multiple strategies may be employed adaptively across a range of ecological settings, in fact extreme variation in feeding patterns are observed in cross-cultural studies of infant feeding (Sellen, 2001a; Wilson, Milner et al. 2006; Sellen, 2009). The goals of this chapter are to model behavioral data and health outcomes to explore the following questions: What factors influence the timing and nature of the weaning transition? Are weaning strategies associated with infant mortality or growth patterns? To what extent do maternally-reported infant feeding practices reflect actual infant feeding behaviors? Data was collected through observation of 133 infants negotiating the weaning transition in a population of forager-horticulturalists, the Tsimane of Bolivia.

3.1 Theoretical Background

This research was grounded in the perspective of life history theory. This analytical framework invoked in studies of humans and other animals to explain inter- and intra-population variation in development, behavior, and reproduction, particularly with respect to resource allocation to life history functions. Fitness is maximized by natural selection when energetic

resources are allocated optimally to competing life history functions (growth, survival, and reproduction) (Gadgil & Bossert, 1970; Smith & Fretwell, 1974; Partridge & Harvey, 1985; Kaplan, Hill et al. 2000; Charnov, 1993). Individuals have a fixed energy budget at a given point in time, therefore trade-offs exist between competing life history functions and investment in one (e.g., growth), comes at a cost to another (e.g., reproduction) (Hill & Hurtado, 1996). When resources are scarce these energetic trade-offs become more pronounced and in risky situations, short-term survival mechanisms are favored at the expense of growth and reproduction (Tschirren & Richner, 2006). Decision-making with respect to energy allocation occurs on physiological and behavioral levels, involving a coordinated response to individual and environmental factors. Under this framework parenting strategies are seen as behavioral strategies shaped by natural selection, adaptive and flexible across socio-ecologic settings, and designed to increase parental fitness by maximizing reproduction and offspring survival.

Parental investment is defined by Trivers as resource allocation to one offspring that diminishes a parent's ability to invest in another (Trivers, 1972). In human mating systems, women bear the energetic burdens of gestation and lactation, throughout life are higher investing parents than males, and cross-culturally provide the majority of offspring care (Campbell, 2002; Konner, 2005). Therefore, maternal physiology and behavior should be shaped by natural selection to employ adaptive parenting strategies: sensitivity and responsiveness to children's needs in a manner that facilitates growth and survival of their offspring (Hrdy, 1999). In particular, adaptive child care strategies reflect trade-offs in maternal investment reflecting each child's mortality risk with other current and future reproductive investments (Ball & Panter-Brick, 2001).

Lactation represents the most energetically costly form of maternal investment (Prentice, Spaaij et al. 1996) and plays a central role in mediating these tradeoffs. Weaning (cessation of breastfeeding) in non-human primates signals the infant's transition to nutritional independence as well as the mother's transition to investment in subsequent offspring (Lee, 1996). In humans, the replacement of breast milk with complementary foods is gradual; complementary feeding can accelerate the resumption of ovulation in mothers and conception may occur while mothers are still partially breastfeeding (Lancaster & Alvarado, 2010). Lactation is costly to mothers because it utilizes significant maternal energy reserves and suppresses reproductive function by

amenorrhea, but it is also essential to infant survival in most ecological contexts (Valeggia & Ellison 2001).

Both mothers and infants face distinct tradeoffs in the years preceding infant nutritional independence. Mothers must attempt to maximize infant survival, balance the energetic burden of lactation, and consider the long- and short-term health consequences of their childcare strategies. Furthermore adaptive maternal decision-making is not limited to care of one infant, but maximize *overall* reproductive success for mothers. Mothers must therefore, partition investment optimally among existing dependent offspring as well as weigh the costs and benefits inherent in current-future reproductive tradeoffs. Infants must convert available resources to costly body and brain growth and maintenance, as well as to develop a complex adaptive immune system that will protect them from pathogens throughout the life course (McDade, 2005). Infants therefore, have an interest in eliciting as much investment as possible from mothers who provide the bulk of their care and are the sole source of nutrients and passive immune defense in the vulnerable first months of life.

Breastfeeding and the Weaning Process

The benefits of breast milk for infant growth and survival are well documented (Cunningham, 1995) and were addressed only briefly here. Human milk has evolved as an adaptation to meet the complex demands of the developing infant that provides adequate macro- and micro-nutrients uniquely adapted to the energetic demands of a growing infant. Breast milk provides passive immune factors to immunologically naive infants, as well as bioactive hormones and growth factors that help regulate histological growth and differentiation and facilitate nutrient transfer in infants (Goldman, 1993; Grosvenor, Picciano et al. 1993; Lönnerdal, 2003). The World Health Organization (WHO) recommends six months of exclusive breastfeeding and two or more years of partial breastfeeding (WHO, 2001). Infants appeared not to process non-breast milk foods adequately prior to six months of age, due to several ontogenic processes including immature morphological development, digestive physiology and immune function (Sellen, 2009).

Mother's milk contains secretory IgA antibodies which (along with as lingering IgE antibodies conferred via the placenta which have a half-life of around six months) allows for immunological protection that is uniquely tailored to the bacterial, viral and parasitic pathogens encountered within her lifetime (Goldman, 1993). These passive defenses create a buffer that

protects the infant who is experiencing substantial energetic demands of brain and body growth. Other immune components like cytokines play a role in modulating the development and learning of infant immune system. Breastfeeding has been shown to reduce respiratory and diarrheal morbidity in infants, which is important in many rural and urban developing world regions where sanitation is substandard and adequate medical care is frequently unavailable (Räihä, 1981; Popkin, Adair et al. 1990; Cunningham, 1995).

Two important milestones are generally used to mark the process: introduction of weaning foods and cessation of breastfeeding. The entire weaning transition which may be broken down into the following three stages that are observed in human societies:

1. *Exclusive breastfeeding*- The period in early infancy in which infant nutrition and passive immunological protection are derived exclusively (or nearly exclusively in the presence of “token” or ritualistic feedings) from the consumption of mother’s milk (Vitzthum, 1994). Nutrient intake via breast milk increases throughout the post-natal phase though suckling frequency may decrease as the infant becomes more efficient at suckling (McDade & Worthman, 1998). The relative benefits of breastfeeding begin to decline as infants approach six months of age because there is a limit to maternal milk output and also because infant energy expenditure and caloric requirements increase rapidly as the infant grows (Dewey, 1997; McDade & Worthman, 1999). While exclusive breastfeeding is uncommon in some modern human societies, it is essential to infant survival in socio-ecologic contexts where modern medicine and sanitation are lacking.
2. *Mixed or transitional feeding*- Transition into this stage is marked by the introduction of liquid and solid weaning foods regularly consumed by infants in conjunction with breast milk. The early stage of mixed feeding can be risky for infants, particularly in settings where weaning foods are nutritionally inadequate and unsanitary conditions increase the risk of infection from contaminated foods (Rowland, Barrell et al. 1978; Kazimi & Kazimi, 1979; Guldan, Zeitlin et al. 1993). Increased gastro-intestinal infection is associated with weaning foods in many societies, which threatens infant survival and may compromise growth (Underwood & Hofvander, 1982; Brown, Black

et al. 1989; Popkin, Adair et al. 1990). Infant foods are often specially prepared to facilitate digestion (Fouts, Hewlett et al. 2001) and presumably to minimize antigen exposure. In some societies, special weaning foods or teas are used. In others children consume adult foods that are processed by boiling, grinding or mastication, which confers saliva-derived s-IgA antibodies that are similar in structure to those found in breast milk (Fitzsimmons, Evans, Pearce, Sheridan, Wientzen & Cole, 1994, Peltó, Zhang & Habicht, 2010). Breastfeeding provides protection against infection and allergy as complementary foods are added to infant diets (Shiva and Nasiri 2003; Dettwyler 2004).

Breastfeeding frequency declines upon introduction of weaning foods, however the rate of decline is difficult to quantify and exhibits tremendous cross-cultural variation. In some modern contexts, supplementation is simultaneous with cessation of breastfeeding (Kazimi & Kazimi, 1979; Whitehead, 1995). In many traditional societies, however mixed feeding is a protracted process (Whitehead, 1995) which adheres to a general mammalian pattern observed in species where mothers give birth to single, altricial infants (Sellen, 2009). Dettwyler argues that a “natural age of weaning” is between three and four years for malnourished populations based on a weaning pattern characteristic of large-bodied mammals in which offspring are weaned when they grow to quadruple their birth weight (Lee, 1996; Dettwyler, 2004). Mixed feeding may last until a child is two or older in traditional societies (Sellen & Smay, 2001).

3. The final stage is that of *nutritional independence*, where the child is capable of digesting (though not procuring) adult foods and has developed immunological memory and the ability to mount defenses to antigenic exposures. In non-human primates, nutritional independence refers to the juvenile’s ability to procure its own foods; in humans, such foraging efficiency is delayed until adolescence or beyond (Kaplan, Hill et al. 2000). Human weanlings receive foods provisioned by mothers, other kin and unrelated alloparents, and in many high fertility societies, mothers of weanlings have already shifted substantial energetic reserves to investment in a subsequent offspring (Lancaster & Alvarado, 2010).

Weaning and Infant Health Outcomes

Morbidity increases upon the introduction of complementary foods, which can disrupt growth and increase mortality risk (McDade & Worthman, 1998), however studies of health outcomes associated with infant feeding practices may be limited in that outcomes are often measured during and shortly after the weaning process occurs. While infectious disease is estimated to account for roughly half of all deaths in children under five worldwide, pathogen exposure at crucial developmental stages in infancy may have protective effects on subsequent survival: In the Philippines, children who experienced higher rates of gastro-intestinal illness as infants exhibited higher IgE concentrations (McDade, Kuzawa et al. 2004) and improved antibody responses to vaccination in adolescence (McDade, Beck et al. 2001b). Others suggested that infectious disease is inevitable in infancy regardless of infant feeding practices, particularly so in areas where sanitation is poor and pathogen burden high (Gray, 1998).

The World Health Organization's Maternal and Child Undernutrition Study Group reported that malnutrition underlies 35% of under-five deaths proximally attributed to infectious disease (Black R., Allen L. et al. 2008) Immune responses are energetically costly and existing malnutrition undermines their efficacy (Chevalier, Sevilla et al. 1994; Panter-Brick, Lunn et al. 2004), therefore mothers in high-mortality contexts may balance the risks of infant under-nutrition with that of infection and may provide complementary foods prior to the six-month recommendation in an attempt to improve infant energy balance (Gray 1998; Wilson, Milner et al. 2006), or to stimulate rapid immunologic maturation, or both.

Growth faltering (defined as a child's growth curve moving downwards (de Onis, Wijnhoven et al. 2004) in infants was frequently reported upon introduction of complementary foods (Lunn P.G., Northrop-Clewes C.A. et al. 1991), when cessation of breastfeeding occurs early (Goto, Panter-Brick et al. 2002) or when bottle feeding is practiced in unhygienic settings (Howrigan, 1988). Growth requires rapid cellular proliferation fueled by the energy metabolism of nutrient resources available to the infant. Requirements for infant brain and body growth are costly; therefore altered growth patterns may reflect resource allocation to immune function (McDade, Beck et al. 2001a; McDade, Reyes-García et al. 2008).

Growth faltering was observed in children throughout the developing world and often becomes evident in the second half of the first year of life, when most infants have just entered the

weaning transition (Lunn P.G., Northrop-Clewes C.A. et al. 1991; Cosminsky, Mhloyi et al. 1993; Valeggia 2002), though it is important to note that some studies have shown no difference in growth between infants exclusively breast-fed for four and six months (Martines, Habicht et al. 1994; Simondon, Delaunay et al. 2003). Others reported that growth was disrupted by weaning, but the results diminish as infants age and are no longer observed when infants reach ten (Winikoff, Castle et al. 1988) and 24 months (Dugdale, 1980). Clearly, many factors may account for variable results in studies linking timing of infant feeding with growth and findings must be situated within distinct socio-ecologic contexts.

Trade-offs between growth and immune function are observed in several species, and are particularly pronounced in environments characterized by resource scarcity and high pathogenicity (Lochmiller & Deerenberg, 2000). In such environments, a heightened demand for investment in immune function may come at a cost to growth. Growth stunting (low height-for-age) is pronounced in Tsimane children with high concentrations of C-reactive protein, an indicator of inflammation (McDade, Reyes-García et al. 2008). Growth stunting is observed in about ½ of indigenous lowland South American children and probably reflects a combination of long-term mild/moderate nutritional stress as well as chronic immune-stimulation (Foster, Byron et al. 2005). Acute and chronic infections compromise growth by inhibiting nutrient acquisition and from increased metabolic requirements due to energetically costly upregulation of immune defenses (Stephensen, 1999). In many impoverished settings, inadequate diet, suboptimal feeding patterns, and chronic infections contribute to growth stunting that continues throughout childhood (Eveleth & Tanner, 1990) resulting in adults of low stature (Howrigan, 1988; Frongillo, 1997; Moffat, 1998).

While infant feeding patterns are often targeted in public health interventions as potential causes of growth faltering, there is substantial evidence that maternal decision-making with respect to infant feeding is driven by the individual needs and growth of each infant. In several mammalian models in which offspring are weaned when they achieve a certain threshold body size, (e.g., quadruple the infant's birth weight) (Lee, 1996). A relationship between weaning and infant length was found in a study of weaning and growth conducted in India (Padmadas, Hutter et al. 2002), however causality was not clearly established in the study of Indian children.

In a study of Senegalese toddlers, prolonged breastfeeding improved linear growth after controlling for height-for-age in early infancy (Simondon, Simondon et al. 2001). In a study of

110 Papua New Guinean mother-infant pairs, Tracer concluded that mothers intensively breastfed infants with poor anthropometric indices, including height (Tracer, 2009). Reverse causality has been considered in other studies exploring the relationship between growth and infant feeding (Marquis, Habicht et al. 1997; Fawzi, Herrera et al. 1998; Simondon & Simondon, 1998). Few health researchers have addressed the theoretical implications of this pattern, which suggests that infant feeding patterns are maternal investment strategies that are driven by (rather than drivers) of infant condition.

Most studies of infant feeding and child health outcomes do not employ evolutionary theoretical perspectives pertaining to parental decision-making. Parental investment theory suggested that parents should bias investment into offspring most capable of translating investment into future reproductive success (Trivers & Willard, 1973; Irons, 1998). This parental assessment may be made based on offspring quality, or the likelihood that an individual offspring will survive to reproduce successfully (Irons, 1998). Ethnographically, Scheper-Hughes (1992) has described diminished maternal investment in Brazilian infants deemed unwilling (or unlikely) to survive. In Tracer's study, the opposite result was found as mothers were observed to engage in more intensive breastfeeding of smaller infants. He speculated that maternal mortality risk, which is high, and cultural ideals regarding family size, may influence parental investment strategies in the Au. While maternal disinvestment in infants that are unlikely to survive is frequently observed and may be adaptive under certain circumstances, principals of energy allocation suggest that the relationship between offspring quality and parental investment should not be simple and linear. Healthy and rapidly-growing infants may require less investment to be successful than offspring with a lower probability of survivorship. There are therefore diminishing returns to continued investment in high-quality offspring. Accelerating the weaning process in such infants may maximize maternal fitness in that mothers are able to return to ovulation sooner, and because fast-growing infants benefit from extra energy afforded by complementary foods (Gray, 1998).

Variation in Infant Feeding and Care

Cross-cultural variations in infant feeding practices have been scrutinized for the role they play in shaping infant and child health trajectories across a variety of socio-ecologic landscapes (Wilson & Milner, et al. 2006, Gray, 1998, Dettwyler, 1987, Howrigan, 1988, Dugdale, 1980). Researchers have demonstrated tremendous cross-cultural variation in infant feeding practices and

draw attention to the complexity of the weaning process, which appears to be mediated by a suite of cultural, demographic, and ecological factors (McDade & Worthman, 1998, Dettwyler, 1992). Mothers in subsistence societies are generally reported in ethnographic literature to practice indulgent caregiving behaviors, characterized by close maternal-infant proximity, swaddling co-sleeping, and frequent, on-demand breastfeeding for the first several months of the infant's life (Dettwyler, 1987; Vitzthum, 1994; Nelson, Schiefenhoewel et al. 2000). These practices contrast markedly with infant care strategies in industrialized societies. In the United States and many other industrialized societies, breastfeeding is often a short duration and in many cases not initiated at all (Stuart-Macadam & Dettwyler 1995; Wiley & Allen, 2009). Even when breastfeeding is practiced in industrialized societies, long periods of maternal-infant separation have become normal and therefore breastfeeding bouts are infrequent. Co-sleeping is not practiced in many industrialized societies, in fact it is not recommended by the American Academy of Pediatrics because it is a risk factor for Sudden Infant Death Syndrome (McKenna & McDade 2005).

It has long been observed that infant care practices in industrialized countries do not adhere to the evolutionary biology of infancy (Bruner, 1972; Barash, 1986; Ball, 2007). Levine observed that cross-cultural infant care practices reflect broader socio-cultural systems, which often differ vastly from infant development environments of human evolutionary history (1998). Indulgent infant caregiving behaviors are practiced in most subsistence societies, particularly those reliant on foraging, and probably reflect a variety of selection pressures under which humans evolved. Where resources are scarce, sanitation is poor and infant mortality high, mothers must adopt strategies that enhance infant survival, particularly during the risky weaning transition, and these strategies are expected to be flexible across a variety of contexts.

Sellen's cross-cultural analysis of the Human Relations Area Files revealed weak correlations between mode of subsistence and infant feeding patterns in 133 traditional human societies; notably, breastfeeding duration was longer and introduction of complementary foods earlier in populations relying on foraged foods for subsistence as compared to those dependent on agriculture or animal husbandry (Sellen & Smay, 2001). It is unclear why subsistence strategies alone might influence weaning patterns; however each subsistence type encompasses an underlying suite of interconnected factors that influence maternal caretaking strategies. Food security and infant infectious disease mortality are shaped by socio-ecological conditions and processes that may affect foraging, agricultural and pastoral communities in different ways

(Cohen, 1989; Kramer & Greaves, 2007). For example, if infant mortality is higher in actively foraging societies than in agricultural societies, as suggested by cross-cultural analysis of demographic data, then prolonged breastfeeding in such societies may confer a higher relative protective benefit to infants (Hewlett, 1991).

Given the importance of breastfeeding for infant survival, it is tempting to assume that women living in traditional societies and other high infant-mortality contexts should employ infant feeding behaviors that maximize offspring survival. This often appears to be the case, particularly with respect to breastfeeding duration, which can be quite long (three years or more) in some traditional societies such as the Bofi (Fouts, Hewlett et al. 2001) and the !Kung (Konner, 1977). However, several investigators have reported early supplementation of infants and short overall breastfeeding durations in traditional societies (Raphael & Davis, 1985; Howrigan, 1988; Dettwyler, 1992; Lawrence & Lawrence, 1994). In rural Zimbabwe, 68% of mothers reported providing complementary foods to infants before they reached six months of age (Cosminsky, Mhloyi et al. 1993), and 57% of Makushi mothers in Guyana reported exclusively breastfeeding for less than six months. In 70% of the cross-cultural sample in Sellen's study, infants were fed non-breast milk liquids or solids earlier than six months of age (Sellen, 2001a). Howrigan reports the proliferation of bottle feeding in Yucatan Maya communities despite notable adverse effects on child health (Howrigan, 1988) and in many societies, antibody-rich colostrum is discarded. Such observations have prompted speculation on the causes underlying "suboptimal" infant feeding practices. In some settings, the presence of modern medical facilities (particularly antibiotics and oral hydration therapy to treat infant gastro-intestinal illness) buffer infants from the survival costs of nonadaptive feeding practices, even when community sanitation remains poor (Howrigan, 1988; Gurven, Kaplan et al. 2007).

Theoretically, complementary food introduction should occur when infant energetic requirements exceed those derived from breast milk can provide. In reality, multiple factors influence this process, and the costs and benefits of initiating complementary foods are shaped by cultural ideologies and ecological conditions. Factors such as household wealth, household composition (Howrigan, 1988), maternal education (Kazimi & Kazimi, 1979), maternal social support (Raphael & Davis, 1985) maternal experience (Dettwyler, 1987; Zeitlin, 1991), maternal illness or pregnancy (Cosminsky, Mhloyi et al. 1993) and availability of alloparents (Quinlan, 2003) as well as maternal workload (Van Esterik, 2002; Ghosh, Mascie-Taylor et al. 2006) have

been shown to influence maternal decision-making with respect to infant feeding. While these results are compelling and it is tempting to draw conclusions, these relationships are far from universally consistent in direction and magnitude. Issues of methodology, phenotypic correlations (covariance among individual traits such as body size) and confounding variables are always present in quantitative studies of human variation.

To wean optimally, mothers are expected to assess costs and benefits with respect to infant condition, and consider potential reproductive payoffs from early weaning and diminishing returns associated with extended investment in individual offspring. Mothers are sensitive to the individual needs of infants and exhibit flexible infant feeding strategies based on factors such as infant growth, developmental stage and activity level (Dettwyler, 2004). Proximate factors (such as availability of resources and social support) act as potential constraints that can further influence maternal decision-making. Adaptive behavioral optima are difficult to determine, and cultural ideologies (ideas, beliefs and assumptions) regarding suitable infant feeding strategies also shape maternal behaviors (Cosminsky, Mhloyi et al. 1993). Changing economic realities in transitioning populations (such as maternal employment) may present obstacles to traditional infant feeding practices, initiating cultural change (Winikoff, Castle et al. 1988). Temporal changes in infant feeding, such as those reported by Howrigan in the Yucatan Mayan communities, reflect changing demographics, subsistence strategies and effects of globalization (Dugdale, 1980; Raphael & Davis, 1985; Howrigan, 1988).

Quantifying the Weaning Transition

Markers of weaning stage (age of introduction of complementary foods and age of breastfeeding cessation) are often used to predict child health outcomes (Goto, Panter-Brick et al. 2002) and are useful when data are collected near to the actual consumption/weaning event. Retrospective interviews are also used (Wilson, Milner et al. 2006) but care must be taken in designing and conducting interviews in populations where mothers may be innumerate or when there is no clear distinction between token and complementary feeding. While Quinlan and colleagues among others found maternal recall to be remarkably accurate (Quandt, 1998; Quinlan, 2003). Other investigators have observed discordance between reported and actual infant feeding practices (Vitzthum, 1994); mothers may confound actual behaviors with cultural ideals and report later introduction of complementary foods than actually occurred (Cosminsky, Mhloyi et al. 1993).

Only observational studies can ascertain breastfeeding structure by quantifying varied aspects of nursing behaviors (Vitzthum, 1992). Detailed focal-follows of infants are particularly valuable and have been used in many studies of infant feeding in behavior (Konner & Worthman, 1980; Vitzthum, 1992; Panter-Brick, 1995; Gray, 1998), however methodological variation hinders cross-cultural comparison across studies (Vitzthum, 1994). Time-allocation methodologies are also used to test hypotheses regarding human behavior based on direct observation of everyday activities within the communities of the population of interest (Gross, 1984; Hames, 1992). Time allocation has been used to quantify paternal investment (Winking 2005), maternal investment (Panter-Brick 1995; Bove, Valeggia et al. 2002), children's labor (Kramer 2005), and children's nutritional outcomes (Sharman, 1970; Hawkes, O'Connell et al. 1997). While useful, such data are rarely collected because behavioral observation is extremely logistically difficult and time-consuming. In this chapter, time-allocation observational data are used to model the weaning transition in 133 Tsimane infants in Bolivia and attempt to link observational weaning data with infant mortality and growth patterns. Contradictions between interview and observational approaches to the study of weaning patterns are also explored. The specific hypotheses to be tested are described below.

Hypotheses

- 1) *The timing and nature of the weaning transition will vary across communities experiencing different infant mortality rates; weaning will be extended in communities where infectious disease morbidity is high, and accelerated in communities where infectious disease morbidity is low.*

In order to optimize both infant survival and maternal fitness, mothers will employ adaptive strategies in their infant feeding practices. Infant feeding practices should reflect infant mortality risk, which varies across Tsimane community types. Breastfeeding provides essential nutrients and passive immunity; therefore we expect weaning to be delayed in communities where infant infectious disease mortality is high. Breastfeeding is calorically costly for mothers and also suppresses ovulation. As there may be diminishing returns to intensive nursing where infant mortality is low, we expect to see accelerated weaning in those communities.

2) *Large infants will experience an accelerated weaning process compared to smaller infants.*

Infant feeding behaviors should reflect not just infant mortality risk, but also infant growth patterns. According to the threshold models of weaning, larger, faster-growing infants will achieve threshold body sizes at earlier ages (Ellison 2001). They also require more nutrients to fuel rapid growth, and thus will outstrip maternal energetic resources (breast milk energy) at younger ages and require additional nutrients from supplementary foods (Dewey, 1997). By accelerating the weaning process in rapidly growing infants, mothers are also able to invest in future reproductive opportunities by reducing the energetic burden of lactation, which leads to the resumption of ovulation.

3.2 Study Population

Overview

The Tsimane are ~8000 forager-horticulturalists inhabiting 80-100 villages the Maniqui River system between San Borja and the town of San Ignacio de Mojos (VAIPO, 1998; INE, 2003). The Tsimane practice a combination of economic activities that blend traditional subsistence strategies with wage labor and market exchange. Tsimane live in villages composed of extended household clusters, each of which typically contains three or four residences composed of consanguineal and/or affinal kin. Tsimane women marry, on average, by age 16 (Rucas, Gurven et al. 2006), reach menarche at 13.9 years and have their first birth by age 18.6 (Walker, Gurven et al. 2006). Marriages are fairly monogamous and stable, though a small number (10%) of polygynous marriages exist (Winking et al. 2007).

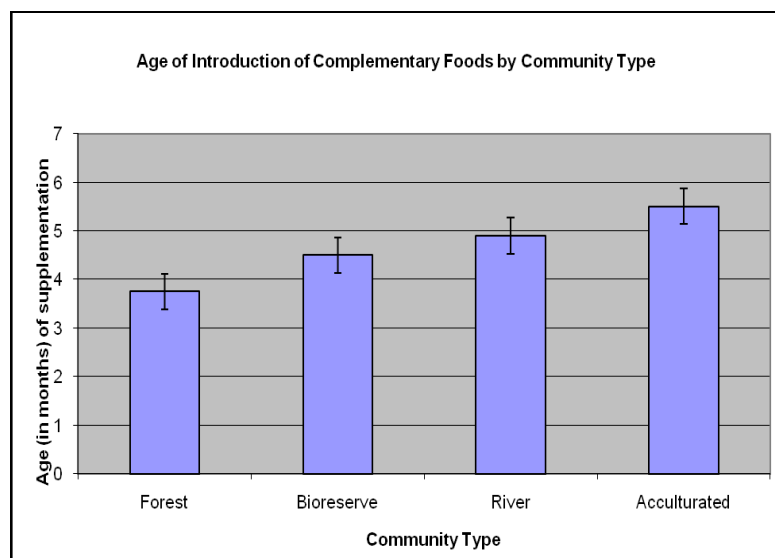
The Tsimane are a natural fertility population and most women spend their entire reproductive lifespan in a cycle of pregnancy and lactation. The Tsimane infant mortality rate (IMR) is higher than the Bolivian national average, averaging around 12.6% from 1990-2002 and varies across communities (Gurven, Kaplan et al. 2007). As in many tropical settings, household sanitation is poor in Tsimane villages, which contributes to profound susceptibility to infections that are major causes of death in infancy and childhood (Gurven, 2007). Tsimane children exhibit growth stunting, which is characteristic of South American indigenous populations (Foster, Byron et al. 2005; Godoy, Nyberg et al. 2010).

Infant Care and Feeding in the Tsimane

Tsimane infants are worn close to the mother in slings or rocked in a hammock while mothers engage in household tasks, and nurse exclusively and on-demand in the first few months of life. The ensuing period of mixed breastfeeding and complementary feeding is prolonged and varies across mother-infant pairs. Some Tsimane mothers breast-feed throughout a portion of a subsequent pregnancy, but most infants are weaned by the time their younger sibling is born. Breastfeeding can last for three years or more, but most often ceases by two years of age. Their pattern of on-demand breastfeeding followed by early introduction of complementary foods and prolonged partial breastfeeding is frequently observed in subsistence societies (Sellen & Smay, 2001).

Data from exploratory interviews of 81 mothers of infants suggest that Tsimane infants begin consuming complementary foods between three and six months of age (mean=4.66 months, see Chapter II for more details on the interview sample and results). There was variation by community type in the reported age of introduction of complementary foods (see Figure 3.1), but the differences were not statistically significant. Most Tsimane mothers begin the weaning process using complementary foods such as boiled or masticated plantain, though a small number of mothers reported using masticated meat or fish or boiled manioc.

Figure 3.1. Reported age of introduction of complementary foods by community type with standard error bars.



Choice of Study Population

The Tsimane are an ideal population for this study because they continue to invest much time and energy in foraging and other subsistence activities, and their population size is appropriate for to obtain a representative sample of infants at different stages of the weaning transition. It is easy to observe mother-infant interactions in this population because women rarely engage in wage labor outside the home. Women's subsistence tasks tend to be compatible with infant care so mothers are in close contact with infants throughout the day. Infants nurse frequently and for long durations (approximately 24 months) and bottle-feeding is not practiced. Finally, the Tsimane villages included in this study exhibit a range of levels of acculturation, allowing me to examine infant feeding practices in a relatively genetically homogenous population experiencing recent shifts in environmental pressures.

Community Types

The infants in the consumption cohort (n=133), were subjects of time allocation observational studies for a one-year period. They come from nine communities which are divided into three community types: acculturated, river, and forest. For more detailed descriptions of the study communities, see Appendix B and C. For the purposes of this study the community types are ranked by acculturation on the basis of the following criteria: distance from town, number of families, presence/absence of a school, percent of adults who speak Spanish, and infant mortality rate (see Table 3.1). Reported age at introduction of complementary foods increases with acculturation rank (see Figure 3.1). CDC Z-Scores are reported in rather Tsimane Z-Scores in Table 3.1 to illustrate that Tsimane children are small in weight and height compared to the U.S. baseline.

In general, the most acculturated communities are close to town, have the most families, the lowest infant mortality rates, have high quality schools, and a high percent of Spanish-speaking adults. The interior forest villages are among the most inaccessible Tsimane communities and access to medicine or medical facilities is rare. Spanish is rarely spoken in the forest communities, which have few families and high infant mortality rates. At the time of the time-allocation study, only one of three of the forest communities sampled had a school. The upriver communities in this sample are quite far from San Borja, but some individuals travel to town frequently when weather

conditions are favorable. Downriver communities are closer to town and it is possible to walk in a short day if river travel is not possible. Infant mortality in the river communities is higher than in acculturated communities, but lower than in forest communities. About half of the adults in river communities speak Spanish, and all of the river communities had schools that varied substantially in quality.

Table 3.1. Summary of characteristics of the study communities with acculturation rankings

Community Type	Mean Distance from SB (km)	Mean # Families	School/ total	% Spanish Speaking Adults	Mean Years School (adults)	IMR*	Mean age of introduction of CFs (months)	Level of acculturation
Acculturated	27	56	1/1	72%	2.8	8.5	5.6	high
Forest	60	22	1/3	35%	0.4	17.1	3.8	low
River	36	29	6/6	55%	1.5	14.7	4.7	medium

*Infant mortality data is taken from Gurven, Kaplan et al. 2007. Bioreserve IMR was not published, but upriver IMRs are similar to those calculated for the bioreserve based on preliminary analysis of demographic interviews.

Inclusion Criteria

Infants were included in the study if they met the following criteria: 1) they were aged 0-36 months at the time of the observations, 2) their birthdates were confirmed, 3) there existed ten or more behavioral observations in the dataset, and 4) there was at least one observation of a consumption event. A consumption event is defined as any observation of nursing or eating or drinking complementary foods, which are defined for the purpose of this study as any non-breast milk food or liquid, including water. The resulting sample consisted of 69 female and 64 male infants aged 0-36 months. There were 35 infants from acculturated communities, 30 from forest communities, and 68 from river communities. In total there were 862 consumption observations for the 133 infants, 526 (61%) of which were nursing observations. There were a total of 108 mothers of the 133 infants in the consumption cohort: 84 mothers had one infant in the cohort, 23 mothers had two infants in the consumption cohort and one woman had three. The woman who had three infants in the cohort lived in two different communities (both riverine) when time allocation data were collected (first in 2002-2003, then in 2006-2007).

3.3 Methods

Data Collection

Data were collected under the auspices of the Tsimane Health and Life History Project which has operated in the Beni department of northeast Bolivia since 2001 under the joint directorship of Michael Gurven (University of California Santa Barbara) and Hillard Kaplan (University of New Mexico). In this chapter, time allocation observations and anthropometric data collected at medical team visits were employed to investigate infant feeding patterns.

Time Allocation

Time allocation data was collected by Bolivian and North American anthropologists in nine Tsimane' communities using scan-sampling techniques (Winking, 2005; Winking, 2009) Direct observation is an extremely time consuming and logistically complex method of data collection, but is superior to interview techniques that are subject to recall bias as well as miscommunication between interviewers and research subjects due to language barriers and different cultural perceptions regarding infant care and feeding. Data were collected from Apere and Cuverene, both forest communities and Cosincho and Munday, both upriver communities, from 2003-2004. Tacuaral, the only acculturated community, and Jamanchi, a forest community, were sampled from 2005-2006. Cedral, Chacal, and Puerto Triunfo, all downriver communities, were sampled from 2006-2007.

Over three years of time allocation data collection, a large database was compiled on the time Tsimane adults and children devote to subsistence and domestic tasks, school and wage labor, and childcare and leisure activities. Two and three-hour house-hold "time blocks" were conducted in household clusters based on proximity which closely matched kinship (Winking, 2005). In the forest, river, and acculturated communities, these clusters were sampled randomly without replacement at 7:00 a.m., 10:00 a.m., 1:00 p.m., and 4:00 p.m. in 3-hour time blocks, covering all hours from 7:00 a.m. to 7:00 p.m. In the bioreserve communities the sample scheme was slightly altered: clusters were sampled randomly without replacement at 7:00 a.m., 9:00 a.m., 11:00 a.m., 1:00 p.m., 3:00 p.m., and 5:00 p.m. in 2-hour time blocks, covering all hours from 7:00 a.m. to 7:00 p.m. During time blocks, instantaneous scans were taken every half hour, in which the

activity, location, and social group of all individuals within the cluster (as well as visitors) were recorded, resulting in four to six time points per person per time block.

The individual's activity was described as best as possible including objects and interactants. Infants were most often coded as being recipients of direct care (being held, nursed, groomed, bathed, fanned, fed, or swung) and the identity of caretakers was noted. Other common infant behaviors included crying, sleeping, and passive or active play. Locations were standardized, allowing for houses, kitchens, yards and fields for each family, and a set of community-specific locales. If infants were outside of the village during the household scans, the location of the infant was ascertained by asking others where the infant's family had gone, or by asking the parents of the infants where they had been upon return. For the purposes of this analysis, only direct, in-camp observations were used.

Anthropometry

Maternal, infant and child anthropometry were collected by the Tsimane Health and Life History Project medical team at biannual medical visits from 2002-2009. The four downriver communities were added to the sample in 2006 and therefore anthropometric measurements are only available for 2006-2009. Maternal and child standing height were measured using a Seca Portable Stadiometer. Infant recumbent length (from crown to heel) was taken using a baby board for infants under the age of two. Maternal and child weight were measured using a Tanita BF680 scale. Young infants were weighed with their mothers and mother's weight was subtracted to obtain infant weights (attempts made to obtain more accurate weights using a Salter Hanging Baby Weigher were abandoned if infants became distressed). Infants were weighed naked and without shoes.

Demography

Infant and maternal demographic data and maternal reproductive histories were obtained as part of the Tsimane Health and Life History Project demographic database. To accurately assess infant ages, Tsimane mothers and fathers were separately asked infant birthdates which then were cross-checked with data from a log maintained by the schoolteacher in some villages and from vaccination records when they were available. For detailed descriptions of demographic data collection methods in the Tsimane, see Gurven, Kaplan et al. 2007.

Data Analysis

The Weaning Model

The PROC NLMIXED procedure in SAS was used to perform a series of random effects logistic regressions using behavioral data to predict the likelihood that each infant would be observed nursing versus consuming complementary foods at each given age (nursing probability). Analyses are limited to time-allocation consumption events recorded for the consumption cohort. This mixed model allowed us to model the rates at which proportion of time spent nursing (versus complementary food consumption) changes over time from 0-36 months while controlling for individual random effects. The model also controlled for infant community, age, and sex.

Nursing Intensity

Observations of infant consumption were recorded over a period of time during which the proportions of nursing and eating were changing, so in this weaning model each observation is a unit of analysis and controls for individual as a random effect. This procedure allows for the output of random effects coefficients (Bayes estimates) which were defined as each individual infant's index of nursing intensity (NI). NI is similar to a Z-Score in that it tells us where infants fall along the continuum of mixed feeding relative to other children who are the same age; or the degree to which infant is ahead (positive NI) or behind (negative NI) population curve in their observed frequency of nursing versus non-breast milk food consumption.

Anthropometry

There were a total of 231 height and weight measurements available for a subset of 108 infants in the consumption cohort. Repeated measures (2-5) were available for 77 of the infants. Z-Scores were calculated to express anthropometric values as the number of standard deviations above or below a reference mean value and are considered the best system of presenting and analyzing anthropometric data (de Onis & Blössner, 1997). Weight-for-age reflects past and present nutritional status (underweight is defined as ≤ -2 Z-Scores below the mean) and height-for-age reflects chronic nutritional conditions (stunting is defined as HAZ ≤ -2 Z-Scores below the mean) (Cosminsky, Mhloyi et al. 1993).

Standardized growth charts are an important comparative tool because they are so widely used, but their use in cross-cultural research is grounded in the assumption that infant growth trajectories are canalized. Cross-cultural comparisons suggest that growth trajectories are in fact variable across populations (Walker, Gurven et al. 2006) and may therefore, confound cross-cultural comparison when using a standardized reference population. There is considerable debate as to whether standardized reference populations are appropriate in evaluating the nutritional status of indigenous children (Stinson, 1996; Santos, 1993).

Using cross-sectional data from all infants aged 0-3 from the Tsimane Health and Life History Project database, in the Tsimane population CDC 2000 Z-Scores decrease rapidly in early infancy (see Figure 3.2). In Figure 3.2, WAZ-Scores for 976 infants and HAZ-Scores for 982 infants are averaged by month. While Z-Scores appear to increase as infants approach 3 years of age, plots of children up to age 7 reveal that Z-Scores level off rather than increase in middle childhood. This is consistent with findings in previous studies in the Tsimane and other South American native populations that show that growth faltering begins in infancy and increases with age. However, this measure compares Tsimane infants to U.S. infants of the same age, so the CDC Z-Scores presented in Figure 3.2 tell us that Tsimane infants rapidly fall behind compared to the U.S. baseline in both weight and height and remain there.

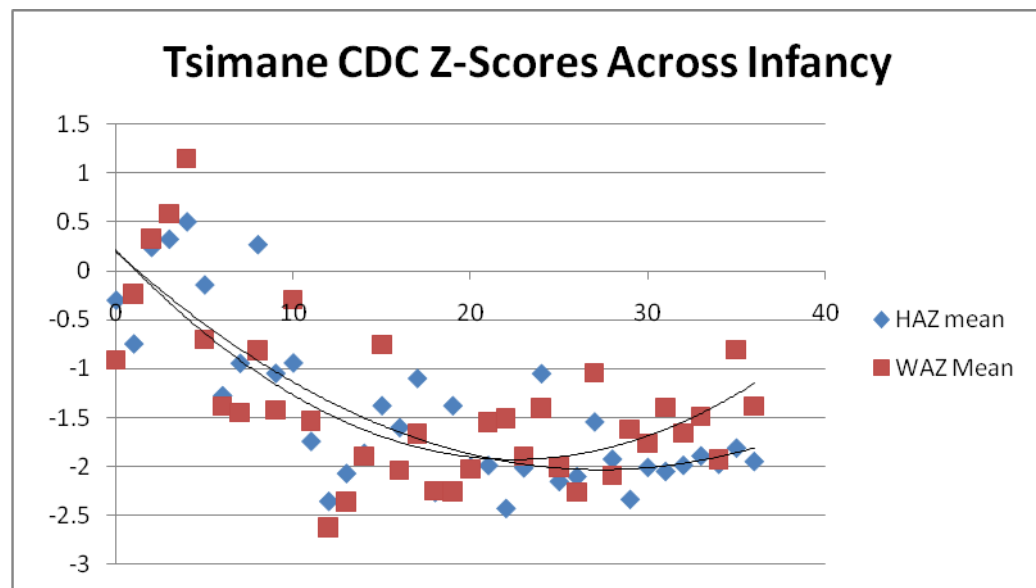
Population-specific Z-Scores provide an alternative by comparing children to a reference mean derived from their own population, therefore eliminating potential biases caused by differences in growth trajectories. In 108 infants from the consumption cohort for which anthropometric data were available, Tsimane-specific and CDC Z-Scores were highly but imperfectly correlated (WAZ $r=.81$, HAZ $r=.78$). Both sets of Z-Scores are used in additional analyses, but only results using the Tsimane baseline (Z2) are reported herein.

The two sets of Z-Scores were calculated as follows:

1. Z-Score 1 (Z1) used the CDC 2000 standards calculated using EpiInfo EpiNutrition software. The CDC scores were based on a reference population of infants and children in the United States. The 2000 CDC Growth Chart reference population included data for both formula-fed and breast-fed infants and data on low birthweight infants but did not include data on very low birth- weight infants.

2. Z-Score 2 (Z2) was generated from the Tsimane Health and Life History Project anthropometric database using Tsimane infants and children aged 0-3. There were 1843 weight measures on n=976 infants and 1851 length/height measures on n=982 infants weight measures as the reference population. The Tsimane-specific Z-Scores were calculated by regressing infant age on infant weight and height. Because the data were heteroscedastic, standardized residuals were used as Z-Scores.

Figure 3.2. Tsimane CDC Z-Scores decline rapidly in early infancy and then level off in the second half of the second year of life.



Anthropometry and Weaning

The relationship between infant anthropometry and breastfeeding frequency was evaluated in two ways. First, the weaning model (dependent variable=probability of nursing) was run for the 108 infants with raw height (in centimeters) and/or weight (in kilograms) included as covariates. Second, generalized estimating equations (GEEs) controlling for PID as repeated subject were conducted to determine if infant weight and height were predictors of NI (nursing intensity) using raw weight and height scores and both CDC (Z1) and Tsimane-specific Z-Scores (Z2). NI was used as an outcome variable because it controls for random effects and the fact that observations of

infant consumption were recorded over a period of time during which the proportions of nursing and eating were changing.

Unfortunately, anthropometric measurements were not available from before the time allocation study period in which the weaning data were collected for all infants. Height and weight measurements existed from prior to the time allocation period for 30 infants, and for 26 infants, height and weight measurements were available from the first three months of life. For the remaining 54 infants, height measurements were only available from after the time allocation study period. A dummy variable was constructed and included in the GEE to account for this sampling problem. If a measurement was conducted before the time allocation study period, or in the first 3 months of the infants life (prior to the age that most Tsimane infants begin to consume complementary foods), they were assigned to Group 1. If the anthropometric measurements were conducted 3-15 months after the time allocation study period, they were assigned to Group 2, and if the anthropometric measurements were conducted 15-36 months after the time allocation study period, they were assigned to Group 3. By comparing the relationship between body size and NI in this way, an attempt was made to better establish the direction of causality. If body size was driving NI, rather than the other way around, then the effect should be strongest in Group 1 and weakest in Group 3.

Maternal factors were also tested for independent effects on NI, nursing probability and child anthropometry to determine if they could confound analyses and should therefore be included as control variables. Maternal age and parity were used to predict NI, WAZ and HAZ-Scores in simple linear regressions as we expected that older mothers with fewer future reproductive opportunities, and lower-parity mothers, might nurse their infants more intensively. Maternal weight and heights are also available from the time allocation study period. If mothers were pregnant at measurement, attempts were made to obtain non-pregnant weights from one year before or after the study period. Maternal weight, height and BMI were used to predict WAZ and HAZ-Scores in simple linear regressions as we expected that larger mothers would have larger infants.

Interview and Observational Approaches

Both interview and observational data were available for a small subset (n=30) of infants. To examine the relationship between reported and observed infant feeding practices, age of

introduction of complementary foods (as reported by mothers) was regressed against nursing intensity (NI). An additional GLM was constructed to control for community type and infant body size (HAZ and WAZ).

3.4 Results

Descriptive

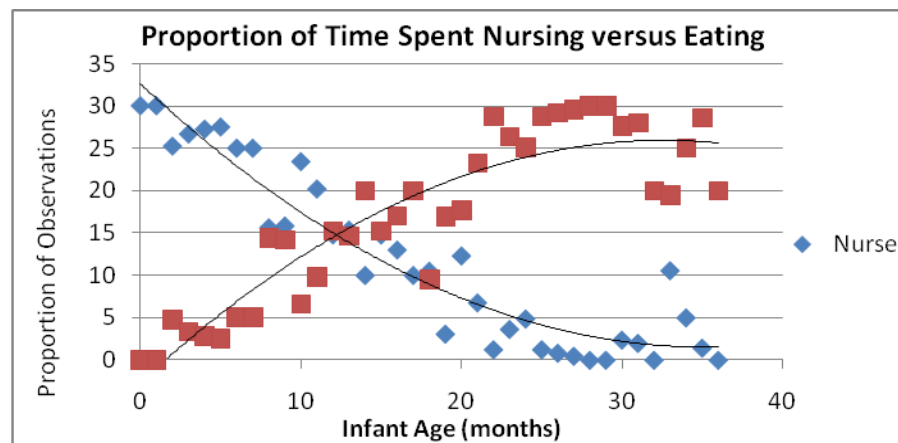
Table 3.2 provides mean demographic and anthropometric characteristics for the 108 mothers and 133 infants in the three community types. Community differences in infant WAZ, HAZ and maternal BMI, maternal weight, maternal height, maternal age and parity were not statistically significant. Average child age at measurement was 21.15 months. Anthropometric characteristics of 108 infants from the consumption cohort are here described using raw weight and height measures and CDC 2000 Z-Scores (Z1). While comparing Tsimane infants to the US baseline may confound infant body size with age (see Methods), CDC Z-Scores are presented here in the descriptive results to situate Tsimane infants within a broader context of infants and facilitate interpretability.

Figure 3.3 shows the proportion of time Tsimane infants were observed in nursing and non-breast milk consumption events. Infants were observed nursing nearly 30% of the time in the first months of life. Non-breast milk food consumption was observed in infants as early as one month of age and increased gradually from 0-36 months. Nursing was observed as late as 36 months, but most infants were fully weaned earlier. Consumption observations exceeded nursing observations when infants reached 13.3 months. After controlling for data coder, there was no direct effect of community type or infant sex on nursing probability.

Table 3.2. Summary of Infant and Maternal Characteristics by Community Type

Community Type	Infant Characteristics (n=133)					Maternal Characteristics (n=108)		
	Consumption cohort (n)	Mean Age	Z-Score (n)	Mean CDC WAZ	Mean CDC HAZ	Mean Age	Mean BMI	Mean Parity
Acculturated	35	21.62	28	-1.90	-1.58	26.20	23.93	4.26
Forest	30	21.31	22	-1.56	-1.63	25.50	22.89	4.37
Riverine	68	20.93	58	-1.62	-1.58	25.99	23.59	4.19
All Infants	133	21.87	108	-1.69	-1.60	25.90	23.47	4.27

Figure 3.3. Observations of nursing and consumption of complementary foods in 133 Tsimane infants



Nursing and eating observations are pooled by month. Nursing and consumption curves cross at 13.3 months.

Hypothesis 1.

The timing and nature of the weaning transition will vary across communities experiencing different infant mortality rates; weaning will be extended in communities where infectious disease morbidity is high, and accelerated in communities where infectious disease morbidity is low.

The weaning model for $y = \text{nurse by age (in months)}$ is presented in Table 3.3. Age was the only variable that was a strong predictor of nursing probability ($p < .0001$). The results for community type were partially in the predicted direction (highest in the high-mortality forest communities) but they were lowest in the river communities (which have higher mortality than the

acculturated communities). Community differences were not significant: Only after controlling for infant height did marginally significant differences in community type emerge (see Table 3.4 under Hypothesis 2); however they were not in the predicted direction. After controlling for height, nursing probability was highest in the river communities. Nursing probability in the river communities was significantly higher than forest and acculturated communities, but the forest and acculturated communities did not differ significantly from each other. With forest communities set as baseline, the odds ratios for nursing probability were calculated: OR=3.84 for infants in the river communities and 1.53 and for infants in acculturated communities. The nearly four-fold difference in nursing probability in forest and river communities is notable and just reaches significance at the $p=.05$ level.

Table 3.3. Weaning Model (random effects logistic regression) for $n=133$ infants after controlling for data coder and random effects. Dependent variable is probability of nursing.

Parameter	Estimate	SE	Pr>t
b0	2.6976	0.9263	.0042
Age infant (months)	-0.1968	0.0160	<.0001
Age=female	-0.3419	0.3347	0.1306
Community=acculturated	-0.2700	0.8349	0.7440
Community=forest	0	n/a	n/a
Community=river	-0.5159	0.9353	0.5882

Hypothesis 2.

Large infants will experience an accelerated weaning process compared to smaller infants.

In this section models are presented in which infant anthropometry was used to predict a) nursing probability (Table 3.4) and b) nursing intensity, or NI (Table 3.5, NI is the Bayes estimate output by the random effects logistic regression model presented in Table 3.3). Infant height, but not weight, was a significant predictor of nursing probability after controlling for age, random effects and coder (see Table 3.4). As predicted, the relationship between height and nursing probability was negative. Marginally significant differences in community type emerged after controlling for height, with nursing probability highest in the river communities.

In the second analysis generalized estimating equations were constructed in which CDC (Z1) and Tsimane-specific Z-Scores (Z2) were used to predict NI. Results were consistent using

both sets of Z-Scores, so only the results from models using Z2 are shown. As in the logistic regression model described above, WAZ was not a significant predictor of NI, but HAZ was a negative, significant predictor of NI (see Table 3.5). Because this analysis regresses a Z-Score (HAZ) on an index that resembles a Z-Score (NI) coefficients are low, but the results are still highly significant. In this model where nursing intensity was the dependent variable, there were no significant differences in NI by community type, but an unexpected sex difference emerged: males had higher NI than female infants. Maternal age, parity and anthropometry did not significantly predict NI. When the age difference between collection of the time allocation data and the anthropometric measures was included in the model to assess causality, Group 3 had the lowest coefficient, as predicted, but Group 2 had the highest coefficient (see Table 3.6). The effect of group was not a significant predictor of nursing probability in Table 3.4 (data not shown), or of NI in Table 3.5.

Table 3.4. Weaning Model (random effects logistic regression) for n=108 infants after controlling for data coder and random effects. Dependent variable is probability of nursing and height is included as a covariate.

Parameter	Estimate	SE	Pr>t
b0	4.6432	1.6925	0.0071
Age infant (months)	-0.1718	0.0173	<0.0001
Raw Height (cm)	-0.0493	0.0232	0.0362
Community=acculturated	0.4256	1.0593	0.6997
Community=forest	0	n/a	n/a
Community=river	1.2192	0.6158	0.0502

Table 3.5. GEE for n=108 infants (dummy variable denotes time elapsed between collection of NI and height measure). Dependent variable, NI, controls for coder and random effects.

Parameter	Estimate	SE	Pr>t
b0	.029	.0404	.467
Sex=female	-.103	.0481	.032
HAZ (Z2)	-0.020	.0053	.000
Time=1	.049	.0388	.206
Time=2	.056	.0132	.074
Time=3	0	n/a	n/a

Interview versus Observational Approaches

No significant correlation existed between reported and observed infant feeding behaviors for the subset of mother-infant pairs for whom both data types were available ($n=30$, $r\text{-squared}=0.032$). There was no significant relationship between reported age of introduction of complementary foods and nursing intensity (NI) before or after controlling for HAZ, community type and infant sex.

3.5 Discussion

The analysis of observational data revealed the complex and protracted nature of the weaning process that is not accurately captured by retrospective interview techniques. In Figure 3.3, infants are observed nursing almost 30% of the time in the first months of life and some continue to nurse up to 36 months of age. Since food consumption was also observed as early as one month and increased gradually with age, there is clearly potential for a very long and variable period of mixed feeding. Weaning is often considered to be two discrete events: the introduction of complementary foods and the cessation of breastfeeding. Observation of Tsimane infants revealed a pattern of weaning that was more like a continuum in which the rate at which breastfeeding declines relative to increasing food consumption can vary tremendously between individuals.

Time spent nursing declines as infants age because they become more efficient at extracting milk from the breast over time, though important questions have been raised regarding the actual extent to which breast milk transfer increases with age (Reilly, Ashworth et al. 2005). In this study it is unfortunately impossible to isolate the effects of this phenomenon from the actual displacement of breastfeeding upon introduction of complementary foods using the data available. While the introduction of complementary foods disrupts breastfeeding structure and can lead to less frequent nursing and accelerated weaning, (Vitzthum 1994), in this study considerable variation in this process is observed across mother-infant pairs.

The Weaning Model

The weaning model was designed to identify whether mortality risk or infant growth patterns play a role in driving variation in the weaning transition. The results of the weaning models presented in Table 3.3 shows little variation in nursing patterns across Tsimane community

types; in fact age was the only significant predictor of nursing probability in the model for 133 infants. For the subset of 108 infants for whom anthropometric data were available, both age and height were significant predictors of nursing probability, and community type became marginally significant. The hypothesis that infant feeding practices would reflect infant mortality rates across Tsimane communities was therefore not supported.

When height was controlled for, the probability of nursing was higher and marginally significant in the river communities when compared to a forest baseline ($p=.0504$). River communities have a higher infant mortality rate (14.7%) than the acculturated communities (8.5%). However, there were not significant differences in nursing probability between acculturated and forest communities (forest communities experience the highest infant mortality rates, 17.1%). This suggests that infant mortality risk was not a strong driver of variation in infant feeding patterns, at least not at the level of community type. More detailed studies should assess nursing patterns with respect to infant mortality risk at the individual level.

The possibility that infants are nursed more intensively in river communities warrants further investigation. Calculation of odds ratios revealed that infants in river communities were 3.84 times more likely to be observed nursing than infants in forest communities, and infants in river communities were 1.5 times more likely to be observed nursing than infants in forest communities. The river sample encompasses both up and downriver communities, though infants from upriver regions compose the majority of the river sample. The upriver communities experience higher prevalence of anemia, and the downriver communities were observed in a lean year when floods destroyed a large number of crops.

It is not clear why mothers should nurse more intensively where anemia is prevalent, as many studies have showed correlations between long exclusive breastfeeding and anemia deficiency in infants. Subtle nutritional and pathogenic pressures may have driven the mothers in this sample of river communities to nurse their infants at higher frequencies. For example, it is plausible that in downriver communities, mothers breastfed more intensively to compensate for increased pathogenicity associated with flooding. However, as this result was tenuous, more research is needed to understand the nature of breastfeeding variation across and within communities. Studies of Tsimane womens' breast milk are underway and might yield clues as to variation in macro-and micronutrient content across community types.

Anthropometric Outcomes

The Tsimane infants were stunted and underweight according to the CDC baseline, which was consistent with previous studies of the Tsimane (Foster, Byron et al. 2005) and in South American indigenous children in general (Stinson 1989; Hodges and Dufour 1991). Infant height was a significant predictor of both nursing probability and nursing intensity (NI). NI shows where individual infants fall along the continuum of mixed feeding relative to other children who are the same age. In both models the relationship between height and nursing was negative; infants who were taller nursed less frequently and thus experienced a more rapid progression through the weaning transition than their shorter counterparts.

It is not possible to ascertain the direction of causality with the observational dataset available. More research is needed to determine if intensively breast-fed infants become stunted, or if mothers alter their breastfeeding strategies according to infant growth patterns. If infant size does drive NI, the relationship should persist across infancy but become attenuated over time. When the time elapsed from collection of the nursing observations and the height measurement was controlled for, the model coefficient was weakest when measurements were taken 15-36 months after the time allocation study period. This finding is compelling but the difference was not statistically significant.

The findings of this study provide tentative support for the hypothesis that Tsimane mothers monitor their infants' statural growth and alter their nursing behaviors by accelerating the weaning process in faster-growing infants. This finding conforms to several mammalian models in which offspring are weaned when they achieve a certain threshold body size (Lee 1996). It makes adaptive sense that the entire process of weaning should be accelerated in children who grow faster, because they are larger and require more daily calories to maintain their larger body size. Mothers' milk quickly becomes insufficient to deliver calories to large infants, thus they may be transitioned to food more quickly (Ellison 2001). While this chapter focused on maternal strategies, Tsimane mothers report that infants play an active role in driving the weaning transition. Faster-growing infants required more nutrients and therefore, may express interest in complementary foods at earlier ages. These findings in the Tsimane add to a body of theoretical and empirical evidence suggesting that growth was one cue mothers used to gauge infant development and to assess their readiness for complementary foods. Future research should explore the role of infant behavioral cues in conjunction with other developmental processes.

The Role of Gender

In generalized estimating equations, female infants had lower nursing intensity (NI) scores than male infants after controlling for age, height and random effects. Sex differences were not observed in the weaning model (where nursing probability was the dependent variable) before or after controlling for height, although the direction of the relationship was consistent in both models. This combination of results suggests that male infants may have higher nursing probability in Tsimane communities; however the effect was not a strong or consistent one. Trivers and Willard proposed that parents should bias investment toward offspring capable of translating parental investment into reproductive success (Trivers and Willard 1973). Demographic interviews of Tsimane men and women indicated that there may be a slight preference for sons in this society, so male-biased investment in infants in some communities could be based in a cultural preference for males that potentially yields higher fitness payoffs.

Alternatively, male infants and children may be less buffered than females from environmental insult (Tanner, 1962; Catalano, Bruckner et al. 2005) and require compensatory parental investment. Evidence from humans (Kruger & Nesse, 2005) and macaques (Muehlenbein & Bribiescas, 2005) demonstrated higher mortality in males than in females. Tsimane infant mortality was not significantly higher in males than females (Gurven, Kaplan et al. 2007), and an extensive review of research on this topic suggested that prenatal, but not postnatal mortality, is higher in males infants than in females (Stinson, 1985). Infant diarrheal and respiratory morbidity, as reported by mothers, were also not different for male and female infants in the consumption cohort. In a preliminary analysis of Tsimane infant anthropometry, however, males did exhibit greater variance in HAZ and WAZ than did females (Gurven, personal communication), and in a study of climate and height in the Tsimane, the TAPS research team found that higher rainfall (or variability in rainfall), particularly in the birth year, was associated with greater height in boys age 2-12 (Godoy, Goodman et al. 2008). The authors suggested that mothers might alter the length and quality of breastfeeding in boys (ibid). Adaptive modulation of investment that favors males makes sense if there are heightened risks for male infants.

Infant development is not only reflected by physical growth, but also in developmental maturation. A recent analysis from a longitudinal study of breastfeeding and cognitive outcomes in Australian children revealed that prolonged breastfeeding was positively associated with

academic success in 10 year-old boys (Oddy, Li et al. 2011). Another series of studies showed that breastfeeding was associated with improved speech and reading abilities in boys but not girls, suggesting that breastfeeding exerts an effect on developmental maturation that is more pronounced in boys (Broad, 1972; Broad, 1975). These findings suggested that there was a connection between gender, breastfeeding, and cognitive development (Oddy, Li et al. 2011). Future research in the Tsimane should explore the possibility that mothers might alter their infant feeding and care strategies in male and female infants, and that they may be responsive to different developmental cues in boys and girls.

Immunologic Calibration

The weaning transition is associated with immunological changes that generate long-term immunological trajectories. It is therefore important to consider the simultaneous roles of immunologic protection and antigenic stimulation in the development of weaning models. By responding to infants' behavioral and developmental cues, mothers may practice optimal feeding strategies and facilitate "immune calibration," (coined by Fessler and Abrams in reference to infant mouthing behaviors (Fessler, 2004), by timing the introduction of complementary foods and subsequent weaning transition so as to promote optimal stimulation of acquired immune defenses.

Infant developmental immunity requires antigenic stimulation at crucial developmental stages in order to develop adequate tolerance and adaptive feedback mechanisms. Allergies and asthma are afflictions associated with inadequate (or untimely) immunological stimulation at crucial developmental stages (Strachan, 1989; Renz & Herz, 2002). If small doses of pathogen exposure are essential to facilitate the maturation of the immune system, the timing and nature of optimal pathogen exposure should vary by infant and be flexible across diverse ecological settings. Limited morbidity in infancy has been associated with longer-term costs of diminished immune capacity in adolescence (McDade, Beck et al. 2001b). Rapidly-growing children, compared to slow-growing peers, may have greater energy available to allocate to a suite of competing life history demands, as demonstrated in McDade's discussion of phenotypic correlations (McDade, 2003). If so, they should also have more energy available to mount an immune response to antigens associated with complementary foods. Where energy is limited, we expect a more pronounced trade-off between growth and immune function.

Maternal fitness encompasses both current and future reproduction, thus mothers must balance the needs of a nursing infant with her own subsistence tasks and care of other dependent children. In addition mothers must maintain adequate energy stores to sustain current activity and to invest in future reproductive opportunities. Therefore, infant feeding decisions must take into account both costs and benefits for individual infants as well as costs and benefits for maternal inclusive fitness. Given realities of costs of prolonged amenorrhea and the reality of maternal depletion in high-fertility, resource scarce settings, theoretically mothers can benefit from providing complementary foods early to infants who are growing rapidly. Mothers cognizant of longer-term health outcomes may then exhibit behaviors often associated with increased infant morbidity (such as early complementary feeding), but only in infants deemed strong enough to handle the pathogenic challenge.

Breastfeeding and Fertility

While growth appears to contribute to variation in nursing patterns in the Tsimane, fertility may also play a role. Tsimane fertility is very high, which is common among contemporary lowland native groups in Latin America undergoing varying levels of acculturation (McSweeney, 2005) and reflects recent changes in diet and activity patterns. The metabolic load hypothesis has been proposed to explain rapidly increasing fertility in the Toba of Argentina: Toba women rapidly resume ovulation despite intensive nursing patterns because of high caloric intake and decreased workload associated with a recent transition to urban lifestyles (Valeggia & Ellison, 2001).

To maximize fitness, mothers must balance investment in the survival of current offspring with future reproductive opportunities, and acceleration of weaning leads to a rapid resumption of ovulation. In Figure 3.3, the predicted curves for nursing and non-breast milk consumption probability cross at 13.3 months of age. While it is not possible to ascertain the relative caloric contribution of these food types using observational data, the changing ratios of breast milk and non-breast milk food consumption across infancy have implications for the resumption of ovulation. When Tsimane total fertility rate (TFR) is averaged across a woman's reproductive lifespan, interbirth intervals range from 2-2.5 years. Interbirth intervals and weaning patterns are linked, however the causal processes underlying this relationship are unclear.

Tsimane women are sometimes observed to nurse well into a subsequent pregnancy and this has been observed in other small-scale societies (Merchant, 1990; Strassman & Warner, 1998)

Still, many Tsimane women claimed that a new pregnancy is a reason to terminate breastfeeding. While breastfeeding is rarely terminated abruptly, mothers may accelerate the weaning process to accommodate a new pregnancy. The interaction of lactation and energetics on fertility outcomes in Tsimane women living under diverse conditions, and the mechanisms underlying amenorrhea and the resumption of fertility, merit examination in future studies.

Conclusions and Directions for Future Research

There is substantial cross-cultural variation in health outcomes associated with infant feeding practices. The complexity of the weaning transition and tremendous variation in ecological conditions made it nearly impossible to standardize research methodologies across populations. This chapter attempts to contribute to the existing literature by modeling observed infant feeding behaviors in a forager-horticulturalist society and to consider the relationships between weaning, infant growth, and infant mortality rates. Results suggested that infant growth plays an important role in determining infant feeding patterns. Community type and infant gender also seem to contribute to variation in weaning patterns in subtle ways.

Relationships between infant growth and infant feeding suggest that health risks associated with early complementary feeding may not result directly from earlier introduction. Concurrent socio-ecologic factors, child condition, and method of complementary feeding may serve to may buffer infants potential risks associated with early supplementation. The WHO recommendation of six months of exclusive breastfeeding has been criticized both because of discrepancies in research findings on infant health outcomes. It also fails to address energetic considerations relevant to maternal fertility and health. If maternal condition strongly influences children's body size, then poor maternal condition poses constraints on child health and growth. Thus attributing poor child health outcomes to "suboptimal" feeding practices may be inappropriate in contexts where there is substantial variation in resources available to mothers. Public health interventions that encourage mothers to conform to the WHO regimen of infant feeding may not only interfere with adaptive processes that balance both infant health outcomes with maternal fitness in resource scarce settings. Furthermore, they can unfairly stigmatize poor women as "bad mothers" if they don't adopt recommended strategies.

This study also offers a methodological contribution by presenting data on infant feeding drawn from a large observational dataset. When interview and observational approaches were

compared in a small sample of mother-infant pairs, earlier complementary feeding was not associated with an accelerated weaning process. Similarly, mothers who introduced complementary foods to infants at later ages did not necessarily nurse more intensively. Future studies should attempt to further assess the validity of maternal reporting with respect to infant feeding patterns, both in the Tsimane and other subsistence societies. Investigations of infant feeding that rely exclusively on retrospective interview data should be interpreted with caution, as we have demonstrated that weaning was a complex and dynamic process not easily captured by the reporting the timing of key weaning transitions.

This observational study was limited in that baseline, pre-complementary food measurements do not exist to control for phenotypic correlations in this cohort of infants. Lack of data on breast milk composition, and actual nutrients derived by infants from breast milk and complementary foods, inhibit our ability to draw robust conclusions on the relative contribution of different food types to infant developmental trajectories. Furthermore, to examine the nature of immune calibration in Tsimane infants and maternal vigilance behaviors, detailed focal follows of infants are needed. Finally, the interactions of maternal condition, fertility, breastfeeding, and infant health require more examination in this population.

CHAPTER IV

THE INFANT THYMUS IN TWO NATIVE SOUTH AMERICAN SOCIETIES

4.1 Theoretical Background

The thymus is the target of much investigation because of its role in immune maturation and the poorly understood nature of its developmental trajectory (McDade & Worthman, 1999; Savino & Dardenne, 2000). A primary lymphoid organ, the thymus enables immunologically vulnerable infants to develop a repertoire of T-lymphocytes that target intracellular viral, bacterial and parasitic infections and cancer. T-lymphocytes are also involved in the coordination of responses involving multiple components of the immune system. Though essential to disease resistance, empirical study of the thymus has progressed with little consideration of the selective pressures shaping this energetically costly organ. In this chapter comparisons of infant thymus and body size in two groups of South American natives are presented. The South American native thymus size values are then compared to published values from urban cohorts of infants in Denmark and Turkey. A life history theoretical perspective was employed to consider how the thymus might develop under conditions of resource scarcity and environmental pathogenicity, and emphasize the need for more investigation of thymic developmental plasticity that spans diverse study populations.

Life History Theory and Human Immune Function

The theoretical approach of this research was grounded in life history theory, an analytical framework invoked in studies of humans and other animals to explain variation in development, behavior, and reproduction. Fitness is maximized by natural selection when energetic resources are allocated optimally to competing life history functions growth, survival, and reproduction (Gadgil & Bossert, 1970; Smith & Fretwell 1974; Kaplan, Hill et al. 2000). Individuals have a

fixed energy budget at a given point in time, therefore trade-offs exist between competing life history functions and investment in one (e.g., growth), comes at a cost to another (e.g., reproduction) (Hill & Hurtado, 1996). When resources are scarce these energetic trade-offs become more pronounced and in risky situations, short-term survival mechanisms are favored at the expense of growth and reproduction (Tschirren & Richner, 2006). Decision-making with respect to energy allocation occurs on both physiological and behavioral levels and involves a coordinated response to both individual condition and environmental factors (McDade, 2003; Stearns, 2005).

Under this framework, immune function is a survival and repair mechanism whose development and maintenance is associated with both learning and energetic costs. These costs are outweighed by the benefit of having an effective pathogen defense system capable of upregulating function in the presence of infection (Sheldon & Verhulst, 1996; Read & Allen, 2000). Ecological immunology applies life history theory to immunologic research by examining variation in immune system development and function across and within species in the context of evolution and of ecology (Norris & Evans, 2000, Sheldon & Verhulst, 1996; McDade, 2003).

Excellent detailed descriptions of immune function are available (Murphy, Travers et al. 2008; Abbas, Lichtman et al. 2009; Abbas & Lichtman, 2011) and here only a brief overview is provided. Human immunity relies on a combination of innate and adaptive defenses. Innate immune factors such as phagocytes, natural killer cells, neutrophils, monocytes, and complement proteins recognize conserved regions that are present on a broad spectrum of pathogens, and are generally thought of as “first line defense,” becoming active against antigens prior to the upregulation of the adaptive response (Goldsby, 2003). Innate immunity lacks the high energetic and learning costs of adaptive immunity and should therefore, be selected for when the costs of upregulating immunity are high (Lochmiller, 2000).

Adaptive immunity consists of the major histocompatibility complex (MHC), also called human leukocyte antigens (HLA), a highly polymorphic region of the genome that plays a critical role in the immune response and disease resistance, cell-mediated immunity with thymus-derived T lymphocytes, and humoral immunity composed of antibody-secreting B lymphocytes residing in peripheral lymphoid organs located throughout the body (Sheldon & Verhulst, 1996). Cellular and humoral immunity generates immunological memory via somatic recombination and mutation. This process renders adaptive immunity highly efficient in eliminating specific antigens and

establishes an adaptive immunological repertoire that is uniquely tailored to the local disease ecology (Tonegawa, 1983; Roitt, Brostoff et al. 2001).

Humoral immunity (antibody dependent immunity) is activated in the presence of parasitic infection and certain types of bacteria. Antigen-specific antibodies are generated by a process of selection and clonal expansion that occurs upon exposure to specific antigens and maintained by antibody secreting B lymphocytes with antigenic memory. A humoral immune response is moderately metabolically costly and associated with increased oxygen consumption and metabolic heat production (Demas, 2003; Demas, 2004). Cellular immunity is invoked in the presence of viral infections, certain bacteria, and cancer. Several distinct types of thymus derived lymphocytes expressing T cell receptors (T lymphocytes) are involved in the cellular immune response. T helper cells (Th cells) are involved in activation and regulation of the immune response, while cytotoxic lymphocytes (CTLs) are capable of killing virus or tumor-infected cells. T lymphocytes secrete cytokines, which are proteins that modulate the immune response by regulating lymphocyte proliferation and activity. Cellular immune function is highly energetically costly and therefore is seriously impaired by malnutrition (Lochmiller & Deerenberg, 2000).

While cellular and humoral immune components orchestrate immune activity with complementary functions, a specific immune response is dominated by either humoral or cellular immune activity. These responses are cross-regulated by T-helper cells (a subset of T lymphocytes) so that activation occurs in the cellular or the humoral immune system while the other remains downregulated (Long, 2004). Th1 helper cells coordinate predominately cell-mediated immune responses, while Th2 helper cells are involved in the humoral immune response (Bona, 2005). The Th1/Th2 paradigm is described in greater detail later in this section.

Infants are born with functional innate immune defenses and proliferative, but naive adaptive immune function, which “learns” antigenic specificity over time upon exposure to environmental pathogens (Goldsby, Kindt et al. 2003; McDade, 2005a). The drawback of this learning system is that immunologically naive infants are vulnerable to disease while adaptive defenses are maturing, and as a result infant infectious disease mortality is quite high in unsanitary, pathogenic environments (McDade, 2003). Across multiple species the upregulation of immune function in response to infection is demonstrated to be energetically demanding (Demas, 2004; Lochmiller & Deerenberg, 2000; Read & Allen, 2000; Sheldon & Verheust, 1996; Norris & Evans,

2000), as evidenced by negative relationships between immune function, reproduction and growth (Tella et al. 2002).

Energetic trade-offs are particularly pronounced in infancy, when energy demands of brain and body growth and maintenance, and immune maturation are substantial. Antigen exposure is inevitable in the postnatal period in pathogenic environments, especially with the introduction of complementary foods (Gray, 1996). Frequent pathogen exposure in infancy leads to chronically upregulated immunity and rapid maturation of adaptive immune defenses (McDade, 2003). Infection in infancy can also impair growth (Martorell, 1980; Bogin, 1999), resulting partially from the costs of immune system upregulation (Panter-Brick, Lunn P.B et al. 2000; McDade, Reyes-García et al. 2008).

The Thymus Gland

The infant thymus is a mass of highly differentiated lymphoid tissues and its volume and function peak in the first year of life (McDade & Worthman, 1999; Weerkamp, de Hass et al. 2005). Located in the upper mediastinum in humans, the thymus is the primary site of T-lymphocyte maturation (Cotman, Brinton et al. 1987) where bone-marrow derived proto-lymphocytes (thymocytes) undergo a process of proliferation followed by positive selection (for MHC recognition) and negative selection for autoreactivity (Abbas & Lichtman, 2011). The screening process is rigorous and only 5% of thymocytes survive to become part of the peripheral T cell population (George and Ritter 1996). Immunological memory generated at this time remains in peripheral circulation throughout life (Sprent 1993). Mature T-lymphocytes are essential to intra-cellular pathogen defense and are involved in the coordination of immune responses across the human life course.

The observation that T-cell manufacture peaks in infancy raises important questions about the nature and design of the thymus, which is also the first organ to present functional loss due to aging (Steinmann, Klaus et al. 1985). Unlike most organs, the thymus gets smaller with age in a process termed involution. Thymic involution progresses gradually across the life course as T-cell production diminishes and functional tissues are replaced with fat. Attempts to establish normal thymic age-related decline have met with methodological problems and substantial variation has been reported in its trajectory (Kendall, Johnson et al. 1980). It has long been maintained that thymic involution begins in adolescence (Muller-Hermelink, Steinman et al. 1982; Tosi, Kraft et

al. 1982) however, recent work suggests that involution of functional thymus tissue begins in infancy (McDade & Worthman, 1999; Miller 2002; Yekeler, Tambag et al. 2004).

Several recent studies have employed ultrasound technology to monitor thymic size and development in infants (Kizilcan, Bilaloglu et al. 1995; Hasselbalch, 1999; Collinson, Moore et al. 2003; Yekeler, Tambag et al. 2004). Many employ a methodological protocol developed by a Danish research team that developed prediction models for thymic development based on longitudinal studies of 37 Danish infants (Hasselbalch, Nielsen et al. 1996; Hasselbalch, 1999). Their thymic index measures correlated with post-mortem thymic weight and volume assessments (Hasselbalch, Nielsen et al. 1996). Thymic size correlates with the proportion of cytotoxic T cells (Jeppesen, Hasselbalch et al. 2004) and with signal-joint T-cell receptor rearrangement circles formed during generation and expression of T-cell receptors in peripheral blood (Moore, Prentice et al. 2009). Thymic size is assumed to correlate with thymic function in general and for the purposes of this chapter; however more research is needed to correlate multiple components of cellular immune function with thymus size, including lymphocyte subsets, cytokines and thymic hormones.

At birth, thymus size correlates strongly with infant weight (Isan, Tarhan et al. 2000; Aaby, Marx et al. 2002; Yekeler, Tambag et al. 2004). Positive correlations were observed between infant body size and thymic size in several populations that persist throughout infancy (Aaby, Marx et al. 2002; Collinson, Moore et al. 2003), though the strength of this relationship diminishes over time (Moore, Prentice et al. 2009) perhaps as the result of postnatal environmental factors. Thymic growth is observed in the first six or so months of life followed by a gradual decline later in infancy (Hasselbalch, 1999; Collinson, Moore et al. 2003; Yekeler, Tambag et al. 2004), and cytometric analysis of infant and child thymic lobes revealed a peak in thymic activity at 3-6 months of age (Weerkamp, de Hass et al. 2005). This pattern of thymic growth and decline may reflect pathogen exposure associated with complementary feeding. Changes in thymus size throughout the weaning process should be examined in further studies within genetically and ecologically diverse populations.

Environmental factors influence thymic development in early infancy and continue to impact its developmental trajectory over time, for example in a study of Danish infants, breastfeeding patterns at four months of age predicted thymic volume up to two years of age (Hasselbalch, Englemann et al. 1999b). In their model exclusively breastfed infants had larger

median thymic index values at most ages than partially breast-fed or formula-fed infants (ibid). In a longitudinal study from the Philippines, infants who grew faster in length in the first year of life had higher production of plasma thymopoietin (a thymic hormone) in adolescence, suggesting that faster-growing infants were in better condition and had more energy available to invest in thymic function as well. More well-controlled, longitudinal studies are needed to better understand long-term immune responses and health outcomes associated with thymic development in infancy.

In addition to changes with age, the thymus is susceptible to involution upon prenatal (Cromi, Ghezzi et al. 2009) and post-natal malnutrition, antigenic assault and stress hormones (Savino and Dardenne 2000; Wilkinson 2000), and prenatal exposure to environmental toxins (Park, Hertz-Picciotto et al. 2008; Moore, Prentice et al. 2009). Thymic ultrasonography has been used as a diagnostic tool to monitor the effects of malnutrition and nutritional rehabilitation on the thymus (Golden, Jackson et al. 1977; Chevalier, 1994) and to assess functional capacity of the thymus in patients experiencing infection (Chevalier, Diagbouga et al. 2002). Some studies have focused on the impacts of malnutrition and infection on the thymus in areas of the developing world where infants experience more nutritionally and pathogenically challenging pre-and postnatal environments than those experienced by infants from privileged Western populations (Aaby, Marx et al. 2002; Chevalier, Diagbouga et al. 2002; Collinson, Moore et al. 2003). These underscore the relevance of the thymus for infant and child health outcomes and emphasize the need to better understand its developmental influences. In Guinea-Bissau, a small thymus at birth and at 6 months were both shown to be strong predictors of child mortality in the first years of life (Aaby, Marx et al. 2002; Garly, Trautner et al. 2008). In hyperseasonal environments, birth in the hungry season, and season of measurement were also associated with thymic size in infancy (Collinson, Moore et al. 2003) and with anergy (immunological non-responsiveness) to delayed-type hypersensitivity testing which measures cellular immune responses to specific antigens (Shell-Duncan, 1995).

While well-nourished humans are capable of mounting adequate cellular immune responses, malnourished individuals exhibit diminished cellular immunity (Koster, Palmer et al. 1987; Chandra, 1992; Shell-Duncan, 1997; Savino, 2002). Malnutrition-induced thymic involution is accompanied by a distinct peripheral T-lymphocyte repertoire: overall diminished T-lymphocytes in circulation, a diminished T- helper cell population, and elevated levels of naïve thymocytes in peripheral circulation (Chandra, 1992; Chevalier, 1994; Nassar, Younis et al. 2007).

The mechanisms underlying the relationship between nutrition and cellular immunity have been the subject of scientific scrutiny (Hoffman-Goetz, 1986; Baqui, Black et al. 1993; Chevalier, 1994; Shell-Duncan, 1997; Savino, 2002); however, most research is devoid of any theoretical exploration of the evolutionary and developmental significance of such a system.

Observing the “selective and managed manner” in which thymic tissue atrophy occurs in malnourished individuals, Prentice speculated that thymic function is not essential to immediate survival once lymphocyte populations are established and therefore may be “switched on and off according to other priorities” (Prentice, 1999). In particular, he argued that the thymus is sacrificed in favor of other essential organs under circumstances of severe malnutrition (Prentice, 1999). Malnutrition-induced thymic involution is often attributed to developmental damage and is mainly researched under circumstances of severe protein-energy malnutrition (Chandra, 1992; Chevalier, 1994). However, since malnutrition and infection are often concurrent, it is difficult to isolate the relative contribution of each to thymic depletion and impaired cellular immune function. Interestingly, one well-controlled study using delayed-type hypersensitivity testing (DTH, consists of intradermal injection of antigen designed to elicit a T-lymphocyte response). showed that in Kenyan pastoralist children, seasonal morbidity had a stronger immuno-suppressive effect than malnutrition (Shell-Duncan, 1995).

The Thymus and Natural Selection

Situating early-life thymic function and development in an evolutionary context may help provide clues to the mechanisms underlying variation in immunologic phenotypes across contemporary human populations. The 34 healthy Danish infants from which prediction models for “normal” thymic development were generated (Hasselbalch, 1999) are far from normal when considered within the context of human evolution. They are from a nutritionally and epidemiologically privileged population and their mothers were most likely well-nourished throughout their pregnancies and enjoyed prenatal health care. Some were breast-fed while others consumed nutrient-fortified formulas (Hasselbalch, 1999), and it is likely that all had access to hygienic, healthy weaning foods, were vaccinated, and experienced no intestinal parasitism and relatively little infectious disease exposure.

Such environments are recent and novel within the context of human evolution, and furthermore, are not even representative of the postnatal experience in many contemporary

societies. Considerable flexibility is observed in growth and reproductive maturation patterns (Walker, Gurven et al. 2006; Kramer, 2008). As plasticity exists in immunologic development and function as well, it is possible that a range of thymic developmental trajectories exist. Establishing norms based on well-nourished infants inhabiting sanitary, urban environments obscures the tremendous cross-cultural variation in selection pressures shaping infant development. While the medical community views a small thymus as pathological, thymic developmental plasticity may be an adaptive response to suboptimal ecological conditions resembling those under which humans evolved.

Natural selection on human immunological profiles occurred largely in the context of a foraging lifestyle. Human ancestors lived in small groups of hunter-gatherers for the majority of their evolutionary history and patterns of disease exposure in ancestral human foraging groups differed greatly from humans in sedentary agrarian societies. Due to smaller population densities and greater mobility, they are not likely to have experienced the acute epidemic diseases that came to plague agrarian societies (Inhorn, 1990; Diamond, 1999). Commensal parasitism (e.g.; typhoid, amoebic dysentery, water-borne parasites) appears to have been common in ancestral human populations (Sprent, 1962) and human immune function should have adapted to chronic infections by parasitic helminths (Hurtado, Frey et al. 2008). Upregulated humoral immune responses are observed under conditions of commensal parasitism and in resource limited settings, this may potentially occur at the expense of other arms of immune function (Blackwell, Snodgrass et al. 2010), in particular costly cellular immune function.

The advent of agriculture was associated with rapid changes in human social organization, subsistence strategies, and patterns of disease exposure. The pathogen environment of the past 10,000 years was probably very powerful in terms of shaping human immunological profiles. The Neolithic period saw an upsurge of RNA viruses, as human population densities began to increase and exposure to domestic animals and sewage became more common (Van Blerkom, 2003). Group size and community sanitation influence the patterning and severity of disease outbreaks, particularly as complex societies emerged some 5,000 years ago (Molnar, 2000).

South American Native Immune Biology and the Th1/Th2 Paradigm

Extant South American foragers and horticulturalists remained isolated from other human groups until relatively recently and maintained a foraging lifestyle. They have been historically observed

to be extremely susceptible to infectious disease morbidity (Salzano & Hurtado, 2004). While more comparative data are needed to determine the nature of South American native immunologic profile, they exhibit an immunologic phenotype characterized by elevated antibody titers and high susceptibility to bacterial and viral infections (Blackwell, Snodgrass et al. 2010). A combination of factors including parasitic infection, trauma, and genetic homozygosity seem to drive the establishment of the South American native immunologic phenotype (Hurtado, Hurtado et al. 2004), which could reflect trade-offs within the immune system as a response to environmental conditions, whereby investment is biased away from energetically expensive defenses toward less costly ones (McDade, 2005; Blackwell, Snodgrass et al. 2010).

Hurtado and others have suggested that infectious disease susceptibility in South American natives reflects Th2-skewed cellular immune responses across the life course (Hurtado, Hurtado et al. 2004; Blackwell, Snodgrass et al. 2010). T helper cells have long been divided into two subsets that were first identified in 1986 by Mossmann and Coffman (1989). Designated T-helper 1 (Th1) and T-helper 2 (Th2) cells, the subsets are distinguished primarily by the cytokines they secrete (Mosmann & Coffman, 1989; Zhu, Yamane et al. 2010) and their respective roles in pathogen defense (Abebe & Bjune, 2009). The subsequent Th1-Th2 paradigm, originally described in murine models and described as follows, predominated in immuno-biological research until recently (Stummvoll, DiPaolo et al. 2008; Takatori, Kanno et al. 2008; Abebe & Bjune, 2009; Paul, 2010).

Th2 cells secrete IL-4, IL-5 and IL-13 cytokines (Kolls 2010) and mediate humoral immune by helping to stimulate antibody production by B lymphocytes (Medzhitov & Janeway, 2000; Romagnani, 2000; Zenewicz, Yancopoulos et al. 2008; Abbas & Lichtman, 2011). Th2 responses are invoked in the presence of extra-cellular pathogens, specifically anti-parasitic and allergic responses (Chen & O'Shea, 2008; Lee, Gudapati et al. 2010), and mucosal inflammation (Zhang, Shen et al. 2009). In contrast, the Th1 cells IFN- γ and mediate cellular immune responses by stimulating macrophage production (Murphy, Travers et al. 2008; Mello, Bechara et al. 2009; Abbas & Lichtman, 2011). A Th1 response also elicits cytotoxic killer cells in the presence of intracellular pathogens (Antelman, Msamanga et al. 2000) and delayed type hypersensitivity (Tang, Rolland et al. 1998; Glimcher & Murphy, 2000). The Th1/Th2 subsets are specialized and cross-regulatory; cytokines secreted by each of these T-helper subsets inhibit responsiveness of the

other subset (Gajewski, Joyce et al. 1989; Yates, Callard et al. 2004; Zenewicz, Yancopoulos et al. 2008; Mello, Bechara et al. 2009; Zhang, Shen et al. 2009).

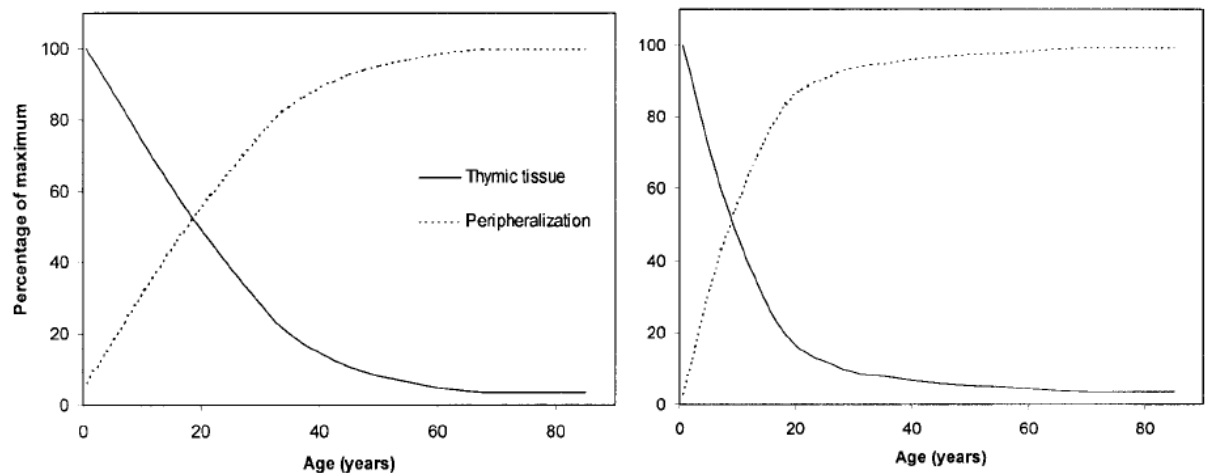
In recent years, the robusticity of the Th1/Th2 paradigm has been questioned (Stummvoll, DiPaolo et al. 2008; Takatori, Kanno et al. 2008; Bluestone, Mackay et al. 2009; Mello, Bechara et al. 2009; Zhang, Shen et al. 2009). Importantly, several other T-cell subsets are now described (Schaerli, Willmann et al. 2000; Shevach 2000; Sakaguchi 2004; Chen & O'Shea, 2008; King 2009; Locksley, 2009; Lee, Gudapati et al. 2010) and several studies have demonstrated the important role of humoral immunity in intra-cellular pathogen defense (Igietseme, Eko et al. 2003; Moore & Hutchings, 2007; McManus & Loukas, 2008; Abebe & Bjune, 2009). Furthermore, the expanding population of T-cell subsets exhibit a substantial degree of functional plasticity and adaptability (Couper, Blount et al. 2008; Bluestone, Mackay et al. 2009). The dichotomous view of Th-lymphocyte differentiation may therefore be insufficient and too simplistic to explain the nature of infectious disease morbidity in South American natives. Regardless, a better understanding of the trade-offs and complexity of interacting immune components is crucial to understanding South American native immune biology (Blackwell, Snodgrass et al. 2010).

By studying thymic size South American native infants, an attempt is made to provide a preliminary, albeit it imperfect lens, on the conditions and selection pressures shaping the biology of cellular immune development. As a particularly costly organ, the thymus should be particularly sensitive to ecologic conditions and develop according to environmental cues of lifetime nutritional availability and disease exposure (George & Ritter, 1996; McDade & Worthman, 1999). In pathogenically protected and nutritionally adequate environments, maintaining a relatively large thymus in infancy seems to be the norm. It is not clear whether there are costs associated with this pattern. It has been proposed that a large thymus could increase the likelihood of autoimmune responses, but so far little empirical data has supported this claim (George & Ritter, 1996; Benn, Jeppesen et al. 2001).

While there is an apparent contradiction between a small thymus and greater demand for immune responsiveness in high-pathogen environments, McDade and Worthman (1999) suggest that in such contexts, more rapid rates of thymic regression and T-lymphocyte peripheralization are to be expected. They present a hypothetical model in which thymic regression and T-cell peripheralization reflect rapid learning and development of the cellular immune repertoire that are necessary for survival in pathogenic environments (see Figure 4.1). A peripheral T-cell population

is potentially more efficient than a thymus in two ways: 1) it is less energetically costly to maintain than a large thymus, 2) mature lymphocyte populations are diverse in their antigenic specificity and are strategically located throughout the body, increasing capacity for antigen recognition and timely response.

Figure 4.1. Theoretical model of regression of thymic tissue and immune system peripheralization under divergent disease and nutritional pressures.



From McDade, TW, 2003. Life History Theory and the Immune System: Steps Toward a Human Ecological Immunology. *Yearbook of Physical Anthropology* 46: 100-125.

The Tsimane of Bolivia and the Pumé of Venezuela are two native South American societies that reside in marginal habitats and are in the early stages of integration into regional market economies. They are subsistence societies, “in which food and other goods are largely produced by the household rather than purchased, and little or no surplus is generated for trade or cash” (Kramer & Greaves, 2007). Agriculture and domestic animal husbandry were only adopted in the last hundred years or so, and are still practiced only sporadically in some communities. They live in villages lacking sanitation systems and inhabit pathogen-dense resource ecologies, suffer chronic malnutrition and have high rates of infant and child mortality compared to their respective national averages (Gurven, Kaplan et al. 2007; Kramer & Greaves, 2007; Godoy, Nyberg et al. 2010).

Despite these similarities it would be incorrect to present them as isolated, primitive, or uniform. The Tsimane and Pumé inhabit unique ecological settings and have different histories of contact with mainstream South American cultures, market economies, and political systems. The

Tsimane are more integrated into the market economy, have schools and some modern medical care is available at least sporadically in many communities located close to town. In contrast the Pumé are less agriculturally dependent, engage less in wage labor, and rely more on hunted and foraged foods. Only one of the Pumé villages had a school that had opened recently and none of the children in the sample had ever seen a doctor. Perhaps most importantly, the Pumé, particularly the savanna Pumé, live in a harsh environment characterized by more resource scarcity than that of the Tsimane. Comparative anthropometric data from the two populations reveals that adult Tsimane men and women are shorter and heavier than Pumé men and women. In particular, Tsimane women have higher body mass index (BMI) than Pumé women (Walker, <http://anthropology.missouri.edu/people/walker.html>).

Hypotheses

Here results are presented from a preliminary, exploratory study of infant thymic and body size in Tsimane and Pumé infants. The following hypotheses are tested:

1. Pumé infants will have smaller body size than Tsimane infants

As we will describe, the Tsimane in general have more access to food resources. They inhabit an environment with more abundant game, practice more intensive agriculture, and have greater access to market foods. The Pumé experience greater resource scarcity than the Tsimane and adults have lower BMI. Therefore, it is expected that Pumé infants will also be smaller than Tsimane infants. They are born to smaller mothers and experience pronounced seasonal resource scarcity, particularly in the savanna communities.

2. Pumé infants will also have smaller thymuses than Tsimane infants

For reasons described for hypothesis 1, it was expected that Pumé infants would be smaller and less well-nourished than the Tsimane infants. We also expect the Pumé infants to have smaller thymic size than Tsimane infants. Thymus size correlates with body size in early infancy, but this relationship diminishes with time. If smaller Pumé mothers give birth to smaller infants, it makes sense that these infants should have smaller thymuses as well. As infancy progresses, we expect to see nutritional influences exerting an impact on the development and function of infant immunity. Because a thymus is energetically expensive to maintain, and often observed to involute under

conditions of malnutrition, we also expect less well-nourished Pumé infants to have smaller thymuses than the Tsimane infants across infancy.

3. Anthropometric measures will be positively correlated with thymus size in Tsimane and Pumé infants.

For reasons described for hypothesis 2, it was expected that anthropometric measures would correlate with thymus size in both the Tsimane and the Pumé. Previous research has documented thymic growth when nutritional conditions are improved in infants and children undergoing immuno-nutritional rehabilitation. If thymus size is directly linked to infant nutritional status, we should see a positive relationship between anthropometric indicators of nutritional status and thymus size in both Tsimane and Pumé infants.

Structure of the Paper

In the body of this chapter a description of each the Tsimane and Pumé ethnic groups is provided to establish an ecological and cultural context for infant development in these populations. This was followed by a description of the methods of data collection and analysis. Results of hypothesis testing are provided along with prediction models of thymic development in the South American native cohorts of infants. The findings were then situated in a broader context by comparing thymic size in the South American native infants with that of cohorts of urban infants from Denmark and Turkey. In the final section results were discussed, followed by speculation on the nature of thymic developmental plasticity, and suggestions directions for future research are discussed.

4.2. Study Populations

The Tsimane

Around 8,000 Tsimane inhabit 80-100 villages in the Maniqui River system between San Borja San Ignacio de Mojos (VAIPO, 1998; INE, 2003). The Tsimane practice a variety of economic activities that blend traditional subsistence strategies with wage labor and market exchange. Much of the diet comes from slash-and-burn horticulture, fishing, hunting, and gathering. Traditional diets are supplemented by market foods which are purchased in San Borja or brought to villages by traveling merchants. Tsimane fertility is high (total fertility rate (TFR) is 8.5 births per woman's lifetime (Kaplan, Gurven et al. 2010) and interbirth intervals short. The

Tsimane infant mortality rate (IMR) is higher than the Bolivian national average, averaging around 12.6% from 1990-2002 (Gurven, Kaplan et al. 2007). Young infants are breastfed exclusively in the first few months and begin to consume complementary foods between three and six months of age. Breastfeeding most often ceases by two years of age. Older infants and small children are often cared for by older siblings and other female kin while mothers participate in subsistence activities.

Household sanitation is poor in Tsimane villages. Only two of the study communities had wells and one went through long periods of dysfunction. Most drinking water is obtained from rivers and creek and rarely boiled. Domestic animals roam freely in the villages and their feces litter pathways between houses, though individual yards are generally kept clean. Flooding is common in the wet season and materials from household latrines presumably are deposited in Tsimane living areas. Food such as fruit and charqui (dried and salted meat and fish) are hung in houses or separate kitchens or outside in the yard for storage, which attracts scavenging animals and flies. Another source of antigen exposure is fermented manioc beer, or *chicha*, whose consumption was observed in infants as young as three month of age.

This combination of factors contributes to profound susceptibility to infections that are major causes of death in infancy and childhood. Parasitic infections are common in children (Tanner et al. 2009) and Tsimane children exhibit elevated levels of c-reactive protein (CRP), indicating a high infectious disease burden (McDade, Leonard et al. 2005b). Anemia is also prevalent among Tsimane children under age 10 (Lindsay KM, Aiello et al. 2003). Tsimane children exhibit growth stunting (Foster, Byron et al. 2005; Godoy, Nyberg et al. 2010) which probably reflects a combination of long-term moderate malnutrition along with energetic trade-offs with chronic immunologic activation (McDade, Reyes-García et al. 2008). Medical care is available in San Borja, but it is a long trip for many Tsimane. Delayed treatment of infants often results in death, as illness often occurs suddenly and progresses quickly. Malnutrition and dehydration underlie much infant disease and inhibit the efficacy of antibiotic treatments. Vaccination teams travel sporadically to villages but the record-keeping is unsystematic and many children are probably only partially vaccinated (Tanner, 2005).

The Pumé

Approximately 8,000 Pumé foragers and horticulturalists are found in the low flat savannas (llanos) of Apure, Venezuela, along and between the Capanaparo, Cinaruco, and Riecito rivers (Greaves, personal communication). The llanos experience a hyperseasonal climate pattern of drought and fire from November to April followed by heavy rain and flooding from May through October. The Pumé diet consists of cultivated and foraged roots, fish, mango, hunted game, and market foods in some communities. Resource acquisition activities range from hunting, fishing and foraging to slash-and burn horticulture, sporadic wage labor, and market exchange. Pumé communities vary with respect to acculturation and subsistence strategies are determined by geographic proximity to rivers and suitable agricultural land. The Pumé recognize these differences and use terms *savanna Pumé* to refer to mobile, central place foragers located in the interior far from major waterways, and *river Pumé* to refer to sedentary forager-horticulturalists located along the major rivers.

Savanna Pumé practice traditional hunting, fishing and foraging practices and engage minimally in agriculture, animal husbandry, and wage labor. Cultivated bitter manioc contributes to less than 10% of the savanna Pumé diet and they experience profound seasonal nutritional stress (Kramer & Greaves, 2010). River Pumé historically have had more contact with outsiders (Petrullo, 1939). Like the savanna Pumé they engage in traditional subsistence practices, but river Pumé experience higher agricultural returns, more abundant fishing and more protein from domestic animals. River Pumé also have increased access to cash and market goods. As a result of these factors river Pumé experience less seasonal variance in food availability and are better nourished than savanna Pumé.

Despite differences in acculturation, river and savanna Pumé inhabit a similar cultural and reproductive environment. Girls marry young and the average age at first birth is 15.5 (Kramer, 2008). During the first six months of a child's life, infants are breastfed on demand. Children are introduced supplemental foods at about six months and weaned by two and a half to three years old. Unlike many other child-development environments where mothers and babies are inseparable for the first six months, Pumé infants are held, cared for and occasionally nursed by alloparents from the day they are born. Pumé live in dirt floor houses and brush shades, and children are permitted to crawl on the ground. Once mobile, children are free to roam throughout the village, though seemingly unsupervised a related adult is always in close proximity.

Infant mortality in river communities is 13.2%, comparable to the Tsimane IMR and significantly higher than the Venezuelan national average of 1.96% (PRB, 1996; Kramer & Greaves, 2007). In savanna communities, infant mortality is 34.6%, nearly three times the river rate (Kramer & Greaves, 2007). The Pumé live in a malarial environment and other significant diseases in the region include measles, yellow fever, Chagas disease, tuberculosis and other respiratory diseases (Lizarralde and Seijas 1991). It is estimated that 10% of savanna children who survive infancy do not survive to reproductive maturity (Kramer, Greaves et al. 2009).

While cause of death is unknown for a sample large enough for analysis, seasonal undernutrition, chronic parasite loads, disease exposure and opportunistic infection clearly have a synergistic effect on the high levels of morbidity and mortality among Pumé infants and children. Infectious disease peaks in the lean season, a time when individuals are likely to be immunologically vulnerable due to inadequate caloric intake (Barreto & Rivas, 2007). Health care workers occasionally enter the more accessible river communities but rarely reach the savanna communities. There were vaccination teams in the savanna in the past, but children there have not received vaccines in the last ten years (Kramer, Greaves et al. 2009).

4.3 Methods

Data Collection

Data on infant length, weight and mid-upper-arm circumference (MUAC) and thymus volume were collected for 86 infants from two groups of native South Americans, the Tsimane of Bolivia (n=57 infants) and the Pumé of Venezuela (n=29 infants). Data were collected in four Tsimane and five Pumé villages from 2006 to 2007. All children aged 0-2 were considered eligible to take part in the growth and thymus study. Participation was based on consent from the child's parents and no other selection criteria were used. The research protocols of this study, including informed consent, were reviewed and approved by the University of New Mexico Institutional Review Board and the University of New Mexico Health Sciences Center Human Research Review Committee.

Age Assessment of Children

Tsimane mothers and fathers were separately asked infant birthdates which then were cross-checked with data from a log maintained by the schoolteacher in one village who documented birthdates in all nearby communities for the purpose of providing national identity

cards for the Tsimane. For detailed descriptions of demographic data collection methods in the Tsimane, see Gurven, Kaplan et al. 2007. The Pumé do not keep written vital records, nor have an absolute means for retrospectively aging older children and adults. However, parents can accurately report the ages of young children by moon or season counts up to four years. All of the Pumé children included in the growth and thymus study were born within the 2005-2007 field season and their mothers were interviewed within at least several months of their birth. For detailed descriptions of demographic data collection methods in the Pumé, see Kramer and Greaves 2007.

Anthropometric Data

Maternal and infant body sizes were described using cross-sectional data. Infant recumbent length (from crown to heel) was taken using a baby board for infants, and maternal standing height was measured using a Seca Portable Stadiometer. Infant upper arm circumference (MUAC) was measured on the left upper arm at the point halfway between the tip of the shoulder and the elbow. Mothers and infants were weighed using a Healthometer digital scale (Pumé) or a Tanita BF680 Scale (Tsimane) to the closest tenth of a kilo. Children who could not stand on their own were weighted by subtracting mother's weight while holding the child. Children were weighed naked and without shoes. Because some of the mothers were pregnant at the time of data collection, non-pregnant weights were used if available from one year before or after the data collection period. Scales used were routinely calibrated against known weights and repeated measures were taken to minimize measurement error.

Thymic Measures

Prior to collection of the thymus data, AV spent one month in intensive training in immuno-nutritional assessment and thymic ultrasonography with Dr. Ricardo Sevilla at the Center for Immuno-Nutritional Rehabilitation at the German Urquidi Hospital in Cochabamba, Bolivia. The field study of infant body and thymus size was piloted in four Tsimane communities from July-December 2005 and five Pumé communities from March-May 2007. Thymic ultrasonography was performed in the method of Chevalier using a solar-powered High Technology, Inc. PU-2200 with a 7.5 MHz linear pediatric probe (Chevalier, 1997; Chevalier, P., 1997). Due to the limitations of solar power and the less developed nature of the right thymic

lobe, only the left lobe was scanned. Infants were scanned prostrate on a flat surface, arms by their side and with a rolled towel placed under the shoulders to achieve protraction of the back for clearer visualization. The sagittal area (SA) of the cross-section of the lobe was assessed and its depth or anterior-posterior dimension (LAP) was measured in centimeters. Body temperature was also measured under the arm as part of the evaluation to control for potential confounding effects of infection which temporarily shrinks thymus size.

Data Analysis

Tsimane and Pumé infant anthropometry and thymus size were plotted by age, compared and tested for significant differences. General linear models were constructed to test for significant demographic and anthropometric predictors of thymic size. South American native infant thymus SA and LAP values were then plotted for comparison with published values from studies conducted in Denmark and Turkey. CDC Z-Scores were calculated using EpiInfo. Native-specific Z-Scores and all additional analyses were conducted using PASW Statistics 18. Analyses are described in greater detail below.

Anthropometric variables

Height-for-age, weight-for-age and MUAC-for-age of Tsimane and Pumé infants were plotted for descriptive purposes and general linear models (GLMs) were constructed to compare anthropometric status in the two populations and also between the savanna and river Pumé infants. For reasons described in Chapter III (Methods) two sets of Z-Scores were used to calculate height-for-age (HAZ), weight-for-age (WAZ) and MUAC-for-age (MUACZ) for additional analyses. Z-Scores they express anthropometric values as the number of standard deviations above or below a reference mean value and are considered the best system of presenting and analyzing anthropometric data (de Onis and Blössner 1997). Weight-for-age reflects past and present nutritional status (underweight is defined as ≤ -2 Z-Scores below the mean) and height-for-age reflects chronic nutritional conditions (stunting is defined as HAZ ≤ -2 Z-Scores below the mean) (Cosminsky, Mhloyi et al. 1993). MUAC is considered a useful measure of child nutritional status as it measures muscle, fat and bone and strongly predicts mortality in infants and children over six months of age (Zemel, Riley et al. 1997). It is also less age-dependent and less affected by swelling than many other measures (Cross, Holden et al. 1995).

One set of Z-Scores (Z1) uses the CDC 2000 standards calculated using EpiInfo EpiNutrition software. The CDC scores are based on a reference population of infants and children in the United States. The 2000 CDC Growth Chart reference population includes data for both formula-fed and breast-fed infants and data on low birthweight infants but do not include data on very low birth- weight infants. MUAC is not considered a useful measure for infants less than 6 months of age and is not calculated for infants aged 0-6 months using EpiInfo EpiNutrition software. The other set of Z-Scores (Z3) was generated using the same method as presented for Z-Score 2 (Z2) using the Tsimane reference population in Chapter 3. In this Chapter, Z3 is calculated using the reference population derived from *both* from the Tsimane and the Pumé anthropometric databases.

Measurements of infants aged 0-24 months were used to calculate Z3 by regressing Tsimane and Pumé infant age on height, weight and MUAC. Standardized residuals are used as Z-Scores. Weight-for-age and MUAC-for-age measurements were heteroscedastic: Variance in MUAC-for age decreased with time, and variance in weight-for-age increased with time. There were a total of 1333 repeated height measures on 854 infants, 1333 repeated weight measures on 848 infants, and 145 repeated MUAC measures on a subset of 116 Tsimane and Pumé infants aged 0-24 months. The CDC (Z1) and Z3 scores were significantly correlated: for HAZ (n=86), $r=.667$, for WAZ (n=84), $r=.549$, and for MUACZ (infants aged 6-14 months only, n=56), $r=.995$.

Hypothesis Testing and Construction of Prediction Models

General linear models were used to test Hypotheses 1, 2 and 3 and to construct prediction models for thymic sagittal area (SA) in the Tsimane and Pumé infants. The specific analysis used to test each hypothesis is listed here:

1) *As they are subject to pronounced resource scarcity, Pumé infants will have smaller body size than Tsimane infants.* To test Hypothesis 1, GLMs were constructed using categorical variable ethnic group (Tsimane or Pumé) and covariate infant age (in months) to predict dependent variables HAZ, WAZ and MUACZ. Both sets of Z-Scores (Z1 and Z3) were used in this analysis. Additional GLMs were constructed using ethnic group and infant age as predictors of height, weight and MUAC using raw values as dependent variables. Results of each set of models were compared for consistency.

2) *As they are subject to pronounced resource scarcity, Pum  infants will also have smaller thymuses than Tsimane infants.* To test Hypothesis 2, General linear models were constructed using categorical variable ethnic group (Tsimane or Pum ) and covariate infant age (in months) to predict dependent variables SA and LAP.

3) *Anthropometric measures will be positively correlated with thymus size in Tsimane and Pum  infants.* To test Hypothesis 3, GLMs were constructed using covariates infant age (in months) and HAZ, WAZ and MUACZ to predict dependent variables SA and LAP. Both sets of Z-Scores were used and results of each set of models were compared for consistency.

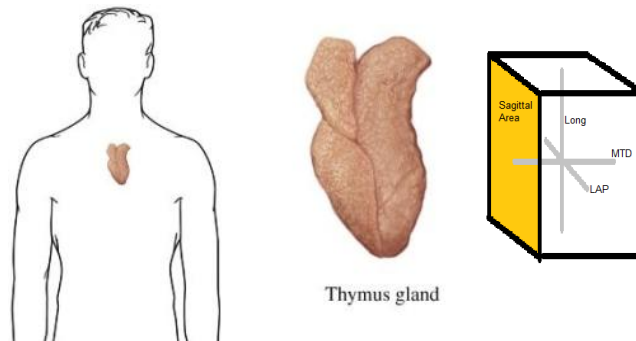
After testing these hypotheses, a prediction model for thymic size in the Tsimane and Pum  infant cohorts was constructed. SA and LAP-for-age were plotted for descriptive purposes and general linear models constructed to test demographic variables (ethnic group=Tsimane or Pum , infant age in months) and anthropometric predictors (HAZ, WAZ and MUACZ) on SA and LAP. Models were constructed both for the combined sample of South American native infants (n=86) and independently for the 57 Tsimane and 29 Pum  infants in the sample. Maternal variables (age, weight and weight-for-age) were tested for independent effects on dependent variables SA, LAP, and anthropometric predictors because maternal condition is known to be a strong predictor of infant condition at birth and later in infancy (Kirksey, Wachs et al. 1994; Medhin, Hanlon et al. 2010). Birth season (wet/dry), infant gender, and presence of fever using the standard cutoff of 38 degrees Celsius (Hsiao & Baker, 2005) were also tested for confounding effects on thymic index, MUAC, WAZ and HAZ.

Comparative Datasets

Data from published ultrasonographic studies of thymic size in three cohorts of infants were compared with the Tsimane and Pum  measures. One study from Istanbul, Turkey (2005) published cross-sectional data on thymic size in a cohort 151 healthy infants aged 0-24 months (Yekeler, Tambag et al. 2005). Thymic dimensions were reported in one-month increments from 1-6 months, then for 6-8 months, 8-10 months, 12-18 months, and 18-24 months. Thymic dimensions left anterior-posterior dimension (LAP), maximal transverse diameter (MTD), the “the

distance between 2 parallel lines passing through the uppermost and lowest borders of the thymus,” (LONG) were reported (ibid). Figure 4.2 provides a diagram of these different thymus dimensions. The 2005 study by Yekeler and Tambag, et al. also published thymic index (TI), an ultrasonographic thymic volume estimate that corresponds linearly to post-mortem thymic width, weight and volume (Hasselbalch, Nielsen et al. 1996). TI is the cube root of the product of MTD and the sagittal area of the larger thymic lobe (Hasselbalch, Nielsen et al. 1996).

Figure 4.2. A representation of the thymus gland. The box to the right represents the dimensions of the thymus that were measured in various studies and described above: LONG, MTD, LAP, and SA.



In another cross-sectional study of infant thymus size, thymic size was measured in 161 healthy infants at the Ankara Hospital in Turkey (Kizilcan, Bilaloglu et al. 1995). Mean values of thymic dimensions (which correspond to LONG, MTD, and LAP in the 2005 study by Yekeler, Tambag et al. 2005) are reported at one-month increments from 0-12 months. In a third study of thymic size in infants, researchers in Copenhagen Denmark (1999) monitored thymus size in 34 infants longitudinally over 24 months (Hasselbalch, Ersböll et al. 1999). The thymus was measured at birth, 4 months, 8 months, 10 months, 12 months, and 24 months, and a prediction model for thymic size at 24 months of age was generated based on breastfeeding status at four months. Only TI values are published in Hasselbalch’s study.

The 2005 study by Yekeler, Tambag et al. provided the most complete set of thymic dimensions. Their Published values from this study are therefore used to construct formulas by which missing dimensions are extrapolated for the other cohorts (see Table 4.1). For the Tsimane and Pumé infants, SA and LAP were measured but there is no MTD value from which to calculate TI. For the 1995 Turkish cohort (Kizilcan, Bilaloglu, et al. 1995), MTD, LONG, RAP, and LAP were published, but neither SA nor TI are reported. LAP correlated with TI ($r=.86$) in the Turkey 2005 cohort. The slope of this regression line as it passes through the origin ($m=1.152$) was multiplied by LAP to calculate an estimate of TI in the Tsimane and Pumé infant cohorts. For the Turkey 1995 cohort, TI was calculated by deriving the cube root of the product of $SA*MTD$.

Published values for left anterior-posterior dimension (LAP, Figure 4.10) were then compared in the two Turkish and two native South American infant cohorts. Published and estimated values of thymic index (TI, see Figure 4.13) were compared in the one Danish, two Turkish, and two South American native cohorts. Each study reported data on infant thymic size at different age increments, so LAP TI values from each infant cohort were collapsed into age categories (0-3 months, 3-6 months, 6-9 months, 9-12 months, 12-15 months, 15-18 months, 18-21 months, and 21-24 months) to facilitate cross-cultural comparison. To assess the external validity of the Tsimane and Pumé thymic measures, the correlation between LAP and SA in these cohorts is compared with the correlation between LAP and SA in the Turkish infants (see Figure 4.11). Because SA values did not appear to have been collected in a systematic manner (the correlation between LAP and SA was different in the Turkish and South American infants and SA was much relatively smaller than LAP), SA comparisons are excluded.

Table 4.1. A description of the calculations used to generate LAP, MTD, SA and TI measures

	LAP	MTD	SA	TI
Tsimane & Pumé (Veile, 2006-2007)	Measured directly	Not measured	Measured directly	Estimated using equation $TI=1.52*LAP$ (derived from Turkey 2005 sample, $r=.86$)
Denmark (Hasselbalch, Ersbøll et al. 1999)	Not reported	Not reported	Not reported	Measured directly
Turkey Kizilcan and Bilaloglu, et al. 1995)	Measured directly	Measured directly	$(TI/MTD)^{1/3} *$	Estimated using equation $SA*MTD$
Turkey (Yekeler and Tambag, et al. 2005)	Measured directly	Measured directly	$(TI/MTD)^{1/3} *$	Measured directly

*The cube root is taken to change it from mm^3 to cm^3 .

4.4. Results

Descriptive Characteristics of Study Population

Descriptive statistics presented in Table 4.2 show differing age distributions for the Tsimane and Pumé samples, with the Pumé sample being biased toward infants of younger ages. Both groups consisted of roughly half male and female infants. There were no significant differences in maternal weight, height or age between the two populations. Tsimane women were slightly heavier, but the difference was not statistically significant, and Tsimane women were also more likely to be pregnant. Weight increased significantly with age in the 86 mothers ($B=.287$, $p=.006$). Z3 (Tsimane and Pumé reference population) are presented in Table 4.2 to compare Tsimane and Pumé means and avoid the confounding effects of Z1 (CDC) and age.

MUACZ, HAZ, and WAZ did not statistically differ between the two cohorts. The Tsimane infants have lower mean HAZ and WAZ-Scores, and Pumé had slightly lower MUAC, but overall growth trajectories are similar (see Figures 4.3, 4.4 and 4.5). Mean left anterior –posterior dimension (LAP) and sagittal area (SA) of the thymus were higher in the Tsimane than in the Pumé, as hypothesized, and the difference is statistically significant (see Figures 4.6, 4.7 and Tables 4.3 and 4.4).

Table 4.2. Sample Characteristics

	Pumé	Tsimane
Total Sample Size	29	57
Age Range (months)	0.16-24	0.65-23.5
Mean Age Infant (months)	8.5	11.3
Median Age Infant (months)	6.8	11.8
Total Girls	15	28
Total Boys	14	29
Mean Age Mother (Years)	26.6	26.3
Mean Weight Mother (Kilos)	50.9	53.2
Mean Height Mother (cm)	150.2	149.5
Mean MUACZ (Z-Score 2) (n=86)	-.3	.0
Mean HAZ (Z-Score 2) (n=86)	0.8	-0.4
Mean WAZ (Z-Score 2) (n=84)	0.3	-0.1
Mean LAP (mm)	9.8	13.5
Mean SA (mm ²)	209.5	262.6

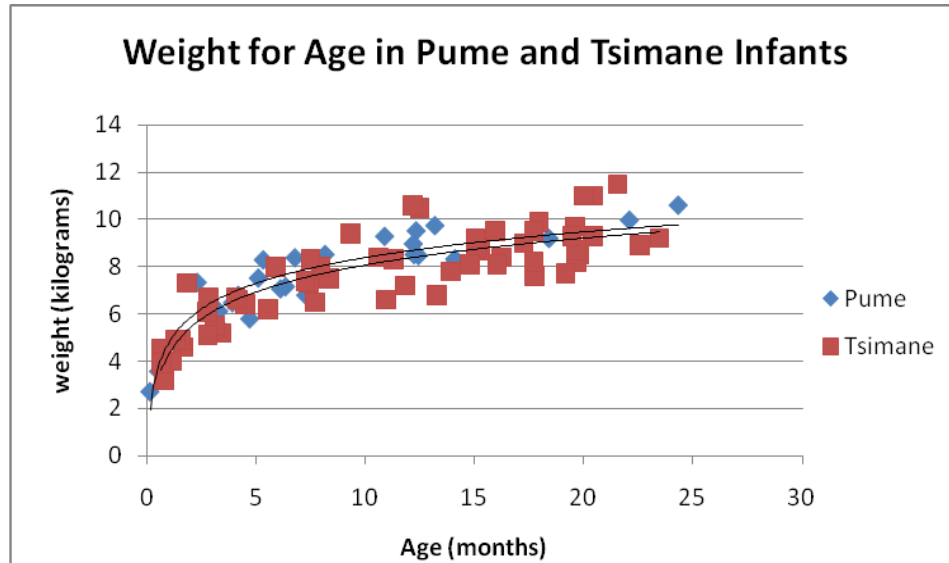
Results of Hypothesis Testing

Hypothesis 1. Pumé infants will have smaller body size than Tsimane infants

Figures 4.3, 4.4 and 4.5 show nearly identical weight, height, and MUAC-for-age curves for the two populations of infants. GLMs revealed that differences in weight, height and MUAC were not statistically significant between the Tsimane and Pumé using both raw values and both

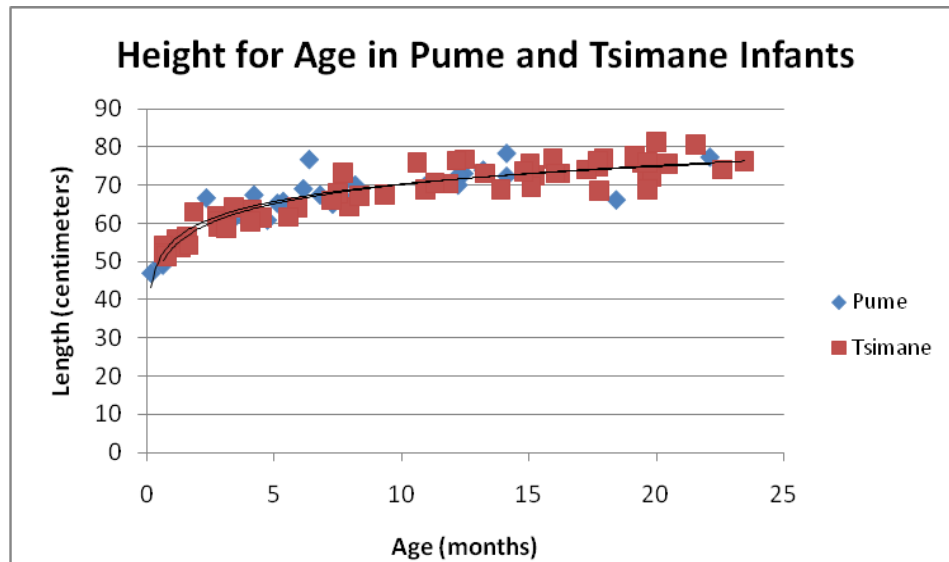
sets of Z-Scores. Unfortunately, the Pumé sample was too small to disaggregate by river and savanna infants, but MUACZ was substantially lower in savanna than river Pumé.

Figure 4.3. Weight-for-age curves in Tsimane and Pumé infants.



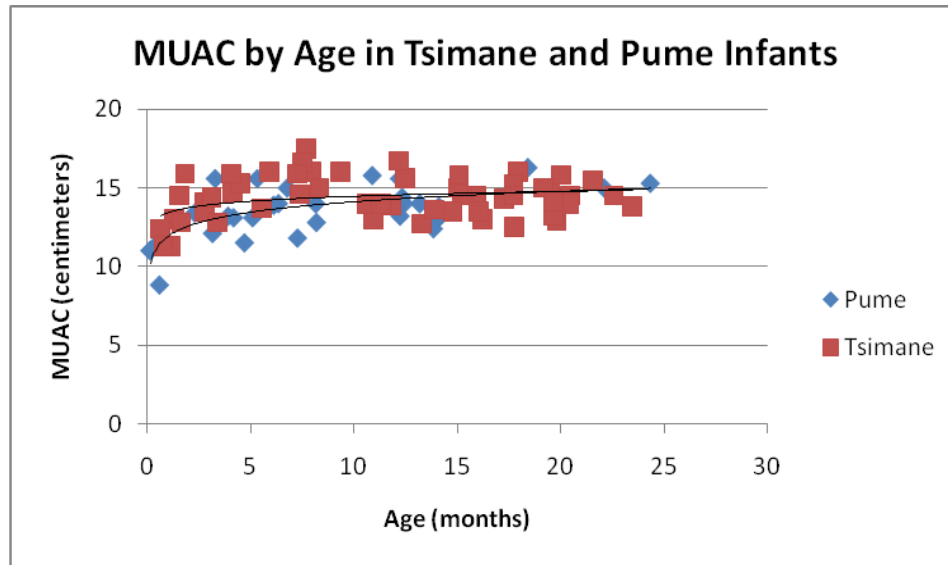
Differences in weight-for-age between the populations were not statistically significant.

Figure 4.4. Height-for-age curves in the Tsimane and Pumé infants



Differences in height-for-age between the populations were not statistically significant.

Figure 4.5. Nearly identical MUAC-for-age curves in the Tsimane and Pumé infants



Differences in MUAC were not statistically significant.

Hypothesis 2. Pumé infants will have smaller thymuses than Tsimane infants.

Figures 4.6 and 4.7 present data on infant left anterior-posterior dimension (LAP) and sagittal area (SA) of the thymus in Tsimane and Pumé infants. The scatter plots in Figures 4.6 and 4.7 show that as hypothesized, Tsimane infants had larger thymuses at all ages than the Pumé infants, and in both cohorts of infants, LAP and SA diminished linearly with age. The difference between LAP and SA in Tsimane and Pumé infants difference was highly statistically significant ($p=.000$, see Tables 4.3 and 4.4). While it appeared there might be an interaction effect between age and group, the effect of an interaction variable was not significant in either model. Adding age-squared to the models presented in Table 4.3 and 4.4 did not improve their model fit.

Figure 4.6. Left anterior-posterior dimension decreased linearly with age in both the Tsimane (r-squared=0.15) and in the Pumé (r-squared=0.42)

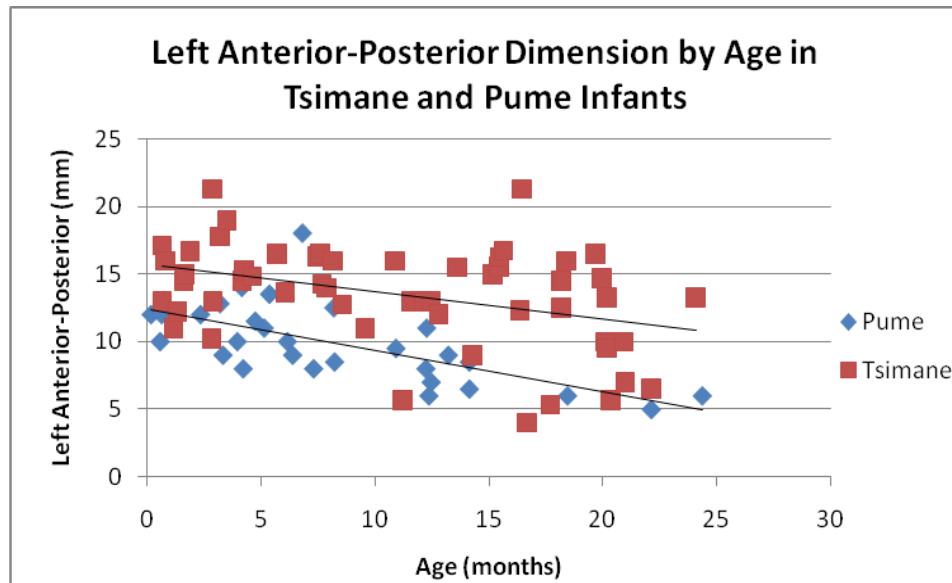


Figure 4.7. Thymus sagittal area decreased linearly with age in both the Tsimane (r-squared=0.39) and in the Pumé (r-squared=0.41)

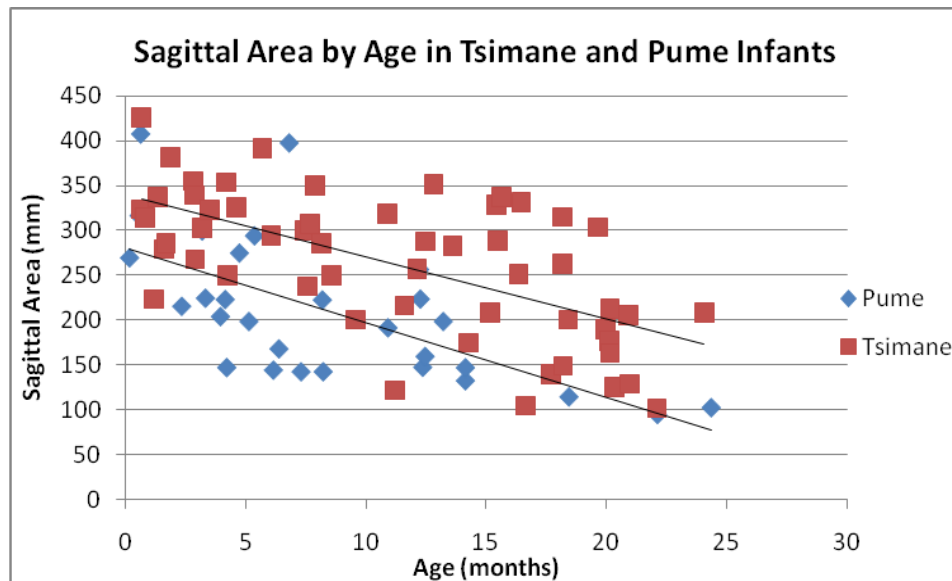


Table 4.3. Significant differences in LAP between Tsimane and Pumé infants after controlling for age

Parameter	B	SE	Sig
Intercept	16.089	.6868	.000
Group=Pumé	-4.201	.7128	.000
Age (months)	-.245	.0491	.000

Table 4.4. Significant differences in SA between Tsimane and Pumé infants after controlling for age

Parameter	B	SE	Sig
Intercept	341.800	13.9036	.000
Group=Pumé	-72.049	14.4312	.000
Age (months)	-7.070	.9933	.000

Hypothesis 3. Infant body size will be positively correlated with thymus size in Tsimane and Pumé infants.

Tables 4.5 and 4.6 reveal the relationships between Z1 (CDC) and Z3 (Tsimane and Pumé reference population) and left anterior-posterior dimension (LAP, Table 4.5) or sagittal area (SA, Table 4.6) in the Tsimane and Pumé infants. WAZ and HAZ were not significant predictors of LAP or SA in the Tsimane and Pumé infants regardless of the Z-Score used. A marginally significant, positive relationship was found between MUACZ and LAP, but only when Z3 was used ($n=86$, $p=.053$, see Table 4.5). MUACZ positively correlated with, but not a significant predictor of SA (using Z-Score 2 and controlling for age) in the cohort of 86 infants ($p=.082$). MUACZ was a highly significant predictor of SA in an analysis restricted to infants aged 6-24 months (using both sets of Z-Scores and controlling for age, $n=56$, $p=.004$).

Because MUACZ was the strongest anthropometric predictor of thymus size, particularly SA, the combined effects of age, ethnic group and MUACZ on SA were modeled for both the entire cohort of 86 infants and the subset of 56 infants aged 6-24 months. In the combined cohort

(n=86), MUACZ became insignificant when group was added to the model (data not shown). A group*MUACZ interaction variable was therefore added to the model, and the interaction variable neared statistical significance ($p=.067$). In the best-fit model (Table 4.7), MUACZ was still a significant predictor of SA in the 86 infants after controlling for age, group, and group*MUACZ. Table 4.8 shows that infant age, ethnic group and MUACZ are significant predictors of SA in the subset of 56 infants aged 6-24 months. After controlling for age and group, MUACZ was still a significant predictor of SA ($p=.010$).

Table 4.5. Relationship between HAZ, WAZ and MUACZ and LAP (controlling for age) in the South American native infant cohort using both CDC and South American native-specific Z-Scores

	Height		Weight		MUAC (0-24 months)		MUAC (6-24 months only)	
	Predicted	Sig	Predicted	Sig	Predicted	Sig	Predicted	Sig
Z1	No effect (n=86)	.927	No effect (n=84)	.213	n/a	n/a	+	.111 (n=56)
Z3	No effect (n=86)	.846	- (n=84)	.511	+	.053 (n=86)	+	.127 (n=56)

Table 4.6. Relationship between HAZ, WAZ and MUACZ and SA (controlling for age) in the South American native infant cohort using both CDC and South American native Z-Scores

	Height		Weight		MUAC (0-24 months)		MUAC (6-24 months only)	
	Predicted	Sig	Predicted	Sig	Predicted	Sig	Predicted	Sig
Z1	No effect (n=86)	.753	No effect (n=85)	.779	n/a	n/a	+	.004 (n=56)
Z3	No effect (n=86)	.417	No effect (n=85)	.609	+	.082 (n=86)	+	.004 (n=56)

Table 4.7. MUACZ (using South American native reference population) is a significant predictor of sagittal area in 86 Tsimane and Pumé infants aged 0-24 months

Parameter	B	Std. error	Sig
Intercept	337.999	13.8459	.000
Age	-6.822	.9978	.000
MUACZ (Z3)	20.210	9.9903	.043
group=Pumé	-73.184	14.4436	.000
MUACZ (Z3) *group	-29.017	15.8593	.067

Table 4.8 MUACZ (using South American native reference population) is a significant predictor of sagittal area in 56 Tsimane and Pumé infants aged 6-24 months

Parameter	B	Std. error	Sig
Intercept	313.844	29.0663	.000
Age	-5.417	1.7682	.002
group=Pumé	-69.651	18.9720	.000
MUACZ (native)	29.965	11.6211	.010

Summary of Hypothesis Testing

Table 4.9. Summary of results from hypothesis testing

Hypothesis	Predicted Direction	Significant	Supported
1. Pumé infants will have smaller body size than Tsimane infants	No	No	Not supported
2. Pumé infants will have smaller thymuses than Tsimane infants	Yes	Yes	Supported
3. Infant body size will be positively correlated with thymus size in Tsimane and Pumé infants.	Yes (MUACZ only)	Yes (MUACZ only)	Partially Supported

Prediction Models by Ethnic Group

For the combined cohort of South American native infants (n=86), the strongest predictors of sagittal area (SA) were infant age and ethnic group (see Table 4.4). MUACZ was also a significant predictor of SA after controlling for age (see Tables 4.7 and 4.8). Controls birth season, gender, presence of fever, and maternal age were not significant predictors of SA after controlling for infant age in either group. Separate analyses of the 57 Tsimane and 29 Pumé infants revealed MUACZ was a statistically significant predictor of SA in Tsimane infants only ($p=.041$), although the correlation was positive in both groups (see Table 4.10).

Separate prediction models were then constructed for the two cohorts (n=57 Tsimane infants and n=29 Pumé infants) to examine the respective effects of age, MUACZ, and maternal condition (operationalized as maternal weight in kilograms). Maternal weight positively associated with SA in the 86 infants. It was not a significant predictor of SA in the Tsimane infants; but approached significance in the Pumé infants after controlling for infant age ($p=.075$, data not shown). In the Tsimane infants, MUACZ was the strongest predictor of SA after controlling for age (see Table 4.10).

In the Pumé infants, the findings were more complex. In a combined model, after controlling for infant age, maternal weight was a significant predictor of SA ($p=.034$, data not shown), but MUACZ was not. Additional analysis revealed that maternal weight was a significant predictor of MUACZ after controlling for age in the Pumé but not the Tsimane (see Table 4.11). Because they were MUACZ and maternal weight were correlated in the Pumé, the best-fit prediction model for SA in Pumé infants emerged when an interaction variable (MUACZ*maternal weight) was included along with infant age, maternal weight and MUACZ (see Table 4.12). The findings presented in Tables 4.4, 4.10, and 4.12, all of which reveal different predictors of SA in the Tsimane and Pumé infants, are summarized in the diagram in Figure 4.8.

Table 4.10. Infant age and MUACZ are predictors of SA in 57 Tsimane infants but not in Pumé infants after controlling for age.

Pumé only

Parameter	B	Std Error	Sig
Intercept	275.489	22.8306	.000
Age (months)	-7.949	2.0629	.000
MUACZ	-5.489	13.2923	.680

Tsimane only

Parameter	B	Std Error	Sig
Intercept	334.140	15.1470	.000
Age (months)	-6.480	1.1369	.000
MUACZ	20.346	9.9754	.041

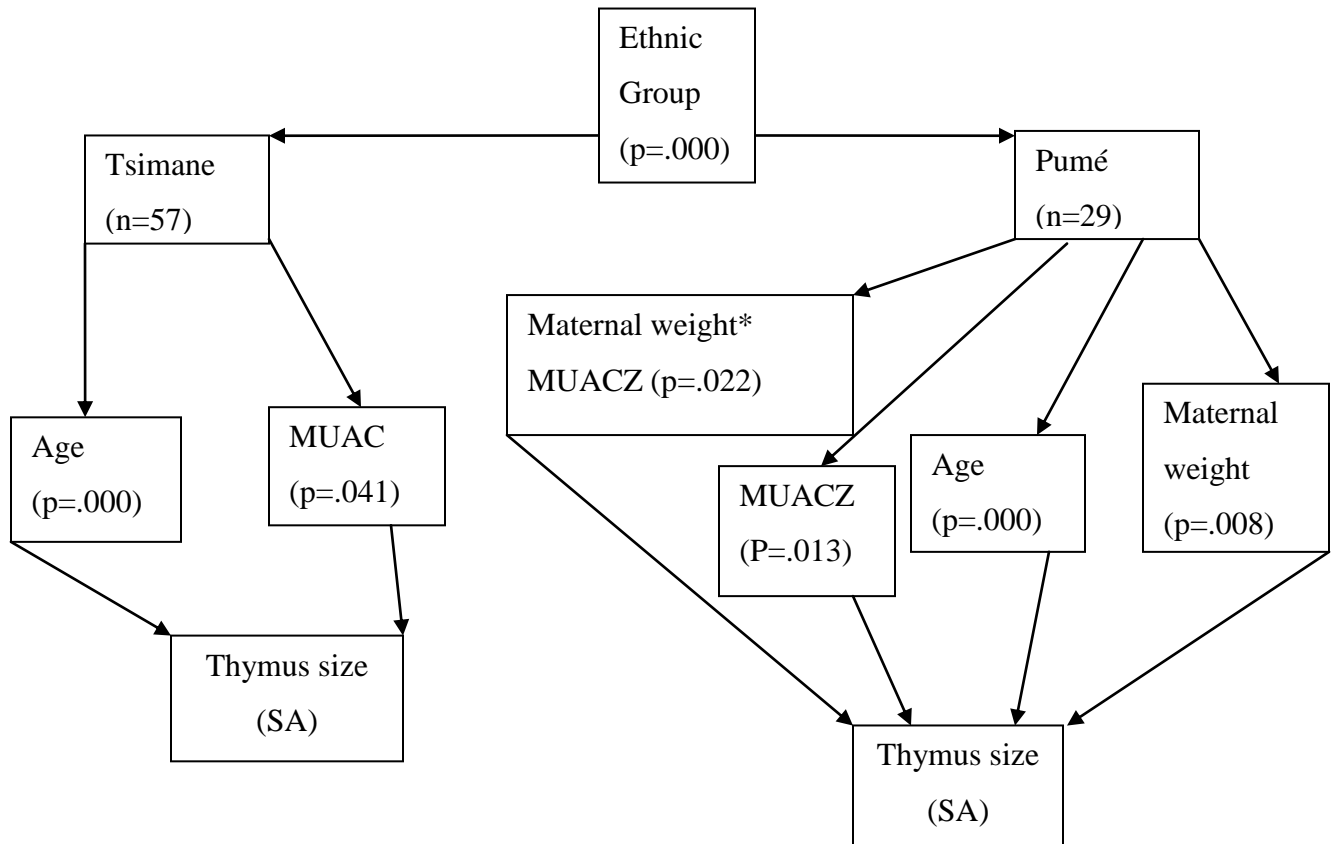
Table 4.11. Maternal weight predicts MUACZ in 29 Pumé infants

Parameter	B	Std Error	Sig
Intercept	-3.143	1.0494	.003
Age (months)	.054	.0252	.032
Maternal weight	.047	.0209	.025

Table 4.12. Predictors of SA in 29 Pumé infants

Parameter	B	Std Error	Sig
Intercept	52.887	81.8110	.518
Age (months)	-7.446	1.8086	.000
Maternal weight	4.072	1.5284	.008
MUACZ	-170.443	68.5028	.013
Maternal weight*MUACZ	3.216	1.3999	.022

Figure 4.8. Flow chart showing most significant predictors of SA in Tsimane and Pumé infants.



Thymus Size in Native South American and Danish and Turkish Urban Infants

Tsimane and Pumé thymus size are here operationalized as left anterior-posterior dimension (or LAP, Figure 4.9), and as estimated thymic index (or TI, Figure 4.11) and compared with published thymic dimensions from infant cohorts in Turkey and Denmark. In Figure 4.9, LAP values are combined for the two Turkish cohorts of infants (Yekeler & Tambag et al. 2005, Zizalcan & Bilalooglu et al. 1995) and also for the Tsimane and Pumé. The overall developmental trajectory of LAP is remarkably similar in the Turkish and South American natives, but LAP is smaller at all ages in the South American native infants. In both cohorts a peak in LAP size is observed in the first six months of life and then it declines from 6-15 months. This decline is

followed by a slight increase in LAP size from 15-18 months in the Turkish infants, and levels off in the South American natives from 15-18 months. In both cohorts there is a gradual decline in LAP from 18-24 months.

In Figure 4.10, the relationship between LAP and SA in the two Turkish cohorts ($r=.84$) and in the South American native infants ($r=.93$) is demonstrated. Differences in the slope of the lines suggest that the method used to collect data in the South American native infants is not fully comparable to those collected by the other researchers using Hasselbalch's method. In particular, SA in the South American natives is smaller than would be predicted using the regression equation for the Turkish populations. Because of this discrepancy and concerns pertaining to external validity, SA values are not included in this cross-cultural comparison.

In Figure 4.11, thymic index (TI) is plotted for three cohorts of infants. TI values were published for the Danish (Hasselbalch's) cohort and for the Turkey 2005 cohort. They were calculated for the Turkey 1995 cohort and estimated for the South American natives as described in the Data Analysis section (see Table 4.1). As expected the urban infants from Denmark and Turkey had larger TI at all ages than the South American native infants. In the first six months, rapid thymic growth is observed in the urban infants, while in the South American native infants, TI increased only slightly in the first three months and then began a gradual decline until 12 months. In the Danish infants, TI decreased notably from 6-12 months as well. In the Turkish cohort, a more gradual decline is observed from 6-15 months. In the Danish and South American native infants, a slight increase is observed from 12-15 months. A gradual increase in TI continues in the Danish infants until 24 months, while a slight decline is observed in the South American native infants from 15-24 months. In the Turkish infants, a notable decline in TI is observed from 9-12 months, followed by a more gradual decline in TI until 24 months.

Figure 4.9. A comparison of the anterior-posterior dimension of the left thymic lobe (LAP) based on direct measures obtained in both Turkish and South American natives aged 0-24 months. LAP was strong predictor of TI ($r=.86$).

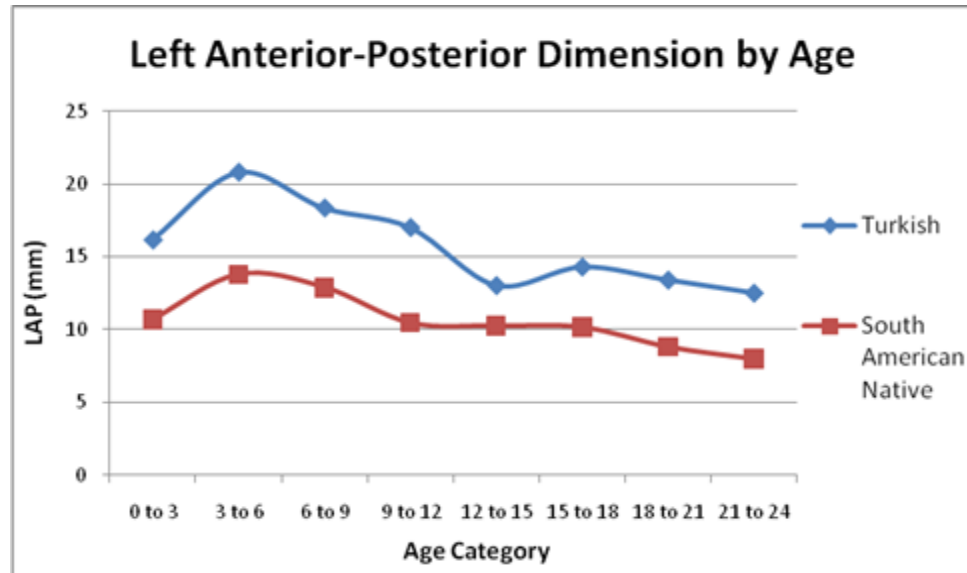


Figure 4.10. Differences in the relationship between LAP and SA differs in the Turkish ($r=.84$) and South American native ($r=.93$) infant cohorts.

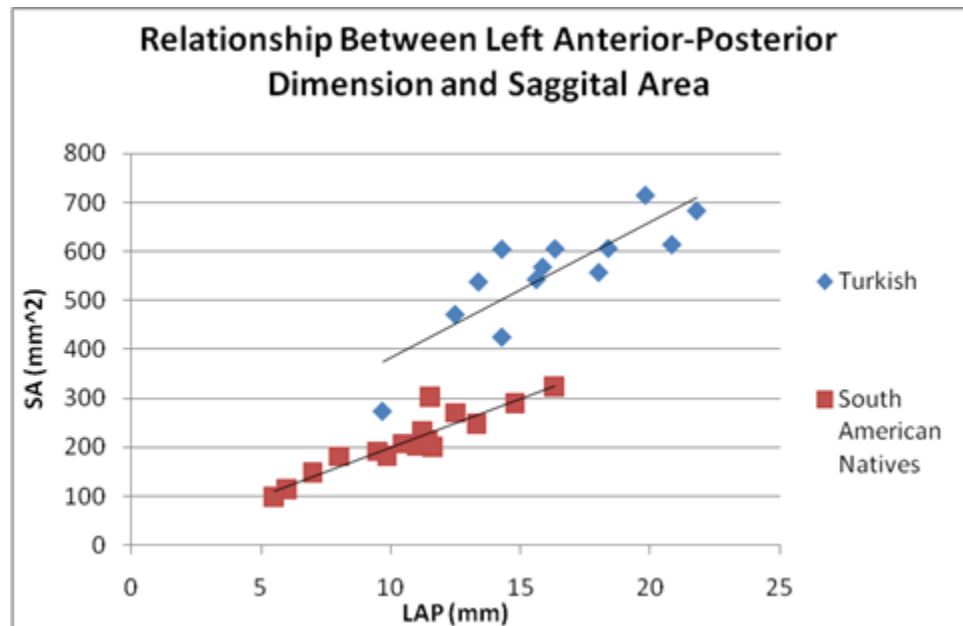
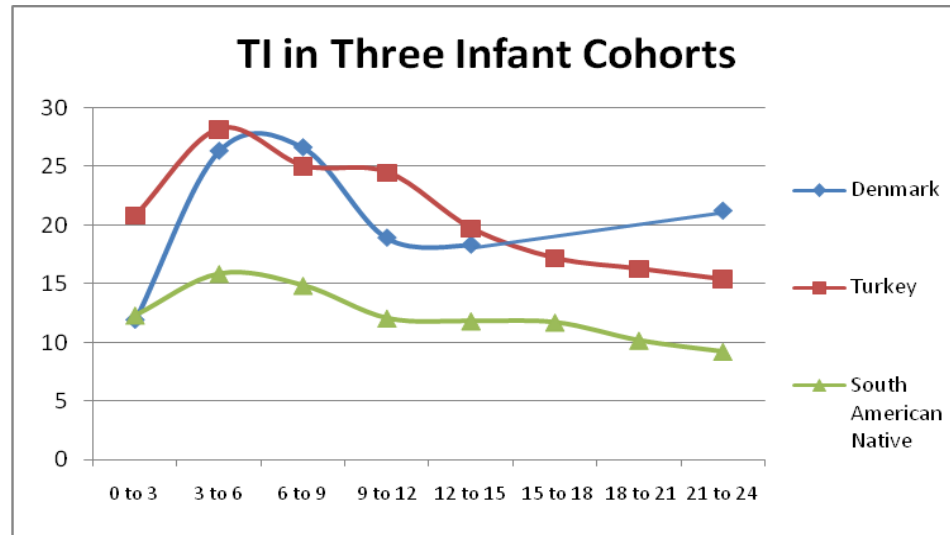


Figure 4.11. A comparison of thymic index in Danish, Turkish and South American native infants. LAP correlated with TI ($r=.86$) in the Turkey 2005 cohort.



4.5 Conclusions

There are six major findings of this study: 1) Despite inhabiting a different subsistence and disease ecology, Pumé and Tsimane infants showed similar physical growth trajectories in weight and height in the first two years of life, 2) Tsimane infants had larger thymic left anterior-posterior dimension (LAP) and sagittal area (SA) at all ages than Pumé infants, 3) Ethnic group and infant age were significant predictors of thymic index in the South American infants, 4) MUACZ was the strongest significant predictor of SA in Tsimane infants after controlling for age, and in the Pumé infants after controlling for age and other confounders, 5) In the Pumé infants, maternal body weight was a predictor of MUACZ and SA, and 6) The South American native infants had smaller thymuses at all ages than the urban infants from Denmark and Turkey, though the overall developmental trajectories showed similarities. The implications of each finding are discussed in detail below, followed by a general discussion of thymic design and a summary and recommendations for future research. To my knowledge, this is the only study of infant thymus size in native South American forager-horticulturalist societies. Results suggest the need to further investigate postnatal environmental influences on immune system development in populations experiencing different pathogenic environments and resource availability.

Results of Hypothesis Testing

Hypothesis 1. Pumé infants will have smaller body size than Tsimane infants

It was not expected that Tsimane and Pumé infants exhibit nearly identical weight-for-age, height-for-age, and MUAC-for age growth curves because the Pumé have much less access to medical care and experience more pronounced resource scarcity than the Tsimane. However, this result may reflect the fact that the Pumé measures were conducted during an energetically favorable period, and that river infants, which compose the majority of the Pumé infant cohort, are expected to be in better condition. Furthermore, the Pumé cohort was younger, and early infancy is a period in which infants are buffered by breast milk from resource scarcity and infectious disease exposure. As discussed in Chapter 3, growth faltering in South American native infants often appears in mid-late infancy, thus very young infants can have relatively higher anthropometric Z-Scores.

In addition to between-group differences that exist between the Tsimane and Pumé, there was substantial within-group heterogeneity between savanna and river Pumé which confound between-group comparisons. While the samples were too small to disaggregate and test statistically, the savanna Pumé children in this sample had much smaller MUACZ than the river Pumé. This was not unexpected since savanna Pumé infants experience more pronounced resource scarcity and were at higher risk of infectious disease morbidity. However, WAZ and HAZ were similar between savanna and river Pumé. These results suggest that WAZ and HAZ don't accurately reflect fat differences or muscle size differences. There also may be variation in body composition between savanna and river Pumé infants, and between Pumé and Tsimane infants, despite similar growth trajectories. Significant differences between Tsimane and Pumé MUAC might appear in a larger sample, particularly if more savanna infants were included.

Hypothesis 2. Pumé infants will have smaller thymuses than Tsimane infants

As predicted, the Pumé infants had smaller thymuses than the Tsimane at all ages (both when thymic left anterior-posterior dimension (LAP), and sagittal area (SA) were compared and the result were highly statistically significant. As Pumé and Tsimane infants had nearly identical weight, length, and MUAC-for-age growth trajectories, the difference cannot be attributed to phenotypic correlations with body size. This suggested that other factors independent of body

composition may underlie differences in thymic size between Tsimane and Pumé infants. The difference in thymus size between the Tsimane and Pumé merits further investigation.

Ethnic differences in thymic size at birth have been observed in other studies (Aaby, Marx et al. 2002) and the possibility that genetic differences influence thymic developmental trajectories cannot be ruled out. Much more research is needed to better understand genetic and epigenetic factors underlying thymic function and immune systems in general. Environmental influences on thymic size have been documented but remain poorly understood. The larger size of Tsimane thymuses may indicate that they have more energy available to invest in competing demands of thymic tissue and growth. This is plausible because in general, Tsimane enjoy greater access to market foods than do the river or savanna Pumé.

Smaller thymic size in Pumé infants could also reflect unmeasured trade-offs occurring on other levels of infant physiology. For example, elevated CRP, in innate immune response, was reported to occur at the expense of growth in Tsimane children (McDade, Reyes-García et al. 2008). Trade-offs do occur between different components of the immune system and should be more pronounced in energetically and pathogenically challenging settings, however it is extremely difficult to isolate environmental effects on multiple components of immune function (McDade 2005c). If the Pumé also experience high levels of infection, trade-offs could conceivably occur at the expense of thymic tissue in the Pumé if they were more pronounced given the resource scarcity of their environment.

CRP and other markers of inflammation are often used to measure immune activity and are assumed to reflect a general level of environmental pathogenicity. Because no biomarkers of inflammation have been collected in the Pumé, it is not possible to draw conclusions with respect to the relative pathogen burden experienced by infants in each society. According to McDade and Worthman's hypothetical model (Figure 4.1), thymic tissue should regress rapidly in contexts of high pathogen burden, and smaller thymus size then reflects maturation and peripheralization of cellular immune system by strategically distributing mature, antigen specific memory lymphocytes throughout the body. The possibility cannot be ruled out that smaller thymus size observed in Pumé infants reflects an adaptive response of accelerated immune maturation under conditions of great infectious disease burden, particularly in the savanna communities where infant mortality is extremely high.

Hypothesis 3. Infant body size will be positively correlated with thymus size in Tsimane and Pumé infants.

Several studies have reported correlations between anthropometric indices and thymus size in infancy (Garly, Trautner et al. 2008; Moore, Prentice et al. 2009). In this study HAZ and WAZ were not significant predictors of thymic left- anterior posterior dimension (LAP) or sagittal area (SA) after controlling for age. Only MUACZ was a predictor of thymic size (SA only) in this cohort of infants. The effect was strongest in infants who were six months old or older. This strong positive relationship between MUACZ and SA persisted after controlling for infant age and ethnic group in the combined cohort of infants when a group*MUACZ variable was added. This suggests that group and MUACZ are correlated, though there were no significant differences in MUACZ between the two groups. There could be subtle differences in body composition between the populations that are driven by very lean savanna Pumé infants that are not reflected in the height and weight-for age Z-Scores. MUACZ measures muscle, fat and bone and strongly predicts mortality in infants and children over six months of age (Zemel, Riley et al. 1997) and is also less age-dependent and less affected by swelling than many other measures (Cross, Holden et al. 1995). As children with larger thymuses exhibit higher survivorship (Aaby, P., Marx, C., Trautner, S., Rudaa, D., Hasselbalch, H., Jensen, H. & Lisse, I., 2002), it makes sense that MUACZ and thymic size would show a strong association.

Prediction Models

The most significant predictors of infant SA in the Tsimane and Pumé were ethnic group and age. Thymic index declined linearly with age in both populations of infants, with decreases in thymic size occurring more rapidly in the Pumé cohort. The strength of age as a predictor suggested that the infant thymus may follow a somewhat canalized, age-related trajectory that is influenced slightly by nutritional and epidemiological conditions. In additional, separate analyses in the Tsimane and Pumé infant cohorts, age is a stronger predictor of sagittal area (SA) than any anthropometric indicator.

Under favorable energetic conditions, relatively high levels of investment in are observed in both growth and immunity because trade-offs are less pronounced. In nutritionally challenging environments, there may be a less straightforward relationship between body size and thymic size because of intervening environmental factors. MUACZ was the strongest marginally significant

predictor of (SA) in the Tsimane after controlling for age. MUACZ was only a significant predictor of SA in the Pumé after controlling for age, maternal weight, and maternal weight*MUACZ. Because maternal weight was also a significant predictor of MUACZ in the Pumé but not the Tsimane, it appears that maternal condition exerts a stronger effect on infant condition in the Pumé than it does in the Tsimane. While a relationship between maternal size and infant condition is not surprising, it is interesting that it is only significant in the Pumé. This finding suggests that maternal condition was more relevant to infant health outcomes under conditions of pronounced resource scarcity, and may reflect the lack of buffer for infants in harsh environments who lack food resources and medical care. A similar relationship between maternal size and infant condition could exist in the Tsimane as well, but intervening factors such as highly available complementary foods and medical care may obscure the relationship.

Thymus Size in Native South American and Danish and Turkish Urban Infants

Comparative plots suggest that the Tsimane and Pumé infants have smaller thymuses and similar thymic developmental trajectories to the infants in the Danish and Turkish cohorts. In general, rapid thymic growth is observed in each Danish, Turkish, and South American native infants in the first six months of life. A roughly similar developmental trajectory is further observed from 6-24 months. The pattern of declining TI from 6-12 months, a brief period of growth or stasis from 12-15 months, and finally a gradual decline from 15-24 months, is consistent in both urban and native South American infants. This suggests that thymic growth may follow a canalized developmental trajectory despite differences in body size.

Peak thymic size in the first six months of life is more pronounced in the urban populations, however, suggesting that thymic development is particularly sensitive to early postnatal environmental conditions. There may be more energy to invest in thymic growth in populations where infants are relatively protected from environmental pathogenicity and chronic upregulation of immune function and may simply reflect an overall high level of energy available to invest in competing life history demands (Hasselbalch, 1999; Iscan, Tarhan et al. 2000). However, studies from rural populations in Guinea-Bissau and Bangladesh also reported rapid thymic growth in early infancy (Collinson, Moore et al. 2003; Moore, Prentice et al. 2009).

While the comparison of urban and South American native infant thymic developmental trajectories is compelling, it is not possible to test the differences statistically. Intra-observer

reliability in thymic ultrasonographic measures is suspect (Aaby, Marx et al. 2002; Collinson, Moore et al. 2003) and some of the differences may stem from intra-observer variation and the consequences of age lumping. The sample sizes for South American natives are small, and some of the trends observed, particularly those at later ages where few infants are represented, may be sample driven. The two cohorts of Turkish infants and South American infants are cross-sectional. Only the Danish cohort was studied longitudinally. Still it is safe to assume that actual differences in thymus size and body size exist between the urban cohorts of infants and the South American native cohorts that may be at least partially attributed to phenotypic correlations (covariance among individual traits such as body size).

Multiple potential confounding factors affect thymus development independent of body size. In addition to being better nourished, the Turkish and Danish infants probably received adequate health care and full courses of vaccines, and most likely inhabit environments that are far more sanitary than those experienced by Tsimane and Pumé infants. As infection generally shrinks the thymus, it would seem that South American native children might experience earlier declines in thymic size if they experience frequent morbidity; unfortunately no direct measures of morbidity are available. It is challenging to isolate the respective roles of pathogen exposure and nutrition in populations inhabiting such drastically different postnatal environments. Still, attempts to control for these correlations and to look more closely at environmental influences on the development of the thymus will contribute to a better understanding of cross-cultural variation in thymic development and long-term health outcomes across diverse contemporary populations.

The timing and nature of pathogen exposure in the South American native infants may uniquely influence thymic development. How antigen exposure in early infancy, particularly helminthic parasites, may contribute to rapid thymic decline is poorly understood. Helminth infestations are rare in urban populations but are commonly observed in rural societies in South America and elsewhere. In Table 4.13, some general factors that characterize the Danish, Turkish and South American native infant cohorts are summarized. The variables listed potentially contribute to thymic developmental trajectories in infancy. While actual measures are available for Tsimane and Pumé body and thymic size and for urban infant thymic size, many of the other variables (pathogen burden, urban infant body size, and vaccination status) are assumed based on results from other studies. While far from conclusive, results of this study suggested that South

American infants experienced an alternative pathway of immune system development as a result of early postnatal factors that could underlie poor health outcomes in these populations.

Table 4.13. General factors that characterize the populations considered in this study that may contribute to thymic developmental trajectories in infancy

Population	Tsimane	Pumé	Urban
pathogen burden	high	high	low
body size	small	small	large
Thymus	middle	smallest	largest
Vaccines	partial	none/little	complete

Does Size Really Matter?

Interpreting the results of this study was hampered by a poor understanding of what it means to have relatively large or small thymus in infancy. Much contemporary medical literature suggests that a large infant thymus is normal and healthy. A small thymus, in comparison to the healthy baseline, was associated with poor health outcomes and suggested developmental insult by malnutrition and/or infection. From an evolutionary perspective, far from being “normal” a large infant thymus can be considered a luxury afforded to those who are exceptionally well-nourished and have experienced minimal pathogen exposure. A large thymus correlated with a large body and had exceptionally high proliferative capacity. These factors combined suggested that a large amount of energy was available to invest in competing life history demands. The fact that adult Tsimane men and women achieve larger body sizes than Pumé men and women suggested that Tsimane infants have more energy to allocate to both immune function and growth, while the Pumé infants invested in growth at the expense of thymic tissue under energy-limited conditions, but the trade-off was reflected in higher mortality and smaller body size. However, taking these results together, it is not clear the meaning of the differences. Results are confounded by problems of within-group heterogeneity, seasonality, and an inadequate understanding of relative pathogenic pressures experienced by the two infant cohorts.

A simple energetic model may not be sufficient as immune function was also shaped by epidemiological conditions. As South American native infants experienced higher levels of pathogen exposure at crucial developmental stages of the immune system, their smaller thymus may represent a more rapidly maturing immunological repertoire. It is possible that South American native infants experienced more rapid T-lymphocyte proliferation because specific pathogens in their environment required a mature cellular immune response. If Pumé infants experienced higher levels of pathogen exposure at crucial developmental stages of the immune system, the smaller thymus in the Pumé may represent a more rapidly maturing immunological repertoire. It is possible that Pumé infants experienced more rapid T-lymphocyte proliferation because specific pathogens in their environment require a mature cellular immune response. For example, studies from Africa have shown high levels of cellular immune responsiveness in infants whose mothers had had malaria while pregnant (Fievet, Ringwald et al. 1996), and Pumé also live in a malarial region while the Tsimane do not. Conversely, larger thymic size in Tsimane infants may reflect a greater need to invest in thymic maintenance given the pathogenic environment.

Limitations of the Study

The South American native sample was small due to logistical constraints. In particular, the Pumé sample is small and the method used to age Pumé infants is less precise than that used to age Tsimane infants. As the savanna Pumé experienced a nearly 35% infant mortality rate, there may be an effect of survivor bias in those communities. Finally, the Pumé measures were taken at the end of the dry season, when foods were most abundant and weight gain was common in reproductive aged females and may translate to higher breast milk quality and better access to complementary foods than would be observed in the resource-scarce wet season.

Directions for Future Research

Clearly, multiple factors influence immunological development and there is a pressing need to develop better causal models leading to better understanding of the nuances of thymic physiology and development and differentiation of immunologic phenotypes. Better understanding of maternal health and antigenic experience before and during pregnancy may be relevant for priming expectations about infant health comes, especially in nutritionally and epidemiologically challenging environments.

It would be beneficial, though logistically challenging, to obtain birth weights to collect longitudinal thymic measures at regular age intervals. Another limitation of this study was its lack of data to measure pathogen exposure in Tsimane and Pumé infants. Measures of inflammation (CRP, etc.) would provide a proxy for innate immune function and would be useful in consideration of trade-offs between immune system components and the establishment of immune-phenotypes. Also, beneficial would be more research on the effect of helminth parasites on immune system differentiation during crucial developmental stages.

As diet and breastfeeding practices are known to influence thymic size, future research should attempt to monitor infant feeding (and other infant care practices) in order to link maternal behaviors and nutritional influences with developing immune system trajectories. Collection of behavioral data is tedious and time-consuming but yields valuable information regarding actual human behaviors not accurately captured by other data collection techniques. It would be important to see if mothers' caretaking strategies potentially modulate immune development in infants. Finally, more collaboration between international researchers studying thymic size in infancy would be useful for establishing more rigorous and standardized methodologies from which to compare cross-cultural datasets.

From a theoretical standpoint there was much to be gained from the study of the thymus. Much research has focused on how pre-and postnatal environments program immune function; less was known about how these affect different health outcomes across diverse contemporary human populations. There was a pressing need to investigate developmental immune processes among South American native populations, who historically have suffered from lack of attention to the unique suite of factors contributing to the health of their populations and their neglected health needs. Finally, more collaboration between international researchers studying thymic size in infancy would be useful for establishing more rigorous and standardized methodologies from which to compare cross-cultural datasets.

CHAPTER V

CONCLUSION

The goal of this dissertation was to explore the evolutionary ecology of infancy in South American native populations, specifically with respect to the nature of the weaning transition, growth patterns, and the thymic developmental trajectories. Within Chapter II, the Tsimane and Pumé study populations were described, and the results of maternal interviews conducted in Tsimane communities regarding preferred infant foods and the timing of introduction of complementary foods presented. In Chapter III, the weaning transition in Tsimane infants was modeled through observation of infant behaviors, and predictions derived from life history theory pertaining to parental investment and infant growth and health were tested. In Chapter IV, thymic developmental plasticity in Tsimane and Pumé infants was explored. Predictions generated from the theoretical perspective of ecological immunology were tested to explore the nature of energetic trade-offs faced by infants across diverse environmental contexts.

5.1 Summary of Results

Infant Feeding Practices in the Tsimane

A breakdown of first infant foods as reported by Tsimane mothers revealed that 79% of women had used plantain as the first complementary food. Masticated meat and fish were also used as first complementary foods by 19% of mothers. There was substantial variation in women's responses regarding the timing of introduction of complementary foods, with a mean reported age of introduction of complementary foods of 4.66 months. Maternal reports of timing of introduction of complementary foods varied at the community level: Mothers from the most acculturated communities reported the latest introduction of complementary foods and mothers from least acculturated communities reporting the earliest introduction of complementary foods.

Maternal reports revealed substantial behavioral flexibility and responsiveness to infant condition and infant cues. Overall, these results do not deviate from infant feeding behaviors reported by mothers across several subsistence societies. Maternal reports did not adhere to observed infant behaviors, which is not surprising as the weaning transition is far more complex than could accurately be captured via retrospective interview techniques.

Anthropometric Reference Populations

Throughout this dissertation, both CDC Z-scores and Z-scores generated from a Tsimane (Chapter 3) or South American native (Tsimane and Pumé) reference population are used. It is demonstrated that South American native infants experience growth faltering with respect to the CDC (US) baseline in the first months of life. This pattern is often observed in infants in the developing world and is probably attributable to a combination of nutritional and epidemiological factors. While analyses in Chapter 3 revealed similar results using different the Z-scores, in Chapter 4 a significant association between MUACZ and thymic sagittal area (SA) was only found when Z-scores based on a South American native infant reference population was used. When CDC Z-scores are applied to South American native infants, they become confounded with age. Using a South American native reference population minimizes this problem and allows us to compare infants to others experiencing similar physical developmental trajectories.

Observation of the Infant Feeding Transition

Examination of infant feeding in the Tsimane infants reveals that weaning is a complex and protracted process in which the rate at which breastfeeding declines relative to increasing food consumption can vary tremendously between individual mother-infant pairs. The strongest predictors of nursing probability were age and height, and community type was marginally significant predictor. The strongest predictors of nursing intensity (NI, a statistical measure that tells us where infants fall along the continuum of mixed feeding relative to other children who are the same age) were height, gender, and age.

The hypothesis that mothers alter their infant feeding strategies in response to these factors by nursing their offspring most intensively where risk of infant mortality by infectious disease is high was not supported. Nursing probability was significantly higher in river communities, one of the community types experiencing high infant mortality, but not in the forest communities where infant mortality was highest.

The hypothesis that large infants should experience an accelerated weaning process was supported in both prediction models for nursing probability and NI. It is not possible to definitively ascertain the direction of causality with the observational data available. However this result provides tentative support for the hypothesis that Tsimane mothers monitor their infants' statural growth and alter their infant feeding behaviors by accelerating the weaning process in faster-growing infants. It is theorized that mothers respond adaptively to infant condition and thereby attempt to maximize their own fitness by monitoring infant growth and accordingly balancing investment in current offspring with future reproductive opportunities. As many Tsimane women report that infants drive the weaning transition, future studies should explore the nature of mother-infant interactions and the role of infant behavioral cues in conjunction with physical indicators of child well-being and growth.

In the prediction model for NI, male infants had higher nursing intensity scores than females after controlling for age, height, coder and random effects. While this finding was not supported in the prediction model for nursing probability, it provides suggestive evidence that male infants receive higher levels of maternal investment. This might happen if males are preferred in a society or because they are more vulnerable in infancy and require compensatory investment. It might also occur if the relative benefits of prolonged nursing are higher in males, or because female infants develop neurological machinery for required for complementary food consumption at earlier ages. This finding was unexpected and warrants further investigation.

Observed infant feeding behaviors did not conform to maternal reports in the small subset of women for whom both data types were available. This suggests that the systematic collection of observational, behavioral data yields a more accurate, detailed and nuanced portrayal of infant feeding practices than are derived using interview techniques. Crude reports of introduction of complementary feeding and breastfeeding duration, based on retrospective interviews, failed to capture the relative contributions of breast milk and non-breast milk foods and how they change across the weaning transition. Future studies should attempt to specifically monitor breastmilk nutrients and energy density, as well as the respective caloric contribution infants derive from complementary foods.

Thymic Development in Infancy

In Chapter 4, three hypotheses were tested: 1) That Tsimane infants would be larger than Pumé infants, 2) That Tsimane infants would have larger thymuses than Pumé infants, and 3) That infant body size would be positively correlated with thymus size in both groups. The study yielded several interesting results that underscore the need to further explore thymic development across a variety of diverse ecological settings.

Hypothesis 1 was not supported: Despite inhabiting a different subsistence and disease ecology, Pumé and Tsimane infants showed similar physical growth trajectories in weight and height in the first two years of life. Hypothesis 2 was supported: Tsimane infants had larger thymuses at all ages than Pumé infants. Hypothesis 3 was partially supported: While there was no significant association between height or weight and thymus size, MUACZ was a significant predictor of sagittal area (but not LAP) in this cohort of native South American infants. However, the significant association between MUACZ and thymus size was only observed when Z-score 2 (South American native reference population). A direct association between MUACZ and thymus size was only observed in the Tsimane when the two groups were analyzed separately.

Differences in thymic size despite no major differences in anthropometric status may suggest that infant care practices buffer Pumé infants from resource scarcity. It also may suggest that Pumé infants invest preferentially in growth at the expense of immunity, at least with respect to thymic tissue, in the energetically demanding infant phase. However within-group heterogeneity that exists between savanna and river Pumé infants may confound between group comparisons with the Tsimane.

Ethnic group and infant age were the strongest predictors of thymic size in the South American infants. While both left anterior-posterior dimension (LAP) and sagittal area (SA) decline linearly with age in both cohorts of infants, additional factors independent of age and ethnic group influence thymic size. It appears that different mechanisms underlie thymic developmental trajectories in the Tsimane and Pumé infants. Only in the Tsimane was a direct and significant relationship between MUACZ and SA found. In the Pumé but not the Tsimane, maternal weight was a predictor of both SA, but only when MUACZ and MUACZ*maternal age were included in the model. This finding suggested a complex interaction between maternal and infant body size and infant thymic size in the Pumé. Maternal condition may be more relevant to

infant health outcomes under conditions of resource scarcity, and may reflect the lack of buffer for infants in harsh environments who lack medical care.

The comparison of thymic size in the Tsimane and Pumé infants with urban infants from Denmark and Turkey revealed methodological issues pertaining to external validity of the thymic dimensions measured. Hasselbalch's method was used in most published cross-sectional studies, and there was no direct way to compare Hasselbalch's thymic index (TI) using the dimensions that were measured in the Tsimane and Pumé. While a formula was constructed to predict TI using left anterior-posterior dimension (LAP), future studies should collect the dimensions utilized by both methods to facilitate comparison with other populations.

Despite methodological weaknesses, results were compelling. As expected, the South American native infants have smaller thymuses than urban infants in Denmark and Turkey. Interestingly the developmental trajectory of the thymus in South American native and urban infants was quite similar using both directly measured values (LAP) and calculated values (TI). In both groups, thymic growth was observed in the first six months of life, though growth was more pronounced in the urban infants. From 6-24 months a gradual decline in thymic size was observed.

5.2 Future Research

Infant Feeding

Upon modeling infant feeding behaviors in a forager-horticulturalist society, it was found that community factors, infant growth, and infant gender interact to determine nursing probability and intensity in the Tsimane. Further investigation is needed, not only to address important theoretical questions, but to consider the relevance of infant feeding practices for maternal-infant health. Little is known regarding the causes and consequences of variation in complementary feeding (quality, quantity, and timing) across diverse environmental contexts and subsistence ecologies.

Poor child health outcomes are often attributed to suboptimal infant feeding patterns, yet parenting strategies that appear maladaptive may make more sense when situated within an evolutionary ecological framework. Infant-focused approaches to community health fail to address the array of trade-offs faced by mothers and particularly mothers in the developing world. Cross-cultural research initiatives exploring infant feeding patterns and health should broaden their

scope to consider the adaptive nature of maternal decision making. Ultimately, there were many potential reasons that mothers might not adhere to WHO's recommended infant feeding regimen. Public health interventions that encourage mothers to conform to the WHO regimen of infant feeding may not only interfere with adaptive processes, they can also unfairly stigmatize poor women as "bad mothers" if they don't adopt recommended strategies.

Future research in the Tsimane should also include the collection of baseline, pre-complementary food measurements to ascertain the direction of causality in the relationship between infant length and nursing intensity. Lack of data on actual nutrients derived by infants from breast milk and complementary foods inhibit our ability to draw robust conclusions on the relative contribution of different food types to infant developmental trajectories. It would also be useful to study the nutritional and immunological properties of Tsimane women's breast milk and to know what they are eating in the different communities, since micronutrients such as iodine, which is high in meat and fish diets and lower in carbohydrate-based diets, may influence infant growth trajectories more than calories per se. Future studies should also attempt to link infant feeding patterns to direct measures of environmental pathogenicity, maternal fertility and long-term child health outcomes. Finally, the interactions of maternal condition, fertility, breastfeeding, and infant health merit further investigation in this population of infants.

The Thymus in Infancy

Infant age and ethnic group were predictors of thymic size in South American native infants. Infant and maternal body size influence thymic size, but do not exert the same effect in the Tsimane and Pumé cohorts. The nature of thymic development and design by natural selection are considered, as well as how energetic trade-offs in infancy may drive variation in thymic developmental trajectories across contemporary populations of infants inhabiting a variety of postnatal environmental settings. Diminished cellular immunity is often observed in South American native populations. The establishment of this immune-phenotype begins in infancy while the immune system is maturing and calibrating to local disease ecologies. Understanding the interactions between pathogenicity and energetics, and their respective roles in shaping infant growth and immunological maturation patterns, is relevant to understanding health outcomes in contemporary South American native populations. Other relevant questions pertaining to South

American immuno-phenotype include the relationship of genetic homozygosity, helminthic infection and the Th2 phenotype.

Future studies should attempt to develop better causal models leading to better understanding of the development and differentiation of immunologic phenotypes. In studies of infants, it would be ideal to obtain birth weights prior to collection of longitudinal measures that encompass all three aspects of immune function: thymic measures, inflammation, and helminthic parasitism, to ascertain their respective roles in driving immune system differentiation during crucial developmental stages. More collaboration between researchers studying the thymus would be useful for establishing more rigorous and standardized methodologies from which to study thymic design and physiology across the life course and to facilitate comparison of cross-cultural datasets.

Immune Calibration and Maternal Vigilance Strategies

As diet and breastfeeding practices are known to influence thymic size, future research initiatives should attempt to draw together methodologies presented in this dissertation as well as to incorporate new ones to further understand of the connections between environment, behavior, growth, and immune system development in infancy and early childhood. In particular, observational studies of infant and maternal behaviors are needed to understand how evolved behavioral strategies can modulate pathogen exposure, and the role these strategies play in contemporary human health.

The implications of early life conditions on the establishment of immune phenotypes are not only relevant in developing world contexts. A large body of evidence suggested that lack of pathogen exposure in crucial developmental phases may lead to immune dysfunction later in life. Strachan's Hygiene Hypothesis, first published in 1989, proposed that increased allergic disorders in industrialized societies may result from altered immune development in individuals who lack early childhood exposure to infectious agents and symbiotic organisms (Strachan 1989). It is now generally accepted that small doses of pathogen exposure are essential to facilitate the maturation of the immune system. Fessler and Abrams provide evidence for the "immunocalibration" whereby infant mouthing behavior functions to "calibrate" the infant's immune system to local disease ecology (Fessler, 2004).

Infectious disease has been a powerful agent of natural selection through evolutionary history and human history has been heavily influenced by disease (Diamond, 1999), therefore human psychology should be evolved to detect and deter costly exposure. Before the invention of antibiotics, vaccines, and anti-bacterial soaps, human populations co-evolved with environmental pathogens. It makes adaptive sense that humans would evolve behavioral strategies for coping with pathogens in the absence of modern medicine. Pathogen avoidance should also be practiced by primary caretakers of vulnerable neonates.

Mothers clearly play a role in the modulation of infant growth and immune system development through adaptive caregiving strategies, however the details of these behavioral mechanisms and the learning process by which maternal strategies are acquired are difficult to elucidate. In pathogenic settings, mothers must be particularly vigilant and protect immunologically vulnerable infants from antigenic exposure. They should therefore embed adaptive pathogen-avoidance behaviors in their infant caretaking strategies with the dual goals of minimizing excessive exposure in the most vulnerable neonates and also of calibrating the infant's immune system to facilitate the timely onset and maturation of infant immune defenses. In subsistence societies it is impossible to eliminate pathogenic exposure altogether, and in high-mortality contexts infants that survive are those who have developed an immune profile that is appropriately calibrated to the local disease ecology.

To draw together the themes described in this dissertation and shed more light on maternal-infant interactions and the ecology of infant development would entail monitoring large cohorts of infants longitudinally, not just with respect to infant feeding, but other infant care practices as well. Infant and maternal behaviors such as immune calibration and vigilance strategies could then be linked to several aspects of developing immune system trajectories. A study of this nature would require detailed studies of infant behavior. Collection of behavioral data is tedious and time-consuming, but as we have shown, yields valuable information regarding actual human behaviors not accurately captured by other data collection techniques.

5.3 Conclusion

In this research ethnographic and quantitative approaches were combined to examine several aspects of infant development from a life history perspective. Important theoretical questions were addressed regarding the nature of maternal-infant behaviors and the interactions of

resource availability, growth and immune function. In general, my results show that maternal behaviors are flexible and appear to be influenced by infant characteristics and community factors, and that thymic developmental trajectories vary across South American native communities despite similarities in infant growth patterns. While grounded in life history theory, this research has important public health implications: it addresses both social and biological aspects of South American native health. Understanding how human behavior and physiology are shaped by natural selection has important implications for health and disease patterns across contemporary human populations.

APPENDICES

APPENDIX A

Data Set	Population	N	Year	Data Type
Infant Feeding/ Health	Tsimane	81	2003, 2006	Interview
Time Allocation	Tsimane	133	2002-2007	Observation
Maternal Demography (Consumption Cohort)	Tsimane	108	2002-2008	Interview
Maternal Demography (Thymus Cohort)	Tsimane	57	2002-2008	Interview
Thymus and Anthropometry (thymus cohort)	Tsimane	57	2006	Physical measure
Anthropometry (consumption cohort)	Tsimane	108	2002-2008	Physical measure
Infant Feeding/ Health	Pumé	29	2007	Interview
Maternal Demography	Pumé	29	2005-2007	Interview
Thymus and Anthropometry	Pumé	29	2007	Physical measure

APPENDIX B

Detailed Description of the Study Villages

The Tsimane infants in this study come from nine communities which are divided into three community types: acculturated, river, and forest. The community types are ranked by acculturation on the basis of the following criteria: distance from town, number of families, presence/absence of a school, percent of adults who speak Spanish, and infant mortality rate. Here detailed descriptions of the community types are provided.

The Acculturated Community

Tacuara del Maito (herein “Tacuaral”) is the only acculturated Tsimane community included in this study. In general, acculturated communities are located on well-traveled roads within two hours of San Borja, have schools and churches, and Spanish fluency is high, especially among men. Tacuaral is located 27 miles from San Borja on a dirt road, and in 2005 had about 56 families. As game is depleted in this region due to deforestation and polluted rivers, wage labor is rapidly overtaking hunting and fishing as a primary subsistence strategy in this community. Wage labor at nearby logging camps, farms, and ranches is common for men. Hunting and fishing trips occur opportunistically when rides in cars or trucks to more game-rich areas become available. Agricultural crops are grown for sale in the regional markets and store-bought foods compose a large portion of the diets of Tsimane living in this village. Travel to San Borja occurs frequently, even daily for many residents as taxis and motorcycles can reach this community easily in the dry season.

The school in Tacuaral is made of concrete and concrete latrines were constructed nearby, but are rarely used for lack of maintenance. There was also a well located in a central area near the school, but during my time there it was not used as it was not properly functioning. At the time of data collection there were three school teachers, one Tsimane man and two non-indigenous women who lived part-time in the community. Tacuaral is also one of the sites of CETA, a traveling high-school that visits monthly, allowing village residents to complete a high school

diploma. Tacuaral also has a church that is led by Tsimane lay leaders who hold services every Sunday.

Most relevant to child health is Tacuaral's close proximity to the hospital and pharmacies in San Borja: they experience the lowest levels of infant mortality of all village types: IMR for infants <1 year is 8.5% and .08% for children aged 1-5 (Gurven and Kaplan et al. 2007). No interview data on infant feeding are available from Tacuaral; however interviews are available for mothers in San Miguel, a comparably integrated community. San Miguel mothers reported introducing complementary foods to infants at a mean 5.6 months of age. Of the San Miguel mothers, 58.8% reported giving infants plantain as a first food while and 41.1% fed their infants meat.

The Bioreserve Communities

Data were collected in the Bioreserve villages: Cedral, Puerto Triunfo, Chacal, and Monte Rosa. Established in 1982, the Reserve is a nationally protected area located along the Maniqui River downstream from San Borja. It encompasses 1,350 km² and is home to roughly 1,200 Tsimane' (Miranda, 1995). Use of the Reserve's resources is restricted to residents for subsistence purposes. Hunting and fishing occur with higher frequency than in the acculturated communities, and large fields of crops are cultivated for sale at local markets. Flooding in recent years (including 2006) has damaged fields and hurt the economic base of many Bioreserve communities. Wage opportunities generally exist outside of the Reserve, as either laborers for ranchers or itinerant river merchants, or through commercial logging. Whole families sometimes leave their villages for prolonged periods to engage in agricultural labor for neighboring Bolivian colonists who are farming and ranching in and around Tsimane territory. Trips to town occur frequently by dugout canoe, which takes about a day. It is also possible to walk about eight hours to the village of Galileo, from which it is a short taxi ride to San Borja. While infant mortality rates for the Bioreserve are as of yet unpublished, results of a preliminary analysis are 12.7% for infants <1 year and 2.3% for children aged 1-5. The sample of women interviewed in Bioreserve communities reported introducing complementary foods to their infants at a mean age of 4.7 months. While 93% of women interviewed provided plantain as their infant's first food, the Bioreserve was the only region where women (two) reported that sweet manioc was their infant's first food, and in both cases it was softened by boiling.

Cedral is one of the larger communities (37 families) and located furthest downstream from San Borja (around 26 miles). Cedral was the only Bioreserve community to have a trained health promoter. During the time of data collection, a concrete school was being built in Cedral that was completed shortly after our research team left. The village had one schoolteacher who was a Tsimane from the nearby acculturated community of San Antonio. Interestingly, the professor's own children lived with relatives in San Antonio during the week so that they could be at the school there with a very popular non-native Bolivian teacher. There were no concrete latrines and no well in Cedral at the time of data collection. Cedral received a gift of solar panels and a large television from the Venezuelan government purportedly to help the Tsimane improve their Spanish language abilities. The television arrived shortly and rapidly began to alter the daily routine of community members, who hurried to watch it each evening.

Puerto Triunfo is a small community (20 households) located between Cedral and Chacal approximately 24 miles downriver from San Borja. A cement well was constructed by the municipal government near the school in Puerto Triunfo during the data collection period and was still functioning when our research team left. A few families reported obtaining their drinking water from the well, however many families whose houses were not near the well continued to obtain their drinking water from the Maniqui River. The village had one Tsimane schoolteacher and the schoolhouse was constructed from traditional housing materials. They were anticipating a shipment of brand-new chairs and desks to arrive from town at the conclusion of the study. Puerto Triunfo was unique among the Bioreserve study communities in housing a small herd of cattle. The herd was owned by a non-native Bolivian and tended by a Puerto Triunfo villager.

Chacal is the largest of the Bioreserve communities (39 houses) and is located 23 miles downriver from San Borja. Chacal was the only community to have both a concrete school and well, both of which were constructed shortly before our arrival to the community. The well was used by the villagers who lived closest to it, by schoolchildren, and by men playing soccer in the large nearby soccer field. Most households continued drawing river water for drinking rather than trek all the way to the well. At one point, a child threw a battery in the well and its use was all but abandoned. Chacal had one Tsimane schoolteacher and an extensive library of children's books in both Spanish and Tsimane, though children were rarely observed reading outside of the classroom.

Monte Rosa is the smallest, and at the time of data collection, most dispersed and least integrated of the four Bioreserve communities. Located approximately 20 miles downstream from

San Borja, Monte Rosa had 21 households at the time of data collection. During my last visit, a new school was being built by the villagers, but only a frame was in place. They intended to fill in mud walls but lacked materials as it had not rained for some time. The school teacher was a non-indigenous Bolivian woman who purportedly lived in the village during the week, but parents complained that she was not present in the village or providing classes consistently. The community had no well at the time of data collection and all of the villagers obtained their water from the river except for one family whose home was very far from the central village. Their house was located next to a small lake that was their water source.

The Forest Communities

Data were collected in forest communities Apere, Cuverene, and Jamanchi, which are among the least acculturated of all Tsimane communities. Cuverene and Apere are located deep in the interior forest and far from the largest river system. Jamanchi is located closer to town, but is categorized as a forest community for reasons we will describe. Most forest communities have no school and little Spanish is spoken. These communities tend to be located near logging roads, which are maintained only as long as logging occurs, so these villages are relatively inaccessible throughout the wet season. Visits to San Borja are sporadic and infrequent, occurring only in the dry season (June-December). Hunting occurs frequently. While most men hunt with shotguns and dogs, several instances of bow-and-arrow hunting were observed in the least acculturated communities, Cuverene and Apere, mostly by young teenage boys and elderly men. Small fields are cultivated in these forest communities, mainly for household consumption. It was common for men to leave their families for weeks or months while working for logging companies. Analyses have shown that infants living in forest communities experience the highest level of infant and child mortality of all community types (17.1% for infants <1 year and 1.7% for children 1-5), probably due to their remote location and lack of access to health care facilities (Gurven and Kaplan et al. 2007). Mothers from least acculturated (forest villages) reported the earliest ages at supplementation with a mean of 3.8 months and 85.7% of mothers in Cuverene and Apere had fed their infants plantain as a first food, with the remaining 14.3% reporting fish.

Cuverene is located 68 miles from San Borja on the Fatima logging road. It was a small community (25 households) with no school, no well and no latrines at the time of data collection. This community was extremely difficult to access even in the dry season, as many rivers crossed

the road and bridges washed out frequently. Apere was located about an hour and a half's walk beyond Cuverene. Located 82 miles from San Borja, Apere also had no school, well or latrines. Drinking water was obtained from small rivers in both of these communities. During the rainy season, no outsiders entered these communities except for one research scientist from the Tsimane Amazonian Panel Study. Children participated in subsistence tasks from an early age since they were not in school, and large, village-wide chicha parties were held frequently in these communities. With the dry season came loggers who sold and traded distilled alcohol to the Tsimane, and many of the men left the community to work for long periods of time.

Jamanchi, the third forest community, is located 31 miles from San Borja in walking distance from Tacuaral. It is classified as a forest community for the purposes of this research because at the time of data collection, the community had recently been established by emigrants from interior villages who wanted their children to have the opportunity to go to school. A school building had been constructed from traditional materials but classes were not yet being held regularly. The community had no latrines or wells and water was obtained from nearby creeks. Infant mortality rates based on demographic interviews more resembled forest than acculturated communities in 2005, it is possible that after five years of access to medical care that IMR is improving.

Upriver Communities

Cosincho and Munday are located along the Maniqui River upstream from San Borja and experience moderate levels of acculturation. They are located roughly two times farther from town than the Bioreserve communities. Travel by river is a one or two day trip with an outboard motor (except during very wet or very dry times). Both upriver communities had schools, and many Tsimane from river communities are engaged in the market economy: rice, plantain, and jatata (woven palm leaves used for roofing materials) are sold and traded for canned goods, clothing, and school supplies. Men, women and children are involved with the production of thatch. Hunting and fishing occur more frequently in the upriver communities than in the acculturated ones, and visits to town are fairly frequent for many river families. Infant mortality rate is 14.7% for infants <1 year and 1.8% for children aged 1-5 (Gurven and Kaplan et al. 2007). Mothers from river communities reported supplementing their infants at 4.7 months of age respectively. Less than

half of mothers from river communities reported that plantain was their infant's first food (46.1%), and the remaining 53.8% reported that it was meat or fish.

Cosincho is located 56 miles upstream from San Borja and at the time of data collection had 40 families. Cosincho had obtained materials to finish constructing a half-built well at end of 2002. Once completed, the well was good for about a year, and even while functioning was only used by the families in the immediate vicinity. The school was made out of concrete and two latrines were located nearby but seldom if ever used. There were two Tsimane schoolteachers, one of whom had an outboard motor which made travel to town convenient. Hunting, fishing and agriculture were practiced as well as production of palm thatch. Sometimes entire household clusters would spend whole days engaged in palm thatch manufacture. A small store was also established by a villager in Cosincho during the data collection period, providing basic household items brought from town.

Nestled along a small tributary to the Maniqui, essentially Munday is located 59 miles from San Borja. It was the smallest community included in this study with households numbering just 14. Munday had no well or latrines at the time of the study and all drinking water came from the river and creek. The school was made out of typical housing materials with a dirt floor and a palm thatch roof. Munday is less acculturated than neighboring Cosincho. Fewer people speak Spanish and men engage in wage labor less frequently.

The Nature of Acculturation in Tsimane Communities

Table 1 summarizes each village's attributes, from which conclusions are drawn regarding the nature of acculturation in Tsimane communities. Forest communities are ranked least acculturated based on education, community sanitation, and distance from town. The up-and down-river communities are ranked moderately acculturated based on the same characteristics, and Tacuaral is ranked highly acculturated. While forager-horticulturalist societies are often described as homogenous, we observe substantial variation between the ten Tsimane study communities; furthermore these communities are observed to be highly dynamic over time. Tsimane have proven resilient to outside forces and adaptable in the face of global change.

APPENDIX C

Summary of characteristics of the study communities with acculturation rankings

Community	Apere	Cedral	Chacal	Cosincho	Cuverene	Jamanchi	Monte Rosa	Munday	Puerto Triunfo	Tacuara del Maipo
Community type	forest	riverine (downriver)	riverine (downriver)	riverine (upriver)	forest	forest	riverine (downriver)	riverine (upriver)	riverine (downriver)	acculturated
Years Sampled	weaning	weaning thymus	weaning thymus	weaning	weaning	weaning	Weaning (no observation) thymus	weaning	weaning thymus	weaning
Data Collected	2002-2003	2007-2008	2007-2008	2002-2003	2002-2003	2005	2007-2008	2002-2003	2007-2008	2005
Number of Households	16	37	39	40	25	25	21	14	20	56
Distance from SB	82	26	23	56	68	31	20	59	24	27
School	none	concrete school	concrete school	concrete school	none	traditional materials	traditional materials	traditional materials	traditional materials	concrete
Latrine	none	none	none	yes but rarely used	none	none	none	none	none	yes but rarely used
Water	river	river	river well	river well	river	river	river	river	river well	river well
Spanish	21%	63%	60%	55%	19%	65%	52%	46%	53%	72%
Years Education	0.1		2.1	1.3	.1	1.05	1.2	1.2	1.9	2.8
Level of acculturation	low	medium	medium	medium	low	low	medium	medium	medium	high

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