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Optimal exclusive breastfeeding duration:
evidence of conflict and congruence in Tsimane mother-infant pairs

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by

Melanie Ann Martin

Committee in charge:

Professor Michael Gurven, Chair

Professor Steven Gaulin

Associate Professor Brooke Scelza

Professor Hillard Kaplan

Professor Claudia Vallengia

December 2015

The dissertation of Melanie Ann Martin is approved.

Steven Gaulin

Hillard Kaplan

Brooke Scelza

Claudia Vaggia

Michael Gurven, Committee Chair

November 2015

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By

Melanie Martin

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ABRIDGED VITA OF MELANIE A. MARTIN

December 2015

EDUCATION

- 2007 BA in Anthropology, University of Puerto Rico, Río Piedras (Magna cum laude)
- 2011 MA in Anthropology, University of California, Santa Barbara (UCSB)
- 2015 Certificate in College and University Teaching, UCSB
- 2015 PhD in Anthropology, UCSB, December 2015 (expected)

PEER-REVIEWED PUBLISHED MANUSCRIPTS & BOOK CHAPTERS

- 2014 Veile A, **Martin M**, McAllister L, Gurven M. Modernization is associated with intensive BF patterns in the Bolivian Amazon. *Soc Sci & Med* 100:148-158.
- 2013 **Martin M**, Blackwell A, Kaplan H, Gurven M. Make new friends but keep the old? Parasite coinfection and comorbidity in Homo sapiens. In: *Primates, Pathogens, & Evolution*. Eds: Brinkworth J & Penchenkina E. Springer Pubs.
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GRANTS, FELLOWSHIPS, HONORS, & AWARDS

- 2013 President's Dissertation Year Fellowship, University of California
- 2013 Graduate Dean's Advancement Fellowship, UCSB
- 2012 Spaulding Service Fellowship, UCSB
- 2012 Doctoral Dissertation Improvement Grant, National Science Foundation (NSF)
- 2012 Dissertation Fieldwork Grant, Wenner-Gren Foundation
- 2010 Honorable Mention, NSF Graduate Research Fellowship
- 2010 Dean's Fellowship, UCSB Central Fellowship
- 2009 Research Experience for Graduates Supplemental Grant, NSF
- 2008 Dean's Fellowship, UCSB Central Fellowships

ABSTRACT

Optimal exclusive breastfeeding duration:

evidence of conflict and congruence in Tsimane mother-infant pairs

By

Melanie Martin

International health organizations recommend six months of exclusive breastfeeding (EBF) as optimal for infant health. Complementary feeding (CF) with any liquids or solids before this age risks increasing infant pathogen exposure and offsetting breast milk intake, which may increase risk of nutritional morbidity and early weaning. Globally, however, most infants begin CF well before six months—including in non-industrialized populations in which breastfeeding initiation and prolonged, on-demand nursing are universal. Why is early CF so common, even among populations in which prolonged EBF would be most protective for infants and feasible for mothers? Epidemiological research generally frames this discordance in terms of barriers to optimal practice and evolutionary research in terms of maternal-infant conflict—i.e. the benefits of reducing EBF costs for mothers may outweigh any risks for infants. An alternate and less-explored hypothesis is that early CF, if introduced without reducing breastfeeding intensity, may ultimately benefit infants.

I examine evidence of maternal-infant conflict and congruence in shaping patterns of early CF among the Tsimane. The Tsimane are an indigenous, high-fertility, high-mortality, forager-horticulturalist population residing in the Bolivian Amazon. Interviews, anthropometric, behavioral, and biomarker data were collected from a mixed-longitudinal sample of Tsimane mother-infant pairs from August 2012 – April 2013. Tsimane mothers exhibited universal and prolonged breastfeeding, with EBF durations of about 4 months on

average. Analysis of predictive factors and outcomes associated with age of CF introduction generally supported a Feeding Augmentation rather than a Feeding Substitution model of early CF. Mothers' reasons for introducing CF more often emphasized perceptions of infant needs than their own time or energy constraints. Earlier CF introduction (0-3 vs. 4-6 months) was associated with increased CF frequency at later ages, but not with subsequently lower frequency of breastfeeding bouts or earlier weaning. Among mothers, shorter EBF durations were not associated with biological indicators of reduced lactational costs. While primiparity and high parity were both associated with age of CF introduction, these factors, and not age of CF introduction, were associated with timing of resumption of menstruation. Finally, while before six months of age infants who were introduced CF earlier were relatively smaller as compared to EBF counterparts, after six months of age mean height-for-age was higher for infants introduced CF at 0-3 vs. 4-6 months. Earlier CF was not associated with greater likelihood of reported illness before or after six months of age.

Among the Tsimane early CF does not appear to benefit mothers at a cost to infants, and likely supplements, but does not supplant intensive breastfeeding. While the Feeding Augmentation Model was supported in this study, the Feeding Substitution Model may have more predictive power in populations in which bottle and formula feeding are more common. However, both models ultimately emphasize variability in optimal EBF durations, dually shaped by the needs of infants *and* mothers. This emphasis need not contradict current recommendations or related public health campaigns, but may be helpful in prompting parallel dialogues about optimal infant feeding practices for individual families.

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Chapter 1: Introduction

1.1. Evolutionary and global health perspectives on infant feeding practices

Maternal postpartum investment in offspring via lactation is a defining feature of mammalian evolution and reproduction (Pond, 1977; Blackburn et al., 1989). Species-typical lactational strategies—encompassing milk composition and yield, the frequency and duration of nursing bouts, the number and sex ratio of simultaneously birthed offspring, and the total duration of lactation—are tightly coupled to species' phylogenetic histories, ecologies, and patterns of growth and development (Hinde and Milligan, 2011). Human lactation and infant development are therefore similarly co-evolved (Humphrey, 2010). However, key aspects of human lactational strategies are highly varied across human populations, likely reflecting increased environmental, ecological, and cultural diversity during human evolution (McDade and Worthman, 1998). In particular, humans exhibit extreme variability in weaning practices—which encompass the timing of complementary food introduction, the relative contribution of breast milk and complementary foods to the weanling diet, and the age at full cessation of breastfeeding. This flexibility is unique even among other primates (Sellen, 2007, 2009). Today, our flexible weaning practices continue to influence differences in infant and maternal reproductive fitness across populations (Vitzthum, 1994), and are relevant to ongoing public health policy and debate (Sellen, 2007).

Human milk contains thousands of nutritional, immunological, hormonal, and other bioactive factors that have been selected over evolutionary time to protect infants against

invading pathogens, while supporting infant growth and immunological, behavioral, and cognitive development (Lönnerdal, 2000; Hamosh, 2001; Schack-Nielsen and Michaelsen, 2007; Ballard and Morrow, 2013). Estimates from high-income populations have shown that energy supplied by exclusive breastfeeding (EBF) is sufficient to support human infant growth through about the first six months of life, beyond which additional energy supplied by complementary foods is necessary to meet growing infant energetic demands (Dewey et al., 1991, 1995; Dewey, 1998, 2001a). Once infants begin complementary feeding (CF), nutritional and bioactive components in human milk continue to buffer infants against nutritional and infectious insults (Goldman et al., 1982, 1983; Dewey, 2001a), and may influence developing metabolic, immune, and behavioral systems (Martin and Sela, 2013). And yet, with adequate supplementation and minimal pathogen exposure, human infants can and do thrive on little to no maternal breastfeeding. Perhaps because of this, there are no established minimums for breast milk intake or breastfeeding duration shown to be critical for any essential infant functioning.

The use of alternatives to maternal breastfeeding is well-documented historically—e.g. wet-nursing (Hrdy, 1992; Maher, 1992), hand-feeding, and vessel feeding from cow horns, cups and spoons (King and Ashworth, 1987)—having long allowed for relatively early weaning or to compensate for maternal absence, death, or lactational failure. However, the spread of manufactured infant formulas, feeding-bottles, and rubber teats beginning in the 19th century, coupled with changing economic and social roles for women, sparked historically unprecedented declines in breastfeeding initiation and duration across populations (King and Ashworth, 1987; Wright, 2001). During this time in the U.S., shortened

breastfeeding durations were increasingly associated with infant mortality, giving rise to the origins of pediatric medicine (Piovanetti, 2001). As public health researchers called for interventions to promote breastfeeding across populations, debates over what breastfeeding practices to recommend ensued (Feachem and Koblinsky, 1984).

In 2002, the World Health Organization and many other national and international health agencies began recommending EBF for the first six months of life, with continued breastfeeding for two years or more (WHO, 2002; Cattaneo et al., 2011). These recommendations explicitly recognize breastfeeding as an “*unequaled way of providing ideal food for the healthy growth and development of infants [and an] integral part of the reproductive process with important health implications for mothers*” (WHO, 2002). In promoting these recommendations, the WHO emphasized that CF introduced too early *or* too late risks nutritional deficits, while continued breastfeeding with safe and energetically adequate complementary foods is necessary to meet evolving nutritional requirements across the first two years of life. Since 2002, reviews of the evidence for these recommendations have found a strong protective effect of breastfeeding against infectious morbidity and mortality in developing countries (Lamberti et al., 2011), and lowered risk of infectious morbidity across scales of economic development (Kramer and Kakuma, 2012). Though evidence is mixed, formula-feeding is increasingly associated with chronic disease at later ages as well, which may reflect differences in bioactive components in human milk vs. formula that alter immune, metabolic, and neurocognitive development (Schack-Nielsen and Michaelsen, 2007; Andersson et al., 2009; Martin and Sela, 2013).

Increased promotion of breastfeeding appears to have improved rates of breastfeeding initiation in many countries since the 1990s, but has had little effect on extending durations of exclusive and total breastfeeding (Lutter and Morrow, 2013). Recent estimates for rates of EBF in infants 0-5 months old average about 25% across lower- and middle-income countries (Black et al., 2008; Arabi et al., 2012; Cai et al., 2012; Lutter and Morrow, 2013), and are generally even lower in high-income populations, e.g. 15% and 17% in Canada and the U.S., respectively (Jones et al., 2011; Jessri et al., 2013). Across populations, concordance with recommended EBF and total breastfeeding duration is inversely associated with measures of economic prosperity (Arabi et al., 2012; Marriott et al., 2012) —suggesting that at the national level, expanded economic opportunities for women, coupled with reduced infectious disease risk and affordable and widely accessible breast milk alternatives, lead to decreased breastfeeding intensity. At the same time, concordance with recommendations *within* populations varies differently across economic scales. Indicators of higher socioeconomic status tend to be associated with shorter EBF durations in low- to middle-income countries (e.g. Adair et al., 1993; González-Cossío et al., 2006; Mahrshahi et al., 2010), but longer EBF durations in high-income countries (e.g. Li et al., 2002; Grummer-Strawn et al., 2008; Jessri et al., 2013). This pattern suggests the economic and opportunity costs and/or social valorization of breast- vs. formula-feeding have likely differently shifted in low- to high-income countries over the last several decades.

At the same time, early CF is also widely observed in so-called “traditional breastfeeding populations”—i.e. populations in which breastfeeding practices have not been disrupted by formula or bottle-feeding, and prolonged, on-demand nursing remains normative (Veile et al.,

2014). Seemingly, in such populations, prolonged durations of EBF would benefit infants at high risk of nutritional or infectious disease, while also remaining socially and economically feasible for mothers. This discordance raises fundamental questions with respect to current infant feeding recommendations: Do the energetic or economic benefits of early CF for mothers significantly outweigh the risks to infants even in traditional breastfeeding populations? Does early CF necessarily increase infant morbidity risks in these populations? Might there be factors other than economic or energetic trade-offs that better predict patterns of early CF in traditional breastfeeding populations?

In this chapter, I review the evolutionary origins of human infant feeding practices and explore the biological validity of current infant feeding recommendations, with particular attention paid to flexibility in the timing of CF introduction and weaning. Variability in the timing of these events is then discussed in terms of maternal-infant fitness conflicts. An evolutionary perspective predicts that early complementary feeding may be favored by mothers if the benefits to them, in reducing the energetic costs of lactation, outweigh increased risk of morbidity for infants. In this review and in subsequent chapters, I will additionally explore evidence for an alternate perspective, in which early CF does not reduce breastfeeding intensity, resulting in no energetic savings to mothers but potentially benefitting infants. Both patterns have relevance to public health debates, as they suggest that “optimal” ages of complementary feeding and weaning can shift depending on the needs of individual mother-infant dyads.

1.2. The evolution of complementary feeding: what is its meaning to weaning?

Mammalian lactation performs at least 3 vital functions: immune protection, nutrition, and fertility regulation (Sellen, 2009). It is also increasingly appreciated that immunological, nutritional, and hormonal factors in maternal milk influence the development of infant immune, cognitive, and metabolic function via several pathways, including interactions with resident gut microbiota (Martin and Sela, 2013). This lactational complexity has evolved over deep time, beginning with protolacteal immunological secretions enhancing egg and/or offspring survival in a Triassic mammalian ancestor (Lefèvre et al., 2010), followed by nutrient-rich secretions, specialized mammary glands, behavioral changes in offspring, and hormonal control of milk secretion and mammary gland development (Blackburn et al., 1989). Species-specific lactation strategies evolved in the last 200 million years following the emergence of this complex lactation system and the subsequent mammalian radiation (Lefèvre et al., 2010).

While the added immunological and nutritional benefits provide clear advantages for offspring (and maternal fitness benefits from enhanced offspring survival), lactation also appears to minimize mechanic and energetic costs of gestation for mothers relative to those of viviparous reptiles (Pond, 1977). Still, lactation is costly for mothers, involving the active conversion and transfer of maternal nutrients and body stores into substrate for infants (Hinde and Milligan, 2011). The energy cost of this conversion is greater than that required by gestation, with most mammalian mothers increasing their energy intake by 2 to 5 times above non-pregnant levels (Lee, 1996). Furthermore, in many mammals, including primates, lactation induces a period of postpartum infertility that modulates birth spacing (Lee, 1996;

McNeilly, 1997). This period of infertility—in humans termed “lactational amenorrhea”—is a facultative mechanism likely selected to optimally allocate energy investment across current and future reproduction. Though the exact proximate mechanisms of lactation-induced infertility have not been determined, growing evidence indicates that a number of hormonal pathways are involved, mediated by both offspring suckling intensity (signaling current infant energy demand) and maternal energy balance (signaling sufficient energy available for investment in future reproduction) (Valeggia and Ellison, 2009). As such, in order to shift somatic investment to future reproduction, mothers can reduce lactational costs either by completely weaning or at least decreasing infant dependency on maternal milk. Offspring themselves will also move away from lactational dependence as they mature and suckling intensity is increasingly offset by independent foraging (and provisioned food in the case of humans).

When and how the shift away from lactational dependence and towards future reproduction for the mother and nutritional independence for the offspring occurs varies according to a host of evolutionary and ecological factors (Lee, 1996). Unsurprisingly, researchers have grappled with the very “meanings of weaning” in trying to define and compare durations of lactational dependence across species. The term “weaning” has alternately been used to denote the complete cessation of suckling, the age at which suckling has been reduced to low levels that no longer inhibit successive conception, the age at which independent foraging accounts for the majority of nutritional intake, and the entire process from first introduction of non-milk foods to full cessation of suckling (Lee et al., 1991; Lee, 1996).

For many species, the timing of the two events which bracket the weaning process—the first introduction of non-milk foods and the complete cessation of suckling—may have only limited impact on maternal reproductive constraints or offspring nutritional independence (Lee, 1996; Humphrey, 2010). Offspring may remain primarily dependent on milk for some time after being introduced to other foods, resulting in relatively no offset in the energetic cost of lactation for mothers. Conversely, offspring may be almost entirely dependent on non-milk foods by the time of full weaning, likely signifying that mothers also have already shifted investment towards future reproduction (i.e. lactation with overlapping ovulation or gestation). Recognizing this, Martin (1984) advocated abandoning all definitions of weaning derived from specific events or thresholds in favor of one that borrowed from Trivers’

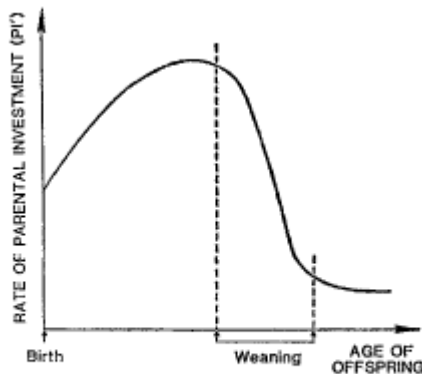


Fig. 1. General relation between the rate of parental investment (PI'), measured in units of parental resources invested per unit time, and age of offspring.

Fig. 1. Reprinted from Martin 1984

of the cumulative index of PI (PI). Weaning can then be viewed as “a phase during which the rate of PI' drops most sharply (Fig. 1), and may be best quantified through assessing changes in rate of milk transfer (Martin, 1984).

concept of parental investment, that is, any investment of biological resources in a current offspring that reduces parental ability to invest in future offspring (Trivers, 1972). As proposed by Martin, a change in the rate of paternal investment can be quantified as the number of units of parental resources invested per unit time, and calculated as the first derivative

However, such measures of weaning rates have not been widely adopted in the literature. While anthropologists generally characterize human weaning as a gradual process (e.g. McDade and Worthman, 1998; Dettwyler, 2004; Sellen, 2007), in the larger body of clinical and epidemiological research, infant feeding practices are typically couched in the terminology of specific events. For wider comparative purposes, I use this latter terminology throughout the dissertation. “CF introduction” refers to the first introduction of non-milk solids or liquids, and “weaning” denotes the full cessation of breastfeeding. In literature reviews and my own data analysis, I also use the term “weaning rate” (or “rate of weaning”) to denote increases in CF frequency that lead to reduced breastfeeding frequency, and by extension reduced rates of milk transfer. “Early CF” (before 6 months of age), is presumed to reduce the rate of milk transfer at a “suboptimal” trajectory, leading to relatively faster and earlier weaning.

As is evident historically and today, humans exhibit considerable variability in these specific weaning events and trajectories. This flexibility, however, derives from co-evolved traits that have diminished the energetic costs and constraints of lactation across human ancestry, namely: the surpluses generated in the context of skills-intensive food production strategies and divisions of labor by sex and age, which facilitate extensive paternal and alloparental investment, resource transfers, and pooled energy budgets (Hawkes et al., 1998; Kaplan et al., 2000; Panter-Brick, 2002; Hrdy, 2007; Hill and Gurven, 2009; Kramer et al., 2009). Enhanced dietary quality and availability may have allowed for earlier CF and weaning relative to other primates, while buffering infants against the risks associated with decreased maternal milk consumption. Decreased breastfeeding investment in turn may have

avored earlier resumption of postpartum ovulation and shorter interbirth intervals relative to other large-bodied primates (Short, 1994; Sellen, 2007; Humphrey, 2010). Researchers have estimated that, on average, human foragers wean 1-3 years earlier and have interbirth intervals that are 1-4 years shorter than those of wild chimpanzees, gorillas, and orangutans (Robson and Wood, 2008). As a cautionary note, primate interbirth intervals calculated from existing life history tables may not have sufficiently accounted for effects of infant mortality or nutritional condition, and varyingly underestimated or overestimated intervals (Borries et al., 2013). Still research generally suggests that humans exhibit shorter interbirth intervals and higher fertility relative to other primates, likely a result of enhanced dietary quality and allomaternal care favoring more flexible or earlier weaning (Galdikas and Wood, 1990; Kaplan et al., 2000; Sellen, 2007; van Noordwijk et al., 2013).

For humans, the flexible weaning process is a paramount example of parent-offspring conflict (Trivers, 1974). Conflict occurs because infants benefit unilaterally from exclusive maternal investment, but mothers must divide their investment across existing and future offspring. These investment decisions may be influenced by maternal condition, reproductive value, available resources, and offspring condition (Fairbanks and McGuire, 1995).

Breastfeeding is a form of costly investment that necessarily trades-off with other forms of fitness investment—i.e. somatic energy available for future reproduction and/or time devoted to nursing that cannot be spent on other fitness-enhancing activities (Hurtado et al., 1992; Panter-Brick, 1993; McDade and Worthman, 1998; Piperata and Dufour, 2007). Notably as compared to other mammals, primate lactation is less costly in terms of metabolic energy costs, but more costly in terms of *time* costs (Charnov and Berrigan, 1993).

In humans, nursing intensity and the relative energetic cost of milk production influence the length of lactational amenorrhea, and therefore mediate the somatic switch to future reproduction. Infant suckling stimulates the release of prolactin, which is necessary for milk production, and may also suppress ovarian function (Martin, 2007; Valeggia and Ellison, 2009). As will be discussed in more detail in Chapter 3, hormonal regulation of fecundity is further mediated by maternal energy balance and the relative metabolic cost of lactation for individual mothers (Ellison, 1994, 2001; Ellison and Valeggia, 2003; Valeggia and Ellison, 2003, 2009). Negative energy balance signals a state of energetic stress that prolongs the duration of lactational amenorrhea, whereas a period of sustained positive energy balance appears to signal sufficient energy to resume postpartum menstruation and ovulatory function (Valeggia and Ellison, 2009).

Early CF that reduces breastfeeding intensity may therefore trigger metabolic and hormonal pathways to resume postpartum menstruation relatively earlier, allowing for relatively earlier conception and, ultimately, higher fertility. Younger/lower parity mothers at the start of their reproductive careers and/or mothers with relatively more resources maybe better able to “afford” the risks of early CF—i.e. through benefits incurred by future reproductive investment and minimized risks to current infants.

However, cultural factors influencing both weaning dimensions and related infant health and nutritional outcomes hugely complicate this picture across human populations. Our understanding of biological constraints operating on human weaning trajectories has been somewhat muddled by the proliferation of modern breast milk alternatives in the 20th century. Are there any universals of human CF and weaning? How much of the variability evident in

those dimensions today might still be influenced by evolved biological relationships? With these questions in mind, I next evaluate evidence of the biological constraints on the timing of CF introduction and weaning. I consider differences in human and non-human primate offspring provisioning, as well as unique characteristics of human lactation and infant physiological development.

1.3. Weaning trajectories in primates

Primate offspring are weaned gradually over variable periods of ‘*transitional feeding*’, which are marked by mixed nutritional dependence on maternal milk and other foods (Sellen, 2009). These foods are acquired by independent foraging and/or shared by adults in the form of passive food transfers or direct provisioning. Most adult-offspring food sharing occurs between mothers and infants, generally with infants taking food from adults, who in turn, passively relinquish it (Brown et al., 2004; Jaeggi and van Schaik, 2011). Provisioning behavior is favored by kin selection, resulting in reduced time to weaning and/or enhanced offspring growth or survival. Adult-offspring food sharing has only been observed in about half of all primate species, including a few prosimians and Old World monkeys, most New World monkeys, and all of the great apes (Brown et al., 2004; Jaeggi and van Schaik, 2011).

Brown et al. (2004) proposed two (non-exclusive) conditions that could favor the evolution of adult-offspring food sharing, finding mixed evidence for both. The *informational hypothesis* predicts that foods that are difficult to acquire or process will be shared in order to facilitate offspring foraging knowledge and skill acquisition. The

nutritional hypothesis predicts foods of particularly high nutritional quality will be shared in order to increase maternal fitness via improved infant growth and survivorship and/or by allowing for earlier weaning and higher fertility. Two studies—a behavioral study of *Pongo* mothers and infants (Jaeggi et al., 2008) and a comparative analysis across primate taxa (Jaeggi and van Schaik, 2011)—have shown that offspring solicitations and food transfers are better predicted by the difficulty of food acquisition than by dietary quality, supporting the informational hypothesis.

There is less evidence in support of the nutritional hypothesis, as transfers among most primates occur too infrequently to be of major nutritional consequence, occur independently of nutritional quality, and/or peak *before* weaning (Jaeggi and Gurven, 2013). However, the nutritional hypothesis does appear supported in callitrichids—who exhibit extensive allomaternal care and feeding around weaning, along with twinning, high growth rates, and relatively short interbirth intervals. While the nutritional hypothesis has not been directly tested in humans, hominin evolution is characterized by increasing dependence on nutritionally dense and difficult to acquire foods, along with extensive allomaternal care and relatively short interbirth intervals (Aiello and Wheeler, 1995; Kaplan et al., 2000).

Interestingly, *direct* provisioning (or proactive food sharing) with offspring has also only been observed in callitrichids and humans (Geary et al., 2004; Jaeggi and Gurven, 2013)—two primate species distinguished by long-term monogamous pair bonds, substantial cooperative breeding, and direct paternal care (Kappeler and van Schaik, 2002; Burkart et al., 2009).

Ultimately, human infant feeding patterns differ from those of other primates in many ways that overlap with uniquely derived human features and life history traits. As reviewed by Sellen (2007, 2009) and van Noordwijk et al. (2013), human offspring, relative to the other great apes, are fatter at birth and weaned earlier, even though they have larger brains, higher energy needs, and remain dependent on direct provisioning from caregivers well into the juvenile period. At the same time, humans have higher fertility, exhibit more intense and systematic allomothering, and supply a greater proportion of infant supplementary food intake through direct provisioning. Thus, the pattern of infant feeding in humans—relatively early onset of complementary feeding, a prolonged mixed period of breast- and complementary feeding, and early weaning—is a uniquely derived variation of primate transitional feeding, which co-evolved with extensive allomaternal care, social foraging, active food sharing, and enhanced food processing and dietary quality shaped in part by increased capacity for cooperation and technological innovation (Sellen, 2007, 2009; van Noordwijk et al., 2013).

1.4. Biological norms for human CF introduction and weaning

While prolonged periods of exclusive and mixed breastfeeding would have been normative during most of human ancestry, there are debates as to what our species typical breastfeeding trajectory is, and the extent to which it may have varied in ancestral populations. From a biological perspective, the lower limit of EBF duration may be the age at which infants can efficiently manipulate, process, digest, and absorb solids and other liquids

outside of human milk. These abilities require the coordination of multiple physiological systems that are not present or fully mature at birth, and appear to develop between 4-6 months of age. For example, the two halves of the mandible are not fully fused until about 5 months of age, deciduous dentition doesn't begin to emerge until about 7 months, and peak adiposity—which may help buffer against weaning-related nutritional insults—is reached at about 6-7 months (reviewed in Humphrey 2010). In terms of infant digestive capabilities, renal capacity and tolerance of non-milk solute loads are limited until about 4 months of age, fat absorption and production of salivary and pancreatic amylase do not reach adult levels until around 6 months of age, and gastric capacity increases from only 10 to 90 ml from birth to 6 months (reviewed in Hendricks and Badruddin, 1992). Additional cross-cultural evidence in support of the notion that the current EBF recommendation might approximate a biological norm comes from an ethnographic review of 42 non-industrialized societies. In this survey, the modal age of CF introduction was 5-6 months, with the average ages of liquid and solid food introduction at 4.5 ± 6.0 and 5.0 ± 4.0 months, respectively (Sellen, 2001).

Several lines of evidence also suggest that six months may be the upper limit at which EBF is sufficient to supply growing infants with all of the energy and nutrients they require. The onset of copious milk production (lactogenesis stage II) occurs generally after 60-72 hours postpartum (Kulski and Hartmann, 1981; Neville and Morton, 2001). Following this period, a mother's rate of milk secretion is primarily determined by infant demand—i.e. suckling rate and mammary-gland emptying (Wilde et al., 1995)—and steadily increases over the first month of lactation (Kent, 2007). With on-demand feeding, milk yield and macronutrient composition remain relatively stable over at least the next six months (Dewey,

2001a; Mandel et al., 2005; Kent et al., 2006), even under conditions of severe maternal malnutrition (Prentice and Prentice, 1995). After about six months, however, increasing energy demands outstrip maximum milk yield, and infant prenatal stores of iron, zinc, and vitamin D (which also has low availability in milk) become depleted, warranting the introduction of complementary foods to sustain optimal growth and development (Dewey, 2001a; Butte et al., 2002).

Once complementary foods are introduced, continued breastfeeding up through the first two years of life does appear to be evolutionarily favored. First, in Sellen's survey of 113 non-industrialized populations (2001), the modal age of weaning was 30 months, with no observation of weaning before 12 months of age. Secondly, infant immune function, postpartum brain growth, and the ability to efficiently process and digest non-milk foods are still immature up to at least two years postpartum (Hendricks and Badruddin, 1992; Holt, 1995; Milligan and Bazinet, 2008). In the latter case, gastric capacity is still only 480 ml at one year, pepsin secretion (necessary for protein absorption in the stomach) does not reach adult levels until 2 years of age, tongue flexibility and chewing ability do not mature until 1 year of age, and manual dexterity continues to develop during the first 2 years of life (reviewed in Hendricks and Badruddin, 1992).

During this time of continued postpartum development, maternal milk would have remained a vital source of immunological and nutritional buffering. For example, after initial decreases during the first three months of lactation, milk concentrations of lactoferrin, lysozyme, and sIgA—all important antimicrobial constituents—remain stable up through two years of lactation (Goldman et al., 1982, 1983). Second, as was shown U.S. infants not

supplemented with formula or cow's milk and gradually introduced CF at 6 months, average volume of milk intake decreased from a maximum of about 875 ml/day after 7 months, but remained at 550 ml/day up through 11-16 months (Dewey et al., 1984). Another study of U.S. infants showed that breastfed infants consumed at least 300 ml/day of milk for up to 30 months under mixed-feeding regimens (Kent, 2007). Under more impoverished conditions, contributions from maternal milk may be more substantial. Previous studies of lactation among Amele mothers (who introduced CF somewhat later at 7-8 months on average), demonstrated that milk production peaked at 9 months and declined slowly thereafter, with production still at more than 50% of peak output at 24 months (Jenkins et al., 1984; Orr-Ewing et al., 1986; Worthman et al., 1993). In terms of milk composition, lactose and protein concentrations remain largely unchanged from 4 to 20 months of lactation (Dewey et al., 1984), fat and total energy concentration remain constant or increase up through 39 months of lactation (Dewey et al., 1984; Mandel et al., 2005), and the composition of specific milk fatty acids may be constant up through 24 months of lactation (Mitoulas et al., 2003; Martin et al., 2012). Studies of children in both developed and developing populations have shown that continued breastfeeding from 11-23 months of age provides between 30-50% of energy requirements, 19-43% of protein requirements, and 25-75% of micronutrient requirements for vitamin A, vitamin B12, calcium, and vitamin C (Dewey et al., 1984; Dewey, 2001a; Onyango et al., 2002).

Beyond this, estimates for a species-typical total duration of breastfeeding are more variable. Biologists have proposed varying physiological thresholds to predict weaning across species, including: the age at first permanent molar eruption (Holly Smith et al., 1994), the

age at which birth weight is quadrupled (Lee et al., 1991), and the age at which offspring attain 1/3 of adult body size (Charnov and Berrigan, 1993). Dettwyler (2004) and Humphrey (2010) have applied these models to humans and estimated a “natural” human weaning age between 2.5 and 7 years. A recent isotopic analysis of North American hunter-gatherer skeletal remains supports this age range, with weaning estimated as early as 1-2 and as late as 5-6 years, for an average of 3.4 years for all specimens analyzed (Eerkens and Bartelink, 2013). Finally, the onset of lactase nonpersistence—the down-regulation of lactase activity that occurs in individuals who do not carry the dominant alleles for adult lactase persistence—appears to be a genetically heterogeneous trait, beginning at varying ages after the first year life (Wang et al., 1998).

Dettwyler (2004) has argued that cultural preferences arising with the proliferation of breast milk alternatives have driven the high prevalence of weaning before 2.5 years observed over the past half century. While this is certainly true, she goes on to argue that humans’ natural weaning age should fall closer to 6-7 years, to compensate for continued physiological development and maturation of immune and neurological functioning (Dettwyler, 2004). However, this duration may more realistically be an upper limit on breastfeeding than a species-typical or evolutionary optimal weaning age for modern *Homo*. Weaning estimates from the mammalian-derived biological models should be cautiously interpreted for the great apes, and particularly humans, as these species do not fit the general mammalian patterns observed for several traits (Harvey and Clutton-Brock, 1985; van Noordwijk et al., 2013). First, in no great apes do weaning ages coincide with eruption of the first molar (Humphrey, 2010). Second, while body size predicts longer durations of gestation

and lactation across primates (Harvey and Clutton-Brock, 1985), the energetic costs of lactation for anthropoid primates are actually lower than would be predicted by body size and are lowest for humans—requiring only about a 30% increase in energy intake as compared to a 50% increase for baboons and the 200-500% increases required by most other mammals (Lee, 1996). When scaling relationships of maternal body mass are restricted to only hominoids (as opposed to all mammals or even all primates), the resulting predicted weaning age for humans is 1,078 days, or about 3 years, for female body mass of 55 kg (Martin, 2007). Third, though the average weaning age of humans is younger relative to the other great apes (Kaplan et al., 2000; Emery Thompson, 2013), it does not correspond with full nutritional independence. Early weaning with continued nutritional dependence is observed in only a few non-human primate species that exhibit allomothering (Brown et al., 2004). Indeed, humans exhibit the longest period of post-weaning nutritional dependence but substantial paternal and inter-generational alloparental care (Sellen, 2007). Finally, in Sellen's (2001) ethnographic survey, weaning ages beyond 3 years were documented in less than 15% of the populations with available information. In sum, the upper estimated range of weaning for humans, while biologically feasible, is incongruous with comparative primate evidence and empirical observations from natural fertility populations suggesting most infants are weaned by 2-3 years of age.

Perhaps more importantly, estimates of any narrowly defined “normal” duration of exclusive or total breastfeeding duration optimal for infant needs assumes constant rates of milk energy availability and infant growth, development, and suckling demand. Variation in individual lactational performance and infant energy or nutrient demands may necessitate

earlier, but still optimal, CF and weaning. For example, as will be discussed in greater detail in subsequent chapters, studies have shown that up to 50% of women (within and across populations of all stages of economic development) report problems maintaining sufficient milk supply to meet infant demand (Tully and Dewey, 1985; Obermeyer and Castle, 1996; Gatti, 2008; Li et al., 2008). Researchers have long contended that only 5-10% of women experience primary insufficient lactation owing to physiological problems (Vahlquist, 1981; Neifert, 2001), with most instances of low milk supply resulting from sociocultural factors that inhibit suckling frequency (Gussler and Briesemeister, 1980; Greiner et al., 1981). However, research increasingly suggests that a number of factors related to infant gestational age and maternal stress and metabolic condition can delay the onset of lactogenesis, resulting in chronic problems with milk supply (Dewey, 2001b; Stuebe, 2014). Regardless of the underlying causes, milk supply that is insufficient to meet infant needs requires supplementation.

Second, maximum milk yield may be set by maternal nutritional conditions during pregnancy (Hinde and Milligan, 2011), infant birth weight, and postpartum maternal energy balance and infant growth trajectories. Variation in these conditions would predict individual-specific optima for age at CF introduction. For example, the *weaning weight hypothesis* suggests that mammalian infant energy needs outstrip the energy yield supplied exclusively through maternal milk when infants have approximately doubled their birth weight (Lee, 1996). For breastfed human infants under optimal conditions, this occurs on average at 5.3—8.8 kg, or anywhere from 3.5—5 months (Humphrey, 2010). Finally, milk macronutrient composition is also more sensitive to variation in maternal factors from 3- 12 months than at

earlier lactational stages (Nommsen et al., 1991), and maternal diet substantially affects availability of milk fatty acids (Yuhas et al., 2006; Brenna et al., 2007; Martin et al., 2012) and vitamins A, B₆, and B₁₂ (Dewey, 2001a; Butte et al., 2002). Unmet availability of these latter micronutrients may be more a limiting factor on the duration of EBF than that of milk energy or protein content, particularly if infant stores are low from birth (Dewey, 2001a). For at least some infants then, pre- or postnatal conditions affecting birth size, milk production, milk composition, and fetal stores of essential nutrients may warrant CF before six months of age. Once complementary foods have been introduced, the contribution of CF to the infant's diet may then varyingly affect infant satiety, suckling intensity, and the subsequent rate of weaning. For example, changes in milk protein, sodium, lactose, calcium, and zinc may occur once milk volume drops below 300-400 ml/day (Dewey et al., 1984; Neville et al., 1991), which may decrease infants' suckling demands and drive "self-led" weaning at any age.

Finally, disease risks and nutritional quality of complementary foods vary across environments, such that different proportions of breastfeeding and CF in the weanling diet will have different effects on infant fitness. The nutritional contribution of breast milk at later ages will be more substantial if high-quality complementary foods are not readily available. For example, the diets of weaned Kenyan toddlers in a recent study were about 68% lower in fat, 55% lower in vitamin A, and 45% lower in niacin as compared to their mixed-breastfeeding counterparts (Onyango et al., 2002). In contrast, formula-feeding appears to promote faster growth (Dewey et al., 1991; Heinig et al., 1993; Cohen et al., 1995), and may thus favor earlier weaning if negative effects on infant health outcomes are not immediately discernible.

Finally, it must also be emphasized that, like current infant feeding recommendations, the biologically derived estimates for species-typical EBF and breastfeeding durations are *infant-centered*. Even when considering variation in infant growth and development, these biological norms do not consider *maternal optimums*. Maternal reproductive interests and energetic demands—including somatic investment in future offspring, energetic investment in existing offspring, and other economic pursuits that maintain maternal and household well-being—exert equal if not greater influence on infant feeding decisions.

1.5. Variation around the optimal duration of EBF: significance for public health

As is evident from the above discussion, EBF and total breastfeeding durations are not rigidly physiologically constrained. Though ancestrally (and still in many populations today) pathogen risk and food insecurity would have selected for generally intense breastfeeding practices, *flexible CF is our evolutionary reaction norm*. Considering this, what proportion of mothers who initiate breastfeeding, but then do not exclusively breastfeed for six months, are actually responding appropriately to their own optima? In populations in which bottle-feeding is common, are most mothers who introduce CF before 6 months really “failing” to meet infant optima because of a lack of knowledge or social support for breastfeeding? The widespread prevalence of early CF among traditional breastfeeding populations contradicts this perspective and demands an alternate explanation. For example, in Sellen’s (2001) survey, non-milk provisioning before 6 months was reported in more than 70% of the populations surveyed. Unfortunately, because most ethnographic accounts did not provide detailed descriptions of CF practices (Sellen, 2001), the relationship of early CF to

subsequent infant health and weaning trajectories in these populations is unclear. Was “traditional” non-milk provisioning at 4-5 months substantial enough to influence subsequent breastfeeding intensity, infant nutritional status, or infant pathogen exposure? What factors contributed to variability around the average—particularly with respect to very early and very late CF introduction?

Without a doubt, the current WHO recommendation of EBF for 6 months is laudable as a simple and easily communicable public health message that is *generally* biologically appropriate for our species. However, the current recommendation may also too rigidly discount the influence of opposing maternal constraints, local variation in infant growth and developmental trajectories, and any possible benefits to early complementary feeding. That is, the current recommendation for EBF may fundamentally exceed the needs and realities of many mother-infant pairs. The inherent danger in this is that many mothers may ignore current recommendations altogether—and in doing so miss out on other relevant information about safe, healthy CF practices, or the nutritional and immunological benefits of prolonged *mixed* breast- and complementary feeding.

In subsequent chapters, this dissertation explores and tests two models to account for early CF in the absence of social and economic factors that promote formula-feeding: the Feeding Substitution Model and the Feeding Augmentation Model. Though the data presented are drawn from a traditional breastfeeding population—Tsimane forager-horticulturalists of Bolivia—the underlying theoretical basis for and conclusions drawn from this work are broadly relevant to maternal and child health research. The first model builds on existing theoretical models that propose maternal fitness trade-offs can predict variation in

breastfeeding decisions (McDade and Worthman, 1998; Tully and Ball, 2013). These models are drawn from parent-offspring conflict theory (Trivers, 1974) and have universal applicability to present-day patterns of breastfeeding variation, but fundamentally rest on the assumption that CF reduces breastfeeding intensity. Thus, the Feeding Substitution Model poses that increased CF benefits mothers by relaxing the energetic costs of breastfeeding, despite increased costs to infants resulting from pathogen exposure and/or suboptimal nutrition. In contrast, the Feeding Augmentation Model proposes that early CF actually benefits infants, with minimal to no effect on breastfeeding intensity. Under this model, early CF supplements, but does not *supplant* infant breast milk intake, in order to buffer infants against poor lactational performance, promote growth, and/or support immune maturation. The Feeding Augmentation Model therefore predicts that early CF results in no energetic savings to mothers, but provides a net benefit to infants relative to the costs of increased pathogen exposure that would result if breastfeeding were reduced.

Subsequent chapters are organized as follows. Chapter 2 describes methods of field data collection and laboratory analysis. Chapter 3 is an ethnographic and quantitative study of Tsimane maternal and infant life from pregnancy through CF introduction and weaning. This chapter will establish the social, economic, and ecological landscapes of Tsimane life that influence variation in maternal and child health and nutrition. Chapter 4 tests if early CF among Tsimane mothers is associated with maternal energetic and reproductive benefits or lactational performance, as would be alternately predicted by the Feeding Substitution and Feeding Augmentation Models. This chapter examines both proximate and ultimate associations, by testing for evidence of maternal energetic savings afforded by early CF, and

for associations between the timing of CF introduction and maternal reproductive fitness.

Chapter 5 tests the balance of infant costs and benefits associated with early CF—in terms of infectious morbidity and nutritional status—that may alternately support either the Feeding Substitution or Feeding Augmentation Model. Lastly, chapter 6 summarizes and discusses the broader implications of findings presented throughout the dissertation, and suggests future directions for research and public policy.

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Chapter 2: Study population and field data collection

Note: this chapter describes the field site, study communities, methods of field data collection, and laboratory analysis. A more detailed description of the study population is given in Chapter 3. Statistical methods for specific data analyses are given concurrently with their respective research aims and results in Chapters 3-5.

2.1 Study population: the Tsimane and their territories

Field work was conducted with the Tsimane in Bolivia. The Tsimane are an indigenous forager-horticulturalist population residing in the northern lowland Beni Department along the Maniqui river system (Figure 1). The current population is estimated at 15,000, distributed over 90 villages of varying size (~80-500, mean \pm SD individuals = 124 \pm 98) (Miner et al., 2014; Reyes-García et al., 2014; Veile et al., 2014). Their largely subsistence-level economy remains centered on slash-and-burn horticulture, hunting, fishing, and foraging, with occasional cash income generated from wage labor, working as a ranch hand, and sales of crops and domesticated animals (Reyes-García et al., 2007; Gurven et al., 2010). The local climate is characterized by relatively constant high and humid temperatures, with dry and wet seasons lasting on average from May – September and October – April, respectively (Godoy 2008). For the present study, I categorize the months of August – October 2012 as the dry season (monthly rain fall of less than 100 cm/month, average 57.4 cm/month), the months of November 2012 - April 2013 as the rainy season (more than 100

cm/month, average 203.4 cm/month), with peak rainfall occurring in December (Table 1).

Previous studies have shown that infectious morbidity and infant mortality are increased during rainy seasons (Gurven 2012). Seasonal rainfall also influences subsistence activities in family fields (*chacos*) and dietary availability of crop and foraging yields. Fields of rice and maize are generally burned and planted in August and harvested in January-February of the following year. Fields of sweet manioc can be planted year-round. Fishing is best during the dry season, when rivers are low and fish migrate to interior lagoons. Hunting is also generally better in the dry season—when animals congregate at known watering holes—though hunters may also be successful in locating prey on high ground during seasonal flooding.

Genetic and linguistic evidence suggests that the Tsimane originally migrated from the highland region into the Piedmont—a forested transitional zone between the Andes and the Amazon—and thereafter remained relatively isolated from neighboring Andean and Amazonian groups (Bert et al., 2001; Corella et al., 2007). Historical accounts place them in the present area in the 1600s, and note that they resisted missionary settlement during the Colonial period as well as participation in the expanding rubber and cattle markets during the 19th century (Chicchón, 1992).

Beginning in the 1950s, national policies aimed at increasing agricultural productivity opened up new roads into Tsimane territories, resulting in increased migration and deforestation that forced many subsequent resettlements and territorial disputes in the area (Bottazzi, 2008). As reviewed by Reyes-Garcia (2014), in 1978 the Bolivia government placed an embargo on logging in the Chimane Forest, but in 1986 ceded 579,000 hectares of the southern Tsimane territories to create a Permanent Production Forest. In 1987, another

135,000 hectares of Chimane Forest around the lower Maniqui River were diverted to create the Estación Biológica del Beni (EBB), a protected area which encompassed some Tsimane villages. A series of decrees in the 1990s formally established Tsimane territorial rights to a 392,220 hectare area along the Maniqui River (the Territorio Indígena Chimane, or TICH), and established the Tierra Indígena Parque Nacional Isiboro-Secure (TIPNIS)—a dual status protected area and indigenous territory (for Tsimane, Moxeño, Yuracaré, and Movima ethnicities) (Reyes-García et al., 2014).

Though the Tsimane have steadily increased integration into the local market economy, they remain relatively insular, with only about 7% of Tsimane living outside designated territories (Godoy et al., 2007). While many villages have schools, the quality and consistency of available education tends to be poor. On average, measures of completed education, literacy, and Spanish fluency are low across the population, especially for women (Godoy et al., 2007). Most Tsimane live in homes constructed from locally sourced forest materials, without plumbing or electricity, and rely on water from rivers, streams, and less frequently from wells constructed centrally or near home. Contraceptive use and access to quality medical care is generally limited, with the result that Tsimane rates of fertility and infectious disease morbidity are higher than rates at the national level (Gurven et al., 2007; Gurven, 2012). In general, resource transfers and cooperation among kin remain vital to Tsimane survival and livelihood (Gurven et al., 2012).

Current Tsimane settlements cluster by regional ecology—i.e. riverine or interfluvial forest settlement—and in relation to the local municipality of San Borja (pop. 45, 692, INE 2011)—i.e. upriver, downriver, near town, and interior forest (Figure 1). These geographic

regions are characterized by differences in diet, physical activity, wage labor participation, disease exposure, health care access, and other aspects of integration with the local *Borjano* economy and culture (Vadez et al., 2004; Gurven et al., 2007, 2013; Undurraga et al., 2010; McAllister et al., 2012; Veile et al., 2014). Despite recent gains in political power at the national and municipal level (Reyes-García et al., 2014), most Tsimane continue to face discrimination and limited opportunities for upward mobility (McAllister et al., 2012).

2.2 Study communities

Nine Tsimane villages were selected as field study communities. The study villages vary in ecology, size, and distance to San Borja (Fig. 1, Table 2). Four of the villages—Monte Rosa, Chacal, Puerto Triunfo, and Cedral—are located downriver at least 29 km away from San Borja, and are only accessible by foot (up to one day travel) or boat (6-12 hours, depending on village and season). Monte Rosa, Puerto Triunfo, and Cedral are located in the protected EBB area. While most household residences in Chacal fall outside of the protected area, the EBB boundaries similarly limit village access and subsistence activities.

The remaining five villages include two downriver villages (Campo Bello and San Antonio), two upriver villages (Santa Anita and Alta Gracia), and one village near town (Manguito) located off a main roadway. These villages are all located less than 20 km from San Borja. Alta Gracia is only accessible by boat (1-2 hours). Campo Bello, San Antonio, and Santa Anita are accessible by boat (2-4 hours) or auto (~1 hour), and Manguito only by auto (~30 minutes). Boat travel observed in the study villages refers to homemade canoes fitted

with a *pequi pequi*—an outboard motor that allows for relatively slow navigation in shallow waters. Auto travel during the field season was also frequently restricted during the rainy season due to poor road conditions.

Incidentally, in September of 2012, the San Borja municipal government, with support of Tsimane villagers, proposed to expand the dirt road from Campo Bello to Chacal. The EBB refused to authorize the expansion, however, because of concerns that it would increase illegal logging in the protected area. At a community meeting in Campo Bello on September 5, 2012, many attending Tsimane, who supported expanding the road, angrily contested the EBB's position, and resolved to have the director fired from his position (which never happened). A large portion of the road leading from Campo Bello to Monte Rosa (outside of the EBB protected area) was eventually cleared with tractors, but was not maintained and had largely grown over and became impassable by January 2013. With the onset of the rainy season, any future plans to expand or maintain the road were at least temporarily abandoned.

2.3 Permissions and Consent

All study protocols were approved by the University of California Santa Barbara Institutional Research Board Human Subjects. Study protocols were also reviewed by Michael Gurven, co-director of the Tsimane Health and Life History Project. The THLHP has worked with the Tsimane since 2002, providing primary health care while collecting biodemographic data on aging and health. Permission to conduct research and provide treatment is granted to the THLHP and affiliates via the local municipal government of San

Borja, the Hospital of San Borja, and annually renewed contracts with the Tsimane governing council (El Gran Consejo Tsimane').

The Gran Consejo Tsimane' and the director of the Estación Biológica de Beni (EBB) additionally reviewed protocols for and authorized the present study in July of 2014. Per existing THLHP protocols, the purpose of the study was then explained to focal villages in community meetings held prior to beginning data collection. Village leaders from Chacal, Cedral, Campo Bello, and Alta Gracia also gave me and my research team permission to live and work in these communities. I accompanied the THLHP mobile biomedical team to the villages of Santa Anita and Manguito.

Individual consent to participate was granted by subjects at the time of interview. Consent was given orally, as the majority of Tsimane women are illiterate. For each initial and follow-up visit, participants were compensated for their time with small care packages that included household goods (e.g. yarn, thread, combs) and over-the-counter medicines (e.g. paracetamol, hydrogen peroxide, salve).

As an aside, in January of 2013, the Director of the EBB suspended all research access to Tsimane villages located in the EBB perimeters (Monte Rosa, Cedral, and Puerto Triunfo). This decision was not a result of any of my actions, but was instead a concession to officials in the Bolivian Ministerio de Medio Ambiente y Agua (MMAyA) who had become increasingly suspicious of U.S.-based research and NGO activities in Bolivia. Following new protocols, I re-applied for permission to work in the EBB and in March of 2013 was granted temporary permission to continue my research by both the EBB and the MMAyA. However, the conditions of the authorization specifically stipulated that I could not collect any

biomedical samples in EBB villages. This condition coupled with the long delay in returning to the EBB villages resulted in a large gap in prospective sampling and truncated data collection for some subjects.

2.4 Field Data Collection

2.4a. Study design and aims

A mixed-longitudinal study was proposed and approved by my Dissertation Committee in the Winter of 2012. Funding was provided by the National Science Foundation (Doctoral Dissertation Improvement Grant # 123-2370) and the Wenner-Gren Foundation (Dissertation Fieldwork Grant). Field data collection included interviews, illness and dietary recalls, maternal and infant anthropometric measures, urinary samples, and behavioral observations. These methods together provide multiple measures by which to assess the complex relationships mediating infant feeding decisions and subsequent maternal and infant outcomes. Specific measures analyzed in subsequent chapters include the following: maternal perceptions of infant condition and reasons for beginning complementary feeding (from ethnographic interviews); infant morbidity, feeding status, and dietary quantity and quality (from illness and dietary recalls); infant nutritional status and growth, and maternal nutritional status and changes in energy balance (from anthropometric measures); maternal C-peptide concentrations (from urine sample, a marker of insulin activity and measure of the metabolic cost of lactation); infant interactions with mothers and alloparents, complementary feeding frequency, and breastfeeding frequency and duration (from participant observation).

Interviews and anthropometrics were collected cross-sectionally from mothers of infants 0-36 months old, to allow for a robust survey of Tsimane infant feeding practices in association with nutritional status and local regional, seasonal, and demographic variation. A subset of infants aged 0-12 months old from the cross-sectional sample was followed prospectively over the next ~6 months to track changes in complementary feeding, maternal metabolic costs, and infant growth and morbidity. Together, the study aims to paint a comprehensive picture of why there is individual variation in the duration of exclusive breastfeeding among Tsimane families, and how this variation in turn affects maternal and infant metabolic and health outcomes.

2.4b. Participant recruitment and cross-sectional sampling

Field data collection was carried out from July 2012 through March 2013, with the help of trained Tsimane and U.S. research assistants (Figure 2). Six villages were initially selected and visited between July and August 2012, with data collection continuing in those villages throughout the duration of the study. These villages were selected because of their variation in distance to San Borja (see Table 2), and aggregate size, which allowed for maximum recruitment of subjects (estimated from census data at ~50 mother-infant pairs) in relatively close proximity. We established moving base camps in three villages (Chacal, Campo Bello, and Cedral), allowing for simultaneous data collection in neighboring villages approximately every three weeks, as follows: (1) Chacal, Monte Rosa, Puerto Triunfo; (2) Cedral, Puerto Triunfo; (3) Campo Bello, San Antonio. This sampling scheme was chosen to minimize time

elapse between collection of biological specimens at participants' homes and the field laboratory at base camp (< 1 hour by bike and < 2 hours by foot, depending on weather conditions). Three additional villages were included in the study and visited between January and March of 2013. These latter villages were chosen to increase representation of more acculturated villages in the sample. Two of these villages (Manguito and Santa Anita) were visited in conjunction with the THLHP mobile team; the third, Manguito, was the natal village of one of my translators.

In all villages, we attempted to locate and recruit all families with children aged 0-3 years to participate. One family declined to participate, and interviews were not successfully coordinated with another 13 families who initially agreed to participate. The final sample included 161 families from 150 households, representing 92% of all resident families.

During in-home visits lasting approximately 1.5 hours, anthropometric measures were taken from participating mothers and infants, and mothers were administered an ethnographic interview and 24-hour dietary recall. Most interviews were conducted in the Tsimane language by one of two Tsimane assistants (Jaime Durbano and Bernabe Nate), with either myself or a U.S. assistant (Geni Garcia) observing and recording answers. I also conducted 10 unassisted interviews (in Spanish) with mothers who were native Spanish speakers and of Yuracaré or mixed-Tsimane ethnicity.

The ethnographic interview consisted of structured short-answer questions about the focal child's birth, breastfeeding, and complementary feeding experiences (see Appendix). The 24-hour dietary recall was used to reconstruct all foods and liquids consumed by the focal child the day prior. The dietary recall was modeled after World Health Organization

guidelines for assessment of age-appropriate Infant and Young Child Feeding (IYCF) practices (WHO, 2008), and adapted to capture local dietary composition and food availability (see Appendix). All interviews were written in Spanish, translated into Tsimane by one of the research assistants, then back-translated into Spanish by the second research assistant to correct any errors in translation.

All anthropometric measures were taken by myself, and included the following: height/length and weight (mothers and children), head circumference (children only), and % body fat, waist and hip circumference, bicep circumference, and skinfold bicep, tricep, subscapular, suprailiac thickness (mothers only). Infant recumbent length was measured to the nearest 0.1 cm using a pediatric measure mat. Maternal and child standing heights were measured to the nearest 0.1 cm using portable stadiometer (Seca 217). Skinfold thickness was measured to the nearest mm (Lange Skinfold Caliper). To minimize logistical difficulties resulting from extensive daily travel between participant homes and study villages, both maternal and child weights were measured to the nearest 0.1 kg with a digital scale (Tanita BF680W Duo Scale), using the tare method to weigh infants-in-arms. The scale was placed on a small raised wooden platform to minimize measurement error on the uneven surfaces of participant homes. As per standard protocols used by the THLHP medical team, all subjects were weighed fully clothed (Tsimane women typically wear lightweight skirts and tops). Infants and young children are typically dressed in only a t-shirt or a t-shirt with lightweight cotton pants (they do not wear diapers). Infants were removed from swaddling materials before measurement. Weight and standing height was measured barefoot. Maternal body fat percentage was estimated from the digital scale via low-level bioelectric impedance.

Standardized z-scores for child weight-for-age, length/height-for-age, and weight-for-length/height were calculated using WHO ANTHRO (v.3.2.2).

2.4c Prospective sampling

Follow-up interviews and anthropometric measurements were conducted with a subsample of 43 families living in the 6 downriver villages. The follow-up sample was restricted to infants aged 0-11 months at an initial visit between September and December of 2012. Seven rounds of follow-up interviews to assess infant feeding status and illness bouts were conducted across the study villages approximately every 3 weeks from October-April. The total number of follow-up interviews per infant varied because some infants were born during the study period ($n = 6$), some families relocated after heavy rains during the fourth round of data collection ($n=15$), and many families were variably absent from their villages during specific follow-up rounds. A total of 143 follow-up interviews were conducted for an average of 4.6 ± 2.0 interviews per child. Anthropometric measurements were taken on the first two follow-up visits for all infants, and then (a) every subsequent visit for exclusively breastfed infants or (b) every other visit for infants who had begun complementary feeding. Including the initial and follow up visits, an average of 4.6 ± 1.3 anthropometric measures per child were collected.

The follow-up interview consisted of the same 24-hour recall administered initially, along with brief questions about activity patterns and maternal or infant illness and medicinal usage during the previous two weeks (see Appendix). Follow-up interviews were conducted

in the participants' homes by one of the Tsimane research assistants, while I recorded participant responses. Anthropometric measures were collected as previously described.

2.4d. Behavioral observations

Fourteen participants additionally consented to participate in focal follows in their homes. . The participants were all part of the follow-up study and lived in the villages of Chacal, Puerto Triunfo, and Monte Rosa. My research assistant and I visited participants 1-2 times each to practice and refine observation methods; a total of 20 practice hours of observation were conducted prior to beginning data collection. Focal follows were arranged with participants one day prior to data collection. Verbal consent to participate was given at this time and again when the observer arrived at the focal subject's home. Due to time travel constraints, observations were always arranged to begin in a participants' home or their *quijodye* (Sp. "chaco", a horticultural field), if the chaco was located adjacent to the home. Participants were instructed to go about any daily activities as they normally would, including leaving the home to work in a distant chaco or visit the home of another family. However, only four observations were conducted at least partially outside of focal subjects' homes: two in nearby chacos, and two at a family member's home. Maternal and infant behaviors in this study are therefore biased towards household activity patterns.

Focal follows were originally planned as four separate 5-hour observations: two to be conducted on non-consecutive days between September-November 2012 (Observation Period 1), and two to be conducted on non-consecutive days between February-March 2013

(Observation Period 2). Owing to problems accessing the study villages after January 2013, however, the second round of observations had to be conducted within a ~2 week period. Owing to these constraints, observations were limited to 3-4 hours with nine subjects.

During focal follows, different sets of selected maternal and infant behaviors were recorded continuously and at 5-minute intervals (see Appendix). Observations were recorded in 3-5 hour time blocks. For interval observations, the observer recorded the infant's activity state, maternal activity state, and infant's proximity (see Appendix) to its mother and father every five minutes. For continuous observations, select infant interactions (along with corresponding actors) were recorded to the nearest second. If the focal mother left her infant with another actor or unattended, the observer stayed with the infant and recorded the mother as absent during that time period. For continuous observations of infants, "holding" was recorded non-exclusively with other activities (e.g. "breastfeeding", "grooming", "playing"). "Breastfeeding" was determined as infant contact with the nipple. Intermittent bursts of suckling separated by less than 10 seconds were recorded as a continuous breastfeeding bout. All instances of complementary feeding were recorded ad libitum in terms of what was given, who fed the infant, approximately how much, and if possible, the duration of the feeding bout. Duration was not recorded if feeding bouts consisted of frequent, intermittent feeding.

During Observation Period 1 (August-November 2012), participants were observed in five hour blocks (one morning and one afternoon observation) on two non-consecutive days. One mother (from Monte Rosa) opted out from additional observations after completing one 5-hour afternoon observation. My research assistant (GG) conducted approximately 70% of the observations during this period. Inter-observer agreement between GG and myself

assessed during four hours of interval observations (4 different behavioral categories observed, 192 observations total) averaged 83% overall, indicating high agreement (Viera and Garrett, 2005), and was substantial within each category (maternal activity, kappa = 0.83; infant interactions, kappa = 0.73; maternal proximity, kappa = 0.77, paternal proximity, kappa = 0.96).

Observation Period 2 was conducted from March-April of 2013 (all observations during this period were conducted by me). As described above, my access to these villages was suspended from January to March of 2013. Owing to time constraints in completing the study, I observed only ten of the original 14 participants during this time period, in 3-4 hour time blocks, and again on two non-consecutive days. Overall, a total of 188 hours of observations were conducted, 129 during Observation Period 1 (9.3 ± 1.8 hours/subject) and 59 during Observation Period 2 (6.3 ± 0.5 hours/subject)).

2.4e. Urine sample collection

At each follow-up interview, maternal subjects were given a sterile specimen collection cup and instructed to give a first morning void urine sample the following day. Samples were collected by a research team member the following day, generally between 7-9 AM. Samples were then transported to the field laboratory in insulated bags within 1-2 hours of collection. At the field laboratory, a researcher agitated the sample and recorded the estimated urine

sample volume, color (clear yellow, yellow, intense yellow, amber), and aspect (clear, slightly opaque, opaque, opaque-turbid). The researcher immediately aliquoted two 1 ml urine samples into sterile cryotubes using a disposable plastic pipette, and then transferred the cryotubes to a liquid nitrogen tank. The remaining portion of the urine specimen was then analyzed using a professional diagnostic urine reagent strip (URS-10, Craig Medical®). Following manufacture protocols for the strip, the researcher recorded diagnostic markers of urine pH, specific gravity, ketones, glucose, protein, leukocytes, nitrites, blood, bilirubin, and urobilinogen. Subjects were informed of any abnormal results and given pharmaceutical treatment as warranted, or referral for further diagnosis and treatment.

Samples were stored in liquid nitrogen until they could be transported to the Centro Nacional de Enfermedades Tropicales (CENETROP) laboratory in Santa Cruz, Bolivia, where they were stored at -20°C. In April 2013, samples were transported to the U.S. on dry ice and stored at the University of California Santa Barbara (UCSB) Human Biodemography Laboratory at -80°C.

2.5 Laboratory analysis of urinary c-peptide

Participant urine samples (n =187) were analyzed for C-peptide concentrations, a validated biomarker of insulin activity. Human C-peptide is a 31 amino acid residue peptide that is cleaved off in the process of synthesizing insulin from pro-insulin. It is secreted in blood on an equimolar basis with insulin, but is not cleared by the liver, and is therefore a more stable measure of endogenous insulin secretion than peripheral insulin levels (Bonser

and Garcia-Webb, 1984). C-peptide concentrations in urine and serum are correlated, though urinary C-peptide may be preferable as a non-invasive biomarker that is also less prone to transient fluctuations than serum C-peptide (Rendell, 1983; Whitten et al., 1998). Though typically used for clinical diagnosis of abnormal insulin or glucose activity, urinary C-peptide has been increasingly used by anthropologists to measure changes in energy balance in humans and non-human primates (e.g. Ellison and Valeggia, 2003; Sherry and Ellison, 2007; Thompson and Knott, 2008). The significance of C-peptide as a biomarker of energy balance in lactating Tsimane mothers will be explained in more detail in Chapter 4.

Urinary C-peptide was assayed at the UCSB Human Biodemography Laboratory, using commercial enzyme immunoassay kits (EIA-1293, C-peptide ELISA, DRG International). All kits used were derived from the same batch. Due to low concentrations of C-Peptide in Tsimane urine, all specimens were diluted 1:10 in assay buffer (instead of the manufacturer recommended 1:20) in order to ensure specimens fell within the range of the standard curve. All other protocols were followed according to manufacturer instructions. All samples corresponding to an individual subject were clustered on the same plate, with multiple individuals on a plate then randomized with respect to lactational stage and village. Within and between-assay coefficients of variation (CVs) for in-house urinary controls were 5.5% and 14.4%, respectively, for the high control (5.2 ng/mL), and 6.3% and 26.7% for the low control (2.1 ng/mL). Urinary C-peptide values were corrected for concentration using specific gravity (Miller et al., 2004; Anestis et al., 2009).

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Chapter 2: Tables and Figures

Figure 1. Map of Tsimane territories and study villages

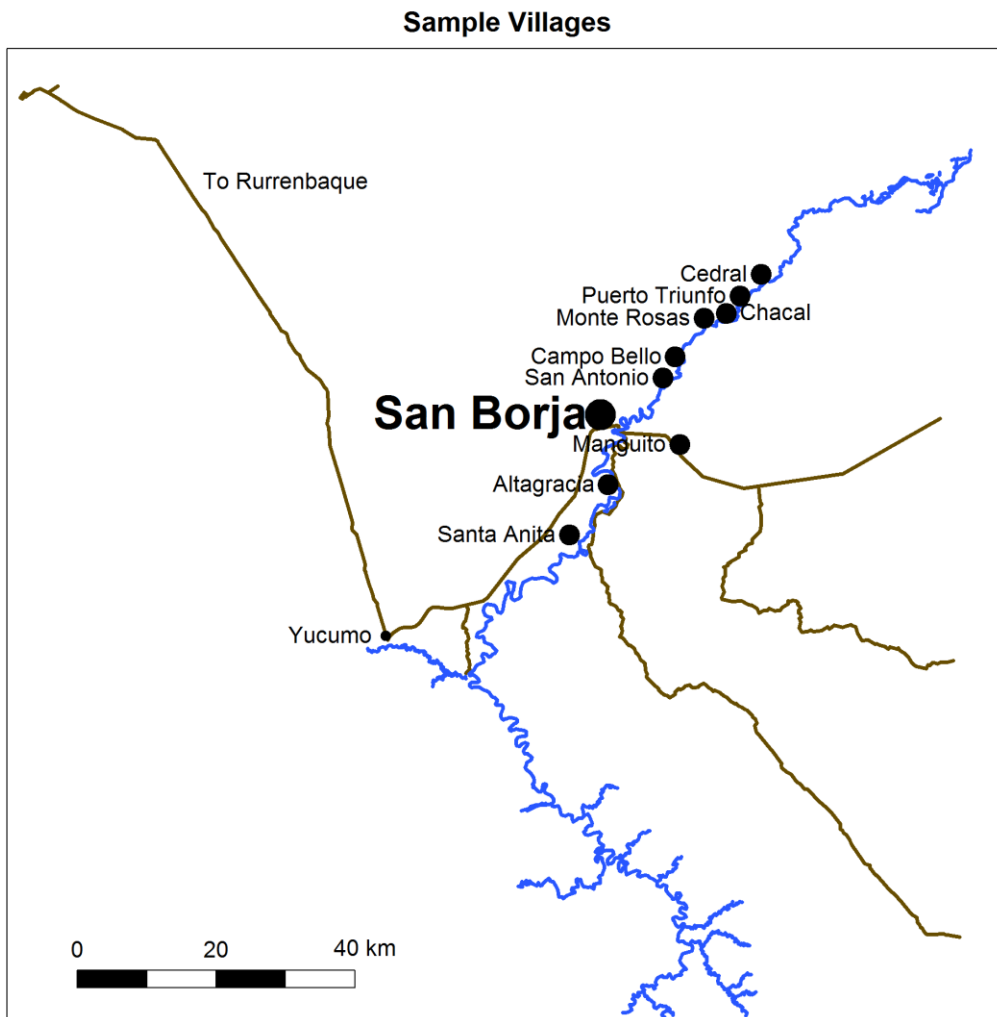


Table 1. Monthly rainfall (cm) in San Borja during field data collection. Source: Sistema de Procesamiento de Datos Meteorológicos (SISMET; www.senamhi.gob.bo)

Month	Rainfall
2012	
August	51.0
September	41.0
October	80.3
November	136.6
December	301.2
2013	
January	168.3
February	292.0
March	205.5
April	116.8

Table 2. Descriptive characteristics of study villages

Village	Dist. SB (km) ¹	Size ²	N ³	Spanish Fluency ⁴	Years Educat. ⁵	Region
Alta Gracia	12	149	18/NA	1	2.0	Near Town/Upriver
Santa Anita	18	115	6/NA	2	3.5	Near Town/Upriver
Manguito	12	170	15/NA	1	3.0	Near Town/Road
San Antonio	14	331	13/6	1	3.0	Near Town/Downriver
Campo Bello	19	292	25/5	0	2.0	Near Town/Downriver
Monte Rosa	30	151	11/12	0	1.0	Remote/Downriver
Chacal	34	253	14/9	1	2.0	Remote/Downriver
Puerto Triunfo	37	101	4/5	1	1.5	Remote/Downriver
Cedral	43	206	6/12	0	2.0	Remote/Downriver

Figure 2. Research Team (From left to right: Bernabe Nate, Melanie Martin, Cody Elwell, Jaime Durbano, and Geni Garcia).



Chapter 3: Tsimane Mothers and Infants: From Womb to Spoon

3.1. Introduction

Researchers affiliated with the Tsimane Health and Life History Project (THLHP) and the Tsimane Amazonian Study Panel (TAPS) have collectively published over 160 scholarly articles related to Tsimane culture, economic production, demography, ecology, and health (see <http://www.unm.edu/~tsimane/> and <http://heller.brandeis.edu/sustainable-international-development/tsimane/working-papers.html> for project descriptions and published works). Many key aspects of the lives of Tsimane women and children have emerged from these studies, and are briefly discussed below. This chapter expands on this earlier work by describing and analyzing variation in two key dimensions of maternal and infant life: (1) pregnancy and childbirth, and (2) breastfeeding, complementary feeding, and weaning. Within these respective dimensions, particular attention is given to the availability of prenatal care and market foods, as these factors may be shifting traditional practices and reproductive decisions that influence maternal and infant health outcomes.

As discussed in Chapter 2, most Tsimane maintain traditional subsistence activities, living in small villages surrounded by kin. Large nuclear families are the main unit of household production, although sharing and cooperation among extended kin are integral to well-being and survival (Gurven et al., 2012). Although there are no formal systems of inheritance, intergenerational transfer of resources does occur, with parents supplying food to offspring's families up through the seventh decade of life (Hooper et al., 2015). The Tsimane are also fairly egalitarian, with little centralized leadership, and community matters

decided through community consensus. To date, however, only men hold formally appointed political positions (von Rueden et al., 2014), and there are clear sexual divisions of labor. Men are primarily responsible for hunting, fishing, heavy agricultural work, household manufacture and repair, wage labor, and commercial interactions, while women are primarily responsible for childcare, domestic animal care, foraging, food processing, weaving/sewing, and other household chores. However, women do fish and participate in agricultural work, and men assist in childcare and domestic chores (Gurven et al., 2009)

While juvenile females (~age 7-14) often assist with care of younger siblings, nieces, and nephews (Winking et al., 2009a), and all children assist in household activities to some extent, in general their lives are unstructured with ample time for play. Most villages have schools, but outside of a few larger, more acculturated villages closer to town, the quality of education tends to be very poor, and attendance is sporadic (Godoy et al., 2007). The Tsimane are also semi-nomadic, with families often traveling together for 2-7 days to hunt and forage in the interior forest, to work in distant fields, to visit kin in neighboring villages, or to sell and buy goods in San Borja (Ellis, 1996; Miner et al., 2014).

On average, Tsimane women reach menarche at 13.9 years (Rucas et al., 2006) and marry and reproduce before age 19 (McAllister et al., 2012). Marriage is recognized by cohabitation, without formal commemoration. Though unions are generally monogamous, 5-10% of marriages are polygynous (mostly sororal). Infidelity is not uncommon, and about 20% of couples separate (Winking et al., 2007, 2009b; Stieglitz et al., 2011). Couples are equally likely to live in natal villages of the husband or wife (Miner et al., 2014), though couples often share natal villages, and may shift residences over time—i.e. from the wife's to

the husband's natal village. The Tsimane are considered a "natural fertility population"; pharmaceutical contraceptives are rarely used and women spend most of their reproductive years either pregnant or lactating. The mean interbirth interval is 30.7 ± 10.6 months, and the average total fertility rate is 9.1, more than double the rate of the surrounding Beni Department (McAllister et al., 2012). As will be discussed in subsequent chapters, intensive breastfeeding provides some amount of contraceptive protection through a prolonged period of lactational amenorrhea. The high fertility rate of Tsimane women is likely a consequence of both the lack of family planning services and the existing agrarian societal structure, which favors high fertility as a source of household labor and a continued marker of high social status (McAllister et al., 2012).

Here, demographic and public health-oriented research begins to paint a fairly bleak portrait of maternal and child wellbeing. The most recent estimate of infant mortality was more than twice that of the Bolivian national rate (13% vs. 5% in 2003) (Gurven et al., 2007a; Gurven, 2012), likely reflecting the influence of high fertility, chronic pathogen exposure, and relatively poor access to medical care. Childhood respiratory and gastrointestinal infections, growth stunting, anemia, and acute inflammation are endemic (Foster et al., 2005; McDade et al., 2005; Gurven et al., 2007a; Tanner et al., 2009; Blackwell et al., 2013; Martin et al., 2013). Women are also threatened by domestic violence and high rates of gynecological morbidity at about~50%, —the latter including sexually-transmitted infections related to partner infidelity (Stieglitz et al., 2011, 2012a; b).

Access to medical care, pharmaceuticals, and market foods have increased for the Tsimane over the last several decades, though regional and temporal differences in this access

persist and have been shown to influence differences in infectious morbidity, infant mortality, and breastfeeding patterns (e.g. Gurven et al., 2007, Veile et al., 2014). Village location, maternal age or parity, and maternal Spanish fluency may varyingly influence access to or use of available prenatal services—which in turn may influence these larger demographic patterns by affecting variation in pregnancy and neonatal outcomes. Similarly, village location, season, and household composition may varyingly affect food availability—which in turn may influence complementary feeding practices such as frequency and diversity of complementary foods that affect infant and young child nutritional status. As such, differences in prenatal care and complementary feeding practices among the Tsimane today may be key factors influencing maternal reproductive outcomes and quantity vs. quality trade-offs.

To explore these relationships, the first section of this chapter combines quantitative and qualitative data to assess differences in access to and use of prenatal care and biomedicine during gestation, labor, and postpartum recovery. The second section combines ethnographic observation with a public health framework to describe Tsimane complementary feeding practices and to test if regional, seasonal, and household variation in those practices influence infant nutritional risks.

3.2. Pregnancy and Birth

3.2a. Aims

As stated above, Tsimane demographic patterns in fertility and infant morbidity have been well documented and earlier ethnographic accounts point to some cultural

preoccupations with the health of pregnant mothers and infants. For example, Ellis (1996) reports that the Tsimane believe the fetus is protected in the womb, and do not discourage prenatal intercourse. However, sexual intercourse during the first 3 – 6 months postpartum is discouraged, as this could harm the mother’s healing womb (Ellis, 1996; Stark et al., 2006) or imbue mothers and infants with an odor (*achijchi*) that is “displeasing to forest spirits” (Huanca, 1999). Paternal extramarital sex is believed to harm the fetus and lead to deformities (Ellis 1996), while a specific disease of severe vomiting and diarrhea in infants (*tseydes*) is also attributed to prolonged paternal absence—especially in relation to an extramarital affair. There are also several pregnancy taboos related to food and ‘contagion magic’—i.e. avoiding meat from animals with antlers or spines (which would harm the baby)—or eating *epoj*, or fused plantains (which can lead to twins, considered dangerous and unlucky) (Ellis, 1996). In fact, the word *epoj* signifies both twinned plantains and twin offspring. Also discussed in earlier ethnographies, ritual familial consumption of *väj* (peach palm fruit) during the rainy season is believed to protect pregnant women and babies; however the flowering plants are believed to be dangerous to women and children, who are forbidden from harvesting them (Huanca, 2006).

Relatively little research, however, has described specific Tsimane practices during gestation and delivery—including both traditional (e.g. midwifery, ethnobotanical or shamanistic medicine) and modern biomedical practice. Access to and preferences for modern pharmaceuticals and prenatal or obstetrical services have likely increased over the past two decades, owing to increasing market integration and national efforts to expand health care to rural and indigenous populations. As reviewed by Silva and Batista (2010),

health care in Bolivia is currently provided through a mix of social security and public and private sector programs and services (including traditional medicines). So-called “first level” services—preventative and outpatient medical care provided through clinics, hospitals, and mobile health promoters—account for 93% of all health services in Bolivia, and nearly all available services in rural areas (Silva and Batista 2010). Since 1994, three national insurance plans have been implemented by the Bolivian government with the specific intention of increasing access to and delivery of maternal and child health services. The current national insurance plan (Seguro Universal Materno Infantil, or SUMI) was implemented in 2003, and has therefore been the principal government program impacting Tsimane maternal and child health care since THLHP data collection began. SUMI provides health care for pregnant women until six months after childbirth and for all children less than five years of age. Services are provided through public health centers and some facilities administered by churches and NGOs. Services covered include ambulatory care, hospitalization, diagnostic services, medical and surgical treatment, and medication for most common pathologies.

Unlike previous plans, SUMI does not guarantee birth control for all women of childbearing age, and has prioritized availability of advanced level services, most of which are only located in urban areas. This design appears to have actually increased urban-rural health disparities and resulted in poorer national health indices over all (Silva and Batista, 2010). As reviewed by Veile et al. (2014), insured services regularly available to Tsimane women and children include prenatal care, vaccinations, nutritional supplements, and, when available, antibiotics and antihelminthics for acute infections. Among villages surveyed in the present study, prenatal screenings were available through four providers: mobile health care

providers that visit Tsimane villages, the hospital in San Borja, a clinic in the town of Galilea (~5 km outside of San Borja, downriver), and a clinic on the outskirts of San Borja (HOREB).

Across villages, however, access to these providers and the quality of services may be very different. First, travel to the hospital in San Borja and the clinic at Galilea requires travel time and money, which generally disadvantages families more distantly located from these providers. In previous fieldwork conducted from 2009-2010, I observed that mobile health care providers made monthly visits to Tsimane villages closer to San Borja, but visited more remote river and interior forest villages only 1-3 times per year—often resulting in incomplete inoculations for children in those villages. Of note, however, during the present study (August 2012- April 2013), a mobile team from the Galilea clinic visited the more remote villages located in the Estación Biológica de Beni (EBB) at least four times, and never once visited the closer downriver villages of San Antonio and Campo Bello (which fall outside the boundaries of the EBB, see map Chapter 2, Figure 1). According to the Galilea doctor, she was able to service the EBB communities solely because of logistical assistance provided by the EBB (boat transportation and gas), whereas no government funds were available to organize service to the closer villages *outside* of the reserve. As the quality of service may vary among different providers, documenting variation in sources of prenatal care may provide insight into growing health disparities across regions. In this study, I expect that the mobile medical team provided the bulk of prenatal services in more remote villages, but still expect rates of prenatal care to be higher among mothers living in closer proximity to San Borja.

Other demographic factors in addition to regional constraints may influence women's motivations or abilities to seek out services. In particular, women who are better educated or more fluent in Spanish may be more comfortable communicating with health care professionals and therefore more likely to seek out services. For example, only in recent years has the hospital permanently staffed a Spanish-Tsimane translator for Tsimane patients (Veile et al., 2014), and there is no such translator at the Galilea clinic. This language barrier may dissuade many lesser-educated Tsimane mothers from seeking out services from these providers. At the same time, more educated or Spanish fluent mothers may be more accepting of biomedical interventions and therefore more motivated to seek out prenatal services. Maternal age and previous birth experience may also varyingly influence mothers' use of prenatal care, though *a priori* predictions with respect to these behaviors are difficult. For example, while younger mothers may be more accepting of biomedical practices, young Tsimane mothers also tend to be shy, which may deter them interacting with male and/or non-Tsimane health care providers. Conversely, previous research has shown that mothers with more dependents are less likely to travel (Miner et al., 2014), and would therefore be less likely to obtain a prenatal screening. At the same time, older mothers may be more likely to have had a previous birth trauma (i.e. miscarriage or stillbirth) and/or better able to detect problems with current pregnancies—which may increase motivation to seek out care.

To explore the above relationships, this section combines ethnographic observation and interviews to estimate the availability and use of prenatal screenings, hospital births, and pharmaceuticals during labor or postpartum recovery. I also examine if use of these services varies in relation to maternal village location, age, and Spanish fluency. Evidence of such

variation in prenatal care may reflect growing inequalities in health care outcomes and/or shifting generational norms indicative of market integration. I close with a brief discussion of how current availability in prenatal care may be improved to better support Tsimane maternal and child health.

3.2b. Methods

Data presented in this section are drawn from responses to semi-structured interviews (Appendix A1) conducted between September 2012 and April 2013. A total of 160 mothers with infants aged 0-3 years, across nine different Tsimane villages, participated in the study (Table 1). See Chapter 2 for additional information on subject recruitment and descriptions of participant study villages. In full interviews conducted with 151 participants, mothers were asked if they had received a *Carnet de Salud de la Madre*, which documents any prenatal screenings they would have received from a hospital, clinic, or visiting health care promoter (Fig. 1). When available, the carnets (i.e. estimated gestation length and delivery date, maternal weight and blood pressure) was recorded with participant consent. Participants were also asked a series of brief open-ended questions about their most recent pregnancy and birth, including emergencies experienced during the pregnancy, where they gave birth, who assisted during labor, and what, if any, over-the-counter and traditional medicines they took or administered to their newborns (see Appendix A1, Section C, questions # 32-46).

Over the course of a prospective follow-up study with a subset of mothers (see Chapter 2), I also repeatedly observed 11 mothers at various stages of gestation. At 0-28 days postpartum, I interviewed nine of those mothers about their birth experiences, and

opportunistically collected weight and length measures from neonates. Infant weight-for-age and height-for-age z-scores were calculated using WHO ANTHRO (v.3.2.2). Descriptive statistics were generated to calculate frequencies of prenatal screenings, emergency prenatal visits, mothers birthing at home or in-hospital, sex and relationship of birth assistants, and use of traditional and pharmaceutical medicines during birth and postpartum recovery.

The likelihood of receiving at least one prenatal screening and the likelihood of using traditional and pharmaceutical medicines during birth or postpartum recovery was evaluated using logistic regression. The following independent variables were assessed: village region, maternal parity, previous offspring death or miscarriage, Spanish fluency, and education. In statistical models, village region was binary coded as either “remote” or “near town” based on general accessibility and distance to San Borja (> 30 km vs. < 20 km) (see Chapter 2). In univariate analysis, the binary category performed better than a continuous measure of distance to San Borja, and also facilitates comparisons with previous studies of maternal and infant health outcomes (e.g. Gurven et al., 2007, McAllister et al., 2013, Veile et al., 2014). In statistical models, I evaluate the total number of births (including offspring who died or were stillborn), rather than maternal age as an independent variable. Although both maternal age and number of births may independently influence prenatal health care behaviors (for reasons discussed above), in this study these factors were highly correlated ($r = 0.87$, $p < 0.001$) and resulted in high variance inflation factor scores when included together in models. Total maternal births rather than total maternal pregnancies were used because several discrepancies among older subjects were noted when comparing miscarriages reported by subjects in this survey to their demographic histories collected in previous years by the

THLHP—suggesting recall error in miscarriage rates (similar problems with offspring births and deaths were not observed).

In addition, because primiparous mothers face higher risks of pregnancy and birth complications, births were grouped as follows to allow for non-linear comparisons across a range of parity categories: primiparous (1 birth, n = 26), low parity (2-3 births, n = 39), prime parity (4-8 births, n = 68), and high parity (9 – 13 births, n = 18). In this sample, the median number of births was four, with 75% of mothers reporting six or fewer births. The high-parity cut off was chosen to reflect parity at or above the average total fertility rate of Tsimane women (9.1, McAllister et al, 2013). These mothers are all over the age of 35 and therefore at or nearing the end of their reproductive careers.

Statistical analyses were conducted using R (ver. 3.0.2). The baseline models for the logistic regressions included terms for village region, parity group, and whether or not a mother reported any previous infant death or miscarriage (acknowledging that miscarriages may be underreported). Subsequent models additively considered Spanish fluency (none vs. minimally conversational), highest education completed, and interaction terms. Final models were selected using AIC and evaluated for multicollinearity using VIF in the *car* package (with 3.5 set as the cut-off for VIF or the squared $\text{GVIF}^{(1/2 * Df)}$ term for categorical variables). In the analyses, Spanish fluency and education were highly correlated with village region and tended to perform poorly in models. Mothers in near town villages accounted for 65% of subjects with conversational Spanish ability, 90% of subjects fluent in Spanish, and 83% of mothers with more than 4 years of completed education. Relationships between the

dependent outcomes and Spanish fluency or education are described in text when not included in statistical models.

3.2c. Prenatal care

Many Tsimane mothers received no prenatal care whatsoever (Table 2), and prenatal care was generally minimal for those who did receive it. Fifty-nine percent (88/151) of mothers reported receiving a *Carnet*. Most of the *Carnets*, particularly in the more remote villages, recorded only one prenatal screening, generally consisting of a simple reproductive history, weight, and blood pressure measurements (see example, Figure 1). Some mothers also reported receiving a 3- month supply of unknown pills, likely prenatal multivitamins or folic acid. Closer to San Borja, mothers were more likely to have had multiple screenings, and one San Antonio mother (a native-Spanish speaker of mixed-ethnicity) received an ultrasound.

Of the mothers who received at least one prenatal screening, 39% were seen by a visiting health care promoter in their own village, while the remaining 61% traveled to a clinic or hospital (Table 2). Mothers living in villages near town were more likely to have received at least one prenatal screening than were mothers in more remote villages, though the difference was only moderately significant (Table 2, chi-square = 2.992, $p = 0.083$). As expected, the majority of prenatal screenings for mothers in villages near town were conducted at the hospital or the Galilea clinic (76%), whereas the majority (77%) of prenatal screenings in more remote villages were conducted by visiting medical teams. As discussed

above, no mobile medical teams visited two of the near town villages at all during the study period; therefore, rates of prenatal screenings in villages closer to town from this period may underestimate rates in other years. Overall, however, only about 23% of the pregnant women surveyed here (33/149) received a prenatal screening from a mobile health care provider.

Though differences were not significant, mothers with at least conversational Spanish fluency (54%, or 81/149 respondents) were somewhat more likely to have received a prenatal screening than were mothers with no Spanish fluency (65% vs. 51%, chi-squared = 2.43, $p = 0.119$), and were somewhat more likely to receive prenatal screenings from a clinic or hospital (68% vs. 50%, chi-squared = 2.09, $p = .148$). Of note, however, all mothers fully fluent in Spanish ($n = 11$) received a prenatal screening, and all of these mothers received care at a clinic or hospital. Looking within regions, mothers in remote villages with at least conversational Spanish fluency were actually somewhat less likely to receive prenatal care than mothers with no Spanish ability (43% vs. 56%, chi-square = 2.43, $p = 0.119$). In contrast for mothers living in villages near town, mothers with at least conversational Spanish fluency were much more likely to receive prenatal screenings than mothers with no Spanish fluency (77% vs. 47%, chi-squared = 7.13, $p = 0.008$). Because clinic and hospital services provided the bulk of prenatal services closer to town, and mothers with better Spanish fluency may be more comfortable interacting with these health care providers, mothers closer to town with at least minimal Spanish fluency may be more inclined to seek out prenatal care than their peers with no Spanish ability.

Forty-nine percent of mothers surveyed here reported at least one child death or miscarriage (35% at least one infant death, 23% at least one miscarriage, 8% have

experienced at least one child death and at least one miscarriage). The frequency of child death or miscarriage did not vary significantly by region (56% vs. 46% for remote vs. near town villages; chi-square = 1.17, $p = 0.279$) or Spanish fluency (51% vs. 49% for no Spanish vs. at least conversational Spanish; chi-square = 0.001, $p = 0.930$), though higher frequencies of infant death/miscarriage were reported with increasing parity (15% for primiparous mothers, 36% 2-3 births, 60% 4-8 births, 94% 9 – 13 births). Overall, prenatal screenings were no more likely among mothers reporting at least one child death/miscarriage than among mothers reporting no child death/miscarriage (50% vs. 52%, chi-square = 0.02, $p = 0.90$).

In a logistic regression model adjusting for village, region and reported infant death/miscarriage, mothers with low parity (2-3 births) were significantly more likely to obtain prenatal screenings than were mothers at prime parity (4 – 8) births (Table 3), while differences between prime and primiparous or high parity mothers were not significant. In subsequent post hoc analysis (not shown), no other significant differences between parity groups were observed. Considered independently, neither maternal Spanish fluency nor education improved model fit as evaluated by AIC. A significant interaction between Spanish fluency and village region was observed, but resulted in high collinearity and large confidence intervals suggesting regression parameters were not accurately estimated. This term was not included in the final model. Interaction terms between village, parity group and offspring death/miscarriage also failed to improve model fit, and therefore the baseline model was kept as the final model.

As the frequency of offspring death/miscarriage increases with parity, and offspring death miscarriage was not a significant predictor of prenatal screenings, it is unlikely that the low parity mothers were particularly motivated to seek out prenatal care due to recent birth traumas. Tentatively, I propose that mothers at low parity may be more likely to receive prenatal care because they are more likely to have other young dependents (~2-5 years of age) still receiving inoculations. That is, they may be more likely to opportunistically receive prenatal care while obtaining health care for their other young dependents. In contrast, primiparous mothers do not have any existing young dependents, while the dependents of higher parity mothers have likely aged out of the inoculation window, since Tsimane interbirth intervals generally increase with age. Future research is required to test this hypothesis.

In interviews, mothers were also asked if they had sought medical attention for a perceived problem with their pregnancies, *independent of* any prenatal visits recorded on a *Carnet* (from here on referred to as “emergency prenatal care”). Thirteen percent of mothers interviewed (19/147) reported seeking such emergency prenatal care (Table 2). Similar to the total sample, most of these mothers (84%) were multiparous (ranging from 2-10 births), with about half (52%) reporting at least one prior infant death or miscarriage. However, nearly all of these mothers (95%) were from villages near town, and 68% reported at least conversational Spanish fluency. A greater proportion of mothers seeking emergency care also obtained a standard prenatal screening, as compared to mothers in the general sample (84% vs. 59%), However, I did not ascertain if emergency visits preceded or followed prenatal screenings. Most of the mothers who reported seeking emergency care did so because of

severe stomach, back, or other pains at 4-8 months postpartum (58%, 11/19). Broadly, the remaining mothers reported seeking emergency care because they feared miscarriage, with half of these cases following a fall or heavy physical activity.

3.2d. Childbirth

The expansion of prenatal services, however modest, has had even less of an impact on Tsimane labor and delivery practices. Only two mothers I interviewed reported giving birth in a hospital (Table 2). Both mothers were multiparous, lived in the relatively accessible and well market-integrated community of Manguito (Figure 1 Chapter 2), and appeared to anticipate complications owing to intense pain. “Yenny”, age 32, began having terrible pains at night. The following morning, with labor progressing, she walked nearly 6 km to Galilea, hopped on the back of a motorbike and rode another 6 km over a bumpy dirt road to the hospital in San Borja. Shortly upon arriving she gave birth, without complications, and was released the following day. “Glinda”, age 33, went to the hospital after experiencing debilitating pain for 1 day. Though her baby was also delivered without complications, doctors told her the baby was premature, and kept the baby under observation in a separate room for about 36 hours. Both she and the baby were given medications (which the subject could not name) and released after 3 days.

Of the remaining mothers who did not give birth in a hospital, 90% labored at home and the other 9% at a residence in another community (usually visiting kin or working on ranches). There are no professional or even informally trained midwives in Tsimane villages,

nor do there appear to be any specific community members within villages who attend the majority of births. In interviews, mothers were asked the following question: “*Ji ba sin buty yocsi in?*” (“who helped you deliver the baby”), and were then asked the age, sex, and relation of any named assistants (Appendix A1). Mothers were not asked to elaborate on the type of assistance provided during delivery, or about any other assistance received during labor or postpartum care.

While limited, the responses to this question suggest that Tsimane mothers solicit assistance from a wide range of helpers. About 10% of respondents (15/147) reported giving birth unassisted (emphatically, in the words of one mother: “nobody helped”). All but one of those mothers birthed at home. Mothers who were assisted during birth reported one to three helpers (median = 2), most frequently kin, their spouses, and affinal kin (Figure 2).

Overwhelmingly, helpers were of comparable age or older than birthing mothers. In only three cases were younger helpers named (a daughter or daughter-in-law). Together subjects’ mothers, spouses, and mothers-in-law accounted for 64% of all helpers named.

Male assistance during birth was not uncommon. While sixty percent of mothers (79/132) were assisted by women only, 27% were helped by men and women, and 14% by men only. Though spouses accounted for most of the male help, other male assistants named included a brother, a grandfather, fathers, and fathers-in-law. And while 77% of husbands (106/132) were reportedly at home during the birth, only 40% (42/106) were specifically named as assistants—suggesting most husbands were present but did not help (or at least did not help much). Interestingly, many mothers I interviewed reported receiving help from a spouse, spousal kin, or a distant relation, even when co-residing with their mothers or older

sisters. Unfortunately, it is not clear from the limited line of questioning which close kin were available to assist at the time of birth, or why, if close female kin were available, mothers still would have preferentially sought out help from their spouses or affinal kin.

Only rarely were non-kin named as assistants. In the most striking case I observed, “Juanita”, a 37-year old mother from Cedral, had been walking back from San Borja and went into active labor as she was passing through San Antonio (about 30 km away from her village). She was in full transition by the time she reached Campo Bello (4 km past San Antonio), and an unrelated Tsimane woman in Campo Bello brought her into her home and attended the birth. My assistant and I came across her and the baby (both healthy and resting under a mosquito net) about an hour after the birth. Juanita had previously reported having to work in her *chaco* throughout her third trimester—despite stomach pains and headaches—because her husband was *to’oty* (“kind of lazy”). That day, she requested we send out a radio message to him; he had recently left her after a drunken rage in which he claimed the baby wasn’t his.

In sum, the variability in sex and relations of reported birth assistants suggests that mothers choose assistants based on some combination of convenience, family composition, kin residence patterns, and individual preference. It is unclear if mothers who relied on help from spouses, affinal kin, or distantly related assistants did so out of necessity, or preference—i.e. if these were individuals that mothers felt particularly comfortable with, had attended previous births, and/or were generally acknowledged as “good at birthing”. Future research should assess both the full suite of potential helpers mothers had access to during

their last birth and the conditions or preferences that ultimately influenced who attended those births.

Additional insight about the labor and birthing process was gleaned through postpartum interviews conducted with 12 mothers pregnant at the start of the prospective study (including Juanita above). Most of these mothers (64%) reported being in labor (“*dolor de parto*”) for 12 hours or less. The other mothers reported labor pains lasting anywhere from 18 hours to 10 days—though I did not attempt to differentiate between “prelabor”, “active labor” and “transitioning” in any responses. During what is presumably the second stage of labor, women grasp onto long strips of rope-like dried bark (*tapi*) hung from the overhead scaffolding in their huts, which they use to stay in a supported, squatting position very low to the ground. Helpers “catch” the baby from behind the mother. The umbilical cord is cut using sharpened wood that is generally used for arrows (*tacuara*, Sp./*ton*’, Tsi.) or housing and other tools (*chuchio*, Sp./*shuru*’, Tsi.). According to one of my research assistants, custom dictates that an older son should cut the umbilical cord so that the baby will grow as well as its existing siblings. While one mother I interviewed did report that an older son (incidentally not named as a helper) did indeed cut her umbilical cord, most mothers said that they themselves, their husbands, or another helper performed this action.

While prenatal care and hospital deliveries remain relatively underutilized, Tsimane mothers were more likely to use over-the-counter pharmaceuticals than traditional medicine during labor or postpartum recovery, largely to alleviate pain (Table 4). Of the women who took pharmaceuticals, 22% specifically reported taking an antibiotic, 20% pain relievers/anti-inflammatories (acetaminophen, diclofenac sodium), and the remaining 48% unknown pills

or intramuscular injections. I did not systematically document the types of traditional medicines used, though Tsimane women commonly use eight different plant species to aid birth or postpartum recovery (Reyes-García, 2001).

Separate logistic regressions were run assessing the likelihood of maternal pharmaceutical and traditional medicinal usage in association with previous offspring death/miscarriage, parity group, and village region. When considered additively, neither maternal Spanish fluency nor education improved model fit and were not included in final models. Village region was not associated with variation in either pharmaceutical or traditional medicine usage (Table 5). Pharmaceutical usage was positively predicted by previous offspring death/miscarriage but was not associated with parity group. No differences in parity groups were observed in post-hoc pairwise comparisons, and no interaction terms were significant or improved model fit. In contrast, traditional medicine usage differed significantly across some parity groups, but did not vary with previous offspring death/miscarriage (Table 5). All other parity groups were significantly less likely to use traditional medicines as compared to high parity mothers [OR (95% CI): primiparous mothers = 0.08 (0.01 – 0.40), $p = 0.002$; low parity mothers = 0.15 (0.04 – 0.57), $p = 0.005$; prime parity mothers = 0.24 (0.08 – 0.76), $p = 0.015$]. In additional pairwise comparisons (not shown), no significant differences between primiparous and early parity mothers were observed.

The Tsimane maternal mortality rate from 1972 to 2012 was estimated at 702 deaths per 100,000 live births (McAllister et al., *in prep*). This rate is higher than recent national rates estimated for Bolivia (which decreased from 510 to 200 per 100,000 live births from

1990 to 2013, UNICEF 2014), but is comparable to an earlier estimate for the Ache (667 per 100,000 from 1940 to 1970) and lower than the rate estimated for the Hadza (1022 per 100,000 from 1985 to 2000 (Blurton-Jones 2013). Owing to the near universal rate of home-births assisted by non-professionals, combined with unsanitary conditions and lack of emergency equipment and medicine, a high risk of childbirth-related mortalities among Tsimane women may be expected. At the same time, the mortality rate is calculated from an actual event rate of 30 deaths out of 4275 live births in 30 years, or approximately 1 per year (McAllister et al., *in prep*). In my interviews, while 26% (39/109) of mothers reported having heard of or known of a woman dying due to childbirth, most of these subjects referenced second hand knowledge of “a woman in another community”. Only five respondents (3%) could name an actual relative who had died during childbirth, and that number included two different sets of sisters who each named the same relative. If we consider that that the Tsimane are a small population of interconnected kin and wider social networks, and that childbirth-related deaths may only directly affect one family per year, Tsimane women may not perceive a high risk of death during childbirth. Tsimane women, therefore, may not be highly motivated to seek out professional help during delivery, particularly given any lingering fears about hospitals or prohibitive costs of accessing such services.

3.2e. Postpartum neonatal care

Although there has been a fair amount of research conducted on reported rates of Tsimane infant morbidity and mortality (Gurven 2007, 2012), the paucity of prenatal care and hospital births has limited systematic study of gestational lengths, birth weights, and other vital birth statistics. Government-supported health care and economic subsidies available for children under 5 years of age have incentivized families to register their infants with SUMI and get birth certificates for them shortly after birth. Of note, the mothers that gave birth during the course of my study recorded the date of birth expressly for this purpose—suggesting that self-reported infant birthdates in recent years are accurate.

Gestational age can be estimated from the reported date of last menstruation, which is recorded on the prenatal health record given out by hospitals, clinics, and visiting medics. However, most prenatal check-ups are performed by visiting health care promoters, and may occur several months after the last date of menstruation, increasing the likelihood of recall error in estimating this date. Similarly, I've observed infants' first postnatal screenings are recorded on their carnets anywhere from within the first week to the first 3 months of life. It is currently impossible, therefore, to accurately estimate the proportion of Tsimane infants that are born prematurely or underweight. During data collection, I was able to opportunistically measure nine neonates within the first two weeks postpartum. The average weight and length of these infants (mean \pm SD 4.1 \pm 4.0 days postpartum) was 3.3 \pm 0.4 kg and 50.2 \pm 1.3 cm, respectively. None of these infants weighed less than 2.5 kg (the "low birth weight" threshold), which is significant given that infants may normally lose up to 10% of their birth weight over the first two weeks of life (Wright, 2004). Using World Health

Organization growth reference standards, the average weight-for-age and length-for-age z-scores for these 10 infants at the time of measurement were -0.22 ± 0.67 and -0.10 ± 0.69 , respectively.

Following birth, mothers customarily stay resting with neonates in a *sajaja* (mosquito net) for 1-4 weeks. The use of traditional medicines for neonates was very rare, reported by only 3% of mothers. However, my questions referred to ingested medicines. A black plant dye called *bi* is typically applied to newborns as a protective coating within the first few days after birth. The dye is obtained from the sap of a native tree species (*Genipa americana*) and remains on the skin for about a week after the initial application (Reyes-García, 2001). The use of *bi* may render the baby invisible to malevolent spirits (Gurven, 2007), ensure that he or she will have dark skin, and/or protect against fungal and other skin infections (Reyes-García 2001). In interviews I conducted in 2009, 69% (36/54) of Tsimane mothers reported applying *bi* to their infants after birth (Martin, unpublished data). Notably, these mothers were all from a fairly large, more market integrated village near San Borja, and therefore the practice may be even more prevalent in more remote villages.

Conversely, 21% (31/150) of mothers reported administering infants some type of pharmaceutical medicine during the first week postpartum: 58% were given an anti-inflammatory (ibuprofen or aspirin), 7% an antibiotic, and the remaining 35% an unspecified medicine. Mothers administered these medicines primarily for fever and flu-like symptoms. Mothers who took medicines themselves during or after childbirth were no more likely to give their infants medicines than those who did not take any medicines (22% vs. 18%, chi-square = 0.237, $p = 0.627$). The frequency of postnatal pharmaceutical administration was

slightly higher among near town villages (24% vs. 16%, chi-square = 1.06, $p = 0.30$).

However in a logistic regression neither village region, parity group, previous offspring death/miscarriage, maternal Spanish fluency, nor maternal education significantly predicted the likelihood of neonatal pharmaceutical administration (results not shown). Additional research with a larger sample size may be needed to ascertain factors predicting either traditional or pharmaceutical medicinal administration to neonates.

3.2f. Concluding remarks

The results presented here demonstrate that most Tsimane mothers receive limited prenatal care, despite initiatives to expand government-subsidized maternal and child healthcare. There is also evidence of emerging inequalities in available care within and across regions: in general mothers who live in villages closer to San Borja are better able to access diverse health care facilities, while within those villages mothers more fluent in Spanish are even more likely seek out prenatal screening and/or emergency prenatal care. These results suggest that more acculturated Tsimane women are either more motivated to seek out healthcare services or are more comfortable using services of non-Tsimane health care providers. Future research with a larger sample might explore how within- and across-region variation in household location, wealth (e.g. cash income, ownership of a motorized boat), and maternal medical histories additionally influence which mothers receive prenatal care, and where, when, and how often.

More research is also needed to better document birth and labor practices, particularly how women choose assistants and what the roles of particular assistants are. The limited responses gleaned from this interview interestingly suggest that laboring women solicit assistance from a variety of relations, and that those assistants are not uniformly women. The high degree of spousal and male participation in the birthing process may reflect the relatively individualistic and egalitarian structure of Tsimane society. A systematic review of ethnographies of labor and childbirth may reveal additional insights into how male participation during labor may vary across cultural and economic dimensions in small-scale societies.

On the surface, the continued prevalence of homebirths and varied patterns of assistance during labor suggest that most Tsimane are fairly experienced in labor, and may not perceive labor and delivery as particularly risky—at least for mothers. As a cautionary note, however, even if only about one Tsimane woman per year dies from childbirth-related complications, rates of miscarriage, still birth, and infant deaths are persistently high across the population (Gurven et al., 2007b, 2012). That is, the nearly universal frequency of homebirths and reliance on non-professional assistance (i.e. doctors or trained midwives) likely contribute to high rates of neonatal mortality. Improved access to prenatal care, and professional care during delivery and postpartum recovery, may reduce rates of premature birth and infant mortality. At the same time, any reduction in maternal mortality owing to an increase in hospital births must be weighed against additional costs at the population level (e.g. increased risk of unnecessary C-sections and related complications). Providing Tsimane women with emergency supplies, training, and education in basic childbirth and postpartum

care may be a more practical solution to decreasing both maternal and neonatal risks associated with childbirth. Furthermore, in this high-fertility population, expansion of prenatal and childbirth services that could improve neonatal outcomes should also be coupled with expanded family planning services. Expansion of such services would reduce risks of unintended consequences associated with improved infant survivability, including higher fertility, shorter interbirth-intervals, and further constraints on household resources available for individual offspring.

3.3. Infant and young child feeding practices

3.3a. Aims

After birth, Tsimane mothers invest considerably in lactation. In previous research (Veile et al., 2014), we characterized the Tsimane as a “traditional breastfeeding population”. Mothers universally initiate breastfeeding and nurse “on demand” day and night (carrying around infants in slings by day and bed-sharing at night). The pace of weaning is protracted and gradual: complementary foods are introduced on average at 4.1 ± 2.0 months of age, the frequency of complementary feeding does not surpass the frequency of breastfeeding until about 13 months of age, and infants are fully weaned at 19.2 ± 7.3 months on average (Veile et al., 2014).

Current international recommendations for age-appropriate infant and young child feeding (IYCF) practices include immediate breastfeeding initiation, exclusive breastfeeding (EBF) for 6 months postpartum, introduction of complementary feeding (CF) between 6-8

months of age, continued breastfeeding with CF from 6 to at least 24 months of age, and age-appropriate minimum frequency and diversity of complementary foods (see Box 1 for definitions) (WHO, 2008). In low and middle-income populations, “suboptimal” breastfeeding—in particular the introduction of CF before six months of age—has been estimated to cause about 1.4 million deaths annually, while suboptimal CF frequency and dietary diversity cause protein and micronutrient deficiencies leading to high rates of morbidity (Black et al., 2008). Other research has shown that suboptimal dietary diversity and meal frequency are associated with poorer growth outcomes (Heidkamp et al., 2013; Marriott et al., 2012b; Menon et al., 2013, Onyango et al., 2014, Saha et al., 2008; but see Jones et al., 2014 for evidence of mixed associations).

Our earlier research (Veile et al., 2014) suggests that Tsimane *breastfeeding* practices are relatively in line with these recommendations, though complementary feeding and weaning practices may not be. Meanwhile, the adequacy of meal frequency and dietary diversity is unknown. Previous research has documented relatively high rates of infant mortality and early childhood stunting among the Tsimane (Foster et al., 2005; Gurven et al., 2007b, 2012). While these poor outcomes are undoubtedly influenced by limited health care access, poor hygiene, and repeated bouts of acute infectious disease, early CF and weaning, and CF quality (i.e. insufficient dietary diversity and meal frequency) may also negatively impact childhood health and nutritional status.

Tsimane adult diets appear energetically sufficient, as studies have shown that less than 1% of adults are underweight (Tanner et al., 2013), and the average BMI of Tsimane and U.S. mothers do not significantly differ (Martin et al., 2012). However, the typical adult diet is not

very diverse, consisting primarily of cultivated plantain, rice, manioc, hunted game and fish, and some fruit and market foods purchased from crop sales or wage labor (Martin et al., 2012). It is unknown if meal frequency or dietary breadth is additionally restricted for young children in any way. Moreover, food security across households may be sensitive to regional and seasonal variation affecting availability of market and foraged foods.

Regional and seasonal factors affecting food availability may influence demographic variation in morbidity and mortality. For example, previous research has shown that rates of infant morbidity and childhood stunting are higher among remote, interior forest villages as compared to villages closer to San Borja (Godoy et al., 2006; Gurven et al., 2007a, 2012). This discrepancy may reflect large differences in access to medical care and market foods, as the road connecting the forest villages to San Borja becomes impassable during the rainy season. Children with more limited access to market foods may be less buffered against seasonal fluctuations in foraging returns or crop yields that could negatively affect dietary sufficiency. Mothers in the forest villages also introduce CF significantly earlier than do mothers in near town villages, which may reflect changing traditions in more acculturated villages that actually favor prolonged exclusive breastfeeding (Veile et al., 2014). In general then, indicators of minimum meal frequency (Box 1) are expected to be more favorable among Tsimane villages closer to town. Conversely, owing to habitat degradation, crowding, and other ecological factors adversely affecting villages closer to town, hunting, fishing, and foraging returns tend to be better with increasing distance from town, which would predict more favorable indicators of minimum dietary diversity in more remote villages.

The region the Tsimane inhabit is also punctuated by distinct dry and wet seasons influencing dietary availability—with the dry season running roughly from May to September and the rainy season from October to April (Godoy et al., 2008). Fishing is best during the dry season, when rivers are low. Hunting is also generally better in the dry season, when prey congregate at known watering holes. During the rainy season, families make frequent trips to the interior in order to fish in lagoons or to hunt prey concentrated at higher elevations, though it is not known if these foraging trips are sufficient to meet family needs. The main staples of the Tsimane diet are plantains, rice, sweet manioc, and maize. Plantain and sweet manioc can be cultivated year round, while rice and maize are harvested annually, beginning in January. Papaya, pineapple, palm nuts, cacao, watermelon, and varietal of yellow squash are also sometimes grown around homes and in family fields, producing periodic yields. Annuals including avocado, citrus, and mango trees are also found scattered around many villages and harvested freely. Other wild fruits are foraged during hunting trips. It is unknown to what extent fluctuations in fish, game, and fruit availability influence the frequency or diversity of complementary foods.

Measures of dietary frequency and diversity calculated from 24-hour dietary recalls correlate generally well with more precise measures of dietary energy and micronutrient composition (Moursi et al., 2008, 2009; Lander et al., 2010). However, specific CF indicators show mixed associations with anthropometric indicators of child nutritional status across populations (Jones et al., 2014). This inconsistency likely reflects within-population heterogeneity in other household factors that influence child health and access to resources—for example maternal height, BMI, household wealth, access to health care, and maternal

education (e.g. Joshi et al., 2012; Senarath et al., 2012a; b; Frojo et al., 2014). For the Tsimane, differences in maternal nutritional status, maternal education and Spanish fluency, and household composition may be indicative of variation in access to resources across households. These factors must also therefore be considered when evaluating the relationship between CF quality and nutritional status.

With the exception of the recent study by Veile et al. (2014) that examined breastfeeding practices, there has been little research on Tsimane IYCF practices, and no tests of specific practices in association with nutritional morbidity. In this section, I describe Tsimane CF practices and traditions. I then present results from a survey of IYCF practices and nutritional status in a cross-sectional sample of Tsimane children aged 0-36 months. IYCF practices are assessed using standardized methods (WHO, 2008) that allow for international comparisons. Subsequent chapters will explicitly test for variability in predictive factors and infant outcomes associated with the timing of CF introduction. This chapter tests if nutritional morbidity is associated with suboptimal CF quality. In order to pinpoint specific risk areas, differences in meal frequency and diversity are examined across region, seasons, and different stages of infancy/early childhood. Results are discussed in terms of targeting culturally-specific interventions to improve Tsimane child nutrition.

3.3b. Methods

From August 2012 to March 2013, the author and assistants visited nine Tsimane communities and attempted to locate and include all families with children aged 0-3 years in

the study. One family that was approached declined to participate and interviews were not successfully coordinated with another 13 families who initially agreed to participate. These 13 families were dispersed across the study villages and other subject characteristics (e.g. maternal age, Spanish fluency, education); interviews were coordinated with families randomly within villages, and therefore failure to include these 13 families is unlikely to systematically bias results in any way. The final sample included 160 families from 150 households, representing 92% of all families with an infant in the target age range and present at the time of visitation. Interviews and anthropometric measurements were conducted at the participants' homes during visits lasting approximately 1.5 hours. Anthropometric measures were collected from 156 participants. See Table 1 for descriptive characteristics of mothers and infants.

Participant interviews were conducted with the mother (the primary caregiver) and consisted of standardized short-answer questions about the focal child's birth, breastfeeding and complementary feeding experiences (Appendix A1), and a 24-hour recall of foods and liquids consumed by the focal child the day prior. The dietary recall used in all interviews was modeled after WHO guidelines for assessment of age-appropriate IYCF practice (WHO, 2008) adapted to adequately capture local dietary composition and food availability (Appendix A2). The recall was used to assess infant feeding practices and calculate indicators for age-appropriate IYCF practices according to WHO criteria (2008, Box 1). Detailed descriptions of study villages, subject recruitment, ethnographic interviews, and methods of anthropometric measurement are given in Chapter 2. Child weight-for-age (WAZ) and

height-for-age (HAZ) z-scores were calculated using World Health Organization international growth reference standards (WHO ANTHRO, v.3.2.2).

Statistical analysis

All statistical analyses were performed using R (ver. 3.0.2). For all non-EBF children (n =131), differences in meal frequency and dietary diversity were analyzed in association with infant age, maternal age, village region (“near town”, < 20 km from San Borja; “remote”, > 30 km from San Borja, and season (“dry”, August-October; “rainy”, November-March). Calculations of WHO indicators revealed that most children achieved minimum meal frequency but less than half achieved minimum dietary diversity (Table 8). The likelihood of achieving minimum dietary diversity was then explored in univariate analyses with the following factors expected to influence child dietary needs, feeding practices, or food availability: child age, sex, birth order, and current breastfeeding status; maternal age, Spanish fluency, education, total number of dependents < age 5 and total household members; village region and season.

A multivariate model was constructed from the following baseline model of factors determined to be significant in univariate analysis: infant age, infant sex, maternal age, highest maternal grade level completed, total number of household members, and season. Additional factors and interaction terms were considered additively using AIC for final model selection.

Of note, in this study three near town villages were visited only during the rainy season, resulting in a high correlation between monthly rainfall and distance to market ($r = -0.634$, p

< .001). Region and season are therefore considered independently in all multivariate analysis. In this sample, maternal age and total births are also highly correlated ($r = .862$, $p < 0.001$). Maternal age was chosen over total births in the multivariate analysis, as the latter may be more predictive of household access to resources or differences in feeding behaviors due to prior experiences. It should also be noted that while parity increases with age, Tsimane household size is dynamic across the life course: younger mothers may live with extended kin (their own or their spouses), while mothers' older dependents begin leaving the household to start their own families as early as age 14. In this sample, household size was only moderately correlated with total births ($r = 0.414$, $p < 0.001$) and maternal age ($r = 0.426$, $p < 0.001$). Variance inflation factors of household size and other factors were assessed in all final multivariate models using the "vif" command of the *car* package.

Separate linear regressions were run to assess variation in child HAZ and WAZ in association with minimum dietary diversity and other factors expected to varyingly influence infant growth, energetic needs, and average dietary availability. A baseline model was constructed from the following factors: infant age group, sex, and breastfeeding status, maternal age and height, total household members. Interaction terms and the following factors were then considered additively using AIC as criteria for best model fit: region and season (considered independently), maternal BMI, parity status (primiparous or multiparous), Spanish fluency, and education. Final models were assessed for multicollinearity and assumptions of normality.

3.3c Ethnographic description of Tsimane breastfeeding and CF practices

When mothers begin complementary feeding, they generally finger- or spoon-feed infants small portions of whatever they themselves are eating—they do not prepare special infant foods. However, the Tsimane diet is generally soft and bland, and readily conducive to infant consumption. Most meals (and subsequently first foods consumed) are “*jo’na*”, which refers to any rice, plantain, or pasta-based stew mixed with meat or fish. Meals are generally eaten over mats on the ground, with family members consuming individual bowls and serving themselves additional helpings as desired. Infants sit in their mothers’ laps and share from their mothers’ plates. While most Tsimane eat three family meals a day, family members and small children snack periodically throughout the day on leftover *jo’na*, plantains, and seasonal fruits.

Liquids and solids/semi-solids are introduced at around the same time, with water, *po’nacdye*, and *chicha* (Spanish; Tsimane *shoc’dye*) generally introduced first. *Po’nacdye* is made by mixing roasted plantains with water. *Chicha* is a home fermented beverage made from boiled and strained manioc, rice, maize, or plantains. ‘*Chicha dulce*’ is strained and served the first day of preparation (fermented < 1 day), and consumed almost daily. ‘*Chicha fuerte*’ is left to ferment 2-3 days, becoming more alcoholic, and generally consumed in a more celebratory fashion (e.g. parties, weekly gatherings). Infants and young children most often consume *chicha dulce*, but are not prohibited from drinking *chicha fuerte* when it is served. Traditionally, liquids are served in hollowed out gourds (*erepas*) and shared communally. These are still the most common drinking vessels served, though mothers also serve infants liquids in plastic mugs and occasionally bottles.

Infant formula and specialty cereals are rarely purchased or prepared for infants, as these generally must be purchased in San Borja and are relatively costly. The cheapest powder formula, packaged in ~400 g cereal boxes and widely available in market kiosks, cost about 40 Bs. (~ \$US 6), equivalent to a full or half-day's wage labor. Higher-quality infant formulas, packaged in ~16 oz cylinder tins and only available in pharmacies, range from 80-120 Bs, and are prohibitively expensive for most families. Through SUMI, infants at least 6 months old were eligible to receive one bag of infant formula per month (a 30-serving bag of rice and milk-based formula, see photo Fig. 3). These could be obtained in San Borja or through visiting health promoters. As discussed in the first section of this chapter, however, such visits occurred sporadically for most villages in this study. When these bags of formula were received, they did not appear to be reserved for the targeted child. I often saw formula eaten in powder form, and shared as a treat by the whole family. Thus, while formula access may be increasing due to the new policy, formula likely does not yet contribute significantly to daily infant consumption.

Mothers are the primary caregivers responsible for feeding children during the first 1-2 years of life, during which time they continue eating from shared plates. Fathers, siblings, and other caregivers also share snacks and drinks with small children. I have observed many children eating independently by age three—including serving themselves at meal times, serving themselves liquids and snacks, and even roasting plantains over open flames.

Premastication

To facilitate consumption of adult foods in early months, mothers may limit infant consumption of stews to ‘*chinsi*’, the clear broth portion of *jo’na*. Mothers also frequently premasticate solid foods for lubrication and safety, generally foods that are deemed too hot, too dry, too tough, or could cause choking (e.g. fish with bones, large gristly pieces of meat). The foods most commonly premasticated were *jo’na*, smoked or fried fish, meat, and roasted plantains. Mothers also reported occasionally giving liquids mouth-to-mouth, generally when they were traveling and didn’t have a drinking vessel handy, or—presumably in cases of illness or dehydration—when they wanted infants to drink “a lot”.

Premastication was common practice, with 84% of Tsimane mothers reporting ever premastinating solids. In total, 44% of children were still given premasticated solids at least on occasion, and 32% the day prior, with frequencies differing across age groups (see Table 6). The practice was most widely observed for children less than 12 months of age, and was rarely reported after 24 months (Table 6). In contrast, giving liquids mouth to mouth was relatively less common, reported by only about half of mothers, and rarely after 12 months of age (Table 6).

As a traditional practice, premastication may be less common among mothers who are younger or market-integrated. To test this, I ran separate logistic regressions on the likelihood of premastinating solids or giving liquids mouth-to-mouth the day prior in association with village region, maternal age, and maternal Spanish fluency (none vs. minimally conversational). Analysis was restricted to non-EBF infants and models were adjusted for infant age and consumption of fish or meat the day prior (for premastication), and evaluated

for multicollinearity. Maternal education was considered additively but did not improve model fit, as determined by AIC. Results are given in Table 7. As expected from the descriptive frequencies given in Table 6, the likelihood of both premastication and liquid mouth-to-mouth feeding decreased with infant age. Eating fish increased odds of premastication, but neither maternal age, Spanish fluency, nor village region significantly predicted these feeding behaviors. These results suggest that, at least in younger infants, these feeding behaviors are fairly prevalent across Tsimane families. Variation in day-to-day occurrence of these feeding behaviors may merely reflect fluctuations in dietary composition, travel, or infant appetite and hydration needs.

3.3d Results: IYCF practices

Indicators for age-appropriate IYCF practice in Tsimane children

Summary descriptive statistics of Tsimane IYCF practices, following WHO (2008) indicators (Box 1) are given in Table 8. Overall, age-appropriate breastfeeding practices were observed for 82 % of subjects. All Tsimane infants are breastfed and introduced complementary foods by 6-8 months, few are bottle-fed, and most are still breastfeeding at 2 years. However only 53% of mothers initiated breastfeeding within 1 hour after birth, and only 64% of infants less than 6 months of age were exclusively breastfed. Only 41% of children were receiving minimal acceptable diets, largely owing to poor dietary diversity. To assess whether these outcomes may be specific risk factors for the Tsimane relative to other Bolivian nationals, I compared IYCF indicators for Tsimane children against composites of

national statistics previously compiled for Bolivia (WHO 2008) (Figures 4 and 5). The Bolivian national rate of minimum dietary diversity (74%) is more favorable than that of the Tsimane, although national rates of early breastfeeding initiation (58%), EBF under 6 months (54%), and minimum acceptable diet (40%) are comparable. However, Bolivian national rates of age-appropriate breastfeeding (65%), minimum meal frequency (52%), continued breastfeeding at 1 year (82%) and 2 years (46%) are all at least 10% lower than those of the Tsimane.

Table 9 describes mean meal frequency and dietary diversity by child age, maternal age, season, and region (non-EBF children aged 0-35 months, n = 131). Mean meal frequency was increased after 0-5 months, but there were no significant differences in meal frequency among the other age groups. Meal diversity at 12-23 and 24-35 months was increased relative to 0-5 and 6-11 months. Both meal frequency and meal diversity were higher during the rainy season (November-April) and lower in more remote villages. However, while 90% of Tsimane children aged 0-23 months achieved minimum meal frequency, less than half (44%) achieved minimum dietary diversity (Table 2). Additional analyses were then performed on dietary diversity, as this may be a more significant risk factor for Tsimane child nutritional health.

Table 10 presents univariate and multivariate analyses of infant, maternal, and household factors associated with the likelihood of achieving minimum dietary diversity. Mean dietary diversity increased with infant age, maternal age, and maternal education, and was higher during the rainy season, but did not vary across household size or infant sex. As assessed by AIC, season was a better predictor of minimum dietary diversity than region.

Addition of interaction terms and other variables examined in univariate analyses did not improve model fit relative to the baseline model.

Differences in consumption of specific food groups were then examined to account for variation in dietary diversity. Figure 6 displays the percentage of each of the seven WHO food groups (Box 1) consumed the day prior for all non-EBF children (n = 131). More than 90% of children consumed basic staples (mainly plantains, rice, manioc, maize, and wheat) and flesh foods (mainly wild game, wild fish, domesticated beef and poultry). Vitamin A-rich fruits and vegetables (primarily papaya, mango, and palm fruits) were more commonly consumed than other fruits and vegetables (primarily citrus, watermelon, and pineapple). Vegetable consumption in these categories was rare—restricted to a local variety of butternut squash and small amounts of vegetables in market foods (e.g. carrots in soups). Consumption of eggs, dairy, and legumes were reported for less than 20% of children. Milk-based formula (in powder form or prepared as liquid) was consumed by 11% of children surveyed, accounting for 67% of dairy products consumed.

Consumption of fruits and vegetables, eggs, and dairy products—while infrequently consumed by Tsimane families in general—may be further restricted for children due to age and seasonal or regional constraints. To test this, consumption of vitamin A-rich fruits/vegetables and other fruits/vegetables were analyzed in association with infant age and season, adjusting for infant sex and maternal age (Table 11). The likelihood of consuming both types of fruits and vegetables was higher during the rainy season and increased with maternal age. Males were more likely than females to consume Vitamin-A rich fruits and vegetables, but not other types of vegetables. Children older than 12 months of age were

more likely to consume non-vitamin A-rich fruits and vegetables as compared to those aged 6-11 months, though this was only a trend for children aged 24-35 months. In identical regression models, none of the above factors significantly predicted either the likelihood of egg or dairy consumption. However, when substituting region for season and holding all other factors constant, the likelihood of dairy consumption was lower in more remote villages (OR = 0.24, 95% CI = 0.05 – 0.81, $p = 0.035$).

I additionally examined variation in consumption of market foods (most frequently sugar, pasta, dried beef, oil, wheat flour, dairy, and foods consumed in San Borja), expecting that these foods would be more frequently consumed in near town villages. For all non-EBF children ($n = 131$), the number of market foods consumed, on average, the day prior was 1.80 ± 1.78 . In an ANOVA adjusting for age group and sex, the number of market foods consumed was significantly lower in remote villages (Est. = -0.82, 95% CI = -1.43; -0.22, $p = 0.008$). Market food consumption did not differ significantly by sex, and in post-hoc analysis, mean differences among age groups were only significant for ages 12-23 vs. 0-5 months (Est. = 1.32, 95% CI = 0.38; 2.27, $p = .006$). The number of market foods consumed was moderately correlated with meal frequency ($r = 0.31$, $p < 0.001$) and meal diversity ($r = 0.24$, $p = 0.006$).

Nutritional status of Tsimane children

Mean WAZ, HAZ, and WHZ and prevalence of low ($< -2SD$) and severely low ($< -3SD$) WAZ, HAZ, and WHZ by age group are given in Table 12. For infants 0-5 months, rates of low and severely low WAZ, HAZ, and WHZ range from 0.0 – 2.2%, signaling a near

absence of undernutrition in this age group. The prevalence of wasting (WHZ < -2SD) remains negligible across all age groups, with no evidence of severe malnutrition. The prevalence of underweight (WAZ < -2SD) increases after six months, but remains steady at about 16% from 12 – 35 months. However, the prevalence of stunting (HAZ < -2SD) increases markedly from 15% at 6-11 months to nearly 50% at 24-35 months. Overall, Tsimane children show a much greater prevalence of stunting when compared against WHO standards (Figure 7), but only a moderately greater prevalence of underweight (Figure 8).

Age groups significantly differed in mean WAZ ($F = 6.773$, $df = 3, 153$, $p < .001$) and HAZ ($F = 20.78$, $df = 3, 153$, $p < .001$) but not WHZ ($F = 1.115$, $df = 3, 152$, $p = .345$). The prevalence of stunting was higher among males as compared to females (41.1% vs. 26.9%), however sex differences in underweight and wasting scores were negligible (13.3% vs. 11.9%, and 1.1% vs. 3.0%, respectively). Table 13 reports results from linear regressions on WAZ and HAZ among children aged 6-35 months in association with minimum dietary diversity and other infant, maternal, and household factors. Adjusting for other covariates, nutritional status as indicated by both HAZ and WAZ did not vary with reported minimum dietary diversity, infant sex, or village region, but was positively associated with maternal age and height. Maternal BMI, education, and Spanish fluency did not improve model fit for either HAZ or WAZ. Adjusting for other covariates, mean HAZ among already weaned children was -1.1 SD lower than that of children who were still breastfeeding. However, there was a significant age group*weaning interaction (Table 13), indicating that mean HAZ was 0.64 SD lower among children weaned before 23 months as compared to those weaned at 24-35 months of age. As no infants were weaned before 12 months of age (Table 7), this

interaction was likely driven by weaning between 12-23 months of age. For WAZ, weaning and the weaning*age group interaction terms substantially reduced model fit (as indicated by AIC), and were subsequently excluded from the final model (Table 12). No other significant interactions among independent variables included in the final models for HAZ or WAZ were observed.

3.3e. Discussion

Nutritional sufficiency of complementary foods varies widely across and within populations owing to regional, demographic, and other household factors (Campos et al., 2010; Marriott et al., 2012a; Senarath et al., 2012a; b). I examined within-population variation in complementary feeding patterns and nutritional status among Tsimane children aged 0-35 months. As is discussed below, poor dietary diversity presents the most significant risk to nutritional sufficiency among Tsimane children, as indicated by international indicators for age-appropriate IYCF practices. Minimum dietary diversity was reported for less than half of Tsimane children aged 6-24 months, and was the primary factor driving low rates of minimum acceptable diet (Table 8). Though the rate of breastfeeding initiation within 1 hour was also relatively low (53%), this indicator does not appear to influence long-term breastfeeding outcomes. Mothers in this study reported universal rates of breastfeeding initiation and breastfeeding at one year, a high rate of breastfeeding at two years (72%), and a low rate of bottle-feeding (11%), confirming previous observations that the Tsimane exhibit generally intensive breastfeeding practices (Veile et al., 2014). The survey does show,

however, that approximately one third of Tsimane infants age 0-5 months were not exclusively breastfed (Table 2), and that the mean reported age of CF introduction was 3.8 months—close to our previously reported estimate of 4.1 months (Veile et al., 2014). Variation in EBF durations and associated effects on child nutritional status and health will be discussed at greater length in Chapters 4 and 5.

Mean meal frequency and dietary diversity were both higher in villages closer to town and during the rainy season. Researchers in Haiti have similarly noted regional differences in the proportion of children achieving minimum dietary diversity, and have emphasized the need for geographically targeted interventions within populations (Heidkamp et al., 2013). In this study, as stated in the methods, season and village region were at least partially confounded by the logistics of sample collection. Though both factors likely contribute independently to observed dietary variability, season may have more of an impact on Tsimane dietary sufficiency, with children more than four times as likely to achieve minimum dietary diversity during the rainy season as during the dry season (Table 10). In this population, flesh foods do not appear to substantially contribute to overall dietary diversity, as diversity was higher during the rainy season (when fishing and hunting yields are lower), and flesh foods were reportedly consumed at a high frequency (90%) by all children across the entire study period (Figure 6).

While overall dietary diversity across the sample was low, dietary diversity was improved by consumption of vitamin A-rich and other fruits and vegetables, eggs, and dairy. Of note, mean dietary diversity was highest from October-January (4.2 ± 0.2 , as compared to 3.4 ± 0.0 in February-March and from August-September 2.8 ± 0.1), which coincided with

seasonal fruiting of avocado, mango, citrus, and palm nuts. The unexpected finding of higher mean dietary diversity among villages closer to town may reflect a relatively greater concentration of fruiting trees in this region, in addition to increased access to dairy products.

The generally low consumption of dairy products by Tsimane children may be expected given that they do not readily have access to domesticated animals and refrigeration. Low prevalence of lactase persistence common among Amerindians (Alzate et al., 1969; Morales et al., 2011), which may further deter Tsimane families from regularly consuming dairy products. In this study, dairy consumption primarily reflected powdered milk and infant formula. Given the relatively high cost of these products and lack of access to clean, potable water for most families, increased consumption of these products would be inadvisable. Instead, Tsimane children's dietary diversity can be more readily improved by promoting increased consumption of widely available but relatively underutilized cultigens and foraged foods. For example, papaya and a local variant of yellow squash were abundant around family homes and may be cultivated year round, but were infrequently consumed by the children in this study. Similarly, many Tsimane families keep chickens, but don't pen them and appear to only search for and consume the eggs on occasion. While a few families cultivated pigeon peas to sell in market— a product introduced several years ago in a pilot intervention program (Leonard and Godoy, 2008) —most Tsimane do not consume legumes. Thus, it is not clear whether low consumption of the above foods reflects specific restrictions for young children or generally low consumption across the population. Regardless, Tsimane families may benefit from educational interventions that emphasize the importance of these foods in increasing young children's nutritional sufficiency.

Tsimane infants aged 0-6 months exhibit almost no signs of nutritional stress (Table 11), likely reflecting that most infants were exclusively or predominantly breastfeeding during this period (Table 8). However, maternal milk yield peaks around 2-4 months of age and is generally insufficient to support optimal infant growth after about six months of age, warranting additional complementary foods (Dewey et al., 1991; Dewey, 1998). The results presented here show a marked increase in stunting from 6-35 months of age (Table 11)—a pattern that is commonly observed in resource-poor populations and attributed to early weaning, cumulative energetic costs of frequent infection, and/or poor quantity or quality of complementary foods (e.g. Castro et al., 2009; Agudo et al., 2010; Marriott et al., 2012b; Onyango et al., 2014). All three of these factors likely contribute to the increased stunting with age previously observed among Tsimane infants aged 0-24 months (Gurven 2012).

Chapters 4 and 5 explicitly test whether shorter durations of EBF contribute to poorer growth outcomes or earlier weaning by reducing breastfeeding intensity. However, even with relatively earlier CF and reduced breastfeeding intensity, breast milk intake likely contributes substantially to nutritional intake during the first two years of life. In this study, most children were still breastfed at two years of age (Table 2), and weaning was associated with poorer nutritional status, particularly if weaning occurred before 24 months of age (Table 7). The negative effects of weaning on growth may reflect the loss of nutritional or immunological buffering from breast milk intake—or both. However, if insufficient CF contributes to growth deficits while children are still breastfeeding, this may compound the negative impacts of weaning.

Protective effects on child nutritional status included maternal height and maternal age. The relationship between maternal height and child nutritional status may reflect genetic inheritance or mothers' own conditions during childhood. The latter could signal some degree of intergenerational wealth inequality across Tsimane households. Notably, maternal age was associated with improved dietary diversity and greater likelihood of consuming fruits and vegetables (Tables 4 and 5), suggesting that older mothers may be generally better able to procure resources for their children. Increased household size had a positive effect on dietary diversity, which may suggest increased dietary breadth owing to intergenerational pooling of resources (Hooper et al., 2015). Children may also share a wider variety of foods with multiple caregivers in larger households. However, dietary household size actually had a significantly negative effect on nutritional status, suggesting that children from larger households have lower net energetic or protein intakes despite relatively greater dietary breadth. Variation in the effects of household size on dietary diversity and nutritional status may also reflect different stages of the maternal life course, as both younger and older Tsimane mothers tend to live in intergenerational households. However, there was no significant interaction between maternal age and household members. Additional research with a larger sample size may be needed to better identify specific household risk factors.

Although minimum dietary diversity was not directly associated with variation in nutritional status in this survey, and regional differences in dietary diversity did not translate to regional differences in nutritional status, it is still possible that poor dietary diversity negatively impacts child nutritional status. First, dietary diversity as measured here reflects only 24-hour intake, whereas dietary diversity may fluctuate daily across Tsimane

households; aggregate measures of dietary diversity are needed to assess longitudinal effects of low dietary diversity on nutritional outcomes. Perhaps more significantly, however, mean dietary diversity was suboptimal (fewer than 4 food groups per day, Box 1) across all demographic and ecological variables, and did not significantly increase after 12 months of age, indicating chronically low diversity of complementary foods (Table 9). In particular, the low intakes of fruits and vegetables, dairy, egg, and legumes (Figure 6) may result in chronic deficiencies in iron, zinc, folate, and A and B complex vitamins, which may contribute to poor immune functioning and increased prevalence of stunting with age (Table 12, Table 13).

It also important to note that while 90% of Tsimane children achieved minimum meal frequency and consumed flesh foods the day prior (Table 2, Figure 4), this survey did not measure actual quantities of foods consumed during meals or snacks. For the Tsimane, CF frequency may not correlate with CF quantity, which may be insufficient to support optimal growth. Tsimane children age 3 and older may be at particular risk of consuming insufficient amounts of complementary foods, as by this age their mothers have likely given birth again, and they are more frequently cared for by older siblings and feeding themselves independently. Indeed, as stated above, the total number of household members was negatively associated with both HAZ and WAZ, which may also indicate that children from larger households may face increased competition for resources or attention during meal times.

3.3f. Concluding remarks

Efforts to improve IYCF practices and nutritional outcomes in specific populations must be culturally-salient and appropriate to the needs and feasible options identified for individual communities (Hadley et al., 2008; Arabi et al., 2012; Daelmans et al., 2013). For populations such as the Tsimane, who already exhibit intensive breastfeeding practices, education and interventions aimed at improving CF quantity and quality may have the biggest impact on early nutrition. This research has identified regional and seasonal risk factors associated with relatively poorer dietary diversity. However, children failed to achieve minimum dietary diversity across regions and seasons, and ultimately variation in child nutritional status was influenced by family level factors, including maternal age, height, and household composition. Therefore, the entire population may benefit from efforts aimed at improving CF quality, while more research is needed to identify specific household factors leading to inequalities in nutritional status.

As compared to the relatively wide and successful implementation of policy and programs to promote optimal breastfeeding practices over the last several decades, relatively little institutional support has been directed at improving CF practices (Lutter and Morrow, 2013). The results presented here suggest that CF quality among the Tsimane can be improved by promoting increased consumption of underutilized foods such as papaya, squash, eggs, peanuts, pigeon peas, and lentils. These foods are already locally cultivated and can be relatively easily incorporated into existing subsistence activities. However, because Tsimane families eat communally and infant diets are only slightly modified from those of their mothers, wider consumption of these foods across the general population must be

promoted. Future research is also needed to determine if sufficient quantities of complementary foods are served to or consumed by children across different age groups, which may suggest additional educational interventions.

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Chapter 3: Tables and Figures

Figure 1. *Carnet de Salud de la Madre* (Tsimane mother from a remote village)

CARNET DE SALUD DE LA MADRE

CARNET PERINATAL - CLAP - OPS / OMS				FECHA DE NACIMIENTO		ALFA BETA		ESTUDIOS		ESTADO CIVIL		CONTROL PRENATAL EN	
NOMBRE: [REDACTED]				dia	mes	año	si	ninguno	primaria	casada	unión estable	G A L I L E A	
DOMICILIO: [REDACTED] ZONA: [REDACTED]				EDAD (años)		< de 16	> de 40	secund	univ	soltera	otro	PARTO EN	
MUNICIPIO: [REDACTED] RED: 06				20 04 18 8		22		años en el mayor nivel				NUMERO H. CLINICA: 200488-PC	
ANTECEDENTES				OBSTETRICOS				EMBARAZO ANTERIOR					
PERSONALES: adm. preconcep. todo folico, cirugía tracto reprod., infertilidad, cardiop. notrop., cond. médica grave.				gestas previas: 00, abortos: 00, vaginales: 00, nacidos vivos: 00, vivien: 00.				FUM: 01, 02, 10, 21, 40; FPP: A, D, A, A, A, D.					
EMBARAZO ANTERIOR: mes, año, menos de 6 meses, más de 5 años.				último < 2500g, último > 4500g, último preclampsia eclampsia.				EMBARAZO PLANEADO: si, no.					
FRACASO METODO ANTICONCEPTIVO: no usaba, condón, DIU, píldora, depo, natural.				EG. CONFIABLE por FUM, MOVIM. FETALES desde mes, año.				ANTIETANICA: si, no, vigente, DOSIS, mes gestación.					
GESTACION ACTUAL				CIGARRILLOS POR DIA: 00, ALCOHOL DROGAS: si, no.				ANTRIEOLA: si, no, previa, no parte.					
NET DE NACIA: si, no. BACTERIAS: si, no. GRUPO: si, no. PAPANICOLAU: si, no.				VORLRRP: < 20 sem, SIFILIS confirmada por FTA.				ESTREPTOCOCCO B: si, no.					
Fecha: 25/07/21, edad gest: 60, PA: 110/70, altura: 150, peso: 49.				IMC: 90.				SIGNOS DE ALARMA, exámenes, tratamientos.					
Hospital en Embarazo: si, no.				Ruptura Membranas Anteparto: si, no.				Presentación: si, no.					
Inicio: espontáneo, inducido, cesar.				Edad Gest al parto: semanas, días.				Medidas: HTA previa, HTA inducida, preclampsia.					

Control a campaña de salud y educación comunitaria.

Table 1: Subject characteristics (n =160)

(Range, mean, SD, where applicable)	N	%
Infant sex		
Male	91	57%
Female	69	43%
Infant age (mos.) (0-35, 13 ± 10)		
0-5	45	28%
6-11	61	38%
12-23	27	17%
24-35	27	17%
Birth interval (mos.) (11 - 164, 33 ± 21)		
No previous birth	26	17%
< 24	43	28%
24 -35	50	32%
36 or more	37	24%
Maternal age (yrs.) (14 - 49, 27 ± 9)		
< 20	33	21%
20 to 29	71	44%
30 to 39	37	23%
40 and over	19	12%
Number of births (1 -13, 5 ± 3)		
No previous birth	26	16%
2 to 5 kids	82	52%
Six or more	51	32%
Spanish fluency		
None	76	48%
Conversational	71	44%
Fluent	13	8%
Education completed (0 - 12, 2 ± 2)		
None	44	28%
1st - 2nd	56	35%
3rd - 5th	52	33%
6th -12th	8	5%
Household members (3 -20, 7 ± 4)		
3 to 5	49	31%
6 to 9	71	45%
10 to 20	38	24%

Table 2. Percentage of Tsimane mothers who received professional medical attention during pregnancy or childbirth

Maternal interview responses	Near Town % yes (n)	Remote % yes (n)	Total % yes (n)
Received <i>Carnet de Salud de la Madre</i>	65% (57/87)	50% (31/62)	59% (87/149)
Where <i>Carnet</i> received			
Galilea clinical	58% (33/57)	10% (3/30)	41% (36/87)
In village (mobile provider)	19% (11/57)	77% (23/30)	39% (34/87)
San Borja Hospital	18% (10/57)	13% (4/30)	17% (14/87)
Horeb clinic	5% (3/57)	--	3% (3/87)
Sought emergency care (not for <i>Carnet</i>)	19% (16/86)	5% (3/61)	13% (147)
Place of birth			
At home	91% (79/87)	87% (55/63)	89% (150)
In another residence	7% (6/87)	13% (8/63)	9% (150)
In hospital	2% (2/87)	--	1% (150)

Table 3. Logistic regression of likelihood of at least one prenatal screening (documented in *Carnet de Salud de la Madre* (n = 149))

Factor	OR (95% CI)	<i>p</i>
Previous infant death or miscarriage*	1.14 (0.53 – 2.44)	0.73
<i>Village residence</i>		
Remote	--	--
Near Town	1.87 (0.94 – 3.73)	0.07
<i>Parity group</i>		
Prime (4-8 births)	--	--
Primiparous	1.78 (0.64 – 4.93)	0.27
Low (2-3 births)	2.49 (1.04 – 5.97)	0.04
High (9-13 births)	1.71 (0.56 – 5.18)	0.35

*Mother reported at least one prior infant death or miscarriage

Figure 2. Proportion of kin types reported to assist with birth (207 total assistants named by 132 maternal subjects).

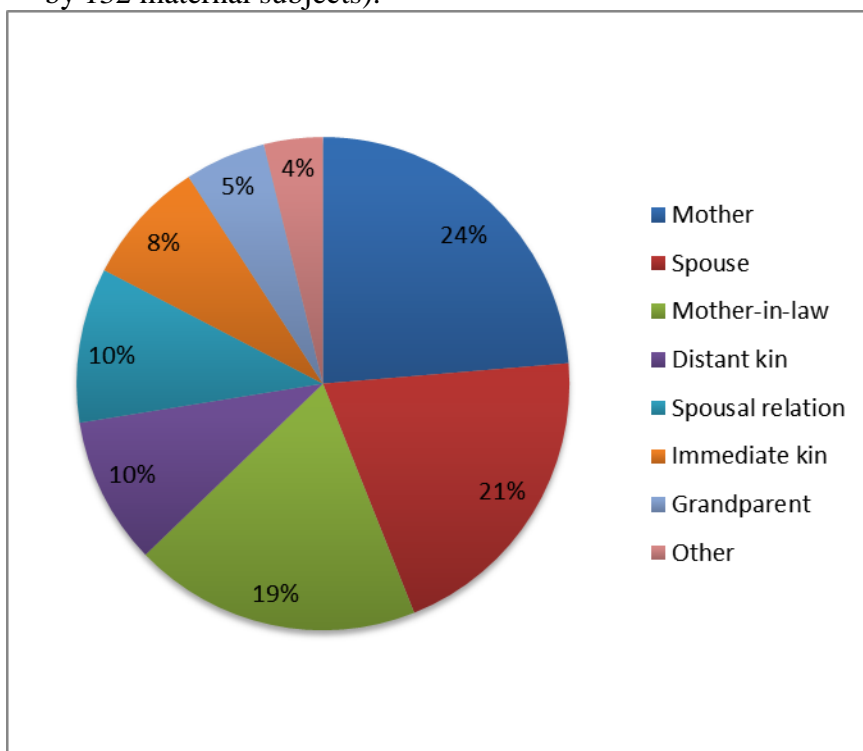


Table 4. Mothers' reported use of pharmaceutical and traditional medicines during labor, delivery, or postpartum recovery. Reasons for taking specified medicine were given as open-ended responses and are grouped together here into five general categories.

Response:	Pharmaceuticals	Traditional Medicine
<i>Any usage</i>	49% (74/150)	30% (45/150)
<i>Reasons for taking specific medicine</i>		
Pain	63% (36/57)	29% (10/35)
Facilitate birth or placental delivery	27% (15/57)	20% (7/35)
Prevent hemorrhaging and infection	10% (6/57)	20% (7/35)
Promote healing/cleansing	--	15% (5/35)
Postpartum contraception	--	17% (6/35)

Table 5. Logistic regression of factors influencing likelihood of maternal pharmaceutical and traditional medicine usage during labor, birth, or postpartum recovery (n = 150)

Factor	Pharmaceutical medicine		Traditional medicine	
	OR (95% CI)	<i>p</i>	OR (95% CI)	<i>p</i>
Previous infant death /miscarriage*	2.40 (1.14 – 5.03)	0.02	0.58 (0.25 – 1.35)	0.21
<i>Village residence</i>				
Remote	--	--		
Near Town	1.24 (0.63 – 2.45)	0.52	0.74 (0.35 – 1.57)	0.44
<i>Parity group</i>				
Prime (4-8 births)	--	--		
Primiparous	1.09 (0.40 – 2.95)	0.86	0.33 (0.10 – 1.16)	0.08
Low (2-3 births)	0.97 (0.42 – 2.22)	0.94	0.60 (0.24 – 1.55)	0.29
High (9-13 births)	0.91 (0.31 – 2.71)	0.87	4.08 (1.31 – 12.71)	0.02

Box 1. WHO (2008) criteria used for assessing infant feeding status and indicators of age-appropriate infant and young child feeding (IYCF) practices. Selected core indicators are based on dietary consumption the day prior, as assessed by 24-hour dietary recall.

Feeding Status	Included	Allowed	Excluded
Exclusive Breastfeeding (EBF)	Breast milk	Oral rehydration salts (ORS), vitamins, minerals, medicines	All other non-milk liquids and solids
Predominant Breastfeeding	Breast milk	Minimal liquids (water, water-based drinks*, ritual fluids, ORS, vitamins, minerals, medicines)	All other liquids and solids (including infant formula and non-human milk)
Complementary feeding (CF)	Breast milk	Any other liquids or solids (including formula and other milks)	NA
Breastfeeding (BF)	Breast milk	Any other liquids or solids (including formula and other milks)	NA
Selected core indicators		Target sample population and description of indicators	
Early initiation of BF		Children 0-24 months breastfed within 1 h of birth	
EBF < 6 months		Children 0-5 months EBF	
Continued BF at 1 year		Children 12-15 months still BF	
Intro CF		Children 6-8 months receiving any complementary foods	
Minimum dietary diversity (MDD)		Children 6-24 months receiving complementary foods from at least 4 out of a possible 7 food groups: (1) staples (grains, roots, tubers and plantains), (2) flesh foods (meat, fish, poultry, organ meats), (3) vitamin A-rich fruits and vegetables, (4) other fruits and vegetables, (5) eggs, (6) legumes and nuts, (7) dairy products	
Minimum meal frequency (MMF)		BF children 6- 8 months receiving at least 2 meals (including snacks) a day; BF children 9-23 months receiving at least 3 meals/snacks a day; non-BF children 6-23 months receiving at least 4 meals/snacks a day	
Minimum acceptable diet		Children 6-23 months receiving MDD and MAD	
Children ever BF		Children 0-23 months ever breastfed	
Continued BF at 2 years		Children 21-24 months still BF	
Age-appropriate BF		Total proportion of children 0-5 months EBF and children 6-23 months BF	
Predominant BF < 6 months		Children 0-5 months predominantly breastfed	
Bottle Feeding		Children 0-24 months fed with a bottle the day prior	

• In this study, infant consumption of sugar water and juices was categorized as “predominant breastfeeding”; consumption of chicha (an essential local staple, consisting of fermented manioc, corn, or rice mixed with water) was categorized as “complementary feeding”

Figure 3. Nutribebe infant formula, provided by the San Juan municipal government.



Table 6. Frequency of premastication by child age

	% (n)
Premasticated solids	
<i>Ever</i>	84 % (106/127)
<i>First food</i>	64 % (80/125)
<i>Still on occasion</i>	44 % (53/120)
0- 5 months	83 % (10/12)
6 – 11 months	76 % (19/25)
12 – 23 months	37 % (22/60)
24 – 36 months	9 % (2/23)
<i>Yesterday</i>	32 %
0- 5 months	47 % (8/17)
6 – 11 months	65 % (17/26)
12 – 23 months	27 % (16/59)
24 – 36 months	4 % (1/24)
Mouth to mouth liquids	
<i>Ever</i>	51 % (65/128)
<i>First liquid</i>	40 % (49/123)
<i>Still on occasion</i>	24 % (15/62)
0- 5 months	83 % (5/6)
6 – 11 months	55 % (6/11)
12 – 23 months	13 % (4/32)
24 – 36 months	0 % (0/13)
<i>Yesterday</i>	6 % (8/132)
0- 5 months	17 % (3/17)
6 – 11 months	15 % (4/26)
12 – 23 months	2 % (1/62)
24 – 36 months	0 % (0/26)

Table 7. Logistic regression of likelihood of pre-masticating or giving liquids mouth-to-mouth the day prior. Dietary information gleaned from 24-hour recall of non-exclusively breastfed children aged 0-35 months.

Factor	Premasticated solids day prior (n = 111)		Liquids mouth-to-mouth day prior (n = 114)	
	OR (95% CI)	<i>p</i>	OR (95% CI)	<i>p</i>
Infant age	0.86 (0.80 – 0.92)	< 0.001	0.78 (0.64 – 0.94)	0.01
Maternal age	0.98 (0.93 – 1.04)	0.545	1.09 (0.98 – 1.21)	0.11
Village region				
<i>Remote</i>	--	--	--	--
<i>Near town</i>	1.76 (0.63 – 4.93)	0.279	0.78 (0.12 – 4.95)	0.80
Spanish fluency				
<i>None</i>	--	--	--	--
<i>Some to fluent</i>	0.76 (0.29 – 1.97)	0.568	0.45 (0.07 – 3.01)	0.41
Ate meat day prior	1.27 (0.46 – 3.55)	0.645	--	--
Ate fish day prior	3.89 (1.42 – 10.61)	0.008	--	--

Table 8. Prevalence of age-appropriate IYCF practices in Tsimane children 0-23 months (by indicator age group).

WHO Indicator (Box 1)	n	%	95 % CI
Early BF initiation (0 -23 months)	131	53.4	(44.5; 62.1)
Children ever BF (0 – 23 months)	133	100.0	(96.5; 1.00)
EBF under 6 months (0 – 6 months)	45	64.4	(48.7; 77.7)
CF 6- 8 months (6 – 8 months)	13	100.0	(71.7; 100.0)
Continued BF at 1 year (12 – 16 months)	18	100.0	(78.1; 100.0)
Continued BF at 2 years (19 – 23 months)	15	72.2	(58.4; 97.7)
Age-appropriate BF (0 – 23 months)	133	82.0	(74.1; 87.9)
Predominant BF (0 – 6 months)	45	11.1	(4.2; 24.9)
Bottle-feeding (0 – 23 months)	133	10.5	(6.1; 17.3)
Min. dietary diversity (6 -23 months)	88	44.3	(33.9; 55.3)
Min. meal frequency (6 – 23 months)	88	89.8	(81.0; 94.9)
Min. acceptable diet (6 – 23 months)	88	40.9	(30.7; 51.9)

Figure 4. Percentages of age-appropriate breastfeeding (BF) practice in Tsimane vs. composite Bolivia national surveys (WHO 2008)

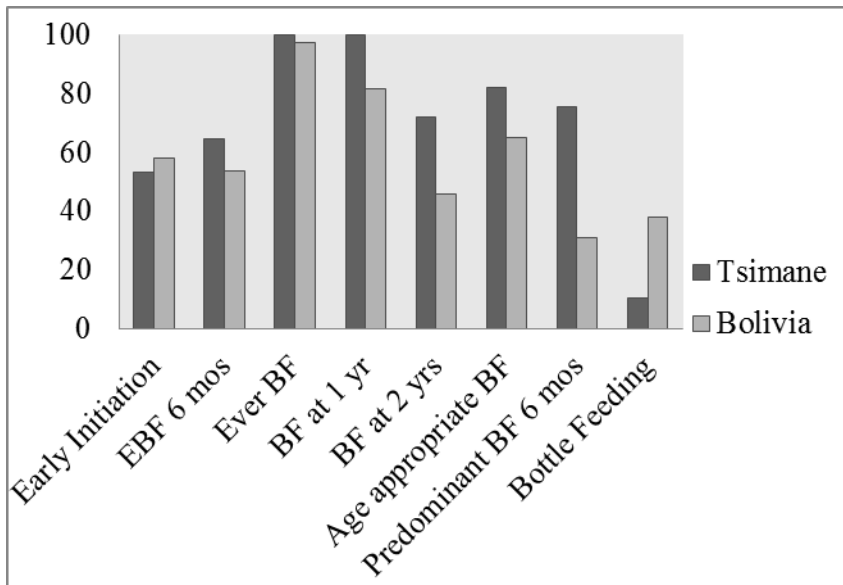


Figure 5. Percentages of age-appropriate CF practice in Tsimane vs. Bolivian national surveys (WHO 2008)

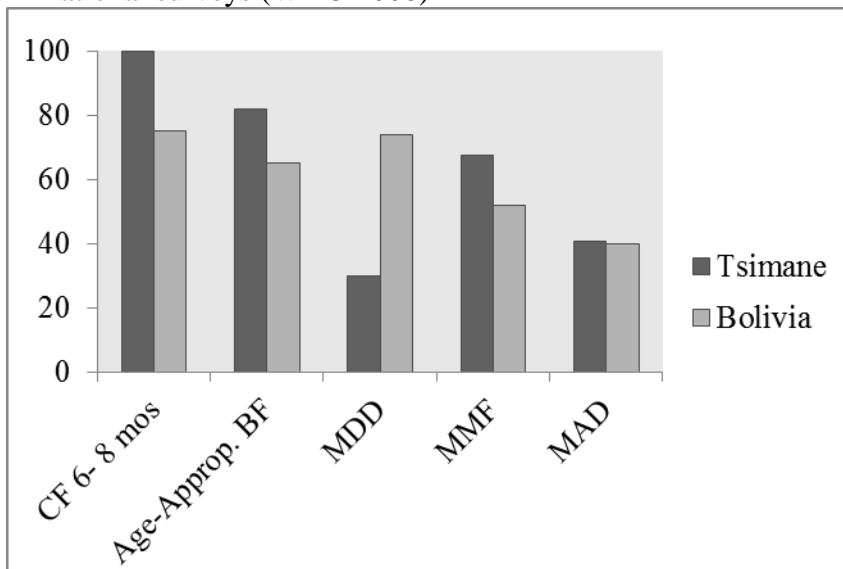


Table 9. Mean meal frequency and dietary diversity (see Box 1 for definitions) by univariate analysis of child age group, maternal age group, season, and region (all non-EBF children aged 0-35 months, n = 131). Means were obtained from post-hoc analysis, with significant differences among factor levels denoted by corresponding superscripts (a, b, c, d).

Factor	N	Mean Meal Frequency	Mean Meal Diversity
All children (mean \pm SD)	131	4.5 \pm 1.9	3.1 \pm 1.2
<i>Child age group (months)</i>			
a) 0 - 5	16	2.4 ^{b*;*c,d***}	1.9 ^{b*;*c***;d*}
b) 6 - 11	27	4.3 ^{a**}	2.9 ^{a,c,d*}
c) 12 - 23	61	4.9 ^{a***}	3.6 ^{a***;b*}
d) 24 - 35	27	4.9 ^{a***}	3.1 ^{a***;b*}
<i>Maternal age group (years)</i>			
a) < 20	25	4.2	2.9
b) 20 - 29	59	4.2	2.1
c) 30 - 39	29	4.8	3.3
d) 40 and over	16	5.3	3.6
<i>Season</i>			
a) Dry (Aug-Oct)	63	4.1 ^{b*}	2.7 ^{b**}
b) Rainy (Nov-Apr)	68	4.8 ^{a*}	3.5 ^{**}
<i>Village distance to town</i>			
a) Near (< 20 km)	79	4.8 ^{b*}	3.4 ^{b**}
b) Remote (> 25 km)	52	3.9 ^{a*}	2.7 ^{**}

*p < 0.05, **p < 0.01, *** p < 0.001. Significance levels were obtained using Tukey HSD multiple comparisons of means tests with 95% family-wise confidence levels.

Table 10. Univariate and multivariate analysis of likelihood of minimum dietary diversity (non-EBF children aged 6-23 months)

Independent Variable	Unadjusted Odds Ratios			Adjusted Odds Ratios		
	OR	95%	<i>p</i>	OR	95%	<i>p</i>
Child Factors						
Child age (months)	1.14	1.04 – 1.25	0.01	1.11	1.00 – 1.24	0.05
<i>Child sex</i>						
Female	1.00	--	--	1.00	--	--
Male	0.79	0.34 – 1.84	0.58	0.69	0.24 – 1.90	0.47
<i>Breastfeeding</i>						
Breastfed	1.00					
Weaned	0.93	0.64 – 1.34	0.69			
Maternal Factors						
Maternal age (yrs)	1.04	0.99 – 1.10	0.11	1.11	1.03 – 1.20	0.01
Living dependents	1.10	0.95 – 1.30	0.22			
# Dependents < 5 yrs	1.03	0.56 – 1.90	0.92			
<i>Spanish fluency</i>						
None	1.00	--	--			
Conversational	1.31	0.55 – 3.12	0.55			
Fluent	1.44	0.16 – 12.97	0.73			
Highest grade completed	1.17	0.96 – 1.44	0.13	1.33	1.00 – 1.85	0.07
Total household	1.08	0.96 – 1.22	0.19	1.01	0.88 – 1.17	0.85
<i>Village region</i>						
Near Town	1.00	--	--			
Remote	0.31	0.12 – 0.77	0.01			
<i>Season</i>						
Dry	1.00	--	--	1.00	--	--
Rainy	4.02	1.67 – 10.24	< 0.01	4.06	1.41 – 12.63	0.01

Figure 6. Percentage of Tsimane children aged 6-23 months (n =131) that consumed specific WHO food groups (see Box 1), as reported by mothers in 24-hour recall.

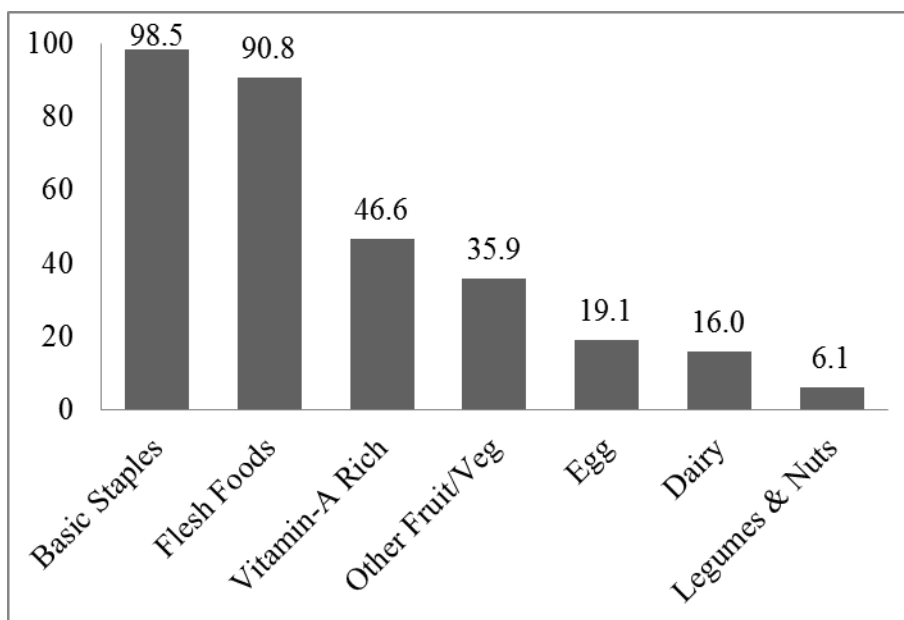


Table 11. Likelihood of consuming fruits and vegetables (all non-EBF children 6-35 months of age, n = 115). Separate logistic regressions were run on vitamin-A rich fruits and vegetables and other fruits and vegetables.

Independent Variable	Vitamin-A Fruits & Veg			Other Fruits & Veg		
	OR	95% CI	<i>p</i>	OR	95% CI	<i>p</i>
<i>Infant age group</i>						
6 – 11 months	1.00	--	--	1.00	--	--
12 – 23 months	1.52	0.54 – 4.36	0.43	4.49	1.49 – 15.94	0.01
24 – 35 months	0.76	0.23 – 2.51	0.66	3.21	0.93 – 12.48	0.07
<i>Infant Sex</i>						
Female	1.00	--	--	--	--	--
Male	2.44	1.05 – 5.89	0.04	1.12	0.50 – 2.58	0.78
Maternal age (years)	1.05	1.00 – 1.11	0.05	1.05	1.00 – 1.10	0.05
<i>Season</i>						
Dry	1.00	--	--	--	--	--
Rainy	5.32	2.32 – 12.98	< 0.01	2.81	1.24 – 6.64	0.02

Table 12. Tsimane children mean, SD, and prevalence of low (< -2SD) and severely low (< -3SD) WAZ, HAZ, and WLZ scores by age group (in months).

Age groups	N	Mean	SD	% < -2SD	(95% CI)	% < -3SD	(95% CI)
Weight-for-age (WAZ)							
(0-5)	45	-0.29	0.84	0.0	(0.0%, 1.1%)	0.0	(0.0%, 1.1%)
(6-11)	26	-0.44	1.05	11.5	(0.0%, 25.7%)	0.0	(0.0%, 1.9%)
(12-23)	61	-0.98	0.96	16.4	(6.3%, 26.5%)	4.9	(0.0%, 11.2%)
(24-35)	25	-0.98	0.97	16.0	(0.0%, 32.4%)	0.0	(0.0%, 2.0%)
Total:	157	-0.71	0.99	10.8	(5.6%, 16.0%)	1.9	(0%, 4.4%)
Length/height-for-age (HAZ)							
(0-5)	45	-0.08	1.07	2.2	(0.0%, 7.6%)	2.2	(0.0%, 7.6%)
(6-11)	27	-0.80	1.10	14.8	(0.0%, 30.1%)	0.0	(0.0%, 1.9%)
(12-23)	60	-1.62	1.39	36.7	(23.6%, 49.7%)	16.7	(6.4%, 26.9%)
(24-35)	25	-2.14	1.14	48.0	(26.4%, 69.6%)	20.0	(2.3%, 37.7%)
Total:	157	-1.12	1.43	24.8	(17.8%, 31.9%)	10.2	(5.1%, 15.2%)
Weight-for-length/height (WHZ)							
(0-5)	45	-0.33	1.05	2.2	(0.0%, 7.6%)	0.0	(0.0%, 1.1%)
(6-11)	26	0.03	1.00	0.0	(0.0%, 1.9%)	0.0	(0.0%, 1.9%)
(12-23)	60	-0.23	1.03	1.7	(0.0%, 5.7%)	0.0	(0.0%, 0.8%)
(24-35)	25	-0.10	1.36	4.0	(0.0%, 13.7%)	0.0	(0.0%, 2.0%)
Total:	156	-0.17	1.02	1.9	(0.0%, 4.4%)	0.0	(0.0%, 0.3%)

Figure 7. Tsimane HAZ scores as compared to WHO standards (age 0-23 months)

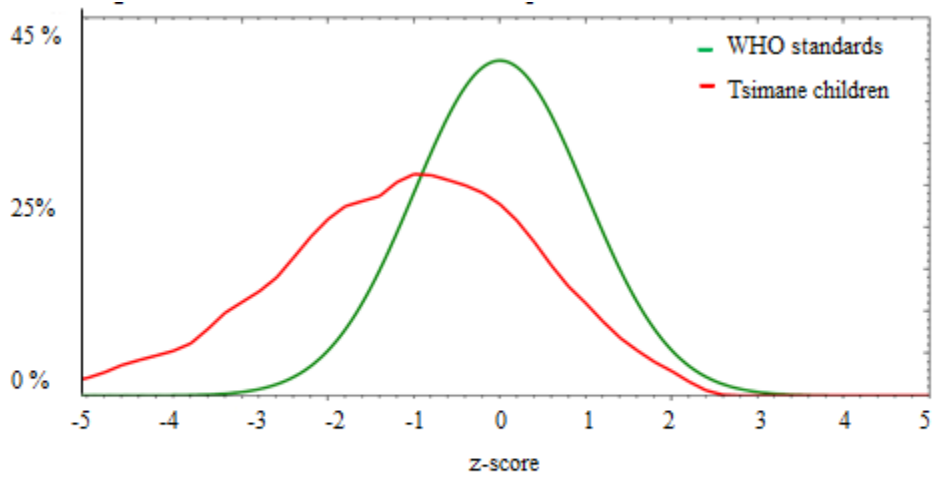


Figure 8. Tsimane WAZ scores as compared to WHO standards (age 0-23 months)

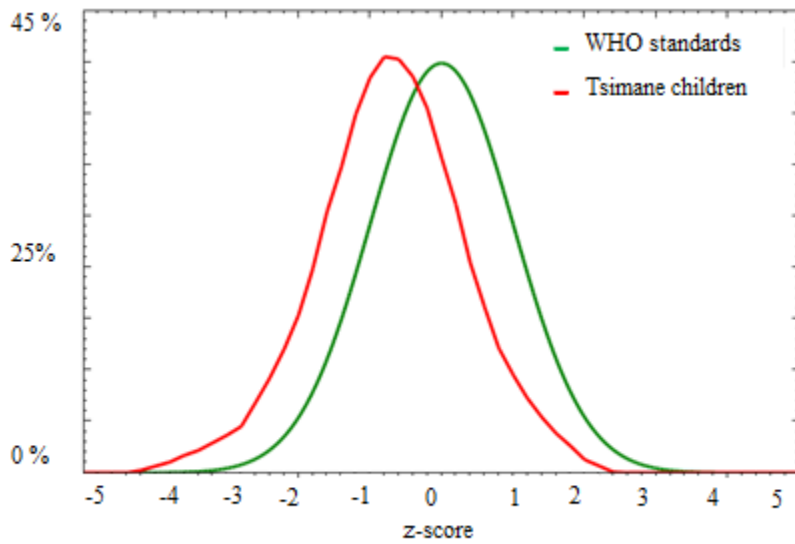


Table 13. Factors associated with HAZ and WAZ in Tsimane children ages 6-35 months (n =110)

Independent Variable	HAZ			WAZ		
	Est.	95% CI	<i>p</i>	Est.	95% CI	<i>p</i>
<i>Infant age group</i>						
6 – 23 months	0.00	--	--	0.00	--	--
24 – 35 months	-1.47	-2.35; -0.59	< 0.01	-0.40	-0.84; 0.04	0.08
<i>Infant sex</i>						
Female	0.00	--	--	0.00	--	--
Male	-0.26	-0.74; 0.21	0.27	-0.23	-0.60; 0.13	0.21
Maternal age (years)	0.04	0.01; 0.07	0.02	0.04	0.01; 0.06	< 0.01
Maternal height (cm)	0.08	0.02; 0.13	0.01	0.05	0.01; 0.09	0.02
Total household	-0.09	-0.16; - 0.02	0.02	-0.06	-0.11; 0.00	0.05
<i>Village Region</i>						
Near Town	0.00	--	--	0.00	--	--
Remote	-0.22	-0.78; 0.34	0.44	-0.14	-0.58; 0.29	0.52
<i>Minimum Dietary Diversity</i>						
No	0.00	--	--			
Yes	-0.08	-0.58; 0.42	0.761	0.00	-0.39; 0.40	0.99
<i>Breastfeeding Status</i>						
Breastfed	0.00	--	--	--	--	--
Weaned	-1.11	-2.03; -0.19	0.018	--	--	--
Age 24- 35 months*weaned	1.75	0.38; 3.12	0.012	--	--	--

Chapter 4: Do shorter EBF durations benefit Tsimane mothers?

4.1 Introduction

Globally, most infants are not “optimally” breastfed according to current recommendations—i.e. exclusive breastfeeding (EBF) for the first 6 months of life followed by complementary feeding (CF) and continued breastfeeding for 2 years or more (WHO 2008). “Suboptimal” CF—defined as the consumption of any non-maternal milk liquids or solids before six months of age (WHO, 2008)—has been shown to increase infant morbidity and mortality risks owing to increased pathogen exposure and reduced breast milk intake, particularly in high-pathogen and resource-limited environments (Hop et al., 2000; Kramer and Kakuma, 2002; Kalanda et al., 2006). Although the quality of complementary foods varies across environments, human milk is specifically adapted to meet the nutritional and immunological needs of developing human infants; therefore, all CF prior to 6 months is considered suboptimal (WHO, 2009). Following this logic, any CF relative to EBF would be indicative of reduced investment in a current infant, even when considering the highest quality complementary foods (e.g. milks from other species or manufactured infant formulas that have been hygienically handled and prepared).

As reviewed in Chapter 1, biological and cross-cultural evidence suggest that about six months of EBF is evolutionarily appropriate for our species, but with an emphasis on *about*. That is, ample variation in EBF durations may be expected owing to maternal reproductive trade-offs, but also differences in infant developmental trajectories and/or or poor lactational performance that necessitate earlier CF. From an evolutionary perspective, however, such

scenarios are not indicative of “suboptimal” EBF durations, but rather shifts in maternal and/or infant optima that favor earlier CF.

Chapter 1 briefly introduced two models that predict variation in EBF duration in association with maternal reproductive interests and infant needs. The first, the Feeding Substitution Model, predicts that relatively earlier CF is favored when the benefits to mothers outweigh the costs to infants. The second, the Feeding Augmentation Model, predicts that relatively earlier CF may actually provide a net benefit to infants, at least up to a certain threshold. Theoretical and empirical support for both of these models is described below, along with relevant aspects of Tsimane reproductive, nutritional, and disease ecologies expected to influence EBF duration. I then describe four hypotheses and component predictions to test the basic premise and underlying mechanistic assumptions of these models. These predictions are evaluated using select data described in Chapter 2, with results and discussion following.

4.1a. Maternal reproductive trade-offs favoring earlier CF

All organisms differently allocate finite resources in order to optimize fitness, resulting in trade-offs between growth, reproductive, and somatic energy investment (Stearns, 1992). Within the category of reproductive investment, additional trade-offs occur between current and future offspring, and the quantity and quality of those offspring (Lack, 1947; Williams, 1966). Humans require considerable parental investment during infancy, have prolonged juvenile stages, and rear offspring from multiple successive births simultaneously (Kramer,

2010). Thus for humans, shifts from current to future reproduction may also facilitate quantity vs. quality investment, depending on the totality of parental resources available to invest per offspring (Borgerhoff Mulder, 2000; Gillespie et al., 2008).

Numerous physiological and behavioral mechanisms in females have evolved to optimize reproductive investment in response to specific socioecological contexts (Blurton Jones, 1986; Voland, 1998; Borgerhoff Mulder, 2000; Vitzthum, 2008; Wasser and Barash, 2015). In particular, fecundity is mediated by hormonal pathways signaling current lactational costs, energy balance, and nutrient stores—i.e. metabolic energy available for future reproduction (Blurton Jones, 1986; Ellison, 1994). Phenomena such as lactational amenorrhea (discussed in greater detail below) and ovulatory suppression that occurs with extreme malnourishment are examples of physiological processes mediating current vs. future investment (Vitzthum, 2009). Fertility is additionally influenced by evolved cognitive mechanisms that track extrinsic risks (e.g. Geronimus et al., 1999) and/or the costs of extra-somatic investment in offspring (Kaplan et al., 2002), allowing parents to adjust reproductive strategies (e.g. age at first intercourse or first reproduction, sexual activity, contraceptive use) to optimally invest in quantity vs. quality of offspring.

In populations in which resources are scarce, extrinsic mortality is high, and extra-somatic capital is not readily accrued or transmitted, energy allocation tends to favor quantity over quality investment—with higher fertility often associated with higher infant mortality, faster life history, smaller body size, and compromised nutritional status of offspring (Strassmann and Gillespie, 2002; Hagen et al., 2006; Walker et al., 2008). In such populations, any improvements in energetic condition or efficiency are invested at a higher

rate in higher fertility, rather than in health, longevity, or quality investment in offspring (Gibson and Mace, 2006; Quinlan, 2007; McAllister et al., 2012). A striking example of improved energy efficiency being allocated to quantity investment was observed among the Arsi, a resource-poor agropastoralist population of Southern Ethiopia. From 1996-2000, village taps were installed that dramatically reduced women's time and energy spent procuring water from distant rivers. Subsequent demographic surveys revealed that these energy savings were converted into higher birth rates, which subsequently reduced available resources in households and increased rates of child malnutrition (Gibson and Mace, 2006). Similarly among Xculoc Mayan women of Mexico, reductions in energy expenditure following the introduction of labor-saving technologies resulted in earlier ages at first birth (Kramer and Mcmillan, 1999).

Flexible complementary feeding may be a key mechanism facilitating such reproductive trade-offs. There is ample evidence linking reduced breastfeeding intensity to shorter durations of postpartum amenorrhea (Howie and McNeilly, 1982; Howie et al., 1982; Huffman, 1984). In natural fertility populations, pregnancy may follow shortly after resumption of menstruation, which often precipitates weaning of the current suckling infant. Relatively early CF may mediate this relationship by reducing nursing intensity and the metabolic cost of lactation, both of which are involved in hormonal regulation of lactational amenorrhea. As reviewed by McNeilly (1997), suckling disrupts release of hypothalamic gonadotropin releasing hormone (GnRH). During normal ovulatory activity, GnRH stimulates secretion of luteinizing hormone (LH), which stimulates secretion of estradiol from developing ovarian follicles. In a positive feedback loop, estradiol stimulates a large

preovulatory surge in LH via GnRH, which induces ovulation (release of an egg from the ovary) and formation of the corpus luteum (necessary for establishing and maintaining pregnancy if the egg is fertilized). Through as yet undetermined mechanisms, suckling interferes with the release of GnRH, ultimately inhibiting follicular development and ovulation. The suckling intensity hypothesis proposes that when suckling declines below some critical level, this inhibitory mechanism is disrupted, and LH secretion increases to levels necessary for normal ovulatory function to resume (McNeilly, 1997).

More recently, however, the *metabolic load model* (Ellison, 1994; Valeggia and Ellison, 2003, 2009) has proposed that breastfeeding suppresses ovulatory function via hormonal signals of the energetic costs of lactation, rather than suckling intensity *per se*. As reviewed by Valeggia and Ellison (2009), multiple hormones—e.g. thyroid hormones, cortisol, and insulin—are dually involved in energy metabolism and reproductive function. The relative availability of these hormones in circulation postpartum serves as a signaling mechanism of energy available for future reproduction, but is influenced by both maternal condition and breastfeeding intensity. Insulin, for example, stimulates production of ovarian hormones and may be involved in GnRH release (Valeggia and Ellison, 2009). Insulin availability, in turn, is expected to be positively correlated with energy intake and baseline maternal condition (Emery Thompson, 2013), but negatively correlated with breastfeeding intensity (Hubinot et al., 1988; Ellison and Valeggia, 2003; Gunderson et al., 2013). Considering the influences of both energy availability and suckling intensity on mediating hormonal signaling, the metabolic load model makes different predictions about the influence of breastfeeding intensity on the duration of lactational amenorrhea. Under this model, given equally high

breastfeeding intensities, a mother in relatively better maternal condition or with higher energy intake would still be expected to resume menstruation earlier than a mother in relatively poorer condition or with limited energy intake—because of the former’s relatively lower net energetic costs.

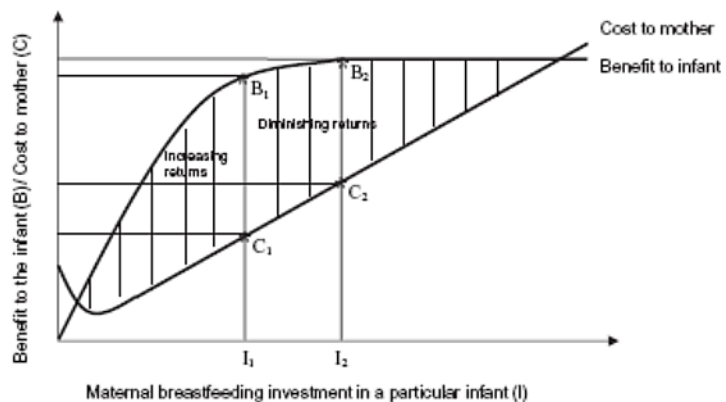
Durations of lactational amenorrhea recorded across human populations are highly varied, reflecting both wide variation in breastfeeding behaviors and maternal condition. Mean durations of postpartum amenorrhea range from 10 – 22 months in rural, well-nourished, traditional breastfeeding populations from Argentina, Papua New Guinea, Bangladesh, and Rwanda (Stern et al., 1986; Worthman et al., 1993; Valeggia and Ellison, 2004). Durations of postpartum amenorrhea among breastfeeding women in urban populations tend to be shorter—about 7 months on average across major cities in China, Guatemala, Australia, New Delhi, Nigeria, Chile, and Sweden (Bhatnagar et al., 1998). In this latter survey, risk of menses was significantly increased by multiple factors indicative of either improved maternal condition or supplementary infant feeding practices. Specific risk factors included lower parity, greater maternal postpartum body mass index (BMI), longer intervals between breastfeeding bouts, introduction of any complementary foods or liquids, reduced frequency and total 24-hour duration of breastfeeding, CF accounting for more than 50% of infant feeds, and full weaning (Bhatnagar et al., 1998).

With the understanding that breastfeeding intensity and maternal condition jointly influence fertility outcomes, several evolutionary-oriented researchers have proposed that variance in EBF durations reflects different resolutions of maternal-offspring conflicts (McDade and Worthman, 1998; McDade, 2001; Sellen, 2007; Vitzthum, 2008; Tully and

Ball, 2013). Broadly, this perspective assumes that infant fitness—in terms of survivorship, growth, immune, and cognitive function—benefits unilaterally from full-term EBF (i.e. EBF to six months). This assumption, at least in part, stems back to Rowland et al.’s (1978) ”Weanling’s Dilemma”, in which high levels of pathogen contamination were found in traditional Gambian complementary foods. The authors concluded from this finding that in developing populations, “*feeding babies with any food other than breast-milk is bound to be hazardous*” (Rowland et al., 1978, pg. 137). McDade and Worthman (1998) extended the Weanling’s Dilemma to account for the maternal metabolic and time costs of EBF. According to their model, maternal fitness may be maximized by relatively shorter durations of EBF, as CF allows them to begin divesting somatic energy and time into future reproduction or other fitness pursuits. At the same time, they stressed that the costs and benefits of EBF for both mothers and infants are context dependent, varying across different, reproductive, cultural, and disease ecologies.

More recently, Tully and Ball (2013) applied parent-offspring conflict theory to formally model optimal breastfeeding investment as a function of trade-offs between maternal costs and infant

Figure 1. Tully and Ball’s (2013) theoretical model of mother-infant breastfeeding trade-offs



benefits. In their model (Fig. 1), reductions in maternal costs or increased perception of

benefits to the infant are expected to increase breastfeeding investment, thereby favoring longer EBF durations. In a given example, increases in structural support for and knowledge about the benefits of breastfeeding (for either maternal or infant health) would be expected to reduce maternal costs and increase perceived benefits, thus shifting maternal optimums towards longer EBF durations (Tully and Ball 2013). However, their interpretation of the model does not address the mediating effects of maternal reproductive fitness on costs of breastfeeding investment. That is, conditions that favor future reproductive investment (e.g. low parity) or quantity over quality investment (e.g. low investment in extra-somatic capital, high extrinsic mortality) may still favor reduced breastfeeding investment.

Thus, in an extension of the above models, I propose the Feeding Substitution Model of CF, which explicitly considers how reproductive value and maternal and infant condition may combine to shift maternal optimums towards shorter EBF durations. First, the Feeding Substitution Model predicts that EBF duration follows an inverse u-shaped curve in relation to maternal reproductive condition—a combination of somatic condition and reproductive value. For example, the high metabolic costs of EBF may favor relatively earlier CF among very nutritionally or physically stressed mothers. Alternately, in environments in which surplus somatic energy is preferentially invested in offspring quantity (e.g., Gibson and Mace, 2006), mothers in relatively better condition may be more likely to shift investment to future reproduction by decreasing breastfeeding costs. In both cases, however, breastfeeding decisions relative to maternal condition will be mediated by reproductive value. That is, decreases in breastfeeding investment, which decrease infant fitness, may only be favored when maternal gains from future reproduction are likely (Tracer, 1996). Consistent with

Williams' 1966 "terminal investment hypothesis", EBF duration and parity are expected to be positively correlated.

Similar relationships between maternal reproductive condition and maternal care have been observed in vervet monkeys. In this study (Fairbanks and McGuire, 1995), rates of maternal rejection were higher among mothers in both marginal and prime reproductive condition as compared to mothers in average condition (with condition assessed as a combination of age, weight, and dominance rank). While maternal reproductive condition has not been systematically associated with EBF duration in human studies, maternal parity and somatic condition may influence the timing of CF introduction through effects on milk synthesis, maternal energy budgets, access to resources, and/or maternal perceptions of infant needs. As reviewed by Hinde and Milligan (2011), milk volume, fat, and protein have been associated with maternal parity, BMI, and adiposity in different populations, though the direction of these relationships are often inconsistent across studies. In the U.S., obesity is more frequently associated with shorter EBF durations, though this relationship may be confounded by socioeconomic status and biological interference with milk synthesis (Li et al., 2002; Nommsen-rivers et al., 2010). Primiparity and adolescence (< 20 years) have also been associated with earlier CF in low and high-income populations (Li et al., 2002; Jones et al., 2011; Kronborg et al., 2014; Balogun et al., 2015), but may be similarly confounded by differences in maternal experience or milk synthesis. The relationship between maternal reproductive condition, milk synthesis, and EBF duration are discussed in more detail below in relation to the Feeding Augmentation Model.

While evidence of maternal reproductive condition influencing EBF duration is mixed, there is ample evidence that maternal time and energy trade-offs influence EBF durations. In small-scale, traditional breastfeeding populations, relatively early CF has been associated with high levels of maternal subsistence activity or wage labor (Nerlove, 1974; Cohen et al., 1995; Tracer, 1996) and specifically maternal labor in combination with allomaternal care (Levine, 1988; Meehan and Roulette, 2013). In both low- and high income populations, maternal work and employment have long been associated with suboptimal breastfeeding practices—from non-initiation of BF to relatively short EBF and total breastfeeding durations (King and Ashworth, 1987; Adair et al., 1993; Dennis, 2002; Baker and Milligan, 2008; Rasheed et al., 2009; Chuang et al., 2010; Mandal et al., 2010; Balogun et al., 2015).

Interestingly, in the last few decades maternal socioeconomic status has been associated with opposing breastfeeding trends in low- vs. high-income populations, reflecting different trajectories of economic development and social and institutional support for breastfeeding. For example, in Dominica and Bangladesh, greater household wealth and alloparental care have been associated with shorter EBF and total breastfeeding durations (Quinlan et al., 2003; Rasheed et al., 2009). While a recent meta-analysis found that peer support increased EBF duration across low and middle-income countries, this effect was significantly reduced when local rates of formula feeding were greater than 10%.—suggesting mixed-messages from health care providers or other peers in influencing EBF duration (Sudfeld et al., 2012). In contrast, in higher-income populations, higher socioeconomic status and maternal education are increasingly associated with longer EBF and total breastfeeding durations (e.g. Fein et al., 2008; Meedya et al., 2010; Jessri et al., 2013; Colen and Ramey, 2014), which may

reflect greater awareness of the benefits of breastfeeding (Heck, Tully and Ball), as well as greater access to structural support mechanisms that explicitly promote prolonged EBF—e.g. in-hospital support for breastfeeding initiation, paid maternity leave, prenatal classes, and lactation consultants (e.g. Britton et al., 2007; Martens, 2012).

These opposing trends suggest that allocation of external resources to support or supplant breastfeeding are also influenced by cultural valorizations of breastfeeding, particularly as they may relate to perceived returns on investment. Notably, Hadley (2010) has challenged the assumption that material and time constraints are the primary barriers to prolonged EBF, arguing that culturally transmitted, “ideational factors” are the primary obstacles to changing health behaviors. However, such ideational factors may have underlying energetic or economic rational—i.e. “cultural” practices that appear suboptimal in a present context may have been biologically or economically meaningful in a past context, and have since been maintained through cultural transmission.

Finally, infant condition is also expected to influence EBF duration, with relatively early CF predicted only when resulting infant costs are marginal relative to the gains in maternal benefits. In general, this predicts that prolonged EBF is favored when risks of early CF are high—i.e. when infants are in relatively poor condition and complementary foods increase pathogen exposure, are relatively scarce, and/or of poor nutritional quality. For infants buffered against these risks (or perceived as buffered against these risks), the benefits of increased EBF duration for infants will be marginal relative to the returns on reducing breastfeeding intensity for mothers (Tully and Ball 2013).

However, quantity-oriented strategies may still favor reduced EBF durations even when the fitness costs to infants are high (i.e. risking death). In one of the more famous examples of the 20th century, the increased promotion and availability of infant formula resulted in drastically different outcomes across economic scales. In higher income populations, increased promotion of formula was matched by improvements in health care and hygiene that decreased infant pathogen exposure and morbidity risks. In conjunction with other social and economic forces shifting the value of breastfeeding (Van Esterik, 1989, 1995; Dettwyler, 1995), the economic costs of EBF increased while infant benefits relative to other alternatives decreased, and breastfeeding rates dropped precipitously—such as would be predicted by Tully and Ball’s (2013) model. In more impoverished populations, however, promotion of infant formula had infamously negative consequences: pathogen exposure, malnutrition, and misuse of formula combined to drastically increase infant morbidity and mortality rates in these populations. This phenomenon subsequently sparked an international outcry, which led to (voluntary) bans on formula advertising in low-income populations that are still in place today (reviewed in Van Esterik 1989). And yet, despite the clear risks to infant health, early CF facilitated by formula and other breast milk alternatives in low-income populations is still prevalent, and continues to be a major focus of global health campaigns (e.g. Black et al., 2008; Lamberti et al., 2011). This latter pattern would be predicted by the Feeding Substitution Model, for example, if maternal benefits in the form of time, energy, and total reproductive success ultimately trumped the immediate costs to infant health and survivorship. As further support for this relationship, it is noted that in Europe prior to the advent of commercial infant formulas, elite and working mothers frequently used wet-nurses

and breast-milk alternatives, despite resulting high infant mortality (Hastrup, 1992; Stuart-Macadam, 1995; Van Esterik, 1995).

In sum, the Feeding Substitution Model proposes that the varying effects of maternal and infant condition on EBF duration observed across populations, along with varying allocation of resources to support EBF, reflect local and individual differences in the net reproductive costs of prolonged EBF. Thus, in predicting individual EBF decisions, all relevant reproductive, economic, and health costs must be considered together.

4.1b. Infant needs favoring earlier CF

While the Feeding Substitution Model of early CF is well supported by both evolutionary logic and the research highlighted above, an alternate explanation considers that early CF is not “suboptimal” for infants either. In reviewing the literature, Meehan et al. (2013) suggest that traditional barriers to prolonged EBF fall broadly into four categories: intensive maternal labor, lack of social support, perceived infant hunger/thirst, and beliefs regarding insufficient milk production. While the first two categories would support the Feeding Substitution Model, the latter two may be honest signals indicative of infant energy needs. Several lines of evidence suggest that supplementation before 6 months of age—and in some cases even beginning at birth—may be necessary to meet infant energy demands, to promote growth and/or immunocompetence, or to compensate for poor lactational performance. This explanation does not deny the risks associated with early CF, but crucially maintains that, for some infants, early CF is purely additive—and that the additive benefits outweigh the costs.

In contrast to the Feeding Substitution Model, the Feeding Augmentation Model predicts that early CF does not interfere with infant breastfeeding intensity, and therefore does not reduce maternal energetic or time costs associated with EBF. Evidence for this model is discussed below, and the remaining chapter simultaneously tests aspects of both models.

First, across populations, the introduction of CF appears highly sensitive to signals of infant growth, health, and development (Orr-Ewing et al., 1986; Marquis et al., 1998; Simondon et al., 2001; Wright et al., 2004). However, as signals filtered through local beliefs about infant growth, temperament, and the influence of breast milk and other foods on infant condition (King and Ashworth, 1987; Harrison et al., 1993; Gray, 1998), it is not always clear if relatively faster or slower growing infants require more energy, or are simply perceived as requiring more energy than supplied by EBF (Reilly and Wells, 2007). Secondly, practices such as colostrum discarding, prelacteal feedings, and early supplementation are widespread in historical and pre-industrial populations (King and Ashworth 1987), and little evidence that such traditional practices are actually harmful (Kusin, 1985). More explicitly, since the official consensus recommending 6 months of EBF emerged in the late 1970s, several researchers have maintained that EBF for 6 months is *not* sufficient to support optimal growth for all infants, particularly under impoverished conditions. In the original formulation of the “Weanling’s Dilemma”, Rowland (1978) noted that some supplementation between 4-6 months was likely necessary to meet growth rates established at the time. In a reply, Waterlow (1981) cited 12 longitudinal studies from non-industrialized and developing populations, all of which demonstrated marked growth faltering in breast-fed infants beginning at three months of age. Importantly in making these comparisons, Waterlow

referenced growth velocities from breastfed infants in the UK, whereas most growth standards at the time inappropriately used data from cross-sectional studies and formula-fed infants—who tend to be larger and grow faster than breastfed infants (Baker et al., 2004). Since that time, high-quality, longitudinal studies using breastfed infants have been convened to establish new reference standards, and it was these studies that determined EBF was sufficient to support optimal growth for 6 months (Dewey, 1998, 2001a; Kramer and Kakuma, 2012). However, subjects in those studies were selected from high socioeconomic households in the U.S., Canada, and Europe, specifically in order to evaluate growth potential under “optimal environmental conditions” (Dewey, 1998). There are no comparable reference standards for the growth of EBF infants under *suboptimal* conditions.

Waterlow (1981) also argued that the wide ranges observed around mean infant weight-for-age, milk volume, energy, and protein would necessarily place some proportion of EBF infants at risk of insufficient nutritional intake, even while acknowledging that established nutritional requirements likely overestimated the needs of breastfed infants. More recently, a European commission—while acknowledging 6 months of EBF as a “desirable goal”—recommended introducing CF between 17 and 26 weeks, finding no evidence that CF between 4-6 months negatively affected growth (Agostoni et al., 2008). Finally, researchers have also have raised concerns about energy and micronutrient deficiencies associated with prolonged EBF (Fewtrell et al., 2007, 2011; Reilly et al., 2007; Nielsen et al., 2011), echoing Waterlow in arguing that individual growth trajectories are too variable for a 6 month EBF recommendation to be ideal in all cases. In the words of Fewtrell (2011):

“Infants exclusively breastfed for six months represent, globally, a small, potentially biased subgroup [that] presumably excludes those perceived by their parents as signaling hunger and so requiring weaning foods earlier. Generalization from this subgroup must therefore be questioned”

It is also worth considering that mothers in resource-poor populations initiate early CF *precisely* in order to accelerate infant growth. Danish infants breastfed for shorter durations and introduced CF before 4 months of age gained more weight during the first year of life than breastfed infants introduced CF after 4 months of age—an effect which did not appear related to birth weight, maternal BMI, or weight gain prior to CF introduction (Baker et al., 2004). Gray (1996) and Meehan et al. (2013) have separately argued that Turkana, Aka, and Ngandu mothers use very early CF (beginning shortly after birth) as a means to buffer infants against nutritional and infectious disease risks. Waterlow (1981) would concur, as he argued that infectious illness increases energy requirements for maintenance, leaving little room for exclusively breastfed infants to catch-up from repeated bouts of disease. The Feeding Augmentation Model proposes that early CF may be initiated without reducing breastfeeding intensity in order to supply infants with additional energy.

The Feeding Augmentation Model further proposes that maternal conditions negatively affecting lactational performance may warrant early CF. This relationship is distinguished from that proposed by the Feeding Substitution Model, however, in positing that mothers continue to breastfeed at their individual maximums. Currently, the WHO recognizes a limited number of medical conditions afflicting both infants and mothers that may warrant

supplementation with breast milk alternatives (WHO, 2009). Recognized infant conditions include genetic disorders that inhibit digestion of galactose, leucine, isoleucine, valine, or phenylalanine (requiring special formulas), birth weight below 1500 g, preterm birth before 32 weeks of gestation, and risk of hypoglycemia or other impaired glucose responses to breastfeeding. Maternal conditions include infection with HIV, HSV-1, or other severe illness, and current use of several medications contraindicated for breastfeeding. Of note, Tully and Ball (2013) cautioned that their trade-off model specifically held for “circumstances in which no medical BF contradictions exist”.

I would further argue, however, that while infants may benefit from supplementation in the presence of medical complications, they would also benefit in cases of low milk supply. As research over the last several decades has shown, insufficient or low milk supply is one of the most common reasons reported worldwide for the early introduction of CF and weaning (Tully and Dewey, 1985; Obermeyer and Castle, 1996; Gatti, 2008; Li et al., 2008). As reported incidences of low milk have generally tracked modernizing infant care and breastfeeding practices (Jeliffe and Jeliffe 1978), many researchers have concluded that the phenomenon is psychosomatic or behavioral in origin. Gussler & Briesemeister (1980), followed by Greiner et al. (1981) were the first to systematically describe “insufficient milk syndrome” as a biocultural phenomenon. They argued that strollers, cribs, bottle feeding, scheduled feedings and other aspects of modern infant care reduce maternal-infant contact and time on the breast, leading to reduced suckling and subsequently down-regulated milk production. Their explanations did not deny the biological reality of low milk, but causally

situated the pathology in a cultural process; that is, culture becomes manifest in behavior which becomes manifest in biology.

While undoubtedly these sociobehavioral processes underlie much of the phenomenon of low milk in the West, they do not account for frequent reports of insufficient milk in traditional breastfeeding populations (Levine, 1988). Indeed, clinical evidence has accumulated indicating several non-behaviorally mediated biological mechanisms that may inhibit milk supply.

First, supplementation shortly after birth may be warranted in the case of delayed onset of lactogenesis stage II—the onset of copious milk secretion that occurs over the first 4 days of lactation (Neville and Morton, 2001). Numerous factors have been associated with delayed onset of lactation, including prolonged labor, caesarean birth, obesity, flat or inverted nipples, parity, high infant birth weight, pre- and late-term birth, certain medications, and maternal stress during labor, birth, or postpartum period (Dewey, 2001b; Stuebe, 2014). The mechanisms underlying these relationships are physiological. For example, in mouse models, maternal adiposity and high-fat diets alter gene and hormonal expression, which impairs mammary development during pregnancy (Rats et al., 1997; Flint et al., 2005). As another example, prematurely born infants often have suckling and latching problems that impede full evacuation of the breast, which is necessary for the onset of lactogenesis (Neville and Morton, 2001; Manganaro et al., 2007).

Secondly—as discussed in Chapter 1—while milk production is generally considered robust to poor maternal condition (Prentice and Prentice, 1995; Hinde and Milligan, 2011), maternal parity, dietary insufficiencies, and stress may alter milk macronutrient,

micronutrient, and hormonal composition in ways that could influence infant growth, temperament, or satiety (Prentice et al., 1984; Whitehead, 1995; Webb-Girard et al., 2012; Hinde et al., 2014). As most mothers gauge milk sufficiency through infant cues, the terms “insufficient” or “low milk supply” need not necessarily apply only to some threshold of milk volume, but rather any established breastfeeding intensity that mothers perceive as insufficient for *their* infants. Here, the Feeding Augmentation Model is differentiated from the Feeding Substitution Model in assuming that mothers maintain their maximum breastfeeding intensity even if initiating early CF because of low milk quality or quantity—i.e. CF does not supplant milk intake. In support of the Feeding Augmentation Model, there are several accounts in the ethnographic literature of mothers introducing CF because of low milk but continuing to breastfeed on demand for prolonged durations (Levine, 1988; Cosminsky et al., 1993; Gray, 1998; Moffat, 2002; Agudo et al., 2010).

To summarize, the models make different predictions about infant fitness as a function of energy intake and the benefits of EBF. In the Feeding Substitution Model (Fig. 2), infant fitness during the first six months of life is constant and maximized by EBF, which assumes an infant is receiving a mother’s maximum milk yield. Infants incur fitness costs with the suboptimal introduction of CF, which displaces

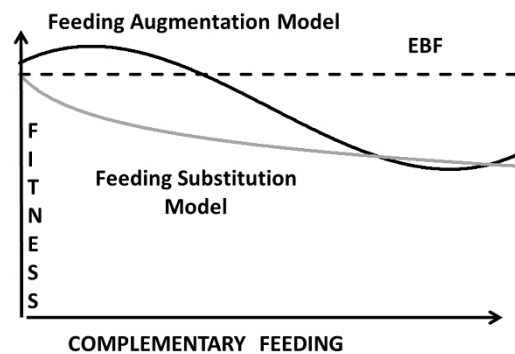


Figure 2. Effects of early CF on infant fitness as predicted by models.

breast milk intake and results in poorer quality diet, increased pathogen exposure, and/or the decreased availability of other benefits of breastfeeding (e.g. immunological and pre- and

probiotic constituents, hormones, bonding). Infant fitness costs will stabilize as energy supplied by increased CF compensates for (or even surpasses) that of displaced milk intake, however maximum infant fitness achieved with any amount of CF will always be lower than that achieved through EBF. In the Feeding Augmentation Model (Fig. 2), infant fitness is maximized by the addition of CF while maintaining maximum milk yield. Beyond a certain threshold of CF, however, continued increases in CF may eventually displace milk intake and reduce infant fitness relative to EBF, at which case outcomes are indistinguishable from the Feeding Substitution Model.

The models also make different predictions about maternal benefits from early CF in the form of energy savings. The Feeding Substitution Model predicts decreasing breastfeeding costs with increasing CF, while the Feeding Augmentation Model predicts no change in breastfeeding costs up to the threshold at which CF begins to displace milk intake. However, the net effect of increasing or decreasing breastfeeding intensity is not expected to directly impact maternal fitness as it does infant fitness, since maternal fitness is a function of reproductive value, maternal condition, and infant fitness (all of which may also vary with breastfeeding intensity).

As a point of clarification, the Feeding Substitution and Feeding Augmentation Models of CF are contrasted in terms of the fitness effects of CF during the first six months of lactation. Both models assume that breastfeeding is initiated after birth, and make predictions about the immediate and downward consequences of introducing CF before six months of age—i.e. before increased CF would be necessary to support continued growth even under

optimal environmental conditions. The consequences of delayed CF or insufficient CF after 6 months are not addressed here.

4.1c Early CF among Tsimane mothers: Substitution or Augmentation?

Existing research on Tsimane infant feeding practices preliminarily supports both models. In support of the Feeding Augmentation Model, it is noted that prolonged, on-demand nursing is normative across the population, despite the observation of frequent and relatively early CF (Veile et al., 2014). As shown in Chapter 3 (Table 8), none of the infants surveyed in this study were weaned before one year of age, and less than 30% weaned before the age of two, suggesting widespread early CF has little effect on late breastfeeding behaviors. In pilot interviews I conducted in 2009 (unpublished data), Tsimane mothers often reported low milk or poor infant growth as reasons for introducing CF. Low milk supply was a commonly acknowledged problem, with most mothers aware of ways to increase milk supply even if they had not experienced the problem themselves.

A priori empirical support for the Feeding Substitution Model is more indirect. First, Tsimane families are limited in their ability to invest in extra-somatic capital, while their subsistence economy and social structure continue to favor high fertility (McAllister et al., 2012). Neonatal and infant mortality rates are high and associated with shorter interbirth intervals, infanticide is not uncommon, and growth appears to falter significantly around the time of weaning (Gurven, 2012; McAllister et al., 2012). Assuming that Tsimane families are indeed investing in offspring quantity over quality, mothers in relatively better condition may

use early CF to mediate this strategy. High parity-for-age has also been associated with earlier weaning ages (Veile et al., 2014), suggesting that, with the evidence above, higher parity trades-off against offspring quality. It is also expected that reductions in maternal energy budgets will be used to support quantity rather than quality investment. For example, with increasing parity, there are more juveniles available to care for younger siblings and contribute to household production (Kramer and Greaves, 2010; Kramer, 2011). Among the Tsimane, the proportion of direct care provided by mothers begins to decrease after the first six months of life (Winking et al., 2009). Though allomothers and fathers may compensate for decreased direct maternal care to some extent, total time spent with caregivers decreases from 55% in infancy to just 20% by three years of age (Winking et al., 2009). This suggests a pattern of rapidly decreasing maternal investment that also supports greater quantity at the expense of quality investments.

To evaluate each of the models among the Tsimane, I have generated the following hypotheses and predictions:

Hypothesis 1A (H_{1A}): *Earlier CF is favored when mothers benefit from reduced breastfeeding costs, allowing for earlier shifts in future reproductive investment*

P_{1A.1}: Mothers' reasons for introducing CF will reflect their own energetic or time constraints.

P_{1A.2}: Early CF will be predicted by factors favoring earlier investment in future reproduction: relatively better maternal condition, increased access to resources, increased allomaternal care, high reproductive value, and better infant condition.

P_{1A.3}: Early CF will predict earlier resumption of postpartum menstruation.

P_{1A.4}: Early CF will predict earlier weaning.

Hypothesis 1B (H_{1B}): Earlier CF that does not supplant breast milk intake is favored when infant demands are not met by EBF.

P_{1B.1}: Mothers' reported reasons for introducing CF will reflect perceived infant needs

P_{1B.2}: Early CF will be predicted by reported low milk supply.

P_{1B.3}: Early CF will not reduce time to first postpartum menstruation

P_{1B.4}: Early CF will not be associated with earlier weaning.

Tests of the above predictions will examine additional variables expected to influence feeding decisions, though I do not make *a priori* predictions about these relationships. For example, village distance to San Borja will be tested as proxy for market integration and access to resources. As discussed in Chapter 3 and elsewhere (e.g. Gurven et al., 2007; Gurven, 2012), villages closer to San Borja have better access to health care and market foods, buffering against nutritional and disease risks, which should favor earlier CF. However, in a comparative study of reported CF across regions (Veile et al., 2014), we found that mean age at CF introduction was actually later in more market-integrated villages, which may reflect increased exposure to public health messages promoting EBF for 6 months. I will also test for effects of infant sex and birth season, as these may affect growth and therefore timing of CF introduction.

I will also examine non-linear relationships between parity and age of CF introduction. While the Feeding Substitution Model predicts that lower parity will favor earlier CF, primiparity imposes particular constraints on maternal condition, increasing risks of premature birth, low birth weight, and lactational failure (e.g. Dewey et al., 2003; Ananth et al., 2007). These constraints may favor earlier CF to buffer against infant risks, providing indirect support for the Feeding Augmentation Model. Conversely, while the Feeding Substitution Model predicts that high parity (low reproductive value) will favor later CF, high parity may also be associated with maternal depletion, favoring earlier CF. Primiparity and high parity may also be related to allomaternal care, which among the Tsimane is most frequently provided by juvenile females—either maternal sisters or older offspring (Winking et al., 2009). Primiparous Tsimane mothers are more likely to live with their or their spouses' kin (and therefore more likely to shift infant care and feeding to grandmothers or sisters), while higher parity mothers are more likely to have juvenile female offspring able to care for and feed infants.

I do not make specific predictions about how the maternal or infant factors examined in the Feeding Substitution Model may provide evidence for the Feeding Augmentation Model. In the Feeding Substitution Model, factors indicative of greater maternal reproductive value, somatic condition, and resource access are expected to directly favor earlier CF in order to reduce breastfeeding costs. Although under the Feeding Augmentation Model factors such as primiparity, high parity, and birth season may operate indirectly to influence age at CF via lactational performance or infant growth in some cases, such relationships are not uniformly predicted. The major distinction between (and test of) the two models arises in the

subsequent outcomes predicted by early CF. In the Feeding Substitution Model early CF reduces maternal breastfeeding costs at some cost to infant fitness. In the Feeding Augmentation Model, early CF has no effect on maternal breastfeeding costs and a positive effect on infant fitness (Fig. 2). In fact, under the Feeding Augmentation Model, breastfeeding costs following CF introduction may even increase if mothers are in poor condition, resulting in relatively delayed resumption of postpartum menstruation.

In order to better discriminate between the models, I have generated two additional hypotheses to test the mechanistic assumptions of the Feeding Substitution Model—i.e. that early CF reduces maternal costs by reducing maternal breastfeeding intensity, resulting in time or metabolic energy savings. Supporting evidence for each hypothesis and component predictions are given below. The assumptions of both models with respect to infant costs (i.e., is early CF costly or beneficial to infants) are tested in Chapter 5. See Table 1 for a summary of hypotheses and predictions tested in this chapter.

4.1d Tests of Assumptions of the Feeding Substitution Model

Milk synthesis is directly related to suckling frequency by positive feedback mechanisms; the more an infant suckles, the more milk a mother produces (Egli and Newton, 1961; Kent, 2007). Breastfed infants self-regulate feeding and will reduce the frequency of suckling if they are satiated with non-milk liquids or foods. This can lead to continued decreases in milk production, requiring increased supplementation to maintain energy intakes. On average, one kcal of complementary food or liquid displaces 0.6-1.7 kcal of milk

(Dewey, 2000). In an early randomized control experiment conducted by Cohen et al. (1994), low-income Honduran mothers were assigned to one of three groups. Two groups were given high-quality commercial complementary foods (jars of canned baby food) to feed to their infants twice per day beginning at 16 weeks, with one group instructed to continue nursing at pre-provisioning intensity, and the other instructed to nurse ad libitum. The third group continued EBF to at least 26 weeks. From 16-26 weeks postpartum, breastfeeding frequency, duration, and breast milk intake declined significantly in both of the CF groups. In infants, total energy intake from milk decreased by 40% and 68%, respectively, in the two CF groups, but was compensated for by the energy supplied from complementary food (Cohen et al., 1994). In sum, the study effectively demonstrated that relatively modest amounts of CF (two jars of baby food per day) can lead to rapid offsets in breast milk production, even when mothers strive to maintain high intensity breastfeeding.

Currently, there is no direct or anecdotal evidence that Tsimane families use commercial baby foods. Early CF with formula or powdered milk is evident but limited (see Chapter 3), and likely not used consistently within families owing to cost and lack of accessibility. Before six months of age, Tsimane infants are most often consuming *chicha*, plantain mixed with water, and stew (Chapter 3). These liquid and semi-solid feedings may be energetically sufficient enough to offset breast milk intake. The Feeding Substitution Model therefore predicts subsequent decreased breastfeeding intensity and increased intake of complementary foods with relatively earlier CF introduction. In contrast, the Feeding Augmentation Model assumes that CF intake will increase after introduction, but the age at CF introduction will not affect subsequent breastfeeding intensity. The predictions below are tested using 24-hour

recalls of Tsimane infant dietary intake from the mixed-longitudinal sample, and behavioral observations from a subset of the prospective sample (see Methods Chapter 2):

Hypothesis 2_A: Earlier CF accelerates weaning via relatively increased CF frequency and reduced breastfeeding intensity.

P_{2A.1}: Age-adjusted CF intake rates reported in 24-hour recall will be higher among infants introduced CF relatively earlier.

P_{2A.2}: Adjusting for infant age, relatively earlier CF will be associated with increased mean frequency of CF/hr, decreased mean breastfeeding frequency/hr, and decreased total time breastfeeding/hr.

Hypothesis 2_B: Earlier CF increases CF frequency without reducing breastfeeding intensity.

P_{2B.1}: Age-adjusted CF intake rates reported in 24-hour recall will be higher among infants introduced CF relatively earlier.

P_{2B.2}: Adjusting for infant age, relatively earlier CF will be associated with increased mean frequency of CF/hr, but no change in mean breastfeeding frequency/hr or time breastfeeding/hr.

Lactation imposes metabolic costs on mothers that are also expected to be reduced by earlier CF. Isotopic methods, such as doubly labeled water or the deuterated water dose to mother method, are the current gold standard for assessing maternal energetic costs of

lactation and volume of breast milk transfer (Coward, 1984; Goldberg et al., 1991; Ainslie et al., 2003), followed by 24-hour infant test weighing (Neville et al., 1988). However, isotopic methods were prohibitively expensive for the present study, and 24-hour test weighing of on-demand nursing infants in field settings is highly impractical (Miller et al., 2012). To test predicted decreases in the metabolic costs of lactation associated with age of CF introduction, I therefore examine changes in maternal postpartum energy balance and urinary C-peptide concentrations.

To meet the metabolic demands of lactation, a mother can increase food intake, mobilize nutrient stores in tissues, increase metabolic efficiency, reduce energy expenditure, or some combination of the above (Butte and Hopkinson, 1998). As reviewed by Butte and Hopkinson (1998), high estrogen levels in human females during pregnancy promote gluteofemoral fat deposition. During lactation, that fat is mobilized from the trunks and thighs, a process that may be mediated by corresponding declines in estrogen levels. In addition, neuroendocrine and other biochemical pathways coordinate to ensure preferential uptake of maternal nutrients by the mammary gland. The stimulation of prolactin by suckling reduces lipogenesis in the liver and adipose tissue, increases insulin sensitivity in the mammary gland, and decreases insulin sensitivity in muscle and adipose tissue. Combined with heightened lipolysis, these mechanisms constrain maternal peripheral fat storage and preferentially direct insulin uptake to the mammary gland (Butte and Hopkinson, 1998).

In a closed system, the above mechanisms should lead to consistent postpartum reductions in body weight and adiposity. In offsetting breastfeeding intensity, relatively earlier CF would thus be expected to downregulate mobilization of fat stores, leading to

subsequently earlier stabilized or positive changes in maternal energy balance. If, however, mothers introduce early CF without offsetting breastfeeding intensity, they would be expected to remain at negative energy balance.

In the literature, early CF—even total substitution of breastfeeding with formula feeding—is not consistently associated with decreased postpartum weight loss across populations (Brewer and Bates, 1988; Butte and Hopkinson, 1998; Valeggia and Ellison, 2009). While this discrepancy may at least in part reflect undocumented differences in reasons for CF, CF intensity, or breastfeeding intensity across studies, variation in other compensatory mechanisms affecting postpartum energy balance—particularly energy expenditure and intake—are major confounding factors (Butte and Hopkinson, 1998). In their non-systematic review, Butte and Hopkinson (1998) noted, for example, that the strongest predictor of postpartum weight loss is prepartum weight gain, with the result that mothers in more affluent populations tend to lose the most weight postpartum. However, a more recent analysis of standardized survey data collected across 65 countries demonstrated substantial buffering of postpartum weight loss in higher-income households (Hruschka and Hagaman, 2015)—as would be expected with relatively greater postpartum energy intake or decreased energy expenditure.

To further substantiate relationships between age of CF introduction and maternal energy costs, I will also examine changes in maternal urinary c-peptide concentrations associated with age of CF introduction. C-peptide (‘connecting’ peptide) is a protein released during conversion of proinsulin to insulin by pancreatic beta cells. It is produced on an equimolar basis with insulin, but is not broken down by the liver (as insulin is); it is not

cleared in a concentration-dependent fashion, and it is not significantly affected by age, sex, obesity or diabetic status (Emery Thompson and Knott, 2008). A small, consistent fraction of C-peptide is excreted in urine. Field collection and use of C-peptide levels as a biomarker for energy balance have been established for both humans (Ellison and Valeggia, 2003; Valeggia and Ellison, 2009) and non-human primates (Sherry and Ellison, 2007; Emery Thompson and Knott, 2008; Emery Thompson et al., 2012).

Owing to preferential uptake of insulin by the mammary gland during lactation, circulating insulin levels of lactating women are decreased relative to those of non-lactating women (Butte et al., 1999), and are negatively associated with breastfeeding duration and intensity (Hubinot et al., 1988; Gunderson et al., 2013). As a biomarker of baseline insulin production, urinary C-peptide concentrations are expected to rise as increased CF reduces breastfeeding intensity, signaling a declining metabolic load of lactation (Ellison and Valeggia, 2003). Owing to the role of glucose and insulin in lactation, C-peptide may be a more accurate measure of changes in lactational costs than are changes in body composition postpartum (Butte et al., 1999). As a note of caution however, changes in C-peptide concentrations cannot be ascribed to specific sources of change in energy balance—i.e., relatively lower C-peptide levels may signal decreased caloric intake or increased physical activity (Bergouignan et al., 2009). Expected increases in urinary C-peptide following earlier CF introduction may therefore be similarly confounded by individual differences in energy intake or expenditure.

Though these caveats must be considered in final interpretations of results, the Feeding Substitution model predicts that earlier CF, in offsetting breastfeeding costs, will be

associated with relatively reduced postpartum weight and fat loss (indicative of stabilizing energy balance), and higher urinary C-peptide concentrations (indicative of increased glucose availability). The following predictions will be tested using repeated maternal anthropometric measures and urinary C-peptide levels collected from the prospective sample:

Hypothesis 3A: Earlier CF is associated with reduced maternal metabolic costs

P_{3A.1} Following CF introduction, mean maternal weight/BMI will be greater among mothers who introduced CF relatively earlier

P_{3A.2} Following CF introduction, mean urinary C-peptide concentrations will be greater among mothers who introduced CF relatively earlier.

Hypothesis 3B: Earlier CF is not associated with reduced maternal metabolic costs

P_{3B.1} Following CF introduction, mean maternal weight/BMI among mothers who introduced CF relatively earlier will not significantly differ from, or will be less than, that of mothers who introduced CF relatively later.

P_{3B.2} Following CF introduction, mean urinary C-peptide concentrations among mothers who introduced CF relatively earlier will not significantly differ from, or will be less than, that of mothers who introduced CF relatively later.

4.2. Methods

4.2a. Sample and description of terms

Between August 2012 and March 2013, a total of 161 Tsimane mother-infant pairs were interviewed and measured. At time of interview, 30 infants were EBF, 106 had already begun CF, and 25 were already weaned. As described in Chapter 2, multiple follow-up dietary recalls, anthropometrics, and maternal urine samples were collected from 44 of the dyads with infants who were first interviewed from September- December 2012 and were less than 12 months of age at the time (see Table 2 for subject characteristics of full sample and subjects sampled only cross-sectionally vs. prospectively). Among the infants sampled prospectively, 20 were EBF and 24 were CF when first interviewed. Over the course of the prospective study, 15 of the EBF infants transitioned to CF, and all infants were still breastfeeding at the end of the study. Repeated follow-up visits to collect dietary recalls, anthropometric measures, and urine samples were conducted approximately every 3 weeks. Owing to subject absence and other logistical difficulties (described in Chapter 2), the number of visits and interval between visits per subject varied greatly. The resulting sample was unbalanced across age ranges and measurement intervals, and therefore samples sizes vary across specific statistical analyses below. See Appendix, Figure 1 and Figure 2 for visual representation of sampling by subject and age at interview.

Using WHO (2008) criteria (see Chapter 3, Box 2), EBF status was defined as consumption of breast milk only, and CF status defined as breastfeeding with any solids or other additional liquids (including formula and powdered milk). The WHO (2008) standards

define “predominant breastfeeding” as breastfeeding with additional minimal water, water-based drinks, and ritual fluids. In this study, Tsimane infant consumption of sugar water and juice (which for the Tsimane tends to be very diluted) was categorized as “predominant breastfeeding “. Consumption of *payuje* (Sp., boiled plantain mixed with water) or *chicha shocdye* (Sp., an essential local staple, consisting of fermented manioc, corn, rice, or plantain mixed with water) was categorized as “complementary feeding” due to the greater energetic content of these beverages. For infants who were already CF at the start of the study, age at CF introduction (in months) was reported in ethnographic interviews ($n = 131$, mean \pm SD = 3.8 ± 2.0). For infants who transitioned from EBF to CF over the course of the prospective study, age at CF introduction was reported by mothers after the first change in feeding status was recorded in repeated dietary recalls ($n = 15$, mean \pm SD = 3.5 ± 1.2). Descriptive statistics were generated for reported mean \pm SD ages of CF introduction and weaning by region, individual villages, infant sex, age group, and maternal parity group, along with mothers’ reported reasons for introducing CF. Statistical methods used to test specific hypotheses are described below. All statistical analyses were performed in R (ver. 3.0.2).

4.2b. Statistical methods H_{1A/1B}

Earlier CF favors greater investment in future reproduction Time to CF introduction (in days) was analyzed by survival analysis, using the *survival* and *smoothHR* packages in R. As estimated by Kaplan-Meier survival, mean \pm SE time to CF introduction for infants in the prospective study was 135 ± 8 days (n observations = 30, n events = 15), which was slightly

higher than mean time to CF introduction reported through recall in ethnographic interviews for other subjects (130 ± 5 days, n observations = 131, n events = 131). However differences were not significant (log rank test $p = 0.722$). Mean time to CF introduction when restricted to observed CF or CF reported within six months of age at interview was 127 ± 7.4 days. This estimate was lower than the mean time to CF introduction if more than six months had elapsed since age at CF was reported (135 ± 6.2 days), though again differences were not significant (Log rank test $p = 0.12$). The full dataset ($n = 159$ records), combining reported and observed ages at CF, was therefore used in a Cox proportional hazards model.

I employ Cox proportional hazards analysis to model the effects of maternal reproductive factors on likelihood of early CF ($P_{1A.2}$). I start with a baseline model that includes the factors expected to influence maternal reproductive trade-offs in terms of maternal condition, access to resources, and/or energy budgets: maternal height, village region (near town vs. remote), number of household females over the age of 7 (following Winking et al., 2009), and parity. Parity was fit with a penalized spline to examine nonlinear effects. Infant sex and birth season (dry vs. rainy) were also included in the baseline model as these may affect infant growth, energy demands, and perceptions of infant condition. A control variable indicating the time elapse since the reported age at CF introduction was also included. Time-dependent covariates related to maternal and infant condition (i.e. maternal weight, fat, BMI, infant length, weight) were not evaluated because of the time elapse between an infants' age at interview and age at CF introduction for many subjects. Nor could these covariates be separately evaluated in the prospective sample due to unbalanced measurement intervals and small sample size. A best fit Cox proportional hazards model was

selected using backwards stepwise selection with AICc, and evaluated for proportional hazards assumptions, influential observations, and nonlinearity of continuous variables. The final model was then fit using the *smoothHR* package in R, which computes optimal degrees of freedom as specified by AICc, and estimates log hazard ratios and corresponding confidence intervals for continuous variables introduced nonlinearly (Meira-Machado et al., 2013).

I then employed OLS regression to test if perceived low milk supply predicted a relatively age of CF introduction ($P_{1B.2}$). Since low milk was reported in conjunction with reasons for introducing CF, this test was restricted to non-EBF infants only ($n = 127$). The OLS regression model was run on age at CF introduction (in months), and included the same predictive factors used in the final Cox proportional hazards model above with an additional binary variable coded for whether or not CF was introduced owing to low milk supply. Interaction terms, model assumptions and collinearity of coefficients were also examined.

Maternal reproductive status (pregnant, lactational amenorrhea, cycling) was reported in initial and all prospective interviews. For mothers followed prospectively, onset of menstruation was confirmed if a subject reported menstrual bleeding in 3 subsequent interviews (generally covering at least two additional menstrual cycles). One subject was excluded from subsequent analysis of postpartum amenorrhea because of indeterminate onset of menstruation. In initial interviews, 53% of mothers interviewed (85/160) were no longer amenorrheic (28% of these mothers were already pregnant). Maternal recall was used to estimate duration of lactational amenorrhea for 86% of the mothers (73/85) who had already resumed menstruation. Of mothers followed prospectively, 15% (7/46) were cycling at time

of initial interviews, 28% (13/46) resumed menstruation during the course of the study, and 57% (26/46) were still amenorrheic at the end of the study. The mean \pm SD duration of lactational amenorrhea was 11.2 ± 4.6 months as reported in ethnographic interviews ($n = 73$) and 9.9 ± 3.5 months as observed during the prospective study ($n = 13$). The mean difference in reported vs. observed duration was not significant ($F = 0.92$, $p = 0.339$), and data from the initial and prospective interviews (including censored cases) were combined to estimate time to first postpartum menstruation by survival analysis.

A Cox proportional hazards model was used to test the effect of early CF on duration of lactational amenorrhea ($P_{1A.3/1B.3}$). Analysis was restricted to mothers who had already begun CF, as CF preceded the onset of postpartum menstruation by at least one month in 96% (83/86) of subjects with known duration of lactational amenorrhea. A baseline Cox proportional hazards model was constructed using the following predictor variables: a spline for maternal parity, maternal height, number of females in household over the age of 7, infant age at time of interview, infant sex, birth season, village region, and age at CF introduction. A control variable for the recall period between date of interview and reported date of onset of first postpartum menstruation was also included. As above, the best fit model was selected using backwards stepwise selection with AICc and evaluated for proportional hazards assumptions, influential observations, and nonlinearity of continuous variables. The final model was then fit using the *smoothHR* package in R. Owing to differences in a sample size and collinearity, a separate model replacing age at CF introduction with a binary term for whether or not CF was introduced because of low milk was also run.

To assess P_{1A.4/1B.4} relating timing of CF to weaning, we analyze weaning events reported by maternal recall in ethnographic interviews. Age at weaning was reported for 21/25 of weaned infants, with no infants reportedly weaned before 12 months of age. Survival analysis of time to weaning was therefore restricted to infants 12-35 months of age, with mean weaning age estimated using Kaplan-Meier survival analysis (n = 100, n events = 21). The mean time elapse since infant age at interview and reported age at weaning was 6.3 months. To test for an effect of early CF on weaning, a baseline model was constructed with early CF introduction (0-3 months) and the following additional variables considered in backwards stepwise selection: infant sex, birth season, mother's current pregnancy status, parity, height, duration of lactational amenorrhea less than one year, the number of female allomothers age 7 or older in the household, whether or not CF was introduced because of low milk supply, and a control term for time elapse since infant age at interview and reported age at weaning. A nonlinear term for parity was not significant. A nonlinear term for household female allomothers was significant, but relatively few subjects were represented among households with more than 2 females. A categorical variable was created to compare households with no additional female allomothers to households with one and households with 2 or more female allomothers. Final models were evaluated for proportional hazards assumptions, influential observations, and nonlinearity of continuous variables.

4.2c. Statistical methods $H_{2A/B}$

Dietary recalls from initial and follow-up interviews were used to test if age at CF introduction influenced subsequent CF intake ($P_{2A.1/2B.2}$). For each recall, the total number of meals and snacks were summed as a measure of total solid feedings. While mothers also reported different liquids consumed by their infants, they often could not remember or accurately estimate the total number of liquid feedings. However, removing liquid feedings would bias the analysis, as many infants consume only liquids when CF is first introduced. I therefore calculated a conservative measure of “CF intake” as the sum total number of solid feedings (meals and snacks), and unique liquids consumed the day prior. It is expected that this measure underestimates total 24-hour number of feeding bouts in 24-hour CF frequency. A linear mixed-effects model combining initial interviews from all infants with repeated dietary recalls of prospectively followed infants did not converge. Separate statistical analyses were therefore performed on the following samples: (1) dietary recalls of all non-EBF infants aged 0-35 months from initial interviews ($n = 125$); (2) all non-EBF infants from the prospective sample with two or more dietary recalls ($n = 138$ observations, 34 subjects, ages 1 -16 months).

From initial dietary recalls, a linear regression on CF intake by age at CF introduction was performed. The following additional variables were controlled for: infant age at time of interview, village region (shown to significantly affect meal frequency and dietary diversity in Chapter 2, Table 9 and Table 10), maternal age (significantly predicted meal frequency and dietary diversity in Chapter 2; significant predictor of age of CF introduction in the Feeding Substitution Model), and whether CF was introduced owing to low milk supply (significant

predictor of age of CF introduction in the Feeding Augmentation Model). Owing to collinearity with region and maternal age, respectively, season and parity were considered in alternate models, but resulted in poorer fits. Age at CF introduction (in months) and infant age (in months) were centered at sample means to examine the interaction of age at CF introduction with infant age and low milk. Two observations were excluded in the final model after evaluating for assumptions of normality and influential observations.

For repeated dietary recalls of all infants in the prospective study, a linear-mixed effects model fit by REML was run using the same variables specified in the linear regression model above. The mixed-effects model was fit with a random intercept and slope for subject ID and age at each interview. Alternate models considering season as a random slope and fixed effect were also evaluated but did not explain any additional variance and resulted in poorer model fit. The *lme4* package in R was used for mixed-effects modeling.

Behavioral observations of 14 mother-infant pairs were then analyzed to assess whether or not age of CF introduction was associated with changes in CF frequency ($P_{2A.1/2B.1}$), or changes in breastfeeding frequency and breastfeeding duration ($P_{2A.2/2B.2}$). All families observed were from remote villages; 4 females and 10 male infants were observed. As described in Chapter 2, observations were conducted in the morning and afternoon on non-consecutive days, in 3 – 5 hour intervals, during two observation periods (Period 1: September – December; Period 2: March – April). Owing to logistical difficulties described in Chapter 2, not all mothers were observed again in Period 2, and observation intervals during this period were limited to 3-4 hours. A total of 187 hours of observation were conducted across all subjects (mean \pm SD of 13 ± 4 hours per subject). During both periods,

observations were conducted between 7am – 5 pm and always commencing in subjects’ homes (with subjects subsequently followed if they went to their fields or the home of another Tsimane).

During observation intervals, infant activity states and their proximity to their mothers and fathers were recorded in 5-minute intervals. Proximity was coded as “direct contact”, “within reach”, “in sight”, or “out of sight”. See the Appendix for a complete list of infant activity state codes. Durations of all infant-caregiver interactions, including breastfeeding, were recorded continuously to the nearest minute. Following Valeggia (2004), separate breastfeeding bouts were designated as more than 10 seconds elapsed between bouts. Summary statistics of proximity, infant activity states, and durations of caregiver interactions were calculated across all observations for all subjects.

Differences in breastfeeding frequency, total breastfeeding duration per hour, and CF frequency per hour were then examined across subjects to test for predicted variation owing to age at CF introduction. Owing to differences in total observation time per interval across subjects, the following standardized summary measures were calculated. All breastfeeding bouts and durations of bouts per subject per interval were summed and divided by total hours of observation in the interval to produce measures of mean breastfeeding frequency per hour and mean time spent breastfeeding per hour per interval. Bouts of CF were recorded *ad libitum*, but not timed owing to intermittent nature of feeding. Mean CF frequency/hr was calculated as described above for mean breastfeeding frequency/hr.

Linear mixed-effects models fit by REML were then run using the *lme4* package in R to examine the effect of early CF on each of the standardized summary measures. All models

were restricted to observation intervals from non-EBF infants ($n = 13$, 39 observation intervals), with a random intercept entered for subject. Age at CF introduction was centered at the sample mean (3.3 months). Models were adjusted for infant age at time of observation interval (centered at the sample mean of 9.1 months), and the percentage of time asleep during the observation interval (estimated from concurrent 5-minute interval observations from the same observation). Interactions between infant age and age at CF introduction were considered, and models were evaluated for assumptions of normality and linearity. Differences in behavioral outcomes among specific age groups or across individual observation intervals could not be robustly evaluated, as only 7 of the 14 non-EBF infants were observed during both periods. In preliminary analyses, the time of day of observation interval (AM or PM) had no effect on outcome variables. Only two mothers in this subsample introduced CF because of low milk, so this variable was not considered in statistical analysis.

4.2d. Statistical methods H_{3A/B}

The prospective sample included 18 mothers with at least two anthropometric measures between September 2012 and March 2013, who were EBF at initial interviews (mean 45 ± 37 days, median 28 days). At least two urine samples were collected from 15 of those mothers (as described in Chapter 2, no urine samples were collected from subjects in three of the study villages after January). At the end of the study, 14/18 (78%) of the EBF mothers had introduced CF. Seven of the 18 initially EBF mothers (39%) exclusively breastfed for 0-3 months, 10/18 (56%) exclusively breastfed for at least 4 months, and one was still EBF at 72

days on her last visit. Unfortunately, multiple anthropometric measures both before and after the introduction of CF were only available for 3 women. For most women who transitioned from EBF to CF, only one pre- or post-CF anthropometric or C-peptide measure was available. These imbalances precluded robust longitudinal analysis of changes in maternal weight and urinary C-peptide before and after introduction of CF. For mothers who were EBF at the start of observations, differences in maternal BMI and urinary C-peptide associated with duration of EBF are evaluated graphically and using descriptive statistics.

Mixed-effects linear regression was used to evaluate changes in BMI following CF introduction. The sample was restricted to 30 subjects with at least two anthropometric measures post-CF introduction (130 measurements total). After evaluation of correlations between BMI and independent variables of interest, a baseline model was constructed with a random intercept for subject and the following fixed effects: month postpartum, month of CF introduction, parity, region, and infant sex. Subsequent models using maximum likelihood estimation were then run testing for additional significant covariates (whether or not CF was introduced because of low milk, total household members, and maternal age) and interaction terms. To facilitate interpretation of a significant interaction between parity and month of CF introduction, parity was categorized into primiparous, prime (2 – 7 births), and high parity (8 + births) groups (based on previously observed non-linear relationship and sample quartiles), and month of CF introduction was centered at the sample mean (3.09 months). The final model was selected based on minimum AICc values, rerun and fit by REML estimation, and evaluated for collinearity and assumptions of normality.

Mixed-effects linear regression was also used to evaluate changes in urinary C-peptide concentrations following CF introduction. Owing to high within-subject variability in C-peptide values and the time elapse between measures, I first conservatively removed all sample outliers from further consideration prior to statistical analysis. This excluded 11 samples that were 1.5 times above the interquartile range of the sample distribution (mean value of outliers = 56.72 ng/mL, range 38.7 – 93.8). Two additional samples with relatively low C-peptide values (0.103 and 0.298 ng/mL), and corresponding low specific gravity (< 0.005) and high interassay CVs in laboratory analysis were also removed. The remaining sample was then restricted to subjects with at least two samples following CF introduction (26 subjects, 105 samples). For these subjects, the mean time elapse between sampling intervals was 30.9 ± 16.5 days (range 11 – 115 days). C-peptide values were log transformed ($\log(1+x)$) for statistical analysis to approximate a normal distribution.

The same mixed-effect model used to assess changes in maternal BMI was then used as a baseline model to examine differences in log C-peptide values post-CF introduction. For this model, month of CF introduction was centered at the sample mean of 2.92 months, and maternal BMI was included as an additional time-varying covariate. As a note, because anthropometric measures were not collected at every urine sampling interval, 44 C-peptide measures had no anthropometric measures corresponding to the date of sample collection. For these sampling intervals, missing anthropometric measures were estimated by calculating a rate of change in BMI from the preceding and following sampling intervals, and extrapolating to the date of urine sample collection. No covariates or interaction terms in the baseline model were significantly associated with C-peptide values. Subsequent models

examining additional covariates (here including season of sample collection) and interaction terms were also considered. Removal of model terms slightly improved model fit as determined by AICc, but failed to reveal any significant predictors of variation in C-peptide levels. Results below therefore present the nonsignificant findings of the baseline model.

4.3 Results

4.3a Results P_{1.1}: Reasons for CF introduction and weaning

Table 3 provides the mean \pm SD age of CF introduction (combining reported or observed age) and age at weaning (reported) for all subjects by infant age group, sex, maternal parity group, village, and village region. Frequency of CF at 0-3 months (“early CF”) and weaning age by categorical variables is also given. The median (IQR) age of CF introduction was 4 (3-6) months. The median (IQR) age of weaning was 19 (15-24) months. Additional variation in CF introduction and weaning is examined in survival analyses below.

Tsimane mothers’ reported reasons for introducing CF were grouped into nine broad categories. Frequency of grouped responses is shown in Figure 3. Broadly, 56% of mothers reported following infant cues of hunger, temperament, or development in deciding when to introduce CF—either perceiving that their infants were big, growing, or developmentally ready for food (e.g. grabbing at food), or that their infants were not growing well and needed supplementation. Responses that infants were crying or hungry were often interchangeable and stated in conjunction with the infant “asking for food”. These responses may represent maternal perceptions of either developmental readiness or poor growth. At the same time,

several mothers expressed that exposure to food via taste, sight, or smell subsequently leads babies to want food and cry for it. One mother cautioned that infants who begin feeding at 3-4 months subsequently “*no dejan las mamas a trabajar*”—they don’t leave their mothers alone, and always cry for food, whereas infants who begin CF later don’t do that, and can feed themselves. Of note, mothers used several different terms to describe infant crying (*shavnaqui, shavñye, tari, váti, vara’shaqui*), all of which denote some form of wailing, crying with tears, loud crying, and incessant crying. As is shown below in results from behavioral observations, infant crying among the Tsimane is very rare. Loud persistent crying may be a relatively unusual behavior, and therefore its sudden onset may be—or may be understandably be interpreted as—an honest signal of interest in food or energetic needs.

Across all non-EBF mothers (n = 140), mothers who perceived their infants as small at birth (relative to other Tsimane newborns) were no more likely to introduce CF before 4 months of age than mothers who perceived their infants as the same size or larger than other Tsimane newborns (42% vs 38%, chi-square = 0.05, p = 0.814). However, mothers who reported their infants as small for their age *at the time of CF introduction* were significantly more likely to have introduced CF before 4 months of age as compared to mothers who reported their infants as similar to or larger than other same-aged infants at the time of CF introduction (59% vs. 31%, chi-square = 8.05, p = 0.004, n=128).

Mothers were also asked to recall developmental milestones at the age of CF introduction: 33% of infants reportedly could not support their own heads, 30% could not grasp objects, and 37% could not sit without support. Since these developmental milestones are generally reached between 2-4 months of age (CDC, UNICEF), these

proportions are consistent with findings from interviews that about 25% of Tsimane infants are introduced CF between 0-3 months of age. At the other end of the spectrum, only 30% of infants reportedly had at least one erupted tooth at the time of CF introduction, and only 7% could sit unsupported. These developmental milestones are generally not observed until at least six months of age, similarly aligning with findings that about 25% of infants reportedly introduced CF at 6-7 months.

Of the mothers who did not cite an infant cue as a reason for introducing CF, 17% cited their own condition, 11% reported “no reason/just because”, and 10% reported that someone else first fed their infants (Figure 3). The majority of mothers citing problems with their own condition (92%) reported that they had low milk supply—in their own words they had “no breast milk” (*itsij tashin*) or were “dry” (*chañej*). The mothers reporting CF introduction because of low milk were no more likely to report delayed initiation of breastfeeding (after 24 hours) than were mothers who introduced CF for other reasons (chi-square = 0.71, $p = 0.39$). However, 95% of mothers reporting low milk introduced CF before 4 months of age, compared to just 32% of mothers who reported introducing CF for other reasons (chi-square = 30.41, $p < 0.001$). These mothers were also significantly more likely to report their infants as small for their age at the time of CF introduction, as compared to mothers who introduced CF for other reasons (71% vs. 28%, chi-square = 15.24, $p < 0.001$). Finally, the frequency of CF introduction owing to low milk supply differed significantly across parity groups (chi-square = 14.37, $p = 0.002$) (Figure 4). After fitting a generalized linear model to low milk by parity group, a Tukey’s HSD test revealed that differences in the likelihood of introducing CF because of low milk were only significantly higher for high as compared to low parity

mothers (Est. = 2.74, $p = 0.008$), and marginally higher for high as compared to prime parity mothers (Est. = 1.37, $p = 0.087$).

Discussions with mothers reporting low-milk induced early CF further suggest that they did indeed sustain chronic problems with lactational sufficiency. Most of these mothers (15/24) introduced liquids (*payuje*, *chicha*, and sometimes formula or powdered milk) within the first few weeks postpartum, and gauged low milk from infant cues such as crying or low weight. These mothers varyingly reported that milk supply problems self-resolved or persisted and required intermittent supplementation, but all continued breastfeeding. Several of the mothers also reported taking active steps to increase milk supply, including drinking more liquids and massaging their breasts with salt or warm papaya skins. The latter remedies suggest that at least some Tsimane mothers struggle with mastitis.

In ethnographic interviews, 40% of Tsimane mothers (49/125) also reported receiving advice to introduce CF, but did not explicitly state this advice as the reason they introduced CF. Of these mothers, 46% were advised to begin CF by their own mothers or mother-in-law, 31% by other kin, 8% by other Tsimane, and 14% by a *napo* (a non-Tsimane Bolivian national). Mothers were advised to begin CF for many of the same reasons cited above (Fig.3), i.e., because the infant was crying or hungry (59%), to promote growth or good health (13%), because the infant was small or sick (11%), because the mother had no milk (9%), or because of general recommendations. Primiparous mothers did not report receiving advice to begin CF any more frequently than did multiparous mothers (40% vs. 43%, chi-square = 0.0005, $p < 0.98$), though lower parity mothers (1-3 births) reported receiving such

advice more frequently than mothers with four or more births (51% vs. 33%, chi-square = 3.35, $p = 0.067$).

In a separate line of questioning, 41% (60/146) of mothers (including those still EBF) reported getting professional advice from a doctor, nurse, or other health care worker about when to feed their infants. However, only 53% of these mothers reported that health professionals recommended introducing CF at 6 months; 25% reported hearing that CF should be later than 6 months, 17% reported hearing CF should be earlier, the remaining 5% did not recall any specific recommended age of CF. In total, only 30% of mothers who received professional advice (16/54) introduced CF at the age they were reportedly recommended: 69% introduced CF earlier than they had been recommended, and 1% introduced CF later. Lower vs. higher parity mothers did not significantly differ in the frequency at which they reported receiving professional advice about infant feeding (38% vs. 47%, chi-square = 0.776, $p = 0.378$).

I did not systematically ask mothers about reasons for weaning, though 60% of pregnant mothers (15/24) had weaned their infants by the time of interview. As has been previously reported for the Tsimane and other natural fertility populations, mothers often wean breastfeeding infants when they become pregnant again (Veile et al., 2014). In this study, all mothers who had weaned their infants had already resumed cycling. Among pregnant mothers, five (21%) reported weaning 1-17 months prior to estimated time of conception, eight (33%) weaned 0-6 months after estimated time of conception, and nine (38%) had yet to wean (age at weaning was unknown for 2 pregnant mothers). This suggests that in total at least 70% of pregnant mothers weaned after conception. In *ad lib* responses to

questions about weaning, some mothers described a gradual infant-led weaning process, with infants only nursing at night. Others reported that a prolonged absence or illness had inadvertently led to weaning, while a few reported taking active measures to wean, such as withholding nursing or putting medicinal ointment on their breasts to deter children from nursing. Anecdotally, in interviews I conducted in 2009 (unpublished data), a few mothers described purposefully weaning their infants because breastfeeding was “*cari*” (Sp. *costa*)—literally, “it’s expensive”.

4.3b Results P_{1A.2}/IB.2: Predictors of timing of CF introduction

As determined by Kaplan-Meier survival analysis, the overall mean \pm SE time to CF introduction for all observations ($n = 161$, n events = 146) was 132.6 ± 4.9 days, or approximately 4.4 months. The best fit Cox proportional hazards model for time to CF (P_{1A.2}) included maternal height, time elapse since reported or observed CF introduction, and parity. Taller women are more likely to initiate early CF: each centimeter gain in maternal height was associated with a 5% decrease in CF risk (HR = 0.95; 95% CI = 0.92 – 0.99; $p = 0.020$), adjusting for parity and the time elapse between infant age at interview and reported age at CF (HR = 0.99; 95% CI = 0.99 – 1.00, $p = 0.12$). The penalized spline for parity was also significant ($p = 0.006$), resulting in a u-shaped hazard curve suggesting that women of lowest and highest parity tend to initiate CF relatively early (Fig. 5). Although CF hazard decreased after a first birth, the log hazard ratio for primiparous mothers was not significant (LnHR = 0.37, 95% CI = -0.07 - 0.81). Confidence intervals for log hazard ratios of CF were only significantly increased from the mean after 9 births (LnHR = 0.45, 95% CI = 0.01 - 0.89).

Village region, infant sex, and birth season did not affect time to CF and did not improve model fit. The number of females over the age of 7 did not improve model fit when entered as continuous linear term, a nonlinear term, or as a categorical variable. A model substituting maternal age instead of parity resulted in a poorer model fit, though height and the penalized spline term for maternal age were significant. The resulting hazard curve was similar to the one observed for parity, with minimum hazard of CF observed at age 28 (Fig. 6). Log hazard ratios for CF were significantly increased for mothers aged 14 (LnHR = 0.69, 95% CI = 0.01 - 1.37) and 15 years old (LnHR = 0.62, 95% CI = 0.03 - 1.21), and then again after age 37 (LnHR = 0.40, 95% CI = 0.04 - 0.75).

In a linear regression adjusting for confounders ($P_{1B.2}$), mothers who reported introducing CF because of low milk began CF on average 3.4 months earlier than those who introduced CF because of other reasons (Table 4). As above, maternal height was associated with delayed CF introduction, and primiparity with earlier CF introduction (Table 4). High parity (9 or more births) was not associated with timing of CF introduction, however, which may reflect the greater frequency at which these mothers reported introducing CF because of low milk. There were no significant interactions between parity group and low milk or height.

4.3c Results $P_{1A.3/1B.3}$: Early CF and resumption of postpartum menstruation

As determined by Kaplan-Meier survival analysis, the mean \pm SE time to onset of first postpartum menstruation was 420.9 ± 18.7 days, or approximately 14 months postpartum (148 observations, 98 events). Restricting the sample to non-EBF infants, a Cox proportional

hazards model was run to test if early CF was associated with shorter duration of lactational amenorrhea ($P_{1A.3/1B.3}$) (n observations = 130, n events = 85). Adjusting for covariates in the final model, the hazard for resumption of postpartum menstruation was not associated with early CF (< 4 months) but was reduced by approximately 47% among mothers who gave birth during the rainy season (Table 5, Model 1). A nonlinear parity term was also significant, with the log hazard curve (Fig. 7) showing a U-shaped curve with minimum hazard at seven births, similar to the curve produced for time to CF introduction (Fig. 5). Log hazard ratios for resumption of postpartum menstruation significantly increased from primiparity (LnHR = 1.02, 95% CI = 2.97 – 1.74) through three births (LnHR = 0.78, 95% CI – 0.11 – 1.46), and then again after 10 births (LnHR = 1.07, 95% CI = 0.94 – 2.74). In the second model, adjusting for other covariates, there was a moderately significant increase in the hazard of for resumption of postpartum menstruation (77%) among mothers who reported introducing CF because of low milk supply,(Figure 5, Model 2). Other model terms remained largely unchanged.

Analyzing just women for whom first onset of menstruation was observed or reported (n = 86), the mean \pm SD duration of lactational amenorrhea was 11.0 ± 4.4 months (range 3 – 27 months). Mean duration of lactational amenorrhea among pregnant and non-pregnant mothers was, respectively, 13.7 ± 5.8 months (range 5 – 27) and 11.1 ± 4.5 months (range 3 – 26 months). Among mothers 12-35 months postpartum (n = 109), 38% resumed menstruation before one year. The percentage of mothers who resumed menstruation before one year was not significantly different between pregnant and non-pregnant cycling mothers (50% vs. 35%, chi-square = 0.53, p = 0.465). Frequency of resumption of menstruation before one year did

not significantly differ among mothers who introduced CF at 0 – 3 vs. 4 – 7 months (46% vs. 32%, chi-square = 1.81, $p = 0.178$); or between mothers who introduced CF because of low milk or for other reasons (53% vs. 34%, chi-square = 1.43, $p = 0.230$). However, the frequency of mothers resuming menstruation before one year was significantly different across the parity groups suggested by the survival analysis, and increased with parity: 1-3 births = 23%; 4-8 births = 45%; 9 or more births = 62% (chi-square = 8.62, $p = 0.014$). To test whether the effect of parity on lactational amenorrhea was mediated by CF introduction, I ran a logistic regression on the likelihood of resumption of menstruation before one year, using terms from the survival analysis and an interaction term for early CF and parity group (not shown). The resulting large confidence intervals suggested the interaction terms could not be accurately estimated, however.

4.3d. Results P_{1A.4/1B.4}: Early CF and weaning

The mean \pm SE time to weaning, as determined by Kaplan-Meier survival analysis for infants 12-35 months of age, was 824.1 ± 36.5 days, or approximately 27 months. A Cox proportional hazards model was run to test for an effect of age at CF introduction on time to weaning (P_{1A.4/1B.4}). Adjusting for other model terms retained by backwards selection, early CF introduction (less than 3 months of age) had no effect on weaning hazard (Table 6). Having one female allomother reduced weaning hazard by 78%, though two or more had no effect. Weaning hazard was reduced by 64% in remote villages, and increased more than 5-fold by a current pregnancy. Neither a linear nor non-linear term for parity was retained in the

model by backwards selection. The survival function from the final model is plotted in Figure 8.

4.3e. Results P_{2A.1/2B.1}: Age of CF and subsequent CF intake

Table 7 reports the results of a linear regression on 24-hour CF intake (the total of each meal, snack, and type of liquid consumed the day prior) across all non-EBF infants surveyed in initial interviews. Infants introduced CF because of low milk were no more likely to be consuming more complementary foods than infants introduced CF for other reasons. While age at CF introduction did not independently influence CF intake, the interaction between age and age at CF introduction was significant. Relatively earlier CF is associated with slightly decreased CF intake at earlier ages, but increasing CF intake at later ages (Fig. 9). According to the model, CF intakes predicted for 8-month old Tsimane infants introduced CF two months before, at, or after the average age of CF introduction (4.3 months), would be estimated at 5.8, 6.25, and 6.7 total meals, snacks, and unique liquids per day. In contrast, CF intakes predicted for a 28-month old Tsimane infants introduced CF two months before, at, or two months after the average age of CF introduction would be estimated at 9.0, 8.4, and 7.8 total meals, snacks, and unique liquids per day, respectively. For those infants introduced CF at the tail ends of the distribution (approximately 3 months before and after the mean age of 4.3 months), subsequent CF intakes at 11 months are estimated at 9.5 and 7.2 total meals, snacks, and unique liquids per day.. Adjusting for covariates, CF intake was positively

associated with maternal age, and infants in remote villages consumed about one less meal, snack or unique liquid per day than infants in villages near town.

Examination of differences in CF intake across repeated dietary recalls from the prospective sample produced similar results (Table 8). Adjusting for other covariates, CF intake decreased in remote villages by 1.6 meals or liquids per day, and was not associated with low milk. In this model maternal age and the interaction between age and age at CF introduction were only moderately significant.

4.3f. Results P_{2A.1.2B.1}: Age of CF and subsequent CF and breastfeeding behaviors

Summary results of infant activity states and proximity to mothers and fathers calculated across all interval observations are presented in Figures 10 and 11. Infants spent 84% of daytime hours at home in close proximity to their mothers (in arms, within reach, or within sight). In contrast, infants spent only 28% of this time in close proximity to their fathers, largely owing to paternal absence from the home during daytime hours. Infant activity was minimal, with infants spending most of their time stationary (sitting or prone) but awake and observant (52%), or sleeping (33%). Infants were engaged in more active or exploratory behaviors such as crawling, standing, and walking (with and without help) only 13% of the time. Infants were observed crying across only 1% of all hours of observation.

For all non-EBF infants observed (n = 13, ages 2 – 16 months) the mean age of CF introduction was 3.3 ± 1.3 months (range 2 – 6 months). At least one CF bout was observed in 31/44 (75%) of observation intervals, with a mean \pm SD frequency of 4.5 ± 2.6 (range 1 -

10) total CF bouts per observation interval. The mean frequency of CF per hour across all hours of observation for all subjects was 0.8 ± 0.7 (range 0 – 2.5).

At least one breastfeeding bout was observed across all observation intervals for all subjects ($n = 44$). The mean \pm SD frequency of total breastfeeding bouts per observation interval was 8.3 ± 4.6 (range 2 – 23). The mean \pm SD total duration of all breastfeeding bouts per observation interval was 22.3 ± 11.9 minutes (range 8.0 – 59.7 minutes). Across all hours of observations for all subjects, the mean frequency of breastfeeding per hour was 2.1 ± 1.4 (range 0.6 – 7.7), and the mean time spent breastfeeding per hour was 5.4 ± 3.0 minutes. The total mean duration of breastfeeding bouts, for each bout observed across all subjects ($n = 366$), was 2.7 ± 2.4 minutes (range 0.07 – 16.6 minutes).

Differences in mean CF frequency/hr, mean breastfeeding frequency/hr, and total breastfeeding time/hr across all observation intervals of non-EBF infants ($n = 39$) were assessed in separate linear mixed-effects models (Tables 9 and 10). Across all models, very little variance was explained by within-subject effects. Age of CF introduction had no effect on summary measures of either CF or breastfeeding behavior (Table 9, Table 10). Both mean CF frequency/hr and mean breastfeeding frequency/hr were increased with infant age, while infant age had no effect on mean time spent breastfeeding/hr. The percent of time spent asleep during the interval had a slight negative effect on CF frequency, but did not affect breastfeeding behaviors. No significant interactions between age and age at CF introduction were observed in any models. Additional covariates (infant sex, maternal age, and season) were considered additively but did not affect model outcomes. However, small sample sizes were likely a limiting factor.

Separate models were also run substituting a categorical variable for infant age (0-5 and 12-16 months vs. 6-11 months as the reference group). In these models, mean CF fq/hr was significantly lower for infants 0-5 months and later age groups, though differences between infants 6-11 and 12-16 months were not significant. In contrast mean breastfeeding fq/hr was significantly higher for infants 12-16 months as compared to younger age groups, while differences between infants 0-5 and 6-11 months old were not significant. Thus, the relationship between increasing CF and breastfeeding frequency with age were alternately driven by low CF frequency at young ages, and higher breastfeeding frequencies at later ages. Breastfeeding time/hr did not vary across categorical age groups. Two breastfeeding observations from relatively older infants were outliers in the dataset: one observation of 7.7 bouts/hr for a 16 month old, and one observation of 6 bouts/hr in a 12 month old. However, the age-associated increase in breastfeeding fq/hr remained significant when these two observations were excluded from the model (Est. = 0.13, $p = 0.023$).

The number of summary observations per subject were too small and unbalanced to assess longitudinal changes across all subjects. Differences in frequency and time spent breastfeeding per hour both before and after CF introduction were only available for two subjects, one introduced CF at 3 months and one at 5 months. For the first infant (introduced CF at 3 months), breastfeeding frequency from 2 months old (while still EBF) to 7 months old increased from 3.6 to 6.1 bouts/hr, and mean breastfeeding time increased from 0.9 to 1.5 minutes per hour. For the second infant (introduced CF at 5 months), mean breastfeeding frequency from 3 to 8 months old decreased from 10.7 to 7.5 bouts/hr, and mean breastfeeding time decreased from 2.4 to 2.1 minutes per hour. The mean percentage of time

each infant was asleep during each observation period (as determined from interval observations) decreased from approximately 70% to 40% between 2 and 7 months for the first infant, and decreased from approximately 40% to 20% between 3 and 8 months for the second infant.

I also examined differences in breastfeeding behaviors for infants observed twice during each observation period ($n = 9$, 142 hours of observation), averaging the summary measures for each interval in each period per subject. Mean differences in breastfeeding behaviors between the two periods were compared by paired t-tests (Table 11). Across all infants, mean breastfeeding fq/hr, but not time spent breastfeeding, significantly increased from Period 1 to Period 2. Whether infants were introduced CF before or after 4 months of age had no effect on change in breastfeeding frequency or time spent breastfeeding between Period 1 and Period 2 (Table 11).

4.3g. Results: changes in BMI among EBF mothers

There were eleven EBF mothers with at least two anthropometric measures between 0-3 months postpartum (n measures = 32). Of these, only 3/11 (27%) subsequently introduced CF before 4 months of age; the remaining 8 mothers subsequently introduced CF or were still EBF at 4-6 months postpartum. For each of these mothers, the net change in weight between measurements was standardized to the time elapsed between measurements to estimate a weekly rate of change in postpartum weight. Between 0-3 months postpartum, weight was decreasing for 5 mothers (mean \pm SD -0.16 ± 0.10 kg/wk) and increasing for 6 mothers

(mean \pm SD 0.14 ± 0.14 kg/wk). There were no obvious patterns in increasing or decreasing postpartum weight changes with respect to minimum EBF duration (0-3 vs. 4 or more months), infant sex, maternal age, parity, or region.

Changes in maternal BMI postpartum from 0-6 months were then examined for all mothers who were EBF at the start of observations, and then separately for all mothers post-CF introduction. Mean changes in individual BMI across measures from 0-6 months postpartum were decreasing for 72% (13/18) of mothers initially EBF (Figure 12). A linear fit of BMI by month postpartum among all initially EBF mothers shows a slightly higher slope among mothers with relatively longer EBF durations (Figure 13). Across individuals, mean changes in BMI and weight across 0-6 months postpartum for mothers who subsequently exclusively breastfed for at least four months were -0.28 and -0.65 kg, respectively, and were -0.25 and -0.60, respectively, for mothers who subsequently exclusively breastfed for less than 4 months. However, the small, unbalanced dataset precluded more robust statistical analysis of changes in BMI or weight across these individuals.

4.3h. Results P_{3A./3B.11}: Age of CF and maternal BMI

After CF introduction, BMI continued to decrease across measures for 67% of mothers, as is shown in individual slopes in Figure 14. A mixed-effects linear regression was run to examine changes in BMI in association with age of CF introduction and other variables. The final model included terms for month postpartum, infant sex, age at CF introduction, whether or not CF was introduced because of low milk, parity group (primiparous, prime, and high), and a parity*age at CF introduction interaction (Table 12). The parity groups performed

better in the model than total parity or a binary term for primiparous vs. multiparous mothers. Maternal age and region did not affect BMI and resulted in poorer model fit. There were no significant interactions between month postpartum and age at complementary feeding, month postpartum and parity, low milk and month postpartum, or low milk and parity.

Across parity groups, relatively earlier CF introduction was associated with lower BMI, while delays in CF introduction were associated with higher BMI in multiparous, but not primiparous mothers (Table 12, Fig. 15). For example, primiparous mothers who introduced CF one month earlier than the mean (3.08 months) are predicted to have a BMI of 21.2 at 8 months postpartum, as compared to a predicted BMI of 21.7 for mothers who introduced CF one month later than the mean—a difference of only 0.5. In comparison, the predicted BMIs of high parity mothers who introduce CF one month earlier vs. one month later than the mean are 24.7 and 27.6, respectively—a difference of 1.9 that would differently classify these mothers into normal and overweight categories. Mean BMI of mothers who introduced CF because of low milk was a 3.15 units higher than that of mothers who introduced CF for other reasons—an effect also large enough to span BMI categories. Adjusting for other covariates, mean BMI was also lower in mothers of sons, and on average decreased by 0.11 per month postpartum (Table 12).

4.3i. Changes in maternal C-peptide among EBF mothers

The grand mean of individual mean C-peptide concentrations for EBF mothers between 0-3 months (n subjects = 10; n measures = 33) was 10.7 ± 5.0 ng/mL (range 3.2 – 19.7).

Individual mean C-peptide concentrations of the EBF mothers did not clearly vary in relation to estimated weekly rate of postpartum weight change, EBF duration, infant sex, maternal age, parity, or region. Across all mothers who were EBF at the start of observations, there were no evident trends in individual changes in urinary C-peptide concentrations by month postpartum or subsequent maximum duration of EBF (< 4 months or \geq 4 months) (Fig. 16). Across all subjects, the slope of C-peptide by month postpartum appears higher among mothers with EBF durations of at least 4 months (Fig. 17), though this could not be statistically evaluated given the small, unbalanced dataset.

4.3j. Results P_{3A.2/3B.2}: Age of CF and maternal C-peptide

Following introduction of CF, individual changes in C-peptide concentrations are highly variable (Fig. 18), with no evident increasing linear trends by month postpartum (Fig. 19). Across all data points, mean \pm SE C-peptide concentrations appear to increase from birth to about 10 to 12 months postpartum, and then subsequently decrease (Fig. 20). Figure 21 shows monthly means by age of CF introduction, with data truncated to 12 months postpartum (data were too sparse to compare means after 12 months). In this graph, mean C-peptide concentrations clearly peak at 10 months postpartum among mothers with relatively longer EBF durations. Mean C-peptide concentrations across month postpartum are generally lower and more oscillating among mothers with shorter EBF durations.

A similar model run on maternal BMI post-CF introduction was fit to log C-peptide concentrations post-CF introduction, with maternal BMI group (overweight or not) included

as additional variable. In this model, the interaction between parity and age at CF introduction was not significant and was excluded. No model terms were significantly associated with log C-peptide concentrations (Table 13). No significant predictors were found when removing model terms through backwards selection. Additional models (not shown) considering region, season, and other interaction terms also failed to demonstrate any significant variation in log C-peptide concentrations. Finally, a separate mixed-effects model was run across all subjects' C-peptide measures (n observations = 155, groups = 37), replacing terms for CF introduction and low milk-induced CF with a term for EBF status. In this model, C-peptide concentrations were higher for mothers of sons (Est. = 0.36, $p = 0.048$), but did not vary with feeding status, month postpartum, parity, or BMI classification at the start of observations.

4.4. Discussion

Two models were proposed to explain patterns of early CF among the Tsimane. The Feeding Substitution Model proposes that early CF benefits maternal reproductive fitness through energetic savings, while the Feeding Augmentation Model proposes that early CF may be necessitated by relatively poor maternal or infant condition, but does not supplant breast milk intake and does not result in any energetic savings for mothers. Original predictions from both models and summaries of results observed are given in Table 1.

Results from ethnographic interviews and regression models provide mixed support for both models. Mothers' reported reasons for introducing CF at any age largely reflect concern

for infant needs, not their own time or energy constraints, supporting the Feeding Augmentation Model (P_{1B.1}). Tsimane mothers who perceived their infants as small for their age were also more likely to introduce CF earlier than mothers who perceived their infants as larger or average for their age. This finding is consistent with research conducted with Aka mothers, who view early supplementation as “good parenting”—a practice that benefits, not risks, infant health (Meehan and Roulette, 2013). Furthermore, low milk supply was the most frequently reported maternal constraint necessitating CF introduction, and was significantly associated with earlier CF (Table 4, P_{1B.2}). Owing to sampling methods, infant size or growth could not be evaluated as predictors of time to CF in survival analysis, though neither infant sex nor birth season were associated with age of CF introduction.

There is limited evidence supporting a relationship between maternal reproductive trade-offs and earlier CF (P_{1A.2}). Age at CF introduction was positively correlated with maternal height, suggesting Tsimane mothers in relatively better condition are better able to invest in prolonged EBF. While primiparous mothers did introduce CF earlier than mothers with 2-8 births (Figure 5, Table 4), mothers with 9 or more births were also more likely to introduce CF earlier than mothers of prime parity..

The link between high parity and early CF may reflect the high prevalence of low milk reported by these mothers (Figure 4), particularly as the relationship was not significant in the linear regression when low milk was added (Table 4). Different mechanisms may favor relatively earlier CF at low and high parity—for example, maternal inexperience and reproductive trade-offs may operate more frequently at low parity, while poor lactational performance operates more often at higher parity.

Both advanced age and parity may negatively impact milk synthesis. For example in the U.S., advanced maternal age has been linked to delayed onset of lactogenesis, which may result from age-associated increases in glucose intolerance and inflammation (Nommsen-Rivers et al., 2012). I have not found any evidence in the literature of premenopausal factors inhibiting milk synthesis, though this may also be a factor in the high parity Tsimane mothers. On the other hand, very high parity (10 or more births) has previously been associated with low concentrations of endogenously synthesized fatty acids (Prentice et al., 1989). Maternal depletion may negatively affect other aspects of milk composition that influence infant satiety or growth, which may then be interpreted as insufficient milk supply. Finally, it is well established that calcium stores become increasingly depleted with age and successive bouts of lactation (Horst et al., 1997; Nordin, 1997). Recent research has demonstrated low bone mineral density among lactating pre-menopausal Tsimane mothers, and particularly so for mothers with five or more children spaced close together (Stieglitz et al., 2015). With sustained calcium secretion at high parity, homeostatic mechanisms may downregulate milk supply to conserve essential maternal function. This hypothesis may be assessed in future research examining Tsimane milk composition.

The results presented here deviate somewhat from those of a previous study (Veile et al., 2014), in which we found that CF introduction occurred earlier in remote Tsimane forest villages as compared to other village regions (Mission, near town, river). We had predicted earlier CF would be more common in villages near town, owing to relatively higher maternal and infant condition as well as acculturating influences that may favor lower breastfeeding intensity. The opposite finding suggested to us instead that improvements in condition and/or

relatively greater access to resources and health care may have influenced more intensive breastfeeding behaviors in villages closer to town (Veile et al. 2014). In the present study, CF introduction did not vary systematically between the remote downriver villages and villages closer to town (Table 3). Region was also eliminated as a predictive factor during model selection for both the Cox Proportional Hazards and linear regression models of age of CF introduction. However, as remote forest villages were not part of the present study, this result is still in agreement with our earlier study, in which differences in CF introduction between river and near town villages were not significant (Veile et al., 2014). Also relevant to our earlier study, while 40% of the mothers I interviewed here reported receiving some advice about CF from health care professionals, less than a third of those mothers actually introduced CF at the age they had been recommended. These responses indicate that exposure to public health messages may have increased knowledge about recommended EBF durations, but ultimately not influenced behavior. If this is the case, the relatively shorter EBF durations previously observed for the forest communities may reflect relatively poorer maternal or infant condition.

Other results from this study suggest that maternal reproductive trade-offs predict shorter durations of postpartum amenorrhea and earlier weaning, though early CF does not appear to directly mediate these relationships. Age of CF introduction was not an independent predictor of either earlier resumption of menses or earlier weaning (Table 5, Table 6). However, mothers who reported introducing CF because of low milk were more likely to introduce CF in the first few months postpartum, and low milk did predict earlier resumption of menstruation. Similarly, primiparity and high parity were associated with

shorter durations of EBF and postpartum amenorrhea (Figures 5 and 7). Previous research has shown a similar u-shaped effect of age on fecundity in natural fertility populations (Strassmann, 1997), suggesting the age effect on onset of menstruation may be independent of EBF duration. Weaning was not associated with parity in this study, but was predicted by current pregnancy (Table 6). Our earlier research showed higher parity-for-age was associated with earlier weaning (Veile et al., 2014). However, weaning ages in that study were reported for next oldest siblings of nursing infants, and may not be comparable to relationships estimated by survival analysis. In summary, primiparous and high parity mothers—who tend to introduce CF earlier—resume menstruation earlier, which may be associated with earlier pregnancy and then weaning, though a causal pathway cannot be conclusively determined.

Previous research has shown that maternal weight loss and infant mortality risks are increased for the Tsimane during the rainy season (Gurven 2012, Gurven et al., *in prep*). In other subsistence-scale populations, researchers have observed variation in milk output (Whitehead et al., 1978), postpartum weight loss (Panter-Brick and C, 1993)(Panter Brick 1993), and ovulatory function (Jasińska and Ellison, 1998; Vitzthum et al., 2009) consistent with seasonal increases in maternal workloads and decreases in energy intake. Here, relatively longer durations of postpartum amenorrhea were predicted for mothers who gave birth during the rainy season (Table 5). Tsimane mothers giving birth during the rainy season may sustain longer periods of negative energy balance—e.g. due to compromised prepartum weight gain, greater postpartum weight loss, and/or higher energetic costs of lactation — which would delay resumption on menstruation, consistent with the metabolic load model (Valeggia and Ellison, 2009). Weaning hazard was also decreased with distance from town

and increased by the number of female alloparents present. Thus, consistent with the Feeding Substitution model, Tsimane mothers in better condition and with greater access to resources or alloparental help may be better able to “afford” earlier disinvestment in breastfeeding. Again though, this relationship only evident at later stages of lactation, and may not be mediated by earlier CF.

While infants introduced CF earlier showed relatively greater CF intake with age (Tables 6, 7, 9; Figure 9), additional analyses of breastfeeding intensity after CF introduction largely support the Feeding Augmentation Model. As predicted by both models, earlier CF was associated with greater subsequent intake of complementary foods, though the increase was moderate (1-2 more meals/snacks or unique liquids per day), and may not be sufficient enough to offset breastfeeding frequency. The modest amounts of CF may further explain the lack of effect of early CF on duration of postpartum amenorrhea, as menses risk may not significantly increase until CF surpasses 50% of feeds (Bhatnagar et al., 1998; Dada et al., 2002) and time spent breastfeeding significantly decreases (Dewey et al., 1997).

Introduction of CF because of low milk supply did not predict subsequently increased CF intake relative to other reasons for introducing CF (Tables 7 and 8). This finding is consistent with the Feeding Augmentation Model in suggesting that Tsimane mothers with low milk continue to breastfeed at their maximum intensity. Moreover, breastfeeding intensity (as measured by mean frequency/hr or time spent nursing/hr) did not vary among non-EBF infants according to their age at CF introduction. Early supplementation similarly had no effect on mean breastfeeding frequency among Aka or Ngandu mothers (Meehan and Roulette, 2013). Observations of Tsimane breastfeeding behaviors further suggest that

mothers do not decrease their breastfeeding intensity over at least the first year of life. Breastfeeding frequency actually appears to increase across infancy, perhaps as Tsimane infants become more active and their demands (energetic, immunological, or emotional) increase. Of note, the two highest mean breastfeeding frequencies calculated for any specific interval (6 and 7.7 bouts/hour) were observed for two relatively older infants in the sample (at 13 and 16 months, respectively). These infants were frequently reported as sick during follow-up interviews, and were both classified as undernourished at measures taken within 2 weeks of these breastfeeding observations (HAZ of -3.94 and -2.12, respectively). However, even removing these two data points from the analysis, the effect of age on increasing breastfeeding frequency remained significant. Breastfeeding intensity has been shown to be similarly sustained across the first 10 months of lactation in Ribhera mothers (Piperata and Mattern, 2011), and up through the second year of lactation in Tamang mothers (Panter-brick, 1989; Panter-Brick, 1991). The frequent, short nursing bouts observed for Tsimane mothers are also typical of those observed in other traditional breastfeeding populations (!Kung, Stern et al., 1986; Toba, Valeggia and Ellison, 2004; Au and Gnau, Tracer, 2009; Ribhera, Piperata and Mattern, 2011).

Unfortunately there was insufficient data (see Appendix) to test if differences in postpartum weight loss or C-peptide concentrations while EBF predicted the timing of CF introduction. Changes in BMI after CF-introduction do not clearly support one model over the other. The Tsimane mothers surveyed here showed continued decreasing BMI up through at least the first year postpartum, consistent with previous studies (Brewer and Bates, 1988; Butte et al., 1999; Dwivedi and Dixit, 2012; Hruschka and Hagaman, 2015). Mothers who

introduced CF because of low milk had higher BMIs, on average, than mothers who introduced CF for other reasons. This relationship may in part be driven by mothers at high parity, who have the highest BMIs and are more likely to report introducing CF because of low milk. At any rate, the finding suggests that reported low milk supply among the Tsimane is not the result of poorer maternal energy availability, as was observed in relation to milk production among Gambian mothers (Whitehead et al., 1978). As discussed above, obesity, age, and parity may all affect onset of lactogenesis or subsequent milk synthesis, therefore it is not surprising that low milk is associated with higher BMI across Tsimane mothers.

Though not a significant independent relationship, relatively longer durations of EBF were associated with higher BMI after CF introduction (Table 12). However, delayed CF introduction was not associated with higher BMI among primiparous mothers—who had significantly lower BMI on average than multiparous mothers (Figure 15). Since BMI continued to decrease across subjects after CF introduction (Figure 13), and most individual slopes continued to show decreasing or unchanging BMI (Fig. 14), the relationship between age of CF introduction and parity was likely mediated by prepartum BMI (i.e. it is unlikely that later CF *resulted in* increased weight gain among multiparous mothers). Recent research has shown that Tsimane mothers do not pay obvious net energetic costs for high parity or shorter interbirth intervals—in effect showing minimal evidence of parity-associated maternal depletion (Gurven et al., *in prep*). Therefore, multiparous mothers, who have higher energy reserves to begin with, may be best able to sustain prolonged EBF durations owing to their relatively lower metabolic loads. Conversely, primiparous mothers would face higher energy deficits with prolonged EBF, which may favor earlier CF in this group. Thus,

relatively poorer maternal condition may favor earlier CF only among primiparous mothers—though whether this ultimately supports the Feeding Substitution or Feeding Augmentation Model would depend on subsequent changes in breastfeeding behaviors.

Levels of C-peptide following CF introduction were not relatively increased by age of CF introduction or even month postpartum. Very little variation in C-peptide concentrations within and across subjects (Figures 16 – 19) was explained in the model (Table 13).

However, the high interindividual variability in C-peptide concentrations is consistent with variability previously observed for lactating Toba women (Ellison and Valeggia, 2003; Valeggia and Ellison, 2009) and lactating chimpanzees (Emery Thompson et al., 2012). Emery Thompson et al.'s (2012) study, which also used a linear mixed-effects model, similarly did not observe any monotonic increase in C-peptide over the course of lactation.

A linear mixed-effects model may not have been appropriate here, however, given the non-linear patterns observed across months postpartum (Figures 20 and 21). Alternate modeling methods will be evaluated in future analysis. The analysis was also limited given unbalanced data collection across sampling intervals and stages of lactation (Appendix). Previously, researchers have examined longitudinal changes in C-peptide relative to key events by standardizing the individual measures before and after those events by individual means. For example, Valeggia and Ellison (2009) standardized subjects' repeated individual C-peptide values during lactational amenorrhea to each subjects' mean value following resumption of menstruation, and were thus able to track increases in C-peptide relative to this event. Similarly, Emery Thompson and Knott (2008) standardized individual measures from male chimpanzees during and after a fruiting season to each subjects' total mean, and was

thus able to compare differences across seasons. Unfortunately, the present data were not sufficiently balanced across a large enough number of subjects to similarly standardize and compare individual measures to subject means before and after CF introduction, or even in relation to particular stages of lactation (see Appendix).

However, the pattern of mean C-peptide levels by month of lactation across subjects observed here (Figures 20 and 21) is consistent with the pattern observed for Toba women (Ellison and Valeggia, 2003; Valeggia and Ellison, 2009). Tsimane mothers' C-peptide levels gradually rise to a peak at 10- 12 months postpartum, and then subsequently decline. Survival analysis estimated the mean month of onset of postpartum menstruation at 14 months in the mixed-longitudinal sample. Therefore, the difference between mean peak C-peptide concentrations and resumption of menses roughly approximates the 3 month difference between these events observed for Toba women (Valeggia and Ellison, 2009). To better investigate this, a future study design should prospectively follow a sample that is less stratified across lactational stages and/or assess C-peptide values averaged over 2-3 days per interval to better account for interindividual variation.

Finally, some discussion of methods in relation to maternal recall bias and definitions and measurement of CF is also warranted. First, it should be noted that mean age at weaning as estimated by survival analysis was approximately 27 months, notably later than the average weaning age of approximately 19 months as estimated solely from maternal recall here (Table 3), and in our previous study (Veile et al., 2014). Moreover, the time elapse since reported onset of menstruation and age at weaning increased the hazard ratios for both of these outcomes (Tables 5 and 6), suggesting maternal recall may underestimate duration of

postpartum amenorrhea and age at weaning. However, since Cox proportional hazards models for onset of menstruation and age at weaning controlled for time elapsed since reported events, and significant covariates differed in both models, recall error is unlikely to have systematically biased those results.

In contrast to the discrepancies observed above for age at weaning, EBF duration as estimated from survival analysis (4.4 months) and maternal interviews in this and our previous study (3.8 months and 4.1 months in Veile et al., 2014) are all largely congruent. However in this study, reported ages of CF introduction did vary with infant age at time of interview (Table 3), and the time elapsed since reported age of CF introduction reduced the hazard curve for time to CF—suggesting a recall bias towards reporting somewhat longer EBF durations. Though the Tsimane record birth dates, they do not keep calendars and most families do not officially mark birthdays. Mothers of older children may report beginning CF at “*choc año*” (half a year) because they are familiar with feeding recommendations or because it is a relatively easier age to reference and recall.

The present study also classified CF on the basis of any liquid or solid feedings, according to WHO (2008) specifications (see Chapter 3, Box 1). Although mothers were asked about first foods and liquids in the ethnographic interviews, the repeated 24-hour dietary recalls may have been more accurate in recording liquid feedings or small portions of solid feedings than mothers would have remembered at later recall. As an example, a 15 year old mother from a remote village first reported her 5 month old as exclusively breastfed, but when systematically questioned during the 24-hour dietary recall reported giving her baby small amounts of water, *po 'nadye'* (plantain and water), sugar cane juice, and some cracker.

Upon further questioning, she reported giving liquids and solids *on occasion* beginning at 2 and 4 months of age, respectively. Such infrequent feedings may be more likely to be captured in 24-hour dietary recalls—and would classify an infant as non-exclusively breastfed—but may not be remembered by mothers recalling first feedings one to two years later. These discrepancies support the current WHO guidelines for assessing feeding practices based on 24-hour recall.

Perhaps more importantly, the example above may be indicative of a general pattern by which Tsimane infants are sporadically introduced small amounts of complementary foods at earlier months. In another example, two infants followed prospectively were also observed to switch back and forth between EBF and CF between the ages of one and four months. Though in analysis these subjects were classified as non-EBF following the first instance of CF reported, these cases demonstrate that relatively early CF (i.e. before four months of age) may be much more limited and sporadic than CF when introduced later in infancy. Currently, however, there are no standardized classification systems of infant feeding status based on graduations of CF quantity. The WHO categories of EBF, CF, and even predominant breastfeeding are likely overly broad for use in analyzing feeding practices among the Tsimane and other traditional breastfeeding populations. Moreover, classification systems—and by extrapolation public health messages—that frame any amount of CF relatively to EBF as risky may not be appropriate for populations in which gradual but “early” CF introduction does not displace milk intake. Indeed, comparative mammalian evidence indicates that for many large-bodied, slowly-developing mammals with multi-year lactation, the onset of infant feeding does not lead to decreased maternal milk intake (van Noordwijk et al., 2013). Early

CF among traditional breastfeeding populations such as the Tsimane may be largely indicative of gradual introduction of complementary foods that—as proposed by the Feeding Augmentation Model—supplements but does not supplant frequent and on-demand breastfeeding. The next chapter will further test this idea by examining the degree to which relatively earlier CF is costly to or benefits infants.

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Chapter 4: Tables and Figures

Table 1. Summary of predictions and results

<i>Is early CF favored by maternal reproductive trade-offs (H_{1A}) or infant needs not met by EBF (H_{1B})</i>			
Factors evaluated	Predicted effect on timing of CF introduction		Observed relationship
	Feeding Model:		
	Substitution	Augmentation	
Maternal reproductive condition/resources ($P_{1A.2}$)			
Parity	Increases	NA	U-shaped
Maternal height	Decreases	NA	Increases
Near market village	Decreases	NA	No association
# of female allomothers	Decreases	NA	No association
Low milk supply* ($P_{1B.2}$)	Decreases	Decreases	Decreases
<i>Does early CF benefit mothers through future reproduction ($H_{1A/1B}$) or reduced breastfeeding costs ($H_{2A/B}$- $H_{3A/B}$)</i>			
Outcome evaluated	Predicted effect of earlier CF		Observed relationship
	Feeding Model:		
	Substitution	Augmentation	
Time to first postpartum menstruation ($P_{1A.3/1B.3}$)	Decreased	No difference/increased	No association (decreased by low milk*)
Time to weaning ($P_{1A.4/1B.4}$)	Decreased	No difference	No effect
CF intake ($P_{2A.1/2B.1}$)	Increased	Increased	Modest increase only at later ages
CF frequency ($P_{2A.1/2B.1}$)	Increased	Increased	No association
Breastfeeding frequency ($P_{2A.2/2B.2}$)	Decreased	No difference	No association
Breastfeeding duration ($P_{2A.2/2B.2}$)	Decreased	No difference	No association
Maternal BMI ($P_{3A.1/3B.1}$)	Increased	No difference/decreased	Increased with high parity (decreased by low milk*)
Maternal C-peptide ($P_{3A.2/3B.2}$)	Increased	No difference	No association

*Refers to “low milk” as reported reason for introducing CF

Table 2. Descriptive characteristics of maternal and infant subjects at initial interviews. Summary characteristics are provided separately for subjects who provided only an initial interview and those who participated in the prospective follow-up study.

Descriptor (Range, mean \pm SD)	All Subjects Surveyed		Initial Interview Only		Prospective Follow-Up	
	N	%	N	%	N	%
Infant sex	161		117		44	
Male	92	57.1	62	53.0	30	68.1
Female	69	42.8	55	47.0	14	31.8
Infant age (mos.)	(0-35, 13.8 \pm 9.8)		(0-35, 17.4 \pm 9.0)		(0-10, 4.3 \pm 10.5)	
0-5	46	28.5	17	14.5	29	65.9
6-11	27	16.8	12	10.3	15	34.1
12-23	61	37.9	61	52.1	-	-
24-35	27	16.8	27	23.1	-	-
Maternal age (yrs.)	(14-49, 26.9 \pm 8.5)		(14-49, 26.7 \pm 8.3)		(14-45, 27.6 \pm 9.1)	
< 20	33	20.4	24	20.5	9	20.5
20 to 29	71	44.1	52	44.4	19	43.1
30 to 39	38	23.6	29	24.8	9	20.5
40 and over	19	11.8	12	10.3	7	15.9
Number of births	(1-13, 5 \pm 3)		(1-13, 4 \pm 3)		(1-12, 5 \pm 3)	
No previous birth	27	16.8	20	17.1	7	15.9
2 to 8 births	115	71.4	82	70.1	33	75.0
9 or more	19	11.8	15	12.8	4	9.1
Spanish fluency						
None	72	45.9	50	44.2	22	50.0
Conversational	72	45.9	52	46.0	20	45.5
Fluent	13	8.2	11	9.9	2	4.5
Education (yrs.)	(0 - 12, 2 \pm 2)		(0 - 12, 2 \pm 2)		(0-6, 2 \pm 2)	
None	39	25.0	28	25.0	11	25.0
1st - 2nd	56	35.9	38	33.9	18	40.9
3rd - 5th	53	34.0	39	34.8	14	31.8
6th -12th	8	5.1	7	6.3	1	2.3
Region						
Near Town	88	54.7	78	66.7	10	22.7
Remote	73	45.3	39	33.3	34	77.2

Table 3. Descriptive characteristics of CF and weaning across subjects (n = 146). Age at CF was reported in first interview or observed during prospective study. All ages at weaning were reported from ethnographic interview.

	Age at CF introduction (months)				Age at weaning (months)			
	n	Range	Mean ± SD	% CF < 4 mos	n	Range	Mean ± SD	% weaned
All subjects	146	0 – 7	3.8 ± 2.0	41.2	21	9 – 31	19.1 ± 5.9	16.7
Region/Village								
Near Town	80	0 – 7	3.9 ± 2.2	37.5	15	10 – 31	18.8 ± 5.2	20.0
Alta Gracia	18	0 – 7	2.9 ± 2.6	55.5	3	12 – 26	18.7 ± 7.0	16.7
Campo Bello	26	0 – 7	4.2 ± 2.1	34.6	3	12 – 20	15.7 ± 4.0	11.5
Manguito	14	0 – 6	4.1 ± 2.1	28.6	3	18 – 21	19.0 ± 1.7	21.4
San Antonio	17	0 – 7	4.6 ± 1.7	23.5	4	9 – 26	19.0 ± 7.7	29.4
Santa Anita	5	0 – 6	3.4 ± 2.6	60.0	2	22 – 24	23.0 ± 1.4	40.0
Remote	66	0 – 6	3.6 ± 1.7	47.0	6	9 – 26	20.0 ± 8.0	12.9
Cedral	15	0 – 5	3.1 ± 1.4	60.0	1	12	NA	13.3
Chacal	20	0 – 6	4.3 ± 1.7	30.0	2	24 – 31	27.5 ± 4.9	16.0
Monte Rosa	23	0 – 6	3.3 ± 1.7	52.2	3	10 – 24	17.7 ± 7.1	13.6
Puerto Triunfo	8	1 – 6	3.5 ± 1.6	50.0	--	--	--	0.0
Infant age group								
0 – 5 months	28	0 – 5	2.4 ± 1.5	75.0	--	--	--	0.0
6 – 11 months	30	0 – 6	3.6 ± 2.1	46.7	--	--	--	0.0
12 – 23 months	61	0 – 6	4.1 ± 1.9	31.1	7	10 – 22	16.4 ± 4.4	13.1
24 – 36 months	27	0 – 7	4.6 ± 1.9	25.9	14	9 – 31	20.5 ± 6.3	62.9
Infant sex								
Male	65	0 – 7	3.8 ± 2.0	43.2	13	9 – 31	19.3 ± 6.6	18.8
Female	81	0 – 7	3.7 ± 2.0	40.0	8	12 – 26	18.8 ± 5.1	13.8
Maternal parity group								
Primiparous	26	0 – 6	3.4 ± 2.0	46.1	4	9 – 31	16.8 ± 10.1	24.0
Low (2 – 3 births)	38	0 – 7	4.2 ± 1.7	31.6	6	12 – 26	19.3 ± 5.0	16.3
Prime (4 – 8 births)	65	0 – 7	4.0 ± 2.1	40.0	9	12 – 26	20.3 ± 4.5	15.4
High (9 – 13 births)	17	0 – 5	2.5 ± 1.9	64.7	2	12 – 24	18.0 ± 8.5	11.8

Figure 3. Reasons reported for introducing complementary feeding (n = 142).

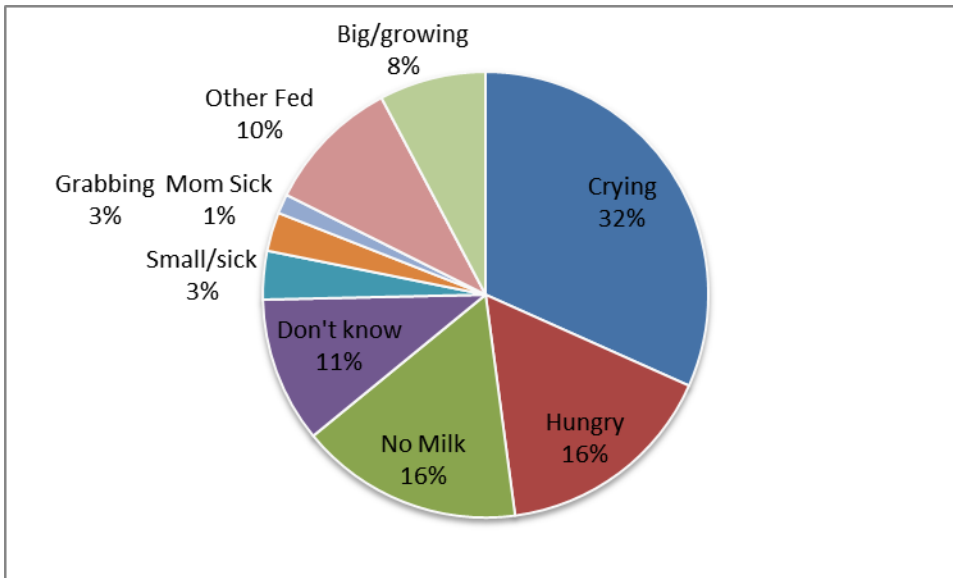


Figure 4. Percent of mothers by parity group reporting introducing CF because of low milk supply (n = 137). Parity group: primiparous = 1 group, low = 2-3 births, prime = 4 – 8 births, high = 9 – 13 births.

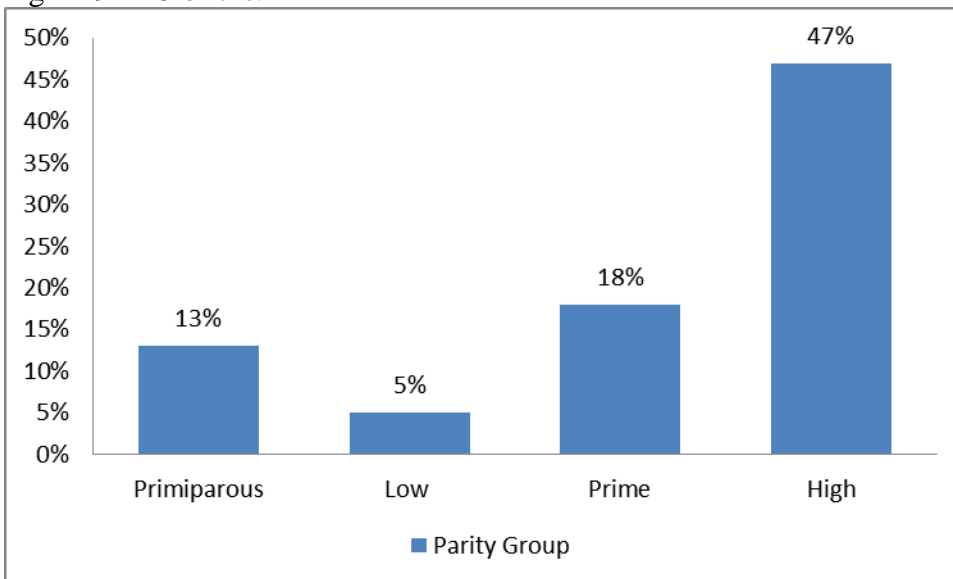


Fig. 5 Relative hazards of CF by parity (log hazard ratio)

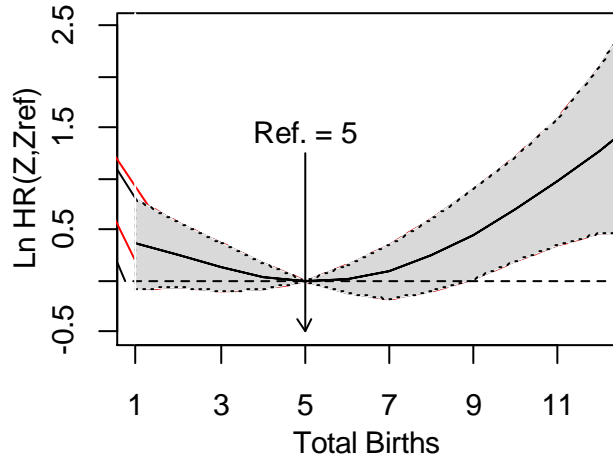


Fig. 6 CF hazard by maternal age

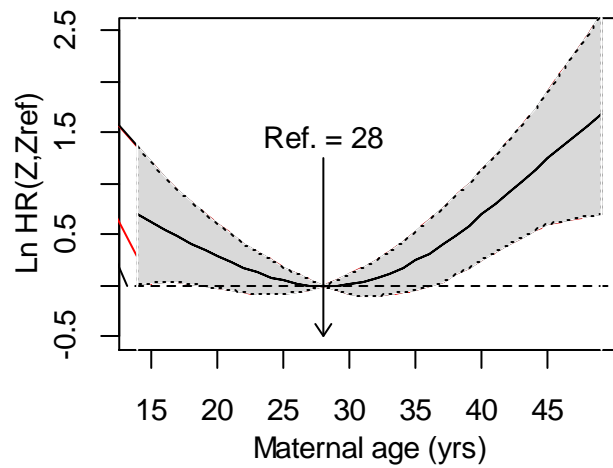


Table 4. Linear regression of effect of low milk supply on age at CF introduction (months), adjusting for time (in days) since CF introduction, maternal height, and parity group. Sample is restricted to infants post-CF introduction (n = 127).

	Est. (95% CI)	<i>p</i>
CF because of low milk		
<i>No</i>	--	--
<i>Yes</i>	-3.43 (-4.15; -2.72)	<0.001
Parity group		
<i>Prime (2 – 8 births)</i>	--	
<i>Primiparous</i>	-0.85 (-1.56; -0.14)	0.018
<i>High parity (9+)</i>	-0.32 (-1.17; 0.51)	0.437
Maternal height (cm)	0.09 (0.04; 0.15)	<0.001
Time since CF intro	1.00 (1.00; 1.96)	0.486

Table 5. Cox proportional hazards on time to resumption of menstruation

Predictor	Model 1 (n observations = 130, n events = 85)		Model 2 (n observations = 118, n events = 81)	
	Risk ratio (95% CI)	<i>p</i>	Risk ratio (95% CI)	<i>p</i>
CF Low Milk	--	--	--	--
<i>No</i>	--	--	--	--
<i>Yes</i>	--	--	1.769 (0.953 – 3.282)	0.070
Age CF introduction			--	--
≥ 4 months	--	--	--	--
0 - 3 months	1.310 (0.817 – 2.100)	0.260	--	--
Parity (nonlinear)	NA	< 0.001	NA	<0.001
Birth Season				
<i>Dry</i>	--	--	--	--
<i>Rainy</i>	0.535 (0.335 – 0.854)	0.008	0.526 (0.323 – 0.857)	0.010
Time since onset of menstruation (days)	1.002 (0.997 – 1.001)	< 0.001	1.002 (0.997 – 1.001)	<0.001

Fig. 7 PPM hazard by parity (from Model 1, Table 5)

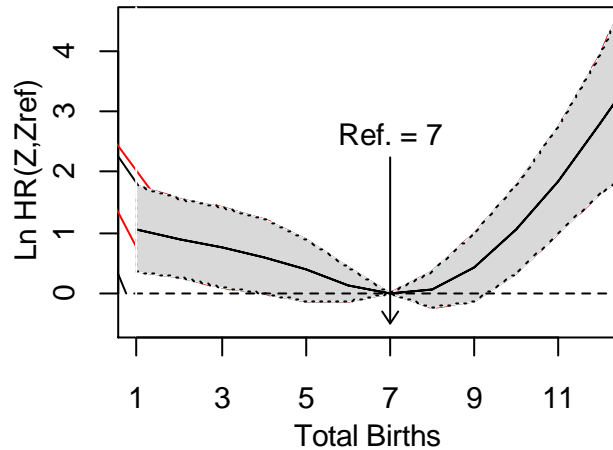


Table 6. Cox proportional hazards model of time to weaning by current pregnancy status, number of females age 7 or older in household, region, and age at CF introduction. Model was adjusted by time elapse since current infant age and reported weaning age (n observations = 100, events = 21)

Predictor	Risk Ratio (95% CI)	<i>p</i>
Age at CF Introduction		
≥ 4 months	--	--
0 – 3 months	2.017 (0.495 – 8.157)	0.325
Current pregnancy	5.318 (1.323 – 21.375)	0.018
Number of females ≥ age 7		
None	--	--
One	0.219 (0.052 – 0.919)	0.038
Two or more	1.636 (0.429 – 6.242)	0.470
Region		
Near Town	--	--
Remote	0.360 (0.06 – 2.09)	0.067
Time since weaning (days)	1.008 (1.005 – 1.011)	<0.001

Figure 8. Estimated survival function for the Cox regression of time to weaning on model predictors (Table 5).

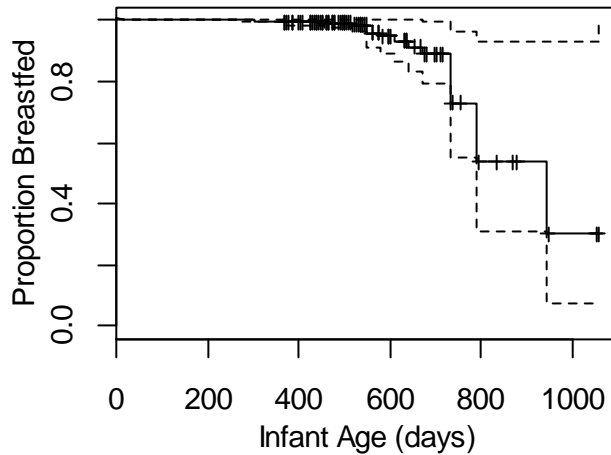


Table 7. Linear regression of CF intake the day prior (total meals, snacks, and unique liquids consumed). Dietary measures were reported by 24-hour recall in initial interviews with all non-EBF infants aged 0 -35 months (n = 125). Model is adjusted for infant age, region, maternal age, and an interaction term between age and age at CF introduction. Infant age and age at CF introduction were centered at sample means (16.7 months and 4.3 months, respectively).

	Est. (95% CI)	<i>p</i>
Age of CF introduction (centered)	0.01 (-0.23; 0.27)	0.887
Introduce CF because of low milk		
<i>No</i>		--
<i>Yes</i>	0.56 (-0.90; 2.04)	0.446
Infant age (centered)	0.10 (0.05; 0.15)	< 0.001
Infant age*Age CF introduction	-0.02 (-0.04; -0.005)	0.016
Maternal age (yrs)	0.06 (0.01; 0.11)	0.016
Region		
<i>Near Town</i>	--	--
<i>Remote</i>	-0.88 (-1.69; -0.06)	0.034

F = 8.47, *p* < 0.001, R² = 0.26

Figure 9. Predicted effect of interaction between infant age (centered at 16.7 months) and age of CF introduction (centered at 4.3 months) on subsequent CF intake. Predicted values generated from linear regression model in Table 7.

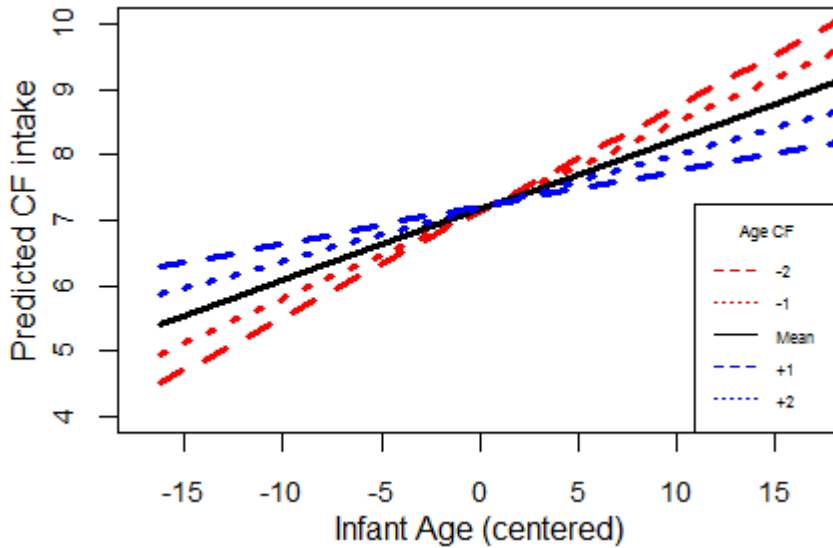


Table 8. Linear mixed-effect model on total liquids and solids consumed day prior by age at CF introduction. Dietary measures were reported in repeated 24-hour recalls with all subjects followed prospectively (n observations = 133, n subjects = 32). Model was constructed with a random slope and intercept for infant age and subject ID. Infant age and age at CF introduction were centered at sample means (8.5 months and 3.6 months, respectively)

Fixed effects	Est. (95% CI)	<i>p</i>
Age of CF introduction (centered)	-0.26 (-0.56; 0.05)	0.126
Introduce CF because of low milk		
<i>No</i>	--	--
<i>Yes</i>	0.30 (-1.01; 1.55)	0.666
Infant age (centered)	0.32 (0.14 – 0.50)	0.002
Infant age*Age CF introduction	-0.11 (-0.22; -0.01)	0.065
Maternal age (yrs)	0.05 (0.00; 0.10)	0.073
Region		
<i>Near Town</i>	--	--
<i>Remote</i>	-1.58 (-2.46; -0.66)	0.007
Random effects	Variance/SD	
Subject	7.21/ 2.69	
Infant age	0.11/0.33	
Residual	3.50/1.87	

Figure 10. Infant proximity to mothers and fathers during daylight hours across all in home observation intervals (n = 2227). Direct contact = in arms or within reach.

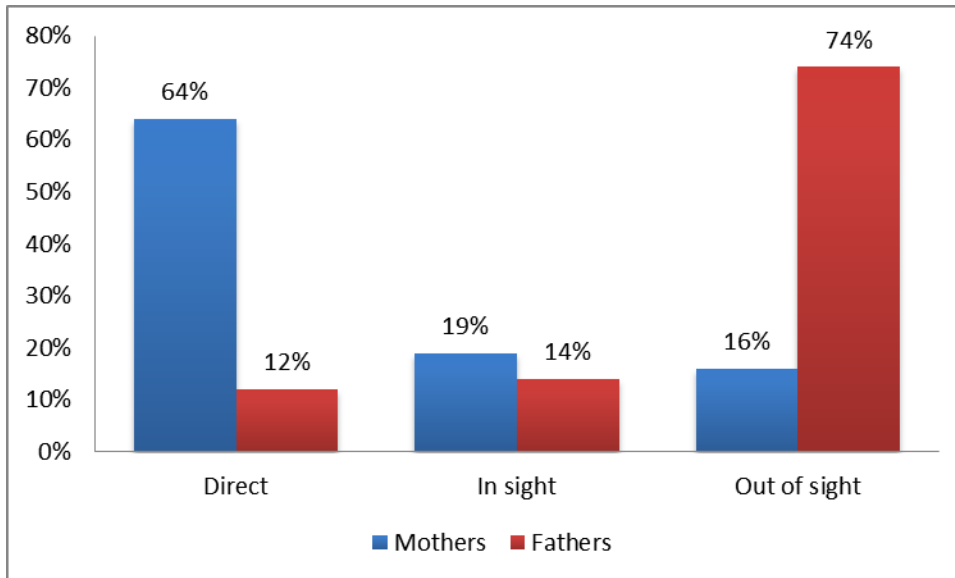


Figure 11. Infant activity states as proportion of time observed in specified activity across all daytime in home observations intervals (n = 2227).

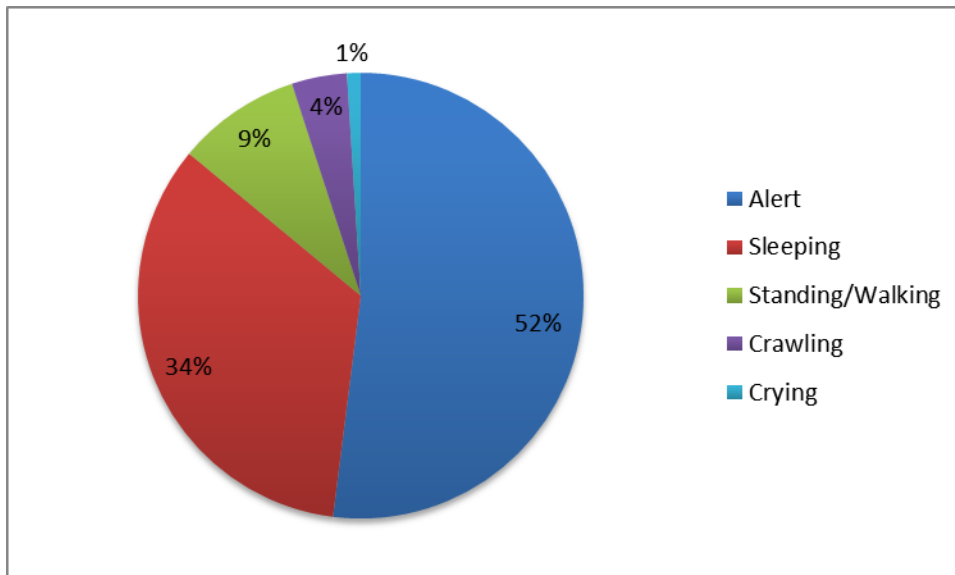


Table 9. Mixed-effects linear regression on CF frequency/hr. CF frequency/hr were calculated from 3-5 hour observation blocks of non-EBF infants aged 2-16 months old (n observations = 39; n subjects = 13). Models included a random slope for subject ID. Infant age and age at CF introduction were centered at sample means (9.1 and 3.3 months)

Fixed Effects	Est. (95% CI)	<i>p</i>
Age at CF introduction (centered)	-0.15 (-0.32; 0.03)	0.146
Age (centered)	0.13 (0.07; 0.18)	<0.001
% Time asleep	-0.01 (-0.02; -0.00)	0.024
Random effects	Variance/SD	
Infant ID	0.08/0.29	
Residual	0.19/0.44	

Table 10. Mixed-effects linear regression models on breastfeeding frequency/hr and total time breastfeeding/hr. Models included a random slope and intercept for infant age and infant ID*. Infant age and age at CF introduction were centered at sample means (9.1 and 3.3 months)

	Breastfeeding fq/hr		Time spent breastfeeding/hr	
	Est. (95% CI)	<i>p</i>	Est. (95% CI)	<i>p</i>
Age at CF (centered)	-0.14 (-0.52; 0.25)	0.511	-0.05 (-0.89; 0.80)	0.91
Infant age (centered)	0.24 (0.11; 0.37)	0.001	0.00 (-0.34; 0.34)	0.98
% Time asleep	-0.01 (-0.03; 0.01)	0.420	-0.01 (-0.07; 0.05)	0.83
Random effects	Variance/ SD		Variance/SD	
Infant ID	0.32/ 0.56		0.02 /0.14	
Residual	1.19/ 1.09		9.97/3.16	

Table 11. Paired t-tests of mean difference in standardized breastfeeding frequency/hr and time spent breastfeeding/hr between observation periods by age at CF (n infants = 9; n CF at 0-3 months = 5; n CF at 4-6 months = 4). * $p = 0.015$

Group	Mean breastfeeding fq/hr		Mean breastfeeding time/hr	
	Period 1	Period 2	Period 1	Period 2
Introduce CF 0 -3 months	1.2 ± 0.5	2.5 ± 1.3 ^{NS}	5.1 ± 2.6	5.8 ± 3.2 ^{NS}
Introduce CF 4 -6 months	2.2 ± 0.8	3.1 ± 1.9 ^{NS}	6.4 ± 3.1	5.8 ± 1.1 ^{NS}
All	1.6 ± 0.8	2.8 ± 1.5*	5.7 ± 2.7	5.8 ± 2.4 ^{NS}

Figure 12. Individual slopes of changes in BMI from 0 – 6 months postpartum among mothers EBF at start of observation (n = 18). Individual lines are color coded by final observed or reported duration of EBF (< 4 months or ≥ 4 months)

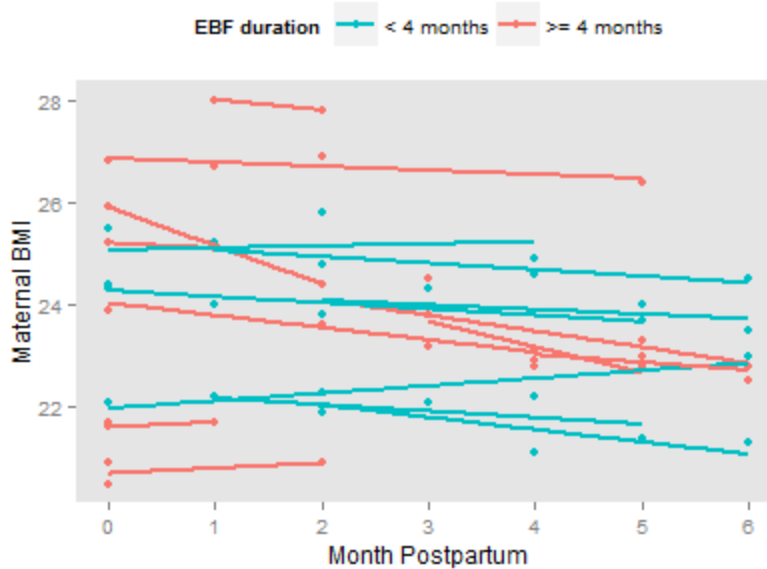


Figure 13. Linear fit of BMI from 0 – 6 months postpartum, across all mothers EBF at start of observations. Separate lines are fit by maximum EBF duration (< 4 or >= 4 months).

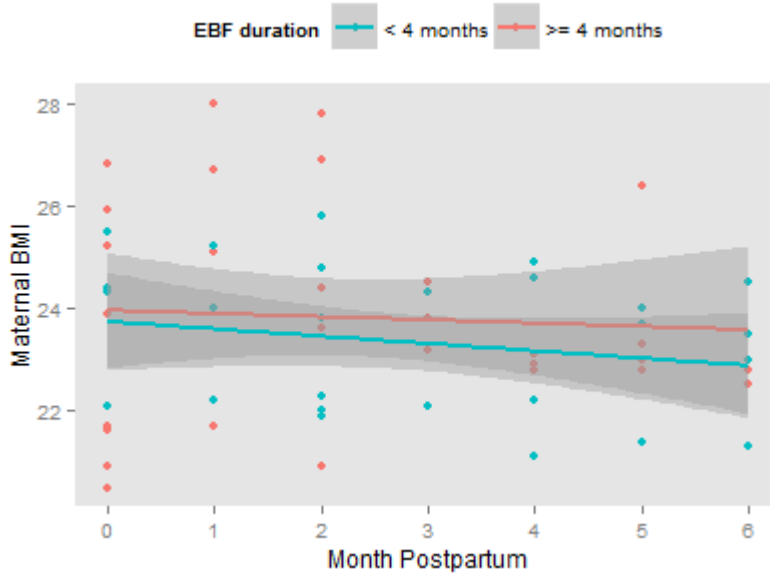


Figure 14. Individual slopes of changes in maternal BMI after CF introduction (n = 34), by month postpartum and age at CF introduction

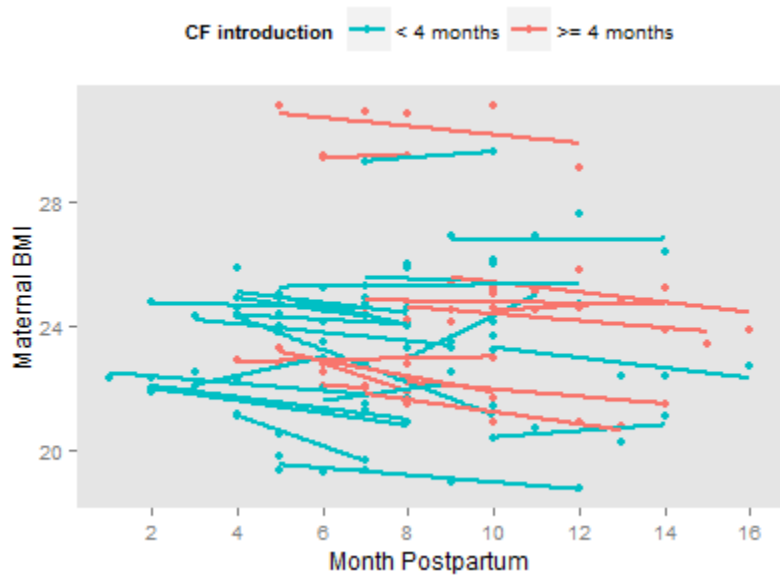


Table 12. Mixed-effects model of maternal BMI after CF introduction (n observations = 130, n subjects = 30). Model included a random intercept for subject; age of CF introduction was centered at mean (3.09 months) and parity grouped by category (primiparous, prime = 2-7 births, high = 8 – 12 births).

Factor	Est. (95% CI)	<i>p</i>
Age CF months (centered)	1.24 (0.70; 1.79)	< 0.001
CF because of low milk		
<i>No</i>	--	--
<i>Yes</i>	3.15 (1.42; 4.86)	0.004
Maternal parity		
<i>Prime</i>	--	--
<i>Primiparous</i>	-2.10 (-3.82; -0.39)	0.044
<i>High</i>	2.66 (1.09; 4.24)	0.007
Parity*age CF		
<i>Prime*age CF</i>	--	--
<i>Primiparous*age CF</i>	-1.00 (-1.82; -0.16)	0.049
<i>High parity*age CF</i>	0.22 (-0.84; 1.28)	0.723
Month postpartum	-0.11 (-0.16; -0.06)	< 0.001
Infant Sex		
<i>Female</i>	--	--
<i>Male</i>	-1.16 (-2.52; 0.20)	0.015
Random Effects	Variance/SD	
<i>Subject</i>	3.23/1.80	
<i>Residuals</i>	0.29/0.54	

Figure 15. Maternal BMI post-CF introduction, by parity and age of CF interaction. Slopes are calculated from linear mixed-effects model (Table 12), with month at CF introduction centered at population mean (3.09 months). Random effects held at zero, other model terms held at reference level or mean.

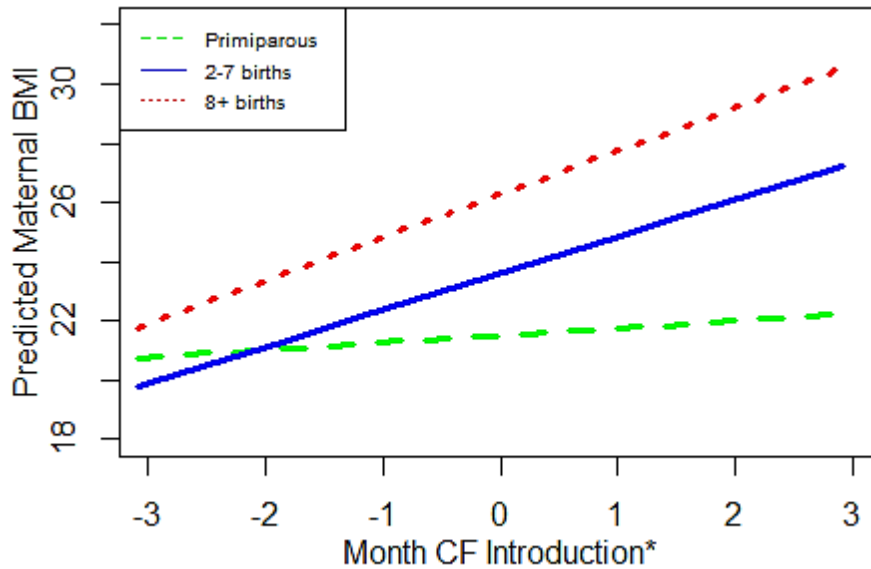


Figure 16. Individual spaghetti plots of urinary c-peptide concentrations from 0-6 months postpartum. Subjects were EBF at start of observation and are color-coded by age later observed or reported introducing CF.

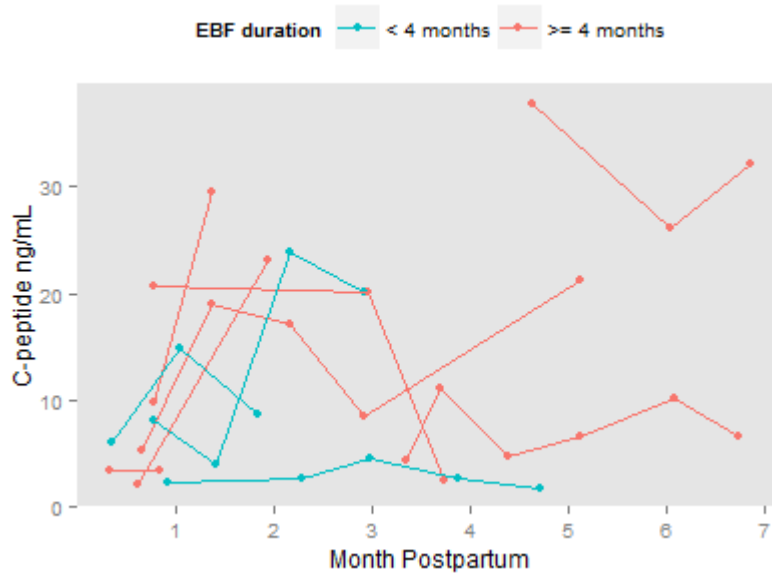


Figure 17. Linear fit of urinary C-peptide by month postpartum for all mothers EBF at start of observations. Separate lines are fit for maximum EBF duration (< 4 vs. \geq 4 months).

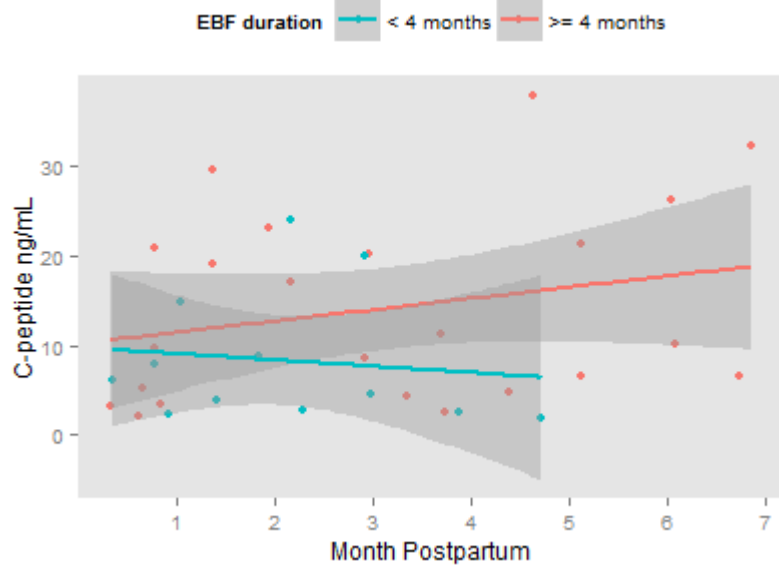


Figure 18. *The C-peptide monster.* Individual spaghetti plots of urinary C-peptide concentration by month postpartum following CF introduction.

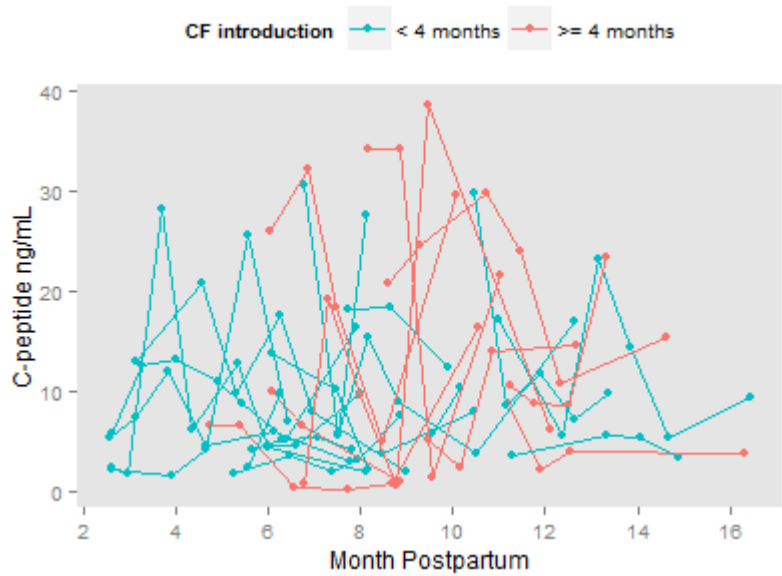


Figure 19. Linear fit of urinary C-peptide by month postpartum after CF introduction. Separate lines are fit for age at CF introduction (< 4 vs. \geq 4 months).

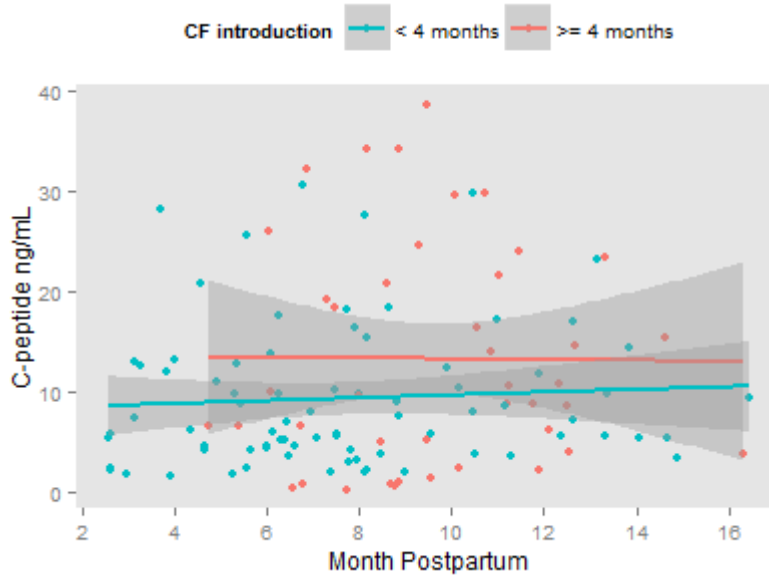


Figure 20. Mean \pm SE urinary C-peptide concentrations by month postpartum, following CF introduction.

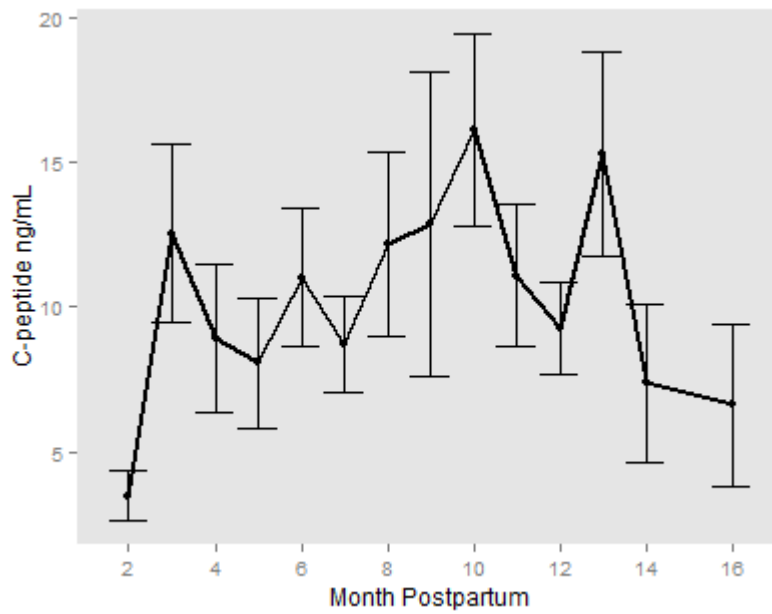


Figure 21. Mean \pm SE urinary C-peptide concentrations from 6 -12 months postpartum, by age at CF introduction (< 4 months vs. \geq 4 months).

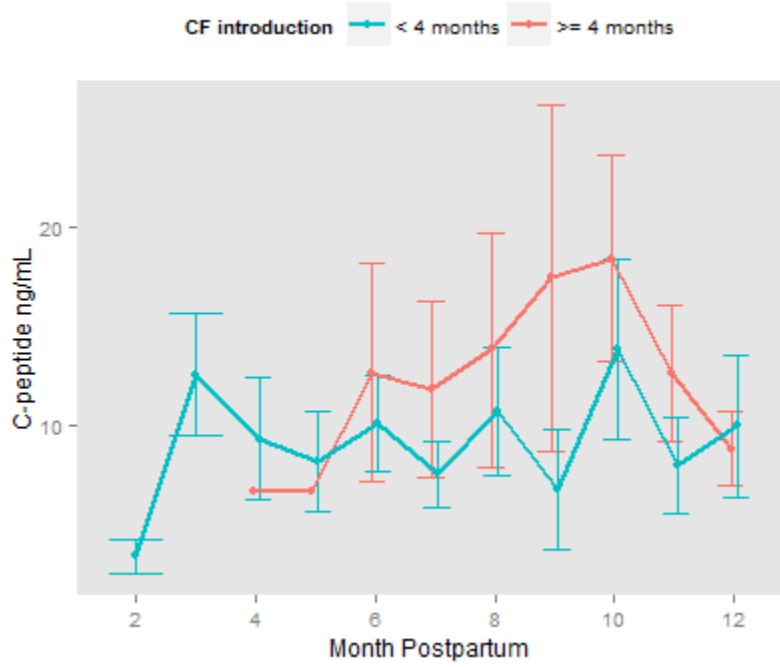


Table 13. Linear mixed-effects model of changes in log C-peptide concentrations after CF introduction (n observations = 106, n subjects = 26). Sample was restricted to subjects with 2 or more C-peptide measures up through 12 months postpartum. Age of CF introduction was centered at sample mean (2.92 months). Model included a random intercept for subject ID.

Factor	Est. (95% CI)	<i>p</i>
Age CF (centered)	0.08 (-0.80; 0.93)	0.326
CF because of low milk		
<i>No</i>	--	--
<i>Yes</i>	0.23 (-0.27; 0.70)	0.458
Maternal parity		
<i>Prime</i>		
<i>Primiparous</i>	-0.01 (-0.44; 0.98)	0.963
<i>High</i>	0.19	0.622
Month postpartum	0.02	0.653
BMI group*		
< 25	--	--
≥ 25	-0.20 (-0.08; 0.29)	0.285
Infant sex		
<i>Female</i>	--	--
<i>Male</i>	0.21	0.397
Random Effects	Variance/SD	
<i>Subject</i>	0.12/0.35	
<i>Residuals</i>	0.56/0.75	

*based on BMI at first observation

Chapter 5: Are shorter EBF durations costly to infants?

5.1 Introduction

Exclusive breastfeeding (EBF) for the first 6 months of life is recommended to reduce risks of infant infectious morbidity and to best support infant growth under optimal conditions (Dewey et al., 1991; Nielsen et al., 2011). Complementary feeding (CF) with any amount of liquids or solids before 6 months of age may introduce novel pathogens (Rowland et al., 1978; McDade and Worthman, 1998) and/or interfere with the initiation or continuation of breastfeeding by displacing infant breast milk intake (Cohen et al., 1994; Haisma et al., 2003; da Costa et al., 2010). In many epidemiological studies, CF before six months of age (from here on referred to as “early CF”) is therefore considered an “inappropriate” or “suboptimal” feeding practice relative to six months of EBF (Saha et al., 2008; Sinhababu et al., 2010; Senarath et al., 2012).

However, infant and young child (from here on “infant”) outcomes associated with early CF vary widely across populations. In lower-income populations, early CF has been consistently associated with increased infectious disease risks (e.g. Khadivzadeh and Parsai, 2004; Kalanda et al., 2006), although the relationship to nutritional status has been mixed (e.g. Gupta et al., 2007; Menon et al., 2013; Jones et al., 2014). Inconsistent effects of early CF on nutritional outcomes across populations may be attributed to differences in quantity or quality of complementary foods (Brown, 1997; Hop et al., 2000; Kalanda et al., 2006; Lander et al., 2010), subsequent duration of breastfeeding (Kramer et al., 2002), and reverse

causality, in which faster growing infants are introduced foods earlier (Marquis et al., 1997; Reilly et al., 2007; Frojo et al., 2014).

Any expectation of risk associated with early CF must therefore be contextualized in terms of CF patterns, motivations, and ultimate effects on breastfeeding practices. This is particularly true for many rural, subsistence-scale populations, in which traditional foods or liquids are commonly introduced before 6 months of age, yet without appearing to reduce subsequent breastfeeding intensity, breast milk intake, or total breastfeeding duration (Jenkins et al., 1984; Kusin et al., 1985; Orr-Ewing et al., 1986; Gray, 1996; Sellen, 2001; Meehan and Roulette, 2013). In these contexts, CF may be introduced ritually or with the express purpose of benefitting infants, with no perceived risks to infants. For example, the Aka and Turkana believe early CF within the first few months of life is necessary to relieve infant thirst and hunger or promote weight gain (Gray, 1998; Meehan and Roulette, 2013). In high-pathogen environments early supplementation may actually buffer infants against the energy losses incurred through constant infectious exposure (Waterlow, 1981). EBF to six months without iron supplementation may also increase risk of infant anemia if maternal stores are suboptimal (Kramer and Kakuma, 2012). More recently it has been argued that introducing common allergenic foods between 4-6 months may promote immunotolerance (Prescott et al., 2008; Wennergren, 2009; Koplin et al., 2010).

To date, however, there has been limited systematic research on growth and morbidity outcomes in association with gradual CF introduction. The Tsimane are an indigenous Amerindian population residing in the Bolivian lowlands. All infants are breastfed frequently and on demand, with mean age at weaning around 27 months of age (Chapter 4). The average

age of CF introduction is around 4 months of age, but the rate of CF does not appear to surpass the rate of breastfeeding until about 13 months of age (Veile et al., 2014). Chapter 4 of this dissertation further demonstrated that early CF was associated with only modestly increased CF at later ages, no change in breastfeeding duration or frequency up through the first year of life, no increased hazard of earlier weaning, and no reduction in maternal energetic costs. Tsimane mothers most often reported infant needs as primary reasons for introducing CF, and CF within the first few months of life was frequently associated with perceived low milk supply. These combined results provided support for a Feeding Augmentation Model of early CF, which posed that early CF may supplement but not supplant breastfeeding—for example if CF quantity is small and/or infant energy demands surpass energy supplied by maximum lactational supply. Extended to infant outcomes, this model would further predict a neutral or positive association between early CF and subsequent nutritional status, and a neutral or negative association between early CF and morbidity risk.

However, the Tsimane live in a high-pathogen environment and typically do not apply rigorous standards of hygiene when preparing and serving complementary foods—e.g. hands are not washed before preparation or serving, water is locally sourced and rarely boiled, and food is not refrigerated. Therefore any amount of early CF—even if it does not displace breast milk intake— may increase pathogen exposure, as has been previously shown for indigenous populations with intensive breastfeeding practices (Mata et al., 1976; Rowland et al., 1978). Infection and/or associated energetic costs of infection and immune responses, particularly at young ages, may result in weight loss and/or cumulative growth deficits (Lutter

et al., 1992; Villalpando and Lopez-Alarcon, 2000; Blackwell et al., 2010). For the Tsimane, any increase in infections owing to early CF may compound the effects of generalized pathogen exposure, weaning, and limited health care access contributing to early life morbidity, mortality, and growth stunting (Gurven et al., 2007; Blackwell et al., 2011; Gurven, 2012).

As proposed by the Feeding Supplementation Model of early CF, any such risks posed to infants may still be outweighed by the benefits to mothers, presuming that energy otherwise invested in EBF could be diverted to other existing or future offspring. That is, early CF may be a strategy demanded by quantity investment in offspring, and as such, may vary with different stages of maternal reproduction. For example, mothers with many existing young offspring may have less time available for EBF, while mothers at earlier stages of their reproductive careers may benefit if reduced EBF durations lead to reduced durations of lactational amenorrhea. While Chapter 4 showed that analysis of breastfeeding behaviors and maternal energy costs following CF introduction ultimately supported the Feeding Augmentation Model, analysis of infant outcomes associated with early CF may provide more support for the Feeding Supplementation Model. In order to further investigate this, two potential indicators of quantity over quality investment—relatively short previous interbirth interval and high birth order—will also be explored in relation to nutritional status and morbidity risks, independently and in conjunction with early CF.

Short interbirth intervals, primiparity, and high parity may indirectly increase risks of infant mortality through multiple, often disputed, mechanisms, including lower gestational length and birthweight, low maternal nutritional reserves, increased competition for resources

among siblings, and/or increased pathogen exposure from other siblings (Haaga, 1989; DaVanzo et al., 2008). The World Health Organization (WHO) currently recommends a minimum birth to pregnancy (BTP) interval of 24 months to minimize adverse maternal, perinatal, neonatal, and infant health outcomes (World Health Organization, 2005), which equates to a minimum recommended interbirth interval (IBI) of 33 months. High parity is defined by the WHO as 5 or more pregnancies with gestation ages of ≥ 20 weeks, though “grand multiparity” has alternately been defined as 7+, or 8+ births and “extreme multiparity” as 10+ births (Aliyu et al., 2005).

By any of these metrics, reproduction among Tsimane women is accelerated: the average age at first birth is 18.3 ± 2.6 years; average total fertility rate is 9.1 births, and the mean IBI for Tsimane mothers following previous child surviving to one year is 29.6 ± 10.0 months (McAllister et al. 2012, *in prep*). Higher infant mortality among multiparous Tsimane mothers has been associated with higher parity, lower maternal BMI, and shorter IBIs in combination with limited medical care access (Kaplan et al., 2015). In Chapter 4 of this dissertation, relative hazards of time of CF introduction and resumption of postpartum menstruation were both increased by primiparity and higher parity. However, maternal BMI following CF introduction was negatively associated with primiparity and positively associated with high parity (8-12 births). Thus, for the Tsimane, IBIs of < 33 months and primiparity are expected to have negative effects on infant nutritional status and morbidity risks, though expected associations with higher birth order are unclear owing to the positive relationship between high parity and maternal BMI.

This chapter uses the same mixed-longitudinal sample used in Chapter 4 to test if and how early CF, interbirth interval, and birth order are associated with Tsimane infant nutritional status and reported incidences of infectious illness. The following factors known to impact infant nutritional and morbidity status are also considered in models: maternal height (Ozaltin et al., 2010; Frojo et al., 2014); village proximity to market (associated with improved dietary diversity, medical care access, and lower morbidity and mortality Chapter 3, Gurven 2007, 2012); birth during the rainy season (associated with increased morbidity risk, Gurven 2012); weaning status (associated with nutritional status, Chapter 3), and infant sex. As an additional measure of robustness, infant weight-for-age and height-for-age are separately evaluated using two growth standards: the international WHO standards (WHO, 2006) and within population-standards derived from a larger Tsimane sample (Blackwell et al., *in progress*).

5.2 Methods

Data collection

This chapter analyzes anthropometric data and illness recalls from the mixed-longitudinal sample previously described in Chapters 2 and 4. Anthropometric measures were collected during initial interviews with 156 subjects, and in follow-up interviews with a subsample of 41 infants who were less than one year of age at the time of initial interview. Infants followed prospectively were visited approximately every 3 weeks after initial interview. Following two rounds of data collection in October and November, prospective

anthropometric measures were collected on every other visit. As described in Chapter 2, the number of visits per subject varied by subject birth date and owing to logistical difficulties and participant absence during village visits (see Appendix for visual map of prospective sampling). Subjects followed prospectively contributed 2-6 measures total (mean \pm SD = 3.2 \pm 1.4 measures per subject). A total of 287 anthropometric measures are included in the mixed-longitudinal sample (156 measures from initial interviews, and 131 follow-up measures).

Mothers participating in the prospective study were asked to recall infant illnesses over the last two weeks at every visit. A total of 141 illness recall interviews were collected from the participating 41 mother-infant pairs (per child range 1 -7, average 4.6 \pm 2.0). In interviews, a mother was asked if her infant was currently ill, what symptoms he/she was exhibiting, and how long symptoms had persisted. She was also asked if over the last two weeks her infant had experienced any bouts of cough, diarrhea, or flu, and for how long (including additional bouts if these symptoms had been reported as a current illness). She was then asked to describe any other illness symptoms and their duration, as well as any medications given (see Appendix for full interview).

Statistical analysis

There was not sufficient data to analyze individual outcomes for infants before and after introducing CF (see Subject Sampling Timeline, Appendix). Variation in nutritional status and illness bouts were analyzed with respect to EBF vs. CF status at time of interview

for infants 0- 5 months of age, and with respect to reported or observed age of CF introduction (0-3 vs. 4-6 months) for infants 6- 36 months of age.

For nutritional outcomes, subject length-for-age z scores (LAZ) and weight-for-age z scores (WAZ) were calculated using WHO ANTHRO (ver. 3.2.2). Weight-for-length was not evaluated owing to the low prevalence of wasting ($WLZ < -2$ SD) in this population (Chapter 3). Generalized estimating equations were fit for LAZ and WAZ in each age group using the *geepack* package (Højsgaard et al., 2006) in R (ver. 3.2.0). Statistical models excluded measurements from two subjects with unknown previous IBIs and six measurements from one subject at 5-12 months whose low HAZ scores appeared to have undue model influence (-3.9 to -4.5). For each outcome in each age group, a baseline model was constructed consisting of the relevant CF predictor, infant age (centered at the mean for that age group), maternal height, and a nominal category for the previous IBI: first birth, < 33 months (reference category), and ≥ 33 months (IBI was used rather than BTP interval owing to the lack of adequate gestational age estimates among subjects). The following predictors were then considered additively and evaluated using QIC values generated with the *MuMIn* package (Barton, 2015): a CF and age interaction, a CF and IBI group interaction, infant birth season (rainy or dry), weaning status (for infants 6-35 months of age), village region (remote or close to market), and infant sex. Confidence intervals (CI) were calculated from GEE robust standard errors; reported p values are drawn from the Wald statistic.

Separate models were run to test for associations between nutritional status and IBI vs. birth order, owing to limited sample sizes and overlapping first birth categories. Birth order and IBI were only weakly correlated among non-firstborn offspring ($r = 0.16$, $p = 0.07$),

however children with birth orders of 8+ were more likely to have IBIs of at least 33 months than children of birth order 2-7 (33% vs 15%, chi-squared = 4.2, $p = 0.04$). A nominal category was used to test for non-linear effects of first and high order birth: first born, 2 – 7 (reference category), and birth order 8 or higher. This high birth order category represents the upper tertile for non-first born offspring and is comparable to the high parity category used in Chapter 4.

Finally, Tsimane and other indigenous Amazonian populations are considerably shorter and lighter as compared to WHO reference standards derived from more affluent populations (Blackwell et al., *in prep*, Urlacher et al., 2015). While nutritional scores calculated from these standards are important for comparative purposes, the exaggerated prevalence of growth stunting may not accurately capture within-population variance likely to influence Tsimane mothers' perceptions of infant growth and related feeding decisions. For this reason, within-population z-scores were also calculated using Tsimane reference LMS (Lambda-Mu-Sigma) curves (Blackwell et al., *in prep*). These reference standards are based on 25,160 mixed-longitudinal observations (n subjects = 8,539) collected by the Tsimane Health and History Project. Methods used for creating the Tsimane within-population reference standards were identical to those previously described for the Shuar (Urlacher et al., 2015). For both age groups, each best fit model for HAZ and WAZ was run using the z scores calculated from Tsimane standards.

To assess morbidity outcomes from 2 week recalls in infants followed prospectively, each different infectious symptom reported (cough, flu, diarrhea, other) was binary coded as

“yes” or “no” and all positive scores were summed. Current and previous bouts of the same symptom were both counted if they were reported as separate incidences during the two week span. This resulted in a total possible range of 0-8 separate illness bouts for the two week period. Symptoms were further classified as indicative of gastrointestinal (GI) or respiratory infection. Due to the ubiquity of respiratory symptoms reported (discussed below), only GI symptoms were analyzed further.

The number of illness bouts and likelihood of GI symptoms exhibited in the past 2 weeks were fit with generalized linear mixed models (*lme4* package), using Poisson and binomial distributions, respectively, the Gauss-Hermite Quadrature (GHQ) method, and random intercepts for subjects. Due to small sample sizes, the baseline model for each outcome for each age group consisted of only the relevant CF term and infant age, with the following variables then considered additively and evaluated using AICc: WAZ and HAZ at time of interview, IBI category, birth order category, birth season, village region, infant sex, and interactions between CF and age, CF and IBI, and CF and birth order. No infants were weaned during the prospective study.

5.3 Results

Descriptive statistics of infant subjects are given in Table 1. Figures 1-4 depict individual changes in length and weight from infants measured at least twice from 0 – 5 months (n = 14) and 6-16 months (n = 28). Plots differentiate among infants introduced CF at 0-3 vs. 4 – 6 months of age.

Figure 5 contrasts the distribution of subject HAZ and WAZ scores by WHO and Tsimane within-population standards. Though Tsimane and WHO generated HAZ and WAZ scores were highly correlated across individuals (HAZ $r = 0.84$, $p < 0.001$, WAZ $r = 0.91$, $p < 0.001$), mean z scores are shifted right and variance is substantially reduced when calculated from Tsimane standards (Figure 5). Using the Tsimane standards, mean \pm SD HAZ and WAZ scores across all subjects were 0.17 ± 0.61 and 0.02 ± 0.67 , respectively. In contrast, mean HAZ and WAZ scores using WHO standards were -1.13 ± 1.43 and -0.71 ± 0.99 , respectively. Mean HAZ and WAZ scores are not appreciably lower in older age groups using Tsimane as compared to WHO reference standards (Table 2). Using Tsimane standards, no subjects fell below -2 SD for HAZ or WAZ, whereas the total prevalence of HAZ and WAZ scores ≤ -2 SD using WHO standards was 35.0% and 12.7%, respectively.

Association of feeding status with nutritional status at 0-5 months

Adjusting for other covariates, the interaction between infant age and CF status was negatively associated with infant HAZ ($\beta = -0.28$, $p < 0.01$; Table 3). Between approximately 0-2 months of age, EBF infants are predicted to have lower mean HAZ than CF infants, whereas from approximately 3-5 months of age, predicted mean HAZ is higher for EBF infants (Figure 6). Holding other variables constant (at mean or reference category), the model predicts that at approximately one month of age EBF infants would be at the 33rd percentile for height and CF infants at the 47th percentile for height. Conversely, the model predicts that at approximately five months of age, EBF infants would be at the 71st percentile

for height, and CF infants at the 41st percentile. An interaction between CF and IBI group was not significant; region, sex, and birth season were not associated with HAZ and did not improve model fit. The age and CF interaction in relation to infant HAZ was robust to substitution of parity group for IBI category (Table 4), and using within-population Tsimane generated z-scores (Table 5).

There was no association between infant WAZ and CF status for this age group, though mean WAZ was higher among infants with IBIs of 33 months or greater as compared to less than 33 months (Table 3). No additional variables tested were associated with WAZ and interactions between CF and age and CF and IBI group were not significant. When z-scores were calculated using Tsimane reference standards, mean WAZ was lower for CF as compared to EBF infants (Table 5). Birth order group was not significantly associated with WAZ when substituted for IBI category, and resulted in lower AICc in models run on HAZ and WAZ (Table 3, Table 4).

Association of early CF with nutritional status at 6-35 months

Adjusting for other covariates, relatively earlier reported age of CF introduction (0-3 vs. 4-6 months) was associated with greater mean HAZ at 6-35 months (Table 6). Holding other variables at mean levels, the model predicts that infants nine months of age and introduced CF at 0-3 vs. 4-6 months of age would fall, respectively, in the 29th and 14th percentiles for height. Although no significant interaction was observed, the relationship between age of CF introduction and HAZ may become more negligible with age. For

example at 24 months of age, the model predicts that children introduced CF at 0-3 months vs. 4-6 months would fall into the 7th and 2nd percentiles for height, respectively. First born children and children with IBIs ≥ 33 months had higher mean HAZ than those with IBIs < 33 months. In this age group, mean HAZ was also reduced with age, lower for infants born in the rainy as compared to the dry season, and positively associated with maternal height. Weaning status was not significantly associated with HAZ but improved model fit as determined by QIC (Table 6). There was no significant interaction between CF and IBI group, nor were region or infant sex associated with WAZ.

The relationships observed between HAZ and early CF, subject age, maternal height, and birth season were robust to substitution of IBI category with parity group, though this substitution resulted in a poorer model fit (Table 7). When z-scores were computed with Tsimane standards, associations between HAZ and CF status, IBI category, and birth season remained consistent; however, HAZ was no longer associated with subject age, while a negative association with weaning was significant (Table 8).

Early CF was not associated with infant WAZ using WHO reference standards (Table 6), nor was an interaction between age and CF significant. However, for infants introduced CF at 0-3 months of age, mean WAZ was significantly greater if their previous IBI was ≥ 33 months. Adjusting for other covariates, mean WAZ from 6-35 months of age was also reduced with subject age and positively associated with maternal height (Table 6). Using Tsimane reference standards, age was no longer associated with WAZ, though the association with maternal height and the interaction between CF and IBI group remained significant. Birth order group was not associated with WAZ independently when substituted for IBI

group, though for infants introduced CF at 0-3 months of age, there was a trend towards lower WAZ among first born infant as compared to birth order 2-7 (Table 7).

Association of feeding status or age of CF introduction with reported illness

In follow-up interviews of infants in the prospective study, at least one infectious symptom was reported for the previous two weeks in 95% of interviews (133/140). Reported symptoms indicative of infectious illness ranged from acute cough and diarrhea, to fever, flu, or general malaise, to skin and eye infections. The sample mean of total infectious symptoms per 2-week recall period per child was 2.1 ± 0.8 . Bouts of GI illness (e.g., diarrhea, vomiting) were reported in 55% of interviews (77/140), with 94% (134/140) of infants reported to have at least one bout of GI illness over the course of the study. Bouts of respiratory illness (e.g. cough, flu), were reported in 84% of interviews, with all infants reported to have at least one bout over the course of the study, and 94% (134/140) reported to have at least two bouts. Differences in reported respiratory illness bouts were not analyzed further due to their ubiquity.

No differences in total number of illness bouts or the likelihood of GI symptoms in the previous two weeks were observed with respect to CF vs. EBF status for infants 0-5 months of age (Table 9). Total number of illness bouts did not vary by infant age or WAZ, nor was any of the variance in reported illness bouts explained by individual random effects. No additional variables or interactions tested were significantly associated with illness bouts and did not improve model fit. Odds of reported GI symptoms were lower by 74% with each 1

SD increase in WAZ (Table 9), but were not associated with age nor any other variables tested.

For infants 6-16 months of age, there was a trend towards increased reported illness bouts among infants introduced CF at 0-3 months of age (Table 10). No variance in illness bouts was explained by individual random effects, age, or WAZ. No other variables or interactions tested were significantly associated with WAZ or improved model fit. There was a trend towards increasing odds of GI symptoms with age for infants introduced CF at 0-3 vs. 4-6 months (Table 10). No other variables or interactions tested were associated with GI symptoms.

5.4 Discussion

Nutrition and morbidity were evaluated in association with early CF—defined as CF vs. EBF status in infants < 6 months old, and introduction of CF at 0-3 months vs. 4-6 months in non-EBF children 6-36 months old. Nutritional status was evaluated using both WHO and Tsimane within-population standards. I first discuss all results from models that employed WHO growth standards. Following this, I compare results generated from models of nutritional outcomes that separately applied WHO and Tsimane standards.

In this study, associations between early CF and nutritional and morbidity outcomes were mixed across age groups. Neither WAZ nor the likelihood of reported GI symptoms were associated with early CF in any age group in this study. Age-associated increases in

HAZ from 0-5 months, however, were modified by early CF (Fig. 1). In the first few months of life, early CF was associated with greater HAZ relative to EBF, though between 3-5 months of age, this relationship was reversed. Owing to the mixed-longitudinal sample, it is unclear if this pattern reflects cumulative negative effects of CF (resulting from offsets in breast milk intake and/or increased pathogen exposure), or existing differences in nutritional status at or close to age of CF introduction. Given results from Chapter 4, which suggest early CF does not reduce breastfeeding intensity, and the lack of association between early CF and reported morbidity presented here (Table 9 and Table 10), there is little evidence to support the former interpretation. Similarly, researchers in Senegal previously observed lower nutritional status but no differences in subsequent growth when comparing CF versus predominantly breastfed infants at 2-3 months (Simondon and Simondon, 1997). They argued that lower nutritional status by 2-3 months was unlikely to have resulted from negative effects of CF up to that point, and therefore associations between early CF and lower HAZ at later ages reflected reverse causality. In contrast, to the extent that the results may have been influenced by cross-sectional sampling, it is plausible that the interaction predicted in Figure 6 reflects shifting motivations for introducing CF at different ages. For example, mothers introducing complementary foods at 0-2 months of age may be responding to perceived accelerated growth, while mothers introducing CF at 3-5 months may respond to perceived poor growth.

In the 6-36 month age group, mean HAZ was actually higher among children reportedly introduced CF relatively earlier. The predicted differences in this group, however, did not span more than one major percentile for height, and continued to diminish with age. Recall

error in reporting age of CF introduction for older infants may also have biased results. Thus, the positive association between early CF and HAZ after 6 months may have limited clinical relevance or, at the very least, suggests that any negative effects of early CF on length are not sustained at later ages.

The results suggest a more consistently positive effect of longer IBIs on nutritional status. Evidence linking IBI to poorer child nutritional outcomes has been mixed, though this may reflect inconsistent methodologies across studies (World Health Organization, 2005; Dewey and Cohen, 2007). Here, in support of the WHO recommendations (2005), IBIs of 33 months or greater were positively associated with WAZ in Tsimane infants 0-5 months of age and with HAZ in children 6-35 months of age. As the majority of children surveyed had IBIs < 33 months (Table 1), relatively shorter IBIs may be a factor influencing high rates of childhood stunting previously observed for this population (Foster et al., 2005; Blackwell et al., 2011), in addition to other genetic and environmental characteristics of Amazonian populations that may favor smaller body sizes (Urlacher et al., 2015).

In alternate models, substitution of IBI group with birth order group consistently produced poorer model fits but did not affect other model relationships (Tables 3-4, 6-7). First born effects on HAZ and WAZ were generally duplicated but weaker. High birth order was positively associated with WAZ in infants 6-35 months of age, which may reflect a protective effect of maternal BMI—shown to increase with parity in this and previous Tsimane studies (Chapter 4, Kaplan et al., 2014, Gurven et al., *in prep*).

Other factors associated with either HAZ or WAZ at different age groups included infant age, maternal height, and birth season. As is frequently observed for lower income

populations (Shrimptom et al., 2010; Onyango et al., 2014) mean Tsimane HAZ and WAZ decline with age when compared to international standards. Maternal height was positively associated with HAZ and WAZ only in the 6-36 month age group, and may suggest that individual genetic or household factors begin to exert stronger influences on achieved growth with age (Frojo et al., 2014; Urlacher et al., 2015). The negative association between rainy season births and HAZ in this study may support previous findings that infants born during this season have poorer outcomes (Gurven, 2012).

Using Tsimane growth standards, all subjects in this study fell within 2 SD of mean height and weight for age, including at later ages when the prevalence of stunting using international standards substantially increases (Chapter 3, Table 11). In general, models of variation in nutritional outcomes were robust to the different reference standards used, despite differences in sampling distributions that would affect estimates of standard errors. Moving from WHO to Tsimane standards altered the *p*-values for some independent associations but did not substantially alter parameter estimates (e.g. early CF on WAZ from 0-5 months, Tables 3 and 6; age on HAZ and WAZ from 6-35 months, Tables 6 and 8). Key findings replicated across reference populations included the interaction between age and CF status (0-5 months), a positive association between early CF and HAZ (6-35 months), and a positive association between IBI \geq 33 months and WAZ (0-5 months) and HAZ (6-35 months). These relationships therefore do not appear to be driven solely by subjects at the extreme low ends of the WHO distribution (who would not be considered “small for age” by within-population standards).

Relative to EBF, CF was not associated with number of illness bouts or GI symptoms for infants 0-5 months old (Table 9). Before 6 months of age, risks associated with introducing CF may be negligible for Tsimane infants because CF during this period is relatively minimal and/or buffered by continued intensive breastfeeding and pre-mastication. These practices may reduce infection risks associated with CF through antimicrobial and other immunomodulatory properties of breast milk and saliva (Pelto et al., 2010, Han et al., *in revision*). After six months of age, there was weak evidence that infants introduced CF earlier experienced higher reported morbidity (Table 10). Morbidity risks associated with early CF may only be apparent at later ages once CF has substantially supplanted breastfeeding intensity and pre-mastication is no longer practiced. Alternately, as discussed above, age-associated increases in risk may be evidence of recall biasing in cross-sectional sampling, rather than cumulative effects of early CF. At later ages, mothers may be more likely to remember introducing CF at 0-3 months of age if at the time they had perceived low milk supply, small birth weight, or poor growth in infants—and those infants may be more generally disease prone at later ages. Neither IBI nor birth order group were retained in models of illness bouts or GI symptoms for either age group.

Chapter 3 introduced two models to explain patterns of early CF among the Tsimane. The Feeding Augmentation Model posited that early CF benefits infants without offsetting breastfeeding intensity. The Feeding Supplementation Model posited instead that early CF is introduced precisely to reduce the energetic costs of EBF, which would benefit future maternal reproductive investment at some cost to the current infant. Analyses of changes in breastfeeding frequency and duration, maternal BMI, and urinary C-peptide concentrations

after CF introduction largely suggested that breastfeeding intensity was not compromised by early CF and therefore the Feeding Augmentation Model was generally supported. In the current study, it was not possible to directly assess changes in infant growth and disease exposure before and after introduction of CF—though evidence of poorer nutritional or morbidity outcomes associated with early CF would be taken as support for the Feeding Supplementation Model.

Results from this study do not clearly support one model over the other, though interpretations are limited by the mixed-longitudinal design of the study. Associations between early CF and poorer nutritional and morbidity outcomes may imply direct and lasting costs of early CF, or a combination of reverse causality and recall bias in reporting or observing age at CF introduction at later stages of infancy. Despite this ambiguity, it is noted that differences in HAZ among infants introduced CF relatively earlier or later were minimal regardless of the direction, and ultimately diminished with age, while early CF was not associated with any immediate increases in illness bouts before six months of age. For populations such as the Tsimane, in which CF introduction does not lead to rapid reductions in breastfeeding intensity or early weaning, risks of early CF may therefore be minimal. Further confirmation that early CF does not pose significant risks to Tsimane infant nutritional and infectious morbidity would require a longitudinal design and examination of more discrete biomarkers of breast milk intake, complementary food intake, infectious exposure, and immune responses. Meanwhile, to offset any risk of increased pathogen exposure from early or later CF, enhanced education and support is recommended to promote safe, nutritious complementary foods to breastfeeding Tsimane children of all ages.

5.5 Conclusions

This study examined associations between early CF, nutritional status, and symptoms of infectious disease among Tsimane children aged 0 -35 months. Early CF was not associated with WAZ at any age. A significant association between early CF and low HAZ from 0-5 months was reversed from 6-35 months. The magnitude of differences in HAZ associated with early CF was minimal in both groups, however, and continued to decrease with age. Early CF was associated with increased reported rates of infectious illness after six months of age, but contrary to expectations, not in earlier months surrounding CF introduction. Minimum recommended IBIs of 33 months were positively associated with nutritional status in both age groups. Associations between early CF, IBI, and nutritional status were robust to use of WHO and within-population Tsimane reference standards. Results from this study, coupled with earlier research suggesting that early CF does not reduce breastfeeding intensity or total duration, tentatively suggest that for the Tsimane nutritional and morbidity risks associated with early CF are minimal and do not result in lasting nutritional deficits.

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Chapter 5: Tables and Figures

Table 1. Descriptive characteristics of infant subjects and households at initial observation (subsample of infants followed prospectively also given)

	All Subjects	Prospective Subjects	Initial only
	n = 156	n = 41	n = 115
	Mean ± SD (Range)	Mean ± SD (Range)	Mean ± SD
	n (%)	n (%)	(Range) n (%)
Sex			
Male	89 (57.1%)	27 (65.8%)	53 (46.1%)
Female	67 (42.9%)	14 (34.1%)	62 (53.9%)
Birth Season			
Dry	76 (48.7%)	20 (48.8%)	56 (48.7%)
Rainy	80 (51.3%)	21(51.2%)	59 (51.3%)
Birth order	4.5 ± 2.9 (1 – 13)	5.0 ± 3.1 (1 – 12)	4.3 ± 2.9 (1 – 13)
1	27 (17.3%)	6 (14.6%)	21 (18.3%)
2-3	39 (25.0%)	7 (17.1%)	32 (27.8%)
4-7	64 (41.0%)	20 (48.8%)	44 (38.3%)
≥ 8	26 (16.7%)	8 (19.5%)	18 (15.6%)
IBI (months)	33.1 ± 21.4 (10.7 – 164.6)	37.6 ± 25.2 (14.3 – 160.6)	31.3 ± 19.7 (10.7 – 164.6)
First born	27 (17.5%)	6 (14.6%)	21(18.6%)
< 33	87 (56.5%)	22 (53.7%)	65(57.5%)
≥ 33	40 (25.6%)	13 (31.7%)	27 (23.9%)
CF	3.7 ± 2.0 (0 – 7)	3.2 ± 1.7 (0 – 6)	3.9 ± 1.7 (0 – 7)
introduction*			
EBF 0 – 3 mos*	61(41.2%)	22 (56.4%)	39 (35.8%)
EBF ≥ 4 mos*	87(58.7%)	17 (43.6%)	70 (64.2%)
Mat. age (yrs)	27.3 ± 8.5 (14.1 – 49.7)	27.9 ± 9.0 (14.1 – 45.3)	27.0 ± 8.2 (14.5 – 49.7)
Household size	7.7 ± 3.8 (3 – 20)	7.7 ± 3.7 (3 – 17)	7.7 ± 3.8 (3 – 20)
Village Region			
Near town	87(55.8%)	10(24.4%)	77 (67.0%)
Remote	69(44.2%)	31(75.6%)	38 (33.0%)

Table 2. Mean ± SD HAZ and WAZ by age group, separately calculated using Tsimane within-population and WHO reference standards

Age (months)	n	HAZ		WAZ	
		Tsimane	WHO	Tsimane	WHO
0 - 5	38	0.17 ± 0.41	-0.09 ± 1.12	-0.04 ± 0.56	-0.27 ± 0.85
6 - 11	56	0.16 ± 0.51	-0.79 ± 1.00	0.24 ± 0.65	-0.44 ± 1.05
12 - 23	25	0.23 ± 0.71	-1.62 ± 1.39	-0.02 ± 0.68	-0.98 ± 0.96
24 -35	27	0.04 ± 0.75	-2.14 ± 1.36	0.02 ± 0.87	-1.10 ± 0.97

Figure 1. Spaghetti plots of individual changes in length for infants measured at least twice from 0-5 months of age (n = 14). Plots are colored according to whether observed or reported EBF duration was before or after 4 months of age.

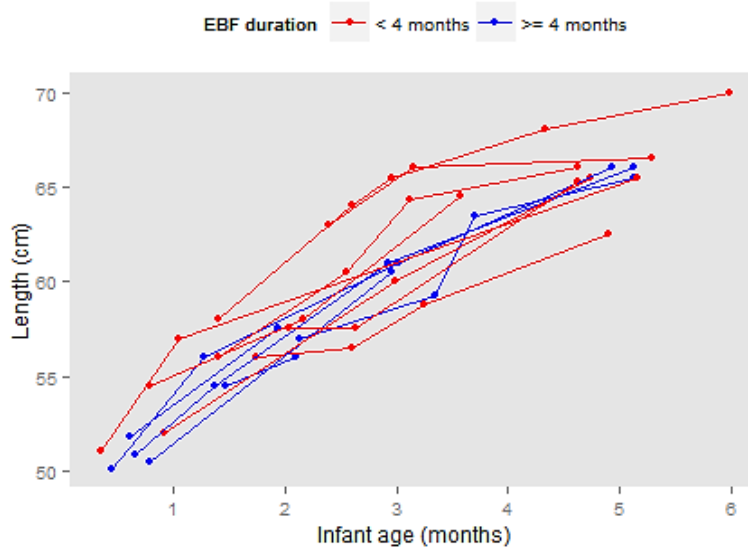


Figure 2. Spaghetti plots of individual changes in weight for infants measured at least twice from 0-5 months of age (n = 14). Plots are colored according to whether observed or reported EBF duration was before or after 4 months of age.

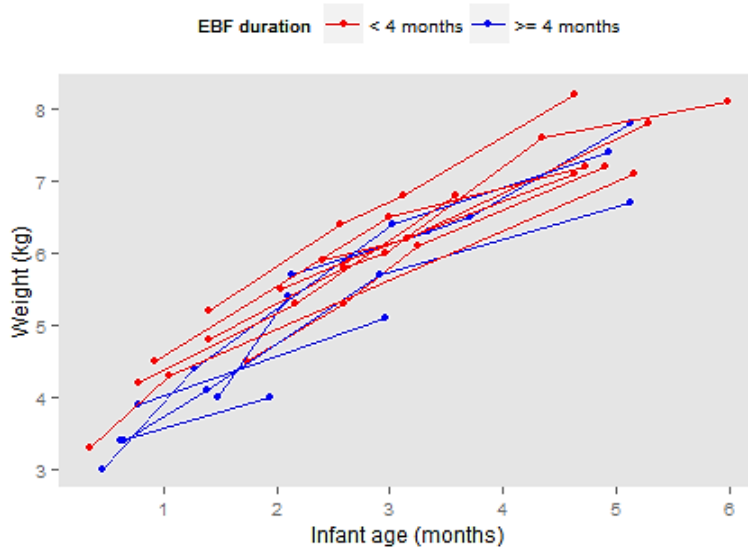


Figure 3. Spaghetti plots of individual changes in length for infants measured at least twice from 6- 16 months of age (n = 28). Plots are colored according to whether observed or reported EBF duration was before or after 4 months of age.

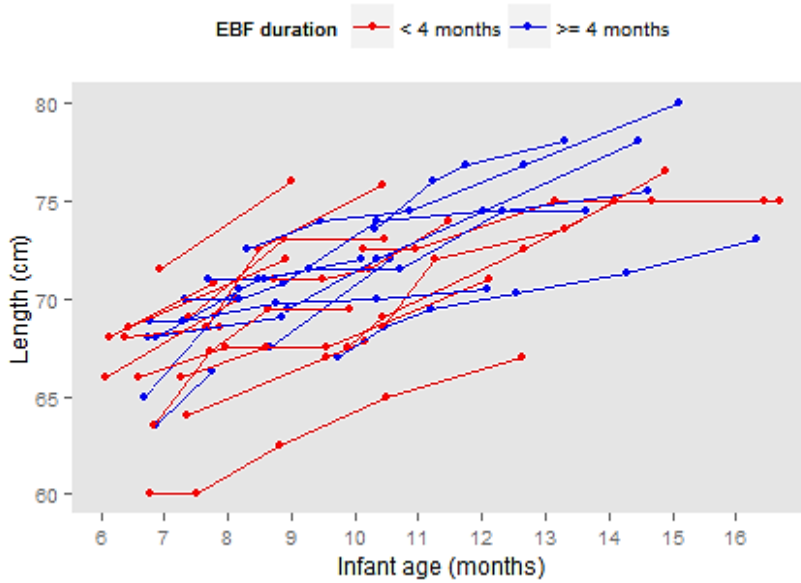


Figure 4. Spaghetti plots of individual changes in weight for infants measured at least twice from 6- 16 months of age (n = 28). Plots are colored according to whether observed or reported EBF duration was before or after 4 months of age.

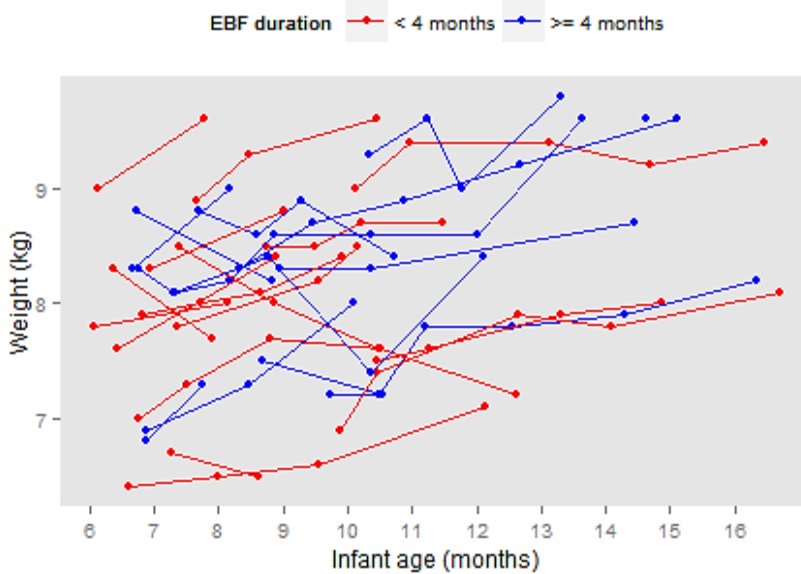


Figure 5. Density plots of HAZ and WAZ score distributions from all subjects at initial interviews (n = 156). Plots contrast scores as calculated according to WHO and Tsimane LMS standards.

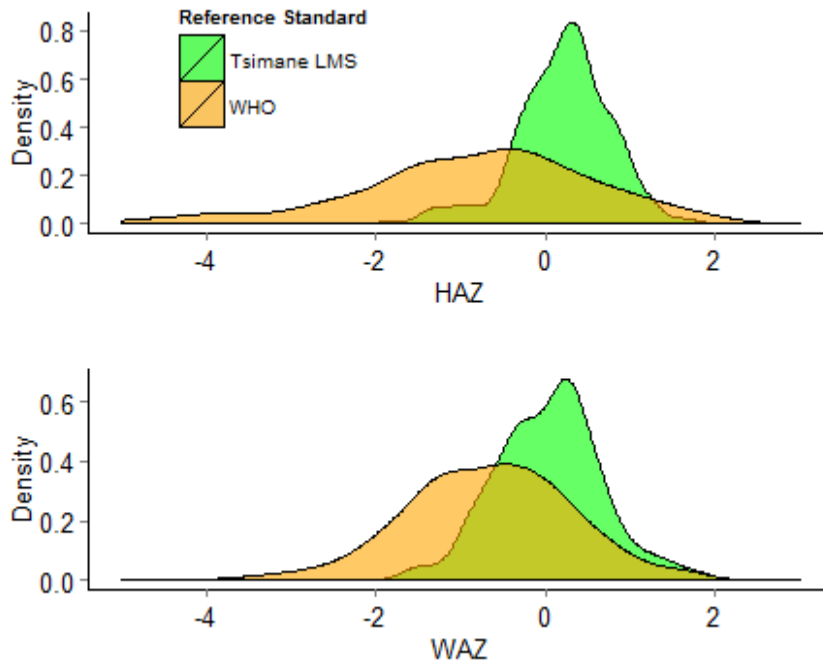


Table 3. Association of CF vs. EBF status on HAZ and WAZ in a mixed-longitudinal sample of infants **0-5 months** old (40 subjects, 82 observations). Separate GEE models were fit for HAZ and WAZ and adjusted for infant age (centered at sample mean, approximately 3.18 months), preceding interbirth interval category, maternal height, and CF*age interaction.

	HAZ	WAZ
Predictor	Est. ± SE (95% CI)	Est. ± SE (95% CI)
Feeding Status		
EBF	--	--
CF	-0.27 ± 0.23 (-0.72; 0.17)	-0.22 ± 0.16 (-0.53; 0.10)
Age (centered)	0.25 ± 0.06 (0.14; 0.36)***	0.04 ± 0.06 (-0.07; 0.15)
Previous IBI		
< 33 months	--	--
First born	0.68 ± 0.34 (0.01; 1.34)*	0.38 ± 0.37 (-0.33; 1.10)
≥ 33 months	0.37 ± 0.29 (-0.20; 0.93)	0.74 ± 0.27 (0.21; 1.28)**
Mat. height (cm)	0.03 ± 0.02 (-0.02; 0.07)	0.02 ± 0.02 (-0.02; 0.07)
CF*age	-0.28 ± 0.11 (-0.50; -0.07)**	--
Alpha	0.59 ± 0.11	0.57 ± 0.10
QIC	-8.98	-27.4

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

Fig. 6 Association between EBF vs. CF status on HAZ by age (infants 0-5months)

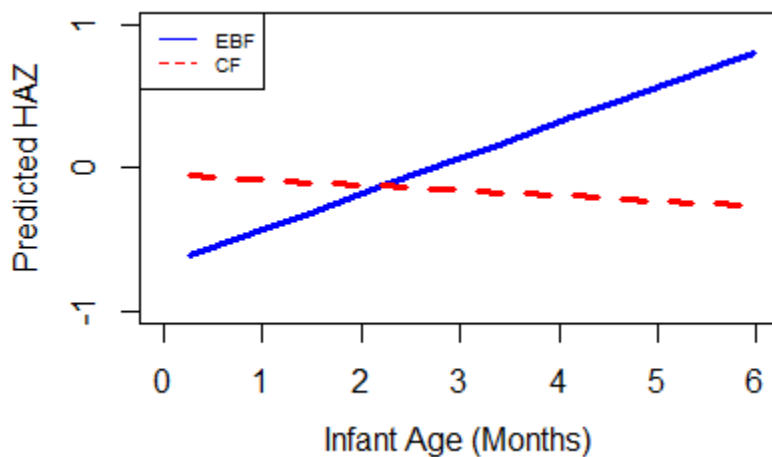


Table 4. Association of CF vs. EBF status on HAZ and WAZ in a mixed-longitudinal sample of infants **0-5 months** old (40 subjects, 82 observations). Alternate GEE models were fit for HAZ and WAZ substituting birth order for IBI category, and adjusting for infant age (centered at sample mean of 3.27 months), maternal height, and CF*age interaction.

	HAZ	WAZ
Predictor	Est. ± SE (95% CI)	Est. ± SE (95% CI)
Feeding Status		
EBF	--	--
CF	-0.21 ± 0.22 (-0.64; 0.22)	-0.19 ± 0.16 (-0.50; 0.12)
Age (centered)	0.25 ± 0.05 (0.15; 0.36)***	0.05 ± 0.05 (-0.06; 0.15)
Birth Order		
2- 7	--	--
First born	0.61 ± 0.32 (-0.02; 1.24) ^t	0.14 ± 0.38 (-0.60; 0.88)
8+	0.44 ± 0.36 (-0.27; 1.15)	0.18 ± 0.27 (-0.35; 0.72)
Mat. height (cm)	0.32 ± 0.03 (-0.02; 0.09)	0.02 ± 0.03 (-0.04; 0.08)
CF*age	-0.32 ± 0.11 (-0.53; -0.11)**	--
Alpha	0.79 ± 0.11	0.71 ± 0.09
QIC	-5.16	-14.0

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ^ttrend $p < .1$

Table 5. Best fit GEE models for HAZ and WAZ of infants aged 0 – 5 months (see Table 2) run using z-scores generated from within-population Tsimane standards.

	HAZ	WAZ
Predictor	Est. ± SE (95% CI)	Est. ± SE (95% CI)
Feeding Status		
EBF	--	--
CF	-0.05 ± 0.08 (-0.21; 0.11)	-0.21 ± 0.10 (-0.42; 0.01)*
Age (centered)	0.10 ± 0.02 (0.07; 0.14)***	0.14 ± 0.04 (-0.07; 0.21)***
IBI category		
< 33 months	--	--
First birth	0.18 ± 0.14 (-0.09; 0.45)	0.14 ± 0.25 (-0.35; 0.62)
≥ 33 months	0.14 ± 0.11 (-0.08; 0.36)	0.44 ± 0.16 (0.13; 0.75)**
Mat. height (cm)	0.01 ± 0.01 (-0.01; 0.02)	0.02 ± 0.01 (-0.01; 0.05)
CF*age	-0.10 ± 0.04 (-0.17; -0.03)**	--
Alpha corr par	0.65 ± 0.13	0.43 ± 0.18

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ^ttrend $p < .10$

Table 6. Association of age of CF introduction (0-3 months vs. 4 -6 months) on HAZ and WAZ in a mixed-longitudinal sample of children **6-35 months** (130 subjects, 202 observations). Separate GEE models were fit for HAZ and WAZ and adjusted for child age (centered at sample mean of 14.6 months), preceding interbirth interval category, maternal height, birth season (rainy or dry), and weaning status.

	HAZ	WAZ
Predictor	Est. ± SE (95% CI)	Est. ± SE (95% CI)
CF age		
4 – 6 months	--	--
0 – 3 months	0.51 ± 0.22 (0.08; 0.93)*	0.03 ± 0.22 (-0.39; 0.45)
Age (centered)	-0.06 ± 0.02 (-0.09; -0.03)***	-0.02 ± 0.01 (-0.04; -0.001)*
IBI category		
< 33 months	--	--
First born	0.50 ± 0.27 (-0.04; 1.03) [†]	0.14 ± 0.20 (-0.24; 0.53)
≥ 33 months	0.63 ± 0.23 (0.18; 1.09)**	-0.36 ± 0.29 (-0.93; 0.21)
Mat. height (cm)	0.08 ± 0.02 (0.04; 0.11)***	0.07 ± 0.01 (0.04; 0.09)***
Birth Season		
Dry	--	--
Rainy	-0.44 ± 0.20 (-0.83; -0.04)*	-0.18 ± 0.15 (-0.48; 0.12)
Weaned		
No	--	--
Yes	-0.42 ± 0.30 (-1.0; 0.15)	-0.07 ± 0.24 (-0.53; 0.40)
CF 0-3* First born	--	-0.41 ± 0.47 (-1.33; 0.49)
CF 0-3* IBI ≥ 33 mos.	--	1.21 ± 0.35 (0.52; 1.91)***
Alpha	0.83 ± 0.20	0.34 ± 0.17
QIC	61.8	-50.7

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

Table 7. Association of CF vs. EBF status on HAZ and WAZ in a mixed-longitudinal sample of infants **6-35 months** old (130 subjects, 202 observations). Alternate GEE models were fit for HAZ and WAZ substituting birth order for IBI category, and adjusting for infant age (centered at sample mean of 3.27 months), maternal height, and CF*age interaction.

	HAZ	WAZ
Predictor	Est. ± SE (95% CI)	Est. ± SE (95% CI)
CF age		
4 – 6 months	--	--
0 – 3 months	0.52 ± 0.21 (0.10; 0.94)*	0.45 ± 0.20 (0.05; 0.85)*
Age (centered)	-0.06 ± 0.02 (-0.11; -0.02)**	-0.03 ± 0.01 (-0.05; -0.01)**
Birth Order		
2- 7	--	--
First born	0.39 ± 0.27 (-0.14; 0.91)	0.34 ± 0.19 (-0.03; 0.71) [†]
8+	0.51 ± 0.30 (-0.09; 1.09) [†]	0.73 ± 0.35 (0.04; 1.42)*
Mat. height (cm)	0.09 ± 0.02 (0.05; 0.13)***	0.07 ± 0.02 (0.04; 0.10)***
Birth Season		
Dry	--	--
Rainy	-0.42 ± 0.21 (-0.84; -0.01)*	-0.17 ± 0.16 (-0.49; 0.15)*
Weaned		
No	--	--
Yes	-0.26 ± 0.32 (-0.89; 0.37)	0.03 ± 0.22 (-0.40; 0.45)
CF 0-3*First born	--	-0.86 ± 0.49 (-1.81; 0.09) [†]
CF 0-3* Birth order 8+	--	-0.42 ± 0.49 (-1.39; 0.55)
Alpha	0.92 ± 0.26	0.59 ± 0.22
QIC	77.1	-27

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, [†]trend $p < .10$

Table 8. Best fit GEE models for HAZ and WAZ of infants aged 6-35 months (see Table 3) run using z-scores generated from within-population Tsimane standards

Predictor	HAZ	WAZ
	Est. ± SE (95% CI)	Est. ± SE (95% CI)
CF age		
4 – 6 months	--	--
0 – 3 months	0.22 ± 0.11 (0.01; 0.43)*	0.08 ± 0.15 (-0.21; 0.37)
Age (centered)	0.01 ± 0.01 (-0.01; 0.02)	-0.00 ± 0.01 (-0.02; 0.01)
IBI category		
< 33 months	--	--
First birth	0.24 ± 0.14 (-0.03; 0.50) ^t	0.07 ± 0.14 (-0.20; 0.35)
≥ 33 months	0.32 ± 0.12 (0.09; 0.54)**	-0.18 ± 0.20 (-0.58; 0.21)
Mat. height (cm)	0.04 ± 0.01 (0.02; 0.06)***	0.05 ± 0.01 (0.03; 0.07)***
Birth Season		
Dry	--	--
Rainy	-0.19 ± 0.10 (-0.39; 0.01) ^t	-0.08 ± 0.11 (-0.29; 0.12)
Weaned		
No	--	--
Yes	-0.40 ± 0.16 (-0.72; -0.09)*	-0.11 ± 0.18 (-0.46; 0.25)
CF 0-3*First born	--	-0.28 ± 0.32 (-0.90; 0.34)
CF 0-3* IBI ≥ 33 mos	--	0.74 ± 0.25 (0.26; 1.22)**
Alpha	0.68 ± 0.15	0.42 ± 0.20

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ^ttrend $p < .10$

Table 9. Association of CF with number of illness bouts and likelihood of GI symptoms in the past two weeks for subjects **0-5 months** of age. GLMM models with a random intercept for subject age were fit for illness bouts and GI symptoms, with Poisson and binomial distributions, respectively (22 subjects, 50 observations). Infant age was centered at sample mean (approximately 3.2 months).

	Illness Bouts Est. (95% CI)	GI symptoms (0-5 mos.) OR (95% CI)
Feeding Status		
EBF	--	--
CF	0.05 (-0.43; 0.53)	0.79 (0.19 – 3.06)
Age (centered at mean)	0.07 (-0.07; 0.21)	0.98 (0.56 – 1.71)
WAZ	-0.04 (-0.29; 0.22)	0.26 (0.08 – 0.87)*
Random effects var/SD	0/0	1.1/1.05

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

Table 10. Association of age of CF introduction with the number of illness bouts and likelihood of GI symptoms in the past two weeks for subjects **6-16 months** of age. GLMM models with a random intercept for subject age were fit for illness bouts and GI symptoms, with Poisson and binomial distributions, respectively (33 subjects, 88 observations). Infant age was centered at sample mean of 9.8 months.

	Illness Bouts Est. (95% CI)	GI symptoms (6-16 mos.) OR (95% CI)
Age CF introduction	--	
4 – 6 months		
0 – 3 months	0.26 (-0.04; 0.56) ^t	0.93 (0.37; 2.38)
Age (centered)	-0.02 (-0.07; 0.04)	0.90 (0.68; 1.18)
WAZ	-0.09 (-0.26; 0.07)	0.88 (0.51; 1.49)
CF 0-3 months*age	--	1.42 (0.98; 2.07) ^t
Random effects var/SD	0/0	0.03/0.19

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

Conclusion

Collectively, anthropological research on human infant feeding practices is substantial. This body of scholarship includes studies on the evolution of human lactational strategies, variation in milk composition across women and lactational stages, ethnographic studies of traditional breastfeeding practices, complementary feeding (CF) practices (i.e. provisioning of any non-maternal milk liquids and solids), and variability in ages at weaning. The latter two areas further encompass variation in the timing and type of complementary foods introduced, the relative contribution of breastfeeding and CF to infant energy intake across lactational stages (e.g. the relative pace of weaning), and the age at full cessation of breastfeeding.

Breastfeeding research has increased even more in the last few decades, following solidification of international recommendations for optimal infant feeding practices and related public health aims beginning in the 1990s (Labbok, 2013). This dissertation has considered the following recommendations broadly and in evaluating infant feeding practices among the Tsimane: (1) exclusive breastfeeding (EBF) for the first six months of life; (2) appropriate frequency and diversity of complementary foods after six months of age, and (3) breastfeeding with complementary feeding (CF) for two years or more. Of these, the emphasis on six months of EBF as a universal standard of optimal feeding practices has in particular catalyzed research on predictive factors associated with variation in CF and the short- and long-term consequences for infants and/or mothers. As an example, a quick PubMed search for articles published between 1950 and 1989 and containing the terms “complementary feeding” or “infant feeding” returns, respectively, 12 and 801 articles. The

same searches performed for the years 1990 to 2015 return, respectively, 623 and 3228 articles—an increase of nearly 400% for the two terms combined.

A complete analysis of that body of literature is beyond the scope of this dissertation. However, one consistent pattern emerges with even a cursory review of the literature: globally, EBF to six months is perplexingly rare (e.g. Sellen, 2007; Cai et al., 2012; Gupta et al., 2013). This holds whether the population in question has seen dramatic increases in breastfeeding initiation in recent decades following public health efforts (Lutter and Morrow, 2013; Sinha et al., 2015) or populations such as the Tsimane in which intensive and prolonged breastfeeding has always been standard practice (Sellen, 2001; Veile et al., 2014).

A second notable observation is that much of the literature on EBF durations is divided between research on infant-centered outcomes and maternal-centered factors that predict those outcomes. Infant-centered *factors* are less systematically evaluated in predicting CF practices—even though mothers in diverse settings frequently cite infant growth and cues of hunger or illness as primary factors motivating CF decisions (e.g. Jenkins et al., 1984; Marquis et al., 1997; Dennis, 2002). Meanwhile, maternal-centered *outcomes*—such as health concerns, time and energy savings following CF introduction—are too often considered as barriers to infant optima, rather than a dually negotiated optima for the dyad. Even less frequently are all four of the above perspectives integrated in a single study. For this reason, the primary aim of this dissertation has been to the push-pull of all of these dynamics in determining individual EBF durations and associated outcomes.

Through synthesis and empirical testing, this dissertation makes several novel contributions to research on human infant feeding practices. First, I reviewed and synthesized

literature across disparate academic fields in evaluating the biological and evolutionary appropriateness of current feeding recommendations for human infants. Next, I collapsed the many individual and societal factors associated with varying breastfeeding practices under two theoretical models to characterize patterns of early CF: the Feeding Substitution Model and the Feeding Augmentation Model. The Feeding Substitution Model draws from existing theoretical extensions of parent-offspring conflict theory in proposing that maternal fitness trade-offs will predict variation in EBF duration (McDade and Worthman, 1998; Tully and Ball, 2013). This model also incorporates the standard epidemiological perspective that early CF risks offsetting breast milk intake and increasing infant pathogen exposure. The Feeding Augmentation Model proposes in contrast that early CF may actually benefit infants, with minimal to no effect on breastfeeding intensity. Finally, the models were tested by generating alternate predictions of predictive factors and outcomes associated with early CF among the Tsimane. These analyses incorporated mixed-methods to measure differences in breastfeeding intensity, CF intake, and associated maternal and infant energetic outcomes. Results of the most significant findings presented in this dissertation are summarized below.

Summary of main findings

Chapter 1 considered whether the recommendations for six months of EBF and at least two years of breastfeeding were consistent with patterns of human infant development and changes in milk composition and yield across lactation—that is, if the recommendations really are evolutionarily appropriate for our species, as has been previously suggested (Sellen, 2007). Evidence of infant physiological and digestive maturation does suggest that complementary foods and liquids given before 4 months of age may not be well tolerated. On

the opposite end, decreased availability of some milk nutrients and evidence of growth faltering with continued EBF beyond six months of age supports an upper limit of EBF for about six months. Ethnographic evidence further indicated that 4-6 months of EBF is typical for non-industrial populations, though very early CF (in the first few days or weeks of life) is not uncommon in these societies. Finally, some variability in EBF duration should be expected given differences in infant energy demands, growth trajectories, and maternal lactation performance. With respect to total breastfeeding duration, lactational research does indicate 2-3 years of breastfeeding would provide important buffering against nutritional and infectious risks during this continued period of growth and immune maturation. As supported by paleoanthropological and ethnographic evidence, weaning at about 2-3 years also appears typical for our species, though weaning ages of 1 to 6 years have been estimated or observed for extant and extinct populations. In sum, the age-based recommendations do approximate evolutionary norms, but if too rigidly interpreted or adhered to, belie both the evolved flexibility of human feeding practices and potential variability in infant optima that might warrant earlier CF or weaning.

Chapters 3-5 examined variability in infant feeding practices among the Tsimane. A systematic survey of infant feeding practices presented in Chapter 3 supports previous characterizations of Tsimane breastfeeding practices as “intensive” (Veile et al., 2014). Tsimane infants are universally breastfed and generally not weaned before two years of age, but introduced CF at around four months of age. Chapters 4 and 5 tested whether this pattern of early CF among the Tsimane was best characterized by the Feeding Substitution or the Feeding Augmentation Model, with results generally supporting the latter. First, mothers’

stated reasons for introducing CF more often emphasized perceptions of infant needs than their own time or energy constraints. Perceived low milk supply was a common reason for reporting very early introduction of CF (often in the first few weeks of life), and was more frequently reported by higher parity mothers. However, introduction of CF because of low milk was not associated with subsequently increased CF intake, suggesting mothers maintained breastfeeding intensity to support their own maximum milk yield, even if these yields were insufficient.

Overall, relatively earlier CF introduction (0-3 vs. 4-6 months) was associated with increased CF frequency at later ages, but not with subsequently lower frequency or duration of breastfeeding bouts, and not with early weaning. Together, these results suggest that early CF among the Tsimane is not energetically sufficient enough to result in immediate offsets in breastfeeding frequency, and has little impact on the overall pace of weaning. Similarly for mothers, shorter EBF durations were not associated with increased urinary C-peptide concentrations or greater BMI—further indication that early CF did not substantially reduce lactational costs.

Somewhat surprisingly, both primiparous *and* high parity mothers began CF relatively earliest. Both primiparity and high parity—but not age of CF introduction—also predicted earlier resumption of menstruation. Parallel results suggest different mechanisms mediating these relationships that are independent of EBF duration. For example, high parity mothers had higher BMI across months following CF introduction, which may be consistent with the metabolic load hypothesis. However, high parity mothers were also more likely to report low milk supply. These subjects may have been driving the relationship between high parity and

EBF duration, whereas the relationship between high parity and postpartum menstruation is driven independently by high parity mothers' relatively greater energetic condition. The mechanism driving earlier menstruation among primiparous mothers, however, is not clear, given their consistently lower BMI following CF introduction.

Analysis of infant outcomes do not strongly suggest benefits associated with early CF, but nor do they provide any evidence of substantially increased risk that would support a model of decreasing breastfeeding intensity following CF introduction. Earlier CF did not appear to lead to subsequently reduced breastfeeding frequency or duration per hour. Before six months of age infants introduced CF were relatively smaller as compared to EBF counterparts. After six months of age, mean height-for-age was higher for infants introduced CF at 0-3 vs. 4-6 months, however estimated differences in growth percentiles may be of little clinical relevance. Finally, early CF was not associated with greater likelihood of reported illness before or after six months of age.

Results presented in Chapter 3 indicate other feeding practices that may increase risks of infant nutritional morbidity. Meal frequency and dietary diversity was not related to child nutritional status, though variation in these measures from 24-hour recall may reflect transient differences in dietary availability, rather than consistent differences in dietary access across households that would manifest as accumulated differences in nutritional status. However, from 12-23 months of age the prevalence of stunting and wasting increases, while at the same time there is no increase in meal frequency relative to 6-11 months of age. Thus, while early, gradual CF appears to have minimal impact on infant morbidity precisely because it does not offset breast milk intake, such limited CF may lead to insufficient energy

intake if extended well past six months of age. In addition, infants weaned before two years of age were significantly smaller than their still breastfed counterparts, which may suggest compounded effects of relatively early weaning and insufficient intake of complementary foods. Diversity of complementary foods was also below recommended minimums across Tsimane households. However, diversity was higher in villages closer to market and during the rainy season, which may result from greater access to market foods and annually fruiting trees. It was suggested that readily cultivated fruits, vegetables, and legumes could be more frequently incorporated into Tsimane infant diets.

Considering all of the above, the optimal age of CF introduction *for infants* appears to fall between 4-6 months of age, though the recommendation of EBF for six months rather rigidly pushes those optima to 6 months for *all* infants. In traditional breastfeeding populations such as the Tsimane, early CF appears to have no effect on breastfeeding intensity, but may be biologically warranted given low milk supply and/or as indicated by infant growth and development. Moreover, if breastfeeding intensity is sustained as CF is introduced, early CF is unlikely to affect maternal fecundity and interbirth intervals. If these findings are correct, then EBF duration for the Tsimane would not be a strong domain of parent-offspring conflict. In this population, shorter EBF durations appear to represent the congruence, not conflict, of maternal and offspring fitness interests. Moreover, as long as early CF poses little risk to Tsimane infants, and intensive breastfeeding practices remain normative, promotion of six months of EBF is unlikely to greatly improve infant health outcomes. Efforts to improve early life nutrition through education and intervention should be appropriate to the most pressing needs and feasible options available to specific

populations (Hadley et al., 2008; Arabi et al., 2012; Daelmans et al., 2013). For the Tsimane, improvements in early life nutrition may be most impacted by promotion of hygienic CF practices, increased frequency of CF after one year of age, and greater diversity of complementary foods.

Study limitations

The conclusions above are limited by a relatively small, unbalanced data set and a sampling design that did not allow for longitudinal analysis across set intervals. Specifically, repeated sampling across a larger cohort from birth through at least the first year of life is needed to assess individual infant growth trajectories before and after CF. Given that mean resumption of postpartum menstruation was predicted at 14 months, more uniform longitudinal sampling up to at least 18 months postpartum is also needed to more robustly assess individual changes in maternal energy balance before and after CF introduction and in association with postpartum fecundity. In this study, urinary C-peptide concentrations had limited utility as a measure of lactational costs, resulting from the unbalanced sampling design in combination with high inter-individual variability in this biomarker. For future study designs, rather than shorten the intervals of urine sampling, collection across uniform lactational stages with repeated sampling around a given measurement date would allow for assessment of concentrations over time with better control of daily oscillations in individuals (e.g., daily sampling of each subject for three-five days every month). Finally, a larger longitudinal sample is needed to test for interactions between parity, CF introduction, and maternal BMI in influencing the resumption of postpartum menstruation. For the present study, follow-up analysis of maternal subjects' subsequent interpregnancy and interbirth

intervals may be helpful in testing whether or not the non-linear relationship between parity and postpartum menstruation extended to relatively earlier conception and successful delivery.

Measurements of infant morbidity were dependent on 2-week recall, which is imprecise and subject to recall error. Results of these analyses should be cautiously interpreted.

Tsimane complementary foods are not hygienically handled or prepared, and should be considered a vessel for disease risk until future research more accurately gauges their pathogenic potential. Future research might also consider whether pathogen exposure through early CF is mitigated by immunological properties of breast milk and/or stimulation and upregulation of infant immune responses. Fecal samples collected from infants in this study are currently being analyzed for changes in infant gut microbial composition and fecal neopterin levels. These results can be compared against dietary and morbidity recalls in order to more robustly assess if early CF exposure (1) altered gut microbial function to an extent that would indicate substantial dietary shift and (2) increased intestinal inflammation to a degree indicative of increased pathogen exposure.

A few final precautions are urged in interpreting the conclusions presented here and in extrapolating results to other populations. First, EBF is a form of costly investment in infant fitness, but it is not the only or even *costliest* measure of maternal investment. While EBF and subsequent breastfeeding decisions may be influenced by mother's reproductive interests, they are also be influenced by trade-offs among different types of maternal investment, which in turn may be necessitated by larger cultural forces. Most pressingly, mothers who work and labor outside of the home may do so at the expense of lactational investment in infants, but

still provide critical support for those infants, other offspring, and family members. Second, the absence of nutritional and morbidity risks associated with early CF in the Tsimane should not be taken as evidence that there are no risks. Third, evidence of more flexible EBF optima and evidence that very early CF is common cross-culturally should not be taken as evidence that very early or rapid *weaning* is evolutionarily appropriate or minimally costly. The Feeding Augmentation Model of early CF only holds as long as CF does not supplant daily, 24-hour breast milk intake. The Feeding Substitution Model may have stronger explanatory power in populations in which bottle and formula-feeding are more common.

Finally, and is discussed in greater detail below, more precise measures of breast milk and complementary food intake are needed to confirm that breastfeeding intensity is not reduced by early CF in the Tsimane. Such measures would also allow for characterization of Tsimane infant breast milk and complementary food intake across all stages of lactation, necessary to confirm whether CF at later ages is generally energetically sufficient.

Future research and broader impacts

In general, all research on infant feeding practices would benefit from more precise quantification of mixed-feeding. Most quantitative research on variation in infant energy intake has focused on differences between breast- and formula fed infants (Garza and Butte, 1990; Dewey et al., 1991; Kramer et al., 2004). Comparatively little research has examined more subtle shifts in energy intakes across later lactational stages and/or in combination with different frequencies and energetic densities of CF. Only a handful of isotopic studies have attempted to quantify changes in infant breast milk intake with advancing age and CF intake (Orr-Ewing et al., 1986; Galpin et al., 2007; da Costa et al., 2010; Nielsen et al., 2011; Wells et

al., 2012). Isotopic analysis of nitrogen levels in infant hair samples are frequently used to estimate breastfeeding and weaning in skeletal populations (Tsutaya and Yoneda, 2012, 2013), but may be equally important as a non-invasive measure of breastfeeding to CF ratio in living populations. Incorporation of observed or reported breastfeeding behaviors with isotopic data may provide further insight into what types of nursing patterns consistently predict more discrete ratios of breast milk to CF intake.

Currently, there are no definitional criteria for IYCF practices that account for differences in quantity of CF relative to breastfeeding. The category of “predominant” breastfeeding, as defined by the WHO is also overly narrow, allowing for water and juice but no formula, solids, or semi-solids in any quantity. By this definition, few Tsimane infants were classified as predominantly breastfeeding, though my own observations and analysis would suggest otherwise. These definitions of exclusive and predominant breastfeeding may be predictive of breastfeeding intensity and duration in populations in which bottle and formula-feeding have radically reduced breastfeeding rates, but they are not predictive of breastfeeding intensity or duration in populations that exhibit traditionally intensive breastfeeding practices.

Thus, setting aside the question of which infant outcomes can or should be appropriately compared across populations, current standards for comparing infant feeding practices across populations are grossly inadequate to evaluate variation in mixed-feeding. To the extent that some degree of CF can be additive and not supplemental, CF is not an appropriate proxy for breastfeeding intensity. The absence of categories based on graduations of breastfeeding and CF fundamentally hinders our understanding of human lactational biology. For example, in both intensively breastfeeding U.S. and Amele women (Dewey

1984, Worthman), steady decreases in milk production were observed about a month following CF introduction (at 6 and 7-8 months, respectively). Are such declines mediated solely by offsets in nursing intensity, however minimal, or do other intrinsic mechanisms force reductions in peak milk production? Across advancing stages of lactation, are there some changes in milk composition that are invariably constrained by maternal biology as opposed to suckling frequency? Are there any biological upper limits for breastfeeding that are independent of the weaning process?

Here, it is worth re-emphasizing a point from the comparative primate review in Chapter 1: while human offspring are unique (excepting callitrichids) in the extent to which they consume directly provisioned vs. independently foraged complementary foods, all primate offspring exhibit transitional feeding stages marked by gradual reductions in maternal milk intake. Yet to the best of my knowledge, very little is known about the pace of this transition in non-human primates, let alone how individually variable it might be. Future research on patterns of transitional feeding in non-human primates may be informative in establishing quantities of CF that differentiate substitution and augmentation of maternal milk intake.

I also argue that the overly broad definition of CF has limited utility in predicting differences in infant and maternal outcomes that vary with breastfeeding or CF exposure. It is more than reasonable to expect that poorer infectious and nutritional outcomes associated with shorter EBF durations actually reflect dose-dependent relationships with CF or total duration of breastfeeding. As to this latter point, there is a relative paucity of research on health outcomes associated with the wide variation in total breastfeeding duration—

especially prolonged breastfeeding for two years or more. As an example, in a recent review of protective effects of breastfeeding on various health outcomes, only one meta-analysis reviewed included an estimated effect for prolonged breastfeeding duration—in this case a reduced risk of obesity for 7-9 months of breastfeeding as compared to any breastfeeding (Dieterich et al., 2013). A more recent review and meta-analysis has established significantly increased mortality risks for non-breastfed as compared to breastfed infants at 6-23 months (Sankar et al., 2015). However, more research on specific outcomes associated with discrete differences in breastfeeding trajectories is needed, particularly given the many societal and individual confounds that influence breastfeeding status at later ages.

Emphasis on the ratio of breastfeeding to CF would help formulate research questions better targeted to specific areas of risk. Across and within populations, these questions can be pursued at two levels of inquiry: (1) to what extent do broad deviations from evolutionary breastfeeding norms affect infant outcomes? (2) What are the risks of differences in age-specific intensity of breastfeeding to CF, given comparable environmental exposures and quality of complementary foods? Those same two lines of questioning would apply to studies of maternal health and reproductive outcomes, given the influence of breastfeeding on maternal hormonal, metabolic, and immune systems. If some period of prolonged breastfeeding is evolved for our species, how has the shift towards non-initiation and short breastfeeding durations (e.g. about 3 months in the U.S.) affected indices of maternal reproductive health at the population level? Do individual differences in breastfeeding intensity and duration—mediated by the timing and subsequent pace of CF—measurably influence fecundity, postpartum energy balance, and immune regulation? Can we establish

safe ranges of mixed-feeding and minimum benefits for varying durations of total breastfeeding? For families weighing recommendations of “optimal” breastfeeding against their own individual circumstances, such research may be particularly welcome.

In conclusion, I argue that CF before six months should not be an automatic proxy for risk, as risk ultimately depends on subsequent changes in breastfeeding intensity (including rate of weaning) and pathogen exposure. Understandably, such an individualized and nuanced message is not readily amenable to public health messages or policy aims. I do not advocate for a return to previous recommendations of 4-6 months as other researchers have (Reilly and Wells, 2005; Agostoni et al., 2008; Fewtrell et al., 2011). Goals to increase rates of EBF to six months require policies that promote intensive breastfeeding, from Baby Friendly Hospital initiatives that encourage universal breastfeeding initiation and successful establishment of milk supply, to workplace and government family leave policies that promote sustained breastfeeding after birth. Movements away from advocacy and policy that promote EBF for six months risk losing the positive effect that these policies have on increasing BF initiation and continuation of breastfeeding more broadly (Lutter and Morrow, 2013; Sinha et al., 2015). These practices save lives and decrease disease burdens globally (Black et al., 2008; Lim et al., 2012; Sankar et al., 2015).

Six months may be an overly rigid and even somewhat arbitrary marker to measure EBF durations against, but it is still a valid indicator of general breastfeeding practices and the adequacy of policies to promote breastfeeding at the population level. That said, this cut-off should not be used as an automatic gauge of optimal feeding for any *individual* mother or infant. Allegorically, the current prevalence of overweight and obesity in the U.S. may be an

appropriate indicator of generally poor health (or chronic health risks) in the population, but the difference between a BMI of 23.5 (“normal”) and 24.5 (“overweight”) is likely not a difference of clinical importance for any individual. In evaluating individual health, low or high BMI are only meaningful indicators of individual health when considered in conjunction with other indicators of metabolic health and/or longitudinal changes (i.e. rapid or sustained weight loss or weight gain). Thus, rather than abandon the six month EBF recommendation in policy and public health messages, there should be space among families, communities, and in pediatrician-patient relationships for parallel conversations about developmental-based feeding responses. Indeed, some researchers have advocated for emphasizing cues of “developmental readiness” between 4 and 10 months of age to help clinicians and parents in making infant feeding decisions (Strassmann and Gillespie, 2002; Cattaneo et al., 2011).

Mothers ultimately make breastfeeding decisions that negotiate the needs of their infants, themselves, and their families. While the two models proposed in this dissertation emphasize different motivations for early CF, they both offer a framework for understanding optimal EBF durations as individually variable and dually shaped by the needs of infants *and* mothers. The models can be practically extended by categorizing particular CF practices as primarily maternal- or infant-centric, which in turn may be useful in better directing strategies to meet needs of particular families or broader communities. For example, if early CF is primarily infant-centric (e.g. owing to low milk supply), interventions to promote EBF may be inappropriate or less effective than interventions that more directly facilitate access to health care and safe, nutritious complementary foods. Maternal-centric patterns of early CF, in turn, may be better framed in terms of balancing maternal needs, rather than “barriers” to

optimal infant feeding. As long as breastfeeding alternatives are safe and energetically sufficient, messages promoting mixed-breastfeeding may be appealing to mothers who are unable or unwilling to exclusively breastfeed for six months. In the long run, flexible approaches to breastfeeding promotion may better approximate the evolved flexibility of complementary feeding in humans, and may have a more positive impact on rates of breastfeeding duration than do rigid recommended timelines of EBF.

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Appendix

A1. Ethnographic Interview

Entrevista Preliminar Infantes

Comunidad: _____ Entrevistador: _____ Fecha: _____
Nombre Mama: _____ Apellidos: _____ MidPID: _____
Nombre Infante _____ Apellidos: _____ MidPID: _____
Infante (1) Leche exclusiva (2) Chupa y come (3) Dejó de chupar
Padres de la mama vive en comunidad SI / NO Padres del marido viven en comunidad SI / NO
Census/Demog: # hijos vivos _____ # de partos _____ # abortos _____ Edad 1ra
menses _____
Diferencias notado: # hijos vivos _____ # de partos _____ # abortos _____ Edad 1ra
menses _____
Razones para las diferencias _____
Nivel de
escuela _____
Nivel castellano _____
Parientes de la casa _____

A. Preguntas de alimentación del bebe

1. Cuando nació tu bebe le dio a chupar inmediatamente, o esperaste una hora, 2 horas, o medio día, o un día, o mas que un día? *Aty najj ya mu ava 'mi vatyequej buty so 'meti mi tashin mi o bisaqui mi yiris hora are 'pärä hora are 'choc mayedye are 'yiris mayedye are pärä mayedye?*
 - a. Inmediatamente
 - b. _____ horas
 - c. _____ días

➔ Si el bebe todavía no come y sigue chupando no más: pasa a las pregunta 17

➔ **Ya come:** Ahora quiero que tú piensas en cuando (nombre bebe) todavía estaba chupando mucho pero empezó a comer y tomar poco a poco cosas coma la chicha, sopita, plátano, y *payuje*. Las preguntas que te hago son sobre cuando tu bebe empezó a comer por primera vez. *Mu tiyac chutiya mi ava 'tum mi jedye buty so 'metemi are 'chinsi jo 'na are 'cävdyis, are po 'nacdye, are 'shocdye tara 'cävdyiti are 'jedye 'cuij rä 'chaäs ji 'je 'be yetinte. Tsun ra 'codyeyac oijcan tupudye 'tash che 'dyem jiquej mu ava 'mi tiyacchuti tsi 'yi judyeya säcsi me jujuijya 'mu yeccoij tsi 'ij mu'.*

2. Que fue la primera cosa que (nombre bebe) comió? *Jedye buty säcsete tashche (nombre bebe)?*
3. Cuantos meses tenía (nombre bebe) cuando comió (respuesta#3) por la primera vez? *Juñucsi ca ivaj chitij (nombre bebe) so 'metej mi (respuesta#3)?*
4. A las _____ meses, cuando empezabas a dar (respuesta#3) a (nombre bebe), pusiste esta (respuesta#3) en tu boca antes de dárselo? SI NO *Tash che 'mi mu 'ava 'mi jun 'buyi 'buty sometemi säcsedye ava mi are çhu can si cacha 'nac si so 'metej mi ava 'mi? Cacha 'nac are jam?*
 - a. **Si dice si:** Sigues poniendo (respuesta#3) en tu boca antes de dárselo? SI NO *Yecoj buty mi çhu can si so 'ma qui ava 'mi? Yecoj are jam?*
 - i. **Si dice si:** A cuantos meses vas a dejar de poner (respuesta#3) en tu boca antes de dárselo? *Juñucsi buty ivaj ava mi tyityij paj qui jam 'somete mi çhu can si säcsedye 'mi?*
 - ii. **Si dice no:** A cuantos meses dejaste de poner (respuesta#3) en tu boca antes de dárselo? *Juñucsi buty ivaj ava 'mi tyitij jam jisäcsetemi çhu can si säcsedye '?*
5. Que tomó primero (nombre bebe): *chicha, gaurapo, payuje, o agua pura?* *Jedye ca 'tyei tashche ava 'mi (_____): are shoc 'dye are guarapo are payuje are jedjej oj 'ni?*

6. Cuantos meses tenía (**nombre bebe**) cuando tomó (**respuesta#5**) por primera vez? *Juñucsi ca ivaj chitij (nombre bebe) tash che' tyeij (respuesta#5)?*
7. Cuando empezabas a dar (**respuesta#5**) a (**nombre bebe**), pusiste este (**respuesta#5**) en tu boca antes de dárselo? SI NO *Jun si chuc somete (respuesta#5) ava mi chu can ca' so' metej mi? chu can are jam?*
- a. **Si dice si:** Sigues poniendo la (**respuesta#5**) en tu boca antes de dárselo? SI NO *Yecoj buty mi chu can si so' ma qui ava' mi? Yecoj are jam? Yecoj are jam?*
- i. **Si dice si:** A cuantos meses vas a dejar de poner (**respuesta#5**) en tu boca antes de dárselo? *Juñucsi buty ivaj ava mi tyityij paj qui jam' somete mi chu can si (respuesta#5) mi?*
- ii. **Si dice no:** Cuantos meses tuvo (**nombre bebe**) cuando dejaste de poner (**respuesta#5**) en tu boca antes de dárselo? *Juñucsi buty ivaj ava' mi tyitij jam ji' tyetej mi chu can si (respuesta#5)?*

➔ Las siguientes preguntas deben referir a lo que se le dio al bebe primero: el alimento o el líquido. Si la mama dice que empezó a dar ambos al mismo edad, entonces refiere al alimento en estas preguntas.

8. Porque empezaste a las ___X___ meses a dar (x comida) a (**nombre bebe**)? *Jun buty mi tiyacchuti _____ ivaj ava' mi jibebeyeti?*
9. A las ___X___ meses, cuando empezaste a dar _____ a (**nombre bebe**), como te pareció tu bebe? Era *chiquito*, era *grande*, o era *igual* a los otros bebes de ___X___ meses que has visto? *Aty tiyacchuti ya' mi ivaj ava' mi _____ jibebeyeti, ju' jñij ca cavete mi? Are miquty mu' are' dartyi are' vajme' qui in yocyi tum _____ yity ivaj?*
10. Y a las ___X___ meses (**nombre bebe**), *lloró mucho*, *lloró poco*, *jamás lloró*, o *lloró igual* como los otros bebes de ___X___ meses que has visto? *Aty _____ ivaj tyityi, are' taririj are' dam tari are' jam yiri' tari are' vajme' tari yocyi tum ava' mo' in _____ yity tum ivaj?*
11. A las ___X___ meses cuando (**nombre bebe**) empezó a comer ___X___ ya tenía dientes? SI TODAVIA NO *Aty _____ X _____ ivaj tyityi ava' mi aty yacchuti säcsiya mu, aty ca' vo' dyin' yi ya' mu'. Vo' dyinyi ya' are jam bi?*
- a. **Si dice si:** Cuantos dientes tenía? *Juñucsi dyem ca' vo' dyin' mu' are jam chi?*
12. A las ___X___ meses cuando (**nombre bebe**) empezó a comer, todavía no podía soportar su cuello, o solo con ayuda podía soportarlo o por si mismo podía sopartarlo? *Aty _____ ya' ivaj aty tiyacchuti ya' sacsı, jam bi buty cui' chejeyaqui tujmu' are' notacdyetum che' jetinte mi in are' aty chejeyaqui?*
13. Podía agarrar cosas por la mano o todavía no? *Tupu jedye tapje' judyeya joquej uña' Tapeje' are jam bi?*
14. Y todavía no podía sentarse, o solo con ayuda podía sentarse, o por si mismo podía sentarse? *Are jam bi pıtsqıenyi cui' are notacdyetum pıtsqıenjetinte are' cui' aty yirity pıtsqıenyi mu?*
15. Alguien te dijo que tenía que alimentar a (**nombre bebe**) a las ___X___ meses? SI NO *Are yocsi jin in paj qui jisacsetemi ava mi' _____ ivaj yity ya? Jin ca' in are' jam?*

Si dice si:

- a. Quien era que te lo dijo? (Apunta el nombre, sexo, relación, y si era relación de su familia o del lado de su marido. Si no era una relación apunta quien era). *Tyi ca jimi?*
- b. Te dijo porque tenias que hacerlo? SI NO *Jun' buty jin' in paj qui me jem' Jin ca in' are' jam jin' in?*
- i. **Si dice si:** Que era la razón que te dijo? *Jun' buty jin' in paj qui jisacsıqui ava' mi?*
16. Quien te ayuda lo mas en cuidar a tu bebe? *Tyi adac anic mujucha' cajcabun ava' mi?*

Las preguntas #17 - #19 solo aplican a bebes que siguen chupando:

17. Jamás tu bebe dejo de chupar porque estaba enfermo? _____ ? SI NO? *Jam adac yiri' mu ava' mi farajje tsyedye japacjoij juy ya mu o are' rej ya mu? Farajje tsyedye are jam?*

Si dice si:

- a. Cuando ocurrió eso? *Jundy ya ca jique mo?*
- b. Por cuantos días dejo de chupar? *Juñuc si ca mayedye aty jam tsi' yi mu?*
- c. Tenia mas o menos leche si no estaba chupando? *Chantaqui ca tashin mi are' dam dye maj fi-i'?*
- d. Hiciste algo para ayudarle chupar? *Jedye ca mejemi pa qui tsi' yeban' ve ava' mi?*
- e. Le diste mas comida porque no estaba chupando tanto? *Jam tsi' ya jiquej dam dyeca dai jisacsetem?*
18. Jämdye adac chantqui tashin mi?

Si dice si:

- a. Jundyeya' jique?
 - b. *Juñucsi aty qui mayedye jam jitsietem?*
 - c. Jedye ca mejem paj qui fi eban tashin mi?
19. *Ahora que tu bebe tiene (xxx edad), como sabes cuando quiere comer?* Quim aty (nombre bebe) (_ x _) ivaj/yomodye, junbu adac codaqui ma'je ya säcsi judyeya' säcsi?

→ Las preguntas #20-21 solo aplican a mamás quienes tenían los carnets blancos en cualquier momento (aun si no hay foto)

20. Recibiste este carnet blanco en tu comunidad, o en la clínica de Galilea, o en el hospital, o en otro lugar? *Juqecami mo'ca carnet jaibas comunidad ya are Galilea ya are hospital can are yocvue?*
21. Cuando recibiste este carnet blanco, el doctor te dijo a cuantos meses debes empezar a alimentar a tu bebe? SI NO
Aty jequeya mi carnet mo jaibas jinca doctor junci chuc tyiacchuti jisäcsaqui ava mi? Jin ca doctor are jam?
a. Cuantos meses te dijo el doctor? *Junucsi ca ivaj jin ca doctor?*
22. Has asistido a un taller sobre la buena alimentación de los bebés? SI NO *Site ca' mi yiris jitchäyitidye jumbuyi buty jäm jisäcsacve ava in? Site ca' are jam?*
23. Alguna vez (un/otro) doctor te aviso como a alimentar bien a tu bebe? SI NO *Me ca jin doctor in jäm ra jisäcsete mu ava'mi? Are jin doctor are jam?*
24. Has escuchado a otras personas que no sean Tsimanes hablando sobre la buena alimentación de los bebés? SI NO
Se'vac si adac mi yocsi in jam chätidye jumbuyi buty jäm jisäcsacve ava in? Se'vac si adac mi are jam?
25. **Si dice "si" a cualquiera de las preguntas #21-24:** Esta persona dijo a cuantos meses las mamás deben empezar a alimentar a sus bebés aunque siguen dando pecho? SI NO *Oij mumsi'in pëye'in jun'si buty mo'nono'in paj qui yacchuti jisäcsaqui ava mo'in yejcoij juij ji'tsiyetem. Jin ca'yocsi in are jam?*
a. **Si dice si:** Según a esta persona, a cuantos meses se debe empezar a alimentar a los bebés aunque siguen chupando? *Munsis dyij jedye mo'in juñucsi buty tupu ivaj ji'säcsac mu ava me' juij ya yeccoij tsi'yi ya' mu?*

→ Si la mamá dijo en las preguntas # 21-24 que si escuchó de un edad específico, compara este edad con su respuesta de #3 ó #6

26. La edad en que la mamá empezó a alimentarle era: Igual Más Temprano Más Tarde
- a. **Si son iguales:** Entonces, a las X meses empezaste a alimentar a (nombre infante) con X porque escuchaste que debías hacerlo? Si fue por eso que empezaste o no, no fue por eso que empezaste? *Tiyacchuti ya' mi so'maqui säcsedye ava' mi aty _____ ivaj chitij ya' mu caçaj na jin yocsi' in paj qui me jisäcsaqui va' mi? Medyes qui me tiyacchuti mi are jam?*
 - b. **Si la mamá empezó más temprano:** Entonces pensaste que tenías que empezar a alimentar a (nombre infante) antes de las X meses? SI NO *Dyij yi ca'mi so'mete ra' _____ ava'mi jam bi dyem _____ ivaj tyit tij. Dyij yi mi are jam?*
 - i. **Si dice si o no:** Entonces porque empezaste a alimentarlo a las X meses? *Jun buty caij mi tiyacchuti so'maqui säcsedye ava'mi aty _____ ivaj tyiti ya' mu'.*
 - c. **Si la mamá empezó más tarde:** Entonces porque esperabas hasta las X meses para empezar a alimentar tu bebe? *Jun dash bise mi _____ ivaj paj qui yacchuti jisäsaqui ava'mi?*

B. Actividades de la mamá:

27. Donde queda tu chaco? Queda cerquita a la casa o más lejos? *Jana adac quijjodye'mi? Are tyei ya aca'mi are' moch?*
28. Cuantos horas hay que caminar para llegar a tu chaco? Media hora, una hora, dos hora, tres hora? *Juñucsi ca hora venjoij quijjodye' çan mi? Choc hora are' yiris hora are' pära hora are' chibin hora?*

29. Hoy es _____. En la última semana, entre el _____ pasado y ayer, trabajaste en tu chaco? *Oij' quin' _____*. *Yocsi cân semana judyeya yocsi cân _____ jiyi'si judyeya munja', caritaqui ca quijodye cân mi?*
 SI NO *Caritaqui ca quijodye cân mi are jam?*

a. Si dice si: Cuantos días trabajaste en tu chaco? *Juñucsi ca mayedye caritaqui mi quijodye' can mi?*

30. En la última semana, entre el _____ pasado y ayer, te fuiste de esta comunidad, que sea para trabajar o pasear o visitar a otra gente? SI NO *Yocsi cân semana judyeya yocsi cân _____ jiyi'si judyeya munja' shupqui ca oyas comunidad yas mi?* *Move ca' jicaj mi are jam?*

Si dice si:

a. A donde fuiste? *Jana' ca jicaj mi?*

b. Por cuantos días fuiste? *Juñucsi ca mayedye move mi?*

c. Que hiciste? *Juntaqui ca move mi?*

d. Llevaste (nombre bebe) contigo? SI NO *Ava tum dyeca mi? Are' ava tum mi are jam?*

i. Si dice no: Quien le cuidó a (nombre bebe) entonces? *Tyi ca' çuj cabun ava' mi?*

e. Se fue tu marido contigo? SI NO *Vämyti tum dyeca mi? Are vomtyi mi are jam?*

31. En la última semana, entre el _____ pasado y ayer, se fue tu marido de esta comunidad mientras tú quedaste aquí? SI NO *Oij' quin' _____*. *Yocsi cân semana judyeya yocsi cân _____ jiyi'si judyeya munja' shupqui ya' vämyti mi uyaty comunidad yaty mi na tacya'ji'yacati' mi oya'?* *Muve ca' jicaj mü are jam?*

Si dice si:

a. A donde se fue? *Jana' ca jicaj mu?*

b. Por cuantos días se fue? *Juñucsi ca mayedye muve?*

c. Que hizo tu marido? *Juntaqui ca muve mu?*

C. El Parto

32. Mientras estabas embarazada con (nombre bebe) tenías que irte al hospital, Galilea o HOREB debido a un problema con el embarazo? SI NO *Aty ya' jajtsisya' mi coque ca' hospital mi are galileache jicaj mi are Horebya mo' ya ca arere' dye mi jatsis dyem' jiquej mi? Move jicaj mi are jam?*

Si dice si:

a. Que problema hubo con el embarazo? *Jedye' ca carijsis mi yoqui dyem' jajtsis ya' jiquej mi?*

b. Que te hicieron los doctores? *Jun' jin ca doctor in?*

33. Tu bebe se nació en tu comunidad o en hospital? *Mo' ava mi na' ij comunidad ya are hospital can?*

→ Si era en comunidad (preguntas 34-35)

34. Alguien te ayudo durante el parto? SI NO *Ji ba sin buty yocsi in? Ji ba sin in are jam?*

Si dice si:

a. Tenías ayudantes mujeres, hombres o los dos? *Are pen in are son in are pären' dye in?*

b. Quien te ayudó? *Tyi ca mu in in?* (Apunta el nombre, sexo, relación, y si era relación de su familia o del lado de su marido. Si no era una relación apunta quien era).

35. Tu marido estaba presente durante el parto o no? *Mu' ya vamtyi mi are jam?*

***** **Pasa a la pregunta**

39*****

→ Si era en hospital (preguntas 36-38)

36. Cuantos días quedaste en el hospital? *Junucsi ca mayedye me buyi hospital can mi?*

37. Después del parto el doctor te dio una receta para medicamentos para ti? SI NO *Aty bas dyi ya jiquej mi so' min ca mu doctor yiris papel receta midyes? Are so' min are jam?*

→ Si dice si

a. Que tipo de medicamentos eran? Pastillas, inyectables o crema *Junis ca mo piñidye? Pastilla, are pöchtacdye are ta' yacdyes?*

b. Para que era el medicamento? Para dolor, para una infección o para falta de nutrientes? *Jedyeyes ca somi piñidye? Are areiredyes are japocjodyes are pandyedyes?*

c. Recuerda si los medicamentos eran antibióticos, vitaminas o paracetamol? *Dyij tac se baj mi mo in piñidye in antibiótico are vitamina are paracetamol?*

d. Recuerda el nombre de esos medicamentos? SI NO *Dyij ye ba mi tij mo piñidyes? Are dyij ye ba are jam?*

e. Nombre recordado: _____

- f. Conseguiste esos medicamentos? SI NO *Dacaqui ca 'mi mo piñidye yoctyi'ya? Are daque' mi are jam' dacaquim?*
- g. Cuantos días te dijo el doctor tomar a estos medicamentos? Un día no mas, 2 o 3 días, 7 días, 10 días, 2 semanas o mas? *Junucsi jin ca mayedye mo doctor paj qui tyei mi mo piñidye? Are yiris mayedye are 2 mayedye are 3 mayedye are 7 yamedye are 10 mayedye are 2 semanas are dam dye 2 semana?*
38. Después del parto el doctor te dio una receta para medicamentos para tu bebe? SI NO *Aty bas dyi ya jiquej mi so 'min ca mu doctor yiris papel receta avadyes mi? Are so 'min are jam?*
- Si dice si
- a. Que tipo de medicamentos eran? Pastillas, inyectables o crema *Junis ca mo piñidye? Pastilla, are pochtacdye are ta'yacdyes?*
- b. Para que era el medicamento? Para dolor, para una infección o para falta de nutrientes? *Jedyeyes ca somi piñidye? Are areiredyes are japocjodyes are pandyedyes?*
- c. Recuerda si los medicamentos eran antibióticos, vitaminas o paracetamol? *Dyij tac se baj mi mo in piñidye in antibiótico are vitamina are paracetamol?*
- d. Recuerda el nombre de esos medicamentos? SI NO *Dyij ye ba mi tij mo piñidyes? Are dyij ye ba are jam?*
- e. Nombre recordado: _____
- f. Conseguiste esos medicamentos? SI NO *Dacaqui ca 'mi mo piñidye yoctyi'ya? Are daque' mi are jam' dacaquim?*
- g. Cuantos días te dijo el doctor a dar estos medicamentos a tu bebe? Un día no mas, 2 o 3 días, 7 días, 10 días, 2 semanas o mas? *Juñuc si jin ca 'mayedye mo doctor paj qui so 'maqui mo piñidye ava 'mi? Are yiris mayedye are 2 mayedye are 3 mayedye are 7 yamedye are 10 mayedye are 2 semanas are dam dye 2 semana?*
- *****
39. Después del parto tomaste cualquier medicamento del pueblo (sin tener receta)? SI NO *Aty basdyiya' mi tyei ca piñidye mi badyecansi (itsis receta)? Are tyei ca are jam?*
- Si dice si:
- a. Que medicamentos tomaste? *Jedye ca tyei piñidye mi badyecansi?*
- b. Por cuantos días tomaste estos medicamentos? Un día no mas, 2 o 3 días, 7 días, 10 días, 2 semanas o mas? *Juñuc si ca mayedye tyei mi mo piñidye badyecansi? Are yiris mayedye are 2 mayedye are 3 mayedye are 7 yamedye are 10 mayedye are 2 semanas are dam dye 2 semana?*
40. Después del parto tomaste cualquiera medicina del monte? SI NO *Tyei ca mi mo piñidye dārācansi aty basdyi ya mi? Are tyei mi are jam?*
- Si dice si:
- a. Que medicina del monte tomaste? *Jedye ca tij mo piñidyes dārācansi tyei si mi?*
- b. Si dice si: Por cuantos días tomaste esta medicina? Un día no mas, 2 o 3 días, 7 días, 10 días, 2 semanas o mas? *Junucsi ca mayedye tyei mi mo piñidye dārācansi? Are yiris mayedye are 2 mayedye are 3 mayedye are 7 yamedye are 10 mayedye are 2 semanas are dam dye 2 semana?*
41. Después de se nació (nombre bebe), le diste cualquier medicamento del pueblo (sin tener receta)? SI NO *Aty ya na ij ava' mi jityequicam piñidye badyecansi? Are jityequicam are jam?*
- Si dice si:
- a. Que medicamento le diste? *Jedye ca piñidye jichete mi?*
- b. Si dice si: Por cuantos días le diste estos medicamentos? Un día no mas, 2 o 3 días, 7 días, 10 días, 2 semanas o mas? *Junucsi ca mayedye jichete mi mo piñidye badyecansi? Are yiris mayedye are 2 mayedye are 3 mayedye are 7 yamedye are 10 mayedye are 2 semanas are dam dye 2 semana?*
42. Después de se nació (nombre bebe), le diste cualquiera medicina del monte? SI NO *Aty ya na ij ava' mi jityequicam piñidye dārācansi? Are tyei mi are jam?*
- Si dice si:
- a. Que medicina del monte le diste? *Jedye ca tij mo piñidyes dārācansi jichetes mi?*
- b. Si dice si: Por cuantos días le diste esta medicina? Un día no mas, 2 o 3 días, 7 días, 10 días, 2 semanas o mas? *Junucsi ca mayedye jichetes mi mo piñidye dārācansi? Are yiris mayedye are 2 mayedye are 3 mayedye are 7 yamedye are 10 mayedye are 2 semanas are dam dye 2 semana?*
43. Cuando se nació (nombre bebe), como se pareció? Era muy chiquito, era bastante grande, o era igual a los otros bebes recién nacidos que has visto? *Juñij ca 'nauj ava' mi yoctyi tum. Are dam miqūtyi, are dām dārti na ij are vajme' qui mundyē in?*

44. Ahora como se parece (nombre bebe)? Es muy chiquito, es muy grande, o es igual como los otros bebes de X meses/años que has visto? *Juñij quim cavaqui ava' mi yoctyi tum _____ yity tum ivaj? Are dam miqúity, are dām dārtyi na ij are vajme' qui mundye in?*

45. Y se enferma *mucho*, se enferma *poco*, jamás se enferma, o se enferma *igual* a los otros bebes X meses/años? *Juñij ca quim' cavaqui ava' mi yoctyi tum _____ X _____ yity tum ivaj? Are' arereij fer, are dam momo' areireij are' vaj cats yoctyi tum in arereij.*

46. Había una mujer en tu familia que se murió después del parto porque de un problema con el parto? SI NO *Me buty mo ya chātidy mi sāni aty basdyi ya vaty que jun coi jedye jijij? Are sāni are jam?*

Si dice si:

- Quien era? *Tyi ca' mo?*
- Hace cuantos años se murió ella? *Jedye ca yomodye jique sāni mo?*
- Que provocó su muerte? *Jun bu yi ca sāni?*

D. Enfermedades del bebe

47. Como esta tu bebe hoy día? Sano o enfermo? *Juñi ava mi hoy mayedye? Are rāsh are areirei are japacjoi?*

Si dice enfermo:

- Que síntomas tiene? *Jedye ca areirei mo are japacjoi mo? _____*
- Por cuantos días esta con _____? *Junucsi ca mayedye _____?*
- Estas dándole tratamiento? SI NO *Piñitute buty in? Are piñitute are jam?*
 - Si dice si:** Medicamentos o medicina del monte? *Mo pinidye' bādyecansi are dārācansi?*

Medicamentos

- Que tipo de medicamentos son? Pastillas, inyectables, crema, medicina para gusanos u otra? *Junis ca mo piñidye badyecansi? Pastilla, are pōch tac dye are pomada are oyadyes are yocsi? _____*
- Por cuantos días esta dándole estos medicamentos? _____ *Juñuc si buty mayedye jichete mi mo piñidye badyecansi mu ava mi?*
- Tienes estos medicamento o su paquete para mostrarme? *Mu'ya ca oij piñidyas paper mo ji'cavacdyes mi. Mu'yam are itsij mi?*
 - Si dice si:** Escribe el nombre del medicamento mostrado aquí: _____
 - Si dice no:** Recuerda el nombre de esos medicamentos? SI NO *Dyij ye ba mi tij mo piñidyas? Are dyij ye ba are jam?*
 - Nombre recordado: _____

Medicina del monte

- Que medicina del monte? *Jedye' ca tij mo pinidye' dārācansi? _____*
- Por cuantos días estas dándole esta medicina? *Junucsi buty mayedye jichete mi mo piñidye dārācansi mu ava mi?*

E. Enfermedades de la mama

48. Como sientas hoy día? Sana o enferma? *Ju'ñi to'o mi? Are rāsh are areirei are japacjoi?*

Si dice enferma:

- Que síntomas tienes? *Jedye ca areirei mo are japacjoi mo mi?*
- Por cuantos días esta con _____? *Junucsi ca mayedye _____?*
- Estas tomando tratamiento? SI NO *Tyei' ca pinidye' mi? Are tyei are jam tyei?*
 - Si dice si:** Medicamentos o medicina del monte? *Mo pinidye' bādyecansi are dārācansi?*

Medicamentos

- Que tipo de medicamentos son? Pastillas, inyectables, crema, medicina para gusanos u otra? *Junis ca mo piñidye badyecansi? Pastilla, are pōch tac dye are pomada are oyadyes are yocsi? _____*
- Por cuantos días esta dándole estos medicamentos? _____ *Juñuc si buty mayedye tyei ca mi mo piñidye badyecansi?*
- Tienes estos medicamento o su paquete para mostrarme? *Mu'ya ca oij piñidyas paper mo ji'cavacdyes mi. Mu'yam are itsij mi?*
 - Si dice si:** Escribe el nombre del medicamento mostrado aquí: _____

b. **Si dice no:** Recuerda el nombre de esos medicamentos? SI NO *Dyij ye ba mi tij mo piñides? Are dyij ye ba are jam?*

i. Nombre recordado: _____

Medicina del monte

4. Que medicina del monte? *Jedye' ca tij mo pinidye' dārācansi?* _____

5. Por cuantos días estas dándole esta medicina? *Junucsi buty mayedye tyei ca mi mo piñidye dārācansi?*

49. Estas con tu regla ahora? SI NO *Ja'pacjoba' buty mi? Are ja'pacjoba' mi are jam?*

50. Ha regresado tu regla desde se nació (nombre bebe)? SI TODAVIA NO *Aty ca ja'pacjoba'mi juñdye ya ca yoqui dyem na ij ya ava mi?*

Si dice si

a. Hace cuantos meses regreso tu regla? (también confirma la edad del bebe de cuando regresó)

b. Show lunar calender: Anoche la luna era _____. Como era la luna la ultima vez que tuviste tu regla: llena, media llena, nueva, o no salió? _____ *Are yocsi can ja'pacjoba' mi aty dyuts ya ivaj are' aradye dyuts are' na ejban ya are tomodye çan?*

51. Uso contraceptivo? Si/No

a. Badycansi o Duracansi

A2. 24-Hour Dietary Recall

DIETA DE LA BEBE

Nombre Mama: _____ Apellidos: _____ MidPID: _____
 Nombre Infante _____ Apellidos: _____ MidPID: _____
 Sexo infante _____ Edad infante _____
 Comunidad: _____ Entrevistador: _____
 Fecha: _____ Hora: _____

Voy a preguntarte sobre todo lo que (nombre bebe) tomó y comió ayer, desde que se despertó en la mañana hasta que se acostó por la noche.

1. Como se alimentó (nombre bebe) ayer: (1) chupó leche no más, o (2) chupó y se comió, o (3) no chupo nada de leche y se comió no más?
 - a. Si responde (1): (Nombre bebe) comió o tomó algo más que la leche en las ultimas 3 días?
 - b. Si responde (3): (Nombre bebe) no chupó nada de leche en las ultimas 3 días?
 - i. Clarifica con la mama: Entonces tu bebe ya dejo de chupar o sigue chupando de vez en cuando?
 - ii. Hace cuando (nombre bebe) dejó de chupar?
2. ¿Ayer le diste a (nombre bebe) jarabe? (1) Sí (2) No (8) No sabe. *Munja ca' so'mete mi jarabe ava' mi? (1) so'mete (2) jam so'mete (3) jam chi*
3. ¿Ayer le diste a (nombre bebe) suero? (1) Sí (2) No (8) No sabe *Munja ca' so'mete mi suero ava' mi? (1) so'mete (2) jam so'mete (3) jam chi*
4. ¿Ayer le diste a (nombre bebe) uña de gato? (1) Sí (2) No (8) No sabe *Munja ca' so'mete mi ovetos ojñi ava' mi? (1) so'mete (2) jam so'mete (3) jam chi*

5	LIQUIDOS	S	No	N	Pedir a la mama cuantas veces tomó (<u>nombre bebe</u>) este liquido	
A	¿Ayer tu bebe tomó agua pura? <i>Jityete ca o'jni munja mi?</i>	1	0	8	A. (1) <i>Junucsi qui ca jityetem o'jni? ()</i> <i>are jam chi ()</i>	a. Tomo de cuchara, taza, erepa o galon? b. Cantidad tomado cada vez: c. Cantidad tomado en total:
B	¿Gaurapo? Qué tipo? (agua con azúcar, yupi, refresco, otro) <i>Jityete ca refresco munja mi? Juñis ca refresco? Escribelo:</i>	1	0	8	B. 1) <i>Junucsi qui ca jityetem o'jni? ()</i> <i>are jam chi ()</i>	a. Tomo de cuchara, taza, erepa o galon? b. Cantidad tomado cada vez: c. Cantidad tomado en total:
C	Chicha de yuca, chicha de pere, po'nacdye yuca, po'nacdye pere, chicha de maíz, chicha de arroz? <i>Shoc'dye o'yi, shoc'dye pere, po'nacdye o'yi, po'nacdye pere, co'ratiyi, are shoc'dye arrosh</i>	1	0	8	C. 1) <i>Junucsi qui ca jityetem o'jni? ()</i> <i>are jam chi ()</i>	a. Tomo de cuchara, taza, erepa o galon? b. Cantidad tomado cada vez: c. Cantidad tomado en total:
D	Payuje? <i>Payuje?</i>	1	0	8	D. 1) <i>Junucsi qui ca jityetem o'jni? ()</i> <i>are jam chi ()</i>	a a. Tomo de cuchara, taza, erepa o galon? b. Cantidad tomado cada vez: c. Cantidad tomado en total:
E	¿Leche de vaca, leche en polvo, o yogur?	1	0	8	D. 1) <i>Junucsi qui ca jityetem o'jni? ()</i>	a. a. Tomo de cuchara, taza, erepa o galon?

					<i>are jam chi ()</i>	b. Cantidad tomado cada vez: c. Cantidad tomado en total:
F	Formula? <i>Jityete ca formula munja mi?</i>	1	0	8	E. 1) <i>Junucsi qui ca jityetem o'jni? ()</i> <i>are jam chi ()</i>	a. a. Tomo de cuchara, taza, erepa o galon? b. Cantidad tomado cada vez: c. Cantidad tomado en total:
G	Jugo de caña? <i>Fi viros?</i>	1	0	8	F. 1) <i>Junucsi qui ca jityetem o'jni? ()</i> <i>are jam chi ()</i>	a. a. Tomo de cuchara, taza, erepa o galon? b. Cantidad tomado cada vez: c. Cantidad tomado en total:
H	Soda? Café? <i>Soda? Cafê?</i>	1	0	8	G. 1) <i>Junucsi qui ca jityetem o'jni? ()</i> <i>are jam chi ()</i>	a a. Tomo de cuchara, taza, erepa o galon? b. Cantidad tomado cada vez: c. Cantidad tomado en total:
E	Cualquier otro líquido? <i>Are yocsi jedye jityete mi munja? Jedye ca?</i> Escribelo:	1	0	8	H. 1) <i>Junucsi qui ca jityetem o'jni? ()</i> <i>are jam chi ()</i>	a. a. Tomo de cuchara, taza, erepa o galon? b. Cantidad tomado cada vez: c. Cantidad tomado en total:

5. Antes de que tu bebe tomó (líquidos reportados) los pusiste en tu boca primero, antes de dárselo? *Are chu can are jam?*
a. **Si dice si:** Con todos los líquidos dados lo hiciste?
b. **Si dice no:** Con cuales líquidos lo hiciste y con cuales no lo hiciste?

ALIMENTOS SOLIDOS Y SEMI-SOLIDOS INCLUSIVO A JONA Y CHINSI

Paso 1: Pide a la mama que comió su bebe durante el día, desde se despierta su bebe por la mañana hasta que se acosta por la noche. Para cada alimento, pide también (1) como se preparaba (al fuego, hervido, ahumado, frito en aceite, frito en manteca, crudo); (2) si había echado sal, manteca o aceite; (3) si el bebe comió su propio plato o compartió con la mama; (4) la cantidad total dado estimado por cuchara, erepa o plato. Circula el alimento mencionada por la mama en la parte del “Paso 2” a que corresponde y marca “SI” para eso grupo.

Paso 2: Pasa por la lista de alimentos por grupo y otra vez pide a la mama si su bebe comió cualquier de esos otros alimentos ayer. Igual para cada alimento nuevo que recuerda la mama, pídelo también (1) como se preparaba (al fuego, hervido, ahumado, frito en aceite, frito en manteca, crudo); (2) si había echado sal, manteca o aceite; (3) si el bebe comió su propio plato o compartió con la mama; (4) la cantidad total estimado por cuchara, erepa o plato.

	<p>PASO 1</p> <p>a) Piensa en cuando tu bebe se despertó ayer. ¿Se desayunó con algo despues de despertar? ¿Dígame todo que comió cuando se despertó. <i>SIGUE</i> ¿Nada más? <i>HASTA LA MAMA DICE NADA MAS.</i></p> <p>b) Comió algo más durante la mañana, después de despertar y desayunar? <i>DICE QUE SI:</i> Que más se comió por la mañana. <i>SIGUE</i> ¿Nada mas? <i>HASTA LA MAMA DICE NADA MAS.</i></p> <p>c) Comió algo por el mediodía, para el almuerzo? Que comió por el mediodía? <i>DICE QUE SI:</i> Dígame todo que se comió por el mediodía. <i>SIGUE</i> ¿Nada mas? <i>HASTA LA MAMA DICE NADA MAS.</i></p> <p>d) Comió algo por la tarde? <i>DICE QUE SI:</i> Dígame todo que se comió por la tarde. <i>SIGUE</i> ¿Nada mas? <i>HASTA LA MAMA DICE NADA MAS.</i></p> <p>e) Comió algo con el anochecer? <i>DICE QUE SI:</i> Dígame todo que se comió con el anochecer. <i>SIGUE</i> ¿Nada mas? <i>HASTA LA MAMA DICE NADA MAS.</i></p> <p>f) Comió algo por la noche, antes de acostarse? <i>DICE QUE SI:</i> Dígame todo que se comió con el anochecer. <i>SIGUE</i> ¿Nada mas? <i>HASTA LA MAMA DICE NADA MAS.</i></p> <p>g)</p>
	<p>PASO 2</p> <p>Ayer tu bebe comió algo de:</p>
13)	<p>Alimentos de granos básicos (1) SI (2) NO (3) NO SABE</p> <p>a) ARROZ <i>arros</i> b) FIDEO c) PAN d) BIZCOCHO e) GALLETAS DE SAL <i>galletas jise'quis</i> f) PAN DE ARROZ <i>tara bizcocho</i> g) HARINA FRITA <i>harina chaca' nactyi</i></p>
14)	<p>Alimentos básicos verduras y frutas (1) SI (2) NO (3) NO SABE</p> <p>a) PLATANO VERDE <i>pújsi'</i> b) PLATANO MEDIO MADURO <i>váshsi'</i> c) PLATANO MADURO <i>ijtsis</i> d) YUCA <i>o'yi</i> e) CHOCLO f) OTROS RAIZES DEL PARIENTE (AJIPA, CAMOTE BLANCO, ETC.) <i>cojço are' binca' are' ca'in are ijtsidyei'si are çhames</i></p>

15)	Frutas ricas en Vitamina A	(1) SI	(2) NO	(3) NO SABE
	a) MANGO <i>manco</i> b) PAPAYA <i>pofi</i> c) MANAI <i>váij</i> d) OTRO			
16)	Frutas no ricas en Vitamina A	(1) SI	(2) NO	(3) NO SABE
	a) TORONJA b) NARANJA c) SANDIA d) CHEREMOYA e) PINA <i>merequi</i> f) COCO g) PALTA h) CEBOLLA i) TOMATE j) OTRA FRUTA DEL MONTE (PAQUIO, PACAI, ACHACHAIRU, GUAYABA)			
17)	Verduras ricas en Vitamina A	(1) SI	(2) NO	(3) NO SABE
	a) ZAPAYA <i>shobo</i> b) ZANAHORIA			
18)	Carne (<i>escriba la clase</i>)	(1) SI	(2) NO	(3) NO SABE
	a) CARNE <i>shush</i> b) GALLINA <i>atava</i> c) AVE <i>jaichtyi</i> d) TORTUGA <i>meme</i> e) PETA <i>qui'bo</i> f) COLA DE LARGATO <i>co'shifores codi mu</i>			
19)	Organos	(1) SI	(2) NO	(3) NO SABE
	a) HIGADO <i>nacaty</i> b) RINONES <i>ca-i-dye</i> c) CORAZON <i>cojtyi</i> d) BOFE <i>shababaty</i> e) OTRO			
20)	Pescado (<i>escriba la clase</i>) <i>Con espina o sin espina?</i>	(1) SI	(2) NO	(3) NO SABE
21)	Huevos	(1) SI	(2) NO	(3) NO SABE
	a) GALLINA <i>atavas</i> b) PATO <i>patos</i> c) PETA <i>qui'bo</i> d) TORTUGA <i>meme</i> e) LARGATO <i>co'shifores</i> f) OTRO			
22)	Legumbres	(1) SI	(2) NO	(3) NO SABE
	a) FRIJOLES <i>co'rishi</i> b) GRANDULES <i>gandul co'rishi</i> c) LENTEJAS <i>lentejas</i> d) SEMILLA DE MANI <i>dabaj</i>			
23)	Dulces preparados	(1) SI	(2) NO	(3) NO SABE

	a) Galletas dulces b) Cofiti c) Chupetas
L	Grasa (1) SI (2) NO (3) NO SABE La jona que comió tu bebe ayer tenia manteca o aceite echado? Si o no? Comio manteca con el platano o la yuca? <i>Mo jona jebe si ava mi munja chãdyetumsi'ca'are aceitetumsi'ca'?</i> <i>Chocojvaqui chãdye pere tum are' oyi tum?</i> NO HAY QUE ESTIMAR
M	Sal (1) SI (2) NO (3) NO SABE Echaste sal a la jona o shush o tabedye que comió tu bebe ayer? <i>Bijtyca jicoj jona can mi are' shush bij tyem are tabedye bij tyem?</i> NO HAY QUE ESTIMAR
N	Ayer tu bebe comió queso? <i>Munja ca' jebe' ava' mi queso?</i> (1) SI (2) NO (3) NO SABE
O	Ayer tu bebe comió lechuga? <i>Munja ca' jebe' ava' mi lechuga?</i> (1) SI (2) NO (3) NO SABE
P	Cualquier otro alimentos (escribelo)

6. Antes de que tu bebe comió (alimentos reportados), tú los pusiste en tu boca primero, antes de dárselo? *Are çhu can are jam?*
- Si dice si:** Con todos los alimentos lo hiciste?
 - Si dice no:** Con cuales alimentos lo hiciste y con cuales no lo hiciste?

A3. Prospective Follow-Up Interview

Prospective Follow-Up Interview

Entrevista de Ronda Mama y Infante

Comunidad: _____ Entrevistador: _____ Fecha: _____
Nombre Mama: _____ Apellidos: _____ MidPID: _____
Nombre Infante _____ Apellidos: _____ MidPID: _____
Estado de alimentación del infante previo: (1) Leche exclusiva (2) Chupa y come (3) Dejó de chupar
Estado de alimentación del infante ayer: (1) Leche exclusiva (2) Chupa y come (3) Dejó de chupar
Cambio de estado de alimentación? SI NO Alimentos solidos y semi-solidos Solo liquidos Ambos
Estado reproductiva de mama previo: (1) Amenorrhea de lactancia (2) ciclando (3) embarazada
Estado reproductiva de mama hoy: (1) Amenorrhea de lactancia (2) ciclando (3) embarazada

Compleja las hojas de antropometría y dieta infantil antes de proceder. Pon “NS” como respuesta a cualquiera pregunta si la mama dice “no sabe” (*jam chi*) y “NR” como respuesta a cualquiera pregunta si la mama no responde.

➔ Si el bebe todavía no come y sigue chupando no más: pasa a las pregunta 8

➔ A. Alimentación del bebe. Si en la última entrevista el bebe chupó no mas y ahora la mama dice que ya le dio a comer o tomar algo, sigue con las preguntas 1-7

52. Que fue la primera cosa que le dio a comer (nombre bebe)? *Jedye buty säcsete tashche* (nombre bebe)?

53. Hace cuantos días le dio a comer (respuesta #1)?

54. Antes de dar a tu bebe (respuesta #1) por la primera vez, pusiste la comida tu boca antes de dárselo? SI NO *Tash che'mi mu'ava'mi jun'buyi'buty sometemi säcsedye ava mi are çhu can si cache'nac si so'metej mi ava'mi? Cache'nac are jam?*

55. Ahora que (nombre bebe) ha empezado a comer, le has dado a tomar chicha, gaurapo, payuje o agua también? (Circulo todos que le ha dado)

56. Pones (chicha, guarapo, payuje, agua) en la boca antes de dárselo?

57. Porque empezaste a dar (respuesta #1) a (nombre bebe)? *Jun buty mi tiyacchuti _____ ivaj ava'mi _____ jjebeyetemi?*

58. Alguien te dijo que tenias que alimentar a (nombre bebe)? SI NO *Are yocsi jin in paj qui jisacsetemi ava mi'? Jin ca' in are' jam?*

Si dice si:

a. Quien era que te lo dijo? (Apunta el nombre, sexo, relación, y si era relación de su familia o del lado de su marido. Si no era una relación apunta quien era). *Tyi ca jimi?*

b. Te dijo porque tenias que hacerlo? SI NO *Jun'buty jin'in paj qui me jem'? Jin ca in' are' jam jin' in?*

i. Si dice si: Que era la razón que te dijo? *Jun'buty jin'in paj qui jisäcsaqui ava'mi?*

B. Actividades de la familia

59. *Yocsi çan semana judyeya yocsi çan _____ jiyi'si judyeya munja', caritaqui ca quijodye çan mi?* SI NO *Caritaqui ca quijodye çan mi are jam?*

a. Si dice si: *Juñucsi ca mayedye caritaqui mi quijodye' can mi?*

60. *Yocsi çan semana judyeya yocsi çan _____ jiyi'si judyeya munja' shupqui ca oyas comunidad yas mi?* *Move ca' jicajj mi are jam?*

Si dice si:

- a. *Jana' ca jįcaįj mi?*
- b. *Juñucsi ca mayedye move mi?*
- c. *Juntaqui ca move mi?*
- d. *Ava tum dyeca mi? Are' ava tum mi are jam?*
 - i. Si dice no: *Tyi ca' ćuj cabun ava' mi?*
- e. *Vämtyi tum dyeca mi? Are vomtyi mi are jam?*

61. *Oįj' quin' _____ . Yocsi ćan semana judyeya yocsi ćan _____ jįyi'si judyeya munja' shupqui ya'vämtyi mi uyaty comunidad yaty mi na tacya'ji'yacati'mi oya'?' Muve ca'įcaįj mu are jam?*

Si dice si:

- a. *Jana' ca jįcaįj mu?*
- b. *Juñucsi ca mayedye muve?*
- c. *ca muve mu?*

C. Enfermedades del bebe

62. *Juñi ava mi hoy mayedye? Are rāsh are areirei are japacjoi?*

Si dice enfermo:

- a. *Jedye ca areirei mo are japacoįj mo? _____*
- b. *Junucsi ca mayedye _____ ?*
- c. *Piñitute buty in? Are piñitute are jam?*
 - i. *Mo pinidyē' bādyecansi are dārācansi?*

Medicamentos

- 1. *Junis ca mo piñidyē bādyecansi? Pastilla, are jarabe are pōch tac dye are pomada are oyadyes are yocsi? _____*
- 2. *si buty mayedye jichete mi mo piñidyē bādyecansi mu ava mi? _____*
- 3. *Mų'ya ca oįj piñidyēs paper mo ji'cavacdyēs mi. Mu'yam are įtsįj mi?*
 - a. **Si dice si:** nombre del medicamento mostrado: _____
 - b. **Si dice no:** *Dyįj ye ba mi tij mo piñidyēs? Are dyįj ye ba are jam?*
 - i. Nombre recordado: _____

Medicina del monte

- 4. *Jedye' ca tij mo pinidyē' dārācansi? _____*
- 5. *Junucsi buty mayedye jichete mi mo piñidyē dārācansi mu ava mi?*

63. *Jeñe yocsi can _____ semanas atsįj ya tśun tasch che eje vacdye ca tśun ava mi? Are tśun are jam?*

Si dice si:

- a. *Junucsi ca mayedye eje vacdye ava mi?*
- b. *Somete ca' pinidyē' ava mi? Somete are jam somete?*
 - i. Si: *Mo pinidyē' bādyecansi are dārācansi?*

***Si dice medicamentos:**

- 1. *Junis' ca mo' pinidyē' bādyecansi? Are paracetemol are jarabe are antibiōtico are pōch tacdye are oyadyes are yocsi?*
- 2. *Mų'ya ca mo pinidyē mi are chā'vās mo'ya paj qui ji'cave mi? Mo'ya are įtsįj?*
 - a. SI: Nombre del medicamento mostrado: _____
 - b. NO: Aun conoce el nombre del medicamento?
SI _____ NO _____
- 3. *Junucsi' ca mayedye somete ava mi pinidyē' bādyecansi?*

64. *Jeñe yocsi can _____ semanas atsįj ya tśun tasch che tśun buty vāsvacdye'?' Are tśun are jam?*

Si dice si

- a. *Por cuantos días estuvo con diarrea?*
- b. *Somete ca' pinidyē' mi? Somete are jam somete?*
 - i. **Si dice si:** *Mo pinidyē' bādyecansi are dārācansi?*

***Si dice medicamentos**

- 1. *Junis' ca mo' pinidyē' bādyecansi? Are paracetemol are jarabe are antibiōtico are pōch tacdye are oyadyes are yocsi?*
- 2. *Mų'ya ca mo pinidyē mi are chā'vās mo'ya paj qui ji'cave mi? Mo'ya are įtsįj?*
 - a. SI: Nombre del medicamento: _____

- b. NO: Aun conoce el nombre del medicamento?
SI _____ NO _____
3. *Junucsi' ca mayedye somete ava mi pinidyé' bädyecansi?*

65. *Jeñe yocsi can _____ semanas atsij ya tsun tasch che ajai ca ava mi? Are ajai ava mi are jam?*

Si dice si:

- a. Por cuantos días estuvo con gripe?
b. *Somete ca' pinidyé' ava mi? Somete are jam somete?*
i. **Si dice si:** *Mo pinidyé' bädyecansi are däräcansi?*

***Si dice medicamentos**

1. *Junis' ca mo' pinidyé' bädyecansi? Are paracetamol are antibiótico are poch tacdye are oyadyes are yocsi?*
2. *Mu'ya ca mo pinidyé mi are chä'väs mo'ya paj qui ji'cave mi? Mo'ya are itsij?*
a. SI: Nombre del medicamento: _____
b. NO: Aun conoce el nombre del medicamento? SI _____ NO _____
3. *Junucsi' ca mayedye somete ava mi pinidyé' bädyecansi?*

66. *Me' ca tsu mo' yocsi japacyodye somete ava mi jarabe are antibióticos are poch mi are oyedyes tyei mi jeñe _____ semanas atsij ya tsun tasch che? Are somete antibiotics are jam. Are poch tacdye are jam. Are somete oyedyes mi are jam?*

Si dice si:

- a. *Jedyé' ca japacjodye mo ya ava mi jiquej?*
b. Por cuantos días tenía esos síntomas?
c. *Mu'ya ca mo pinidyé mi are chä'väs mo'ya paj qui ji'cave mi? Mo'ya are itsij?*
i. SI: Nombre del medicamento: _____
ii. NO: Aun conoce el nombre del medicamento? SI _____ NO _____
d. *Junucsi' ca mayedye somete ava mi pinidyé' bädyecansi?*
e. *Jun'dye ya' ca' aty jam somete ava mi mo pinidyé' bädyecansi?*

67. Desde la última vez que te visitamos, le dificultaba tu bebe a chupar? SI NO?

Si dice si:

- a. No podía chupar o solo chupo poco?
b. Dime porque no podía chupa o solo chupo poco?
c. Sigue chupando poco? SI NO
i. **Si dice si:** Por cuantos días no está chupando tanto?
ii. **Si dice no:** Por cuantos días no podía chupar tanto?
d. Hiciste algo para ayudarle chupar? SI NO
i. **Si dice si:** que hiciste?
e. Estas dándole más comida, chicha, payuje o otros líquidos porque no estaba chupando tanto? SI NO
f. Piensas que tienes menos leche porque tu bebe no está chupando tanto? SI NO

D. Enfermedades de la mama

68. *Ju'ni to'o mi? Are räsh are areirei are japacjoi?*

Si dice enferma:

- a. *Jedye ca areirei mo are japacjoi mo mi?*
b. *Junucsi ca mayedye _____ ?*
c. *Tyei' ca pinidyé' mi? Are tyei are jam tyei?*
i. **Si dice si:** *Mo pinidyé' bädyecansi are däräcansi?*

Medicamentos

1. *Junis ca mo piñidyé bädyecansi? Pastilla, are poch tac dye are pomada are yocsi?*
2. *Juñuc si buty mayedyé tyei ca mi mo piñidyé bädyecansi?*
3. *Mu'ya ca oij piñidyés paper mo ji'cavacdyes mi. Mu'yam are itsij mi?*
a. **Si dice si:** Nombre del medicamento: _____
b. **Si dice no:** *Dyij ye ba mi tij mo piñidyés? Are dyij ye ba are jam?*
i. Nombre recordado: _____

Medicina del monte

4. *Jedyé' ca tij mo pinidyé' däräcansi?*
5. *Junucsi buty mayedyé tyei ca mi mo piñidyé däräcansi?*

69. Jeñe yocsi can _____ semanas atsij ya tšun tasch che eje vacdye ca tšun mi? Are tšun are jam?

Si dice si:

- a. Junucsi ca mayedye eje vacdye mi?
- b. Tyei' ca' pinidye' mi? Tyei are jam tyei?
 - i. Si dice si: Mo pinidye' bädvecansi are däräcansi?

*Si dice medicamentos:

1. Junis' ca mo' pinidye' bädvecansi? Are paracetamol are jarabe are antibiótico are pôch tacdye are oyadyes are yocsi?
2. ca mo pinidye mi are chä'väs mo'ya paj qui ji'cave mi? Mo'ya are itsij?
 - a. SI: Nombre del medicamento mostrado aquí: _____
 - b. NO: Aun conoce el nombre del medicamento?
SI _____ NO _____
3. Junucsi' ca mayedye tyei mi pinidye' bädvecansi?

70. Jeñe yocsi can _____ semanas atsij ya tšun tasch che tšun buty väsvacdye'? Are tšun are jam?

Si dice si:

- a. Por cuantos días estuviste con diarrea?
- b. Tyei' ca' pinidye' mi? Tyei are jam tyei?
 - i. Si: Mo pinidye' bädvecansi are däräcansi?

*Si dice medicamentos

1. Junis' ca mo' pinidye' bädvecansi? Are paracetamol are jarabe are antibiótico are pôch tacdye are oyadyes are yocsi?
2. NO Mu'ya ca mo pinidye mi are chä'väs mo'ya paj qui ji'cave mi? Mo'ya are itsij?
 - a. SI: Nombre del medicamento mostrado aquí: _____
 - b. NO: Aun conoce el nombre del medicamento? SI _____ NO _____
3. Junucsi' ca mayedye tyei mi pinidye' bädvecansi?

71. Jeñe yocsi can _____ semanas atsij ya tšun tasch che ajai ca mi? Are ajai mi are jam?

Si dice si:

- a. Por cuantos días estuviste con gripe?
- b. Tyei' ca' pinidye' mi? Tyei are jam tyei?
 - i. Si: Mo pinidye' bädvecansi are däräcansi?

*Si dice medicamentos

1. Junis' ca mo' pinidye' bädvecansi? Are paracetamol are jarabe are antibiótico are pôch tacdye are oyadyes are yocsi?
2. Mu'ya ca mo pinidye mi are chä'väs mo'ya paj qui ji'cave mi? Mo'ya are itsij?
 - a. SI: Nombre del medicamento mostrado aquí: _____
 - b. NO: Aun conoce el nombre del medicamento?
SI _____ NO _____
3. Junucsi' ca mayedye tyei mi pinidye' bädvecansi?

72. Me' ca tšu mo' yocsi japacyodye tyei' ca mi antibióticos are pôch mi are oyedyes tyei mi jeñe _____ semanas atsij ya tšun tasch che? Are tyei antibiotics are jam. Are pôch tacdye are jam. Are tyei oyedyes mi are jam?

Si dice si:

- a. Jedye' ca japacjodye mo ya mi jiquej?
- b. Por cuantos días estuviste con esos síntomas?
- c. Mu'ya ca mo pinidye mi are chä'väs mo'ya paj qui ji'cave mi? Mo'ya are itsij?
 - i. SI: Escribe el nombre del medicamento mostrado aquí: _____
 - ii. NO: Aun conoce el nombre del medicamento? SI _____ NO _____
- d. Junucsi' ca mayedye tyei mi pinidye' bädvecansi?

73. Jeñe yocsi can _____ semanas atsij ya tšun tasch che taca buty çoqui are'redye mi tashin? Are taca are jam?

Si dice si:

- a. Yoqui dyem tšun ya areredye jitsi yaqui ava mi? Are are'rei mi are jam?
- b. Taca are'rei mi? Are taca are jam?
- c.
 - i. Si dice si: Junucsi' ca mayedye arerei mi?
 - ii. Si dice no: Junucsi' ca mayedye arerei mi?
 - iii. Si dice no: Jundye ya ca jiquej ji'jiyaqui mo are'redye?

74. *Jeñe yocsi can _____ semanas atsij ya t̄sun tasch che japacjoijmi juñis êui t̄sun areredye jitsi yaqui ava mi?*

Si dice si

a. *Jedye ca areredye'?*

b. *Junucsi' ca mayedye arerei' mi?*

c. *Yej coi' ca' arerei'?* Yej coi' are jam?

i. NO: Hace cuantos días te quito el problema?

75. *Ja'pacjoba' buty mi? Ivaj j̄j̄ cai'si? Are ja'pacjoba' mi are jam?*

76. *Yoqui dyem aty atsij ya t̄sun ja'pacjoba' mi? Are ja'pacjoba' mi are jam?*

77. Fechas de ultima menstruación (estimada calendario/reportada): ____/____/____ - ____/____/____

78. Uso contraceptivo? Si/No

a. Badyecansi o Duracansi

A4. Behavioral Observation Codes

Mom state	Code	Infant state	Code
Dormiendo	D	Dormiendo	D
Sentado	S	Alerto (lying down)	AL
Parado	P	Sienta con ayuda	SA
Squatting	SQ	Sienta a si mismo	SM
Caminando	C	Gatea (vodoidoi)	V
Absent*	AB	Para con ayuda	PA
		Para a si mismo	PM
		Camina con ayuda	CA
		Camina a si mismo	CM
		Llora	LLO
M/D Proximity	Code		
Direct		0	
Arms reach		1	
Within sight		2	
Out of sight		3	
Mom action	Code	Infant Interaction	Code
Talking (charlar)	C	Held by	Hold
Prepare food	PF	Rocked by	Rock
Make sarai	S	Groomed by	GR
Make mats (shumi)	SH	Played with by	Play
Make jatata	J	Breastfed	BF
Taca (pound rice/other food)	T	Watched by	W
Eat	E		
Wash clothes (putaqui)	P		
Clean house/dishes (limpiar)	L		
Sew/mend clothes (ropa)	R		
Chop with axe (hacha)	H		
Machete	M		
Carry load (llevar)	LL		
Misc household	MH		
Groom self	GS		
Feed Dependent	FD		
Groom Dependent	GD		
Hold Dependent	HD		
Play Dependent	PD		
Watch Dependent	WD		
Allomothe	ALLO		

Prospective Subject Sampling Schedules

Figure 1. Infant anthropometric measures collected for each subject, by feeding status and age (months)

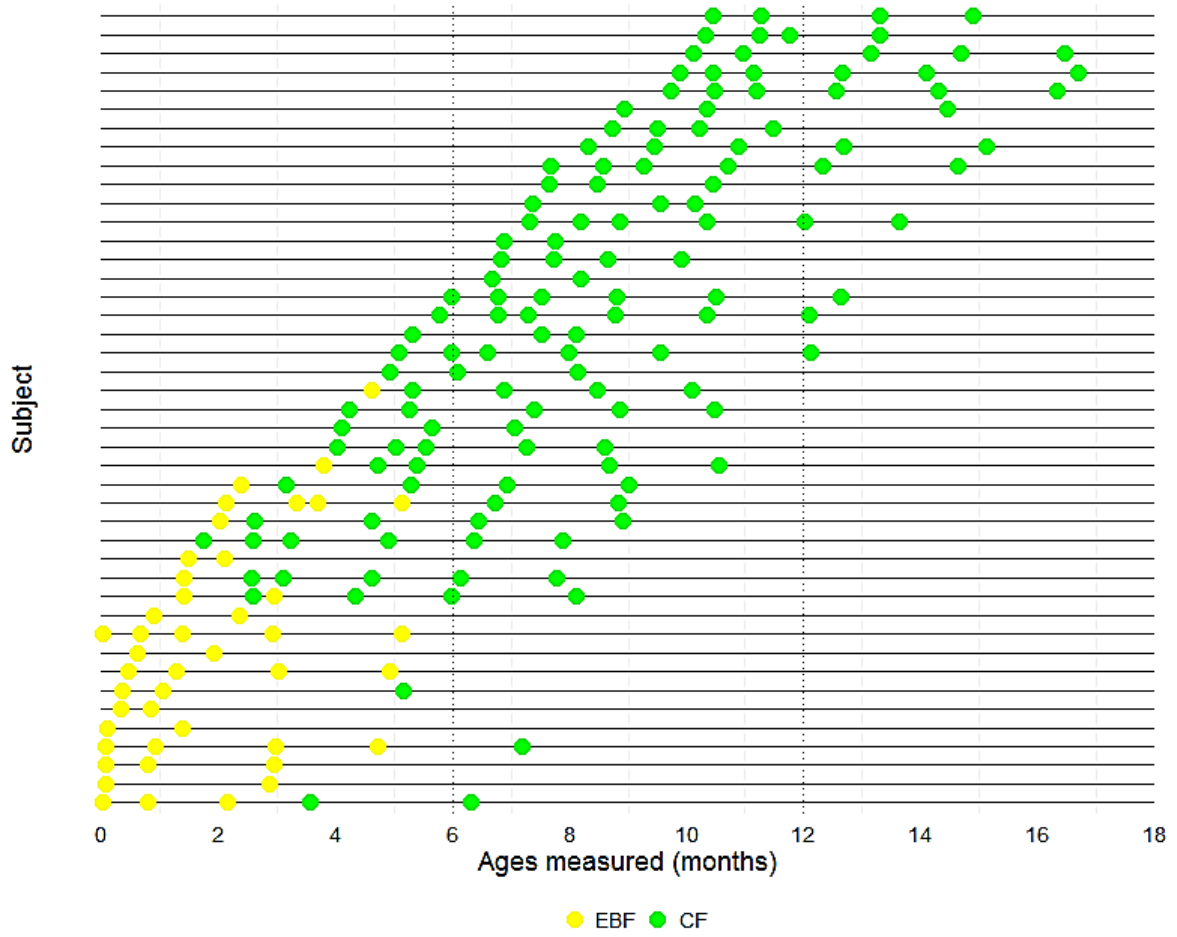


Figure 2. Maternal anthropometric and urine samples collected for each subject, by feeding status and month postpartum

